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# Knowledge gaps in research and control of porcine circovirus 2 (PCV2) infections

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**Abstract**

Porcine circovirus type 2 (PCV2) remains one of the most important pathogens in swine production, associated with a spectrum of clinical conditions collectively termed PCV2-associated diseases. Despite the remarkable success of vaccination programs, which have drastically reduced the incidence of systemic disease and reproductive disorders, PCV2 continues to circulate globally in both domestic and wild swine populations. Its high evolutionary rate, capacity for recombination, and broad host plasticity raise ongoing concerns regarding viral persistence and long-term control. This review synthesizes current knowledge and identifies critical research gaps that hinder the development of sustainable PCV2 control strategies. While vaccines effectively mitigate clinical disease, they do not fully prevent infection or virus shedding, thereby allowing continued circulation and genetic diversification. The biological consequences of this viral evolution—including potential impacts on cross-protection, virulence, and vaccine escape—remain insufficiently understood. Similarly, the role of host immunity, co-infections, and environmental or management factors in modulating disease outcomes is incompletely characterized. A deeper understanding of the mechanisms underlying PCV2 pathogenesis, including immune modulation and determinants of subclinical versus clinical outcomes, is urgently needed. Diagnostic approaches have also evolved, with molecular techniques such as quantitative PCR largely replacing histopathology and immunohistochemistry. While highly sensitive, these methods cannot establish causal relationships between viral presence and disease, underscoring the need for integrated diagnostic frameworks. In addition, harmonized thresholds for viral load quantification and standardized serological assays to assess protective immunity are lacking, limiting comparability across studies and surveillance systems. Future priorities should include the development of next-generation vaccines capable of inducing sterilizing immunity, investigation of optimal vaccination schedules in the context of maternally derived antibodies, and exploration of innovative vaccine delivery platforms. Furthermore, integrated surveillance strategies combining molecular epidemiology, wildlife monitoring, and international data sharing will be essential to track viral evolution and detect potential vaccine breakthroughs. Addressing these knowledge gaps will require coordinated efforts across fundamental, applied, and translational research, aligned with the needs of veterinarians and the swine industry. Only through such an integrated agenda can the sector advance from disease control towards the long-term goal of PCV2 elimination.

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## Introduction

Porcine circovirus 2 (PCV2) remains a globally significant pathogen in swine production, primarily due to its involvement in PCV2-associated diseases (PCV2-AD) [1], particularly PCV2-systemic disease (PCV2-SD) and PCV2-subclinical infection (PCV2-SI), which have led to substantial economic losses in the last three decades [2]. Despite the successful implementation of vaccination strategies and an improved understanding of the virus-host interaction, several critical knowledge gaps persist that hinder more effective long-term control, prevention, and the eventual elimination of the virus from swine herds.

Although PCV2 vaccines have shown to be a game-changer in terms of disease control in pigs [3], multiple gaps remain in understanding pathogenesis, evolution, immunology, and long-term control strategies of this viral infection. Addressing these gaps is essential not only for improving animal health and productivity but also for ensuring the sustainability of current control measures in the face of a genetically dynamic and globally distributed pathogen.

## Genetic diversity of PCV2

PCV2 possesses a closed circular, single-stranded, ambisense DNA (ssDNA) genome of 1767–1768 nucleotides. Several open reading frames (ORFs) have been identified, although only two seem to be essential for viral viability. ORF1 encodes, through alternative splicing, the Rep and Rep' proteins, which are involved in viral replication. ORF2 encodes the Cap protein, the sole structural component of the viral capsid, which plays a key role in determining viral tropism and represents the main target of the host immune response [4].

Other open reading frames (ORFs 3 to 6) have been shown to be transcribed and expressed. Although they are not essential for PCV2 viability, they are believed to contribute to viral pathogenesis. ORF3 protein is relatively well characterized in terms of its involvement in pathogenesis. In contrast, the remaining ORFs have been more recently identified, and their roles remain less defined. Overall, these accessory proteins are thought to modulate apoptosis, host cell signalling pathways, and immune responses ([5–8].

