REVIEW
J Vet Intern Med 2009;23:1151–1163

Porcine Circovirus Type 2 and Porcine Circovirus-Associated Disease

J. Gillespie, T. Opriessnig, X.J. Meng, K. Pelzer, and V. Buechner-Maxwell

Porcine circovirus type 2 (PCV2) belongs to the viral family Circoviridae and to the genus *Circovirus*. Circoviruses are small, single-stranded nonenveloped DNA viruses that have an unsegmented circular genome. PCV2 is the primary causative agent of several syndromes collectively known as porcine circovirus-associated disease (PCVAD). Many of the syndromes associated with PCVAD are a result of coinfection with PCV2 virus and other agents such as *Mycoplasma* and porcine reproductive and respiratory syndrome virus. PCV2 infection is present in every major swine-producing country in the world, and the number of identified cases of PCVAD is rapidly increasing. In the United States, the disease has cost producers an average of 3–4 dollars per pig with peak losses ranging up to 20 dollars per pig. The importance of this disease has stimulated investigations aimed at identifying risk factors associated with infection and minimizing these risks through modified management practices and development of vaccination strategies. This paper provides an overview of current knowledge relating to PCV2 and PCVAD with an emphasis on information relevant to the swine veterinarian.

Key words: Epidemiology; Immunohistochemistry; Infectious diseases; Microbiology; Respiratory tract; Viral virulence mechanisms; Virology general.

Porcine circovirus type 2 (PCV2) is the primary causative agent of several syndromes collectively known as porcine circovirus-associated disease (PCVAD). Although many other common organisms contribute to the clinical signs associated with PCVAD, PCV2 is the common link among the diseases and therefore it is vital to understand the biology of the virus for control of the disease. PCVAD is a globally emerging disease that is having a huge impact on swine-producing countries and is arguably the most economically important disease affecting the global swine industry today. The British Pig Executive national herd mortality data for finisher pigs in 2006 found that mortality increased from 3.3 to 6.5% when PCVAD began affecting herds. Fifty percent of affected farms had mortality rates over 9.7%.[1]

PCV2 infection is present in every major swine-producing country in the world and the number of cases of PCVAD is rapidly increasing. In 1998, University of Guelph's Animal Health Laboratory reported a pathologic diagnosis of <20 cases. In contrast, 350 cases were reported in 2005 by the same laboratory and these numbers continue to increase.[2]

In the United States, the disease has cost producers an average of 3–4 dollars per pig with peak losses ranging up to 20 dollars per pig. The importance of this disease has stimulated investigations aimed at identifying risk factors associated with infection and minimizing these risks through modified management practices and development of vaccination strategies. This paper provides an overview of current knowledge relating to PCV2 and PCVAD with an emphasis on information relevant to the swine veterinarian.

Abbreviations:

AASV	American Association of Swine Veterinarians
GAG	glycosaminoglycans
IHC	immunohistochemistry
ISH	in situ hybridization
nt	nucleotide
ORF	open reading frame
PCV	porcine circovirus
PCV1	porcine circovirus type 1
PCV2	porcine circovirus type 2
PCVAD	porcine circovirus-associated disease
PDNS	porcine dermatitis and nephropathy syndrome
PMWS	postweaning multisystemic wasting syndrome
PPV	porcine parvovirus
PRRSV	porcine reproductive and respiratory syndrome virus
USDA	U.S. Department of Agriculture

History of the Porcine Circovirus (PCV)

There exist 2 phenotypically different but genetically related strains of PCVs.[3,4] Porcine circovirus type 1 (PCV1) was originally discovered in 1974 as a persistent contaminant of the porcine kidney PK-15 cell line ATCC CCL-33.[5] This virus is widespread in the swine population but does not cause clinical disease and is nonpathogenic in swine.[6–8] PCV2 was discovered in association with postweaning multisystemic wasting syndrome (PMWS) in Canadian weaning piglets in 1991.[7,9–11] It is the smallest known freely replicating virus in vertebrates.[12] PCV2 has been recognized as the primary causative agent of PMWS,[13] now known as PCVAD since the name was modified in March 2006 by the American Association of Swine Veterinarians (AASV).

After Harding and Clark[11] defined the syndrome in 1997, it became clear that PCVAD was the cause of many

From the Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Corresponding author: Virginia Buechner-Maxwell, DVM, MS, Diplomate ACVIM, Professor, Virginia Polytechnic Institute and State University, Duck Pond Drive, Blacksburg, VA 24061-0342; e-mail: bmax@vt.edu.

Submitted June 18, 2009; Revised August 2, 2009; Accepted August 12, 2009.

10.1111/j.1939-1676.2009.0389.x

losses in pig herds in all the major swine-producing countries.[3,7,10,13–15] Retrospective studies of pig serum samples found PCV2-specific antibodies as early as 1969 in Belgium,[16] 1970 in the United Kingdom,[15] 1973 in Ireland,[17] and 1985 in Canada and Spain.[18,19] PCV2 antibodies were also identified in 13.6% of tissues collected from Canadian pigs in 1985. Virus-positive samples increased to 72.4% in 1989, and then leveled off at 66.7% in 1997.[18] In Northern Ireland, 69.1% of serum samples were antibody positive in 1973, 55% in 1984, 100% in 1988, and 92.1% in 1999.[17]

Retrospective studies on archived pig tissues in the United Kingdom diagnosed PCVAD in 68 cases between 1970 and 1997.[15] Sequence analysis of the virus from those tissues showed a high sequence identity to isolates from a pig diagnosed in the year 2000 with another disease associated with PCV2, porcine dermatitis and nephropathy syndrome (PDNS). This indicated that the virus changed very little during the 30-year period.[15] Serology has demonstrated that PCV2 antibodies are present globally in most swine herds and up to 100% of individual pigs within those herds [17,18,20]; this includes herds in the United States.[20]

Current State of PCVAD

PCVAD is considered an emerging disease and the incidence of this disease has increased dramatically over the past years. In Part II of the Reference of Swine Health and Health Management in the United States published in the year 2000 by the U.S. Department of Agriculture (USDA),[21] PCVAD prevalence was determined by USDA veterinarians who collected data from commercial herds of 100 or more pigs accounting for nearly 94% of the U.S. pig inventory (Table 1).

Of nursery age pigs, the percentage of sites where PCVAD was known or suspected to have caused sickness or mortality in 1 or more pigs during the previous 12 months in farms with <2,000 animals was 4.4%, farms with 2,000–10,000 animals had 10.4%, and >10,000 animals reported 20.9% for an overall of 5.7%. Approximately 30% of animals were diagnosed by a veterinarian or diagnostic laboratory. In grower and finisher pigs, small farms had 2.3%, medium farms had 8.8%, and large farms had 12.4% affected pigs for an overall prevalence of 3.6%. Approximately 54% of the pigs reported to have PCVAD were diagnosed by a veterinarian or diagnostic laboratory.

When the same report was published in the year 2006, which again accounted for 94% of the U.S. pig population,[22] prevalence in nursery pigs on small farms (<2,000 animals) was 21.5%, medium farms (2,000–5,000 animals) was 12.5%, and large farms (>5,000 animals) was 39.6% for an overall of 22.3%. Approximately 60% were diagnosed by a veterinarian. In growers and finishers, small farms reported 25.0%, medium farms 35.4%, and large farms 59.9% for an overall of 31.3%. Approximately 70% were diagnosed by a veterinarian or diagnostic laboratory.

The prevalence of porcine dermatologic and nephropathy syndrome was also described for the 1st time in the 2006 report, and in nursery pigs of small farms the prevalence was 3.3%, medium farms 0.0%, and large farms 3.4% for an overall of 2.9%. In growers and finishers the numbers were 1.6% for small, 10.4% for medium, and 23.9% for large farms, overall prevalence was 6.0%. The age of onset for PCVAD affected pigs ranged from 8.9 to 16.3 weeks (Table 1).

Taxonomy

PCV1 and PCV2 belong to the family Circoviridae[7,10,13,23] and to the genus *Circovirus*.[24] Other known viruses in this genus are canary circovirus, goose circovirus, pigeon circovirus, and psittacine beak and feather disease virus. Another genus in the circoviridae family, *Gyrovirus*, includes chicken anemia virus.[24] The *Gyrovirus* genus is distinct in that it has a negative sense genome and larger virions than is typical for circovirus.[25] Circoviruses are host specific, most of which are avian, or have a relatively narrow host range. Several species produce lymphoid depletion in infected hosts whereas others cause subclinical infection.[24] PCV1 is most closely related to the beak and feather disease virus.[26] Several human circoviruses also exist and include the torque teno virus (TTV), which is related to the swine TTV, TTV-like mini virus, and the SEN virus. The human circoviruses have not been definitively linked to any disease in humans.[27–30]

Table 1. Prevalence of PCVAD by type of operation, farm size, and year.[21,22]

		Farm Size			
Year	Age Group	<2,000	2,000–10,000	>10,000	Overall (%)
2000	Nursery	4.4	10.4	20.9	5.7
	Grower and finisher	2.3	8.8	12.4	3.6
		Farm Size			
		<2,000	2,000–5,000	>5,000	Overall (%)
2006	Nursery	21.5	12.5	39.6	22.3
	Grower and finisher	25.0	35.4	59.9	31.1
PDNS	Nursery	3.3	0.0	3.4	2.9
	Grower and finisher	1.6	10.4	23.9	6.0

PCVAD, porcine circovirus-associated disease; PDNS, porcine dermatitis and nephropathy syndrome.

