

PORCINE CIRCOVIRUS 3



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SUMMARY

Etiology

- Porcine circovirus (PCV) is a non-enveloped DNA virus with a single-stranded circular genome. It belongs to the genus *Circovirus* in the family *Circoviridae*.
- Two species, PCV1 and PCV2, are currently recognized. While taxonomy is still debated, four distinct clades have been identified: PCV2a, PCV2b, PCV2c and PCV2d. Epidemiological studies suggest that PCV2d (mPCV2b) has displaced PCV2b as the dominant genotype in recent years.
- Researchers at Kansas State University have proposed a third species, PCV3, isolated from diseased sows and mummified fetuses.

Cleaning and Disinfection

- In the laboratory, potassium peroxymonosulfate and sodium chloride (Virkon®S), sodium hypochlorite (Clorox® Bleach), and sodium hydroxide appear to be the most effective virucidal agents against PCV2. Chlorhexidine, ethanol, aldehydes, and iodine products are generally not effective disinfectants for PCV.
- Specific information on the disinfection of PCV3 is not available.

Epidemiology

- Domestic and feral swine are susceptible to PCV1 and PCV2 infection. Both species of virus are present worldwide in areas where swine are raised.
- Evidence of PCV has been found in other species, including humans, although virus replication and transmission within these species has not been reported. Attempts to produce disease experimentally in non-porcine species have been inconsistent, and there are no reports of human illness associated with PCV.
- The novel virus described as PCV3 was isolated from a sow farm in North Carolina in 2015. Incidence of disease at this facility was reported at 12.5%. Deaths occurred among fetuses and sows, although exact numbers are unknown.
- Morbidity and mortality associated with PCV2 vary, due to the multifactorial nature of the disease and differing clinical presentations. In piglets with postweaning multisystemic wasting syndrome (PMWS), morbidity ranges from 4–60%, while mortality is slightly lower at 4–20%. Rates of reproductive failure are also varied, with farrowing rates below 60% and litters of up to 75% mummified fetuses or stillborn piglets reported.

Transmission

- Transmission of PCV2 occurs by direct contact, and virus can be detected in most bodily secretions. Oronasal is considered to be the primary route. The mode of PCV3 transmission among infected sows has not yet been determined.
- Vertical transmission can occur if the dam is infected during gestation or inseminated with infected semen. Infection spreads from fetus-to-fetus in utero. Vertical transmission has been seen with PCV2 and PCV3 infection.

Infection in Swine/Pathogenesis

- PCV depends on host cell DNA polymerases and replicates in actively dividing cells such as lymphocytes, macrophages, endothelial and epithelial cells. PCV2 co-infection with other pathogens is common, and immune stimulation appears to play a role in enhancing virus replication in target cells.
- Subclinical infection is most common. Porcine circovirus-associated disease (PCVAD)/porcine circovirus disease (PCVD) is multifactorial, and systemic, respiratory, enteric, and reproductive presentations are possible. The virus is one factor linked to PMWS, a multifactorial disease of pigs two-to-four months-of-age, as well as porcine dermatitis and nephropathy syndrome (PDNS).
- The only two disease presentations associated with PCV3 infection thus far are reproductive failure and PDNS. No co-infection with other pathogens has been reported.

Diagnosis

- Clinical assessment is important for diagnosis due to the ubiquitous presence of PCV2 in swine populations. Confirmation of PCVAD/PCVD depends on a combination of clinical signs, characteristic gross and microscopic lesions, and detection of the virus in the tissues.
- Quantitative polymerase chain reaction assay (qPCR) is useful in the identification and quantification of PCV2 and PCV3 in tissues or serum. Virus thresholds to suggest disease causality vary significantly between laboratories, and qPCR alone is not sufficient for individual diagnosis.
- Immunohistochemistry (IHC) and in situ hybridization are utilized to detect PCV2 in tissues and used in conjunction with qPCR.
- Metagenomic sequencing was used by Kansas State University researchers in the discovery of PCV3.

Immunity

- Many pigs worldwide have anti-PCV2 antibodies indicative of previous exposure.
- Seroconversion occurs in both subclinical and clinical cases. Symptomatic pigs may demonstrate decreased humoral immunity, specifically with fewer neutralizing antibodies, higher levels of viremia, and increased viral shedding.
- Several PCV2 vaccines are commercially available in the U.S. and have significantly reduced post-weaning mortality.

Prevention and Control

- Herd diagnoses are based on increased levels of clinical signs and postweaning mortality, herd history, diagnosis of PCV in multiple individuals, and ruling out other common swine pathogens. Laboratory diagnosis is especially important on farms with PCVAD/PCVD in pigs that have already been vaccinated for PCV2 or farms where vaccination programs are not meeting expectations.