PCV2 exhibits a high evolutionary rate (~ $10^{-4}$–$10^{-3}$ substitutions/site/year), which has resulted in remarkable genetic variability, including at the within-host level, where complex subpopulation structures—potentially associated with clinical outcomes and host immunity—have been identified [9–12]. Based on the analysis of the ORF2 gene, eight genotypes (PCV2a to PCV2h) have been formally defined [13]. However, the continuous viral evolution, along with the increasing availability of sequences has revealed a more complex and nuanced genetic landscape, posing challenges to any schematic classification system.

Sequences of PCV2 DNA have been sequenced and divided into different genotypes.

- Major genotypes, including PCV2a, PCV2b, and PCV2d, which are characterized by their widespread distribution and persistence over time.
- Minor genotypes, include PCV2c, PCV2e, PCV2f, PCV2g and PCV2h, have been detected only sporadically. However, an increasing number of countries are now reporting their circulation. Whether this reflects a genuine expansion of these genotypes or merely a bias resulting from the growing availability of sequence data remains to be clarified.

Major PCV2 genotypes gave rise to distinct waves over time. The first genotype shift occurred around 2000 and was evident by a replacement of PCV2a towards PCV2b. The second genotype shift was observed in 2010 and replaced PCV2b towards PCV2d and is now known as the second genotype shift [10]. However, more recent evidence suggests an ongoing, largely underestimated cocirculation of PCV2d combined with ongoing evolution of PCV2a and PCV2b. Prevalence fluctuations of these two latter genotypes after their initial decline may result from the interaction with host population immunity, both natural and vaccine induced [14]. The reasons underlying such genotype epidemiological patterns remain largely speculative, and the relationship between genetic variability and cross-protection, particularly in the context of vaccine-induced immunity, continues to be debated. Although there is consensus that PCV2 behaves as a single serotype and that immunity is broadly cross-protective, evidence of genotype- and strain-specific epitopes has also been reported [15, 16]. Epidemiological data support the hypothesis of at least partial or differential cross-protection, which, while potentially insufficient to affect clinical disease outcomes in properly immunized animals, may nonetheless play a role under suboptimal conditions or influence genotype fitness when assessed on a broader, population-level scale [14, 17–19].

Differential virulence among strains and genotypes has been proposed, and variability in the cellular uptake capacity of PCV2 strains has also been observed,

particularly in relation to the distribution of positive charges on the capsid [20]. Notably, the experimentally determined amino acid positions involved in this process were confirmed to be under evolutionary pressure through bioinformatic analyses based on extensive sequence datasets, including different hosts [21]. These data suggest their functional relevance in modulating viral fitness and potentially contributing to viral replication efficiency and virulence.

No specific virulence markers or consistent phylogenetic associations have been identified for PCV2. Experimental studies remain limited by the multifactorial nature of PCV2-associated diseases, while epidemiological investigations are constrained by their observational nature and the lack of standardized and detailed data on disease severity and other virulence indicators [22]. Furthermore, most molecular epidemiology studies focus on ORF2, whereas other ORFs may significantly contribute to virulence. The specific impact of subtle genetic variability within PCV2 strains is virtually unknown. Solving these knowledge gaps may also provide insights into the role of recombination, predominantly occurring in the intergenic region, and the impact of coinfection with multiple genotypes in shaping the biological characteristics and clinical outcome of PCV2 infection [23–25].

The effect of host-related factors on viral evolution remains a largely unexplored area. Notably, the growing—though still limited, body of knowledge on PCV2 genotype and variant circulation in wild and rural swine populations, as well as in closely related species, provides evidence that the host, or more likely, the structure and dynamics of host populations, may influence viral circulation and evolution. Moreover, certain genotypes have been preferentially detected in wild or rural populations, suggesting a possible host tropism [21, 26–28]. Whether this pattern is driven by biological determinants or by epidemiological factors remains to be elucidated.

