

Review Article

“Porcine Circovirus Infections: Current Knowledge and Future Perspectives in Disease Control”

ABSTRACT

Porcine circovirus (PCV) is a significant viral pathogen affecting swine herds worldwide, leading to considerable economic losses in the global pork industry. This review provides a comprehensive analysis of the current knowledge on porcine circoviruses, focusing on two main species, PCV2 and PCV3, which are associated with disease in pigs. The article discusses the virology, pathogenesis, and epidemiology of these viruses, highlighting their genetic diversity and evolution. Key clinical manifestations of PCV-associated diseases (PCVAD), including postweaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS), are explored in detail. Moreover, the review delves into the host immune responses to PCV infection, including the role of both humoral and cell-mediated immunity. The effectiveness of current vaccines against PCV2 and the challenges posed by PCV3, which has no available vaccine, are critically examined. In addition, the paper addresses diagnostic methods, emphasizing molecular techniques for early and accurate detection of PCV infections. Finally, the review highlights future research directions, including the need for novel vaccines, improved diagnostic tools, and a deeper understanding of PCV3 pathogenesis. This comprehensive review aims to serve as a resource for veterinarians, researchers, and industry stakeholders to better understand and mitigate the impact of PCV on swine health.

Keywords: *Porcine circovirus (PCV), PCV-associated diseases (PCVAD), pigs, vaccines*

1. INTRODUCTION

1.1. Historical Background

Comment [D1]: DELETE

Comment [D2]: delete

Comment [D3]: delet

Comment [D4]: DELETE ADD DISCUSS

Comment [D5]: delete

The story of Porcine Circoviruses (PCVs) began with an unexpected discovery in 1974 when researchers identified a viral contaminant in the continuous porcine kidney cell line, PK-15 (ATCC-CCL31). This virus, later named Porcine Circovirus type 1 (PCV1), was found to be non-pathogenic to pigs, leading to its classification as a relatively benign entity (Allan *et al.*, 1995). However, the narrative took a darker turn in 1997 with the isolation of Porcine Circovirus type 2 (PCV2). Unlike its predecessor, PCV2 was linked to a range of clinical conditions in growing pigs, including Post weaning Multisystemic Wasting Syndrome (PMWS), respiratory and enteric diseases, as well as Porcine Dermatitis and Nephropathy Syndrome (PDNS).

The emergence of PCV2 marked the beginning of a global challenge for swine health, with PMWS being recognized as the most severe of the Porcine Circovirus-Associated Diseases (PCVAD). Although antibodies to PCV2 were detected in pig sera as early as 1985 in Belgium, it wasn't until 1991 in western Canada that the clinical disease was first described (Segalés & Domingo, 2002). PMWS primarily affects post-weaned pigs aged 7 to 15 weeks, with clinical signs such as wasting, unthriftiness, respiratory distress, diarrhea, and, occasionally, jaundice. The rapid onset of these symptoms, often within 3 to 7 days, and the subsequent weight loss frequently prove fatal (Madec *et al.*, 2008).

The narrative of PCVs continued to evolve with the identification of Porcine Circovirus type 3 (PCV3) in 2015, discovered in pigs suffering from PDNS, reproductive failure, myocarditis, or systemic inflammation (Palinski *et al.*, 2016). Interestingly, PCV3 was also found in healthy pigs, suggesting a complex interplay between the virus and its host (Klaumann *et al.*, 2018). The most recent chapter in this ongoing saga unfolded in April 2019, when a novel Porcine Circovirus type 4 (PCV4) was identified in pigs with severe clinical disease in Hunan province, China. This discovery added another layer to the intricate story of PCVs (Zhang *et al.*, 2020).

1.2. Microbiological and Genomic Characteristics

Porcine Circoviruses belong to the family Circoviridae, known for their small, non-enveloped icosahedral structures and circular single-stranded DNA genomes. The Circoviridae family is divided into two genera: Circovirus and

Cyclovirus. The viral particles are remarkably small, with diameters ranging from 12 to 20.7 nm (Rosario et al., 2017). Within this family, PCV1 and PCV2 genomes consist of 1,759 and 1,768 nucleotides, respectively, encoding two major open reading frames (ORFs) (Gillespie et al., 2009). PCV3, however, boasts the largest genome among them, with approximately 2,000 nucleotides (Fux et al., 2018). PCV4, with 1,770 nucleotides, shares the highest genomic identity with mink Circovirus (66.9%) but has lower similarity (43.2%–51.5%) with other PCV genomes (Zhang et al., 2020).

Comment [D6]: italics

Comment [D7]: ITALICS

The genome of PCV1 and PCV2 includes two key ORFs: ORF1, which encodes the replication proteins Rep and Rep'; and ORF2, responsible for the capsid protein. PCV2, in particular, contains 11 ORFs, with seven encoding proteins larger than 5 kDa (Lv et al., 2014). Notably, ORF3, identified in 2005, encodes a nonstructural protein implicated in inducing apoptosis by activating caspase-8 and caspase-3 pathways (Liu et al., 2020). Additionally, ORF4, which overlaps with ORF3, plays a role in suppressing caspase activity and modulating CD4+ and CD8+ T lymphocytes, thereby helping to prevent virus-induced apoptosis without being essential for viral replication (Gao et al., 2014).

Comment [D8]: Italics

Comment [D9]: italics

Comment [D10]: italics

1.3. Taxonomy and Nomenclature

The International Committee on Taxonomy of Viruses (ICTV) classifies the Circoviridae family into two genera: Circovirus and Cyclovirus (Rosario et al., 2017). While Circovirus species are predominantly found in vertebrates, Cycloviruses have been identified in both vertebrates and invertebrates (Rosario et al., 2012). Until 2010, pigs were the only mammals known to be affected by Circoviruses, apart from avian species. However, advances in viral metagenomics and PCR techniques have since revealed Circovirus genomes in a variety of unconventional hosts, including freshwater fishes, minks, dogs, chimpanzees, humans, and bats (Wu et al., 2016).

Comment [D11]: italics

Comment [D12]: italics

Comment [D13]: Italics

Circoviridae is closely related to the Nanoviridae family shown similar step-loop structure at the origin of replication and exhibit commonalities in their replication proteins (Todd et al., 1997). Gibbs and Weiller (1999) proposed that Circovirus may have originated from a plant Nanovirus that infected a vertebrate host,

Comment [D14]: Italics

subsequently undergoing recombination with a vertebrate-infecting RNA virus, possibly a Calicivirus.

Focusing on PCV2, this virus has been classified into multiple genotypes based on differences in ORF2. Initially, PCV2 was divided into five strains: PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e (Olvera *et al.*, 2007; Xiao *et al.*, 2015; Davies *et al.*, 2016). Further studies revealed additional subtypes, including PCV2d-1 and PCV2d-2 (Xiao *et al.*, 2015), with retrospective analyses identifying another genotype, PCV2f, in 2017 (Bao *et al.*, 2018). In 2018, Franzo & Segalés proposed two new genotypes, PCV2g and PCV2h (Franzo and Segalés, 2018). Additionally, Olvera, Cortey, & Segalés suggested that PCV2 might be divided into two groups with eight clusters (1A–1C and 2A–2E) (Olvera *et al.*, 2007)

Comment [D15]: italics

Comment [D16]: ITALICS

Comment [D17]: ITALICS

Comment [D18]:

Comment [D19]: Italics

2. EPIDEMIOLOGY

2.1 Transmission

Globally, a shift in the primary genotypes of Porcine Circovirus Type 2 (PCV2) from PCV2a to PCV2b has been observed across various nations, a transition often linked to more severe clinical manifestations of the disease. The virus spreads rapidly within swine populations through both horizontal and vertical transmission pathways. While airborne transmission remains a possibility, direct contact is the most efficient route of infection, particularly as the virus is present in contaminated respiratory, digestive, and urinary secretions. The likelihood of transmission diminishes significantly with increasing distance between infected and susceptible pigs, emphasizing the role of close proximity in the spread of the virus (Rose *et al.* 2012).

