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Review

Recent progress on porcine circovirus type 3

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ABSTRACT

Porcine circovirus 3 (PCV3) is a newly identified virus that belongs to the genus *Circovirus* in the family *Circoviridae*. Since the first identification of PCV3 in domestic swine in 2016 in the USA, exciting progress on PCV3 has emphasized the importance of the virus. The aim of this review is to present recent advances in the molecular characteristics, epidemiology, and pathogenesis of PCV3. The virus spreads widely throughout almost all tissues of pig and wild boar in various countries, with a gradual increase of the infection. PCV3 is a pathogen associated with porcine dermatitis and nephropathy syndrome (PDNS)-like clinical signs, reproductive failure, and cardiac and multiorgan inflammation. Furthermore, PCV3 has been detected in other animals and ticks, suggesting that PCV3 possesses cross-species transmission abilities and has an unexpectedly broad distribution and circulation in the wild, where these animals may serve as potential reservoirs for PCV3 and pose a threat to the swine industry or even to humans. Moreover, several detection methods, which can specifically detect PCV3 or differentiate PCV3 from the other viruses, are also reviewed. The present review provides updated knowledge on PCV3-related research. Identification of the prevailing strain of PCV3 and its reservoirs is essential for researchers to understand PCV3 infections and PCV3-related diseases.

1. Introduction

Porcine circoviruses (PCVs) are members of the genus *Circovirus* of the family *Circoviridae*, characterized as single-stranded circular DNA viruses enclosed in an icosahedral virion with an approximately 17 nm diameter (Ouyang et al., 2019; Ren et al., 2016). To date, three types of PCVs have been discovered, including porcine circovirus type 1 (PCV1), porcine circovirus type 2 (PCV2) and porcine circovirus type 3 (PCV3).

PCV1 was discovered in 1974 as a contaminant of a porcine kidney cell line (PK-15). PCV1 infection is common in pigs, and pigs can produce antibodies against this virus (Calsamiglia et al., 2002; Finsterbusch and Mankertz, 2009). PCV2 is the main pathogen of porcine circovirus diseases and porcine circovirus-associated diseases (PCVD/PCVAD), which present clinically as PCV2 systemic disease (PCV2-SD, also known as postweaning multisystemic wasting syndrome, PMWS), respiratory and enteric disease, and porcine dermatitis and nephropathy syndrome (PDNS), and reproductive failure (Ouyang et al., 2019; Ren et al., 2016). However, the exact mechanism of PCVD/PCVAD has not been elucidated thus far. Furthermore, PCV2 DNA is usually detected in the PDNS pigs, whereas PCV2 antigen is not detected in some sick animals (Drolet et al., 1999; Palinski et al., 2016; Segales et al., 1998; Thibault et al., 1998), suggesting that other factors may be associated with the disease. However, most of the PCV2-negative PDNS tissue samples have been found to be PCV3-positive (Palinski et al., 2016). These results suggest that PCV3, as a potential pathogen, deserves further investigation.

PCV3 is a virus that was newly identified in 2016 in the USA in sows with PDNS-like clinical signs or cardiac and multiorgan inflammation (Palinski et al., 2016; Phan et al., 2016). Rolling-circle amplification, followed by Sanger sequencing, showed that the viral genome is a 2000-nucleotide (nt) circular genome containing three open reading frames (ORFs) (Palinski et al., 2016). Two ORFs, encoding replicase and capsid protein, show 55 and 35% identity, respectively, to proteins from bat circovirus (Li et al., 2018; Phan et al., 2016; Yuzhakov et al., 2018) and 31–48% identity to those of PCV1 and PCV2 (Ouyang et al., 2019; Palinski et al., 2016; Phan et al., 2016), indicating that PCV3 is distinct from the circoviruses reported previously. Since 2016, the virus has gradually been detected in many countries, including Japan, South Korea, China, Sweden, Russia, Thailand, Brazil, Denmark, Italy and Spain (Faccini et al., 2017; Franzo et al., 2018a; Hayashi et al., 2018; Kim et al., 2018b; Sukmak et al., 2019; Sun et al., 2018; Tochetto et al., 2018; Ye et al., 2018; Yuzhakov et al., 2018), indicating that PCV3 is distributed worldwide. The purpose of this review is to provide updated knowledge on PCV3 infection and PCV3-related research.