- Limiting PCVAD/PCVD depends on vaccination of piglets and/or sows plus controlling other factors that may influence and enhance PCV infection. Good nutrition, strict biosecurity, adequate space and ventilation, separation by age and sex, and vaccination for other co-infecting pathogens are all beneficial.

Gaps in Preparedness

- The abundance of known genomes and partial sequences, variations in disease presentation, and worldwide presence of PCV complicate efforts to study the virus and present data in a uniform way. Likewise, pathogenesis is not completely understood. Further study of host genetics and susceptibility, timing of infection (i.e., impact of waning maternal antibody on early PCV2 infection as well as disease co-factors, both infectious and non-infectious), and virus genotype may offer more clues.
- Widespread PCV2 vaccination has been successful in lessening the economic impact of this disease in the U.S. However, continued vigilance is necessary to identify PCV variants that may impact vaccine efficacy such as PCV3. Future needs include the development of a PCV3 infectious clone for the purpose of reproducing clinical disease, confirmation that existing PCV2 vaccines do not protect against PCV3, and subsequent development and testing of PCV3 vaccines.
- In addition, relatively little is known about PCV3 or the connection between PCV and porcine dermatitis and nephropathy syndrome (PDNS). Investigation into the prevalence of this virus in U.S. swine herds is also needed.

OVERVIEW

Porcine circovirus (PCV) is one of the smallest DNA viruses known to infect mammals and has been extensively studied in recent years. It is non-enveloped with a single-stranded circular genome and belongs to the genus *Circovirus*, family *Circoviridae*. The abundance of known genomes and partial sequences, worldwide presence of the virus, and presence of recombinant strains have presented unique challenges in PCV taxonomy. First described in 1974, PCV1 was found to be a nonpathogenic contaminant of a porcine kidney cell line. In the mid-1990s, PCV2 was discovered and became associated with multiple economically significant disease syndromes in swine. While taxonomy is still debated, four distinct clades have been identified: PCV2a, PCV2b, PCV2c and PCV2d. Epidemiological studies suggest that PCV2d (mPCV2b) has displaced PCV2b as the dominant genotype. Researchers at Kansas State University have recently isolated a potentially new species, PCV3. PCV1 and PCV2 are discussed briefly in this document to provide background information; however, PCV3 is the focus of this review.

The systemic, respiratory, enteric, and reproductive diseases caused by PCV infection are known collectively as porcine circovirus-associated disease (PCVAD)/porcine circovirus disease (PCVD). In North America, the term PCVAD is generally preferred, while PCVD is used primarily in Europe. Terminology proposed by Segalés¹ to describe the clinical presentations of PCVAD/PCVD includes the following:

- PCV2-systemic disease (PCV2-SD), also known as postweaning multisystemic wasting syndrome (PMWS),
- Porcine dermatitis and nephropathy syndrome (PDNS),
- PCV2 lung disease (PCV2-LD), also known as proliferative and necrotizing pneumonia (PNP),
- PCV2 reproductive disease (PCV2-RD),
- PCV2 enteric disease (PCV2-ED), and
- PCV2 subclinical infection (PCV2-SI).

Both PCV1 and PCV2 resist inactivation at high temperature, and cold temperatures may also contribute to virus survival. PCV2 retains some infectivity over a wide pH range, from 2–12. In the laboratory, potassium peroxydisulfate and sodium chloride (Virkon[®]S), sodium hypochlorite (Clorox[®] Bleach), and sodium hydroxide appear to be the most effective virucidal agents against PCV2. Other potentially effective products include quaternary ammonium compounds (e.g., Roccal[®] D Plus, Fulsan[®]) and phenolics (e.g., Tek-Trol[®] 1-Stroke Environ[®]). Chlorhexidine, ethanol, aldehydes, and iodine products are generally not effective disinfectants for PCV. No information was found specifically on the survival or disinfection of PCV3.

Domestic and feral swine are susceptible to PCV1 and PCV2 infection, while PCV3 has only been reported in domestic swine thus far. Mice and rats in the vicinity of pig farms can also become infected with, and transmit, PCV. While PCV has been detected in farmed mink, buffaloes, cattle, and farmed shellfish, its ability to cause disease in other species is uncertain. The virus has been detected in human feces, although neither viral replication in humans nor a specific immune response to PCV have been proven. Humans and non-porcine species potentially acquire the virus from consumption of contaminated water, or beef or pork products, but there are no reports of human illness associated with PCV.

While PCV1 and PCV2 are found throughout the world, PCV3 has only been isolated from pigs on a single farm in North Carolina. The incidence of disease associated with PCV3 at this facility was 12.5%. Morbidity associated with PCV2 systemic disease varies from 4–30%, and mortality ranges from 4–20%. Piglets are most commonly affected when levels of maternal antibodies are declining or inadequate. In cases of PDNS, morbidity can reach nearly 100% in pigs over three months, while only 50% of younger

pigs are affected. Reproductive failure from PCV2 infection can drop farrowing rates below 60%. Vaccines have been available in the U.S. since 2006 and have significantly reduced mortality due to PCV2 infection.

