VIRULENCE
2024, VOL. 15, NO. 1, 2439524
https://doi.org/10.1080/21505594.2024.2439524

Taylor & Francis
Taylor & Francis Group

REVIEW

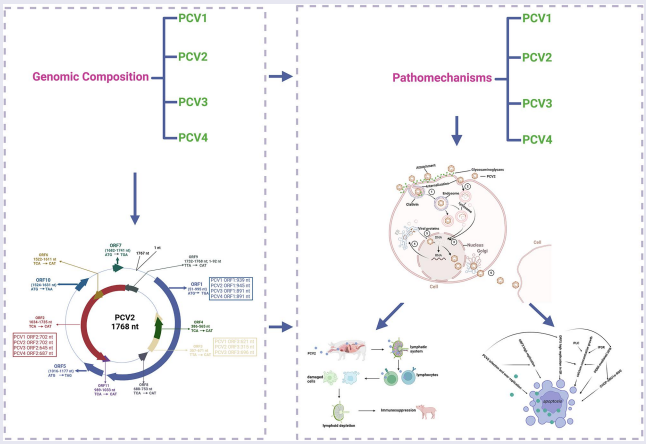
Genomic composition and pathomechanisms of porcine circoviruses: A review

Yong-Yu Gao[a], Qian Wang[b], Han-Wen Li[c], Shuang Zhang[a], Jian Zhao[d], Di Bao[a], Han Zhao[a], Kai Wang[a], Gui-Xue Hu[a], and Feng-Shan Gao [e,f]

[a]College of Animal Medicine, Jilin Agricultural University, Changchun, China; [b]The Third Affiliated Hospital of Changchun University of Traditional Chinese Medicine, Changchun, Jilin, China; [c]College of Life Sciences, Nankai University, Tianjing, China; [d]ChangChun Sino Biotechnology CO. LTD, Changchun, Jilin, China; [e]College of Life and Health, Dalian University, Dalian, China; [f]The Dalian Animal Virus Antigen Epitope Screening and Protein Engineering Drug Developing Key Laboratory, Dalian, China

ABSTRACT

Porcine circovirus (PCV) belongs to the genus *Circovirus* within the family *Circoviridae*; it has the smallest genome and a complicated classification system comprising PCV1, PCV2, PCV3, and PCV4. Most types of these viruses can cause animals to develop serious diseases; in pigs in particular, it may manifest as postweaning multisystemic wasting syndrome (PMWS), reproductive failure, porcine dermatitis and nephropathy syndrome (PDNS), congenital tremors (CTs), proliferative and necrotizing pneumonia (PNP), lymphoid injury, and immunosuppression. Different types of PCVs cause different types of diseases and sometimes feature no pathogenicity; these various PCV types are associated with different pathomechanisms in animals. In this review, the genomic composition and systemic pathomechanisms of porcine circoviruses are introduced, and future research prospects are discussed.



ARTICLE HISTORY
Received 23 February 2024
Revised 1 November 2024
Accepted 3 December 2024

KEYWORDS
Porcine circovirus; genomic composition; pathomechanisms; lymphoid injury; autophagy

Introduction

Porcine circovirus (PCV) is a non-enveloped, single-stranded, circular DNA virus with icosahedral symmetry. When the virus replicates, a double-stranded DNA transition occurs and a loose circular superhelix molecule is formed [1–3]. In most cases, this is a single-stranded DNA molecule, and its genome is very small (approximately 2000 nucleotides [nt]), making it one of the smallest animal viruses found thus far [4]. The virus was first discovered in porcine kidney epithelial cells PK-15 by Tischer et al. in 1974 [5], but it was considered to be a cellular pollutant due to the limited conditions of that time. In 1982, this cellular pollutant was identified as a virion and named porcine circovirus [6]. In 1998, Ellis et al. [7] isolated a different virus from PK-15 cell culture contaminants derived from pigs with post-weaning multisystemic wasting syndrome (PMWS), which can cause disease in pigs. In the same year, Meehan et al. [8] first proposed that PCV should be divided into PCV1 and PCV2; of these two types, PCV2 can cause PMWS in weaned piglets. In 2016, Phan et al. [9] discovered a new type of porcine circovirus, PCV3, in diseased tissues. In 2020, Zhang et al. [10] first reported a novel porcine circovirus in

CONTACT Gui-Xue Hu huguixue901103@163.com; Feng-Shan Gao gfsh0626@126.com
Supplemental data for this article can be accessed online at https://doi.org/10.1080/21505594.2024.2439524

Hunan, China. Its genome is similar to that of the mink circovirus and can cause enteritis, porcine dermatitis, and nephropathy syndrome (PDNS). This virus was tentatively named PCV4. PCV is currently divided into four species: PCV1, PCV2, PCV3, and PCV4 [10]. Among these species, PCV1 is non-pathogenic. PCV2 causes a series of symptoms such as PMWS, PDNS, granulomatous enteritis, and reproductive disorders in sows, which may lead to immunosuppression and piglet death, thereby causing great harm to the pig industry [11]. PCV3 is a newly discovered porcine circovirus. Although its detailed pathomechanism is not completely clear, it has been reported that PCV3 is related to PDNS, respiratory diseases, diarrhoea, and reproductive failure [12,13]. The pathomechanism of PCV4 has not yet been elucidated. The pathomechanism of PCV is related to the genomic composition of the virus [14–16].

Genomic composition of PCV

Porcine circovirus belongs to the family *Circoviridae* and genus *Circovirus*. It comprises single-stranded circular DNA with no envelope, icosahedral symmetry [17], and an average diameter of 17 nm [18]. It is one of the smallest known animal viruses [4]. The genomic compositions and sizes of PCV1-PCV4 are different.

PCV1

The whole genome of PCV1 comprises 1758–1760 nucleotides (nt) [19,20]. PCV1 contains seven open reading frames (ORFs), of which ORF1 and ORF2 are the two largest [21]. ORF1 is located on the sense strand and is 939 nt in size [22]. It mainly encodes the replication (Rep) protein with a predicted size of 312 amino acids [23], which is involved in viral replication. Rep proteins have glycosylation modification sites at positions 20–22 [24]. The product of the spliced Rep transcript is termed Rep′ and has 168 amino acids. Rep and Rep′ together comprise the functional replication initiator factor of PCV1 [23]. The genome length of ORF2 is 702 or 705 nt, and it is located on the anti-sense strand of the viral genome and encodes viral capsid protein (Cap). The Cap protein is composed of 233 or 234 amino acids and is the main immunogenic protein of PCV1 [22,25]. PCV1 ORF3 has a genome length of 621 bp and a molecular weight of 23.2 kDa and encodes 206 amino acids. It has been reported that the first 104 amino acids of PCV1 ORF3 contain a domain capable of inducing cell death, whereas the C terminus of PCV1 ORF3 contains a domain possibly responsible for enhancing cell death under laboratory conditions [26]. The functions of PCV1 ORF4–ORF7 have not yet been reported in detail.

PCV2

Most genomes of PCV2 are between 1766 and 1768 nt in length [27,28] and encompass several gene subtypes, but a minority have a length of 1777 [29], 1778 [30], or 1779 nt [31]. In 2008, PCV2 was divided into three genotypes by the European Union Porcine Circovirus Committee: PCV2a, PCV2b, and PCV2c. In 2009, Wang et al. [32] classified PCV2 strains in China into five genotypes: PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e, a relatively well-accepted genotype classification for PCV2. PCV2 has since been further divided into eight genotypes: PCV2a–2 h [33]. Recently, a novel genotype, PCV2i, was identified in the United States, but whether PCV2i should be considered a new genotype warrants further studies of its epidemiology and pathogenesis studies, including the use of more sequences in the analysis [34].

PCV2 contains 11 ORFs, of which ORF1, ORF5, ORF7, and ORF10 are all located on the sense strand of the viral genome, and the other seven ORFs are located on the anti-sense strand. ORF2 and ORF3 are the main open reading frames (ORFs), as shown in Figure 1.

The full-length 945 nt ORF1 encodes 314 amino acids and translates into full-length replicase Rep proteins with a molecular weight of approximately 35.8 kDa and is then spliced into a frame-shifted version Rep' (178 aa) [35], which is essential for initiating PCV2 replication [36]. ORF1 is located in a sense strand direction and is close to the starting site of the PCV2 genome. Rep consists of ORF1 and contains three N-glycosylation sites. The first site is located at amino acids 23–25 (NPS), the second site is located at amino acids 256–258 (DQT), and the third site is located at amino acids 286–288 (DAT). Among these, a glycosylation site mutation in the first two sites can reduce viral replication, and a mutation in the third site can promote viral replication [37]. The Rep protein contains three conserved motifs and is the starting site for rolling-loop replication; it has a P-loop structure containing dNTP. These structural changes lead to changes in virus replication [38]. The Rep protein cannot initiate porcine circovirus replication alone but interacts with Rep' to initiate viral replication.

ORF2 at a full length of 702, 705, 708, or 717 nt encodes 233, 234, 235, or 238 amino acids, respectively [39–41]. This gene mainly encodes the virus capsid protein (Cap), with a molecular weight of approximately 30 kDa [42], located on the anti-sense strand

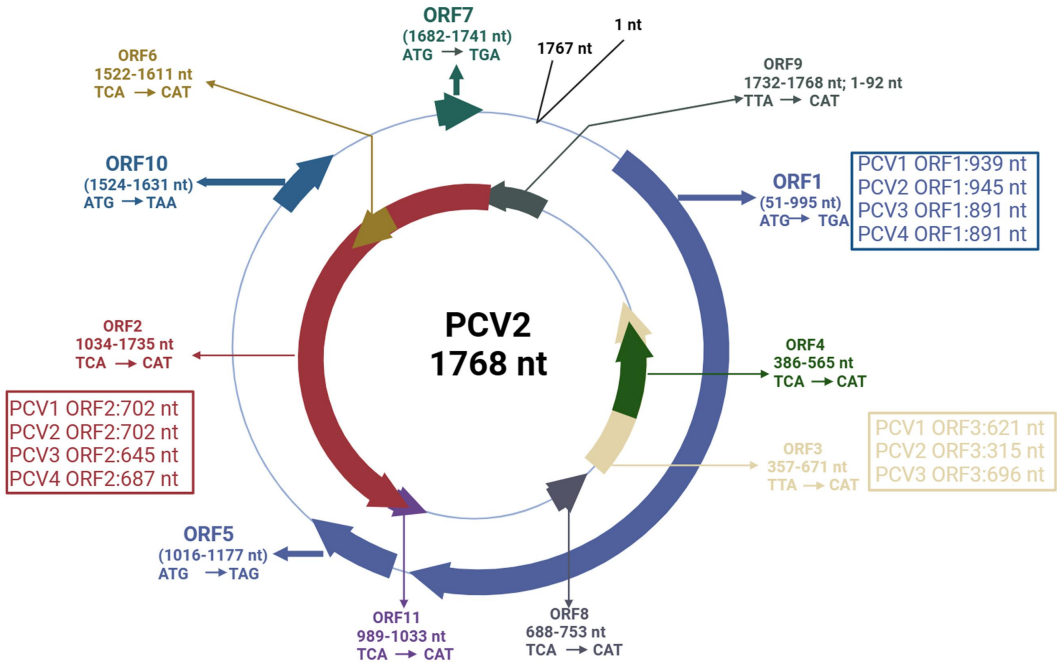


Figure 1. The genome structure of PCV2 based on accession no. AF027217 and the corresponding genome of PCV1, PCV3, and PCV4. (taken from the studies carried out in China by Dai Zhenjiang and Zeng Zhiyong et al., and Wang et al. [58], and slightly modified). Blue, orange, and yellow boxes show different sizes of ORF1, ORF2, and ORF3 for different types of PCV species.

of DNA, and can recognize the virus receptor of host cells and is closely related to viral infections. This protein can be used to develop vaccines and detect the presence of target proteins using serological methods. The amino terminal of the Cap protein is an arginine-rich region that is highly conserved and related to the nuclear localization of the protein. The Cap protein has a glycosylation site located at amino acids 143–145 of the PCV2 Cap [43]. Recently, some researchers have suggested that changes in the Cap protein gene may affect the pathogenicity of the virus. For example, mutation of proline (P) to alanine (A) at position 110 and arginine (R) to serine (S) at position 119 of the Cap protein increases the replication ability of the virus in cells and decreases its pathogenicity in animals [44].