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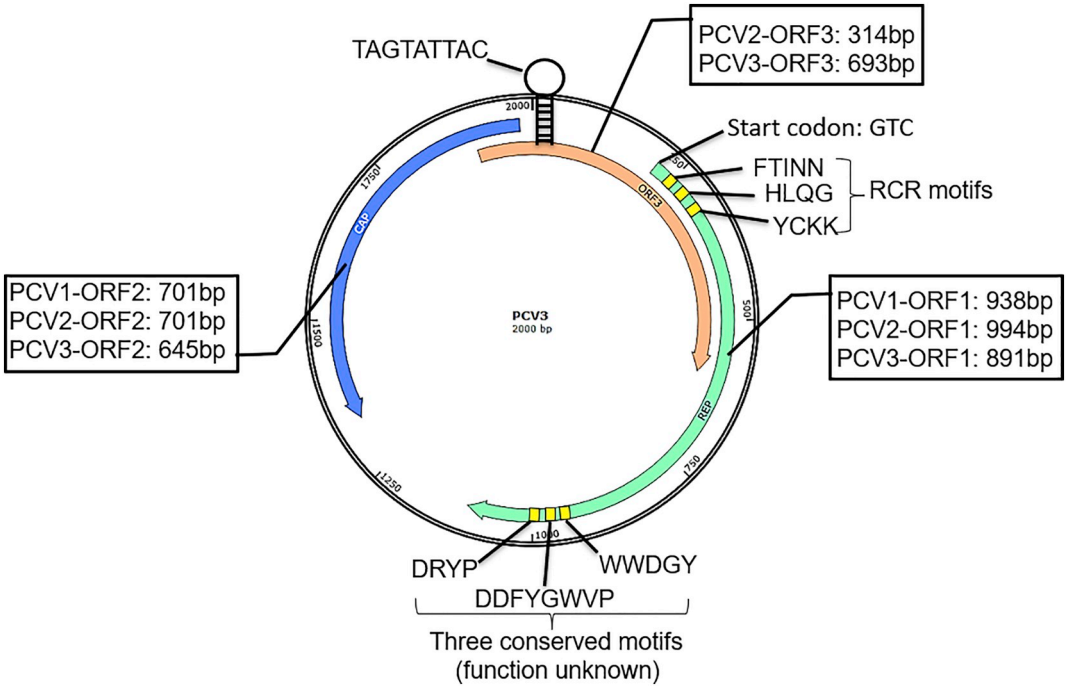

Fig. 1. The arrangement of the PCV3 genome according to previously described results (Palinski et al., 2016; Phan et al., 2016). The PCV3 genome is a 2000-nt single-stranded circular DNA genome containing three predicted ORFs that are different from those of PCV1 and PCV2. The stem-loop of PCV3 contains a conserved sequence (TAGTATTAC) located within the 235-nt 5′ intergenic region between ORF1 and ORF2, which serves as an origin of replication (*ori*) during rolling circle replication (RCR). A GTC start codon is located at the 5′ end of the Rep gene, and the Rep gene contains three RCR motifs, FTINN, HLQG and YCKK.

2. Molecular characteristics, genetic diversity and evolutionary history

2.1. Molecular characteristics

The PCV3 genome is a 2000-nt single-stranded circular DNA with a GC content of 50%, containing three predicted ORFs (Palinski et al., 2016; Phan et al., 2016). The arrangement of the PCV3 genome is similar to that of PCV1 and PCV2, with ORF1 and ORF2 oriented in inverse directions and a stem-loop (Fig. 1) (Palinski et al., 2016; Phan et al., 2016). The stem-loop contains a conserved sequence (TAGTAT TAC) located within the 235-nt 5′ intergenic region between ORF1 and ORF2, which serves as an origin of replication (*ori*) during rolling circle replication (RCR) (Palinski et al., 2016; Phan et al., 2016). However, at the amino acid level, the full-length sequence of PCV3 is only 31 and 48% homologous to that of PCV1 and PCV2, respectively (Ye et al., 2018).

