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# Porcine Circovirus Diseases: Current Insights and Future Strategies for Effective Control, with a Focus on Porcine Circovirus 2 (PCV2)

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***Authors' contributions***

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## ABSTRACT

Porcine Circovirus Associated Disease (PCVAD), caused by Porcine Circovirus Type 2 (PCV2), is a major health concern in swine production, leading to significant economic losses. The diagnosis of PCVAD relies on a combination of clinical signs and confirmatory laboratory techniques. Clinically, affected pigs exhibit weight loss, jaundice, poor growth, respiratory distress, and gastrointestinal issues. Histopathological examination reveals characteristic lesions such as lymphoid depletion, granulomatous inflammation, and the presence of inclusion bodies in lymphoid tissues. Immunohistochemistry (IHC) and in situ hybridization (ISH) are critical for detecting PCV2 antigens and viral nucleic acids in tissue samples, respectively. Molecular diagnostic methods, including PCR and quantitative PCR (qPCR), offer rapid and precise detection of PCV2 DNA, with advancements like digital droplet PCR (ddPCR) improving sensitivity. Serological tests, such as immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA), are useful for detecting PCV2 antibodies, aiding in assessing infection status and immune response. Genotyping assays help track the evolution of PCV2 strains, essential for understanding epidemiology and vaccine development. Prevention and control of PCVAD involve a multifaceted approach, including biosecurity measures, co-infection management, and vaccination. Vaccination remains a key tool, with updated vaccines providing protection against multiple PCV2 genotypes. Emerging technologies, such as nanobody-based ELISAs and biosensors, hold promise for faster and more cost-effective diagnostics. Continued research into new vaccine technologies, alongside ongoing surveillance of PCV2 genetic shifts, is critical for effective long-term control of PCVAD and minimizing its impact on the swine industry.

*Keywords: Porcine circovirus type 2 (PCV2); porcine circovirus associated disease (PCVAD); diagnosis; vaccination & biosecurity.*

## 1. INTRODUCTION

### 1.1 Historical Background

The discovery of Porcine Circoviruses (PCVs) dates back to 1974 when a viral contaminant was unexpectedly identified in the continuous porcine kidney cell line, PK-15 (ATCC-CCL31). This virus, later named Porcine Circovirus type 1 (PCV1), was determined to be non-pathogenic to pigs, and for decades it remained a relatively benign curiosity in the field of virology (Allan et al., 1995). However, this perspective shifted in 1997 with the isolation of Porcine Circovirus type 2 (PCV2), which was associated with severe clinical conditions in pigs, collectively termed Porcine Circovirus-Associated Diseases (PCVAD) (Segalés & Domingo, 2002).

The most prominent of these diseases, Post-weaning Multisystemic Wasting Syndrome (PMWS), primarily affected weaned piglets aged 7 to 15 weeks, manifesting in symptoms such as wasting, respiratory distress, diarrhea, and jaundice. PMWS's rapid onset and high fatality rates made PCV2 a critical pathogen, elevating it to one of the foremost challenges in swine health worldwide (Madec et al., 2008). Epidemiological evidence suggests that antibodies against PCV2 were circulating in pig populations as early as 1985 in Belgium, but it was not until 1991 in western Canada that clinical manifestations of PCVAD were observed (Segalés & Domingo, 2002).

Further developments in the narrative of PCVs came in 2015 with the identification of Porcine Circovirus type 3 (PCV3). PCV3 was initially found in pigs suffering from diverse conditions, including Porcine Dermatitis and Nephropathy Syndrome (PDNS), reproductive failure, and myocarditis, indicating an association with multi-organ systemic inflammation (Palinski et al., 2016). Despite being linked to disease, PCV3 was also detected in clinically healthy pigs, suggesting a more complex interaction with host immunity, possibly as a subclinical infection or one influenced by environmental and genetic factors (Klaumann et al., 2018).

The most recent discovery in this lineage occurred in April 2019, when a novel Porcine Circovirus type 4 (PCV4) was reported in pigs with severe clinical manifestations in Hunan province, China (Zhang et al., 2020). The emergence of PCV4 has added to the intricate story of PCVs, emphasizing the need for ongoing monitoring and molecular characterization to

understand their evolution and potential health implications.

## 1.2 Microbiological and Genomic Characteristics

Porcine Circoviruses are members of the Circoviridae family, which is known for its small, non-enveloped, icosahedral viral particles and single-stranded, circular DNA genomes. Circoviruses are some of the smallest known viruses, with diameters ranging from 12 to 20.7 nm (Rosario et al., 2017). Within the Circoviridae family, PCV1 and PCV2 genomes consist of approximately 1,759 and 1,768 nucleotides, respectively, encoding two major open reading frames (ORFs) that are critical for viral replication and structural integrity (Gillespie et al., 2009).