Circoviridae is most closely related to the family Nanoviridae, which includes plant viruses. These viruses share a step loop structure at the origin of replication and show similarities in the replication proteins.[31] These similarities are also shared by the plant Geminiviruses, and it is speculated that circovirus may be the genetic link between the 2 plant virus families.[26] It has been proposed that an ancestor of PCV1 may have been a plant nanovirus that infected a vertebrate host and recombined with a vertebrate-infecting RNA virus, which was most likely a calicivirus.[32]

PCV2 has been divided into 2 distinct genotypes. They have been named PCV2-group 1 and PCV2-group 2.[4] There is no difference in pathogenesis between the 2 genotypes, but the viruses differ in size with PCV2-group 1 being 1,767 nucleotides (nt) and PCV2-group 2 being 1,768 nt.[4] PCV2-group 1 is further divided into 3 clades and PCV2-group 2 divided into 5 clades.[4] Historically, only PCV2-group 2 isolates were found in the United States; but in late 2005 several outbreaks of higher than normal mortality (5–50%) were reported in Kansas, North Carolina, and Iowa. These outbreaks were found to be associated with PCV2-group 1 isolates.[33]

About the same time as PCV2 was being grouped into group 1 and group 2 isolates, North American laboratories proposed grouping PCV2 into North American isolates, or PCV2a, and European-like isolates, or PCV2b.[34] PCV2b falls into PCV2-group 1 and PCV2a falls into PCV2-group 2.[34]

Genomic Organization

PCV1 and PCV2 have a small, nonenveloped icosahedral virion[5] with a single-stranded, circular DNA genome of 1,759 nt and 1,767–1,768 nt in size, respectively.[3,31] They contain 2 major open reading frames (ORFs) encoded in an antisense direction[24] (Fig 1). ORF1 encodes for viral replication proteins (Rep), and ORF2 encodes for the capsid protein (Cap), which contains the immunodominant antigenic epitopes.[31,35–38] ORF1 is very similar between PCV1 and PCV2 with 83% nucleotide and 86% amino acid identity between them.[39] ORF1 is alternatively spliced into 8 RNA strands in PCV1 and 5 RNA strands in PCV2. Only 2 RNA strands, Rep and Rep′, are essential for virus replication.[36,40,41] PCV1 and PCV2 share 67% nucleotide and 65% amino acid sequence identity in ORF2.[39] A 3rd ORF has been described in PCV2 and it has been suggested that ORF3 is involved with apoptosis,[42] but this report has not been able to be verified by independent laboratories. Overall, PCV1 and PCV2 share 76% nucleotide sequence homology and are similarly organized.[3,43]

Virus Life Cycle

The mechanisms of PCV2 cell recognition, attachment, and entry are currently being researched and not well understood. It is believed that PCV2 uses a relatively common cell receptor, because viral replication and PCV2 antigen has been found in many different cell types.[44] PCV2 binds to heparin sulfate and chondroitin sulfate, which are glycosaminoglycans (GAGs), as a 1st step of attachment.[45] However, as PCV2 is found in cells that lack GAGs, it is thought that another coreceptor is also used for viral entry.[45]

The hallmark lesion of PCV2 infection is lymphoid depletion with histiocytic replacement. In affected lymph organs, dendritic cells, and macrophages that replace the lymphocytes contain large amounts of PCV2 virus.[7,46,47] There is no viral degradation in these cells, and because dendritic cells are highly mobile, it is thought that dendritic mobility may be a method of viral dissemination in tissues.[48] It is still unknown how PCV2 causes a reduction in lymphocytes. Hypotheses include induced apoptosis, decreased lymphocyte production in the bone marrow, or reduced lymphocyte proliferation in secondary lymphoid tissue.[23]

Because PCV2 encodes for only 2 major proteins, it is thought that the virus relies on its host cell for protein expression and for replication. The virus requires an actively replicating cell, specifically in the S-phase,[49] for DNA replication via a DNA polymerase. PCV2 replication is speculated to involve a rolling-circle method.[50,51] It was determined in porcine kidney (PK-15) cells that the 1st detectable protein produced postinfection is the Cap protein, which was localized in the perinuclear area

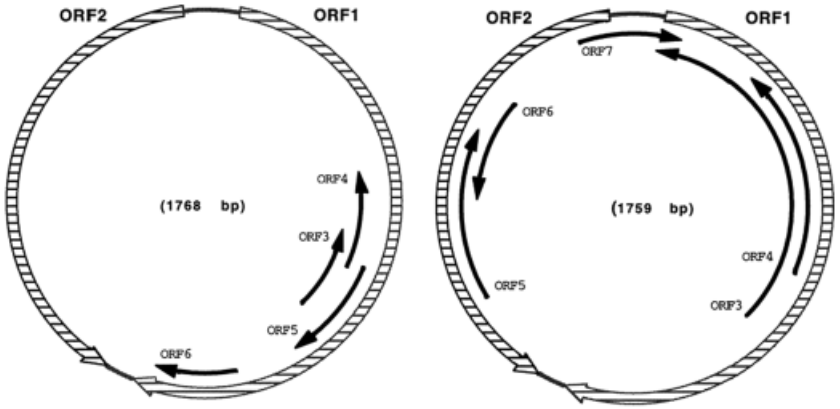

Fig 1. Genomic organization of PCV2.

of the cell.[52] At 12 hours postinoculation, both Cap and Rep were detectable in the nucleus. By 36 hours postinoculation, the viral titer had stabilized indicating that the virus had completed replication.[52] There is currently no information on how the capsid is made, how the virus assembles or how it is released from infected cells.

Viral Transmission

PCV2 can be transmitted in several ways. The main route is by oro-nasal contact with infected feces,[13] contact with infected urine, or directly with infected pigs.[53,54] PCV2 is shed in respiratory secretions, oral secretions, urinary secretions, and feces in both clinically affected as well as in infected but apparently healthy pigs. Clinically affected pigs shed virus in higher quantity compared with infected but clinically healthy pigs.[55] PCV2 can also be transmitted vertically (from the sow to the piglets) through the placenta causing persistently infected piglets at birth, but this method of transmission appears to be rare.[56–59] PCV2 has also been shown to be shed in colostrums,[60] but whether this can result in an infection is still being investigated. Recent work has shown that PCV2-infected pork products (lymphoid tissue, skeletal muscle, and bone marrow), when fed to naive piglets for 3 days, resulted in viremia and seroconversion to PCV2 in all of the piglets.[61]

PCV2 is also shed in semen and in experimental studies seminal virus shedding was detected as early as 5 days postinoculation.[62–64] Shedding in naturally infected boars appears to be low and sporadic.[62] The greatest amount of virus appears in the seminal fluid and nonsperm fraction.[65] Boars that are persistently infected may continue to shed the virus in semen and semen samples were found to be positive in boars up to 71 weeks of age. Samples collected from boars ranging from 71 to 149 weeks of age were not found to shed virus in semen.[62] The virus did not appear to affect the percentages of live and morphologically normal sperm.[62] Recent evidence has shown that PCV2 virus present in the semen is infectious when injected IP into pigs, but failed to seroconvert gilts that were artificially inseminated. In addition, all pigs born of the gilts were negative for PCV2 antibodies.[66,67]

The incubation period in experimentally infected pigs ranges from 2 to 4 weeks.[54,68–70] Once inside the host, PCV2 1st infects the tonsils and lymph nodes of the head and begins replicating.[71] PCV2 also infects B cells[72] that likely causes dissemination throughout the body via the lymphatic system. PCV2 has been detected in the spleen, Peyer's patches, and many lymph nodes.[73] PCV2 then starts replicating in T cells and peripheral blood mononuclear cells.[72,74] Viremia in pigs is detectable between 7 and 14 days postinoculation. PCV2 has the ability to cause a prolonged infection, with viral DNA detectable in pigs up to 125 days postinoculation in experimental infections.[53,54,68–70,75–77]

Host Immunity

Because most breeding age sows are seropositive for PCV2, most piglets are born with maternal antibodies against PCV2.[78] In weaned piglets, the mean half life of antibodies is 19 days. Antibody levels will wane at 4–6 weeks in pigs with initially low levels of antibody, at 6–10 weeks with moderate antibody levels, and by 8.5–13.5 weeks in pigs with high antibody levels.[20] Piglets do not typically demonstrate clinical signs of disease before 4 weeks of age, suggesting that maternally derived antibodies are protective.[78–81] Experimental studies found that maternal antibody protection is dependent on the level of maternal antibodies present. High levels of maternal antibodies are more protective than low levels, but do not completely prevent infection, whereas low levels of antibodies did not provide any protection against infection.[78]

Two- to 3-month-old pigs are capable of producing an antibody response to PCV2 infection, but this response is not completely protective as these pigs can still develop viremia.[82–84] Experimental infections show that pigs seroconvert between 14 and 28 days postinfection (DPI).[68,69,76,85] By 10 DPI, pigs can develop neutralizing antibodies that increase in titer up to 21 DPI.[86] Neutralizing antibodies were detected in naturally infected Belgian pigs by 10 weeks of age, and in Danish pigs at 3 weeks of age. Pigs that develop PCVAD have low or undetectable levels of neutralizing antibodies.[86]

PCV2 pathogenesis appears to be related to the immunomodulatory effects of the virus. PCV2 infection results in a decreased expression of B-cell growth factor IL-4 and the cytotoxic T cell and macrophage-activating cytokine IL-2.[87] This results in a decreased proliferation of lymphocytes and the interferon antiviral response[88] although causing an increase in expression of proinflammatory cytokines IL-1B and IL-8.[87]

PCV is believed to be a species-specific virus; however, antibodies to PCV1 have been detected in mice, cattle, and humans,[89] but currently PCV2 is not considered to be a zoonotic disease. However, with the advent of xenotransplantation using porcine organs, the risk of implanting PCV2 infected organs into immunocompromised xenograft recipients should be investigated.