Comment [D20]: italics

Piglets are particularly vulnerable to PCV2 infection while still in utero, and continuous exposure occurs post-birth due to contact with infected sows and contaminated farrowing environments. Interestingly, maternal immunity does not appear to influence the transmission of PCV2 to piglets or the viral load in sows (Dvorak *et al.* 2013). The virus is notably resilient to environmental conditions, allowing it to persist in farm environments and posing a continuous risk of infection. Elements such as farm personnel, management tools, and facilities, even without

Comment [D21]: Italics

direct animal contact, can contribute significantly to the maintenance and spread of PCV2 on and off the farm (López-Lorenzo *et al.* 2019).

Comment [D22]: Italics

PCV2 can also be shed through colostrum and semen, though the extent to which these serve as sources of infection is still not fully understood (Park *et al.* 2005). Experimental studies have demonstrated that feeding native piglets PCV2-infected tissues, such as lymphoid tissue, skeletal muscle, and bone marrow, can lead to viremia and seroconversion, underscoring the ease of transmission via contaminated tissues (Opriessnig *et al.* 2009).

Comment [D23]: Italics

Comment [D24]: Italics

2.2 Factors Influencing PCV2-Related Diseases

PCV2 is the primary causative agent of Porcine Circovirus-associated diseases (PCVAD). However, the progression to clinical disease is influenced by several factors, including viral characteristics, host genetics, co-infections, and immunomodulation.

a. Viral Factors

While PCV2 is associated with various disease syndromes, the viral genomes isolated from different clinical presentations show high sequence homology, with no significant differences between diseased and healthy pigs. This suggests that clinical outcomes are likely influenced by factors other than viral genetics alone (Grierson *et al.* 2004). However, even minor mutations in the viral genome can significantly impact the severity of pathological lesions, as evidenced by studies showing that specific amino acid changes can alter the virus's pathogenic potential (Fenaux *et al.* 2004a). Moreover, pigs infected with heterologous strains (PCV-2a/PCV-2b or PCV-2b/PCV-2a) tend to develop more severe disease than those infected with homologous strains, indicating that strain variation plays a role in disease expression (Harding *et al.* 2010).

Comment [D25]: Italics

Comment [D26]: Italics

Comment [D27]: Italics

b. Host-Dependent Factors

PCV2 can infect pigs across various breeds, with clinical PCVAD observed in multiple purebred and crossbred pigs. However, some studies suggest that certain genetic backgrounds may confer different levels of susceptibility to PCVAD. For

example, a cohort study found that while Duroc, Landrace, and Large White pigs were all susceptible to PCV2 infection, only Landrace pigs developed clinical symptoms and microscopic lesions consistent with PMWS (Fenaux, et al. 2004b).

Comment [D28]: Italics

c. Effects of Co-Infection

PCV2 alone is not sufficient to cause the full spectrum of PCVAD symptoms; co-infections with other pathogens are often necessary to trigger severe clinical disease. Co-infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is particularly common and has been identified as a major risk factor for the development of PCVAD. Other pathogens, such as Porcine Parvovirus (PPV) and *Mycoplasma hyopneumoniae*, also contribute to the exacerbation of PCVAD (Harms et al. 2001). A study on US pigs with PCV2-associated systemic illness found that 52% of cases were co-infected with PRRSV, 36% with *Mycoplasma hyopneumoniae*, and only 2% had a single PCV2 infection, underscoring the importance of co-infections in disease progression (Pallarés et al. 2002).

Comment [D29]: italics

Comment [D30]: Italics

d. Effects of Immunomodulation

Immunostimulation and immunosuppression are critical factors in the progression of PCV2 infection to PCVAD. Studies have shown that immunostimulation, such as vaccination, can accelerate disease progression in PCV2-infected pigs. For instance, pigs that were immunostimulated and subsequently infected with PCV2 developed symptomatic PCVAD, suggesting that the timing and type of immunization can influence disease outcomes (Krakowka et al. 2001). On the other hand, immunosuppression can also exacerbate PCV2 infection. For example, pigs treated with cyclosporine, an immunosuppressive agent, showed enhanced PCV2 replication and viral dissemination, although inflammatory lesions typical of PCVAD were absent, indicating that the host immune response plays a key role in lesion development (Krakowka et al. 2002).

Comment [D31]: Italics

Comment [D32]: Italics

3. PATHOGENESIS

The pathogenesis of Porcine Circovirus 2 (PCV2) infection and the specific cell types that facilitate its replication remain incompletely understood. In pigs

manifesting clinical signs of PCVAD (Porcine Circovirus Associated Disease), lymphoid depletion and peripheral blood lymphopenia are frequently observed. Immunohistochemistry (IHC) and in situ hybridization (ISH) techniques have revealed that high concentrations of PCV2 antigens or nucleic acids within the cytoplasm of macrophages and dendritic cells often replace lymphocytes in depleted lymphoid follicles (Allan and Ellis 2000).

In vitro studies investigating the role of monocytes and macrophages in PCV2 replication have shown that while PCV2 persists in the cytoplasm of these cells, active viral replication does not occur (Gilpin *et al.* 2003). Similarly, Vincent *et al.* found no evidence of viral replication within dendritic cells in vitro; however, the virus remained viable, suggesting that dendritic cells could serve as carriers, disseminating the virus throughout the host due to their migratory nature (Vincent *et al.* 2003).

Comment [D33]: italics

Comment [D34]: italics

PCV2 infection and replication within lymphoid tissues can severely disrupt the follicular architecture, leading to lymphoid depletion and replacement by histiocytic cells (Opriessnig *et al.* 2007). The severity of lymphoid depletion correlates positively with the amount of PCV2 antigen present in affected tissues. PCV2 replication has been documented in multiple tissues, including bronchial and inguinal lymph nodes, tonsils, lungs, liver, kidneys, spleen, and thymus. However, the precise mechanisms contributing to the significant lymphoid depletion observed in infected pigs are not fully understood and may involve additional, yet unidentified, processes beyond direct viral replication and cell lysis (Darwich and Mateu 2012).

Comment [D35]: Italics

Notably, studies on pigs inoculated with an ORF3-null PCV2 mutant demonstrated delayed seroconversion and lower serum viral loads; however, there was no significant difference in histological or gross lesion severity, nor in the amount of PCV2-specific antigen in tissues compared to those infected with wild-type PCV2. This suggests that while ORF3 is not essential for PCV2 replication in pigs, its role in apoptosis or overall pathogenesis remains unclear (Juhan *et al.* 2010).

Comment [D36]: Italics

Further research revealed that cultured monocytes, pulmonary macrophages, and monocyte-derived macrophages could internalize PCV2 in vitro, with the presence of PCV2-specific antigens in their cytoplasm, despite the absence of

evidence for efficient viral replication in these cells (Gilpin *et al.* 2003). Consequently, these immune cells are likely not the primary sites of PCV2 replication. However, PCV2 replication has been detected in T and B cells and monocytes from peripheral blood mononuclear cells, as well as in bronchial lymph nodes of infected pigs (Yu *et al.* 2009).