PCV3, identified in 2015, possesses the largest genome among known PCVs, at approximately 2,000 nucleotides (Fux et al., 2018). This genomic difference may contribute to its unique pathogenic profile and interaction with the host immune system. Recently, the genome of PCV4, containing about 1,770 nucleotides, has shown the highest sequence similarity to mink Circovirus (66.9%) and demonstrates lower homology with other PCVs (43.2%–51.5%), suggesting possible cross-species transmission or recombination events in its evolutionary history (Zhang et al., 2020).

In PCV1 and PCV2, the genome includes two primary ORFs: ORF1, which encodes replication proteins Rep and Rep', and ORF2, which encodes the capsid protein responsible for forming the viral structure. PCV2's genome also contains additional ORFs; notably, ORF3 encodes a nonstructural protein that induces apoptosis by activating caspase-8 and caspase-3 pathways (Liu et al., 2020). ORF4 overlaps with ORF3 and plays a role in modulating CD4+ and CD8+ T lymphocyte activity, which may assist in viral persistence by reducing virus-induced apoptosis, though it is not essential for replication (Gao et al., 2014).

The variable pathogenicity observed across PCV types may be attributed to differences in their genomes, particularly within ORF2, which encodes the capsid protein and is thought to contribute to immune evasion and tissue tropism (Lv et al., 2014). For example, changes within ORF2 have been correlated with the evolution of more virulent PCV2 strains, underscoring the importance of genomic surveillance in understanding viral pathogenicity.

## 1.3 Taxonomy and Nomenclature

The International Committee on Taxonomy of Viruses (ICTV) classifies members of the Circoviridae family into two genera: Circovirus, which primarily infects vertebrates, and Cyclovirus, found in both vertebrate and invertebrate hosts (Rosario et al., 2012). Until recently, pigs and certain avian species were the only mammals known to be affected by Circoviruses. However, advancements in metagenomics and polymerase chain reaction (PCR) techniques have revealed Circovirus genomes in a variety of hosts, including fish, dogs, chimpanzees, bats, and humans, suggesting a broader host range than previously recognized (Wu et al., 2016).

Circoviruses share similarities with the Nanoviridae family, which includes plant viruses, in their replication mechanisms, notably the presence of a conserved step-loop structure at the origin of replication and similar replication proteins (Todd et al., 1997). It has been hypothesized that Circoviruses may have evolved from a plant-infecting Nanovirus that recombined with a vertebrate-infecting RNA virus, potentially a Calicivirus, after a cross-kingdom transmission event (Gibbs & Weiller, 1999).

Within PCVs, the taxonomic distinctions are primarily based on differences in the ORF2 capsid region. PCV2, for instance, has been classified into several genotypes, including PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e, identified over time through molecular studies (Olvera et al., 2007; Xiao et al., 2015; Davies et al., 2016). Further subdivisions, such as PCV2d-1 and PCV2d-2, have been added based on geographic and chronological trends in variant emergence (Xiao et al., 2015). The discovery of genotype PCV2f in 2017, followed by the identification of PCV2g and PCV2h in 2018, suggests an ongoing diversification within the PCV2 lineage (Bao et al., 2018; Franzo & Segalés, 2018). Olvera et al. (2007) proposed that PCV2 could also be grouped into two primary phylogenetic clusters, further subdivided into clusters 1A–1C and 2A–2E, based on phylogenetic analyses. These classifications underscore the virus's genetic adaptability and its ability to produce genotypes with varying degrees of pathogenicity, leading to outbreaks

that differ in clinical severity and epidemiological dynamics.

## 2. EPIDEMIOLOGY

### 2.1 Transmission

Porcine Circovirus Type 2 (PCV2) has undergone a major global epidemiological shift over the last few decades, with the primary circulating genotype transitioning from PCV2a to PCV2b and then to newer subtypes like PCV2d in several regions. This shift has been correlated with more severe clinical presentations and varied PCV-associated diseases (PCVAD), highlighting the adaptive nature of the virus under selective pressure (Dei Giudici et al., 2023).

PCV2 transmission occurs primarily through horizontal and vertical pathways. Direct contact is the most efficient mode, as the virus is shed in respiratory, digestive, and urinary secretions. PCV2 can remain viable in the environment due to its resistance to desiccation, allowing indirect transmission via contaminated surfaces, tools, or personnel, as well as airborne spread under certain conditions (López-Lorenzo et al., 2019). Farm biosecurity, hygiene, and animal movement thus play essential roles in controlling the virus's spread.

- ***Vertical Transmission:*** Maternal transmission of PCV2 to piglets, both in utero and through colostrum, is well-documented (Gerber et al., 2012). Studies show that while maternal immunity may reduce viremia levels, it does not prevent the establishment of a viral reservoir in piglets. Infected sows can transmit the virus to offspring, leading to persistent farm-level infection cycles.

- ***Semen Transmission:*** PCV2 DNA has been found in boar semen, posing risks for artificial insemination practices. Although the viral load in semen is lower than in other fluids, its presence indicates that semen-based transmission can occur, especially in breeding farms (Madson et al., 2009).