Factors that Modulate Diseases Caused by PCV2

PCV2 infection is characterized by having a high prevalence of infection but low morbidity, and thus not all animals infected with PCV2 will develop clinical signs of PCVAD.[90–92] Seroprevalence in commercial herds in some countries is near 100%.[92,93] Although most pigs in a herd will become viremic, only 5–30% of susceptible pigs will show clinical signs of PCV2.[90,92] There are 4 main factors essential in the expression of PCV2-related diseases: viral effects, host effects, and the effects of coinfection and immunomodulation.[23]

Viral Factors

Although PCV2 is capable of causing several distinct disease syndromes, there are no significant differences in the virus genomes recovered from the different syndromes. Sequence analysis of PCV2 from PCVAD-affected pigs, and pigs with clinically unapparent infec-

tion, showed 95.6–100% sequence homology and no distinct patterns of sequence variations were evident between the 2 groups. This has led to the belief that there are other factors affecting the expression of disease.[94] It has been demonstrated that PCV2 isolated from pigs without disease can cause PCVAD under experimental conditions.[95,96]

It was shown that 2 amino acid mutations in the PCV2 genome significantly altered the gross and histopathologic lesions seen in pigs, indicating that only minor alterations in the viral genome are required to alter the function of the virus.[97] In the Canadian outbreak of 2004, a change in virus type was demonstrated that caused a much more severe disease characterized by pulmonary edema, granulomatous enteritis, more severe lymphoid depletion, and lymphoid necrosis.[98] Subsequent reports of the introduction of PCV2b into the United States were associated with severe outbreaks in Kansas, Iowa, and North Carolina in 2006.[33] It is unknown if the increased prevalence of PCV2b is associated with a change in virulence, new introduction to the area, or other factors that allowed an increase in replication of this viral type.

Host Factors

All breeds of pigs appear to be susceptible to infection, and clinical disease has been observed in many purebred and crossbred pigs (PG Halbur, unpublished data). However, studies have shown differences in susceptibility in different breeds of pigs.[99,100] Differences in the type of adaptive immune response against PCV2 in different pigs may explain the host variation in the outcome of infection.[101] There are significant differences in the replication patterns of PCV2 in alveolar macrophages from different conventionally crossbred pigs.[52]

Coinfection

Although PCV2 is required to cause the characteristic lymphoid depletion of PCVAD, many strains likely require a cofactor to cause the full spectrum of clinical signs associated with PCVAD. Coinfection with several other viral and bacterial pathogens has been shown to cause an increase in incidence and a markedly more severe clinical course of disease. The agent implicated as creating the greatest risk is porcine reproductive and respiratory syndrome virus (PRRSV).[102] Other agents include porcine parvovirus (PPV),[68,75,103–105] *Mycoplasma hyopneumoniae*,[106] and very recently the TTV, which singly is not associated with disease but present in many pig populations.[107,108] A retrospective analysis of the number of PCVAD cases in which there were coinfections was performed by the Iowa State Veterinary Diagnostic Laboratory. The results showed that more than 98% of pigs had coinfections. Specifically, 52% were coinfected with PRRSV, 36% with *M. hyopneumoniae*, 15% with PPV, 14% with bacterial septicemia, 7.6% with bacterial pneumonia, and 5.4% with swine influenza virus. A single PCV2 infection occurred in only 1.9% of the cases.[109]

Immunomodulation

Part of the pathogenesis of coinfection causing more severe disease may be associated with immunostimulation before PCV2 infection. One study showed that pigs that were immunostimulated with keyhole limpet hemocyanin developed clinical PCVAD when infected with PCV2.[85] There is also mounting evidence that common adjuvanted vaccination regimens may actually enhance the development of PCVAD.[110–112] In pigs vaccinated with the same antigen, but different adjuvants, the oil-in-water adjuvant was shown to cause a longer length of viremia, increased amounts of PCV2 in serum and tissue, and more severe lymphoid depletion when compared with pigs vaccinated with aqueous and aluminum hydroxide products.[113] Similarly, vaccination with *M. hyopneumoniae* or *Actinobaccilus pleuropneumoniae* vaccines followed by immediate infection with PCV2 in specific pathogen-free pigs caused a significant increase in viremia duration and more severe histopathologic lesions than in nonvaccinated pigs.[112]

The effects of immunosuppression on disease caused by PCV2 have also been studied. Infection of pigs with PCV2 after injection with cyclosporine caused an increase in PCV2 replication, and a higher titer of virus compared with controls, but the pigs did not develop clinical PCVAD.[114] In another study, pigs treated with dexamethasone before PCV2 infection developed a granulomatous lymphadenitis that was not observed in pigs inoculated with PCV2 alone.[115] In addition, a series of studies indicate that cell-mediated immunity plays an important role in protection.[45,78,101,116] A proportion of pigs vaccinated with a live PCV1-2 chimeric vaccine developed only low levels of antibody and yet the vaccinated pigs were fully protected against subsequent challenges with PCV2.[116,117]

PCVAD

PCVAD recently replaced the older name of PMWS. The name PCVAD was adopted to be inclusive of all the recognized syndromes associated with PCV2 infection.[118] According to the AASV, PCVAD can be subclinical or include 1 or more clinical manifestations including multisystemic disease with weight loss and high mortality, respiratory disease, porcine dermatologic and nephropathy syndrome, enteric signs including diarrhea, and reproductive disorders on an individual or herd basis.[119] Distinguishing the different forms of PCVAD can be accomplished by observation of gross or histopathologic characteristic lesions in the intestines, lungs, and lymphoid tissue.[23]

Syndromes

PMWS

The most significant manifestation of PCVAD is the multisystemic syndrome. This syndrome has been recognized in wild boars, but the source of the infection is believed to be the domestic pig.[120] This disease affects pigs between 7 and 16 weeks old in the United States and

5–12 weeks old in Europe.[7,121,122] This age difference is most likely related to variation in management practices and vaccination timing between producers in the United States and Europe.[112] Morbidity is associated with the development of viremia and lymphopenia in piglets followed by the clinical manifestations of disease. Mortality is usually around 10%[7,11,121] (range 4–20%),[121] but can reach 50%.[7,11] Because the clinical course of wasting and decreased economic efficiency can be prolonged, 70–80%[121] of pigs that develop PCVAD are subsequently euthanized.

Clinical signs of PCVAD include wasting with progressive weight loss, lethargy, dark-colored diarrhea, lymphadenopathy, and paleness or jaundice. The main characteristic histopathologic lesions are lymphoid depletion with histiocyte replacement in lymphoid tissues, and intracytoplasmic inclusion bodies.[6,7,11,13,71,123] Early signs of reduced weight gain, ill-thrift, pale skin, and rough hair coat often go unnoticed or are misdiagnosed. Later signs include dyspnea, tachypnea, anemia, diarrhea, and jaundice.[11] Pigs can also have coughing and gastric ulceration, which most likely contributes to the anemia. On necropsy, the lungs fail to collapse and are mottled, tan colored, and in chronic cases some kidneys have white streaks or spots.[23] Affected pigs also have enlargement of the superficial inguinal, submandibular, mesenteric, and mediastinal lymph nodes.[71] Granulomatous lesions can also be found in the lungs, liver, kidney, heart, and intestines.[23]

A scoring system has been developed that estimates the severity of disease based on the extent of lymphatic tissue involvement. The 7 lymphoid tissues that are evaluated for the purpose of scoring include the tracheobronchial lymph nodes, the mesenteric lymph node, the mediastinal lymph nodes, the superficial inguinal lymph nodes, the external iliac lymph nodes, the tonsils, and the spleen. This system accounts for the severity of lesions, the amount of PCV2 antigen and the distribution of the lesions. Scores are assigned and range from 0 to 9.[106] Although this system is useful for classifying the severity of disease, it is impractical for field necropsies.