Comment [D37]: Italics

Comment [D38]: italics

Additionally, PCV2 genomic DNA contains five oligodeoxynucleotide (ODN) sequences, four of which enhance IFN- α production, while one inhibits it. The interaction between these immunomodulatory regions of PCV2 DNA and host cells may play a critical role in the pathogenesis of PCV2 infection (Hasslung *et al.* 2003).

Comment [D39]: Delete

Comment [D40]: Italics

4. PCV-2 ASSOCIATED SYNDROMES

The term "postweaning multisystemic wasting syndrome" (PMWS) has been updated to "porcine circovirus associated disease" (PCVAD) to encompass all PCV-2-related conditions. According to the American Association of Swine Veterinarians (AASV), PCVAD can be subclinical or manifest in various clinical forms, including respiratory distress, porcine dermatologic and nephropathy syndrome, enteric signs like diarrhea, and reproductive disorders, either individually or across herds.

4.1. Postweaning Multisystemic Wasting Syndrome (PMWS)

PMWS is the most prominent clinical manifestation of PCVAD, primarily affecting post-weaning pigs. It is characterized by post-weaning mortality, reduced growth, poor feed efficiency, and increased antibiotic use (Baekbo *et al.* 2012). The morbidity of PMWS is closely linked to viremia and lymphopenia, alongside the presentation of clinical symptoms. Mortality rates in affected herds range from 10% to 50% (Segalés and Domingo 2002). PMWS is most commonly observed in pigs aged 2 to 3.5 months, though cases have been reported in pigs as young as 1 month and as old as 6 months (Allan *et al.* 1995).

Comment [D41]: Italics

Comment [D42]: Italics

Clinical signs include wasting with progressive weight loss, lethargy, dark-colored feces, lymphadenopathy, and pallor or jaundice. Histopathologically, lymphoid tissues show lymphocytic depletion and histiocytic replacement, along with

intracytoplasmic inclusion bodies (Allan et al. 1995; Segalés and Domingo 2002; Segalés et al. 2005). Early clinical signs, such as weight loss, malnutrition, pallor, and a rough hair coat, may go unnoticed or be misdiagnosed. More advanced symptoms include dyspnea, tachypnea, anemia, diarrhea, and jaundice (Harding and Clark 1997). Coughing and gastric ulcers are also common, contributing to anemia. Necropsy findings often reveal mottled, brown lungs, and kidneys with white streaks or patches in chronic cases. Granulomatous lesions may be present in the lungs, liver, kidneys, heart, and intestines (Opriessnig et al. 2007). The superficial inguinal, submandibular, mesenteric, and mediastinal lymph nodes are typically enlarged in affected pigs (Rosell et al. 1999).

Comment [D43]: italics

Comment [D44]: Italics

Comment [D45]: Italics

Comment [D46]: Italics

4.2. PCV-2 Associated Enteritis

PCV2-associated enteritis is an emerging condition, predominantly affecting pigs aged 8 to 16 weeks. Clinically, this enteritis resembles subacute or chronic ileitis linked with *Lawsonia intracellularis*. Affected piglets often exhibit diarrhea, stunted growth, and increased mortality. The intestinal mucosa becomes significantly thickened, and the mesenteric lymph nodes are enlarged. Histopathologically, lesions in Peyer's patches include granulomatous enteritis and classic PCV-2 lesions, which are absent in other lymphoid tissues. Differentiation between *Lawsonia* and PCV-2 infections is achievable through histopathology (Jensen et al. 2006).

Comment [D47]: Italics

4.3. PCV-2 Associated Reproductive Failure

Since its initial observation by West et al. in western Canada in 1999, numerous cases of PCV2-associated reproductive failure have been reported (O'Connor et al. 2001). Affected farms report increased rates of abortions, stillbirths, fetal mummification, and pre-weaning mortality, particularly in gilt startups or new herds. The hallmark lesion in stillborn and neonatal pigs from field cases is nonsuppurative to necrotizing or fibrosing myocarditis, coupled with high levels of PCV2 antigen (Mikami et al. 2005). Experimental intrauterine infection with PCV2 has demonstrated viral replication within fetal tissues, with the heart identified as the primary site of replication. Viral replication was significantly higher in fetuses infected at 57 days of gestation compared to those infected at 75 or 92 days. Twenty-

Comment [D48]: Italics

Comment [D49]: Italics

one days post-infection, all fetuses were deceased, with those infected at 57 days exhibiting lesions such as edema, liver enlargement, and congestion (Sanchez *et al.* 2001). Another study involving 37 fetuses from three sows injected intramuscularly at 86, 92, and 93 days of gestation resulted in 24 normal pigs and 13 mummified, stillborn, or weak-born pigs, further demonstrating PCV2's capacity to infect late-term fetuses and induce reproductive anomalies (Johnson *et al.* 2002).

Comment [D50]: Italics

Comment [D51]: Italics

4.4. Porcine Dermatitis and Nephropathy Syndrome (PDNS)

First identified in the United Kingdom in 1993, PDNS is a severe condition later linked to PCV-2 in 2000 (Rosell *et al.* 2000). Clinically, PDNS is characterized by raised purple skin lesions that evolve into multifocal red-purple scabs with black centers, typically on the hind legs, accompanied by fever and lethargy. Macroscopically, the kidneys appear enlarged, tan, and waxy, with petechial hemorrhages. Microscopically, PDNS is marked by systemic vasculitis with dermal and epidermal necrosis, alongside necrotizing and fibrinous glomerulonephritis. The signature lesions of PDNS, generalized vasculitis, and glomerulonephritis, suggest a type III hypersensitivity reaction, characterized by the deposition of antigen-antibody complexes or immune aggregates within specific tissues (Thibault *et al.* 1998).

Comment [D52]: Italics

Comment [D53]: Italics

5. DIAGNOSIS

Clinical signs alone can provide a provisional diagnosis of Porcine Circovirus Associated Disease (PCVAD). Common symptoms include weight loss, reduced growth rate, pallor or jaundice, and general ill thrift. Infected pigs may also exhibit respiratory distress, coughing, and dark-colored diarrhea. Diagnosis can be confirmed by detecting the presence of PCV2 antigen in multiple lymphoid tissues or in a combination of one lymphoid tissue and another organ system, such as the lungs, liver, kidney, or gut (Opriessnig *et al.* 2007). The amount of antigen present and the scoring of lesions allow for staging the infection.

Comment [D54]: Italics

For a definitive diagnosis of PCVAD, the presence of characteristic lesions in affected organs must be associated with PCV2 antigens or nucleic acids. Immunohistochemistry (IHC) and in situ hybridization (ISH) are considered the gold standards for identifying PCV2 as part of a PCVAD diagnosis (Sorden 2000). IHC is

more sensitive and produces more vivid staining than ISH, although it is less precise. Moreover, IHC is less expensive and has a quicker turnaround time. Microscopic abnormalities associated with PCVAD include syncytial cells in lymph nodes, Peyer's patches, and the lamina propria of the intestinal villi. Macrophages may also contain clearly defined, spherical, and basophilic cytoplasmic inclusion bodies (Rosell *et al.* 1999).

Comment [D55]: Italics

An immunofluorescence assay (IFA) based on the open reading frame (ORF) 2 protein was described by Racine *et al.* in 2006. When compared to the ORF2 protein-based IFA test, the traditional full PCV2-based IFA assay showed only 57.1 percent relative sensitivity (Racine *et al.* 2004). Inter laboratory testing comparing IFA and immunoperoxidase monolayer assay (IPMA) results on the same 20 serum samples from different laboratories across Europe and Canada revealed significant differences in titers. IPMA yielded larger titers than IFA, and using para formaldehyde as a fixative produced higher titers than acetone or ethyl alcohol (McNair *et al.* 2004).