Oronasal contact is the primary route of transmission of PCV2. The virus can be found in respiratory secretions, urine, feces, milk, colostrum, and semen. Vertical transmission of PCV2 occurs in swine if no maternal antibodies are present to protect piglets from infection. Specifically, fetuses can become infected if the dam is inseminated with infected semen or otherwise exposed to the virus during pregnancy. Vertical transmission has also been seen with PCV3 infection. Fomites, contaminated feed and biologics, hypodermic needles, and biting insects may also play a role in spreading infection among pigs.

Known clinical signs associated with PCV3 infection are limited to reproductive disease and PDNS-like skin lesions. Some affected sows died acutely, and others aborted or gave birth to mummified fetuses. The current clinical picture of PCV2 is more complex and depends on the age of the pig and other unknown factors. Subclinically infected pigs may show only a decreased average daily weight gain, while those with clinical disease are more severely affected. Typically in pigs between two- and four-months-of-age, the virus can cause wasting, diarrhea, lymphadenopathy, respiratory distress, and pallor or icterus. Older pigs more often present with PDNS signs and lesions, including anorexia, depression, stiff gait, glomerular nephritis, and irregular skin lesions that are red-to-purple or dark and crusted and commonly occur around the hind limbs and perineum. These lesions appear to be a result of systemic necrotizing vasculitis. Sows infected with PCV2 often appear clinically normal themselves, but will have mummified, macerated, stillborn, or weak-born piglets. Placentas and lymphoid tissues of sows are usually normal.

Clinical assessment must accompany any laboratory testing; detection of virus or antibodies alone are not sufficient to make a diagnosis. Quantitative polymerase chain reaction assays (qPCR) can easily identify and quantify the virus in serum of viremic animals and be used to get a low cost clinical picture of a herd. However, an individual diagnosis will ideally include immunohistochemistry (IHC) or in-situ hybridization (ISH), considered the gold standard for detecting the virus in tissues. Lymphoid tissue is commonly used, or fetal myocardium in the case of reproductive disease. Serology alone is of limited value, due to the presence of subclinical infection and the ubiquitous nature of the virus, although testing is not confounded by vaccination. Metagenomic sequencing was used by Kansas State University researchers in the discovery of PCV3.

Vaccines against PCV2 have been commercially available in the U.S. since 2006, and vaccination programs have been very successful in controlling outbreaks of disease. However, continued vigilance is necessary to identify PCV variants that may impact vaccine efficacy such as PCV3. Future needs include the development of a PCV3 infectious clone for the purpose of reproducing clinical disease, confirmation that existing PCV2 vaccines do not protect against PCV3, and subsequent development and testing of PCV3 vaccines.

The control and elimination of co-infecting pathogens is essential while the true nature of their influence on PCV infection is unknown. Further epidemiological and pathogenesis studies are also warranted to understand the distribution and patterns of PCVAD/PCVD in swine worldwide. Lastly, a global consensus must be reached in the taxonomic classification and description of various disease manifestations to allow future research to move forward in a faster, more cohesive manner.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Porcine circovirus (PCV) is a small non-enveloped virus with a single stranded, circular DNA genome in the genus *Circovirus*, family *Circoviridae*. It utilizes an ambisense transcription strategy and contains four open reading frames (ORFs). Originally discovered in 1974 as a cell culture contaminant, porcine circovirus type 1 (PCV1) is generally considered to be nonpathogenic. The identification of porcine circovirus type 2 (PCV2) followed in 1997. The systemic, respiratory, enteric, and reproductive manifestations of PCV infection are known collectively as porcine circovirus-associated disease (PCVAD)/porcine circovirus disease (PCVD).¹ In North America, the term PCVAD is generally preferred, while PCVD is used primarily in Europe. Kansas State University researchers have recently discovered a third species, porcine circovirus type 3 (PCV3).² PCV1 and PCV2 are discussed briefly in this document to provide background information; however, PCV3 is the focus of this review.

Terminology proposed by Segalés¹ to describe the clinical presentations of PCVAD/PCVD includes the following:

- PCV2-systemic disease (PCV2-SD), also known as postweaning multisystemic wasting syndrome (PMWS),
- Porcine dermatitis and nephropathy syndrome (PDNS),
- PCV2 lung disease (PCV2-LD), also known as proliferative and necrotizing pneumonia (PNP),
- PCV2 reproductive disease (PCV2-RD),
- PCV2 enteric disease (PCV2-ED), and
- PCV2 subclinical infection (PCV2-SI).