ORF1 of PCV3 encodes a 296–297 amino acid (aa) replicase (Rep), which is 54–55% identical to the Rep of bat circovirus and 48% identical to that of PCV2 (Palinski et al., 2016; Phan et al., 2016). The Rep of PCV3 contains three RCR motifs, FTINN, HLQG and YCKK (Palinski et al., 2016; Phan et al., 2016). Furthermore, a non-ATG start codon, GTC, is located at the 5′ end of the Rep gene (Palinski et al., 2016; Phan et al., 2016). ORF2 of PCV3 encodes a 214 aa capsid (Cap), which is 35% and 36% identical to the Cap of bat circovirus and PCV2 at the DNA level, while the Cap is 24 and 26% homologous to that of PCV1 and PVC2, at the amino acid level, respectively (Palinski et al., 2016; Phan et al., 2016). The ORF3 protein contains 231 predicted amino acids, but the initiation codon and the function of the protein are currently unknown (Palinski et al., 2016; Phan et al., 2016).

2.2. Genetic diversity and evolutionary history

PCV3 is widely distributed in pig populations worldwide (Faccini et al., 2017; Franzo et al., 2018a; Hayashi et al., 2018; Kim et al., 2018b; Sukmak et al., 2019; Sun et al., 2018; Tochetto et al., 2018; Ye et al., 2018; Yuzhakov et al., 2018). PCV3 can be divided into three major clades (PCV3a, PCV3b and PCV3c) based on the full-length genomic DNA sequence or on the presence of two amino acid mutations (A24V and R27K) in the Cap protein (Chen et al., 2019a; Fu et al., 2018; Li et al., 2018; Ouyang et al., 2019; Qi et al., 2019). However, PCV3 strains share high sequence homology based on the sequences of available strains from different countries, with 94.44–100% nucleotide homologies and 96.3–100% amino acid sequence homologies (Hayashi et al., 2018; Qi et al., 2019; Saraiva et al., 2019; Sukmak et al., 2019; Yuzhakov et al., 2018). Sequence comparisons showed that mutations of PCV3 are mainly located in the Cap protein, especially at positions 8–10, 24, 27, 77, 137 and 150 (Li et al., 2018; Qi et al., 2019). These results indicate that PCV3 is relatively genetically stable and has no relationship with the geographical origin of the virus strains. However, two Colombian strains (GenBank no. MH327784 and MH327785) identified recently have been divided into subgroups a1 and a2 of the PCV3a clade based on the full-length genomic sequences (Vargas-Bermudez et al., 2019). Furthermore, a group from China also found that the PCV3a clade can be subdivided into two stable subclades (PCV3a-1 and PCV3a-2) and an intermediate clade (PCV3a-IM) (Li et al., 2018). Li et al. found that PCV3 has a low codon usage bias, which may be affected by natural selection, mutation pressure, and dinucleotide composition, while natural selection plays a dominant role in the selection force (Li et al., 2018). These results suggest that the genetic diversity of PCV3 tends to increase in the swine population.

Although PCV3 was identified in 2016 in the USA, retrospective studies have shown that PCV3 is closely related to bat circovirus (Li et al., 2018; Ouyang et al., 2019; Phan et al., 2016; Yuzhakov et al., 2018). The earliest cases of PCV3 can be traced back to 2009 in the United States, 1966 in China, and as early as 2006 in Brazil and Thailand (Fu et al., 2018; Saraiva et al., 2019; Sun et al., 2018; Zhao et al., 2018), suggesting that PCV3 may have originated in 1966 or even earlier. Thus, it has been hypothesized that PCV3 may be an old virus that has evolved from bats and then gradually adapted to pigs and other

animals (Yuzhakov et al., 2018).