Comment [D56]: Italics

Comment [D57]: Italics

Comment [D58]: Italics

Polymerase Chain Reaction (PCR) amplification of PCV2 DNA from serum or plasma, although not a replacement for existing diagnostic methods, has been suggested as a useful tool for early detection of PMWS in live animals (Caprioli *et al.* 2006). Multiplex real-time PCR and PCR-REBA (Reverse Blot Hybridization Assay) are molecular tests that offer efficient, quick, and convenient detection of PCV2, as well as differentiation between PCV2 genotypes, directly from clinical samples within approximately 2–3 hours (Wang *et al.* 2020). A qPCR based on a TaqMan probe, developed by Henriques *et al.*, achieved 100% sensitivity and specificity for PCV2 detection (Henriques *et al.* 2018). A Real-Time PCR technique established by Chang *et al.* in 2010 enabled the diagnosis of PCV-1 and PCV-2 infections and viral load measurement in field samples within 45 minutes, following viral DNA extraction using a commercial kit.

Comment [D59]: Delete this line

Comment [D60]: delete

Comment [D61]: Italics

Comment [D62]: Italics

Comment [D63]: Italics

Comment [D64]: Italics

Comment [D65]: Italics

The enzyme-linked immunosorbent assay (ELISA) is another effective method for identifying and quantifying antibodies in the blood. The timing of PCV2 infection can be inferred from comparing IgG and IgM levels. A higher IgM value than IgG indicates early active infection (within the first 21 days post-inoculation), a lower IgM value than IgG suggests active infection (between 20 and 50 days post-

inoculation), and a high IgG value with a negative IgM indicates a late or resolving infection (approximately 2 months post-inoculation) (Opriessnig et al. 2007). A recombinant capsid protein-based ELISA for indirect detection of PCV-2 antibodies, using a nuclear localization signal-truncated capsid protein produced in *E. coli* (CAP ELISA), was developed and validated in 2008. This assay demonstrated diagnostic sensitivity (DSN) and specificity (DSP) of 95.3% and 93.9%, respectively, compared to IFA, and 93.3% and 84.2%, respectively, compared to a PCV-2-based ELISA (Shang et al. 2008).

Comment [D66]: Italics

Yin et al. (2010) developed an ELISA based on a truncated soluble ORF-2 protein expressed in *E. coli* for detecting PCV-2 antibodies in domestic pigs. This test, known as Tc ELISA, showed diagnostic sensitivity, specificity, and accuracy of 88.6%, 90.7%, and 89.4%, respectively, when compared to IFA. In 2011, Huang et al. developed a monoclonal antibody (Mab)-based blocking ELISA to detect serum neutralizing antibodies against PCV-2. The detector antibody was a Mab with neutralizing activity, created by immunizing with a recombinant capsid protein of PCV-2 synthesized in insect cells (Huang et al. 2011).

Comment [D67]: Italics

Comment [D68]: Italics

In 2012, Jittimaneet et al. developed an in-house indirect ELISA using a recombinant nuclear localization signal-truncated capsid (rntCap) protein of PCV-2 produced in *E. coli*. This ELISA, compared to IPMA, achieved diagnostic sensitivity, specificity, and accuracy of 98.33%, 93.33%, and 96.67%, respectively, with an optimal cutoff optical density (OD) of 0.330 based on ROC curve analysis (Jittimaneet et al. 2012). In 2021, Mu et al. developed a competitive ELISA (cELISA) based on a nanobody-horseradish peroxidase fusion protein for detecting anti-PCV2 antibodies in clinical porcine serum. This method proved to be a quick, low-cost, reliable, and effective approach for evaluating current PCV2 vaccine efficacy and diagnosing PCV2 infection indirectly (Mu et al. 2021).

In terms of distinguishing between PCV2 genotypes, an ORF2-based PCR-RFLP assay using HinfI, HinPII, KpnI, MseI, and RsaI enzymes was developed in 2000 to differentiate between PCV2 isolates (PCV2A, B, C, D, and E) (Hamel et al. 2000). Additionally, a PCR-RFLP test using the NcoI enzyme to distinguish between PCV1 and PCV2 was described by Fenaux et al. (2000). In 2005, Wen et al. described another ORF2-based PCR-RFLP assay utilizing Sau2AI, BanII, NspI, XbaI, and CfrI

enzymes, capable of distinguishing between nine different PCV2 patterns (Wen et al. 2005).

6. PREVENTION AND CONTROL

Pre-Vaccine Era Management: Before the development of the PCV2 vaccine, the management of Porcine Circovirus Associated Disease (PCVAD) focused on reducing stress, minimizing coinfections, and stimulating the immune system to slow down the progression of PCV2. The primary approach emphasized good manufacturing practices to eliminate contributing factors. Treatment was largely supportive, tailored to the clinical signs exhibited by individual animals. Given the high rate of coinfections, the choice of treatment was also dependent on identifying and addressing other pathogens present.

20-Point Control Plan: On severely affected farms, a comprehensive 20-point plan was proposed for controlling PCVAD. The key elements of this plan were summarized in four "golden guidelines":

1. **Limit Pig-to-Pig Contact:** Reduce the opportunities for disease transmission between animals.
2. **Reduce Stress:** Implement practices to minimize stress, which can exacerbate the disease.
3. **Maintain Good Hygiene:** Ensure cleanliness in housing and handling to lower the risk of infection.
4. **Adhere to a Good Feeding Regime:** Provide adequate nutrition to support immune function and overall health (Madec et al. 1999).

Comment [D69]: italics

Disinfection and Isolation: The use of effective disinfectants in cleaning buildings and transportation vehicles is crucial in controlling the spread of PCV2 (Royer et al. 2001). Despite rigorous isolation practices, outbreaks can still occur on farms. Vaccination against *Mycoplasma hyopneumoniae* or *Actinobacillus pleuropneumoniae* two to four weeks before PCV2 infection has shown efficacy in preventing lesions associated with PCV2 (Opriessnig et al. 2003).

Comment [D70]: Italics

Comment [D71]: Italics

Addressing Co-infections: Preventing PCV2-related disorders also involves treating bacterial infections and managing cofactors. Chlortetracyclines, for instance, have been effective in treating *M. hyopneumoniae* infections in pigs co-infected with PCV2 (Chlortetracycline is Effective in Reducing Lesions in Pigs Co-infected with Mycoplasma Hyopneumoniae and Porcine Circovirus Type 2).

Housing and Management: Proper housing management is crucial for disease prevention. This includes stress relief practices, maintaining hygiene, avoiding mixing pigs of different ages, and implementing all-in/all-out management systems. These practices have proven effective in controlling the disease. Other options, such as immunized serum therapy, have practical limitations, while depopulation has generally been ineffective due to the virus's high environmental resistance (Royer et al. 2001).

Comment [D72]: Italics

Potential Transmission Vectors: There is uncertainty about whether PCV2 can be transmitted by insects or wild animals. While circoviruses are generally highly species-specific, it is unlikely that animals other than feral boars would pose a significant risk of transmitting PCV2 to domestic pig herds (Ramamoorthy and Meng 2009).

Risk Factors: Risk factors for developing PCV2 infection include:

- Co-infection with Porcine Parvovirus (PPV) or Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).
- Housing in large pens compared to small pens for weaners.
- Increased levels of crossbreeding.
- Vaccination against PRRSV (López-Soria et al. 2005).

