PORCINE CIRCOVIRUS 3

Swine Health Information Center

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SUMMARY

IMPORTANCE

- Porcine circovirus 3 (PCV3) is an emerging pathogen of swine. It has been associated with signs like those caused by porcine circovirus 2 (PCV2).
- Individual case definitions for PCV3-associated reproductive disease and systemic disease have been proposed to standardize diagnostic criteria.

PUBLIC HEALTH

- Porcine circoviruses (PCVs), including PCV3, are not considered to be zoonotic.

INFECTION IN SWINE

- PCV 3 has been associated with neurological disease, reproductive failure, respiratory disease, enteric disease, and porcine dermatitis and nephropathy syndrome (PDNS). However, only a few studies have demonstrated the presence of PCV3 in lesions.
- In pathogenicity studies, PCV3 inoculation does not consistently lead to development of disease.

TREATMENT

- There is no treatment for pigs infected with PCV3.

CLEANING AND DISINFECTION

- Circoviruses are stable in the environment. In the laboratory, potassium peroxymonosulfate and sodium chloride, sodium hypochlorite, and sodium hydroxide are the most effective for PCV2.
- Specific information on the disinfection of PCV3 is not available.

PREVENTION AND CONTROL

- PCV2 prevention is based on factors that can influence susceptibility. These include good nutrition, biosecurity, and vaccination.
- No specific control measures have been described for PCV3.

TRANSMISSION

- Both PCV2 and PCV3 spread through vertical and horizontal transmission. Virus is shed in most secretions and excretions. Direct contact is the route of most significance but spread can also occur via fomites and ingestion.

PATHOGENESIS

- Little is known about PCV3 pathogenesis. PCV3 utilizes a clathrin- and dynamin-2-mediated endocytic pathway. PCVs generally replicate in actively dividing cells.

DIAGNOSIS

- PCV3 was first identified by metagenomic sequencing. Many polymerase chain reaction (PCR) assays have been developed for PCV3 in the research setting. Most are based on the Cap protein. Virus can be demonstrated in lesions by immunohistochemistry (IHC) or in situ hybridization (ISH). PCV3 has only recently been isolated in cell culture (PK-15 cells).
- Although antibodies are not diagnostic for PCV3, a few enzyme-linked immunosorbent assays (ELISAs) have been described to detect recombinant Cap protein.
- PCV3 can be detected in many tissues and organs. Heart, lung, and lymphoid tissue are important for histology and IHC or ISH. When PDNS is suspected, skin lesions should be submitted, and in cases of reproductive failure, fetal tissues should be submitted. Oral fluids can be used with some PCR assays.

EPIDEMIOLOGY

- PCV3 is found in wild and domestic pigs. Antibodies to PCV3 have been detected in dogs, cattle, and mice. PCV3-positive mosquitoes have been found. Wild ruminants and ticks may also be reservoirs.
- PCV3 is found in many swine-producing regions of the world. Epidemiological studies have found that infection is widespread, with prevalence up to 100%. Little is known about the course of natural infection. A few studies have shown that prevalence is highest in piglets/weaners and decreases with age, but PCV3 has been detected in pigs up to 23 weeks of age.

ETIOLOGY

- PCV3 belongs to the family *Circoviridae*. Members are very small, non-enveloped viruses with a circular, covalently closed, single-stranded DNA genome.
- Currently, there are four recognized circoviruses of swine, designated PCV1–4. PCV3 is distinctly different from other PCVs and is closely related to canine and bat circoviruses.

HISTORY IN SWINE

- PCV1 was identified in 1974 as a contaminant in a pig kidney cell line. In 1997, PCV2 was recognized as the cause of a novel wasting disease affecting Canadian pigs. In 2016, PCV3 was detected in tissues from sows from North Carolina that died acutely with PDNS-like clinical signs and aborted fetuses. PCV4 was discovered in samples from pigs with respiratory disease, enteric disease, and PDNS in China in 2019.
- Evidence suggests that known PCVs have been circulating in swine long before they were first detected.

IMMUNITY

- The PCV3 antibody response is mainly due to IgG, which can be detected experimentally at seven days post-infection. The duration of immunity is unknown.
- There is no cross-protection between PCV2 and PCV3. There are no commercially available PCV3 vaccines, but vaccination is available through veterinary prescription.