## Epidemiology and transmission

### Circulation of PCV2 over time

PCV2 was formally recognized in the late 1990 s, but retrospective analyses of archived samples revealed its circulation in domestic pig populations decades earlier. Retrospective in situ hybridization (ISH) and polymerase chain reaction (PCR) analyses of porcine tissue samples from Northern Germany identified PCV2 DNA as early as 1962 [29]. Genetic analysis of archived Brazilian samples identified a PCV2 isolate from 1967, representing the oldest confirmed PCV2 genome sequence outside Europe [30]. The earliest evidence of PCV2 in North America dates to 1973 in Mexico [31]. By the 1980 s, the virus was likely endemic in many countries, while the earliest evidence from Asia comes only from the 2000s. More recently, PCV2 has also been detected in pigs from several African countries [32]. However, based on phylogenetic studies, it is very likely that PCV2 has been circulating in swine for a much longer period [10], apparently without causing disease or, eventually, contributing silently to existing disease burden. However, given the short time span for which high-quality sequences are available and the evident sampling biases, our understanding of the initial phases of PCV2 circulation, including the geographical location, remains extremely limited. Although phylogeographic analyses tentatively point to an Asian origin for the three main genotypes, and wild boar as the initial host population, these inferences should be treated with great caution due to model assumptions, uneven sampling, and the abovementioned temporal limitations in sequence availability [21, 33].

#### *PCV2 shedding*

PCV2 is shed via multiple routes over extended periods, making horizontal transmission the primary mode of spread. Vertical transmission also plays an important role at herd level, although its exact impact is difficult to quantify. Due to its moderate-to-high transmissibility and environmental resilience, PCV2 can persist in herds without external introduction [34]. Widespread vaccination has reduced prevalence in most major pig-producing countries, but it is assumed that PCV2 remains present and continues to evolve in all pig farms [2].

#### *PCV2 in domestic and feral pigs across the Globe*

Domestic pigs are the primary hosts, but substantial evidence indicates that wild boar and feral pigs are also susceptible to the infection and may serve as reservoirs of PCV2 worldwide. The virus is prevalent across their populations on all continents where they occur [35–40]. In Namibia, PCV2 has also been detected in warthogs, one of several species native to Africa [27]. Similarly, Brazilian white-lipped and collared peccaries, which are closely related to pigs, were found to harbour PCV2 DNA [41]. While prevalence in vaccinated pig populations is declining, infection rates remain high in wild boar, exceeding 76% in some regions [42–44]. Their role as reservoirs and potential vectors of interregional spread highlights the need for integrated wildlife-livestock surveillance.

#### *Direct and indirect transmission*

Transmission of porcine viruses between pigs and free-living pigs is well documented and can occur via direct or indirect contact. Also, PCV2 has been detected in rodents [45] and flies [46]. PCV2 nucleotide sequences obtained from mice and rats captured on Brazilian farms were highly similar to those from pigs, and immunohistochemistry confirmed PCV2 in rodent spleen, lung, and kidney tissues [45]. Experimental infection of mice produced microscopic lesions, seroconversion, or both

[47]. These findings suggest rodents may contribute to PCV2 maintenance and transmission in the field, while flies most likely act as mechanical vectors [46]. However, the precise role of non-suid species in the transmission of PCV2 is not known but probably low or negligible. In scenarios where vaccination reduces infectious pressure in domestic pigs this may be lower.