Comment [D73]: Italics

Risk Reduction Strategies: Strategies that have been shown to reduce the risk of PCV2 infection include:

- Long idle periods between groups of pigs.
- Regular treatment of ectoparasites.
- Thorough barn and crate cleaning.
- Internal and external biosecurity measures.

- Vaccination against atrophic rhinitis

7. VACCINATION

Early Attempts: One of the initial strategies in developing a vaccine against PCVAD involved the use of a killed Porcine Parvovirus (PPV) vaccine. Given the frequent co-infection of pigs with both PPV and PCV2, it was hypothesized that early immunization might prevent PCVAD. However, while a killed Parvovirus-Leptospira-Erysipelothrix (PLE) vaccine was effective in preventing PPV viremia, it did not prevent clinical Post-Weaning Multisystemic Wasting Syndrome (PMWS) or reduce the severity of lymphoid depletion in pigs co-infected with PCV2 and PPV (Opriessnig et al. 2004).

Comment [D74]: Italics

Chimeric PCV1-2 Vaccine: A significant development was the creation of a chimeric PCV1-2 virus, which involved cloning the immunogenic ORF2 capsid gene of the pathogenic PCV2 into the non-pathogenic PCV1 genomic backbone. This chimeric virus induced a specific antibody response to the PCV2 capsid antigen, though it weakened the pigs (Fenaux et al. 2004b). Further research showed that the attenuated chimeric PCV1-2 live virus and its infectious DNA clone could generate protective immunity against PCV2 infection when administered intramuscularly, suggesting its potential as an effective vaccine (Fenaux et al. 2003).

Comment [D75]: Italics

Comment [D76]: Italics

Subunit and DNA Vaccines: Protection against PCV2 was also explored through other approaches. Two trials demonstrated that pigs were protected against a PCV2 challenge after vaccination with either plasmids encoding the Orf2 protein (DNA vaccine) or a baculovirus-expressed Orf2 protein sub-unit vaccine. The sub-unit vaccine provided better protection than the DNA vaccine, as PCV2 replication was completely suppressed (Blanchard et al. 2003). Additionally, the gene encoding the PCV2 capsid protein was cloned into a DNA vaccination plasmid, and when BALB/c mice were vaccinated three times, all produced antibodies against PCV2 (Kamstrup et al. 2004).

Comment [D77]: Italics

Commercial Vaccines: One of the first commercially available PCV2 vaccines was CIRCOVAC, an inactivated PCV2 vaccine with an oil-based adjuvant for breeding

animals. It is administered intramuscularly in two injections, 3–4 weeks apart, with subsequent doses given during each gestation period (Opriessnig et al. 2007).

Comment [D78]: Italics

Baculovirus-Based Vaccines: A capsid-based sub-unit vaccine manufactured by Intervet Inc/Schering-Plough Animal Health, known as Circumvent PCV in the United States and Canada, and Porcilis PCV in Europe and Asia, is administered intramuscularly to piglets at least three weeks old, with a booster dose given at six weeks of age (Opriessnig et al. 2007). Similarly, Ingelvac® CircoFLEX, produced using a baculovirus vector system to express the PCV2 ORF2 gene in insect cells, showed significant reductions in clinical symptoms, mortality, and lesions in vaccinated pigs compared to non-vaccinated pigs. Field trials further demonstrated that vaccination against PCV2 alone could significantly improve the growth performance of pigs suffering from Porcine Respiratory Disease Complex (PRDC), as evidenced by lower mortality rates, improved average daily weight gain, and shorter time to market (Fachinger et al. 2008).

Comment [D79]: Italics

Comment [D80]: Italics

Other Vaccines: Suvaxyn® PCV2 has also shown high efficacy and safety in field investigations, reducing mortality in vaccinated pigs to 1.0–2.0%, compared to 8–10% in non-vaccinated pigs. The vaccine was well-tolerated, with no adverse effects observed in immunized pigs. It has also been shown to provide protection against PCV2 infection in piglets, even in the presence of maternal antibodies (Opriessnig et al. 2008). The live version of the chimeric PCV1-2 vaccine has demonstrated genetic stability in inoculated pigs and presents a promising option for live PCV2 vaccination (Gillespie et al. 2008).

Comment [D81]: italics

Comment [D82]: Italics

Efficacy and Economic Impact: Accumulating field data has consistently confirmed the efficacy of these commercial vaccines, as indicated by improved average daily weight gain (ADG) and economic benefits in vaccinated pigs. Given the role of PCV2 as a co-infecting agent, vaccination against PCV2 is also believed to provide additional benefits in protecting against other pathogens (Chae 2016).

8. CONCLUSION

Porcine circoviruses (PCVs), specifically PCV1 and PCV2, have significant implications for swine health and the pork industry. While PCV1 is generally considered non-pathogenic, PCV2 is associated with a range of diseases collectively referred to as porcine circovirus-associated diseases (PCVAD). These diseases can lead to substantial economic losses due to decreased productivity, increased mortality, and the costs of management and control measures.

Advancements in understanding the molecular biology, transmission, and pathogenesis of PCVs have facilitated the development of effective vaccines, which have significantly reduced the prevalence and severity of PCVAD in many regions. However, the emergence of new strains and the potential for recombination events underscore the need for continued surveillance, research, and adaptation of vaccination strategies. Ongoing research is essential to fully elucidate the complex interactions between the virus, host, and environment that contribute to PCVAD. This knowledge will be crucial for improving control measures, developing next-generation vaccines, and ultimately mitigating the impact of these viruses on the global swine industry.

REFERENCES:

1. Allan GM, McNeilly F, Cassidy JP, Reilly GAC, Adair B, Ellis WA, McNulty MS. 1995. Pathogenesis of porcine circovirus; experimental infections of colostrum deprived piglets and examination of pig foetal material. *Vet Microbiol.* 44(1):49–64. [https://doi.org/10.1016/0378-1135\(94\)00136-K](https://doi.org/10.1016/0378-1135(94)00136-K)
2. Segalés J, Domingo M. 2002. Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. *Vet Q.* 24(3):109–124. <https://doi.org/10.1080/01652176.2002.9695132>
3. Madec F, Rose N, Grasland B, Cariolet R, Jestin A. 2008. Post-Weaning Multisystemic Wasting Syndrome and Other PCV2-Related Problems in Pigs: a 12-Year Experience. *Transbound Emerg Dis.* 55(7):273–283. <https://doi.org/10.1111/j.1865-1682.2008.01035.x>
4. Palinski R, Piñeyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM. 2016. A Novel Porcine Circovirus Distantly Related to Known Circoviruses Is Associated with Porcine Dermatitis and Nephropathy Syndrome and