1.2 Strain Variability

Studies comparing known complete genomes of PCV1 and PCV2 suggest that pathogenicity of PCV2 may be related to its significantly greater genetic diversity and substitution rate compared to PCV1.³ It possesses the highest recorded rate of nucleotide substitution of any single-stranded DNA virus.⁴ The capsid protein, determined by the ORF2 nucleotide sequence,⁵ provides the primary differentiation between PCV types. Isolates of PCV1 and PCV2 share less than 80% homology,⁶ and the capsid proteins of PCV2 and PCV3 share approximately 35% amino acid identity.² Historically, there are two major genetically distinct subgroups of PCV2, each further divided into subgroups. These major subgroups, PCV2a and PCV2b, are determined by their DNA sequences and do not have a clear association with disease presentation.⁶ They are thought to have diverged from a common ancestor approximately 100 years ago and still share greater than 93% genetic similarity.⁷ While PCV2a was initially the most dominant genotype, a global shift to predominantly PCV2b occurred around 2003.⁸

Alternatively, recent proposals have sought to reclassify PCV2 into only four genotypes, PCV2a–d.^{4,5} Conserved reference sequences and/or identification of marker positions within the ORF2, rather than genetic distance, appear to support this realignment.⁵ Unique PCV2 strains identified in 2010 were designated PCV2d⁹, and similar viruses isolated in North¹⁰ and South America¹¹ have been designated mutant PCV2b (mPCV2b). Epidemiological studies suggest that PCV2d (mPCV2b) has displaced PCV2b as the dominant genotype in recent years.⁴

Classification reshuffling will likely continue, in part, due to the increasing recognition of recombinant strains of PCV2.⁵ The same pig can be co-infected with multiple strains of the same or different genotypes, allowing for exchange of genetic material. Evidence of viral recombination has been seen in

vivo and in vitro.⁷ One such recombinant virus has a genome containing an ORF1 from PCV1 and ORF2 from PCV2a.¹² Distinct genotypes of the newly identified PCV3 have not been reported, and it is unclear where this novel virus will fit into future classification schemes.

2. Cleaning and Disinfection

2.1 Survival

Both PCV1 and PCV2 are resistant to inactivation at high temperatures. The stability of PCV1 at 70°C (158°F) for 15 minutes, and PCV2 at 75°C (167°F) for 15 minutes or 56°C (133°F) for one hour, suggests that the virus may remain infectious at high ambient temperatures in the environment.⁷ Cold temperatures may also contribute to survival. It has been shown that PCV2b can survive and remain infectious in fresh pork for two days post-inoculation (DPI) at room temperature (25°C/77°F), six DPI at refrigeration temperature (4°C/39.2°F), and 30 DPI at freezer temperature (-20°C/-4°F).¹³ Additionally, PCV1 is stable at a pH of three, while PCV2 retains some infectivity at a pH as low as two and as high as 11 or 12.⁷

No information was found on the survival of PCV3.

2.2 Disinfection

In the laboratory, potassium peroxymonosulfate and sodium chloride (Virkon®S), sodium hypochlorite (Clorox® Bleach), and sodium hydroxide appear to be the most effective virucidal agents against PCV2. Other potentially effective products include quaternary ammonium compounds (e.g., Roccal® D Plus, Fulsan®) and phenolics (e.g., Tek-Trol®, 1-Stroke Environ®). Chlorhexidine, ethanol, aldehydes, and iodine products are generally not effective disinfectants for PCV.¹⁴⁻¹⁶

No information was found on the disinfection of PCV3.

3. Epidemiology

3.1 Species Affected

Domestic and feral swine are susceptible to PCV infection, and isolates from both pig populations are almost identical to each other.¹⁷ In one study 26% of feral swine showed serological evidence of PCV2 infection.¹⁸ Mice and rats found near swine herds can be infected with PCV2 and transmit the virus; however, rodent populations outside of pig farms are not known to carry PCV. The exact role of rodents as a potential host or mechanical vector is not clear.⁷ While it was initially thought that non-porcine species were not susceptible to PCV infection, evidence now exists for cross-species transmission. Though studies are limited, PCV2 has been detected in farmed minks with diarrhea,¹⁹ beef products,²⁰ buffaloes,¹⁷ and farmed shellfish.²¹ Moderate clinical signs, viremia, and seroconversion were observed in a small group of calves experimentally infected with PCV2. Clinical signs included diarrhea, swelling of superficial lymph nodes, and mild dyspnea; however, characteristic wasting commonly seen in affected piglets was not seen in any of the calves.²² Experimental infection in other livestock species has not consistently produced disease.²³