GAPS IN PREPAREDNESS

- PCV3 has been associated with many PCVAD-like clinical signs. The strongest evidence of causality lies with PCV3-systemic and PCV3-reproductive disease. Like PCV2, many PCV3 infections are subclinical, and infection with other swine pathogens is common.
- To better understand the impact of PCV3 on the swine industry, more evidence is needed on pathogenesis, host genomics, viral genomics, and immunology.

LITERATURE REVIEW: PORCINE CIRCOVIRUS 3

IMPORTANCE

Porcine circovirus 3 (PCV3) is an emerging swine pathogen with potential economic importance.[1] It has been associated with signs similar to those caused by porcine circovirus 2 (PCV2). However, many infections seem to be subclinical, and PCV3 pathogenicity studies have had mixed results. Saporiti and colleagues have recently proposed individual case definitions for PCV3-associated reproductive disease and systemic disease to standardize diagnostic criteria.

PUBLIC HEALTH

PCVs are not considered to be zoonotic. PCV3 transmission has occurred in baboons receiving heart transplants from PCV3-positive pigs. However, experimental infection of human embryonic kidney cells has been unsuccessful.[2]

Anti-PCV antibodies detected in humans, mice, and cattle are most likely due to a similar virus, not PCV.[3] Although PCV nucleic acids have been detected in vaccines produced for humans and pigs, this is thought to be due to poor quality control.[4]

INFECTION IN SWINE

PCV 1 and PCV2

Porcine circovirus 1 (PCV1) is nonpathogenic in swine.[4] In the 1990s, PCV2 was associated with postweaning multisystemic wasting syndrome (PMWS) in pigs 2–4 months of age. Clinical signs of PMWS include enlarged subcutaneous lymph nodes, wasting, diarrhea, respiratory distress, pallor, and occasionally icterus.[4] Lymphoid depletion and lymphohistiocytic or granulocytic inflammation are components of diagnosis.[4] Although PCV2 is a primary pathogen, most infections are subclinical.[4,5] Co-infection with other swine pathogens is common.[6] Collectively, PCV2 infections are now known as porcine circovirus-associated disease (PCVAD).[5] Clinical manifestations include systemic disease (PCV2-SD, formerly PMWS), porcine dermatitis and nephropathy syndrome (PDNS), lung disease (PCV2-LD), reproductive disease (PCV2-RD), enteric disease (PCV2-ED), and subclinical infection (PCV2-SI).[5]

PCV3

PCV3 was first detected in sows with signs of PDNS (red-to-purple or dark crusted skin lesions, often on the hind limbs and perineum, along with enlarged and pale kidneys with petechiae) and aborted mummified fetuses.[4] The affected farm had chronic reproductive problems, including high sow mortality and below-average conception rates.[7] Histological lesions included bronchointerstitial pneumonia, peribronchiolar and perivascular cuffing (lymphocytes, plasma cells), and intraluminal edema in the alveoli. Necrotizing vasculitis was seen in the skin, with fibroid changes and neutrophilic infiltration, hemorrhage, and fibrin exudation.[7] The lymph nodes showed diffuse granulomatous lymphadenitis with moderate lymphoid depletion (histiocytes and multinucleated giant cells).[7] Diffuse membrane proliferative glomerulonephritis was seen in the kidneys.[7] Ten sows from the outbreak were also torque teno virus (TTV)-positive.[7] The role of TTV is unclear, but it has previously been linked to PCV2 infection.[8] Investigators also screened archived samples from PDNS cases that were PCV-2-negative by immunohistochemistry (IHC); many were positive for PCV3 by quantitative polymerase chain reaction (qPCR). A subset also tested positive via IHC.[7]