Comment [D83]: multisystemic

- Reproductive Failure. *J Virol.* 91(1):e01879-16.
<https://doi.org/10.1128/JVI.01879-16>
5. Klaumann F, Correa-Fiz F, Franzo G, Sibila M, Núñez JI, Segalés J. 2018. Current Knowledge on Porcine circovirus 3 (PCV-3): A Novel Virus With a Yet Unknown Impact on the Swine Industry. *Front Vet Sci* [Internet]. [accessed 2022 Oct 3] 5.
<https://www.frontiersin.org/articles/10.3389/fvets.2018.00315>
 6. Zhang H-H, Hu W-Q, Li J-Y, Liu T-N, Zhou J-Y, Opriessnig T, Xiao C-T. 2020. Novel circovirus species identified in farmed pigs designated as Porcine circovirus 4, Hunan province, China. *Transbound Emerg Dis.* 67(3):1057–1061. <https://doi.org/10.1111/tbed.13446>
 7. Rosario K, Breitbart M, Harrach B, Segalés J, Delwart E, Biagini P, Varsani A. 2017. Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. *Arch Virol.* 162(5):1447–1463. <https://doi.org/10.1007/s00705-017-3247-y>
 8. Gillespie J, Opriessnig T, Meng X j., Pelzer K, Buechner-Maxwell V. 2009. Porcine Circovirus Type 2 and Porcine Circovirus-Associated Disease. *J Vet Intern Med.* 23(6):1151–1163. <https://doi.org/10.1111/j.1939-1676.2009.0389.x>
 9. Fux R, Söckler C, Link EK, Renken C, Krejci R, Sutter G, Ritzmann M, Eddicks M. 2018. Full genome characterization of porcine circovirus type 3 isolates reveals the existence of two distinct groups of virus strains. *Virol J.* 15(1):25. <https://doi.org/10.1186/s12985-018-0929-3>
 10. Lv Q, Guo K, Zhang Y. 2014. Current understanding of genomic DNA of porcine circovirus type 2. *Virus Genes.* 49(1):1–10. <https://doi.org/10.1007/s11262-014-1099-z>
 11. Liu C, Liu Y, Feng H, Zhao B, Chen Y, Huang H, Wang P, Deng R, Zhang G. 2020. PCV cap proteins fused with calreticulin expressed into polymers in *Escherichia coli* with high immunogenicity in mice. *BMC Vet Res.* 16(1):313. <https://doi.org/10.1186/s12917-020-02527-9>
 12. Gao Z, Dong Q, Jiang Y, Opriessnig T, Wang J, Quan Y, Yang Z. 2014. ORF4-protein deficient PCV2 mutants enhance virus-induced apoptosis and show differential expression of mRNAs in vitro. *Virus Res.* 183:56–62. <https://doi.org/10.1016/j.virusres.2014.01.024>

13. Rosario K, Duffy S, Breitbart M. 2012. A field guide to eukaryotic circular single-stranded DNA viruses: insights gained from metagenomics. *Arch Virol.* 157(10):1851–1871. <https://doi.org/10.1007/s00705-012-1391-y>
14. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, Qian Z, Dong J, Sun L, Zhu Y, et al. 2016. Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. *ISME J.* 10(3):609–620. <https://doi.org/10.1038/ismej.2015.138>
15. Todd D, Meehan BM, McNulty MS, Creelan JL. 1997. Sequence of porcine circovirus DNA: affinities with plant circoviruses. *J Gen Virol.* 78(1):221–227. <https://doi.org/10.1099/0022-1317-78-1-221>
16. Gibbs MJ, Weiller GF. 1999. Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus. *Proc Natl Acad Sci.* 96(14):8022–8027. <https://doi.org/10.1073/pnas.96.14.8022>
17. Olvera A, Cortey M, Segalés J. 2007. Molecular evolution of porcine circovirus type 2 genomes: Phylogeny and clonality. *Virology.* 357(2):175–185. <https://doi.org/10.1016/j.virol.2006.07.047>
18. Xiao C-T, Halbur PG, Opriessnig T. 2015. Global molecular genetic analysis of porcine circovirus type 2 (PCV2) sequences confirms the presence of four main PCV2 genotypes and reveals a rapid increase of PCV2d. *J Gen Virol.* 96(7):1830–1841. <https://doi.org/10.1099/vir.0.000100>
19. Davies B, Wang X, Dvorak CMT, Marthaler D, Murtaugh MP. 2016. Diagnostic phylogenetics reveals a new Porcine circovirus 2 cluster. *Virus Res.* 217:32–37. <https://doi.org/10.1016/j.virusres.2016.02.010>
20. Bao F, Mi S, Luo Q, Guo H, Tu C, Zhu G, Gong W. 2018. Retrospective study of porcine circovirus type 2 infection reveals a novel genotype PCV2f. *Transbound Emerg Dis.* 65(2):432–440. <https://doi.org/10.1111/tbed.12721>
21. Franzo G, Segalés J. 2018. Porcine circovirus 2 (PCV-2) genotype update and proposal of a new genotyping methodology. Sola C, editor. *PLOS ONE.* 13(12):e0208585. <https://doi.org/10.1371/journal.pone.0208585>
22. Rose N, Opriessnig T, Grasland B, Jestin A. 2012. Epidemiology and transmission of porcine circovirus type 2 (PCV2). *Virus Res.* 164(1):78–89. <https://doi.org/10.1016/j.virusres.2011.12.002>
23. Dvorak CMT, Lilla MP, Baker SR, Murtaugh MP. 2013. Multiple routes of porcine circovirus type 2 transmission to piglets in the presence of maternal

- immunity. *Vet Microbiol.* 166(3–4):365–374.
<https://doi.org/10.1016/j.vetmic.2013.06.011>
24. López-Lorenzo G, Díaz-Cao JM, Prieto A, López-Novo C, López CM, Díaz P, Rodríguez-Vega V, Díez-Baños P, Fernández G. 2019. Environmental distribution of Porcine Circovirus Type 2 (PCV2) in swine herds with natural infection. *Sci Rep.* 9(1):14816. <https://doi.org/10.1038/s41598-019-51473-6>
25. Park J-S, Kim J, Ha Y, Jung K, Choi C, Lim J-K, Kim S-H, Chae C. 2005. Birth Abnormalities in Pregnant Sows Infected Intranasally with Porcine Circovirus 2. *J Comp Pathol.* 132(2):139–144.
<https://doi.org/10.1016/j.jcpa.2004.09.003>
26. Opriessnig T, Patterson AR, Meng X-J, Halbur PG. 2009. Porcine circovirus type 2 in muscle and bone marrow is infectious and transmissible to naïve pigs by oral consumption. *Vet Microbiol.* 133(1–2):54–64.
<https://doi.org/10.1016/j.vetmic.2008.06.018>
27. Grierson SS, King DP, Wellenberg GJ, Banks M. 2004. Genome sequence analysis of 10 Dutch porcine circovirus type 2 (PCV-2) isolates from a PMWS case-control study. *Res Vet Sci.* 77(3):265–268.
<https://doi.org/10.1016/j.rvsc.2004.04.008>
28. Fenaux M, Opriessnig T, Halbur PG, Elvinger F, Meng XJ. 2004a. 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.* 78(24):13440–13446. <https://doi.org/10.1128/JVI.78.24.13440-13446.2004>
29. Harding JCS, Ellis JA, McIntosh KA, Krakowka S. 2010. Dual heterologous porcine circovirus genogroup 2a/2b infection induces severe disease in germ-free pigs. *Vet Microbiol.* 145(3):209–219.
<https://doi.org/10.1016/j.vetmic.2010.03.026>
30. Fenaux M, Opriessnig T, Halbur PG, Elvinger F, Meng XJ. 2004b. A Chimeric Porcine Circovirus (PCV) with the Immunogenic Capsid Gene of the Pathogenic PCV Type 2 (PCV2) Cloned into the Genomic Backbone of the Non-pathogenic PCV1 Induces Protective Immunity against PCV2 Infection in Pigs. *J Virol.* 78(12):6297–6303. <https://doi.org/10.1128/JVI.78.12.6297-6303.2004>