ORF3 is located in the ORF1 region of the virus genome, but its transcription direction is opposite to that of ORF1. The genome length of PCV2 ORF3 is 315 nt, encoding 104 amino acids with a molecular weight of 11.9 kDa [35]. Studies have found that ORF3 is not related to viral replication but is instead related to virus-induced apoptosis [45,46]. Some researchers have speculated that ORF3 is not the only factor that determines viral virulence. Fenaux et al. [47] chimerized the Cap genes along with their ORF3 of PCV1 and PCV2 to construct four strains. Through experiments on pigs, it was found that all strains showed a positive conversion of PCV2; however, the animals did not show any clinical symptoms of PCV2, indicating that neither the ORF3-encoded protein nor the Cap protein is the sole pathogenic factor of PCV2 [44,48]. Other pathogenic factors may also exist within the PCV2 genome.

ORF4 is located in the interior of ORF3, and its transcription direction is the same as that of ORF3. It is 180 nt in size and encodes a protein (59 aa) with a molecular weight of 6.5 kDa [49]. Studies have shown that ORF4 is not necessary for virus replication [49], but when it is deleted with ORF3, the virus cannot replicate normally [45]. This may be due to the fact that both ORF3 and ORF4 are located in ORF1, and the common absence of ORF3 and ORF4 affects the variable cutting function of ORF1 mRNA. He et al. [49] have shown that the protein encoded by ORF4 can inhibit the activity of caspase and the apoptosis of PK-15 cells and has an antagonistic function with ORF3, suggesting that ORF4 May be related to the pathogenicity of the virus. They further confirmed that the ORF4 gene of PCV2 is unrelated to viral replication in infected PK-15 cells but can inhibit the activities of Caspase-3 and Caspase-8 and regulate the number of $CD4^+$ T and $CD8^+$ T cells during PCV2 infection, resulting in a decrease in the numbers of these two types of cellular immune T cells. ORF5 is located on the sense strand of PCV2 in the same

direction as ORF1. The nucleotide comprises 162 or 180 nt and encodes 53 or 59 amino acids with a molecular weight of approximately 6.2–6.6 kDa [24,50]. ORF5 and ORF11 are located between ORF1 and ORF2 and encode short-chain peptides. Lv et al. [51] confirmed that when PCV2 invades cells, ORF5 begins to be expressed at both transcriptional and translational levels. Point mutations have confirmed that ORF5 protein is a necessary factor in the process of infection and replication of PCV2. Lv et al. also confirmed that ORF5 is expressed in the endoplasmic reticulum of the cell and subsequently degraded by the proteasome. ORF5 inhibits the proliferation of porcine alveolar macrophages and prolongs the S phase of the cell cycle. ORF5 protein can also induce endoplasmic reticulum stress and activate nuclear factor kappa-B (NF-κB), leading to the upregulation of interleukin-6 (IL-6) and IL-8. Therefore, ORF5 is likely involved in PCV2 replication [51]. ORF6 is 90 nt in size, encodes 29 amino acids, has a molecular weight of 3.1 kDa, and participates in viral replication [24,52]. ORF7 is only 60 nt in size, encodes 19 amino acids, and has a molecular weight of 1.9 kDa; it also participates in virus replication [24]. The size of ORF8 is 66 nt. It encodes 21 amino acids with a molecular weight of 2.3 kDa and participates in virus replication [24,53]. ORF9 is located on the anti-sense strand of the PCV2 genome and encodes in the same direction as ORF2 and partially overlaps with ORF2. The size of ORF9 is 129 nt, and it encodes 42 amino acids with a molecular weight of 4.6 kDa [24]. The function of ORF9 is still unclear. The size of ORF10 is 108 nt, and it encodes 35 amino acids with a molecular weight of 4.1 kDa. This gene is not involved in viral replication and pathogenicity, and no other functions have been attributed to it [24]. The size of ORF11 is 45 nt, and it encodes 14 amino acids with a molecular weight of 1.8 kDa. It is the smallest coding protein of PCV2. ORF11 is related to virulence, and the deletion of this gene can weaken this virulence [24].

PCV3

With a genome size of 2000 nt and a GC content of 50%, PCV3 is one of the smallest viruses known to replicate in mammals [13]. Based on phylogenetic tree analysis, Chinese PCV3 has been categorized into three different subgroups: 3a–3c. Among these, 3a and American PCV3 reference strains previously occupied the same evolutionary branch [54]. However, there is still some debate about the classification of PCV3 [55,56]. The PCV3 genome contains three main ORFs, ORF1, ORF2, and ORF3. ORF1 and ORF3 are located in the sense strand direction of the genome, whereas ORF2 is located in the anti-sense strand direction of the genome [9]. The genomic size of ORF1 is 891 nt, and it encodes 296 amino acids and a replicase-related protein (Rep) [9]. The starting codon is GTC. The Rep protein contains a P-ring motif (P-loop) that binds to dNTP, and three conserved motifs are involved in the rolling loop replication of PCV3: I (FTINN), II (HLQG), and III (YCKK) [13]. The starting point of the rolling-ring replication of PCV3 was between ORF1 and ORF2. At nucleotide 235 of the genome, the 5' ends of the Rep and Cap genes have a conservative neck-and-loop structure composed of nine nucleotides (TAGTATTAC), also known as a stem-loop. Its sequence is identical to that of the PCV1 stem-loop but has only 55% homology with the PCV2 Cap gene [13]. The genome size of ORF2 is 645 nt. The start codon is ATG, which encodes a 214 amino acid capsid protein (Cap). It is an intracellular protein with no signal peptide and contains two glycosylation sites (S91 and S95) [13]. It is then transcribed and translated using viral DNA. The Cap protein is related to the immunogenicity of PCV3 and is the main structural protein of PCV3. However, the homology between PCV3 and PCV2 Cap proteins is very limited at only 37% [9], and they do not have any homology in terms of Cap protein epitopes, indicating that they do not feature cross-protection. The genome length of ORF3 is 696 nt, and it encodes 231 amino acids; its starting codon is TCG and its transcription direction is the same as that of ORF1 [13]. However, the function of the ORF3 protein remains unclear.

PCV4

The gene size of PCV4 is 1770 nt. It was shown by an ORF finder search (http://wheatomics.sdau.edu.cn/sms2/orf_find.html) to contain two ORFs. ORF1 is located in the sense strand direction, with a genome size of 891 nt, and encodes a protein of 296 amino acids, also known as the Rep protein. ORF2 is located on the anti-sense strand, and its genome size is 687 nt, encoding a protein of 228 amino acids called the Cap protein. The start codon of both ORFs in PCV4 is ATG [57,58]. Similar to other PCVs, there is a highly conserved 11-nucleotide sequence (TTCAGTATTAC) for rolling loop replication at the 5' end of the intergenic region of the PCV4 Rep and Cap proteins, and there is a 16-nucleotide reverse complementary sequence [10,59] on both sides of the motif. Nguyen et al. [57] confirmed that there are three motifs at the N-terminus of the PCV4 Rep protein: FT[LI]NN, PHLQG, and YC[Sx]K. These three motifs function as endonucleases and belong to the endonuclease domain. The

C-terminus of the Rep protein contains helicase domains of GxxxxGKS (dNTP-binding site), DDF, and ITSN. These two domains are highly conserved among different viruses [38]. In addition, it is predicted that the N-terminus of the Cap protein should have a nuclear localization signal (NLS) and a tyrosine motif, which are related to network protein-mediated endocytosis [57]. Recently, the NLS has been identified at amino acid residues 4–37 of the N-terminus of the PCV4 Cap, i.e. at two fragments (NLS-A and NLS-B). The α-Helixes formed by the NLS-A determines the nuclear localization of the Cap protein [60].

Pathomechanisms of PCVs

Pathomechanism of PCV1

PCV1 was first detected in a porcine kidney cell line (PK-15) and has generally been considered non-pathogenic [61,62]. Although PCV1 has no obvious pathogenicity, previous studies have shown that it can cause pathology in the lungs of porcine foetuses under experimental conditions [62]. In addition, results from clinical sample analyses have shown that co-infection of PCV1 with other PCVs [63–65] and other pathogens [66] is widespread. Interestingly, it has been reported that mixed infection with PCV1 and PCV2 generally results in a recombinant strain, but its origin is up for debate [67]. PCV1 has been shown to infect human liver cancer cells under laboratory conditions, which raises concerns for the vaccine safety of PCV1 growth in human hepatocellular carcinoma cells [68,69]. Other studies have shown that, although PCV1 DNA and PCV1 particles have been discovered at all stages of the production of a human rotavirus vaccine, there was no immunological evidence that any infants became infected [70–72], suggesting that PCV1 has so far been non-pathogenic to humans. Additionally, it has been reported that the transformed PCV1 ORF3 can induce cell-specific apoptosis [26], but further studies revealed that PCV1 does not appear to be cytopathogenic for cultured cells or for infected pigs [73]. In summary, there is still no evidence that PCV1 can cause clinical diseases in pigs and humans.

Pathomechanism of PCV2

PCV2 can cause serious diseases in pigs, including PMWS in weaned piglets, reproductive failure, PDNS, congenital tremors (CTs), and proliferative and necrotizing pneumonia (PNP); this is related to the pathomechanism of PCV2. The pathomechanism of PCV2 involves several aspects.

Receptor-mediated PCV2 infection into host cells

If PCV2 infection initiates the occurrence of disease, the first step of PCV2 infection in hosts is the attachment to the surface of susceptible cells. What causes this attachment to the susceptible cell's surface? Misinzo et al. [74] first reported that glycosaminoglycans (GAGs), including heparan sulphate (HS) and chondroitin sulphate B (CS-B), could be PCV2 attachment receptors on host cells, because competition with soluble GAG and/or enzymatic GAG removal reduced PCV2 attachment to the cell surface receptors, and these two GAGs are largely linked to glycoproteins anchored in the plasma membrane of all cell types [75]. In addition, mutant CHO-derived cells lacking either HS or all GAGs showed significantly lower levels of PCV2 infection compared to wild-type CHO cells, indicating that GAGs are PCV2 receptors. They concluded that HS is a major PCV2 receptor compared to CS-B based on competitive inhibition of PCV2 infection, and with soluble GAG and enzymatic removal of GAG from the cell surface. Later, researchers further identified that dextran sulphate (8 kDa) has a higher affinity for PCV2 than heparin (12 kDa), chondroitin sulphate B (41 kDa), hyaluronic acid (1.6 MDa), and dextran (6 kDa). This indicates that polymers high in sulphate content are capable of competing with the PCV2-heparan and therefore have the potential to inhibit PCV2 infection [76]. The fact that PCV2 can still infect mutant CHO cells that do not express GAG indicates that, apart from HS and CS-B, other receptors and cellular surface molecules participate in virus binding and subsequent virus entry [74]. However, no other PCV2 attachment receptors have been identified on the surface of host cells. It should be noted that some compounds with low molecular weight, such as epigallocatechin gallate [77] and sulphated chitosan [78], have been reported to inhibit PCV2 attachment to host cell receptors, which indicates that they could act as anti-infective agents against circovirus. Analysis of the amino acid sequence of the PCV2 capsid protein revealed a putative heparin (an analogue of the heparan sulphate)-binding motif (^{98}IRKVKV^{103}). However, because the motif is not located on the surface of the capsid protein, its role as a binding region is questioned [75,79]. It is speculated that other more exposed regions with lysine and arginine residues may be more suitable targets [75]. Recently, Dhindwal et al. [76] further identified that the binding sites of PCV2 capsid protein to the attachment receptor should contain arginine, lysine, and polar amino acids, because mutation of these amino acids to alanine diminished the binding capacity.