#### *Finding PCV2 in pork and other animals*

Pork and pork-derived products, major protein source worldwide, may also act as transmission vehicles for PCV2 to humans and carnivores. Metagenomic analysis detected PCV2 in pork samples from retail outlets in San Francisco, California [48]. In Brazil, PCV2 DNA was identified in 17.1% of liver samples collected at slaughterhouses [49]. In China, PCV2 DNA was found in 75% of pork samples, 73.9% of cutting board swabs, and 64.3% of meat stall floor swabs from live markets in six border cities, with sequences closely related to Russian and local Chinese farm isolates, suggesting pork trade as a pathway for viral spread [50]. Since pig-to-pig transmission through meat consumption has been demonstrated experimentally [51], interspecies transmission via meat or byproducts is plausible [52]. PCV2 antigens have also been detected in human faeces [53], although the clinical significance remains unclear. PCV2 DNA has additionally been found in carnivores, such as dogs [51], black-backed jackals [54], mink [55], raccoon dogs [56], and foxes [57]. Such detection is likely due to the eventual contents of pork products in the diets of human and carnivores, but the genome plasticity of circoviruses and their eventual cross-species transmission [26] must be a potential concern regarding PCV2 as a pathogen beyond suids. The detection of PCV2 in carnivorous species, especially farmed animals likely fed raw pig byproducts, is easier to explain than its occurrence in ruminants such as goats [58], yaks [59], and buffalo [60]. Experimental infections in calves resulted in seroconversion and PCV2 DNA detection in lymphoid tissues and serum for up to seven weeks, along with clinical signs such as respiratory dysfunction, lymph node enlargement, and diarrhoea [61]. PCV2 has also been detected in wild ruminants, including oryx in Namibia [62] and red deer in Austria [44], possibly due to environmental contamination. Swine manure, commonly used as fertilizer, contains the virus in abundance [63], and PCV2 has been also detected in water sources, including rural surface water, urban deep wells [64], and freshwater habitats overlying the Guarani Aquifer in Brazil [65]. Environmental contamination has also been confirmed in Europe, where PCV2 was found in 41% of blue mussel samples collected from Danish harvesting areas along the North Sea coast of Jutland [66]. It is important to note that the role of non-suid species in the epidemiology of PCV2 remains poorly elucidated, and current evidence suggests they most likely represent epidemiological dead-end hosts resulting from spillover events.

## Pathogenesis of PCV2 infections

### Virus replication in vitro

The replication cycle of PCV2 takes approximately 36 hours, which is extremely slow [67]. This long replication cycle can be explained by difficulties that the virus encounters during different stages. The first difficulty is to enter the target cell. In the continuous cell lines 3D4 and PK15, the PCV2 binding to the cells is mediated by the interaction of positively charged capsomeres with the polysaccharides (glycosaminoglycan family) heparan sulphate and chondroitin sulphate on the plasma membrane [68, 69]. In lymphoblasts and mononuclear phagocytes, two major in vivo target cells, PCV2 is mainly targeting chondroitin sulphate [70, 71]. Recently, it was demonstrated that PCV2 strains are genetically changing over time to bind better to chondroitin sulphate. Indeed, in contrast to PCV2a and PCV2b, PCV2d formed linear regions of clustered basic amino acids in the form of a three-winged windmill on the surface of its capsid trimers [20, 72, 73]. These conformational changes are largely increasing the avidity of PCV2d strain to its in vivo target cells and are facilitating the infection in the host. As most chondroitin sulphate molecules are not linked to a protein core that is internalizing, most viruses get stuck on the outside of the cells [68]. Only a few proteoglycans containing chondroitin sulphate are capable of internalization, with phosphacan being one of them [20, 73]. Following entry, the viral capsid present in endosomes must be cleaved to release the viral genome, a process mediated by serine proteases in T-lymphoblasts. Genetic differences exist in pigs on the need of a pH drop for the activation of the serine proteases and the subsequent release of the viral genome [73]. It is believed that upon cleavage of the capsid, the genome unfolds due to the high DNA pressure and destroy the endosome. Thereafter, the circular genome, having a calculated diameter of 200 nm, is translated towards the nucleus, where nuclear pores have a diameter of 40–50 nm. This constitutes a barrier for the virus, since the circular genome is too large to pass through the nuclear pore complex, differently from other small ssDNA viruses, such as parvoviruses, whose non-circular viral strand can easily cross the nuclear pores. Therefore, the circovirus relies on the breakdown of the nuclear membranes to enter the nucleus, which happens during mitosis. Afterwards, the cellular polymerase synthesizes the complementary strand and genome copies for the progeny [74]. New capsid and replicase/replicase' proteins are produced in the cytoplasm and shuttle back to the nucleus for the formation of new virus particles.

The newly formed viruses become released through cell lysis [75].