31. Pallarés FJ, Halbur PG, Opriessnig T, Sorden SD, Villar D, Janke BH, Yaeger MJ, Larson DJ, Schwartz KJ, Yoon KJ, Hoffman LJ. 2002. Porcine Circovirus Type 2 (PCV-2) Coinfections in US Field Cases of Postweaning Multisystemic Wasting Syndrome (PMWS). *J Vet Diagn Invest.* 14(6):515–519. <https://doi.org/10.1177/104063870201400614>
32. Krakowka S, Ellis JA, McNeilly F, Ringler S, Rings DM, Allan G. 2001. 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.* 38(1):31–42. <https://doi.org/10.1354/vp.38-1-31>
33. Krakowka S, Ellis JA, McNeilly F, Gilpin D, Meehan B, McCullough K, Allan G. 2002. Immunologic Features of Porcine Circovirus Type 2 Infection. *Viral Immunol.* 15(4):567–582. <https://doi.org/10.1089/088282402320914511>
34. Allan GM & Ellis JA. 2000. Porcine Circoviruses: A Review. *J Vet Diagn Invest.* 12(1):3–14. <https://doi.org/10.1177/104063870001200102>
35. Gilpin DF, McCullough K, Meehan BM, McNeilly F, McNair I, Stevenson LS, Foster JC, Ellis JA, Krakowka S, Adair BM, Allan GM. 2003. In vitro studies on the infection and replication of porcine circovirus type 2 in cells of the porcine immune system. *Vet Immunol Immunopathol.* 94(3–4):149–161. [https://doi.org/10.1016/S0165-2427\(03\)00087-4](https://doi.org/10.1016/S0165-2427(03)00087-4)
36. Vincent IE, Carrasco CP, Herrmann B, Meehan BM, Allan GM, Summerfield A, McCullough KC. 2003. Dendritic Cells Harbor Infectious Porcine Circovirus Type 2 in the Absence of Apparent Cell Modulation or Replication of the Virus. *J Virol.* 77(24):13288–13300. <https://doi.org/10.1128/JVI.77.24.13288-13300.2003>
37. Opriessnig T, Meng X-J, Halbur PG. 2007. Porcine Circovirus Type 2–Associated Disease: Update on Current Terminology, Clinical Manifestations, Pathogenesis, Diagnosis, and Intervention Strategies. *J Vet Diagn Invest.* 19(6):591–615. <https://doi.org/10.1177/104063870701900601>
38. Darwich L, Mateu E. 2012. Immunology of porcine circovirus type 2 (PCV2). *Virus Res.* 164(1–2):61–67. <https://doi.org/10.1016/j.virusres.2011.12.003>
39. Juhan NM, LeRoith T, Opriessnig T, Meng XJ. 2010. The open reading frame 3 (ORF3) of porcine circovirus type 2 (PCV2) is dispensable for virus infection but evidence of reduced pathogenicity is limited in pigs infected by

- an ORF3-null PCV2 mutant. *Virus Res.* 147(1):60–66.
<https://doi.org/10.1016/j.virusres.2009.10.007>
40. Yu S, Halbur PG, Thacker E. 2009. Effect of porcine circovirus type 2 infection and replication on activated porcine peripheral blood mononuclear cells in vitro. *Vet Immunol Immunopathol.* 127(3):350–356.
<https://doi.org/10.1016/j.vetimm.2008.10.332>
41. Hasslung FC, Berg M, Allan GM, Meehan BM, McNeilly F, Fossum C. 2003. Identification of a sequence from the genome of porcine circovirus type 2 with an inhibitory effect on IFN- α production by porcine PBMCs. *J Gen Virol.* 84(11):2937–2945. <https://doi.org/10.1099/vir.0.19362-0>
42. Baekbo P, Kristensen CS, Larsen LE. 2012. Porcine Circovirus Diseases: A review of PMWS. *Transbound Emerg Dis.* 59(s1):60–67.
<https://doi.org/10.1111/j.1865-1682.2011.01288.x>
43. Harding JC, Clark EG. 1997. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). *J Swine Health Prod.* 5(5):201–203.
44. Rosell C, Segalés J, Plana-Durán J, Balasch M, Rodríguez-Arrijo GM, Kennedy S, Allan GM, McNeilly F, Latimer KS, Domingo M. 1999. Pathological, Immunohistochemical, and In-situ Hybridization Studies of Natural Cases of Postweaning Multisystemic Wasting Syndrome (PMWS) in Pigs. *J Comp Pathol.* 120(1):59–78. <https://doi.org/10.1053/jcpa.1998.0258>
45. Jensen TK, Vigre H, Svensmark B, Bille-Hansen V. 2006. Distinction between Porcine Circovirus Type 2 Enteritis and Porcine Proliferative Enteropathy caused by *Lawsonia intracellularis*. *J Comp Pathol.* 135(4):176–182. <https://doi.org/10.1016/j.jcpa.2006.08.006>
46. O'Connor B, Gauvreau H, West K, Bogdan J, Ayroud M, Clark EG, Konoby C, Allan G, Ellis JA. 2001. Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. *Can Vet J Rev Veterinaire Can.* 42(7):551–553.
47. Mikami O, Nakajima H, Kawashima K, Yoshii M, Nakajima Y. 2005. Nonsuppurative Myocarditis Caused by Porcine Circovirus Type 2 in a Weak-Born Piglet. *J Vet Med Sci.* 67(7):735–738.
<https://doi.org/10.1292/jvms.67.735>

48. Sanchez RE, Nauwynck HJ, McNeilly F, Allan GM, Pensaert MB. 2001. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. *Vet Microbiol.* 83(2):169–176. [https://doi.org/10.1016/S0378-1135\(01\)00425-4](https://doi.org/10.1016/S0378-1135(01)00425-4)
49. Johnson CS, Joo HS, Direksin K, Yoon K-J, Choi YK. 2002. Experimental in Utero Inoculation of Late-Term Swine Fetuses with Porcine Circovirus Type 2. *J Vet Diagn Invest.* 14(6):507–512. <https://doi.org/10.1177/104063870201400612>
50. Rosell C, Segalés J, Ramos-Vara JA, Folch JM, Rodriguez-Arrijo GM, Duran CO, Balasch M, Plana-Durán J, Domingo M. 2000. Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. *Vet Rec.* 146(2):40–43. <https://doi.org/10.1136/vr.146.2.40>
51. Thibault S, Drolet R, Germain M-C, D’Allaire S, Larochelle R, Magar R. 1998. Cutaneous and Systemic Necrotizing Vasculitis in Swine. *Vet Pathol.* 35(2):108–116. <https://doi.org/10.1177/030098589803500204>
52. Sorden SD. 2000. Update on porcine circovirus and postweaning multisystemic wasting syndrome (PMWS). *J Swine Health Prod.* 8(3):133–136.
53. Racine S, Kheyar A, Gagnon CA, Charbonneau B, Dea S. 2004. Eucaryotic Expression of the Nucleocapsid Protein Gene of Porcine Circovirus Type 2 and Use of the Protein in an Indirect Immunofluorescence Assay for Serological Diagnosis of Postweaning Multisystemic Wasting Syndrome in Pigs. *Clin Vaccine Immunol.* 11(4):736–741. <https://doi.org/10.1128/CDLI.11.4.736-741.2004>
54. McNair I, Marshall M, McNeilly F, Botner A, Ladekjaer-Mikkelsen A-S, Vincent I, Herrmann B, Sanchez R, Rhodes C. 2004. Interlaboratory Testing of Porcine Sera for Antibodies to Porcine Circovirus Type 2. *J Vet Diagn Invest.* 16(2):164–166. <https://doi.org/10.1177/104063870401600214>
55. Wang H, Song JK, Shin S, Kim H. 2020. Comparison of Multiplex Real-Time PCR and PCR-Reverse Blot Hybridization Assays for the Direct and Rapid Detection of Porcine Circovirus Type 2 Genotypes. *Front Vet Sci.* 7:200. <https://doi.org/10.3389/fvets.2020.00200>
56. Henriques AM, Duarte M, Barros SC, Fagulha T, Ramos F, Luís T, Fevereiro M. 2018. Development and validation of a real-time PCR for the detection and