Myocarditis and multisystemic inflammation have also been linked to PCV3 in postweaning pigs.[9] In a 2–3 week-old pig with weight loss and swollen joints, lesions included fibrinous arthritis/synovitis with necrotizing arteriolitis of the synovial capsule, necrotizing arteriolitis in the esophagus, diffuse lymphohistiocytic interstitial pneumonia, and multifocal lymphoplasmacytic and histiocytic myocarditis and arteriolitis.[9] A second pig, 9–10-weeks-old from another state, had a history of respiratory disease and rectal prolapse. Several swine pathogens

were identified, and unexplained perivascular lymphocytic encephalitis and meningitis in the cerebellum was later linked to PCV3.[9] The third pig (19-days old and from another state) presented with severe dyspnea and neurologic disease. Similar lesions were seen as those previously described. In this study, co-infection with porcine astrovirus, rotavirus A, porcine cytomegalovirus, and porcine hemagglutinating encephalomyelitis was documented.[9]

PCV3 nucleic acids have since been detected in pigs with neurological disease,[9-11] reproductive failure,[11-25] respiratory disease,[7,9,18,26-30] enteric disease,[28-31] and PDNS.[7,11,13] PCV3 has recently been associated with death and growth retardation in pigs with caudally rotated (thrown-back) ears.[32,33] PCV3 is also commonly found in healthy pigs.[29,34-41]

Relatively few studies have demonstrated the presence of PCV3 within lesions.[9,11,18,21,23,33,42,43] Based on the lesions most commonly associated with PCV3, heart, lung, and lymphoid tissue appear to be most important for diagnosis.[43]

Published studies on experimental PCV3 infection have had mixed results.

- In 4- and 8-week-old SPF pigs, intranasal inoculation with an infectious PCV3 clone led to **fever, anorexia, diarrhea, and respiratory distress, plus PDNS-like lesions and sudden death** in some pigs. PCV3 was demonstrated in lungs, heart, lymph nodes, liver, kidneys, and small intestines using IHC.[44]
- In 6-week-old CD/CD pigs, PCV3 intranasal and intramuscular inoculation (plus subcutaneous administration of an immunostimulant two days before and after inoculation) **did not lead to development of clinical disease**.[45] However, multisystemic inflammation and perivasculitis were observed via histology, and PCV3 nucleic acids were detected in lesions by ISH.[45]
- In 5-week-old CD/CD pigs, PCV3-positive tissue homogenate was used for intramuscular and intranasal inoculation. Pigs were re-inoculated after seven days. **No clinical signs were seen during the study**. Viremia occurred in PCV3-inoculated pigs at three days post-infection (dpi) and continued until the end of the study. Histology demonstrated multisystemic inflammation and perivasculitis. PCV3 was confirmed in tissues via qPCR and ISH.[46]
- In 4-week-old pigs, PCV3 intranasal inoculation led to moderate clinical signs, including **anorexia, emaciation, and coughing** at 12 dpi. At >12 dpi, **shivering and tachypnea** were seen, plus development of **multifocal papules on the skin**. Histology revealed mucosal epithelial cell necrosis and lymphocyte necrosis in tissue from the small intestine. The presence of PCV3 antigen was confirmed by IHC.[47]
- In 3-week-old CD/CD pigs, PCV3 intranasal and intramuscular inoculation **did not lead to development of clinical disease**. Despite this, PCV3 nucleic acids were detected in many organs/tissues via qPCR, with the highest amounts in lung and inguinal lymph node. PCV3 replication was detected in these tissues by ISH. Histology revealed lymphocyte reduction and inflammatory cell infiltration in the lymph nodes and epithelial cell proliferation, inflammatory cells infiltration, and thickened alveolar septum in the lungs. Passage in PK-15 cells failed.[48]
- In 3-week-old piglets orally inoculated with PCV3-positive intestinal contents, **diarrhea** occurred along with **anorexia** and **depression**. Moderate to severe villus atrophy was observed in the small intestine.[31]

To standardize diagnostic criteria and improve case finding, Saporiti and colleagues[49] proposed case definitions for reproductive and systemic disease caused by PCV3 (see *Table 1*).