The second step of PCV2 infection is the internalization and endocytosis of PCV particles [75]. Two distinct pathways have been reported: clathrin-mediated endocytosis in monocytic and dendritic cells, and caveola-, clathrin-, and dynamin-independent small GTPase-regulated pathways in epithelial cell lines of porcine kidney (PK-15), swine kidney (SK), and swine testicles [76]. The former pathway does not lead to a full infection, while the latter does [75]. It has been reported that some membrane proteoglycans undergo endosomal cycling and are therefore potential internalizing receptors [74,80]. Recently, Ouyang et al. [81] identified that chondroitin sulphate (CS) and dermatan sulphate (DS) could not only have an impact on the binding of the capsids of PCV2, but also play an important role in PCV2 internalization, which indicates that they are potential internalizing receptors. In addition, phosphacan is identified as one of the potential candidates for PCV2 internalization.

After internalization, PCV2 traffics through the endosome-lysosome pathway, with the viral genome escaping from the acidified endosome – lysosome of cells, leaving the viral capsids partially degraded in the lysosomes [82]. A bipartite NLS including NLS-A and B at the N terminus of the capsid protein has been implicated in the guiding of the nucleocapsid into the nucleus for genome replication [76,83]. The PCV2 replicase edited by ORF1 recruits the cellular machinery to initiate rolling circle replication of the PCV2 genome in the nucleus. The newly synthesized PCV2 capsid protein is transported into the nucleus, via its NLS, for genome encapsidation and assembly of infectious virions. The virions then egress from the cell to initiate another cycle of infection [76]. Thus, the PCV2 infection is over, and a series of pathogenic effects are stimulated. The total process of receptor-mediated PCV2 infection into host cells is shown in Figure 2.

Lymphoid depletion and immunosuppression

In the process of PCV2 infection, lymphoid depletion and histiocyte replacement in lymphoid tissue are characteristic injuries. The infection and replication of PCV2 in lymphoid tissue can destroy the structure of lymph nodes, leading to lymphoid depletion, which can then be replaced by tissue cells. However, the destruction of lymphoid follicles and leucopenia associated with PCV2 infection can lead to immunosuppression in pigs. It has been reported that in pigs infected with PCV2, the numbers of interfollicular dendritic cells (DCs), B cells, NK cells, γδ T cells, $CD4^+$ T cells, and $CD8^+$ T cells decrease with the decrease in lymphocytes; this phenomenon is then accompanied by an increase in the number of monocytes and granulocytes [2,69,84–87]. The degree of lymphoid depletion is related to the number of PCV2 antigens detected in infected tissues. Studies [84,85] have shown that PCV2 replication mainly occurs in bronchial and inguinal lymph nodes, tonsils, lungs, liver, kidneys, spleen, and thymus. In addition, the direct replication of the virus and the cleavage of infected cells in infected lymphoid tissue (as well as other indirect and unknown factors) are related to damage in infected pig lymphoid tissue (Figure 3). Understanding the precise mechanism of lymphoid depletion will help control the rate of infection with this virus in the future. He et al. [49] reported that a viral gene named ORF4 from PCV2, which is associated with the transcription and translation of the viral genome, might be related to the lymphoid tissue damage mechanism because ORF4-deficient PCV2 (PCV2 Delta) can cause a higher serum viral load (genomic copies) and more severe lymphoid tissue damage in the spleens of infected mice than in those infected with wild-type PCV2. Recently, Liu et al. [88] identified that a differentially expressed gene, CXCL13, can suppress lymphocyte apoptosis during PCV2 infection in Yorkshire × Landrace (YL) pigs, indicating that the downregulation of CXCL13 in animals could cause lymphoid depletion when PCV2 infection occurs. Further studies are needed to fully clarify the mechanism of lymphoid depletion associated with PCV2 infection causing immunosuppression.

Infection and apoptosis of PCV2

Currently, there is still a significant level of controversy regarding whether PCV2 infection can induce apoptosis. It has been confirmed that a protein encoded by ORF3 in the direction of the ORF1 anti-sense chain in PCV2-infected PK-15 cells can induce apoptosis through the Caspase-3 and Caspase-8 pathways [46], or by increasing the half-life of p53 by blocking ubiquitin-dependent proteasomal degradation of p53 [16]. Interestingly, ORF3 has been found to be truncated in non-pathogenic PCV1-infected cells [25]. Further research using infected mice has shown that the pathogenicity of an ORF3-deficient PCV2 mutant is significantly lower than that of a PCV2 wild strain [45]. In addition, abolishing the function of ORF3 significantly reduces the virulence of PCV2 in pig infection [89]. However, in an independent study using a large number of pigs, it was found that there was no significant difference in the average value of histological damage caused by PCV2 wild-type strain infection and a mutant infection causing the deletion of the ORF3 function. Additionally, no difference was found in the number of specific viral antigens in PCV2-infected

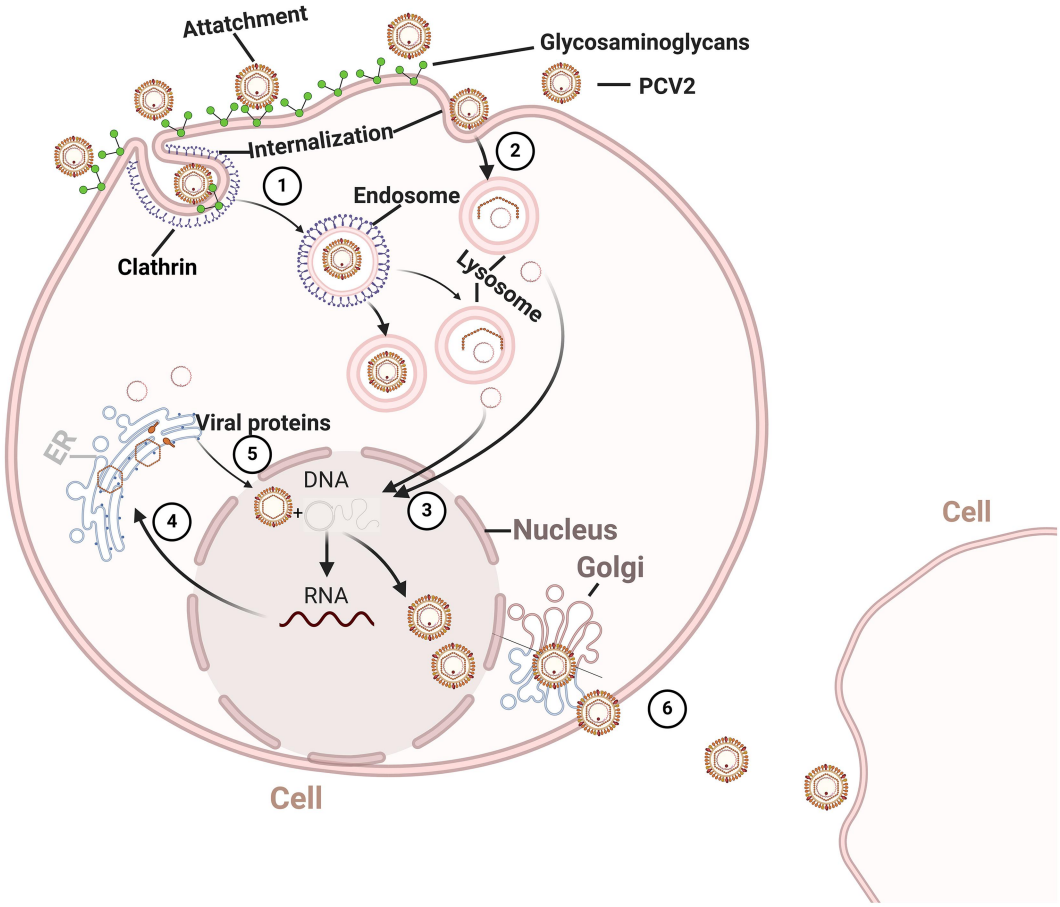


Figure 2. Schematic representation of receptor-mediated PCV2 infection to host cells (slightly modified from Nauwynck et al. [75]). (1) is indicated for the process of internalization via clathrin-mediated endocytosis in monocytic and dendritic cells. In this pathway, only part of PCV2 virions from endosome are uncoated and enter into the followed process of virus assembling and egress by through a lysosome pathway, in which a low pH environment favours in the uncoating for PCV2 virions; (2) is indicated for the process of internalization via caveola-, clathrin-, and dynamin-independent small GTPase-regulated pathways in epithelial cells. In this pathway, full of PCV2 virions enter into the lysosome and uncoated at a low pH, then full of them enter into the process of virus assembling and egress; (3) is indicated for the process that uncoated PCV2 DNA is transported into nucleus of host cells, then initiating the rolling circle replication and transcription of PCV2 genome in nucleus of host cells; (4) is indicated for the process that the transcriptional viral RNA is transported into cytoplasm and synthesized viral proteins in endoplasmic reticulum (ER); (5) is indicated for the process that the synthesized viral proteins are transported into nucleus for genome encapsidation and assembly of infectious virions; (6) is indicated for the process that assembled PCV2 virions egress from the cell to initiate another cycle of infection.

tissues [48]. Therefore, although ORF3 is optional for PCV2 replication, previous data do not fully support the relationship between ORF3 and PCV2-induced apoptosis and pathogenesis. Some studies have identified proapoptotic or apoptosis genes. For example, Shibahara found that the loss of B lymphocytes was related to apoptosis [90]. In addition, some researchers have found that macrophage-related apoptosis occurs in the liver of PCV2-infected mice, although mice are not natural hosts of PCV2 [91]. However, other studies have argued that apoptosis is also involved in the pathogenesis of PCV2. For example, it has been found that lymphoid tissue damage caused by PCV2 infection in pigs is not the result of apoptosis but instead is the result of reduced cell proliferation [92]. Similar studies have reported that apoptosis is not the main mechanism of lymphoid tissue injury or hepatocytopenia in pigs with PMWS [93]. Some scholars have compared pigs with PMWS and healthy pigs and found that the higher the viral load, the more serious the pathological changes and the lower the degree of autophagy in the tissues. The results of that study seem to contradict the view that apoptosis is caused by PCV2 [94]. Gao et al. constructed two mutants of PCV2 ORF4: the start codon mutant M1-PCV2 and the frameshift mutant M2-PCV2. These two mutants did not express ORF4 normally. When the two mutant and wild strains were infected with PK-15 cells, the results showed that both

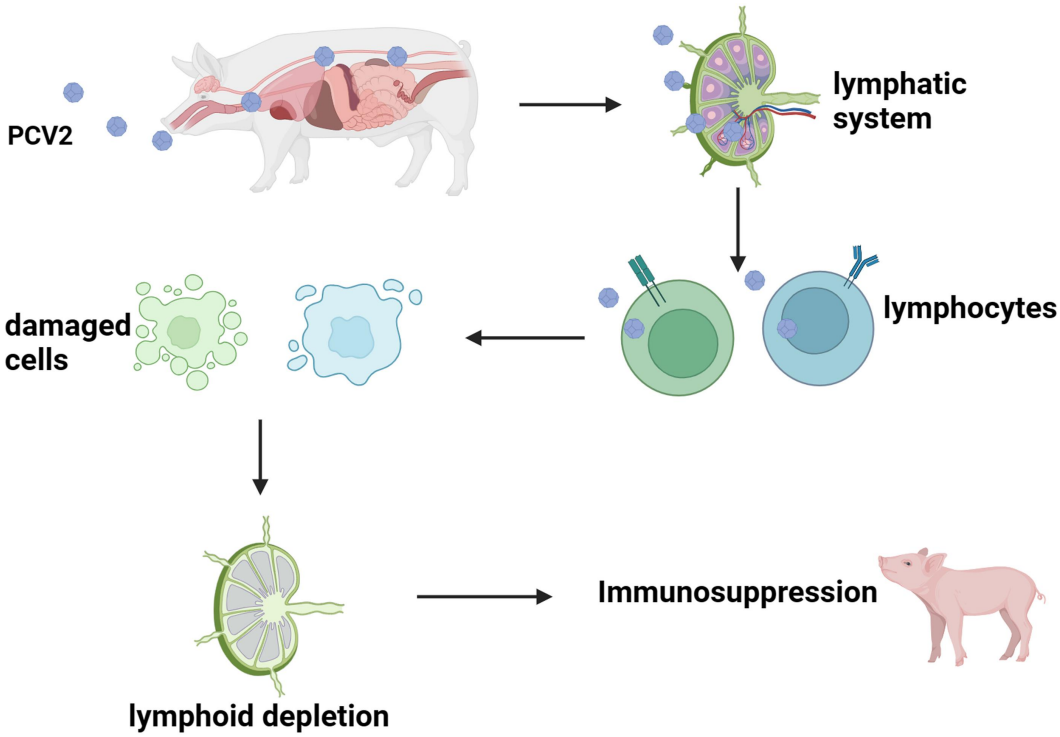


Figure 3. Mechanisms of lymphoid depletion and immunosuppression due to PCV2 infection. Blue dots indicate PCV2 particles.