#### *Virus replication in embryos*

PCV2 has been shown to replicate in embryos with a strong mitotic activity after hatching, causing embryonic death [76, 77]. Experimentally, the clinical consequence is return-to-oestrus. However, the frequency of this outcome under farm conditions is not known; it is very well possible that this is underdiagnosed.

#### *Virus replication in foetuses*

The replication in foetuses depends on the sow's gestation stage [78, 79]. The replication decreases with foetal age. At an early stage, the virus is replicating in multiple organs, with the heart representing the primary target. Replication in myocardiocytes leads to heart failure, foetal death and mummification. When the foetus can raise an immune response, starting from 70 days of gestation, the foetus may survive an infection, resulting in normal, seropositive animals at birth. The frequency of reproductive failure attributed to PCV2 infection under field conditions seems to be low; in fact, in some cases there is well-documented evidence of intrauterine infections without evident clinical signs [80]. Moreover, the precise role of subclinical infections in pregnant sows is poorly understood.

#### *Virus replication in postnatal pigs*

After birth, the replication of the virus is mainly occurring in lymphoid tissues, but also in some epithelial/endothelial cells [81–83]. Upon immune stimulation during co-infections and upon vaccination, viral replication is activated due to the strong blastogenesis, leading to an increased number of susceptible lymphoblasts [84–86]. Curiously, the proportion of epithelial/endothelial cells displaying PCV2 replication is low [83] but probably represents a considerable absolute number when extrapolated to the whole organism. Cells of the monocytic lineage are taking up large amounts of viruses or viral debris but are rather resistant to a productive infection [75, 87]. However, genetic material is accumulated in these cells (monocytes, dendritic cells) for a long time and may cause a dysfunction of these cells [70, 88]. Histopathological changes, characterized by lymphocyte depletion and monocyte infiltration, is associated with a high level of virus replication [81]. Long-term replication of PCV2, as observed during PCV2-SD, is related with the absence of neutralizing antibodies [75, 89, 90]. However, the precise mechanisms by which a pig develops a PCV2-SI or a PCV2-SD are still to be fully elucidated. Different scenarios are suggested to explain the multifactorial nature of PCV2-SD, involving host related factors -immunity in particular-, co-infections, and environmental/managerial determinants, which play a role as potential disease triggering factors, plausibly in combination with viral strain features [3]. Viral load serves as a proxy for the interplay of these factors; PCV2-SI pigs usually have lower viral loads than diseased ones [91].

#### *Virus replication in sows*

Most sows are infected at a young age and are immune at first insemination. When naïve sows become infected at insemination with virus-positive spiked semen, as semen from naturally infected boars is probably too low to infect sows [92], or oronasally during gestation, the virus may replicate in the embryos/foetuses. As abovementioned, this may result in return-to-oestrus or the birth of mummies, stillborn foetuses and immune piglets, depending on the infection timing during gestation.

#### *Virus replication in boars*

Upon experimental infection, boars may shed PCV2 intermittently in their semen [92, 93]. Semen of naturally infected, seropositive boars may be PCV2 positive by PCR; however, it is not clear if the virus is infectious and may cause problems in sows afterwards [94, 95]. Apparently, boars do not develop clinical disease upon PCV2 infection, but one study associated PCV2 with boar reproductive tract lesions [96].

## Diagnosis of PCV2-associated diseases

### Diagnostic criteria

The diagnosis of PCV2-AD in an individual pig is relatively straightforward, with criteria established more than 25 years ago: compatible clinical signs, histopathological lesions in lymphoid tissues (postnatal disease) and foetal myocardium (reproductive disease), and detection of PCV2 genome or antigen within these microscopic lesions [97]. Diagnosing of PCV2-ADs on a herd level can be challenging as most pig herds worldwide are infected with PCV2 (and antibodies and viruses can be found in healthy and diseased pigs). The specific establishment of PCV2-SI should include the detection of the virus in pigs with no overt clinical signs but with decreased average daily weight gain (ADWG) [97]. Not confusing PCV2 detection with disease diagnosis is fundamental.