Table 1. Proposed Diagnostic Criteria for PCV3-Associated Diseases*		
Proposed Name	*Main Clinical Signs*	*Individual Diagnostic Criteria*
PCV-3-reproductive disease (PCV-3-RD)	Late abortion, malformations, mummified fetuses, stillborn fetuses, weak-born piglets	1. Late reproductive problems and higher perinatal mortality 2. Multisystemic lymphoplasmacytic to lymphohistiocytic perivascular inflammation 3. Moderate to high amount of PCV-3 genome in damaged tissues
PCV-3-systemic disease (PCV-3-SD)	Wasting, weight loss, ill thrift or poor-doers, neurological signs	1. Weight loss, rough hair, neurological signs 2. Multisystemic lymphoplasmacytic to lymphohistiocytic perivascular inflammation 3. Moderate to high amount of PCV-3 genome in damaged tissues

*Diagnostic criteria proposed by Saporiti et al. Porcine circovirus 3 (PCV-3) as a causal agent of disease in swine and a proposal of PCV-3 associated disease case definition. *Transbound Emerge Dis*. 2021. doi: 10.1111/tbed.14204

There is no proposed case definition for PCV3-PDNS. For PDNS associated with PCV2, detection of the virus is not a diagnostic requirement. PCV2-PDNS causes hemorrhagic and necrotizing skin lesions (primarily on the hind limbs and perineal area) and/or swollen and pale kidneys with generalized cortical petechiae; systemic necrotizing vasculitis; and necrotizing fibrinous glomerulonephritis.

PCV4

PCV4 has been recently described, and more information is needed to determine whether it causes clinical illness in pigs (see *History in Swine*).

TREATMENT

There is no treatment for pigs infected with PCVs. Antibiotics may be used to treat secondary bacterial infections.

CLEANING AND DISINFECTION

SURVIVAL

Circoviruses are very stable in the environment.[50] PCV1 survives at 70˚C (158˚F) for 15 minutes, and PCV2 survives at 75˚C (167˚F) for 15 minutes or 56˚C (133˚F) for one hour.[4] PCV2 has been found in different water sources, including those treated by chlorination for human or swine consumption.[51]

In fresh pork, PCV2b can survive for two dpi at room temperature, six dpi at refrigeration temperature (4˚C/39.2˚F), and 30 dpi at freezer temperature (-20˚C/-4˚F).[52] PCVs are also resistant to extreme pH. PCV2 retains some infectivity at pH of 2 and pH of 11–12.[4] No information was found on the survival of PCV3.

DISINFECTION

In the laboratory, potassium peroxymonosulfate and sodium chloride, sodium hypochlorite (bleach), and sodium hydroxide appear to be the most effective virucidal agents for PCV2. Other potentially effective products include quaternary ammonium compounds and phenolics. Chlorhexidine, ethanol, aldehydes, and iodine products are generally not effective disinfectants for PCVs.[53-55] No information was found on the disinfection of PCV3.

PREVENTION AND CONTROL

DISEASE REPORTING

PCVs are not OIE-listed. There are no restrictions for the importation of animals from countries or zones affected by PCVs. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION

PCV2 prevention is based on factors that can influence susceptibility. These include good nutrition, biosecurity, and vaccination (see *Immunity*). Since females have a lower risk of developing PMWS, sorting nursery pigs by sex can be beneficial.[4] Increased risk in males may be due to infection following castration and genetic or hormonal influence.[56] Poor management, including overcrowding, lack of ventilation, and frequent commingling, may contribute to disease severity. Management factors linked to lower disease risk include:

- Housing pregnant sows in groups
- Weaning at higher weights
- Vaccinating sows for atrophic rhinitis
- Treating for ectoparasites
- Adding spray-dried plasma to nursery rations[4]

No specific preventive measures have been described for PCV3.

DISEASE CONTROL

There are no specific control measures for PCV3. However, standard biosecurity practices should be in place on all swine premises.

TRANSMISSION

As described by Opriessnig et al.,[6] both PCV2 and PCV3 are spread by vertical and horizontal transmission. PCVs are transmitted mainly through direct contact. Virus can be detected in nasal, ocular, tonsillar, and bronchial secretions, as well as saliva, urine, feces, milk, colostrum, and semen.[4,27] Fomites, contaminated feed, needles, and biting insects may play a role in transmission. Pigs might become infected by eating the raw tissues of viremic animals.[4] Fetal infection can occur if the dam is exposed during pregnancy or inseminated with virus-containing semen. The virus can also be spread fetus-to-fetus, and the timing of in utero infection determines the clinical outcome.[57]

PATHOGENESIS

Circoviruses replicate in actively dividing cells of young animals. PCV infection is enhanced when the immune system is stimulated, and more lymphocytes are available for replication.[58] Likewise, virus replication can occur in other cells with a high mitotic index, such as endothelial cells, epithelial cells, and macrophages.[4]