ORF4-deficient strains could increase the level of ORF3 transcription and apoptosis, which indirectly indicated that ORF4 could inhibit ORF3 transcription and apoptosis induced by PCV2 [95]. Recently, Zhai et al. [96] found that the enhancement of PCV2 replication caused by oxidative stress is related to autophagy during apoptosis, which indirectly indicates that PCV2 is still related to apoptosis. In recent years, Wang et al. [97] studied the mechanism of apoptosis caused by PCV2 infection and found that PCV2 infection can cause endoplasmic reticulum stress, which is characterized by an increase in the concentration of calcium in the endoplasmic reticulum. It was further clarified that the mechanism of apoptosis was activated by phospholipase C (PLC) and the inositol 1,4,5-trisphosphate receptor (IP3R) pathway, thus elucidating the mechanism of apoptosis induced by PCV2 at the molecular level. Zhou et al. [98] further discovered that PCV2 infection increased the expression of the proapoptotic protein C/EBP homologous protein (CHOP) and ER oxidoreductase 1 α (ERO1α). They also identified that the inhibition of CHOP through RNA silencing or the inhibition of ERO1α through short hairpin RNA or EN460 causes the PCV2-induced generation of reactive oxygen species (ROS); it also represses cytosolic calcium levels and rates of apoptosis in PK-15 cells. The overexpression of ERO1α enhances PCV2-induced oxidative stress, caspase-3 cleavage, and apoptosis. Therefore, they hypothesized that PCV2 induces apoptosis through ER stress via CHOP – ERO1α–ROS signalling in host cells. In addition, researchers have reported that ORF5 protein, localized only in the ER, enhances the replication of PCV2 by inducing ER-associated stress, thereby leading to autophagy, protein kinase RNA-like endoplasmic reticulum kinase (PERK)-mediated unfolded protein response (UPR), and, ultimately, apoptosis caused by Ca^{2+} and ROS [2,99]. At present, although there is still controversy, the general consensus among an increasing number of scholars is that PCV2 can cause apoptosis in the later phases of infection and the over-replication of PCV2. Based on the above findings, we can speculate on the mechanisms of infection and apoptosis within PCV2 cells, as shown in Figure 4.

Genetic determinants of the virulence of PCV2

Industry scholars have long been concerned with which of the two genotypes of PCV2, PCV2a or PCV2b, is more likely to cause disease in pigs. Early studies have shown that in aseptic pigs inoculated with PCV2a and PCV2b strains, although the onset of PCV2b is earlier and the morbidity and mortality are higher for PCV2a (100% vs. 25%), the extent of lesions and the antigen

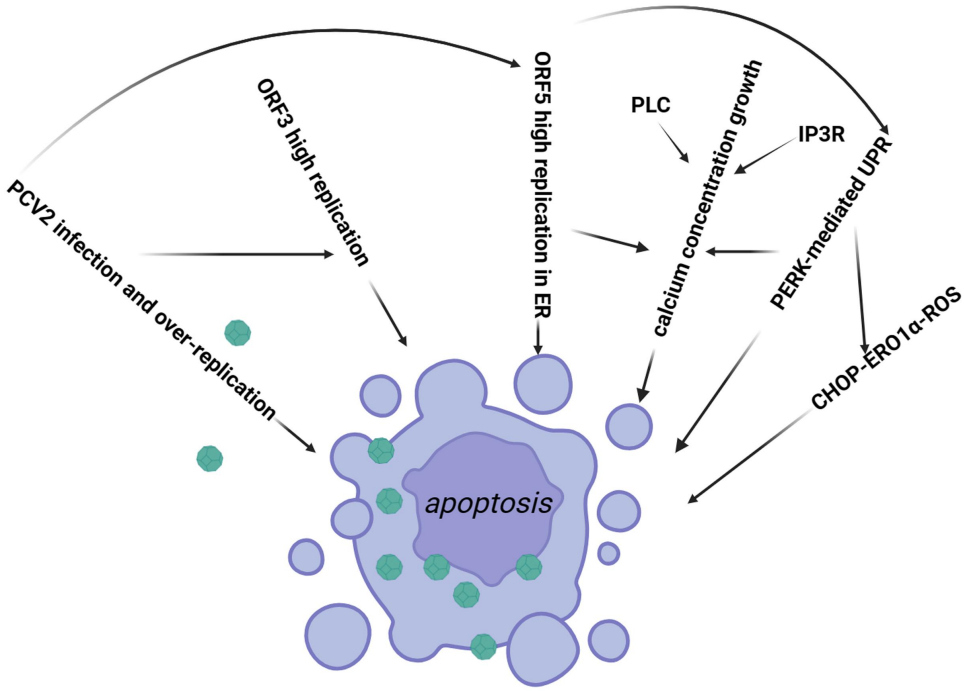


Figure 4. Mechanism of infection and apoptosis of PCV2.

CHOP, C/EBP homologous protein; ERO1α, ER oxidoreductase 1 α; PLC, phospholipase pathway; IP3R, inositol 1,4,5-trisphosphate receptor; PERK, protein kinase RNA-like endoplasmic reticulum kinase; ROS, reactive oxygen species; UPR, unfolded protein response. The green dots indicate PCV2 particles.

load are the same [100]. However, follow-up experiments have confirmed that there is no difference in viral virulence between traditionally raised pigs infected with PCV2a or PCV2b in the laboratory [101]. Therefore, the experimental data obtained thus far do not support a difference in virulence between the two genotypes of PCV2. This is probably due to the fact that the genotype of PCV2 has increasingly changed from PCV2b to porcine circovirus-associated disease (PCVAD) since 2003 [102]. Guo et al. [103] reported that a porcine circovirus type 2b (PCV2b) mutant with 234 amino acids in the capsid protein showed more virulence in vivo when compared with a classical PCV2a/2b strain with 233 amino acids, which indicates that the mutant amino acid site of 234 with lysine (K) could enhance the virulence of the mutant PCV2b strain in vivo. Recently, Oh et al. [104] further compared the differences in virulence among four PCV2 genotypes (2a, 2b, 2d, and 2e) in pigs solely infected with PCV2 and pigs dually infected with PCV2 and *Mycoplasma hyopneumoniae*. They found that, within the single-infection model, PCV2a, PCV2b, and PCV2d were more virulent than PCV2e, while significant differences in virulence were not found among the PCV2a, PCV2b, and PCV2d groups; within the dual-infection model, PCV2d was more virulent than the other three PCV2 genotypes. The results of this study revealed that *Mycoplasma hyopneumoniae* exacerbated the severity of PCV2-associated lymphoid lesions and increased the PCV2 load in the blood and lymph nodes, regardless of the PCV2 genotype. Suh et al. [105] also compared four PCV2 genotypes (2a, 2b, 2d, and 2e) with a single infection and co-infection with PCV2 as well as another swine pathogen: porcine reproductive and respiratory syndrome virus (PRRSV). They discovered that within the single-infection groups, PCV2a, PCV2b, and PCV2d exhibited similar virulence and were more virulent than PCV2e. However, within the dual-infection groups, the combination of PCV2d and PRRSV was more virulent than the other three PCV2 genotypes (2a, 2b, and 2e), each in combination with PRRSV. In addition, the authors suggested that genetic differences in ORF2 of PCV2 May affect the virulence of PCV2 genotypes.

There have been attempts to genetically analyse PCV2 isolates from recovered pigs inoculated with or without PCVAD to link the genetic determinants of the virus to PCVAD [106]. However, scientists have discovered another interesting phenomenon: it is easy to change amino acids in the protective epitopes of capsid proteins isolated from animals infected with PCV2 [107]. In addition, Fenaux et al. [108] found that changes in two amino acids of the capsid protein of PCV2a weakened the virulence of the virus. After 120

passages of PCV2a in porcine kidney cells, it was found that there were two amino acid mutations in the Cap gene: P110A, which appeared in the 30th generation and remained stable in the subsequent passage strain, and R191S, which appeared in the 120th generation. Since then, in vivo and in vitro experiments have confirmed that these two mutation sites can enhance the replication of PCV2 in pigs but weaken the virulence of the virus. This study revealed that the two mutation sites of the PCV2 capsid protein were related to the virulence of the virus. The relationship between the key amino acid epitopes and virulence was further confirmed by Krakowka et al. [109] In that study, genomes were amplified from frozen tissues infected with PCV2 (non-pathogenic) from 1970 to 1971. The ORF1 gene encoding replicase was homologous to the virulent strain PCV2. However, in the collection of the ORF2 gene of the virus, a virulent PCV2 strain, it was found to be between 1331 and 1339 (ACA-GGG-AAC). The corresponding sequence of the virulent PCV2 strain was GCC-ACA-GCC, indicating that the amino acid encoded by nucleotides at this position may be the key epitope determining the virulence of PCV2 [109]. However, to date, no further experiments have confirmed the correlation between the nine-nucleotide sequence and the virulence of PCV2. Wang et al. [110] identified the N-terminal 24RRR26 of Cap to be crucial for binding to p32, a key regulator for porcine circoviral nuclear egress, and mutation of these three arginine residues significantly weakened the replication and pathogenesis of PCV2 in vivo, which indicated that the N-terminal of Cap is crucial for the pathogenesis of PCV2. Recently, Zhang et al. [111] reported that the carboxyl terminus (CT) of the PCV2 Cap also plays a critical role in the evolution, pathogenesis, and proliferation of this virus. They further identified a conserved residue (227 K) in the CT, utilized for virus entry into cells, which indicated that the residue (227 K) might be the key amino acid determining the virulence of PCV2. In addition, Suh et al. [105] observed that there were five amino acid insertions in the CT of ORF2 of PCV2e compared to PCV2a or PCV2b with 233 amino acids, but only four amino acid insertions in PCV2d with 234 amino acids, which could result in a significant decrease in the virulence of the newly discovered genotype of PCV2e. This suggests that the CT of ORF2 in PCV2 dominates the virulence of PCV2.