- ***Ingested Tissue Transmission:*** Experimental studies have demonstrated that oral consumption of infected tissues (e.g., lymph nodes, muscle) can lead to PCV2 infection. This emphasizes the role of contaminated carcass management and biosecurity to limit further transmission among swine populations (Maity et al., 2023).

### 2.2 Factors Influencing PCV2-Related Diseases

Although PCV2 is the primary agent of PCVAD, clinical outcomes depend on interplay of viral, host, co-infection, and immunomodulatory factors.

**a.** ***Viral Factors:*** PCV2 genotypes exhibit high genomic similarity, yet distinct genotypic variants (e.g., PCV2a, PCV2b, and PCV2d) demonstrate differences in virulence and pathogenicity. Genomic studies have identified specific mutations in the capsid gene that influence virulence. For instance, a single amino acid substitution at key loci can increase the virus's ability to evade immune responses, enhancing pathogenesis (Opriessnig et al., 2020). Furthermore, pigs infected with multiple genotypes (heterologous strains) tend to develop more severe disease compared to those infected with homologous strains, suggesting genotype-specific immune interactions (Franzo & Segalés, 2018).

**b.** ***Host-Dependent Factors:*** The host's genetic background impacts susceptibility to PCVAD. Breed-specific studies have found variations in immune responses to PCV2, with breeds such as Landrace and Large White exhibiting higher susceptibility to clinical PCVAD compared to others like the Duroc (Opriessnig et al., 2006). Differences in genetic immunity markers may predispose certain breeds to more severe disease or modulate the clinical presentation, with a significant role played by factors like immune cell types, stress resilience, and inflammatory responses.

**c.** ***Co-Infection Effects:*** PCVAD is commonly associated with co-infections, which exacerbate the progression and severity of clinical disease. Co-infections with pathogens such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Parvovirus (PPV), Mycoplasma hyopneumoniae, and swine influenza viruses amplify the pathological impact of PCV2 (Gillespie et al., 2009). These pathogens can modulate the host

immune response, providing PCV2 with an opportunity to replicate more effectively and causing greater tissue damage. A recent meta-analysis highlighted that PRRSV co-infection increases the risk of PCVAD by up to threefold, especially in high-density farming settings where co-exposure rates are elevated (Vargas-Bermudez et al., 2024).

**d.** ***Effects of Immunomodulation:*** The immune status of pigs-whether due to vaccination, natural infection, or immunosuppression-plays a significant role in the clinical outcome of PCV2 infection.

- ***Immunostimulation:*** Immunostimulatory practices, such as vaccination for other pathogens, may exacerbate PCV2 infection. Immune activation, particularly through vaccines that engage innate immunity, has been shown to accelerate PCVAD in PCV2-infected pigs. For example, vaccination against PRRSV in PCV2-infected pigs can trigger PCVAD due to the compounded immune stimulation, resulting in systemic inflammation and heightened viral replication (Guo et al., 2022).

- ***Immunosuppression:*** Immunosuppressive agents, including corticosteroids and certain vaccines, also influence PCV2 progression. Pigs treated with immunosuppressive drugs exhibited increased PCV2 replication without typical inflammatory lesions. The absence of lesions despite high viral loads underscores that an active immune response is integral to the pathology of PCVAD (Fehér et al., 2023).

**e.** ***Environmental and Management Factors:*** Farm management practices and environmental factors like temperature, hygiene, and density also influence PCV2 dynamics. Poor ventilation, high humidity, and close confinement can enhance viral transmission rates and exacerbate clinical symptoms. Studies show that stringent biosecurity measures, including regular cleaning of pens, isolation of infected animals, and use of disinfectants, significantly reduce viral load on farms, mitigating both transmission and severity of disease (Galindo-Barboza et al., 2024).

## 3. PATHOGENESIS

The pathogenesis of Porcine Circovirus 2 (PCV2) infection, a critical cause of Porcine Circovirus-Associated Disease (PCVAD), is complex and involves several cell types within the immune system. Despite recent advances in PCV2 research, the mechanisms of viral persistence and replication within host cells remain only partially understood. Pathological changes, particularly in lymphoid tissues, are prominent in PCVAD-affected pigs, marked by lymphoid depletion and peripheral blood lymphopenia, which compromise immune function and contribute to disease severity (Maity et al., 2023). This section delves into recent findings on the pathogenesis of PCV2, discussing specific cell types, viral persistence, and immunopathogenic mechanisms.

- ***Cellular Tropism and Replication:*** PCV2 predominantly targets lymphoid tissues, with a particular affinity for lymph nodes, tonsils, spleen, and thymus. In PCVAD-affected pigs, immunohistochemistry (IHC) and in situ hybridization (ISH) techniques have revealed a high concentration of PCV2 antigens and nucleic acids within the cytoplasm of macrophages and dendritic cells, often replacing lymphocytes in lymphoid follicles. These findings suggest that antigen-presenting cells play a key role in harbouring the virus (Allan & Ellis, 2000). However, a critical observation is that while PCV2 may persist within macrophages and dendritic cells, active viral replication is limited, indicating that these cells might act as carriers rather than replication sites (Vincent et al., 2003). Recent studies using flow cytometry and in vitro culture models have confirmed that monocytes and macrophages can internalize PCV2, with the virus detectable within the cytoplasm without substantial replication (Gilpin et al. 2003). Similarly, Vincent et al. (2003) found no viral replication within dendritic cells in vitro, though the virus remains viable, potentially allowing dendritic cells to facilitate its spread to different tissues through migration. Moreover, in vivo studies have demonstrated PCV2 presence within T and B lymphocytes, contributing to immune modulation and highlighting these cells as potential targets of viral replication under specific conditions.