#### *Strategies to detect PCV2 genome by PCR*

Detection of PCV2 genome by real-time quantitative PCR (qPCR) is of wide use for various sample types (including serum, blood, oral fluid, lymph nodes, heart tissues from aborted foetuses, processing fluids and others). Although several studies have proposed viral genome titre cut-off thresholds to discriminate between subclinical infected and diseased pigs [2], absolute quantification may vary depending on the specific qPCR protocol [98]. Validation of these thresholds in different

diagnostic matrices has not been carried out with current commercially available techniques and, therefore, interpretation of qPCR results on these matrices across various age groups in a herd under PCV2 control program is challenging [99]. PCR pen site tests have been developed for several pathogens, but minimal efforts have been placed on PCV2 [100]. Coupling PCV2 detection by molecular methods with genome sequencing (Sanger or next generation sequencing) allows determining the genotypes involved in a particular infection. Although cross-protection among different PCV2 genotypes has been demonstrated [101], genotype-specific epitope differences have also been identified. It remains uncertain whether future immunization strategies will need to be tailored according to the genotype composition of the vaccine.

#### *PCV2 antibody detection*

PCV2 antibody testing is mostly performed using ELISA assays. Antibody detection tests allow assessing the prevalence of PCV2 exposure in a herd and may help monitoring vaccination responses, although this later issue depends on the vaccine type used, since not all of them cause evident seroconversion, especially considering moderate to high maternally derived antibody (MDA) levels at immunization time [102]. ELISA kits may vary regarding their plate coating since they use different antigen composition; therefore, their direct comparability is not possible. Importantly, ELISA tests do not discriminate between neutralizing and non-neutralizing antibodies, so, their results do not constitute correlates of protection against PCV2. Development of easy-to-perform, affordable PCV2 antibody neutralizing tests would be highly desirable to help demonstrating vaccine compliance. Routine vaccination can interfere with antibody diagnostics, since viremia can still occur in a proportion of animals and thus seropositivity should not be considered a proxy of absence of viral circulation. Barns and pig houses are often permanently contaminated with PCV2 [103] and getting PCV2 removed from a site can be challenging or impossible.

#### *Immunohistochemistry (IHC) and in situ hybridization (ISH)*

These assays have long been established as essential diagnostic tools for PCV2-ADs, particularly when combined with histopathological examination [104]. These methodologies enable the detection and localization of PCV2 antigen (IHC) and genome (ISH) within affected cells and tissues, thereby providing critical insights into viral distribution and tissue tropism. Such information is highly valuable for elucidating the role of PCV2 in lesion development and pathogenesis. However, the increasing reliance on faster and more cost-effective molecular techniques, such as quantitative PCR (qPCR), has progressively displaced histopathology and IHC/ISH in routine diagnostics. While qPCR provides sensitive and quantitative detection of viral genomes, its exclusive use may compromise the ability to establish a comprehensive diagnosis of PCV2-ADs, since it lacks the capacity to correlate viral presence with tissue lesions and cellular targets.

#### *Other PCV2 detection methods*

Electron microscopy has previously been used to detect PCV2. However, due to their complexity and cost today it is left as a complementary method and rarely used. Viral isolation is uncommon for PCV2, although it is a fundamental methodology with the purpose to produce viral inoculum for challenge studies.

## Prevention and control

Before the advent of vaccines, PCV2-AD control relied primarily on mitigating risk factors and management conditions that could trigger disease onset [105]. However, from 2006 onwards, the most efficient way of preventing PCV2-ADs worldwide has been through vaccination [106].

The available literature on PCV2 vaccines highlights their critical role in controlling PCV2-ADs. Six commercial PCV2 vaccines are available globally, initially targeting PCV2-SD and now also addressing other PCV2-AD manifestations. In addition, 10 different PCV2 vaccines, including inactivated, subunit, and vectored vaccines, have been developed and are applied in the Chinese market [106]. The efficacy of PCV2 vaccines has been widely studied. Vaccination has been associated with reduction in clinical signs, decreased viral load and improved growth performance and feed conversion rates in vaccinated animals [107, 108]. Safety profiles indicate that adverse reactions to PCV2 vaccines are minimal, with most side effects being mild and transient [106]. However, they do not protect against infection and transmission and allow for (silent) circulation of the virus [109] which in turn may influence the evolution of the virus [110].