Differences in the genetic sensitivity of different pigs to PCV2 infection

Genetic differences in pigs can affect the sensitivity of the virus to host infection. Differences between pig breeds and PCV2 and PCVAD infections have been reported previously. For example, Opriessnig et al. [112] reported that Landrace pigs were more susceptible to PCV2 infection than Duroc and Yorkshire pigs. In addition, some studies found that the degree of pathological changes in Landrace and Pietrain pigs infected with PCV2 was different, and the lymph node lesions in Landrace pigs infected with PCV2 were more serious than those in Pietrain pigs; the difference was statistically significant. The study also found that crossbred Yorkshire and Duroc pigs were more likely to be infected with PCV2 than purebred Pietrain pigs [113]. Studies have shown that under natural feeding conditions, the incidence of PCV2 infection in pig farms is affected by the genetic background of the pigs [114]. Therefore, the genetic characteristics of the host play an important role in the pathogenesis of PCV2 [84]. Li et al. [115] identified breed-dependent differences in porcine susceptibility to PCV2. They discovered that Yorkshire × Landrace (YL) pigs exhibited serious clinical features typifying PCV2 disease, whereas Laiwu (a Chinese indigenous pig breed, LW) pigs showed few clinical symptoms of the disease during PCV2 infection. They further explored the genetic mechanism underlying the difference in resistance to PCV2 infection in YL and LW pigs through an analysis of the changes that occurred in cytokines and some regulated genes within the serum after PCV2 infection. It was found that the serum levels of IL-4, IL-6, IL-8, IL-12, and TGF-β1 in LW pigs and tumour necrosis factor (TNF)-α in YL pigs increased significantly in the early stages of infection, while the levels of IL-10 and interferon (IFN)- γ in YL pigs were greatly increased at 35 days post infection (dpi). RNA-seq analysis identified four upregulated genes (TFPI, SERPNC1, SERPNA1, and SERPNA5) that were enriched in the complement and coagulation cascade pathways in PCV2-infected LW pigs. According to their results, the mRNA expression of SERPNA1, as well as the three genes TGF-β1, TGF-β2, and VEGF (which are regulated by SERPNA1), was significantly increased ($p < 0.05$). It was deduced that susceptibility to PCV2 infection depends on the genetic differences between LW and YL pigs, and SERPNA1 likely plays an important role in the resistance of LW pigs to PCV2 infection. Recently, this group further explored the genetic sensitivity of different pigs to PCV2 infection. They found that the mannose receptor C type 1 (MRC1), a key factor in regulating the body's immune response to resist pathogen invasions, might be associated with susceptibility to PCV2 based on genetic differences in pigs. They discovered that a 14 bp indel

polymorphism "GTTTTTTTTTTTTT" at site – 864 of porcine MRC1 promoter significantly influences gene transcriptional activity both prior to and post PCV2 infection, and the frequency of allele B (with the 14 bp insertion) is more prevalent in pigs resistant to PCV2 infection. Therefore, their discovery suggests that the 14 bp insertion polymorphism in the porcine MRC1 promoter is a potential DNA marker for PCV2 resistance in different breeds of pig [116]. In summary, elucidating the mechanisms of genetic resistance or sensitivity of hosts to PCV2 infection will help us control PCV2 infection and cultivate PCV2-resistant pig breeds in the future.

Immune enhancement of PCV2-related diseases

It is well known that PCV2 infection can cause immunosuppression. However, it is notable that immune stimulation in vitro can induce the development of PCV-related diseases [84]. Adjuvants such as Freund's adjuvant and azithrocyanin, either vaccinated or co-infected with other pathogens such as porcine parvovirus (PPV) and PRRSV, can aggravate PCV2-related diseases [84,117–119]. Other studies have shown that the stimulation of peripheral blood monocytes (PBMCs) with concanavalin A (ConA) can enhance the replication of PCV2 and activate the replication of PCV2 supported by T lymphocytes in vitro [120]. Further studies have shown that the mRNA expression of the PCV2 Cap gene is higher when proliferating or resting cells are stimulated by ConA or *Phytolacca americana* mitogen, suggesting that the enhancement of PCV2 replication does not depend on the mitotic stage of cells [121]. The immune enhancement exhibited by PCV2 may draw attention to other pathogenic immunization programs in pigs. Because PCV-related diseases are often found in healthy pigs, an interesting research question is whether multiple immunizations and vaccine-induced immune enhancement will have negative effects on pigs infected with PCV2 or PCV-related diseases. If so, how to mitigate this impact is another question that should be answered [69,122]. Recently, researchers have revealed that IL-10 is another immunoregulatory cytokine associated with the development of PCV2 infection with high expression. Because IL-10 has anti-inflammatory properties, including hampering excessive inflammation and creating allergic or autoimmune responses in the host, IL-10 might be associated with the immune enhancement of PCV2-related diseases [2]. However, to fully elucidate the mechanism of immune enhancement in PCV2-related diseases, more attention should be paid to this issue.

Co-infection with other pathogens

Previous studies have shown that PCV2 co-infection with other pathogens, such as pseudorabies virus (PRV), classical swine fever virus (CSFV), PRRSV, and porcine epidemic diarrhoea virus (PEDV), usually aggravates these diseases [105,123–125]. This begs the question: What is the secret or mechanism of these co-infections? Further studies have shown that PCV2 infection alone does not influence cell differentiation, antigen processing and presentation, or pro-inflammatory cytokine production in cultured DCs. Similarly, it does not affect IFN-α/tumour necrosis factor α (TNF-α)-induced DC maturation or major histocompatibility complex class II (MHC II) antigen-presenting protein and CD80/86 T-cell co-stimulatory molecule expression. However, once the virus co-infects cells with different pathogens, IFN-α and TNF-α expression is inhibited and the pathogens impair DC maturation, immune cell activation, and direct antiviral effects [2,126]. Zhou et al. [127] explored the synergistic pathogenesis mechanism induced by PRRSV and PCV2 co-infection by detecting the kinetic changes in immune regulatory molecules, inflammatory factors, and immune checkpoint molecules in porcine alveolar macrophages (PAMs) in individuals infected or co-infected with PRRSV and/or PCV2. This research indicated that PCV2 and PRRSV co-infection mainly promoted PRRSV replication, and then inhibited the immune regulatory molecules (IFN-alpha and IFN-gamma) and activated inflammatory factors (TNF-alpha, IL-1beta, IL-10, and TGF-beta) and immune checkpoint molecules (PD-1, LAG-3, CTLA-4, and TIM-3), enhancing the pulmonary lesions through dual infection with PCV2 and PRRSV in PAMs. Recently, Guo et al. [128] reported that PCV2 and Glaesserella parasuis serotype 4 (GPS4) co-infection can activate the TGF-beta and p38/MAPK signalling pathways in vivo and upregulate Snail1, a transcriptional repressor, ultimately down-regulating the expression of ZO-1 and Occludin. This elucidates how PCV2 infection promotes GPS4 to breach the tracheal epithelial barrier and aggravate clinical manifestations. In summary, PCV2 coinfection with other pathogens impairs the immune system of cells [2].

The pathomechanism of PCV3

Since its discovery in 2016, PCV3 has become widespread in many countries such as the United States, Poland, Germany and China [9,129–131]. Pigs infected with PCV3 mainly exhibit PDNS, reproductive disorders, and heart and multiple organ inflammation. The pathogenesis of PCV3 involves the following aspects.

Pathogenicity

Both PCV3 virion and genomic DNA are pathogenic and infective, and can increase the expression of pro-inflammatory cytokines (IL-6, IL-1 β, IFN-γ, IL-23 α, and TNF-α) and chemokine ligand 5 (CCL5) in infected piglets, indicating that the pathogenicity of PCV3 is not only related to PDNS but also to inflammation [12]. After PCV3 infection is initiated, DNA can be detected in almost all animal tissues and body fluids, including the heart, brain, kidneys, spleen, serum, saliva, nasal fluid, peritoneal fluid, pleural effusion, faeces, and semen. PCV3-specific antigens can also be detected in a variety of animal tissues and organs, including the skin, kidneys, lungs, heart, liver, small intestine, lymph nodes, and colostrum [132,133], which can cause myocarditis, nephritis, vasculitis, PDNS, reproductive failure, respiratory diseases, diarrhoea, and so on [61]. Therefore, PCV3 and PCV2 have similar extensive tissue tropisms, and it is speculated that PCV3 has a pathomechanism similar to that of PCV2.

Immunosuppression

The Cap protein of PCV3 is related to viral replication and may be related to PCV3-induced immunosuppression. Zhang et al. [134] found that the PCV3 Cap protein can inhibit DNA-induced IFN-β mRNA transcription and IFN promoter activation. In addition, Cap interacts with the GTPase-activating protein SH3 domain-binding protein 1 (G3BP1) and inhibits the interaction between G3BP1 and cyclic GMP-AMP (cGAMP) synthase (cGAS), suggesting that the interaction between Cap and G3BP1 prevents cGAS from recognizing DNA and inhibits IFN production. Recently, Shen et al. [135] also found that the PCV3 Cap protein can inhibit innate immunity mediated by IFN-β through the interaction between G3BP1 and the signal transducer and activator of transcription 2 (STAT2) protein, thereby leading to immunosuppression. This study also showed that the PCV3 virus can inhibit the host's natural antiviral immune response with the help of its Cap protein, allowing the PCV3 virus to escape. It appears that the immunosuppressive mechanism of PCV3 is different from that of PCV2 because of its different associated viral proteins and host cytokines. However, further studies are needed to fully elucidate the mechanism of PCV3 immunosuppression.

PCV3 infection and autophagy

Geng et al. [136] were the first to study autophagy induced by PCV3. In this study, the recombinant plasmid pCMV-Cap and lentivirus LV-Cap-encoding Cap protein of PCV3 were constructed and transfected into HEK293T cells. Western blotting, laser confocal microscopy, and transmission electron microscopy showed for the first time that the PCV3 Cap protein could induce autophagy by inhibiting the phosphorylation of the mammalian target of rapamycin (mTOR). Although both PCV2 and PCV3 can cause autophagy, the mechanisms involved are different because of the different associated proteins and signalling pathways. There are still few reports on this topic; therefore, further studies are needed to confirm whether PCV3 Cap can induce autophagy or whether other proteins are also associated with autophagy caused by PCV3 infection.

PCV3 immune stimulation

The promotion or induction of other pathogens in the development of disease is related to immune stimulation, which has been shown to be an important part of the pathogenesis of PCV2-related PMWS. Therefore, whether the pathogenesis of PCV3 is also related to immune stimulation requires further discussion. Jiang et al. [12] constructed PCV3 infectious clones using a virus rescue technique. They inoculated 4- and 8-week-old pigs using these PCV3 infectious clones combined with immune stimulator keyhole limpet haemocyanin (KLH). The results showed that both immunization methods could lead to similar symptoms of PDNS in pigs, resulting in a progressive increase in the viral load in the serum and various tissues. The two inoculation methods also significantly upregulated IL-1 β, IL-6, IL-23 α, IFN-γ, TNF-α, and CCL5 in piglets. Although KLH did not show a significant increase in the infectious cloning of PCV3, in general, the addition of KLH immunostimulants was proven to increase the survival rate of animals. However, to date, research has only been conducted at the experimental level, with no reports from the field. In addition, to reveal whether there are other factors associated with immune stimulation in PCV3, further studies must be conducted.

Recently, it has been reported that PCV3 can also cause inflammatory responses, which are activated by the hypoxia-inducible factor-1 pathway, glycolysis, and the AGE/RAGE pathway, using reverse genetics approaches. This has made the inflammatory pathomechanism of PCV3 clearer [137]. Generally speaking, because the discovery of PCV3 was relatively late compared to the discovery of PCV2, its corresponding pathomechanism is still the subject of continuous debate, exploration, and research.

The pathomechanism of PCV4

PCV4 can also cause serious diseases in pigs, including respiratory diseases, diarrhoea, and skin lesions,

suggesting that pigs may develop PDNS [138]. Autopsies have shown that the interlobular septum becomes moderately swollen, the space is filled with transparent exudate, and the lung surface may be dotted with multifocal, variously dark areas [10]. Recently, Zheng et al. [60] identified that the amino acid ^{4}RSRY^{7} in the NLS of the PCV4 Cap was a critical motif affecting the packaging of virus-like particles (VLPs), and could be used for elucidating the infection mechanism of PCV4 and developing subunit vaccines based on VLPs. However, the pathomechanism of PCV4 is still not fully elucidated.