3.2 Zoonotic Potential

One study in the U.S. found PCV in 5% of human adult stool samples and 70% of store-bought pork products tested. A single highly divergent sequence was also found, based on the replication-associated (Rep) protein, suggesting the existence of additional uncharacterized PCV species.²⁴ Discovery of PCV in both human and swine vaccines has also caused concern and led to questions regarding vaccine production.⁷ Despite evidence of PCV existence in humans, viral replication in human cells or an immune response to the virus have not been verified.¹⁷ Similarly, no direct evidence exists of human illness

associated with PCV infection. Anti-PCV antibodies have been detected in humans; however, this may be the result of infection with a similar circovirus and not necessarily indicative of PCV infection.²⁵

3.3 Geographic Distribution

The newly discovered PCV3 was isolated from a sow farm in North Carolina.²⁶ Prior to that, a different recombinant PCV, proposed by researchers to be PCV1/2a or PCV3 was identified by the diagnostic laboratory at the University of Montreal.¹² To date, PCV3 is not recognized as a distinct species by the International Committee on Taxonomy of Viruses²⁷ and its true geographic range is unknown.

In contrast, PCV2 can be found in North America, Asia, Europe, and Oceania in regions where swine are raised. Since its initial discovery in France, it has been recognized as a significant pathogen among swine worldwide.⁶ Still, the prevalence of clinical disease associated with PCV2 is considerably less than its actual presence among populations.¹ While PCV1 can also be found in swine worldwide, its prevalence is reportedly less than that of PCV2.³

3.4 Morbidity and Mortality

To date, only one PCV3 outbreak has been documented on a single farm. The virus was isolated both from mummified fetuses and from sows that died from PDNS. The incidence of disease associated with PCV3 at this facility was 12.5%, or 34 positive samples out of 271.²⁶

Among seven- to 12-week-old-piglets, mortality associated with PMWS is significantly higher in those demonstrating a low serologic titer at seven weeks. This suggests that greater levels of maternal antibodies can offer protection against more severe clinical disease.²⁸ In general, morbidity is variable, from 4–30%, and occasionally as high as 60%. Mortality is slightly lower, between 4–20%.¹ When PDNS is associated with PCV2 infection, morbidity is nearly 100% in pigs over three-months-old and 50% in younger pigs.⁷

Rates of reproductive failure associated with PCV2 infection are varied. The first reported outbreak at a breeding facility resulted in farrowing rates below 60%, of which 20% were beyond 13 weeks of gestation. Farrowing during the peak of the outbreak resulted in litters of approximately 75% mummified fetuses or stillborn piglets. However, not all herds are affected to this extent and there are often other factors contributing to reproductive failure.⁸

Since their introduction in 2006, commercial vaccines for sows, gilts, and piglets have significantly reduced mortality associated with PCV2 infection in the U.S.²³ There is also some indication that disease susceptibility²⁹ and vaccine effectiveness³⁰ is influenced by genetic differences (e.g., breed).

4. Transmission

Transmission of PCV2 is known to occur by direct contact with infected pigs, primarily through oronasal exposure. The virus has been detected in nasal, ocular, tonsillar, and bronchial secretions, as well as saliva, urine, feces, milk, colostrum, and semen.⁷ Fomites, contaminated feed and biologics, hypodermic needles, and biting insects may also play a role in the spread of infection.²³ It has been suggested that transmission to non-porcine species is the result of consumption of infected beef or pork products¹⁹ or water contaminated with pig feces.¹⁷ Likewise, pigs can become infected by eating the raw tissues of viremic animals.⁷

Fetal PCV2 infections can occur if the dam is exposed during pregnancy or inseminated with infected semen. The virus can also be spread fetus-to-fetus, and the timing of in utero infection will determine the clinical outcome.³¹

5. Infection in Swine/Pathogenesis

Circoviruses typically replicate in actively dividing cells of young animals. Enhancement of PCV infection also occurs when the immune system is stimulated and a greater number of lymphocytes are available for the virus to replicate in.⁶ Likewise, virus replication can occur in other cells with a high mitotic index, such as endothelial cells, epithelial cells, and macrophages.⁷

5.1 Clinical Signs

Despite the involvement of PCV2 in several disease presentations, subclinical infection is most common. Pigs with subclinical infection may show decreased average daily weight gain with no other clinical signs or lesions.¹ The virus is one factor responsible for PMWS, which affects piglets typically between two- and four-months-of-age. This multifactorial disease manifests as enlarged subcutaneous lymph nodes, wasting, diarrhea, respiratory distress, pallor, and occasional icterus.⁷

Enteritis or respiratory disease can also be present individually in the absence of lesions in the lymphoid tissues, thus not consistent with the definition PMWS or PCV2 systemic disease. Consequently, PCV2 is also one of several potential contributors to porcine respiratory disease complex (PRDC).¹ Expression of disease can be influenced and enhanced by co-infection with other pathogens, such as porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), *Mycoplasma hyopneumoniae*,⁶ and torque teno virus (TTV).⁷ However, PCV2 is also capable of causing disease independent PRRSV, PPV, or pseudorabies virus (PRV).²⁸ *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, *Staphylococcus* species, and *Streptococcus* species have also been found in swine infected with PCV.²³ Acute disease can cause death within a few days or the disease can take a chronic course resulting in animals that fail to thrive or show a marked delay in reaching market weight.²³