Little is known about PCV3 pathogenesis. One recent study found that PCV3 utilizes a clathrin- and dynamin-2-mediated endocytic pathway, entering both early and late endosomes, the latter of which requires an acidic environment.[59] Another study found that experimentally, PCV infection leads to disruption of gut microbiota.[47]

DIAGNOSIS

PCVs are widely distributed in the global swine population. Diagnosis of PCVAD is based on a combination of clinical signs, characteristic gross and microscopic lesions, and detection of the virus in lesions.[4]

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

The *cap* gene, encoding for the highly conserved Cap protein, is often the basis for PCR. PCR assays that have been described for PCV3 include:

- PCR and qPCR targeting the *cap* gene[12,60,61]
- TaqMan qPCR detecting the *cap* gene[7,10,11,14,16,62,63]
- SYBR green-based qPCR detecting the *rep* gene[64] and *cap* gene[16]

- Direct (no DNA required) and qPCR targeting the *rep* gene[65,66]
- Loop-mediated isothermal amplification (LAMP) assay detecting the *cap* gene[67,68]
- Real-time recombinase polymerase amplification (rt-RPA) assay detecting the *cap* gene [69]
- Colorimetric isothermal multiple-self-matching-initiated amplification (IMSA) detecting the *cap* gene[70]
- Polymerase spiral reaction (PSR) assay detecting the *cap* gene[71]
- Multiplex qPCR to detect and differentiate PCV3 and PCV2[72]
- Multiplex PCR to detect and differentiate PCV1, PCV2, and PCV3[73]
- Duplex qPCR for simultaneous detection of PCV2 and PCV3[74]
- Multiplex qPCR to detect and differentiate PCV3, PCV2a, PCV2b, and PCV2d[75]
- Duplex qPCR to differentiate PCV2 and PCV3[76,77]
- Duplex qPCR to detect PCV2 and PCV3[46]
- Duplex qPCR to detect PCV3 and PCV4[78]
- Duplex qPCR assay to simultaneously detect PCV3 and porcine epidemic diarrhea virus[80]
- Duplex qPCR to simultaneously detect PCV3 and pseudorabies virus[81]
- Duplex qPCR to simultaneously detect PCV3 and classical swine fever virus[82]
- Quadruplex qPCR to differentiate porcine PCVs (PCV1–PCV4)[79]
- Visual loop-mediated isothermal amplification (vLAMP) assay for penside detection of cap gene[83]
- Single multiple cross displacement amplification (P-S-MCDA) assay to detect cap gene[84]
- CRISPR/Cas12a system combined with enzymatic recombinase amplification (ERA) nucleic acid amplification to detect PCV3[85]

In addition to PCR, demonstration of virus within lesions is important for PCV diagnosis. Methods include immunohistochemistry (IHC) and in situ hybridization (ISH).[86] PCV3 was first identified by metagenomic sequencing[7,9] and confirmed in tissues from sows with PDNS-like lesions (by PCR and IHC)[7] and postweaning pigs with weight loss and swollen joints (by ISH).[9] Next-generation sequencing has been described in combination with ISH to detect challenging or emerging pathogens like PCV3.[87]

Porcine kidney cells (PK-15),[88] Vero cells, and other porcine-derived cell lines can be used for isolation of PCV2.[4] Until recently, PCV3 had not been isolated in cell culture. One successful study found that PCV3 did not induce a cytopathic effect in primary porcine kidney cells, but infection was confirmed with ISH.[89] PCV3 has also been isolated from diagnostic case samples (from weak-born, stillborn, or mummified pigs)[11] in PK-15 cells and confirmed by qPCR and next-generation sequencing.[45]

TESTS TO DETECT ANTIBODY

Antibodies are not diagnostic for PCV infection, but they may be useful for confirmation at the herd level. Tests described for PCV3 include several enzyme-linked immunosorbent assays (ELISAs) to detect recombinant Cap protein.[7,45,90-92] One assay utilized both a *cap*-based peptide and a *rep*-based peptide.[93]