Conclusions and prospects

In summary, we systematically uncovered the genomic composition (including each ORF's function) of four PCVs to understand and elucidate their pathomechanisms. However, not all of the functions of the ORFs in the genomes of these four PCVs are completely understood, which hinders our understanding of the PCVs' pathomechanisms. This provides us with a direction for future work. It can be concluded that co-infection with other pathogens is the main cause of PCVAD from PCV2 or even PCV3 and that the pathomechanisms of co-infection with other pathogens should be investigated in greater depth. At present, research on the pathomechanisms of PCVs predominantly focuses on PCV2, whereas research on the pathomechanisms of other types of PCVs needs further improvement. For example, we must question whether there is a gene exchange between PCV1, PCV2, and PCV3, leading to the production of new strains and PCVAD, and whether PCV3 and PCV4 co-infect with other pathogens. These problems should be further investigated to provide better guidance for the prevention and control of PCV.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by the Natural Science Foundation of Jilin Province, China [grant no. 20230101257JC] and the Project for Scientific Technology Development in Jilin Province [grant no. 20200402055NC].

Author contributions

Conceptualization: Y.Y.G. and F.S.G.; writing – original draft preparation: Y.Y.G. and F.S.G.; writing – review and editing: Y.Y.G., Q.W., H.W.L., S.Z., J.Z., D.B., and H.Z.; supervision: K.W., G.X.H., and F.S.G. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ORCID

Feng-Shan Gao http://orcid.org/0000-0003-1646-5227

References

[1] Tischer I, Buhk HJ. Viral DNA from cells infected with porcine circovirus. Zentralbl Bakteriol Mikrobiol Hyg A: Med Microbiol Infect Dis Virol Parasitol. 1988;270 (1–2):280–287. doi: 10.1016/S0176-6724(88)80164-0

[2] Feher E, Jakab F, Banyai K. Mechanisms of circovirus immunosuppression and pathogenesis with a focus on porcine circovirus 2: a review. Vet Q. 2023;43(1):1–18. doi: 10.1080/01652176.2023.2234430

[3] Gao YY, Wang Q, Zhang S, et al. Establishment and preliminary application of duplex fluorescence quantitative PCR for porcine circoviruses type 2 and type 3. Heliyon. 2024;10(11):e31779. doi: 10.1016/j.heliyon.2024.e31779

[4] Huang Y, Chen X, Long Y, et al. Epidemiological analysis from 2018 to 2020 in China and prevention strategy of porcine circovirus type 2. Front Vet Sci. 2021;8:753297. doi: 10.3389/fvets.2021.753297

[5] Tischer I, Rasch R, Tochtermann G. Characterization of papovavirus-and picornavirus-like particles in permanent pig kidney cell lines. Zentralbl Bakteriol Orig A. 1974;226(2):153–167. Available from: https://www.ncbi.nlm.nih.gov/pubmed/4151202

[6] Tischer I, Gelderblom H, Vettermann W, et al. A very small porcine virus with circular single-stranded DNA. Nature. 1982;295(5844):64–66. doi: 10.1038/295064a0

[7] Ellis J, Hassard L, Clark E, et al. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J. 1998;39(1):44–51.

[8] Meehan BM, Jewhurst F, Kennedy D, et al. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. J Gen Virol. 1998;79 (9):2171–2179. doi: 10.1099/0022-1317-79-9-2171

[9] Phan TG, Giannitti F, Rossow S, et al. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virol J. 2016;13(1):184. doi: 10.1186/s12985-016-0642-z

[10] Zhang HH, Hu WQ, Li JY, et al. Novel circovirus species identified in farmed pigs designated as porcine circovirus 4, Hunan province, China. Transbound Emerg Dis. 2020;67(3):1057–1061. doi: 10.1111/tbed.13446

[11] Rodriguez-Carino C, Sanchez-Chardi A, Segales J. Subcellular immunolocalization of porcine circovirus type 2 (PCV2) in lymph nodes from pigs with post-weaning multisystemic wasting syndrome

(PMWS). J Comp Pathol. 2010;142(4):291–299. doi: 10.1016/j.jcpa.2009.12.001

[12] Jiang H, Wang D, Wang J, et al. Induction of porcine dermatitis and nephropathy syndrome in piglets by infection with porcine circovirus type 3. J Virol. 2019;93(4):e02045–18. doi: 10.1128/JVI.02045-18

[13] Palinski R, Pineyro P, Shang P, et al. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol. 2017;91(1):e01879–16. doi: 10.1128/JVI.01879-16

[14] Zhan Y, Yu W, Cai X, et al. The carboxyl terminus of the porcine circovirus type 2 capsid protein is critical to virus-like particle assembly, cell entry, and propagation. J Virol. 2020;94(9). doi: 10.1128/JVI.00042-20

[15] Sirisereewan C, Nguyen TC, Piewbang C, et al. Molecular detection and genetic characterization of porcine circovirus 4 (PCV4) in Thailand during 2019–2020. Sci Rep. 2023;13(1):5168. doi: 10.1038/s41598-023-32382-1

[16] Park SW, Park IB, Kang SJ, et al. Interaction between host cell proteins and open reading frames of porcine circovirus type 2. J Anim Sci Technol. 2023;65(4):698–719. doi: 10.5187/jast.2023.e67

[17] Neira V, Ramos N, Tapia R, et al. Genetic analysis of porcine circovirus type 2 from pigs affected with PMWS in Chile reveals intergenotypic recombination. Virol J. 2017;14(1):191. doi: 10.1186/s12985-017-0850-1

[18] Bian H, Yu C, Wei Y, et al. Purification of porcine circovirus type 2 using an affinity chromatography based on a neutralizing monoclonal antibody against viral capsid protein. Pathogens. 2021;10(12):10. doi: 10.3390/pathogens10121564

[19] Tan CY, Opaskornkul K, Thanawongnuwech R, et al. First molecular detection and complete sequence analysis of porcine circovirus type 3 (PCV3) in Peninsular Malaysia. PLOS ONE. 2020;15(7):e0235832. doi: 10.1371/journal.pone.0235832

[20] Meehan BM, Creelan JL, McNulty MS, et al. Sequence of porcine circovirus DNA: affinities with plant circoviruses. J Gen Virol. 1997;78(1):221–227. doi: 10.1099/0022-1317-78-1-221

[21] Allan G, Krakowka S, Ellis J, et al. Discovery and evolving history of two genetically related but phenotypically different viruses, porcine circoviruses 1 and 2. Virus Res. 2012;164(1–2):4–9. doi: 10.1016/j.virusres.2011.09.013

[22] Feng H, Segales J, Wang F, et al. Comprehensive analysis of codon usage patterns in Chinese porcine circoviruses based on their major protein-coding sequences. Viruses. 2022;14(1):14. doi: 10.3390/v14010081

[23] Mankertz A, Hillenbrand B. Replication of porcine circovirus type 1 requires two proteins encoded by the viral rep gene. Virology. 2001;279(2):429–438. doi: 10.1006/viro.2000.0730

[24] Hamel AL, Lin LL, Nayar GP. Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. J Virol. 1998;72(6):5262–5267. doi: 10.1128/JVI.72.6.5262-5267.1998

[25] Mankertz A, Caliskan R, Hattermann K, et al. Molecular biology of porcine circovirus: analyses of gene expression and viral replication. Vet Microbiol. 2004;98(2):81–88. doi: 10.1016/j.vetmic.2003.10.014

[26] Chaiyakul M, Hsu K, Dardari R, et al. Cytotoxicity of ORF3 proteins from a nonpathogenic and a pathogenic porcine circovirus. J Virol. 2010;84(21):11440–11447. doi: 10.1128/JVI.01030-10

[27] Shang SB, Jin YL, Jiang XT, et al. Fine mapping of antigenic epitopes on capsid proteins of porcine circovirus, and antigenic phenotype of porcine circovirus type 2. Mol Immunol. 2009;46(3):327–334. doi: 10.1016/j.molimm.2008.10.028

[28] Somashekara S, Ravishankar C, Ravindran R, et al. Molecular characterization and phylogenetic analysis of porcine circovirus 2 from Kerala, India. Virusdisease. 2023;34(2):331–338. doi: 10.1007/s13337-023-00814-1

[29] Wei C, Lin Z, Dai A, et al. Emergence of a novel recombinant porcine circovirus type 2 in China: PCV2c and PCV2d recombinant. Transbound Emerg Dis. 2019;66(6):2496–2506. doi: 10.1111/tbed.13307

[30] de Boisseson C, Beven V, Bigarre L, et al. Molecular characterization of porcine circovirus type 2 isolates from post-weaning multisystemic wasting syndrome-affected and non-affected pigs. J Gen Virol. 2004;85(2):293–304. doi: 10.1099/vir.0.19536-0

[31] Cai L, Han X, Hu D, et al. A novel porcine circovirus type 2a strain, 10JS–2, with eleven-nucleotide insertions in the origin of genome replication. J Virol. 2012;86(12):7017. doi: 10.1128/JVI.00760-12

[32] Wang F, Guo X, Ge X, et al. Genetic variation analysis of Chinese strains of porcine circovirus type 2. Virus Res. 2009;145(1):151–156. doi: 10.1016/j.virusres.2009.05.015

[33] Franzo G, Segales J, Sola C. Porcine circovirus 2 (PCV-2) genotype update and proposal of a new genotyping methodology. PLOS ONE. 2018;13(12):e0208585. doi: 10.1371/journal.pone.0208585

[34] Wang Y, Noll L, Lu N, et al. Genetic diversity and prevalence of porcine circovirus type 3 (PCV3) and type 2 (PCV2) in the Midwest of the USA during 2016–2018. Transbound Emerg Dis. 2020;67(3):1284–1294. doi: 10.1111/tbed.13467

[35] Lv QZ, Guo KK, Zhang YM. Current understanding of genomic DNA of porcine circovirus type 2. Virus Genes. 2014;49(1):1–10. doi: 10.1007/s11262-014-1099-z

[36] Mankertz A, Mueller B, Steinfeldt T, et al. New reporter gene-based replication assay reveals exchangeability of replication factors of porcine circovirus types 1 and 2. J Virol. 2003;77(18):9885–9893. doi: 10.1128/JVI.77.18.9885-9893.2003

[37] Shi J, Peng Z, Fu F, et al. Mutant Rep protein of the porcine circovirus type 2 N-glycosylation: 23–25aa, 256–258aa mutation reduced virus replication but 286–288aa mutation enhanced virus replication in PK-15 cells. Vet Microbiol. 2015;177(3–4):370–372. doi: 10.1016/j.vetmic.2015.03.016

[38] Faurez F, Dory D, Grasland B, et al. Replication of porcine circoviruses. Virol J. 2009;6(1):60. doi: 10.1186/1743-422X-6-60

[39] Liu J, Wei C, Dai A, et al. Detection of PCV2e strains in Southeast China. PeerJ. 2018;6:e4476. doi: 10.7717/peerj.4476

[40] Davies B, Wang X, Dvorak CM, et al. Diagnostic phylogenetics reveals a new porcine circovirus 2 cluster. Virus Res. 2016;217:32–37. doi: 10.1016/j.virusres.2016.02.010

[41] Guo LJ, Lu YH, Wei YW, et al. Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. Virol J. 2010;7(1):273. doi: 10.1186/1743-422X-7-273

[42] Nawagitgul P, Morozov I, Bolin SR, et al. Open reading frame 2 of porcine circovirus type 2 encodes a major capsid protein. J Gen Virol. 2000;81(9):2281–2287. doi: 10.1099/0022-1317-81-9-2281

[43] Gu J, Cao R, Zhang Y, et al. Deletion of the single putative N-glycosylation site of the porcine circovirus type 2 cap protein enhances specific immune responses by DNA immunisation in mice. Vet J. 2012;192(3):385–389. doi: 10.1016/j.tvjl.2011.08.005

[44] Fenaux M, Opriessnig T, Halbur PG, et al. 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. 2004;78(12):6297–6303. doi: 10.1128/JVI.78.12.6297-6303.2004

[45] Liu J, Chen I, Du Q, et al. The ORF3 protein of porcine circovirus type 2 is involved in viral pathogenesis in vivo. J Virol. 2006;80(10):5065–5073. doi: 10.1128/JVI.80.10.5065-5073.2006