Nursery, growing, and adult pigs can all be affected by PDNS associated with PCV2 infection. Symptoms include anorexia, depression, stiff gait, and irregular, red-to-purple or dark crusted skin lesions, most often on the hind limbs and perineum.⁷ Acutely affected individuals often die of renal failure within several days after presenting with clinical signs.²³ This disease manifestation has also been seen in sows infected with PCV3, independent of infection with PCV2, PRRSV, or swine influenza virus (SIV).²

Infection with PCV3² and PCV2 have been associated with reproductive failure.⁷ Mummified fetuses from aborting sows with PDNS-like skin lesions were found to contain the novel PCV3 virus, and the affected farm had reported chronic poor reproductive performance.²⁶ Sows with PCV2 infection may abort or have increased rates of mummified, macerated, stillborn, or weak-born piglets. Early embryonic death and increased pre-weaning death may also be observed. The most consistent finding is mummified fetuses of varying size. Dams may present with fever or anorexia, although clinical signs are often absent.³¹ At least in the case of PCV2, reproductive disease is uncommon and mainly affects naïve populations.¹

5.2 Postmortem Lesions

Lesions associated with subclinical PCV2 infection include granulomatous inflammation of lymphoid tissues, lungs, liver, kidney, heart, and intestines.⁶ Histopathology commonly shows corresponding lymphocyte depletion and infiltration by histiocytic cells, with or without intracytoplasmic inclusion bodies.⁷ Nursery pigs have succumbed to heart failure due to acute necrotizing vasculitis or chronic fibrosing myocarditis with chronic vasculitis in the heart, kidney, and lymphoid tissues.¹ Peribronchial fibrosis, fibrinous bronchiolitis, interstitial pneumonia,⁷ purulent bronchopneumonia,²⁸ and granulomatous enteritis¹ can be seen in some cases. The liver may be pale, firm, and either enlarged or atrophied. Lymphocyte depletion contributes to lymph node atrophy in later stages of disease, in addition

to cortical atrophy of the thymus. White spots may be present on the cortex of the kidney.⁷ A moderate to high amount of PCV2 can be found in damaged tissues.¹

Necrotic skin lesions of the rear legs, as well as vasculitis and glomerulonephritis of the kidneys, are associated with PDNS presentation.⁶ Lymph nodes may become red and enlarged due to drainage from hemorrhagic lesions.²³ Enlarged kidneys will appear granular with red pinpoint lesions and renal pelvis edema in acute cases. Lesions are associated with systemic necrotizing vasculitis and will not consistently contain PCV antigen. While skin or renal lesions may occur alone, they more commonly occur together.⁷

In utero PCV2 infection can result in mummified or edematous fetuses with ascites, hydrothorax, and hydropericardium. Other potential lesions include hepatic congestion, cardiac hypertrophy, myocarditis, and mild pneumonia in developing fetuses.¹ Mummified fetuses were collected from the North Carolina farm with reported PCV3 infection;^{2,26} however, specific postmortem lesions have not been described.

6. Diagnosis

6.1 Clinical History

In 1974, PCV1 was discovered as a persistent contaminant in a pig kidney cell line. Although it has been isolated from stillborn piglets, it is widely believed to be apathogenic in swine.⁶ A novel wasting disease affecting Canadian swine was first described in the early 1990s and reached epidemic proportions in countries across the world over the next two decades. PCV2 was identified in 1997. However, the first isolation and complete genome sequence of PCV2 was not reported until 1998. Severe outbreaks of disease in North America were subsequently described beginning in 2004. Despite the sudden global emergence, retrospective evidence exists for PCV2 subclinical infection as early as 1985, with polymerase chain reaction (PCR) evidence of the virus as far back as 1962.³² Phylogenetic studies go even further in suggesting that the virus has infected pigs for at least 100 years.²³ The common presence of PCV among swine populations makes clinical assessment a key factor. Identifying the virus or antibodies against it are not enough to confirm a diagnosis.¹ The confirmation of PCVAD/PCVD depends on a combination of clinical signs, characteristic gross and microscopic lesions, and the detection of the virus in lesions.²³