3. Molecular epidemiology

3.1. Infection rate and susceptible animals

Since the first identification of PCV3 in domestic swine, the molecular epidemiology of PCV3 has been studied extensively (Ouyang et al., 2019; Palinski et al., 2016; Phan et al., 2016). Astonishingly, PCV3 is widely circulating in pig farms of various countries with high infection rates. PCV3 positive rates were 47.8% in nine states of Brazil (Saraiva et al., 2019), 36.70% in samples obtained from 26 pig farms in Thailand from 2006 to 2017 (Sukmak et al., 2019), 9.8% in farm samples in Korea (Kim et al., 2018a), and 9.6% in tissue samples collected from Japanese pig herds in 2016 (Hayashi et al., 2018). In China, many groups have investigated the circulation of PCV3 (Deng et al., 2018; Fu et al., 2018; Ha et al., 2018; Qi et al., 2019; Wang et al., 2017a; Wen et al., 2018; Xu et al., 2018; Zhai et al., 2017; Zhao et al., 2018; Zou et al., 2018). Retrospective surveys have shown that PCV3 positive rates in most Chinese farms were approximately 10–60% between 2013 and 2018, while the rate was up to 100% in some farms (Deng et al., 2018; Fu et al., 2018; Ha et al., 2018; Qi et al., 2019; Wang et al., 2017a; Wen et al., 2018; Xu et al., 2018; Zhai et al., 2017; Zhao et al., 2018; Zou et al., 2018). These results indicate that PCV3 is widely distributed in China and other countries, and the positive rate gradually increased during these years.

PCV3 can infect sick pigs as well as healthy pigs (Qi et al., 2019; Saraiva et al., 2019; Wen et al., 2018; Zhai et al., 2017; Zou et al., 2018). Qi et al. found that PCV3 infection has a close relationship with digestive diseases ($p < .05$) and respiratory diseases ($p < .01$), as 10.4% and 26.6% of samples collected from pigs with digestive disease and respiratory disease were PCV3 positive, respectively (Qi et al., 2019). Furthermore, an epidemiological survey demonstrated that weaned pigs with severe respiratory disease (63.75%) or diarrhea (17.14%) had significantly higher positive rates of PCV3 than weaned pigs with mild respiratory disease (13.14%), nondiarrheal (2.86%) or asymptomatic pigs (1.85%), respectively (Zhai et al., 2017). Moreover, PCV3 has also been detected in samples collected from healthy sows (21.9%) and pig slaughter houses (19.14%) (Wen et al., 2018; Zou et al., 2018). Another group from Brazil found that the PCV3 positive rate in healthy swine was higher (29.8%) than that of sick swine (17.9%) (Saraiva et al., 2019). Additionally, PCV3 can infect pigs of almost all ages, including pigs aged from 1 day to 24 weeks, gilt and multiparous sows (Sukmak et al., 2019). These results indicate that PCV3 infection has no association with animal health conditions, gender or age but is associated with clinical symptoms.

PCV3 DNA has been detected in almost all tissues and fluids, including brain, kidney, heart, spleen, serum, pleural effusion, peritoneal cavity fluid, oral and nasal fluids, feces and semen of the sick piglets or healthy animals (Chen et al., 2017; Collins et al., 2017; Franzo et al., 2018a; Kedkovid et al., 2018; Ku et al., 2017; Kwon et al., 2017; Liu et al., 2018; Yuzhakov et al., 2018). Furthermore, PCV3-specific antigens have been detected in various tissues and organs, including skin, lung, heart, kidney, lymph nodes, spleen, liver, and small intestine, in symptomatic and asymptomatic infected piglets (Jiang et al., 2019a). Moreover, 17 of 38 (44.74%) colostrum samples collected from sows were found to be PCV3 positive (Kedkovid et al., 2018). Additionally, PCV3 DNA has been detected in serum samples collected from commercially imported healthy boars from the United States and western European countries (Feng et al., 2019; Zhang et al., 2019b), which may further enhance global transmission. These results demonstrate that PCV3 is distributed in all types of swine tissues, enabling horizontal and vertical transmission.