- quantification of porcine circovirus type 2. *VirusDisease*. 29(3):355–361. <https://doi.org/10.1007/s13337-018-0476-y>
57. Chang G-N, Hwang J-F, Chen J-T, Tsen H-Y, Wang J-J. 2010. Fast Diagnosis and Quantification for Porcine Circovirus Type 2 (PCV-2) Using Real-Time Polymerase Chain Reaction. *J Microbiol Immunol Infect*. 43(2):85–92. [https://doi.org/10.1016/S1684-1182\(10\)60014-X](https://doi.org/10.1016/S1684-1182(10)60014-X)
58. Shang S-B, Li Y-F, Guo J-Q, Wang Z-T, Chen Q-X, Shen H-G, Zhou J-Y. 2008. Development and validation of a recombinant capsid protein-based ELISA for detection of antibody to porcine circovirus type 2. *Res Vet Sci*. 84(1):150–157. <https://doi.org/10.1016/j.rvsc.2007.02.007>
59. Yin S, Yang S, Tian H, Wu J, Shang Y, Cai X, Liu X. 2010. An ELISA based on a truncated soluble ORF2 protein for the detection of PCV2 antibodies in domestic pigs. *Viol Sin*. 25(3):191–198. <https://doi.org/10.1007/s12250-010-3085-5>
60. Huang LP, Lu YH, Wei YW, Guo LJ, Liu CM. 2011. Identification of one critical amino acid that determines a conformational neutralizing epitope in the capsid protein of porcine circovirus type 2. *BMC Microbiol*. 11(1):188. <https://doi.org/10.1186/1471-2180-11-188>
61. Jittimane S, Nuntawan Na Ayudhya S, Kedkovid R, Teankum K, Suradhat S, Thanawongnuwech R. 2012. An indirect enzyme-linked immunosorbent assay using a recombinant truncated capsid protein of Porcine circovirus-2. *J Vet Diagn Invest*. 24(6):1129–1132. <https://doi.org/10.1177/1040638712461251>
62. Mu Y, Jia C, Zheng X, Zhu H, Zhang X, Xu H, Liu B, Zhao Q, Zhou E-M. 2021. A nanobody-horseradish peroxidase fusion protein-based competitive ELISA for rapid detection of antibodies against porcine circovirus type 2. *J Nanobiotechnology*. 19(1):34. <https://doi.org/10.1186/s12951-021-00778-8>
63. Hamel AL, Lin LL, Sachvie C, Grudeski E, Nayar GP. 2000. PCR detection and characterization of type-2 porcine circovirus. *Can J Vet Res Rev Can Rech Veterinaire*. 64(1):44–52.
64. Fenaux M, Halbur PG, Gill M, Toth TE, Meng X-J. 2000. 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.* 38(7):2494–2503. <https://doi.org/10.1128/JCM.38.7.2494-2503.2000>

Comment [D84]: delete/insert

65. Wen L, Guo X, Yang H. 2005. Genotyping of porcine circovirus type 2 from a variety of clinical conditions in China. *Vet Microbiol.* 110(1–2):141–146. <https://doi.org/10.1016/j.vetmic.2005.07.003>
66. Madec F, Eveno É, Morvan P, Hamon L, Morvan H, Albina E, Truong C, Hutet É, Cariolet R, Arnauld C, Jestin A. 1999. La Maladie de l’Amaigrissement du Porcelet (MAP) en France. *Journ Rech Porc En Fr.* 31:347–354.
67. Royer RL, Nawagitgul P, Halbur PG, Paul PS. 2001. Susceptibility of porcine circovirus type 2 to commercial and laboratory disinfectants. *J Swine Health Prod.* 9(6):281–284.
68. Ramamoorthy S, Meng X-J. 2009. Porcine circoviruses: a minuscule yet mammoth paradox. *Anim Health Res Rev.* 10(1):1–20. <https://doi.org/10.1017/S1466252308001461>
69. López-Soria S, Segalés J, Rose N, Viñas MJ, Blanchard P, Madec F, Jestin A, Casal J, Domingo M. 2005. An exploratory study on risk factors for postweaning multisystemic wasting syndrome (PMWS) in Spain. *Prev Vet Med.* 69(1–2):97–107. <https://doi.org/10.1016/j.prevetmed.2004.11.015>
70. Opriessnig T., Thacker EL, Yu S, Fenaux M, Meng X-J, Halbur PG. 2004. Experimental Reproduction of Postweaning Multisystemic Wasting Syndrome in Pigs by Dual Infection with *Mycoplasma hyopneumoniae* and Porcine Circovirus Type 2. *Vet Pathol.* 41(6):624–640. <https://doi.org/10.1354/vp.41-6-624>
71. Fenaux M, Opriessnig T, Halbur PG, Meng XJ. 2003. Immunogenicity and Pathogenicity of Chimeric Infectious DNA Clones of Pathogenic Porcine Circovirus Type 2 (PCV2) and Nonpathogenic PCV1 in Weanling Pigs. *J Virol.* 77(20):11232–11243. <https://doi.org/10.1128/JVI.77.20.11232-11243.2003>
72. Fenaux M, Opriessnig T, Halbur PG, Elvinger F, Meng XJ. 2004b. 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.* 78(12):6297–6303. <https://doi.org/10.1128/JVI.78.12.6297-6303.2004>
73. Blanchard P, Mahé D, Cariolet R, Keranflec'h A, Baudouard MA, Cordioli P, Albina E, Jestin A. 2003. Protection of swine against post-weaning multisystemic wasting syndrome (PMWS) by porcine circovirus type 2 (PCV2) proteins. *Vaccine.* 21(31):4565–4575. [https://doi.org/10.1016/S0264-410X\(03\)00503-6](https://doi.org/10.1016/S0264-410X(03)00503-6)
74. Kamstrup S, Barfoed AM, Frimann TH, Ladekjær-Mikkelsen A-S, Bøtner A. 2004. Immunization against PCV2 structural protein by DNA vaccination of mice. *Vaccine.* 22(11–12):1358–1361. <https://doi.org/10.1016/j.vaccine.2004.01.032>
75. Opriessnig T, Patterson AR, Elsener J, Meng XJ, Halbur PG. 2008. Influence of Maternal Antibodies on Efficacy of Porcine Circovirus Type 2 (PCV2) Vaccination To Protect Pigs from Experimental Infection with PCV2. *Clin Vaccine Immunol.* 15(3):397–401. <https://doi.org/10.1128/CVI.00416-07>
76. Fachinger V, Bischoff R, Jedidia SB, Saalmüller A, Elbers K. 2008. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. *Vaccine.* 26(11):1488–1499. <https://doi.org/10.1016/j.vaccine.2007.11.053>
77. Gillespie J, Juhan NM, DiCristina J, Key KF, Ramamoorthy S, Meng XJ. 2008. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) is genetically stable in vitro and in vivo. *Vaccine.* 26(33):4231–4236. <https://doi.org/10.1016/j.vaccine.2008.05.051>
78. Chae C. 2016. Porcine respiratory disease complex: Interaction of vaccination and porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, and *Mycoplasma hyopneumoniae*. *Vet J.* 212:1–6. <https://doi.org/10.1016/j.tvjl.2015.10.030>

UNDER PEER REVIEW