PCV2 vaccines available on the global marked consists of traditional inactivated vaccines that contain killed classical or chimeric viruses (Circovac®, Ceva Animal Health; and Fostera™PCV, Zoetis Animal Health) or subunit vaccines developed using baculovirus expressed PCV2 Cap protein (Circoflex™PCV, Boehringer Ingelheim Animal Health; Circumvent® PCV G and Porcilis PCV®, MSD Animal Health; and Cirbloc® M Hyo, CEVA Animal Health) [106]. The subunit vaccines are typically available both as monovalent vaccines against PCV2 and as multivariant vaccines containing also *Mycoplasma hyopneumoniae* antigens. The claims on PCV2 efficacy are identical between monovalent and bivalent versions of

several PCV2 vaccines, fact also observed under experimental conditions [111].

These vaccines require an adjuvant to enhance the immune response, may require booster doses to maintain immunity and the effect may be reduced if piglets are vaccinated in the presence of MDA [102]. In addition to the standard intramuscular vaccinations, PCV2 vaccines have been licensed to be administered by needleless intradermal injection which benefit animal welfare, save resources and decrease the risk of iatrogenic transmission of pathogens between animals/pens [112–114]. Most of the available vaccines are based on the PCV2a genotype, but vaccines containing both genotype PCV2a and PCV2b and, more recently, PCV2d have been licenced. However, there is no convincing evidence that the genotype of the vaccine strain has a major impact on the vaccine efficacy.

The advancements in PCV2 vaccine development during recent years have focused on enhancing efficacy, broadening protection, and improving delivery systems. A wide range of diverse experimental vaccines, including attenuated, chimeric, and nucleic acid-based vaccines have shown promise in enhancing protective immunity; however, they remain at an early stage of development. New multi-epitope subunit vaccines targeting PCV2b variants showed high antibody titres in mice when using various adjuvants, indicating strong potential for further development [115]. Virus-Like Particles (VLPs) incorporating dominant T and B cell epitopes have demonstrated significant immunogenicity and protection in both piglets and mice, reducing viral loads and clinical signs [116]. With the detection of new circoviruses such as PCV3 and PCV4, approaches to develop multivariant vaccines have been launched. These multivalent vaccines combining capsid proteins from PCV2, PCV3, and PCV4 has been shown to induce protective immune responses, addressing the issue of PCV co-infections in swine [117]. Furthermore, more effective adjuvants are being explored to further boost vaccine efficacy especially promoting enhanced activation of the cell mediated immune system [106, 118, 119].

PCV2 exhibits high genetic variability raising concerns about the efficacy of existing vaccines derived from earlier strains [120, 121]. Despite this, current vaccines have demonstrated cross-protection against prevalent genotypes, suggesting that all PCV2 strains belong to a single serotype [101]. While the advancements in PCV2 vaccination are promising, ongoing research is necessary to address the challenges posed by evolving virus strains and to optimize vaccine strategies for effective disease control i.e. by using a combination of vaccines containing different virus strains/genotypes as documented for other vaccines against fast-evolving viruses [122].

While vaccination is a critical tool for controlling PCV2, integrated control strategies are essential for managing the disease effectively [123]. These strategies may include implementing strict biosecurity protocols to prevent the introduction and spread of PCV2 within and between farms. This includes managing the movement of pigs and equipment, controlling access to farms, and thorough sanitation practices [124]. Furthermore, optimization of pig housing, ensuring proper ventilation and density can reduce stress and disease susceptibility. PCV2 is known to have a relatively stable viral structure and can withstand elevated temperatures. PCV2 is also relatively stable across a range of pH levels, surviving in acidic and alkaline conditions and by that resist's standard disinfectants [125, 126]. Moreover, PCV2 is widely distributed in all parts of the infected herds, including areas where pigs have no access [127]. Thus, phase-out of vaccination in herds that test free of PCV2 even over an extended time may possess a high risk of reinfection [128] and is not presently recommended.