Subclinical PCV2 Infection

PCV2 infection can be limited to 1 or 2 lymph nodes in the absence of evidence of clinical disease.[106,124] However, the presence of PCV2 might be associated with a decrease in vaccine efficacy[125] and healthy pigs can still exhibit a necrotizing lymphadenitis.[124,126] The significance of this finding to the pig is unknown, but can cause the carcass to be condemned at slaughter.[23]

PCV2-Associated Enteritis

This syndrome affects piglets from 8 to 16 weeks old and resembles chronic ileitis associated with *Lawsonia intracellularis* infection. Affected piglets have diarrhea, unthriftiness, retarded growth, and increased mortality. Histopathologic lesions include a granulomatous enteritis and characteristic PCV2 lesions in Peyer's patches but not in other lymphoid tissues. At necropsy, mesenteric lymph nodes are enlarged and the intestinal mucosa is grossly thickened. Histopathology is able to easily distinguish between *Lawsonia* versus PCV2 infections.[127]

PCV2-Associated Pneumonia

This syndrome can play a role in porcine respiratory disease complex.[128,129] It affects pigs from 8 to 26 weeks old and is associated with multiple pathogens. The clinical signs include decreased rate of growth, decreased feed efficiency, anorexia, fever, cough, and dyspnea. This can be very similar to systemic infection and there is some overlap of the syndromes. The histopathologic lesions include a granulomatous bronchointerstitial pneumonia with mild to severe necrotizing and ulcerative bronchiolitis and bronchiolar fibrosis. Differentials for the bronchiolitis lesions include swine influenza or porcine respiratory coronavirus infections.[23]

PCV2-Associated Reproductive Failure

This syndrome was first reported in Canada in 1999[130] and typically affects gilts and start-up operations.[131] The clinical signs include increased abortion, still births, fetal mummies, and preweaning mortalities. The histopathologic lesions include a nonsuppurative to necrotizing or fibrosing myocarditis in still born and neonatal pigs.[131] The time of infection determines the clinical course of the disease. Fetuses inoculated at 57 days of gestation had higher viral replication than those infected later in gestation and when killed at 21 days postinoculation had edema, enlarged livers, and congestion. Fetuses inoculated at 75 and 92 days of gestation failed to produce similar lesions or viral loads.[132] Late term infections at 86, 92, and 93 days of gestation caused an increase in reproductive abnormalities including still birth, fetal mummies, and weak piglets.[133] However, data from field cases indicate that most breeding herds appear to be immune to this disease.[23]

PDNS

This syndrome was first described in the United Kingdom in 1993[134] and was associated with PCV2 in the year 2000.[135] This disease is often fatal within 3 days of development and mostly affects grower pigs, but can affect pigs as young as 5 weeks old. Clinical signs include an acute onset of fever, lethargy, and raised purple skin lesions progressing to multifocal red-purple scabs with black centers being most prominent on the rear legs. At necropsy the kidneys are enlarged, tan, and waxy in appearance with petechial hemorrhages. Histopathologically, there is a systemic vasculitis with dermal and epidermal necrosis and necrotizing and fibrinous glomerulonephritis appearing similar to a type 3 hypersensitivity reaction with deposition of antigen-immune complexes in the vascular and glomerular capillary walls.[23] The development of disease is aided by coinfection with PRRSV,[136,137] *Pasteurella multocida*, *Streptococcus suis* types 1 and 2, among others.[138,139] Recently, PDNS was experimentally reproduced with

PRRSV and TTV in PCV2-free pigs.[140] Therefore, PDNS is not always associated with PCV2.

PCV2-Associated Neuropathy

In 2001, PCV2 was associated with pigs born with congenital tremors and a nonsuppurative menigoencaphalitis located in the brain.[43,59,141] More recent reports have associated PCV2 infection with cerebellar lymphohistiocytic vasculitis combined with hemorrhages or with lymphohistiocytic meningitis. PCV2 antigen was found with immunohistochemistry (IHC) in the cytoplasm and nuclei from intralesional perivascular machrophages and endothelial-like cells in the brain tissue.[142] In addition, naturally occurring neurologic disease characterized by opisthotonus, nystagmus, and convulsions was associated with PCV2 infection in pigs ranging from 6 to 8 weeks old in which cerebellar vasculitis was also present.[143] The role of PCV2 in the development of this disease is still under investigation, but may indicate a new spontaneously occurring type of PCV2 disease.

Diagnosis

The diagnosis of PCVAD is based on clinical signs and demonstration of PCV2 antigen in more than 1 lymphoid tissue, or 1 lymphoid tissue and 1 other organ system such as the lungs, liver, kidney or intestine, or in 2 organ systems. If antigen is found only in 1 organ system, then the disease is categorized based on that organ system. If only limited PCV2 antigen is found but there are severe lesions, it is classified as chronic severe PCVAD.[23] Scoring of lesions and the amount of antigen in tissues allows for staging of infection.[23] Diagnosis can be tentatively made based on clinical signs. In a survey of farms experiencing PCVAD disease, the percentage that observed the following clinical signs included wasting (98.1%), diarrhea (77.2%), dyspnea (75.1%), lymphadenopathy (44.8%), central neurologic signs (39.6%), jaundice (37.1%), inappetence (90.4%), and death (96.8%).

Detection of PCV2 antigen or nucleic acid is considered the gold standard for the diagnosis of PCVAD. The best tests for this are polymerase chain reaction, in situ hybridization (ISH) and IHC.[23] There is currently no information on sensitivity and specificity of these tests, but IHC gives more intense staining and is considered more sensitive, but less specific than ISH. IHC is also cheaper to run and has a faster turn around time. However, many labs do not offer IHC, because one of the required reagents is anti-PCV2 antiserum. Although a monoclonal anti-PCV2 is commercially available, definitive diagnosis can still be difficult with that product. The best way to diagnose PCVAD is the identification of the characteristic lesions of the disease. Microscopic lesions associated with PCVAD include syncytial cells in lymph nodes, Peyer's patches, and the lamina propria of intestinal villa. In addition, macrophages have sharply demarcated, spherical, basophilic cytoplasmic inclusion bodies.[71]

Serology can also be performed and is a convenient method for detecting exposure to PCV2 for large numbers of pigs. However, it must be remembered that many clinically healthy pigs are seropositive. Other tests that have been developed include immunofluorescence assay, IgM immunoperoxidase monolayer assay, enzyme-linked immunosorbant assay, virus isolation, electron microscopy, and serum virus neutralization assays.[9,15,23,39,76,89,101,103,144–149] There is currently no field test for the diagnosis of PCVAD.

Management and Classification of Herd Outbreaks

No specific treatment is available for diseases associated with PCV2 infection. In general, treatment of individually affected pigs is supportive only and will vary greatly depending upon the clinical signs that the animal displays. Because many animals are coinfected, choosing appropriate treatment will also depend upon identification of the other agents infecting the animal. In addition, prognosis is dependent upon animal factors, such as age as well as the syndrome that the animal displays. There is currently no data on the existence of PCVAD in pet potbellied pigs, but some veterinarians are recommending PCV2 vaccination to their clients. During initial PCVAD outbreaks, pigs treated with antibiotics actually suffered a higher mortality rate than those not treated, but it is believed that this was more because of spreading the virus with common use needles than any effect by the antibiotics (Dr RB Baker, personal communication, Iowa State University, Ames, IA).

PVC2-related syndromes are of greatest economic concern when they occur in herd populations. To identify and manage outbreaks of PCV2 and PCVAD diseases within a herd, it is important to determine whether the disease is a significant herd problem or is only sporadically causing a herd problem. A definition has been developed to help with this determination. An important herd problem has been defined as an increase in mortality of equal to or more than the mean of historical mortality levels plus 1.66 times the standard deviation. If there are no historical mortality data, a herd problem can be defined as an increase in mortality that exceeds the national or regional level by 50%. In other words, if 50% or more of the pigs from a representative sample are diagnosed with PCVAD and there has been a significant increase in mortality compared with previous mortality data, it is considered a herd problem. However, if $<50\%$ of the pigs are diagnosed with PCVAD, but there has still been an increase in mortality or if more than 50% of the pigs are diagnosed with PCVAD but there has been no increase in mortality, the outbreak may be considered sporadic and not a herd problem.[150]

Prevention of PCV2-Associated Diseases

Prevention of PCVAD can be difficult. Disease outbreaks are reported to occur on farms even with strict isolation practices. It has been shown that vaccination against *M. hyopneumonia* or *A. pleuropneumoniae* 2–4 weeks in advance of infection with PCV2 prevented any lesions associated with PCV2 infection,[112] but the practical

issues of this method make it nearly impossible to accomplish. PPV vaccination does not reduce the severity of PCVAD in coinfected pigs[105] but a combined PPV and swine erysipelas vaccine appeared to protect against PCV2-induced reproductive failure.[151]

Treatment of bacterial infections and prevention of cofactor-associated diseases is also a good practice in preventing PCV2 diseases. Treating *M. hyopneumonia* with chlorotetracyclines was highly effective.[152] Bleach (3–6% sodium hypochlorite) is an effective chemical in killing PCV2, but has unknown field efficacy. The protocol utilized at Iowa State University to disinfect pens is included in Table 2.[23]

Good housing management is critical in disease prevention. It has been shown that reducing stress, paying attention to proper hygiene, preventing mixing of ages, and utilizing all in/all out practices are effective in controlling disease. Other options include immunized serum therapy, which has practical limitations, and depopulation, which has been shown to be ineffective because the virus is very resistant in the environment.[153] Whether PCV2 can be found in insects or wild animals that could possibly transmit disease to pigs is not known. However, because circoviruses are highly species specific, it is unlikely that these animals, excluding feral boars, would pose a threat of PCV2 transmission to domestic herds.[154]

Risk factors for development of infection include PPV or PRRSV infection, large pen sizes versus small pen sizes for weaning piglets, increased levels of cross fostering[155] and vaccination against PRRSV.[156] As such, reduction of these risk factors on farms may be helpful in controlling PCVAD. A study in Canada showed a strong association of increased mortality with *M. hyopneumoniae* infection, PRRSV, diarrhea caused by *Escherichia coli* K88, close proximity to other herds, multiple suppliers, large within-group range in age of pigs and not using spray-dried plasma in 1st nursery rations.[157] Factors that decreased the risk included long empty times between pig groups, regular treatment of external parasites, pen versus crate gestation, internal versus external gilt replacement,[155] and vaccination against atrophic rhinitis.[156]

Vaccine Development and Vaccination

Initial attempts at vaccine development included using a killed PPV vaccine. Because pigs are often coinfected with PPV and PCV2, it was hoped that vaccination at an early age would prevent PCVAD. This approach empirically appeared promising in the field, but a benefit was not confirmed when the vaccine was tested under more controlled conditions.[105,158,159]

Table 2. Iowa State University disinfection protocol

Apply degreaser detergent with foamer at a 1 : 64 dilution (Iowa uses grease-free PV, but any product should work the same)
Leave for 10 minutes
Remove with pressure washer using hot water
Decontaminate with Virkon S[a] at 1 : 30 dilution
Leave for 10 minutes
Rinse with hot water
Before occupancy, fog with Clindox-S[b] at 1 : 5 : 1 dilution and let dry
Rinse with water 6–12 hours after fogging and allow to dry

[a]Antec International (Sudbury, Suffolk, UK).
[b]US Pharmacal Com LLC (Erie, CO).