SAMPLES

PCV3 can be detected in many sample types, including oral fluids.[94] Heart, lung, and lymphoid tissue should always be submitted for histology.[43] In cases of reproductive failure, fetal tissues should be submitted, including the myocardium. For suspected cases of PDNS, diagnostic submissions should include skin lesions.[4]

EPIDEMIOLOGY

SPECIES AFFECTED

As described by Opriessnig et al.,[6] circoviruses are found in mammals, fish, birds, and insects. PCVs are found in

both wild[95-103] and domestic pigs, but clinical disease seems to occur only in the latter (see *Infection in Swine*). PCV2 has been detected in farmed minks with diarrhea,[104] buffaloes,[105] beef products,[106] dogs,[107] farmed shellfish,[108] and flies.[109] While PCV2 antibodies have been found in rodents,[3] few studies have confirmed PCV in field samples.[110]

Species susceptible to PCV3 include dogs,[111,112] cattle,[113] mice,[114] donkeys,[115] and mosquitoes.[116] One study found evidence of PCV3 infection in chamois, roe deer, and ticks (*Ixodes ricinus*).[95] However, others have found that wildlife (including rodents and *Ixodes* spp. ticks,[117] and free-ranging ruminants and lagomorphs)[118] play little to no role in PCV3 epidemiology.

GEOGRAPHIC DISTRIBUTION

PCV1 may have been widespread at one time. PCV2 and PCV3 are currently found in many swine producing regions of the world, including North America,[7,9] South America,[17,22,32,61,119-122] Europe,[13-15,39,40,66,123-126] Africa,[127,128] and Asia.[12,25-27,36,60,129-136] To date, PCV4 has been described only in Asia.[78,137-140]

MORBIDITY AND MORTALITY

The current prevalence of PCV1 is thought to be low, but this is uncertain since PCV1 primers are not included in assays that detect PCV2 and PCV3.[6] Both PCV2 and PCV3 are common in pigs. For PCV3 specifically, up to 100% seroprevalence occurs as described by Ouyang et al.[94] Prevalence of the recently emerged PCV4 is unclear. However, estimates from China show that 5–45% of swine samples are PCV4-positive.[78,138,139] A study from Guangxi Province found that nearly 70% of samples testing positive for PCV4 also contained PCV2 or PCV3.[139]

PCV3 seems to affect pigs of all ages.[132] Prevalence of PCV3 was shown to be highest in fetuses during the last third of gestation.[141] A few studies have demonstrated that prevalence is highest in piglets and/or weaners, then decreases with age.[11,131,142] However, a longitudinal study found that PCV3 infection spanned from 4–23 weeks[143] High seroprevalence has also been documented in healthy multiparous sows and fattening pigs. [144] In boars, age has been correlated with seropositivity.[145] Evidence shows that primiparous sows shed PCV3 at higher levels in colostrum[37,38] and have more PCV3-infected fetuses compared to multiparous sows. Cases of PCV3 often involve co-infection with other swine pathogens, including PCV2.[24,49,94,135,146]

Morbidity for PCVAD is variable but generally low to moderate. However, mortality can be quite high, especially for pigs with PDNS vs. pigs with wasting.[4] Since their introduction in 2006, commercial vaccines for sows, gilts, and piglets have significantly reduced PCV2-associated mortality infection in the United States. There is also some indication that disease susceptibility[147] and vaccination effectiveness are influenced by genetic differences (e.g., breed).

ETIOLOGY

CHARACTERISTICS OF CIRCOVIRUSES

PCVs are members of the family *Circoviridae*. Circoviruses are very small (15–25 nm), non-enveloped viruses that contain a circular, covalently closed, single-stranded DNA genome.[50] As of 2020, the family includes two genera, *Circovirus* and *Cyclovirus*, which contain 29 and 49 species, respectively.[148]

CHARACTERISTICS OF PORCINE CIRCOVIRUSES

Like other circoviruses, PCVs use an ambisense transcription strategy. They have at least two major open reading frames (ORFs). ORF1 encodes the replication-associated protein (Rep, encoded on the virion sense strand), and ORF2 encodes the capsid protein (Cap, encoded on the complementary sense strand).[50]