[46] Liu J, Chen I, Kwang J. Characterization of a previously unidentified viral protein in porcine circovirus type 2-infected cells and its role in virus-induced apoptosis. J Virol. 2005;79(13):8262–8274. doi: 10.1128/JVI.79.13.8262-8274.2005

[47] Fenaux M, Opriessnig T, Halbur PG, et al. Immunogenicity and pathogenicity of chimeric infectious DNA clones of pathogenic porcine circovirus type 2 (PCV2) and nonpathogenic PCV1 in weanling pigs. J Virol. 2003;77(20):11232–11243. doi: 10.1128/JVI.77.20.11232-11243.2003

[48] Juhan NM, LeRoith T, Opriessnig T, et al. 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. 2010;147(1):60–66. doi: 10.1016/j.virusres.2009.10.007

[49] He J, Cao J, Zhou N, et al. Identification and functional analysis of the novel ORF4 protein encoded by porcine circovirus type 2. J Virol. 2013;87(3):1420–1429. doi: 10.1128/JVI.01443-12

[50] Choi CY, Choi YC, Park IB, et al. The ORF5 protein of porcine circovirus type 2 enhances viral replication by dampening type I interferon expression in porcine epithelial cells. Vet Microbiol. 2018;226:50–58. doi: 10.1016/j.vetmic.2018.10.005

[51] Lv Q, Guo K, Xu H, et al. Identification of putative ORF5 protein of porcine circovirus type 2 and functional analysis of GFP-Fused ORF5 protein. PLOS ONE. 2015;10(6):e0127859. doi: 10.1371/journal.pone.0127859

[52] Li D, Wang J, Xu S, et al. Identification and functional analysis of the novel ORF6 protein of porcine circovirus type 2 in vitro. Vet Res Commun. 2018;42(1):1–10. doi: 10.1007/s11259-017-9702-0

[53] Choi J, Stevenson GW, Kiupel M, et al. Sequence analysis of old and new strains of porcine circovirus associated with congenital tremors in pigs and their comparison with strains involved with postweaning multisystemic wasting syndrome. Can J Vet Res. 2002;66(4):217–224.

[54] Li G, Wang H, Wang S, et al. Insights into the genetic and host adaptability of emerging porcine circovirus 3. Virulence. 2018;9(1):1301–1313. doi: 10.1080/21505594.2018.1492863

[55] Franzo G, Segales J. Porcine circovirus 3 (PCV-3) variability: Is it in the virus or in the classification criteria? Virol J. 2023;20(1):26. doi: 10.1186/s12985-023-01984-6

[56] Franzo G, Delwart E, Fux R, et al. Genotyping porcine circovirus 3 (PCV-3) nowadays: does it make sense? Viruses. 2020;12(3):12. doi: 10.3390/v12030265

[57] Nguyen VG, Do HQ, Huynh TM, et al. Molecular-based detection, genetic characterization and phylogenetic analysis of porcine circovirus 4 from Korean domestic swine farms. Transbound Emerg Dis. 2022;69(2):538–548. doi: 10.1111/tbed.14017

[58] Wang D, Mai J, Yang Y, et al. Current knowledge on epidemiology and evolution of novel porcine circovirus 4. Vet Res. 2022;53(1):38. doi: 10.1186/s13567-022-01053-w

[59] Sun W, Du Q, Han Z, et al. Detection and genetic characterization of porcine circovirus 4 (PCV4) in Guangxi, China. Gene. 2021;773:145384. doi: 10.1016/j.gene.2020.145384

[60] Zheng J, Li N, Li X, et al. The nuclear localization signal of porcine circovirus type 4 affects the subcellular localization of the virus capsid and the production of virus-like particles. Int J Mol Sci. 2024;25(5):2459. doi: 10.3390/ijms25052459

[61] Sirisereewan C, Thanawongnuwech R, Kedkovid R. Current understanding of the pathogenesis of porcine circovirus 3. Pathogens. 2022;11(1):64. doi: 10.3390/pathogens11010064

[62] Saha D, Lefebvre DJ, Ducatelle R, et al. Outcome of experimental porcine circovirus type 1 infections in mid-gestational porcine foetuses. BMC Vet Res. 2011;7(1):64. doi: 10.1186/1746-6148-7-64

[63] Yang K, Jiao Z, Zhou D, et al. Development of a multiplex PCR to detect and discriminate porcine circoviruses in clinical specimens. BMC Infect Dis. 2019;19(1):778. doi: 10.1186/s12879-019-4398-0

[64] Hu X, Chen Z, Li Y, et al. Detection of porcine circovirus 1/2/3 and genetic analysis of porcine circovirus 2 in wild boar from Jiangxi Province of China. Animals (Basel). 2022;12(16):12. doi: 10.3390/ani12162021

[65] Chen N, Xiao Y, Li X, et al. Development and application of a quadruplex real-time PCR assay for differential detection of porcine circoviruses (PCV1 to PCV4) in Jiangsu province of China from 2016 to 2020. Transbound Emerg Dis. 2021;68(3):1615–1624. doi: 10.1111/tbed.13833

[66] Chen Y, Shi K, Liu H, et al. Development of a multiplex qRT-pcr assay for detection of African swine fever virus, classical swine fever virus and porcine reproductive and respiratory syndrome virus. J Vet Sci. 2021;22(6):e87. doi: 10.4142/jvs.2021.22.e87

[67] Gagnon CA, Music N, Fontaine G, et al. Emergence of a new type of porcine circovirus in swine (PCV): a type 1 and type 2 PCV recombinant. Vet Microbiol. 2010;144(1–2):18–23. doi: 10.1016/j.vetmic.2009.09.072

[68] Beach NM, Cordoba L, Kenney SP, et al. Productive infection of human hepatocellular carcinoma cells by porcine circovirus type 1. Vaccine. 2011;29 (43):7303–7306. doi: 10.1016/j.vaccine.2011.06.097

[69] Meng XJ. Porcine circovirus type 2 (PCV2): pathogenesis and interaction with the immune system. Annu Rev Anim Biosci. 2013;1(1):43–64. doi: 10.1146/annurev-animal-031412-103720

[70] Opriessnig T, Karuppannan AK, Castro A, et al. Porcine circoviruses: current status, knowledge gaps and challenges. Virus Res. 2020;286:198044. doi: 10.1016/j.virusres.2020.198044

[71] Victoria JG, Wang C, Jones MS, et al. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. J Virol. 2010;84 (12):6033–6040. doi: 10.1128/JVI.02690-09

[72] Dubin G, Toussaint JF, Cassart JP, et al. Investigation of a regulatory agency enquiry into potential porcine circovirus type 1 contamination of the human rotavirus vaccine, Rotarix™. Hum Vaccin Immunother. 2013;9(11):2398–2408. doi: 10.4161/hv.25973

[73] Hough KP, Rogers AM, Zelic M, et al. Transformed cell-specific induction of apoptosis by porcine circovirus type 1 viral protein 3. J Gen Virol. 2015;96 (2):351–359. doi: 10.1099/vir.0.070284-0

[74] Misinzo G, Delputte PL, Meerts P, et al. Porcine circovirus 2 uses heparan sulfate and chondroitin sulfate B glycosaminoglycans as receptors for its attachment to host cells. J Virol. 2006;80(7):3487–3494. doi: 10.1128/JVI.80.7.3487-3494.2006

[75] Nauwynck HJ, Sanchez R, Meerts P, et al. Cell tropism and entry of porcine circovirus 2. Virus Res. 2012;164 (1–2):43–45. doi: 10.1016/j.virusres.2011.11.003

[76] Dhindwal S, Avila B, Feng S, et al. Porcine circovirus 2 uses a multitude of weak binding sites to interact with heparan sulfate, and the interactions do not follow the symmetry of the capsid. J Virol. 2019;93(6). doi: 10.1128/JVI.02222-18

[77] Li J, Song D, Wang S, et al. Antiviral effect of Epigallocatechin Gallate via impairing porcine circovirus type 2 attachment to host cell receptor. Viruses. 2020;12(2):12. doi: 10.3390/v12020176

[78] Jimenez-Arriagada D, Hidalgo AA, Neira V, et al. Low molecular weight sulfated chitosan efficiently reduces infection capacity of porcine circovirus type 2 (PCV2) in PK15 cells. Virol J. 2022;19(1):52. doi: 10.1186/s12985-022-01781-7

[79] Khayat R, Brunn N, Speir JA, et al. The 2.3-angstrom structure of porcine circovirus 2. J Virol. 2011;85 (15):7856–7862. doi: 10.1128/JVI.00737-11

[80] Bernfield M, Gotte M, Park PW, et al. Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem. 1999;68(1):729–777. doi: 10.1146/annurev.biochem.68.1.729

[81] Ouyang Y, Nauwynck HJ, Kibenge FSB. PCV2 uptake by porcine monocytes is strain-dependent and is associated with amino acid characteristics on the capsid surface. Microbiol Spectr. 2023;11(2):e0380522. doi: 10.1128/spectrum.03805-22

[82] Wei R, Van Renne N, Nauwynck HJ. Strain-dependent porcine circovirus type 2 (PCV2) entry and replication in T-Lymphoblasts. Viruses. 2019;11(9):11. doi: 10.3390/v11090813

[83] Wang N, Zhan Y, Wang A, et al. In silico analysis of surface structure variation of PCV2 capsid resulting from loop mutations of its capsid protein (Cap). J Gen Virol. 2016;97(12):3331–3344. doi: 10.1099/jgv.0.000634

[84] Ramamoorthy S, Meng XJ. Porcine circoviruses: a minuscule yet mammoth paradox. Anim Health Res Rev. 2009;10(1):1–20. doi: 10.1017/S1466252308001461

[85] Darwich L, Mateu E. Immunology of porcine circovirus type 2 (PCV2). Virus Res. 2012;164(1–2):61–67. doi: 10.1016/j.virusres.2011.12.003

[86] Darwich L, Segales J, Domingo M, et al. Changes in CD4(+), CD8(+), CD4(+) CD8(+), and immunoglobulin M-positive peripheral blood mononuclear cells of postweaning multisystemic wasting syndrome-affected pigs and age-matched uninfected wasted and healthy pigs correlate with lesions and porcine circovirus type 2 load in lymphoid tissues. Clin Diagn Lab Immunol. 2002;9(2):236–242. doi: 10.1128/cdli.9.2.236-242.2002

[87] Grierson SS, King DP, Tucker AW, et al. Ontogeny of systemic cellular immunity in the neonatal pig: correlation with the development of post-weaning multisystemic wasting syndrome. Vet Immunol Immunopathol. 2007;119(3–4):254–268. doi: 10.1016/j.vetimm.2007.06.004

[88] Liu G, Wang Y, Jiang S, et al. Suppression of lymphocyte apoptosis in spleen by CXCL13 after porcine circovirus type 2 infection and regulatory mechanism of CXCL13 expression in pigs. Vet Res. 2019;50(1):17. doi: 10.1186/s13567-019-0634-2

[89] Karuppannan AK, Liu S, Jia Q, et al. Porcine circovirus type 2 ORF3 protein competes with p53 in binding to Pirh2 and mediates the deregulation of p53 homeostasis. Virology. 2010;398(1):1–11. doi: 10.1016/j.virol.2009.11.028

[90] Shibahara T, Sato K, Ishikawa Y, et al. Porcine circovirus induces B lymphocyte depletion in pigs with wasting disease syndrome. J Vet Med Sci. 2000;62:1125–1131.