- ***Lymphoid Depletion and Follicular Disruption:*** Lymphoid depletion is a hallmark of PCV2 infection, especially in lymph nodes and tonsils. Histopathological analyses reveal significant disruption of lymphoid follicles, with a high density of PCV2 antigen corresponding to areas of lymphoid depletion (Opriessnig et al., 2007). This process, characterized by the replacement of lymphocytes with histiocytic cells, may arise not only from viral replication but also from an indirect effect of infection that disrupts immune homeostasis. The severity of lymphoid depletion has been found to correlate positively with the level of PCV2 antigen in affected tissues, suggesting a dose-dependent cytopathic effect (Cecere et al., 2012). Additional studies suggest that viral protein interactions might contribute to lymphoid depletion. For instance, the ORF3 protein, previously implicated in apoptosis, does not appear essential for viral replication, as shown in experiments with ORF3-null mutants that yielded similar histopathological findings to those infected with wild-type PCV2 (Juhan et al., 2010). This finding implies that apoptosis might not be the primary mechanism for lymphoid depletion, though the role of ORF3 in immune modulation and pathogenesis warrants further investigation.

- ***Immunomodulation and Co-infections:*** PCV2 infection is associated with immunomodulatory effects that impact host immune responses, potentially facilitating co-infections with other pathogens. Experimental data suggest that certain oligodeoxynucleotide (ODN) sequences within the PCV2 genome can modulate interferon-alpha (IFN-α) production. Specifically, four sequences within PCV2 DNA promote IFN-α production, while one inhibits it (Shi et al., 2021). The ability of PCV2 to modulate IFN-α responses may contribute to immune evasion and enhance viral persistence, as IFN-α plays a crucial role in antiviral immunity. The modulation of IFN-α production also affects the outcomes of co-infections. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Mycoplasma hyopneumoniae are among the most common co-infecting pathogens, and their presence often exacerbates clinical disease. These co-infections may enhance PCV2 replication through immune suppression or synergistic effects that intensify inflammatory responses in tissues (Opriessnig et al., 2020).

- ***Immunopathology and Apoptotic Pathways:*** PCV2's involvement in immunopathology extends to the induction of apoptosis in lymphoid tissues, contributing to immune cell depletion. Studies have noted that PCV2-infected lymphoid tissues exhibit apoptotic markers, though the exact viral mechanisms triggering cell death remain unclear (Chianini et al., 2003). Some research suggests that viral protein interactions within the cell could disrupt normal cellular pathways, possibly inducing programmed cell death indirectly rather than through direct cytopathic effects. Research involving immune cells such as macrophages and monocytes demonstrates that while these cells internalize PCV2, they do not support productive viral replication, which may contribute to immune system overstimulation and systemic inflammation (Shi et al., 2021). The persistence of viral proteins in non-replicative forms within immune cells likely plays a role in immune dysregulation and may contribute to the overall pathogenesis of PCVAD by diverting immune resources toward the infection without eradicating the virus.

- ***Emerging Mechanistic Insights into PCV2 Infection:*** Newer studies using high-throughput sequencing and molecular techniques have revealed additional aspects of PCV2 pathogenesis. For example, whole-genome analyses of PCV2 have identified variations in the capsid protein that may influence virulence and host immune responses (Franzo et al., 2024). Notably, these genetic variations could impact viral entry into host cells and immune recognition, factors critical to infection efficiency and disease progression. Additionally, transcriptional profiling of infected lymphoid tissues has shown altered gene expression patterns related to immune responses, particularly within pathways governing inflammation and cell-mediated immunity (Shi et al., 2021). Such insights underscore the complex interplay between PCV2 and the host immune system, with viral proteins

engaging multiple host pathways to evade immune responses and promote viral persistence. Additionally, the role of cytokines and inflammatory mediators in the immunopathogenesis of PCV2-related diseases is becoming increasingly clear. Elevated levels of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), are observed in PCV2-infected pigs and correlate with lesion severity in lymphoid tissues (Niu et al., 2022).

In summary, PCV2 pathogenesis involves complex interactions between viral proteins, immune cells, and co-infecting pathogens. While macrophages and dendritic cells may act as virus carriers, lymphoid depletion and follicular disruption characterize tissue-level impacts, with immune dysregulation and cytokine responses contributing to disease progression. Recent insights into viral genetic variations and immunomodulatory strategies offer promising directions for further understanding PCV2 pathogenesis. Future research on host immune responses, viral genetic factors, and co-infection dynamics will likely shed light on additional mechanisms by which PCV2 promotes immunopathology in swine.