Vaccination against PCV2 has significantly advanced in recent years, leading to effective control of the disease in many swine populations. Continued research into the development of innovative vaccine formulations that can induce sterilizing immunity, combined with comprehensive herd management and biosecurity measures, remains vital to control and prevent PCV2 and its associated diseases. Future efforts should focus on maximizing vaccination efficacy, addressing emerging strains of the virus, and enhancing overall herd health to secure the swine industry against future losses caused by PCV2. A recent speculative review proposed the eventual elimination of PCV2 at local, regional or global scales by means of sustained mass vaccination including two immunizations in piglets, one in gilts at acclimation and twice a year in breeding stock [129].

## Major unresolved areas in PCV2 research

- The incomplete understanding of its infection pathogenesis, particularly the mechanisms by which the virus induces disease in a pig, or by which the ADWG decreases in some pigs but not in others.
- While most pigs infected with PCV2 remain subclinical, a non-characterized subset develops severe disease, suggesting that host immunity, genetic susceptibility, co-infections, and environmental stressors modulate disease expression. The precise role of immune modulation and virus-induced immune dysregulation in disease progression was the subject of many research initiatives before the advent of PCV2 vaccines [130], but due to the excellent vaccine protection this type of investigation almost disappeared after 2006-08.

- Another important research gap relates to the virus's genetic diversity and evolution. PCV2 is characterized by a high mutation rate for a DNA virus, leading to the emergence of multiple genotypes with different epidemiological relevance [33]. More studies are needed to monitor genetic shifts and to understand the biological consequences of viral diversity, particularly regarding cross-protection between genotypes and the possible emergence of new, more virulent strains.
- The role of maternal immunity and its interference with vaccination efficacy also remains insufficiently understood. MDA can interfere with vaccine-induced immunity in piglets [131]; at present, it cannot be ruled out if such effect leads to suboptimal protection and variability in vaccine responses with existing products in the market. Identifying optimal vaccination schedules and understanding the kinetics of MDA decline are crucial for enhancing herd-level immunity.
- While current commercial vaccines significantly reduce clinical disease and viral shedding, current vaccines do not prevent virus replication or infections of pigs completely [106]. This allows PCV2 to persist and circulate silently in vaccinated populations, potentially creating the conditions for the emergence of vaccine escape variants [14, 110], raising the need for more effective or sterilizing vaccines.
- In this latter context, the development of next-generation vaccines, including novel delivery methods (e.g., intranasal or oral) and broader cross-protective formulations, should be a high-priority research direction.
- There is a lack of harmonized, standardized diagnostic tools and protocols for quantifying viral load and assessing vaccine efficacy under field conditions. This hampers the comparability of research outcomes and limits the ability to implement effective surveillance programs.

## Conclusions

PCV2 remains one of the most economically important pathogens in swine production, and although vaccination has drastically reduced the burden of PCV2-ADs, several knowledge gaps continue to challenge both research and control strategies. Critical questions remain regarding the interplay between host immunity, co-infections, and management factors in disease expression, as well as the long-term evolutionary dynamics of viral genotypes under widespread vaccine pressure. Moreover, the lack of harmonized diagnostic criteria and standardized tools across regions limits the comparability of surveillance data and constrains the assessment of true vaccine effectiveness in diverse epidemiological contexts.

From a broader perspective, addressing these gaps requires a coordinated agenda that integrates fundamental research, translational approaches, and eventually policy-level initiatives. Priority should be given to the development of next-generation vaccines that enhance cross-protection, reduce further viral replication and shedding, and ideally move towards sterilizing immunity. In parallel, investment in international surveillance networks and harmonized diagnostic platforms would be essential for tracking viral evolution and detecting potential vaccine breakthroughs. Finally, fostering public–private partnerships and aligning research efforts with the needs of veterinary practitioners and the swine industry will ensure that scientific advances translate effectively into field-level impact. Only through such a holistic approach can the global swine sector move from control towards a potential long-term goal of PCV2 elimination.

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