Currently, there are four recognized circoviruses of swine, designated PCV1–4.[148] PCV2 is highly diverse compared to PCV1, particularly within ORF2.[149] PCV2 is divided into five genotypes (PCV2a-2e).[4] PCV3 is

distinct from PCV1 and PCV2, with only 31% and 48% homology, respectively.[125] However, a high degree of homology occurs between PCV3 viruses. Based on *cap* features, several classification schemes have been suggested, including two (a, b) or three (a, b, c) clades. Clade a has been further divided into either two (a1, a2) or three (a1, a2, a3) subclades.[6] At least six lineages have been known to circulate in North and South America.[150]

Phylogenetic analyses show that PCV3 is related to canine and bat circoviruses.[7,9] Additionally, the PCV3 *cap* gene has similarities to avian ones, suggesting a recombinant origin.[151] It is likely that new PCVs will continue to emerge. Additionally, classification reshuffling will likely continue, in part due to the increasing recognition of recombinant PCVs.[119]

HISTORY IN SWINE

In 1974, a small, spherical virus-like contaminant was detected in a pig kidney cell line (PK-15).[152] The virus, later identified as PCV1, was found to be widespread in pigs but has never been associated with disease.[153] As described by Harding and Clark,[154] a novel wasting disease affecting Canadian pigs emerged in the mid-1990s, reaching epidemic proportions in countries across the world. In 1997, the causative agent was determined to be PCV2, which is antigenically and genetically distinct from PCV1.[155]

In the United States, PCV3 was detected in tissues from sows that died acutely with PDNS-like clinical signs and aborted fetuses in 2016 using metagenomic sequencing.[42] Since that time, PCV3 has been widely found in swine (see *Epidemiology*). PCV4 was discovered in samples from pigs with respiratory disease, enteric disease, and PDNS in China in 2019.[137] Additional PCV4s have since been detected in Asia and are similar to the original isolate.[78,79,138,139,156,157] Current evidence suggests that known PCVs were circulating in swine decades before they were first detected.[6,61,94,136]

IMMUNITY

POST-EXPOSURE

Many swine herds have anti-PCV2 antibodies indicating previous exposure; however, these are not necessarily protective.[4] In piglets, seroconversion occurs as colostral antibody wanes around seven weeks-of-age.[56] PCV2 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 factor in viral clearance.[4]

A few experimental studies have examined the immune response to PCV3 infection.

- In 4–6-week-old CD/CD pigs, PCV3 antibody response (primarily IgM) was detected 7–10 days following intranasal and intramuscular inoculation.[45]
- In 5-week-old CD/CD pigs, an IgG response was dominant, appearing at 7 dpi and persisting at 42 dpi following intramuscular and intranasal inoculation with PCV3.[46]
- In 3-week-old CD/CD pigs, IgG was variable following intranasal and intramuscular inoculation with PCV3. Of the nine piglets tested, four showed an obvious IgG response at 7–10 dpi, one showed an obvious IgG response at 14 dpi, and the remaining four piglets either had a mild IgG response or did not seroconvert by the end of the study (28 dpi).[48]

VACCINES

PCV2 vaccines confer humoral and cellular immunity, reducing mortality while improving average daily weight

gain, feed conversion, and uniformity at slaughter. In the United States, PCV2 prevalence has decreased due to widespread vaccination.[158] There is no cross-protection between PCV2 and PCV3. There are no commercially available PCV3 vaccines, but vaccination is available through veterinary prescription. Experimental vaccines include a recombinant pseudorabies virus expressing the PCV3 Cap protein.[159]

CROSS-PROTECTION

Commercial vaccines confer cross-protection between PCV2 genotypes a–d.[6] However, PCV2 vaccines are not expected to protect against other PCVs due to a lower degree of homology. A recent study found that PCV3 viremia was not related to PCV2 vaccine efficacy.[160] Similarly, a field study found that vaccinating pigs for PCV2 did not affect PCV3 presence.[66]

GAPS IN PREPAREDNESS

PCV3 has been associated with many PCVAD- like clinical signs. The strongest evidence of causality lies with PCV3-systemic and PCV3-reproductive disease. However, like PCV2, many PCV3 infections are subclinical, and infection with other swine pathogens is common. To better understand the impact of PCV3 on the swine industry, more evidence is needed on pathogenesis, host genomics, viral genomics, and immunology.

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