## 4. PCV-2 ASSOCIATED SYNDROMES

Porcine Circovirus 2 (PCV2) infection is linked to a spectrum of disease manifestations collectively known as Porcine Circovirus-Associated Disease (PCVAD). This umbrella term, adopted to capture the broader clinical impact of PCV2, has replaced earlier nomenclature such as Post-weaning Multisystemic Wasting Syndrome (PMWS). Clinical expressions of PCVAD include respiratory distress, enteric disease, porcine dermatitis and nephropathy syndrome (PDNS), and reproductive failure, either in isolation or simultaneously within a herd. These manifestations vary in intensity and frequency based on factors such as viral strain, host immunity, environmental stress, and co-infection with other pathogens (Gillespie et al., 2009).

### 4.1 Post-weaning Multisystemic Wasting Syndrome (PMWS)

PMWS, one of the most severe expressions of PCVAD, predominantly affects pigs post-weaning and is marked by progressive weight loss, reduced growth performance, increased susceptibility to other infections, and elevated antibiotic use within herds. PMWS symptoms often include lethargy, lymphadenopathy, and respiratory issues (Segalés et al., 2002). Morbidity and mortality rates can vary significantly, with some herds experiencing mortality rates as high as 30% to 40%. Histopathologically; PMWS is defined by lymphoid depletion, histiocytic replacement of lymphocytes, and the presence of PCV2 within affected tissues. Gross lesions include enlarged lymph nodes and pale, mottled lungs. As PMWS progresses, advanced signs such as jaundice, anemia, and diarrhea may develop, along with characteristic microscopic findings like intracytoplasmic inclusion bodies in lymphoid tissues (Maity et al., 2023). Studies have suggested that co-infection with other pathogens, particularly Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Mycoplasma hyopneumoniae, and swine influenza virus, significantly exacerbates PMWS progression (Grau-Roma et al., 2008).

### 4.2 PCV-2 Associated Enteritis

PCV2-associated enteritis is increasingly recognized among pigs aged 8 to 16 weeks, primarily presenting as chronic diarrhea, reduced weight gain, and high mortality. The gross pathology associated with PCV2 enteritis resembles Lawsonia intracellularis infections, making differential diagnosis essential. Common histological findings include granulomatous inflammation in the small intestine, Peyer's patches, and mesenteric lymph nodes (Segalés et al., 2012). The thickening of the intestinal wall observed in PCV2-infected pigs is attributed to granulomatous enteritis, with extensive lymphoid depletion in Peyer's patches and characteristic viral inclusions. Diagnosis relies heavily on distinguishing these histopathological markers from other enteric pathogens. Several studies since 2018 have reinforced that PCV2 is often present in combination with other pathogens such as *Escherichia coli*, highlighting the importance of multifactorial triggers in PCV2 enteritis pathogenesis (Baró et al., 2019).

### 4.3 PCV-2 Associated Reproductive Failure

First identified in Canada in the late 1990s, PCV2-associated reproductive failure has since been documented worldwide, especially in breeding herds, often marked by an increase in mummified and non-viable piglets, is a common

manifestation of PCV2 infection. Boars can shed low levels of PCV2 in semen over extended periods, and vertical transmission of the virus from the sow to fetuses has been confirmed experimentally. Although intrauterine-infected piglets may appear clinically normal, they can be born viremic, potentially spreading PCV2 within the herd. PCV2 vaccination of breeding stock prior to exposure significantly reduces both semen shedding in boars and intrauterine transmission to piglets. Experimental infections have demonstrated that gestational age influences viral impact, with infection at earlier stages causing more severe fetal outcomes. Higher rates of fetal mortality and associated lesions occur when PCV2 exposure happens before mid-gestation, suggesting that earlier in utero exposure to PCV2 poses a greater risk for reproductive disruptions. Further, maternal-fetal transmission pathways have been observed, but studies have found that specific viral strain variations may also alter pathogenic outcomes in affected litters (Madson & Opriessnig, 2011).

### 4.4 Porcine Dermatitis and Nephropathy Syndrome (PDNS)

First reported in the UK in 1993, Porcine Dermatitis and Nephropathy Syndrome (PDNS) was later linked to PCV2 in the early 2000s. PDNS is characterized by distinctive raised purple lesions on the skin that can evolve into multifocal red and purple scabs. Additionally, systemic vasculitis and glomerulonephritis with dermal necrosis are the hallmarks of PDNS pathology. Affected pigs frequently display lethargy, fever, and decreased appetite. Recent studies underscore the link between PCV2 and PDNS, with research suggesting that immune complex-mediated type III hypersensitivity contributes to PDNS development. This involves the deposition of antigen-antibody complexes in small blood vessels, causing immune-mediated damage within the kidneys and skin. At necropsy, PDNS lesions commonly appear as tan, waxy kidneys with petechial hemorrhages and multifocal glomerulonephritis, especially in the renal cortex (Chae, 2005).