One of the 1st PCV2 vaccines on the market was Merial's CIRCOVAC (Duluth, GA), an inactivated PCV2 vaccine with oil adjuvant for use in breeding age animals.[23] This vaccine was most extensively used in Europe and it was also available in Canada. The vaccine was successful in reducing PCV2 circulation and shedding in the 1st weeks of life of the piglets born to the vaccinated sows.[160] This vaccine is designed to be given as 2 injections IM 3–4 weeks apart and completed at least 2 weeks before breeding and once at each subsequent gestation.[23]

Another vaccine available in the United States is the Boehringer Ingelheim's Ingelvac CircoFLEX vaccine (Petersurg, VA), a capsid-based subunit vaccine expressed in inactivated baculovirus. This vaccine demonstrated significant decreases in mortality in vaccinated pigs versus unvaccinated pigs on 4 different Canadian finishing sites.[161] This vaccine is to be given in a single dose IM to piglets >3 weeks of age.[23] A field trial for this vaccine recently was reported in the United Kingdom in which 3-week-old pigs were vaccinated. Mortality caused by PCVAD was reduced from 14.3 to 4.6%.[162]

A 3rd vaccine that is produced by Intervet Inc/Schering-Plough Animal Health (Kenilworth, NJ) is also a capsid-based subunit vaccine. This vaccine, expressed in a baculovirus, is marketed under the name Circumvent PCV in the United States and Canada and Porcilis PCV in Europe and Asia. It is designed for vaccination of piglets 3 weeks and older. Circumvent PCV is given in 2 doses 3 weeks apart at 3 and 6 weeks of age given IM.[23] Porcilis PCV only requires 1 dose. Studies including 35,000 pigs on 21 different farms showed that mortality of vaccinated pigs was reduced by 77.5% when compared with unvaccinated pigs.[163]

The 1st USDA fully licensed PCV2 vaccine was licensed for use in pigs 4 weeks of age or older. This genetically engineered chimeric vaccine was created by inserting the immunogenic capsid protein of PCV2 into the genetic backbone of the nonpathogenic PCV1.[97,116,117,164] This chimeric vaccine was shown to be attenuated in pigs and was able to prevent the viremia and lymphopenia associated with PCV2 morbidity.[116,117] This vaccine was named PCV1-2 to indicate its chimeric nature, and was released in July 2006 in a killed form labeled as Suvaxyn PCV2 One dose (Fort Dodge and Wyeth Animal Health, Fort Dodge, IA). The vaccine is given as a single dose IM.

Field studies on Suvaxyn PCV2 One dose indicate that it is very effective and safe. The vaccine is able to decrease the mortality from 8–10% in nonvaccinated pigs to 1.0–2.0% in vaccinated pigs. Safety studies have been completed on over 1,000 pigs in 4 different locations. No adverse reactions were recorded in any of the vaccinated pigs. In addition, experiments have proven that the PCV1-2 vaccine is able to break through maternal antibodies to provide protection in piglets against PCV2 infection. This allows piglets to be vaccinated before

maternal antibodies wane and before the typical age of infection with PCV2.[165] The live version of the chimeric PCV1-2 vaccine has also been demonstrated to be genetically stable in vaccinated pigs,[166] and thus could serve as a good candidate for a live vaccine against PCV2.

Conclusions

Since its initial discovery in 1991, PCV2 and PCVAD have had a significant and adverse impact on the economy of the swine industry. There are currently 7 recognized syndromes related to PCV2 infections. Many of these syndromes are a result of coinfection with PCV2 virus and other agents like *Mycoplasma* and PRRSV. Diagnosis of PCV can occasionally be difficult because of nondescript clinical signs but diagnostic lab tests are available. The current knowledge and research into vaccines is providing relief for the swine producer from the heavy losses associated with the disease. Key points in reducing loses are centered on proper vaccination and management. The reduction in swine loses already being witnessed after the introduction of vaccines in the world market has been significant, and further research to provide even better vaccines will likely continue to reduce economic loss in the world swine market.

References

1. British Pig Executive (BPEX). Pig Yearbook 2006. Kenilworth, Warwickshire, UK: Agriculture and Horticulture Development Board; 2006:47–50.
2. Carman S, McEwen B, DeLay J, et al. Porcine Circovirus type-2 associated disease continued in fall of 2005. Guelph Univ Anim Health Lab Newslett 2006;10:6–7.
3. Fenaux M, Halbur PG, Gill M, et al. Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with postweaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. J Clin Microbiol 2000;38: 2494–2503.
4. Olvera A, Cortey M, Segales J. Molecular evolution of porcine circovirus type 2 genomes: Phylogeny and clonality. Virology 2007;357:175–185.
5. Tischer I, Gelderblom H, Vettermann W, et al. A very small porcine virus with circular single-stranded DNA. Nature 1982;295: 64–66.
6. Allan GM, McNeilly F, Cassidy JP, et al. Pathogenesis of porcine circovirus; experimental infections of colostrum deprived piglets and examination of pig foetal material. Vet Microbiol 1995;44:49–64.
7. Allan GM, Ellis JA. Porcine circoviruses: A review. J Vet Diagn Invest 2000;12:3–14.
8. Tischer I, Mields W, Wolff D, et al. Studies on epidemiology and pathogenicity of porcine circovirus. Arch Virol 1986;91:271–276.
9. Allan GM, McNeilly F, Kennedy S, et al. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. J Vet Diagn Invest 1998;10:3–10.
10. Ellis J, Clark E, Haines D, et al. Porcine circovirus-2 and concurrent infections in the field. Vet Microbiol 2004;98:159–163.
11. Harding J, Clark E. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). J Swine Health Prod 1997;5:201–203.
12. Meehan BM, McNeilly F, Todd D, et al. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. J Gen Virol 1998;79(Part 9):2171–2179.
13. Segales J, Allan GM, Domingo M. Porcine circovirus diseases. Anim Health Res Rev 2005;6:119–142.
14. Allan G, McNeilly F, Meehan B. Isolation and characterization of circoviruses from pigs with wasting syndrome in Spain, Denmark, and Northern Ireland. Vet Microbiol 1999;66:115–123.
15. Grierson SS, King DP, Sandvik T, et al. Detection and genetic typing of type 2 porcine circoviruses in archived pig tissues from the UK. Arch Virol 2004;149:1171–1183.
16. Sanchez R, Nauwynch G, Pensaert M. Proceedings of the International Conference of ssDNA Viruses, Plants, Birds, Pigs, and Primates, St Malo, France, 2001, p. 122.
17. Walker IW, Konoby CA, Jewhurst VA, et al. Development and application of a competitive enzyme-linked immunosorbent assay for the detection of serum antibodies to porcine circovirus type 2. J Vet Diagn Invest 2000;12:400–405.
18. Magar R, Muller P, Larochelle R. Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. Can J Vet Res 2000;64:184–186.
19. Rodriguez-Arrioja GM, Segales J, Rosell C, et al. Retrospective study on porcine circovirus type 2 infection in pigs from 1985 to 1997 in Spain. J Vet Med B Infect Dis Vet Public Health 2003;50:99–101.
20. Opriessnig T, Yu S, Thacker EL, et al. Derivation of porcine circovirus type 2-negative pigs from positive breeding herds. J Swine Health Prod 2004;12:186–191.
21. USDA. Part II: Reference of Swine Health and Health Management in the United States. Fort Collins, CO: USDA:APHIS, VS, CEAH, National Animal Health Monitoring System; 2000, #N3550202. Available at: http://nahms.aphis.usda.gov/swine/swine 2000/Swine2kHi2.pdf. Accessed November 4, 2008.
22. USDA . Swine 2006, Part II: Reference of Swine Health and Health Management Practices in the United States. Fort Collins, CO: USDA:APHIS, VS, CEAH; 2006, #N4791207. Available at: http://nahms.aphis.usda.gov/swine/swine2006/Swine2006_highlightsPt 2.pdf. Accessed November 4, 2008.
23. Opriessnig T, Meng XJ, Halbur PG. Porcine circovirus type 2 associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest 2007;19:591–615.
24. Todd D, Bendinelli M, Biagini P. Circoviridae. In: Fauquet MA, Mayo J, Maniloff U, Desselberger LA, eds. Ball Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses. London: Elsevier Academic Press; 2005:327–334.
25. Gelderblom H, Kling S, Lurz R, et al. Morphological characterization of chicken anaemia agent (CAA). Arch Virol 1989;109: 115–120.
26. Niagro FD, Forsthoefel AN, Lawther RP, et al. Beak and feather disease virus and porcine circovirus genomes: Intermediates between the geminiviruses and plant circoviruses. Arch Virol 1998; 143:1723–1744.
27. Biagini P, Gallian P, Attoui H, et al. Genetic analysis of full-length genomes and subgenomic sequences of TT virus-like mini virus human isolates. J Gen Virol 2001;82:379–383.
28. Miyata H, Tsunoda H, Kazi A, et al. Identification of a novel GC-rich 113-nucleotide region to complete the circular, single-stranded DNA genome of TT virus, the first human circovirus. J Virol 1999;73:3582–3586.
29. Nishizawa T, Okamoto H, Konishi K, et al. A novel DNA virus (TTV) associated with elevated transaminase levels in post-transfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 1997;241:92–97.
30. Takahashi K, Iwasa Y, Hijikata M, et al. Identification of a new human DNA virus (TTV-like mini virus, TLMV)

intermediately related to TT virus and chicken anemia virus. Arch Virol 2000;145:979–993.