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Diagnostic tests have varying efficacy based on the specific type of PCVAD/PCVD.¹ Generally, systemic, respiratory, enteric, and late term reproductive disease can be easily diagnosed by a laboratory in combination with clinical signs. Despite the ability to accurately detect PCV2 in serum of viremic pigs using quantitative PCR (qPCR), the actual viral threshold required for diagnosis tends to vary with each laboratory and technique used. For this reason, histopathology and detection of the virus in tissues are also important.¹ Both immunohistochemistry (IHC) and qPCR are useful in determining the number and distribution of infected cells to assess the role of PCV2 in clinical disease in an individual pig.⁶ A qPCR assay to identify and quantify PCV3 in tissues has also been developed.² In addition to IHC, in-situ hybridization (ISH) is considered the gold standard for detection of PCV2 antigen or DNA in tissues.³¹ Low to no amounts of virus in lymphoid tissues can distinguish PCV2-SI and PDNS from other types of PCVAD/PCVD.¹ A multiplex real-time qPCR (mrtqPCR) is able to detect and differentiate between different PCV2 genotypes, PCV2a and PCV2b.¹²

Several cell lines can be used for virus isolation of PCV, including porcine kidney cells (PK15A),¹² Vero cells, and other porcine derived cell lines.⁷ Immunofluorescence assay (IFA) with anti-PCV2 antibodies and genome sequencing have been used to confirm presence of the virus in cell lines.¹²

Metagenomic sequencing was utilized by Kansas State University in the identification of the new species, PCV3. This method allows for detection of multiple viruses or quasispecies, sequencing from isolated virus or directly from collected samples, and diagnosis in cases of unknown etiology. In the case of PCV3, tested samples from mummified fetuses and sows with PDNS lesions were negative for PCV2 by both PCR and IHC.²⁶

6.3 Tests to Detect Antibody

Serologic assays are available for the detection of anti-PCV2 antibodies; however, their usefulness is questionable due to the ubiquitous nature of the virus. Seroconversion patterns are similar in farms with or without clinical signs.⁷ Indirect IFA, enzyme linked immunosorbent assay (ELISA), and immunoperoxidase staining can be used to detect anti-PCV2 antibodies.²³

6.4 Samples

6.4.1 Preferred Samples

The genome of PCV2 can be detected in lung and lymph nodes with ISH²⁸ and these tissues have also been used to isolate PCV for mrtqPCR.¹² Samples from intestinal mucosa and Peyer's patches can be used for IHC or ISH in cases of enteric disease. Quantification of the virus in lymphoid tissues is commonly achieved by qPCR.¹ Likewise, serum can be used to isolate virus for qPCR, and testing pooled samples this way is an economically viable option for estimating viral load in herds suspected of having subclinical infection.¹

For diagnosis of reproductive disease, fetal myocardium and lymphoid tissue are the most appropriate samples. The virus can also be found in the thymus, spleen, tonsil, and lung. Serology or viremia of the dam is not diagnostic, although serum from pre-colostral liveborn piglets can be used.³¹ Detection of IgG in fetal fluids is not a reliable indicator of in utero infection.¹

6.4.2 Oral Fluids

Bronchial secretions and saliva can harbor PCV2, and fluid from bronchiolar lavage has been used to isolate the virus. However, the use of oral fluids alone is of limited diagnostic value.²³

7. Immunity

7.1 Post-exposure

Viremia associated with PCV2 infection is first detectable at approximately seven DPI, with a peak at 14–21 DPI. Many swine herds worldwide have anti-PCV2 antibodies indicative of previous exposure, although not all antibodies are protective. Pigs will mount a specific immune response two to three weeks after being infected.⁷ In piglets, seroconversion occurs on a timescale typical of other viral infections in swine, with lowest colostral antibody concentration around seven weeks of age.²⁸ Seroconversion occurs in both subclinical and clinical cases, although some studies have shown decreased humoral immunity, and specifically fewer neutralizing antibodies, in symptomatic pigs. This corresponds with a higher concentration of virus in the serum and increased viral shedding among this cohort. Cell-mediated immunity may also be a key factor in viral clearance.⁷ These diseased individuals will typically demonstrate increasing viremia from seven- to 12-weeks-of-age, corresponding to outbreaks of PMWS.²⁸ Piglets younger than four weeks do not usually develop PMWS. Severe, acute cases can result in death a few days after the onset of symptoms, or survivors will begin to recover within 7–10 days.⁷

Higher serum antibody titers have also been identified in pigs with PDNS, compared to clinically healthy pigs or those with systemic disease. This is indirect evidence of the involvement of PCV2 in the type III hypersensitivity reaction responsible for PDNS lesions.²³