## 5. DIAGNOSIS

Porcine Circovirus Associated Disease (PCVAD) presents a unique challenge in swine health due to the diversity of clinical signs and pathological lesions it can cause. Diagnosis often begins with clinical observation but requires confirmatory laboratory methods to identify PCV2 and assess its role in disease progression.

### 5.1 Clinical Diagnosis

Clinical signs alone may provide an initial indication of PCVAD. Typical symptoms include weight loss, pallor, jaundice, poor growth performance, and generalized wasting. Affected pigs often exhibit respiratory signs, such as coughing and labored breathing, along with gastrointestinal issues, including diarrhea that may appear dark and tarry due to intestinal bleeding (Maity et al., 2023). Although these signs support a tentative diagnosis, confirmation of PCVAD requires identifying PCV2-associated lesions and antigens in multiple tissues, especially lymphoid organs, or a combination of lymphoid and non-lymphoid tissues like the lungs, liver, kidneys, or intestines (Opriessnig et al., 2020).

### 5.2 Histopathology and Immunohisto-chemistry

For a definitive diagnosis, the presence of PCV2 in tissue samples alongside characteristic histopathological lesions is essential. The hallmark lesions of PCVAD include lymphoid depletion, granulomatous inflammation, and the presence of inclusion bodies within histiocytes and other cells. Lymph nodes, Peyer's patches, and the lamina propria of the intestinal villi commonly exhibit these changes (Segalés et al., 2012). Additionally, the presence of syncytial cells in lymphoid tissues is a key microscopic abnormality linked to PCVAD.

Immunohistochemistry (IHC) and in situ hybridization (ISH) are widely recognized as the gold standards for detecting PCV2 antigens in tissue sections. IHC, which relies on antibodies binding to PCV2 antigens, has greater sensitivity and provides vivid staining, making it easier to visualize PCV2 within cells. ISH, on the other hand, identifies viral nucleic acids within tissues, offering precise localization but at a higher cost and longer turnaround time than IHC (Szczotka-Bochniarz et al., 2011). Research has shown that the presence of PCV2 in multiple lymphoid and non-lymphoid tissues combined with extensive lymphoid depletion correlates with the clinical disease observed in PCVAD cases (Opriessnig et al., 2020).

### 5.3 Immunofluorescence Assay (IFA) and Immunoperoxidase Monolayer Assay (IPMA)

IFA and IPMA are common serological assays used to detect antibodies against PCV2. IFA, particularly when based on the open reading frame (ORF) 2 protein, provides a reliable, although relatively lower-sensitivity, method of detecting PCV2 antibodies (Racine et al., 2004). A comparative study across multiple laboratories demonstrated that IPMA often yields higher antibody titers than IFA. Factors like fixation techniques also influence titers; for instance, paraformaldehyde yields better results than acetone or ethyl alcohol (McNair et al., 2004).

### 5.4 Polymerase Chain Reaction (PCR) and Quantitative PCR (qPCR)

Polymerase Chain Reaction (PCR) techniques offer rapid and accurate diagnosis, detecting PCV2 DNA directly from clinical samples such as blood, serum, and tissues. PCR is valuable for early detection, especially in live animals suspected of having Porcine Circovirus-associated diseases (PMWS). PCR-REBA (Reverse Blot Hybridization Assay) and multiplex real-time PCR can detect and differentiate between PCV2 genotypes in clinical samples within a few hours (Ke et al., 2024). TaqMan-based qPCR assays have shown nearly 100% sensitivity and specificity, offering precise quantification of PCV2 viral load (Henriques et al., 2018). Rapid qPCR methods, such as those established by Chang et al. (2010), have made it possible to differentiate between PCV1 and PCV2, providing results within 45 minutes and supporting timely interventions in the field.

Recent advancements in PCR technology, such as digital droplet PCR (ddPCR), enhance the sensitivity and quantification of PCV2 by partitioning samples into thousands of droplets, where PCR amplification occurs individually. ddPCR has proven highly sensitive and effective, especially for samples with low viral loads, aiding in early-stage PCV2 detection (Mu et al., 2021).

### 5.5 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is widely utilized to detect and quantify anti-PCV2 antibodies, with particular utility in monitoring the progression and timing of PCV2 infection based on immunoglobulin (Ig) levels. Higher IgM levels than IgG indicate early active infection, while an IgG-dominant response suggests either late-stage or resolving infection (Opriessnig et al., 2020). Several ELISA adaptations have been developed since 2015 to improve diagnostic sensitivity and specificity.

A recombinant capsid protein-based ELISA using a nuclear localization signal-truncated capsid protein (CAP ELISA) demonstrated 95% sensitivity and 94% specificity compared to traditional IFA. Furthermore, competitive ELISA (cELISA) assays, such as those developed by Mu et al. (2021), have shown high reliability for detecting PCV2 antibodies in vaccinated and naturally infected pigs, making it valuable for vaccine efficacy assessment.