31. Meehan BM, Creelan JL, McNulty MS, et al. Sequence of porcine circovirus DNA: Affinities with plant circoviruses. J Gen Virol 1997;78(Part 1):221–227.

32. Gibbs M, Weiller G. Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus. Proc Natl Acad Sci USA 1999;96:8022–8027.

33. Cheung AK, Lager KM, Kohutyuk OI, et al. Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. Arch Virol 2007;152:1035–1044.

34. Gagnon C, Tremblay D, Tijssen P, et al. PCV2 strain variation: What does it mean? Proc Am Assoc Swine Pract 38:535–540.

35. Cheung AK. Transcriptional analysis of porcine circovirus type 2. Virology 2003;305:168–180.

36. Cheung AK. The essential and nonessential transcription units for viral protein synthesis and DNA replication of porcine circovirus type 2. Virology 2003;313:452–459.

37. Lekcharoensuk P, Morozov I, Paul PS, et al. Epitope mapping of the major capsid protein of type 2 porcine circovirus (PCV2) by using chimeric PCV1 and PCV2. J Virol 2004;78:8135–8145.

38. Mankertz A, Mankertz J, Wolf K, et al. Identification of a protein essential for replication of porcine circovirus. J Gen Virol 1998;79(Part 2):381–384.

39. Morozov I, Sirinarumitr T, Sorden SD, et al. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. J Clin Microbiol 1998;36:2535–2541.

40. Cheung AK. Identification of the essential and non-essential transcription units for protein synthesis, DNA replication and infectious virus production of porcine circovirus type 1. Arch Virol 2004;149:975–988.

41. Mankertz A, Hillenbrand B. Replication of porcine circovirus type 1 requires two proteins encoded by the viral rep gene. Virology 2001;279:429–438.

42. Liu J, Chen I, Kwang J. Characterization of a previously unidentified viral protein in porcine circovirus type 2-infected cells and its role in virus-induced apoptosis. J Virol 2005;79:8262–8274.

43. Larochelle R, Magar R, D'Allaire S. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus Res 2002;90:101–112.

44. Darwich L, Segales J, Mateu E. Pathogenesis of postweaning multisystemic wasting syndrome caused by porcine circovirus 2: An immune riddle. Arch Virol 2004;149:857–874.

45. Misinzo G, Delputte PL, Meerts P, et al. Porcine circovirus 2 uses heparan sulfate and chondroitin sulfate B glycosaminoglycans as receptors for its attachment to host cells. J Virol 2006;80:3487–3494.

46. Chianini F, Majo N, Segales J, et al. Immunohistological study of the immune system cells in paraffin-embedded tissues of conventional pigs. Vet Immunol Immunopathol 2001;82:245–255.

47. Sorden SD. Update on porcine circovirus and postweaning multisystemic wasting syndrome (PMWS). Swine Health Prod 2000; 8:133–136.

48. Vincent IE, Carrasco CP, Herrmann B, et al. Dendritic cells harbor infectious porcine circovirus type 2 in the absence of apparent cell modulation or replication of the virus. J Virol 2003;77: 13288–13300.

49. Tischer I, Peters D, Rasch R, et al. Replication of porcine circovirus: Induction by glucosamine and cell cycle dependence. Arch Virol 1987;96:39–57.

50. Mankertz A, Caliskan R, Hattermann K, et al. Molecular biology of porcine circovirus: Analyses of gene expression and viral replication. Vet Microbiol 2004;98:81–88.

51. Tischer I, Buhk HJ. Viral DNA from cells infected with porcine circovirus. Zentralbl Bakteriol Mikrobiol Hyg [A] 1988; 270:280–287.

52. Meerts P, Misinzo G, McNeilly F, et al. Replication kinetics of different porcine circovirus 2 strains in PK-15 cells, fetal cardiomyocytes and macrophages. Arch Virol 2005;150:427–441.

53. Magar R, Larochelle R, Thibault S, et al. Experimental transmission of porcine circovirus type 2 (PCV2) in weaned pigs: A sequential study. J Comp Pathol 2000;123:258–269.

54. Bolin SR, Stoffregen WC, Nayar GP, et al. Postweaning multisystemic wasting syndrome induced after experimental inoculation of cesarean-derived, colostrum-deprived piglets with type 2 porcine circovirus. J Vet Diagn Invest 2001;13:185–194.

55. Segales J, Calsamiglia M, Olvera A, et al. Quantification of porcine circovirus type 2 (PCV2) DNA in serum and tonsillar, nasal, tracheo-bronchial, urinary and faecal swabs of pigs with and without postweaning multisystemic wasting syndrome (PMWS). Vet Microbiol 2005;111:223–229.

56. Maldonado J, Segales J, Martinez-Puig D, et al. Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain. Vet J 2005;169:454–456.

57. Park JS, Kim J, Ha Y, et al. Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. J Comp Pathol 2005;132:139–144.

58. Pensaert MB, Sanchez RE Jr, Ladekjaer-Mikkelsen AS, et al. Viremia and effect of fetal infection with porcine viruses with special reference to porcine circovirus 2 infection. Vet Microbiol 2004;98:175–183.

59. Stevenson GW, Kiupel M, Mittal SK, et al. Tissue distribution and genetic typing of porcine circoviruses in pigs with naturally occurring congenital tremors. J Vet Diagn Invest 2001;13:57–62.

60. Shibata I, Okuda Y, Kitajima K, et al. Shedding of porcine circovirus into colostrum of sows. J Vet Med B Infect Dis Vet Public Health 2006;53:278–280.

61. Opriessnig T, Patterson AR, Meng XJ, et al. Porcine circovirus type 2 in muscle and bone marrow is infectious and transmissible to naive pigs by oral consumption. Vet Microbiol 2009;133:54–64.

62. McIntosh KA, Harding JC, Parker S, et al. Nested polymerase chain reaction detection and duration of porcine circovirus type 2 in semen with sperm morphological analysis from naturally infected boars. J Vet Diagn Invest 2006;18:380–384.

63. Kim J, Han DU, Choi C, et al. Differentiation of porcine circovirus (PCV)-1 and PCV-2 in boar semen using a multiplex nested polymerase chain reaction. J Virol Methods 2001;98:25–31.

64. Larochelle R, Bielanski A, Muller P, et al. PCR detection and evidence of shedding of porcine circovirus type 2 in boar semen. J Clin Microbiol 2000;38:4629–4632.

65. Kim J, Han DU, Choi C, et al. Simultaneous detection and differentiation between porcine circovirus and porcine parvovirus in boar semen by multiplex seminested polymerase chain reaction. J Vet Med Sci 2003;65:741–744.

66. Madson DM, Ramamoorthy S, Kuster C, et al. Characterization of shedding patterns of porcine circovirus types 2a and 2b in experimentally inoculated mature boars. J Vet Diagn Invest 2008;20:725–734.

67. Madson DM, Ramamoorthy S, Kuster C, et al. Infectivity of porcine circovirus type 2 DNA in semen from experimentally-infected boars. Vet Res 2009;40: article 10.

68. Allan GM, Kennedy S, McNeilly F, et al. Experimental reproduction of severe wasting disease by coinfection of pigs with porcine circovirus and porcine parvovirus. J Comp Pathol 1999;121: 1–11.

69. Balasch M, Segales J, Rosell C, et al. Experimental inoculation of conventional pigs with tissue homogenates from pigs with

post-weaning multisystemic wasting syndrome. J Comp Pathol 1999;121:139–148.

70. Harms PA, Sorden SD, Halbur PG, et al. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. Vet Pathol 2001;38:528–539.

71. Rosell C, Segales J, Plana-Duran J, et al. Pathological, immunohistochemical, and in-situ hybridization studies of natural cases of postweaning multisystemic wasting syndrome (PMWS) in pigs. J Comp Pathol 1999;120:59–78.

72. Yu S, Vincent AL, Opriessnig T, et al. In vitro studies on the use of quantitative real-time RT-PCR assay to detect PCV2 replication in peripheral blood mononuclear cells. Presented at the 18th Congress of the International Pig Veterinary Society, Hamburg, Germany, 2004.

73. Choi C, Chae C. In-situ hybridization for the detection of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. J Comp Pathol 1999;121:265–270.