7.2 Vaccines

Several major vaccines are commercially available for PCV2 in swine. They confer humoral and cellular immunity and serve to reduce mortality while improving average daily weight gain, feed conversion, and uniformity at slaughter.²³ Options include inactivated whole virus,³³ virus like particle (VLP),³⁴ a chimeric vaccine containing the ORF1 from PCV1 with the ORF2 responsible for the immunogenic capsid protein of PCV2,³⁵ and an inactivated baculovirus vector combination vaccine that also targets *Mycoplasma hyopneumoniae*.³⁶ A study on PCV2 prevalence in the U.S. was undertaken in 2006 and repeated in 2012 following widespread vaccination. The virus has not been completely eliminated from herds, but viral loads and presence of the virus at the site level and in individual finishing pigs has shown significant decline.⁸ Vaccination has also aided in the recognition of subclinical infection by increasing average daily weight gain in apparently healthy pigs infected with the virus.²³

Despite the overall success of vaccination programs in the U.S., there are still exceptions. An emerging mutant strain of PCV2 was discovered in vaccinated pigs with PCVAD/PCVD in 2012,¹⁰ and sows that died or aborted fetuses after infection with PCV3 had been previously vaccinated against PCV2.²

7.3 Cross-protection

Commercial vaccines against PCV2a can prevent infection caused by PCV2a and PCV2b, indicating that different genotypes are immunologically similar with regard to the Cap protein. Viral loads of both genotypes have been reduced to a similar extent since the initiation of vaccination programs in 2006.⁸

Anti-PCV antibodies have been detected in humans, mice, and cattle, but binding of these non-porcine antibodies is weaker compared to the porcine counterparts. Studies suggest that these are antibodies against similar species-specific circoviruses that share a limited number of antigenic epitopes.²⁵

8. Prevention and Control

Control involves not only limiting PCV infection, but controlling other factors that can enhance PCV infection. Good nutrition and biosecurity practices are key components, and several vaccine types are available to protect against PCV2.⁶ A herd diagnosis should be established based on increased levels of clinical signs and postweaning mortality, also taking into account the herd history. Individual diagnosis in one out of every three-to-five necropsied pigs and ruling out other infectious agents can help to further link PCV to disease causality. Laboratory diagnosis is especially important on farms with signs of PCVAD/PCVD in pigs that have already been vaccinated for PCV2 or farms where vaccination programs are not meeting expectations.¹

Females have a lower risk of developing PMWS, so sorting nursery pigs by sex can be beneficial.⁷ It has been suggested that this increase in disease prevalence among males can be attributed to infections following castration, as well as genetic or hormonal influence.²⁸ Other factors associated with a decreased risk include group housing of pregnant sows, greater minimum weaning weight, vaccinating sows against atrophic rhinitis, treatment of ectoparasites, oxytocin use during farrowing, and use of spray-dried plasma in nursery rations.⁷ Alternatively, overcrowding, poor air quality, and comingling of different age groups may contribute to disease severity.²³

Replication of PCV2 can occur in embryos without a zona pellucida and lead to embryonic death. Transmission during embryo transfer is unlikely if an intact zona pellucida is maintained and internationally approved washing procedures are followed. This allows for the elimination of PCV from a herd with the production of virus-free offspring.³⁷

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

The 2016 OIE Terrestrial Animal Health Code does not cover PCV. There are no recommendations for importation of swine from countries or zones infected with PCV.

10. Gaps in Preparedness

The increasing number of complete PCV genomes and partial sequences has created some confusion surrounding classification of the virus. Further complicated by the global presence of this disease, the lack of consensus and consistency can lead to difficulties in diagnosis, as well as understanding the epidemiology of PCV and factors that contribute to its virulence.⁵ Similarly, the multifactorial nature of infection and variation in disease presentation, complete with differing terminology, complicate efforts to study the virus.¹

The success of PCV2 vaccination programs has helped to reduce the economic impact of systemic disease; however, subclinical infection is now coming forward to assert a greater influence on the industry.¹ It remains unknown what exactly caused PCV2 to make the switch from an endemic virus to one of epidemic proportions in different parts of the world around the same time period. International trade, susceptible pig genetic lines, and spontaneous genotype shifts have all been proposed. Also unclear is why some pigs develop disease while others around them remain subclinically infected, or why variation in clinical disease exists among pig species. Further study of host genetics and susceptibility, timing of infection (i.e., impact of waning maternal antibody on early PCV2 infection as well as disease co-factors, both infectious and non-infectious), and virus genotype may offer more clues. The potential for introduction of new variants following vaccination pressure on PCV2 also warrants further investigation.³²

Lastly, due to its recent discovery, relatively little is known about PCV3 or the connection between PCV and PDNS. Immune complexes associated with a type III hypersensitivity reaction are responsible for the characteristic lesions, but definitive proof that lesions are caused by PCV antigen does not exist.²³ Continued vigilance is necessary to identify PCV variants that may impact vaccine efficacy such as PCV3. Future studies include development of a PCV3 infectious clone for the purpose of reproducing clinical disease, confirmation that existing PCV2 vaccines do not protect against PCV3, subsequent development and testing of PCV3 vaccines, and investigation into the prevalence of this virus in U.S. swine herds.²

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