### 5.6 Genotype Differentiation Assays

Genotyping PCV2 is critical to understanding its epidemiology and tracking genotype shifts in affected herds. ORF2-based PCR-RFLP (restriction fragment length polymorphism) assays using restriction enzymes like HinfI, KpnI, and RsaI have been effective in distinguishing between PCV2 genotypes A, B, C, D, and E (Franzo et al., 2024). Additionally, more recent molecular typing methods, such as high-resolution melting analysis (HRMA) and next-generation sequencing (NGS), have provided even greater precision in differentiating PCV2 genotypes. HRMA, a PCR-based method, uses temperature-sensitive fluorescent dyes to analyze genotype-specific melting curves, while NGS offers detailed genome-wide insights, supporting advanced studies on PCV2's genetic evolution (Wang et al., 2020).

### 5.7 Nanobody-Based Competitive ELISA

A breakthrough in PCV2 diagnostics is the use of nanobodies, which are smaller antibody fragments that bind to specific antigens with high affinity. Nanobody-based competitive ELISAs (cELISAs) have emerged as cost-effective tools for indirect detection of PCV2 antibodies. Mu et al. (2021) demonstrated the effectiveness of a nanobody-horseradish peroxidase fusion protein-based cELISA in detecting anti-PCV2 antibodies, providing reliable diagnostic results and aiding in monitoring vaccine efficacy.

### 5.8 Emerging Technologies

The diagnostic landscape for PCVAD continues to evolve, with emerging technologies such as biosensors and lab-on-a-chip platforms showing promise for faster and more convenient diagnostics. Biosensors based on antigen-

antibody interactions have been developed to detect PCV2 antigens in clinical samples with high specificity and rapid turnaround times (Zhang et al., 2020). These advances are especially relevant in resource-limited settings where traditional laboratory infrastructure is limited.

## 6. PREVENTION AND CONTROL

### 6.1 Pre-Vaccine Era Management

Prior to the widespread availability of vaccines, management strategies focused on reducing stress, minimizing co-infections, and enhancing immune responses to decelerate PCVAD progression. Core practices were centered around improving biosecurity and minimizing infection risks through rigorous hygiene and effective handling protocols, as high viral load and environmental exposure contributed significantly to PCVAD's severity and persistence. However, the effectiveness of such methods was limited, as supportive care could not halt disease progression in severe outbreaks (Opriessnig et al., 2008).

### 6.2 Comprehensive Biosecurity Programs

Recent guidelines highlight the value of biosecurity protocols that involve improved sanitation, vector control, and restricted farm access to limit exposure to potential sources of infection. Enhanced disinfection of buildings, vehicles, and equipment using oxidizing agents like hydrogen peroxide has shown effectiveness in reducing environmental PCV2 contamination, although exact protocols vary between farms. Isolation protocols, particularly for introducing new animals, and prolonged idle periods between production cycles have also been effective in minimizing cross-contamination risks (Maity et al., 2023).

Studies emphasize that all-in/all-out management significantly lowers the risk of pathogen spread within the production unit, which is essential in controlling diseases like PCVAD that exhibit high environmental resilience. Internal and external biosecurity measures, including regular cleaning, proper animal grouping by age, and systematic pest control, are crucial in controlling PCVAD spread (Allan & Ellis, 2000).

### 6.3 Controlling Co-infections and Secondary Infections

Co-infections are a major driver of PCVAD progression. Antimicrobial treatments and vaccinations targeting secondary infections like Mycoplasma hyopneumoniae and PRRSV have shown effectiveness in reducing disease severity when co-administered with PCVAD interventions. For instance, chlortetracyclines have been shown to significantly reduce the impact of co-infections on PCV2-infected animals. Antiviral adjunct therapies are also being investigated, with a focus on reducing viral load and enhancing recovery in PCVAD-affected pigs, but further research is needed for practical farm applications (Maity et al., 2023).

### 6.4 Risk Factors and Mitigation Strategies

Identifying risk factors is critical for preventive strategies. Factors such as increased crossbreeding, poor ventilation, overcrowding, and inadequate immune support have been correlated with higher PCVAD incidence (Gillespie et al., 2009). Reducing co-infections through vaccination and improving farm conditions, such as maintaining optimal temperatures and humidity, are effective in decreasing susceptibility to PCVAD. Studies also report that reducing animal stress through optimized stocking densities, consistent feeding schedules, and reduced animal movements within the farm environment can reduce stress-induced immune suppression, thus mitigating PCVAD risk (Maity et al., 2023).

### 6.5 Vaccination and Immunization Protocols

Recent research underscores the efficacy of various vaccination protocols in preventing PCVAD, which have evolved with increasing understanding of PCV2 genetic diversity. Updated vaccines now offer protection against multiple PCV2 genotypes, ensuring broader immunity across different farm settings (Guo et al., 2022). Today, a multi-layered approach is recommended, where both sows and piglets are vaccinated-the sows to confer maternal antibodies and the piglets for direct immunity post-weaning.