74. Yu S, Opriessnig T, Kitikoon P, et al. Porcine circovirus type 2 (PCV2) distribution and replication in tissues and immune cells in early infected pigs. Vet Immunol Immunopathol 2007;115:261–272.

75. Krakowka S, Ellis JA, Meehan B, et al. Viral wasting syndrome of swine: Experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol 2000;37:254–263.

76. Pogranichnyy RM, Yoon KJ, Harms PA, et al. Characterization of immune response of young pigs to porcine circovirus type 2 infection. Viral Immunol 2000;13:143–153.

77. Rovira A, Balasch M, Segales J, et al. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. J Virol 2002;76: 3232–3239.

78. McKeown NE, Opriessnig T, Thomas P, et al. Effects of porcine circovirus type 2 (PCV2) maternal antibodies on experimental infection of piglets with PCV2. Clin Diagn Lab Immunol 2005;12:1347–1351.

79. Ostanello F, Caprioli A, Di Francesco A, et al. Experimental infection of 3-week-old conventional colostrum-fed pigs with porcine circovirus type 2 and porcine parvovirus. Vet Microbiol 2005;108:179–186.

80. Allan G, McNeilly F, McNair I. Passive transfer of maternal antibodies to PCV2 protects against development of post-weaning multisystemic wasting syndrome (PMWS): Experimental infections and a field study. Pig J 2002;50:59–67.

81. McIntosh KA, Harding JC, Ellis JA, et al. Detection of Porcine circovirus type 2 viremia and seroconversion in naturally infected pigs in a farrow-to-finish barn. Can J Vet Res 2006;70: 58–61.

82. Larochelle R, Magar R, D'Allaire S. Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. Can J Vet Res 2003; 67:114–120.

83. Rodriguez-Arrioja GM, Segales J, Calsamiglia M, et al. Dynamics of porcine circovirus type 2 infection in a herd of pigs with postweaning multisystemic wasting syndrome. Am J Vet Res 2002; 63:354–357.

84. Sibila M, Calsamiglia M, Segales J, et al. Use of a polymerase chain reaction assay and an ELISA to monitor porcine circovirus type 2 infection in pigs from farms with and without postweaning multisystemic wasting syndrome. Am J Vet Res 2004; 65:88–92.

85. Krakowka S, Ellis JA, McNeilly F, et al. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). Vet Pathol 2001;38:31–42.

86. Meerts P, Misinzo G, Lefebvre D, et al. Correlation between the presence of neutralizing antibodies against porcine circovirus 2 (PCV2) and protection against replication of the virus and development of PCV2-associated disease. BMC Vet Res 2006;2: article 6.

87. Darwich L, Pie S, Rovira A, et al. Cytokine mRNA expression profiles in lymphoid tissues of pigs naturally affected by postweaning multisystemic wasting syndrome. J Gen Virol 2003;84: 2117–2125.

88. Vincent IE, Balmelli C, Meehan B, et al. Silencing of natural interferon producing cell activation by porcine circovirus type 2 DNA. Immunology 2007;120:47–56.

89. Tischer I, Bode L, Apodaca J, et al. Presence of antibodies reacting with porcine circovirus in sera of humans, mice, and cattle. Arch Virol 1995;140:1427–1439.

90. Calsamiglia M, Sibila M, Segales J, et al. Detection of porcine circovirus type 2 in different routes of excretion: Possible transmission routes and correlation with presence of postweaning multisystemic syndrome characteristic lesions. Proceedings of the 17th International Pig Veterinary Society Congress, Ames, IA, 2002, 1, p. 28.

91. Liu Q, Wang L, Willison P, et al. Quantitative, competitive PCR analysis of porcine circovirus DNA in serum from pigs with postweaning multisystemic wasting syndrome (PMWS). J Clin Microbiol 2000;38:3474–3477.

92. Sibila M, Calsamiglia M, Seagales J, et al. Detection of porcine circovirus type 2 genome in nasal swabs and serum samples from naturally infected pigs using polymerase chain reaction. Presented at the International Conference on ssDNA Viruses of Plants, Birds, Pigs, and Primates, St Malo, France, European Society of Veterinary Virology, 2001.

93. Rose N, Larour, Le Diguerher G. Serological profile of porcine circovirus type 2 (PCV2) in PMWS affected and non-affected farms. Presented at the International Conference on ssDNA Viruses of Plants, Birds, Pigs, and Primates, St Malo, France, 2001.

94. Grierson SS, King DP, Wellenberg GJ, et al. Genome sequence analysis of 10 Dutch porcine circovirus type 2 (PCV-2) isolates from a PMWS case-control study. Res Vet Sci 2004;77:265–268.

95. Allan G, McNeilly F, Meehan B, et al. Reproduction of postweaning multisystemic wasting syndrome in pigs experimentally inoculated with a Swedish porcine circovirus 2 isolate. J Vet Diagn Invest 2003;15:553–560.

96. Hasslung F, Wallgren P, Ladekjaer-Hansen AS, et al. Experimental reproduction of postweaning multisystemic wasting syndrome (PMWS) in pigs in Sweden and Denmark with a Swedish isolate of porcine circovirus type 2. Vet Microbiol 2005; 106:49–60.

97. Fenaux M, Opriessnig T, Halbur PG, et al. Two amino acid mutations in the capsid protein of type 2 porcine circovirus (PCV2) enhanced PCV2 replication in vitro and attenuated the virus in vivo. J Virol 2004;78:13440–13446.

98. DeLay J, McEwen B, Carman S, et al. Porcine circovirus type 2-associated disease is increasing. AHL Newsletter, 2005, 9, p. 22.

99. Lopez-Soria S, Segales J, Nofrarias M, et al. Genetic influence on the expression of PCV disease. Vet Rec 2004;155:504.

100. Opriessnig T, Fenaux M, Thomas P, et al. Evidence of breed-dependent differences in susceptibility to porcine circovirus type-2-associated disease and lesions. Vet Pathol 2006;43:281–293.

101. Meerts P, Van Gucht S, Cox E, et al. Correlation between type of adaptive immune response against porcine circovirus type 2 and level of virus replication. Viral Immunol 2005;18:333–341.

102. Pogranichniy RM, Yoon KJ, Harms PA, et al. Case-control study on the association of porcine circovirus type 2 and other swine viral pathogens with postweaning multisystemic wasting syndrome. J Vet Diagn Invest 2002;14:449–456.

103. Allan GM, McNeilly F, Meehan BM, et al. A sequential study of experimental infection of pigs with porcine circovirus and porcine parvovirus: Immunostaining of cryostat sections and virus isolation. J Vet Med B Infect Dis Vet Public Health 2000;47:81–94.

104. Kennedy S, Moffett D, McNeilly F, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. J Comp Pathol 2000;122:9–24.

105. Opriessnig T, Fenaux M, Yu S, et al. Effect of porcine parvovirus vaccination on the development of PMWS in segregated early weaned pigs coinfected with type 2 porcine circovirus and porcine parvovirus. Vet Microbiol 2004;98:209–220.

106. Opriessnig T, Thacker EL, Yu S, et al. Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2. Vet Pathol 2004;41:624–640.

107. Ellis JA, Allan G, Krakowka S. Effect of coinfection with genogroup 1 porcine torque teno virus on porcine circovirus type 2-associated postweaning multisystemic wasting syndrome in gnotobiotic pigs. Am J Vet Res 2008;69:1608–1614.

108. McKeown NE, Fenaux M, Halbur PG, et al. Molecular characterization of porcine TT virus, an orphan virus, in pigs from six different countries. Vet Microbiol 2004;104:113–117.

109. Pallares FJ, Halbur PG, Opriessnig T, et al. Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). J Vet Diagn Invest 2002; 14:515–519.

110. Allan G, Mcneilly F, McNair I. Neonatal vaccination for Mycoplasma hyopneumoniae and post-weaning multisystemic wasting syndrome: A field trial. Pig J 2001;48:34–41.

111. Kyriakis SC, Saoulidis K, Lekkas S, et al. The effects of immuno-modulation on the clinical and pathological expression of postweaning multisystemic wasting syndrome. J Comp Pathol 2002; 126:38–46.

112. Opriessnig T, Yu S, Gallup JM, et al. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Vet Pathol 2003;40:521–529.

113. Hoogland MJ, Opriessnig T, Halbur PG. Effects of adjuvants on porcine circovirus type 2-associated lesions. J Swine Health Prod 2006;14:133–139.

114. Krakowka S, Ellis JA, McNeilly F, et al. Immunologic features of porcine circovirus type 2 infection. Viral Immunol 2002;15: 567–582.

115. Kawashima K, Tsunemitsu H, Horino R, et al. Effects of dexamethasone on the pathogenesis of porcine circovirus type 2 infection in piglets. J Comp Pathol 2003;129:294–302.

116. Fenaux M, Opriessnig T, Halbur PG, et al. A chimeric porcine circovirus (PCV) with the immunogenic capsid gene of the pathogenic PCV type 2 (PCV2) cloned into the genomic backbone of the nonpathogenic PCV1 induces protective immunity against PCV2 infection in pigs. J Virol 2004;78:6297–6303.

117. Fenaux M, Opriessnig T, Halbur PG, et al. Immunogenicity and pathogenicity of chimeric infectious DNA clones of pathogenic porcine circovirus type 2 (PCV2) and nonpathogenic PCV1 in weanling pigs. J Virol 2003;77:11232–11243.