[91] Kiupel M, Stevenson GW, Choi J, et al. Viral replication and lesions in BALB/c mice experimentally inoculated with porcine circovirus isolated from a pig with postweaning multisystemic wasting disease. Vet Pathol. 2001;38(1):74–82. doi: 10.1354/vp.38-1-74

[92] Mandrioli L, Sarli G, Panarese S, et al. Apoptosis and proliferative activity in lymph node reaction in postweaning multisystemic wasting syndrome (PMWS). Vet Immunol Immunopathol. 2004;97(1–2):25–37. doi: 10.1016/j.vetimm.2003.08.017

[93] Krakowka S, Ellis J, McNeilly F, et al. Features of cell degeneration and death in hepatic failure and systemic lymphoid depletion characteristic of porcine circovirus-2–associated postweaning multisystemic wasting disease. Vet Pathol. 2004;41(5):471–481. doi: 10.1354/vp.41-5-471

[94] Resendes AR, Majo N, Segales J, et al. Apoptosis in lymphoid organs of pigs naturally infected by porcine circovirus type 2. J Gen Virol. 2004;85(10):2837–2844. doi: 10.1099/vir.0.80221-0

[95] Gao Z, Dong Q, Jiang Y, et al. ORF4-protein deficient PCV2 mutants enhance virus-induced apoptosis and show differential expression of mRNAs in vitro. Virus Res. 2014;183:56–62. doi: 10.1016/j.virusres.2014.01.024

[96] Zhai N, Liu K, Li H, et al. PCV2 replication promoted by oxidative stress is dependent on the regulation of autophagy on apoptosis. Vet Res. 2019;50(1):19. doi: 10.1186/s13567-019-0637-z

[97] Wang S, Li C, Sun P, et al. PCV2 triggers PK-15 cell apoptosis through the plc–IP3R–Ca2+ signaling pathway. Front Microbiol. 2021;12:674907. doi: 10.3389/fmicb.2021.674907

[98] Zhou Y, Zhou X, Dong W, et al. Porcine circovirus type 2 induces CHOP-ERO1α-ros-mediated apoptosis in PK-15 cells. Vet Microbiol. 2022;273:109548. doi: 10.1016/j.vetmic.2022.109548

[99] Lv J, Jiang Y, Feng Q, et al. Porcine circovirus type 2 ORF5 protein induces autophagy to promote viral replication via the perk-eIf2α-ATF4 and mTOR-ERK1/2-AMPK signaling pathways in PK-15 cells. Front Microbiol. 2020;11:320. doi: 10.3389/fmicb.2020.00320

[100] Lager KM, Gauger PC, Vincent AL, et al. Mortality in pigs given porcine circovirus type 2 subgroup 1 and 2 viruses derived from DNA clones. Vet Rec. 2007;161(12):428–429. doi: 10.1136/vr.161.12.428

[101] Opriessnig T, Ramamoorthy S, Madson DM, et al. Differences in virulence among porcine circovirus type 2 isolates are unrelated to cluster type 2a or 2b and prior infection provides heterologous protection. J Gen Virol. 2008;89(10):2482–2491. doi: 10.1099/vir.0.2008/001081-0

[102] Harding JC, Ellis JA, McIntosh KA, et al. Dual heterologous porcine circovirus genogroup 2a/2b infection induces severe disease in germ-free pigs. Vet Microbiol. 2010;145(3–4):209–219. doi: 10.1016/j.vetmic.2010.03.026

[103] Guo L, Fu Y, Wang Y, et al. A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strain. PLOS ONE. 2012;7(7):e41463. doi: 10.1371/journal.pone.0041463

[104] Oh T, Cho H, Suh J, et al. Virulence comparison of four porcine circovirus type 2 (PCV2) genotypes (2a, 2b, 2d and 2e) in pigs single-infected with PCV2 and pigs dual-infected with PCV2 and Mycoplasma hyopneumoniae. J Comp Pathol. 2022;199:88–98. doi: 10.1016/j.jcpa.2022.09.012

[105] Suh J, Oh T, Chae C. Virulence comparison of 4 porcine circovirus type 2 (PCV-2) genotypes: 2a, 2b, 2d, and 2e with a single infection and co-infection with PCV-2 and porcine reproductive and respiratory syndrome virus (PRRSV). Can J Vet Res. 2023;87(1):41–50.

[106] Larochelle R, Magar R, D'Allaire S. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus Res. 2002;90(1–2):101–112. doi: 10.1016/S0168-1702(02)00141-7

[107] Olvera A, Cortey M, Segales J. Molecular evolution of porcine circovirus type 2 genomes: phylogeny and clonality. Virology. 2007;357(2):175–185. doi: 10.1016/j.virol.2006.07.047

[108] Fenaux M, Opriessnig T, Halbur PG, et al. 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. 2004;78(24):13440–13446. doi: 10.1128/JVI.78.24.13440-13446.2004

[109] Krakowka S, Allan G, Ellis J, et al. A nine-base nucleotide sequence in the porcine circovirus type 2 (PCV2) nucleocapsid gene determines viral replication and virulence. Virus Res. 2012;164(1–2):90–99. doi: 10.1016/j.virusres.2011.10.027

[110] Wang T, Du Q, Niu Y, et al. Cellular p32 is a critical regulator of porcine circovirus type 2 nuclear egress. J Virol. 2019;93(23). doi: 10.1128/JVI.00979-19

[111] Zhan Y, Yu W, Cai X, et al. The carboxyl terminus of the porcine circovirus type 2 capsid protein is critical to virus-like particle assembly, cell entry, and propagation. J Virol. 2020;94(9):e00042–20. doi: 10.1128/JVI.00042-20

[112] Opriessnig T, Fenaux M, Thomas P, et al. Evidence of breed-dependent differences in susceptibility to porcine circovirus type-2-associated disease and lesions. Vet Pathol. 2006;43(3):281–293. doi: 10.1354/vp.43-3-281

[113] Opriessnig T, Patterson AR, Madson DM, et al. Difference in severity of porcine circovirus type two-induced pathological lesions between landrace and pietrain pigs. J Anim Sci. 2009;87(5):1582–1590. doi: 10.2527/jas.2008-1390

[114] Lopez-Soria S, Nofrarias M, Calsamiglia M, et al. Post-weaning multisystemic wasting syndrome (PMWS) clinical expression under field conditions is modulated by the pig genetic background. Vet Microbiol. 2011;149(3–4):352–357. doi: 10.1016/j.vetmic.2010.12.009

[115] Li Y, Liu H, Wang P, et al. RNA-Seq analysis reveals genes underlying different disease responses to porcine circovirus type 2 in pigs. PLOS ONE. 2016;11(5):e0155502. doi: 10.1371/journal.pone.0155502

[116] Liu H, Wang C, Liu G, et al. A 14 bp indel polymorphism in the promoter region is associated with different responses to porcine circovirus type 2 infection by regulating MRC1 transcription. Vet Immunol Immunopathol. 2021;234:110202. doi: 10.1016/j.vetimm.2021.110202

[117] Ladekjaer-Mikkelsen AS, Nielsen J, Stadejek T, et al. Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old piglets experimentally infected with porcine circovirus type 2 (PCV2). Vet Microbiol. 2002;89(2–3):97–114. doi: 10.1016/S0378-1135(02)00174-8

[118] Krakowka S, Ellis J, McNeilly F, et al. Mycoplasma hyopneumoniae bacterins and porcine circovirus type 2 (PCV2) infection: induction of postweaning multisystemic wasting syndrome (PMWS) in the gnotobiotic swine model of PCV2-associated disease. Can Vet J. 2007;48(7):716–724.

[119] Allan GM, McNeilly F, Kennedy S, et al. Immunostimulation, PCV-2 and PMWS. Vet Rec. 2000;147(6):170–171.
[120] Yu S, Vincent A, Opriessnig T, et al. Quantification of PCV2 capsid transcript in peripheral blood mononuclear cells (PBMCs) in vitro. Vet Microbiol. 2007;123 (1–3):34–42. doi: 10.1016/j.vetmic.2007.02.021
[121] Yu S, Halbur PG, Thacker E. Effect of porcine circovirus type 2 infection and replication on activated porcine peripheral blood mononuclear cells in vitro. Vet Immunol Immunopathol. 2009;127(3–4):350–356. doi: 10.1016/j.vetimm.2008.10.332
[122] Dunkelberger JR, Serao NVL, Weng Z, et al. Genomic regions associated with host response to porcine reproductive and respiratory syndrome vaccination and co-infection in nursery pigs. BMC Genomics. 2017;18 (1):865. doi: 10.1186/s12864-017-4182-8
[123] Zhang X, Shu X, Bai H, et al. Effect of porcine circovirus type 2 on the severity of lung and brain damage in piglets infected with porcine pseudorabies virus. Vet Microbiol. 2019;237:108394. doi: 10.1016/j.vetmic.2019.108394
[124] Zhang H, Shi H, Wei Y, et al. Impact of porcine circovirus type 2 on porcine epidemic diarrhea virus replication in the IPI-FX cell line depends on the order of infection. Front Microbiol. 2023;14:1162104. doi: 10.3389/fmicb.2023.1162104
[125] Deka D, Barman NN, Deka N, et al. Sero-epidemiology of porcine parvovirus, circovirus, and classical swine fever virus infections in India. Trop Anim Health Prod. 2021;53(1):180. doi: 10.1007/s11250-021-02587-5
[126] Ablasser A, Hur S. Regulation of cGAS- and rlr-mediated immunity to nucleic acids. Nat Immunol. 2020;21(1):17–29. doi: 10.1038/s41590-019-0556-1
[127] Cui Z, Zhou L, Hu X, et al. Immune molecules' mRNA expression in porcine alveolar macrophages Co-infected with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. Viruses. 2023;15(3):15. doi: 10.3390/v15030777
[128] Guo M, Zhang J, Wang Q, et al. Porcine circovirus type 2 and Glaesserella parasuis serotype 4 co-infection activates Snail1 to disrupt the intercellular junctions and facilitate bacteria translocation across the tracheal epithelium. Vet Microbiol. 2024;288:109954. doi: 10.1016/j.vetmic.2023.109954
[129] Stadejek T, Wozniak A, Milek D, et al. First detection of porcine circovirus type 3 on commercial pig farms in Poland. Transbound Emerg Dis. 2017;64 (5):1350–1353. doi: 10.1111/tbed.12672
[130] Prinz C, Stillfried M, Neubert LK, et al. Detection of PCV3 in German wild boars. Virol J. 2019;16(1):25. doi: 10.1186/s12985-019-1133-9
[131] Xu T, Zhang YH, Tian RB, et al. Prevalence and genetic analysis of porcine circovirus type 2 (PCV2) and type 3 (PCV3) between 2018 and 2020 in central China. Infect Genet Evol. 2021;94:105016. doi: 10.1016/j.meegid.2021.105016
[132] Ouyang T, Niu G, Liu X, et al. Recent progress on porcine circovirus type 3. Infect Genet Evol. 2019;73:227–233. doi: 10.1016/j.meegid.2019.05.009
[133] Kedkovid R, Woonwong Y, Arunorat J, et al. Porcine circovirus type 3 (PCV3) shedding in sow colostrum. Vet Microbiol. 2018;220:12–17. doi: 10.1016/j.vetmic.2018.04.032
[134] Zhang P, Shen H, Liu X, et al. Porcine circovirus type 3 cap inhibits type I interferon induction through interaction with G3BP1. Front Vet Sci. 2020;7:594438. doi: 10.3389/fvets.2020.594438
[135] Shen H, Liu X, Zhang P, et al. Porcine circovirus 3 Cap inhibits type I interferon signaling through interaction with STAT2. Virus Res. 2020;275:197804. doi: 10.1016/j.virusres.2019.197804
[136] Geng SC, Li XL, Fang WH. Porcine circovirus 3 capsid protein induces autophagy in HEK293T cells by inhibiting phosphorylation of the mammalian target of rapamycin. J Zhejiang Univ Sci B. 2020;21:560–570.
[137] Chang CC, Wu CY, Wu CM, et al. Cytotoxicity effect and transcriptome analysis of PCV3-infected cells revealed potential viral pathogenic mechanisms. Microb Pathog. 2024;192:106715. doi: 10.1016/j.micpath.2024.106715
[138] Niu G, Zhang X, Ji W, et al. Porcine circovirus 4 rescued from an infectious clone is replicable and pathogenic in vivo. Transbound Emerg Dis. 2022;69 (5):e1632–e41. doi: 10.1111/tbed.14498