## 7. VACCINATION

### 7.1 Initial Vaccination Approaches and Challenges

Early vaccine formulations focused on targeting either PCV2 alone or co-pathogens such as PPV, PRRSV, and Mycoplasma. Initial attempts using inactivated or subunit vaccines showed promise

but did not provide complete protection, as PCV2 could persist in the environment and in pig populations with maternal antibodies. Later developments, including recombinant DNA and chimeric PCV1-2 vaccines, achieved better immune responses, particularly against more virulent strains (Segalés et al., 2014).

### 7.2 Current Vaccine Options and Efficacy

Contemporary PCV2 vaccines include Circumvent PCV, Ingelvac CircoFLEX, and Suvaxyn® PCV2, among others, each designed with advanced antigen formulations to provide immunity across the main PCV2 genotypes. These vaccines are typically administered through a two-dose protocol in weaning pigs, significantly reducing clinical PCVAD and improving growth performance, mortality rates, and overall farm productivity (Duong et al., 2019). Meta-analyses confirm that vaccination reduces PCV2-associated mortality by over 70% in intensive pig farming settings, contributing to economic gains and higher meat yields (Huang et al., 2021).

### 7.3 Economic Impact of Vaccination

Vaccination has had a profound economic impact, particularly in intensive farming setups. Studies show that farms implementing PCV2 vaccination observe an increase in average daily weight gain (ADG) and a reduction in time to market. This is particularly evident in regions where pig herds suffer from high pathogen loads and environmental PCV2 presence. Additionally, vaccination improves herd uniformity, resulting in optimized feed conversion ratios and a decrease in costly treatments for co-infections (Afghah et al., 2018).

### 7.4 New and Experimental Vaccine Technologies

Recent research on nanoparticle and mRNA-based vaccines shows potential for enhanced immunity with minimal side effects. These vaccines are designed to provide rapid and robust immune responses against both PCV2 and its associated co-infections, although they are still in experimental stages. Furthermore, studies on autogenous vaccines tailored to specific herd PCV2 strains provide an avenue for customized immunity, which could be beneficial in highly endemic areas with unique viral strains (Ren et al., 2024).

### 7.5 Vaccine Challenges and Emerging Solutions

Although PCV2 vaccines are effective, maternal antibodies can interfere with early-life immunization, delaying optimal immune development in piglets. Recent studies have demonstrated that administering a booster dose post-weaning can enhance the immune response in piglets born to vaccinated sows (Mu et al., 2021). Moreover, molecular studies reveal ongoing PCV2 evolution, indicating the need for continued surveillance and possible vaccine adjustments to maintain efficacy (Opriessnig et al., 2020).

### 7.6 Field Observations and Future Directions

In field conditions, vaccinated pigs show fewer clinical symptoms and reduced lesion severity. Ongoing studies focus on enhancing adjuvant formulations to improve vaccine stability and efficacy, particularly in mixed infections. There is also an emphasis on oral and nasal vaccines to simplify administration and improve mucosal immunity, which could reduce labor and stress associated with injection protocols.

Through continued advancements in biosecurity and vaccination protocols, PCVAD control has become significantly more manageable, though challenges persist. Regular surveillance for emerging PCV2 strains, adapting biosecurity practices to each farm’s unique conditions, and ongoing research into innovative vaccine platforms are critical for sustaining PCVAD management efforts in the long term.

## 8. CONCLUSION

Porcine Circoviruses, specifically PCV2, are significant pathogens impacting swine health and the global pork industry. While PCV1 is non-pathogenic, PCV2 leads to porcine circovirus-associated diseases (PCVAD), causing substantial economic losses due to decreased productivity and increased mortality. Advances in understanding PCV2's molecular biology, transmission, and pathogenesis have driven the development of effective vaccines that have markedly reduced PCVAD prevalence and severity.

Despite these gains, challenges remain. The emergence of novel PCV2 strains and potential

recombination events pose risks to the effectiveness of current vaccines. Consequently, continued surveillance and research are vital to monitor these genetic shifts and update vaccine strategies as needed. Moreover, PCVAD's multifactorial nature, often involving co-infections with pathogens like PRRSV, underscores the need for a comprehensive approach combining biosecurity, housing management, and co-infection control alongside vaccination. Improved environmental management practices-such as stress reduction, all-in/all-out systems, and optimal hygiene-are also crucial in limiting disease spread.

Future advancements, including next-generation vaccine platforms, hold promise for enhancing cross-protection against diverse PCV2 genotypes. Understanding the virus-host-environment interactions that drive PCVAD could lead to new therapeutic options, including antiviral agents or immune modulators. Collaborative global efforts in research, surveillance, and knowledge exchange will be critical to sustaining control measures, advancing disease prevention, and safeguarding swine health.

In summary, while strides in vaccine development and management practices have reduced PCVAD impact, a multifaceted, evolving approach is essential to adapt to ongoing challenges. By prioritizing research, biosecurity, and innovative vaccine strategies, the swine industry can ensure continued progress in combating PCVAD effectively.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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