118. Allan G, Krakowka S, Ellis J. PCV2: Ticking time bomb? Pig Prog 2002;18:14–15.

119. American Association of Swine Veterinarians (AASV). Porcine circovirus associated disease (PCVAD) case definition, 2006. Available at: http://www.aasp.org/aasv/position-PCVAD.htm. Accessed November 4, 2008.

120. Schulze C, Neumann G, Grutze I, et al. Case report: Porcine circovirus type 2 infection in an European wild boar (*Sus scrofa*) in the state of Brandenburg, Germany. Dtsch Tierarztl Wochenschr 2003;110:426–428.

121. Segales J, Domingo M. Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. Vet Q 2002;24:109–124.

122. Clark E., Postweaning multisystemic wasting syndrome. Proceedings of American Association of Swine Practitioners Quebec City, Canada, 1997, 499–501.

123. Horlen KP, Dritz SS, Nietfeld JC, et al. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. J Am Vet Med Assoc 2008;232: 906–912.

124. Opriessnig T, Janke BH, Halbur PG. Cardiovascular lesions in pigs naturally or experimentally infected with porcine circovirus type 2. J Comp Pathol 2006;134:105–110.

125. Opriessnig T, McKeown NE, Harmon KL, et al. Porcine circovirus type 2 infection decreases the efficacy of a modified live porcine reproductive and respiratory syndrome virus vaccine. Clin Vaccine Immunol 2006;13:923–929.

126. Kim J, Chae C. Necrotising lymphadenitis associated with porcine circovirus type 2 in pigs. Vet Rec 2005;156:177–178.

127. Jensen T, Vigre H, Svensmark B, et al. Distinction between porcine circovirus type 2 enteritis and porcine proliferative enteropathy caused by *Lawsonia intracellularis*. J Comp Pathol 2006;135: 176–182.

128. Harms PA, Halbur PG, Sorden SD. Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infection. J Swine Health Prod 2002;10:27–30.

129. Kim J, Chung HK, Chae C. Association of porcine circovirus 2 with porcine respiratory disease complex. Vet J 2003;166:251–256.

130. West KH, Bystrom JM, Wojnarowicz C, et al. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. J Vet Diagn Invest 1999;11:530–532.

131. Mikami O, Nakajima H, Kawashima K, et al. Nonsuppurative myocarditis caused by porcine circovirus type 2 in a weak-born piglet. J Vet Med Sci 2005;67:735–738.

132. Sanchez RE Jr, Nauwynck HJ, McNeilly F, et al. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. Vet Microbiol 2001;83:169–176.

133. Johnson CS, Joo HS, Direksin K, et al. Experimental in utero inoculation of late-term swine fetuses with porcine circovirus type 2. J Vet Diagn Invest 2002;14:507–512.

134. Smith WJ, Thomson JR, Done S. Dermatitis/nephropathy syndrome of pigs. Vet Rec 1993;132:47.

135. Rosell C, Segales J, Ramos-Vara JA, et al. Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. Vet Rec 2000;146:40–43.

136. Choi C, Chae C. Colocalization of porcine reproductive and respiratory syndrome virus and porcine circovirus 2 in porcine dermatitis and nephrology syndrome by double-labeling technique. Vet Pathol 2001;38:436–441.

137. Thibault S, Drolet R, Germain MC, et al. Cutaneous and systemic necrotizing vasculitis in swine. Vet Pathol 1998;35:108–116.

138. Lainson FA, Aitchison KD, Donachie W, et al. Typing of *Pasteurella multocida* isolated from pigs with and without porcine dermatitis and nephropathy syndrome. J Clin Microbiol 2002;40: 588–593.

139. Thomson JR, Higgins RJ, Smith WJ, et al. Porcine dermatitis and nephropathy syndrome. Clinical and pathological features of cases in the United Kingdom (1993–1998). J Vet Med A Physiol Pathol Clin Med 2002;49:430–437.

140. Krakowka S, Hartunian C, Hamberg A, et al. Evaluation of induction of porcine dermatitis and nephropathy syndrome in gnotobiotic pigs with negative results for porcine circovirus type 2. Am J Vet Res 2008;69:1615–1622.

141. Choi J, Stevenson GW, Kiupel M, et al. Sequence analysis of old and new strains of porcine circovirus associated with congenital tremors in pigs and their comparison with strains involved with postweaning multisystemic wasting syndrome. Can J Vet Res 2002;66:217–224.

142. Correa AM, Zlotowski P, de Barcellos DE, et al. Brain lesions in pigs affected with postweaning multisystemic wasting syndrome. J Vet Diagn Invest 2007;19:109–112.

143. Seeliger FA, Brugmann ML, Kruger L, et al. Porcine circovirus type 2-associated cerebellar vasculitis in postweaning multisystemic wasting syndrome (PMWS)-affected pigs. Vet Pathol 2007;44:621–634.

144. Ellis J, Hassard L, Clark E, et al. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J 1998;39:44–51.

145. Blanchard P, Mahe D, Cariolet R, et al. An ORF2 protein-based ELISA for porcine circovirus type 2 antibodies in post-weaning multisystemic wasting syndrome. Vet Microbiol 2003;94:183–194.

146. Liu C, Ihara T, Nunoya T, et al. Development of an ELISA based on the baculovirus-expressed capsid protein of porcine circovirus type 2 as antigen. J Vet Med Sci 2004;66:237–242.

147. Nawagitgul P, Harms PA, Morozov I, et al. Modified indirect porcine circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV. Clin Diagn Lab Immunol 2002; 9:33–40.

148. Hamel AL, Lin LL, Sachvie C, et al. PCR detection and characterization of type-2 porcine circovirus. Can J Vet Res 2000; 64:44–52.

149. Shibata I, Okuda Y, Yazawa S, et al. PCR detection of porcine circovirus type 2 DNA in whole blood, serum, oropharyngeal swab, nasal swab, and feces from experimentally infected pigs and field cases. J Vet Med Sci 2003;65:405–408.

150. Segales J. Proc Am Assoc Swine Veterinarians: PCV2/PMWS Seminar 2006;12:1–7.

151. Rose N, Blanchard P, Cariolet R, et al. Vaccination of porcine circovirus type 2 (PCV2)-infected sows against porcine parvovirus (PPV) and Erysipelas: Effect on post-weaning multisystemic wasting syndrome (PMWS) and on PCV2 genome load in the offspring. J Comp Pathol 2007;136:133–144.

152. Opriessnig T, Thacker E, Halbur P. Chlortetracycline is effective in reducing lesions in pigs infected with Mycoplasma hyopneumoniae and porcine circovirus type 2. Proc. 19th Int. Pig Vet. Soc. Congr. 2006;2:203.

153. Royer R, Nawagitgul P, Halbur PG, et al. Susceptibility of porcine circovirus type 2 to commercial and laboratory disinfectants. J Swine Health Prod 2001;9:281–284.

154. Ramamoorthy S, Meng XJ. Porcine circovirus: A miniscule yet mammoth paradox. Anim Health Res Rev 2008;9:1–20.

155. Rose N, Larour G, Le Diguerher G, et al. Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. Prev Vet Med 2003;61:209–225.

156. Lopez-Soria S, Segales J, Rose N, et al. An exploratory study on risk factors for postweaning multisystemic wasting syndrome (PMWS) in Spain. Prev Vet Med 2005;69:97–107.

157. Dewey CE, Johnston WT, Gould L, et al. Postweaning mortality in Manitoba swine. Can J Vet Res 2006;70:161–167.

158. Halbur PG. Update on PRRSV in grow-finish. Proc. Swine Dis. Conf. Swine Pract. 2000;8:113–123.

159. Halbur PG. PMWS: Improved models and recent findings of clinical significance. Proc. Swine Dis. Conf. Swine Pract. 2001;9: 162–166.

160. Charreyre C, Beseme S, Brun A, et al. Vaccination strategies for the control of circoviral diseases in pigs. Proc Intern Conf Anim. Circoviruses Assoc. Dis. 2005;26–30.

161. Desrosier R, Clark E, Tremblay D. Pulmonary results with Ingelvac® Circo FLEXä to protect multiple ages of Quebec pigs against PCVAD. Proc Am Assoc Swine Pract. 2007;38:143–145.

162. Von Richthofen I, Woolfenden N, Lischewski A, et al. Field efficacy of a PCV2 vaccine in three week old piglets in the United Kingdom. Fifth Symposium on Emerging and Re-Emerging Pig Diseases Krakow, Poland, 2007, p. 122.

163. Grau AF, Jorgensen J, Thacker B, et al. Field performance of a conditionally licensed vaccine: The US experience. Proc Am Assoc Swine Vet.: PCV2/PMWS Sem. 2007;38:159–161.

164. Fenaux M, Halbur PG, Haqshenas G, et al. Cloned genomic DNA of type 2 porcine circovirus is infectious when injected directly into the liver and lymph nodes of pigs: Characterization of clinical disease, virus distribution, and pathologic lesions. J Virol 2002;76:541–551.

165. Opriessnig T, Patterson AR, Elsener J, et al. Influence of maternal antibodies on efficacy of porcine circovirus type 2 (PCV2) vaccination to protect pigs from experimental infection with PCV2. Clin Vaccine Immunol 2008;15:397–401.

166. Gillespie J, Juhan NM, DiCristina J, et al. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) is genetically stable in vitro and in vivo. Vaccine 2008;26: 4231–4236.