PCV3 has also been detected in different mammalian domestic or wild hosts and ticks (Fig. 2) (Franzo et al., 2019; Franzo et al., 2018b; Jiang et al., 2019b; Prinz et al., 2019; Wang et al., 2019a; Zhang et al., 2018a). The PCV3 infection rate of wild boars was found to be 30 to 50%, which is similar to or higher than that of domestic boars (Klaumann et al., 2018; Klaumann et al., 2019; Prinz et al., 2019). A survey conducted by Franzo et al. showed a PCV3-positive rate of approximately 30% in 187 wild boar serum samples (Franzo et al., 2018b). Notably, no gender difference was observed among wild boars, whereas the infection rates were lower in animals younger than 12 months compared with those of older boars (Franzo et al., 2018b). Notably, PCV3 has been detected in dogs, cattle and mice (Jiang et al., 2019b; Wang et al., 2019a; Zhang et al., 2018a). Zhang et al. found that 4 of 44 dogs that were negative for PCV2 and canine circovirus (CanineCV), were PCV3 positive (Zhang et al., 2018a). Another group detected PCV3 in 74 of 213 cattle serum samples (34.7%) (Wang et al., 2019a). The bovine-derived PCV3 strains can be classified into PCV3a based on the highly homologous Cap gene (98.0–100%) and the full-length genome (97.5–99.8%) of porcine PCV3 reported previously (Wang et al., 2019a). Recently, PCV3 DNA has also been found in all serum samples ($n = 20$) collected from Balb/C and ICR mice (Jiang et al., 2019b). The mouse-origin PCV3 strains show high homology to the complete genome (97.9–98.8%) and the Cap gene (96.9–98.3%) of the references porcine PCV3 strains (Jiang et al., 2019b). PCV-3 was also recently detected for the first time in chamois, roe deer and *Ixodes ricinus* (Franzo et al., 2019). These results demonstrate that PCV3 can infect pigs, wild boar, cattle, mice, dogs, and ticks, suggesting that PCV3 possesses cross-species transmission abilities and has an unexpectedly broad range and circulation in the wild. Wild animals may therefore serve as potential reservoirs for PCV3 and pose a threat to the swine industry. In addition, other groups and we have found that PCV1 and PCV2 can infect human cells (Arteaga-Troncoso et al., 2005; Beach et al., 2011; Hattermann et al., 2004; Liu et al., 2019), and PCV2 DNA has also been detected in samples collected from children who received a live rotavirus vaccine (Esona et al., 2014). Therefore, whether PCV3 can infect human cells needs to be further elucidated.

3.2. Coinfections

Coinfection of PCV2 with other pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), and swine influenza virus (SwIV), is often detected in domestic swine and wild boars (Ouyang et al., 2019). Therefore, coinfections of PCV3 with other viruses have also been investigated by several groups. The results showed that PCV3-positive samples have high coinfection rates with PCV2, PRRSV, porcine epidemic diarrhea virus (PEDV), torque teno sus virus (TTSuV), or porcine parvovirus (PPV) (Table 1) (Ha et al., 2018; Han et al., 2019; Kim et al., 2018b; Sukmak et al., 2019; Zheng et al., 2018a).

Similar to PCV2, PCV3 also commonly coinfects with other disease-causing pathogens in pigs (Kim et al., 2018b). Among these pathogens, PCV3-positive samples were found to have the highest coinfection rate with TTSuV, as 83.3% of PCV3-positive samples were found to be co-infected with TTSuV1, 71.2% were found to be coinfected with TTSuV2, and 50.0% were found to be coinfected with both TTSuV1 and TTSuV2 (Zheng et al., 2018a). Another survey showed that coinfection rates of PCV3 with PCV2 or PRRSV were 61.54% and 61.54%, respectively, while 30.76% of PCV3-positive samples were coinfected with both PCV2 and PRRSV (Sukmak et al., 2019). The coinfection rates of PCV2 and PCV3 are 1.26–39.39% in China (Chen et al., 2019a; Zhang et al., 2018b; Zheng et al., 2018a; Zheng et al., 2017), 28.3% of sampled pigs were found to be coinfected with PCV3 and PCV2 in Korea (Kim et al., 2017), and the coinfection rate of PCV3 with PEDV was identified as 27.27% in piglets suffering from diarrhea on different pig farms in China (Han et al., 2019). These results reveal that coinfections of PCV3 with other agents are common in pig herds. Therefore, further studies need to focus on the coinfection of PCV3 with other pathogens.

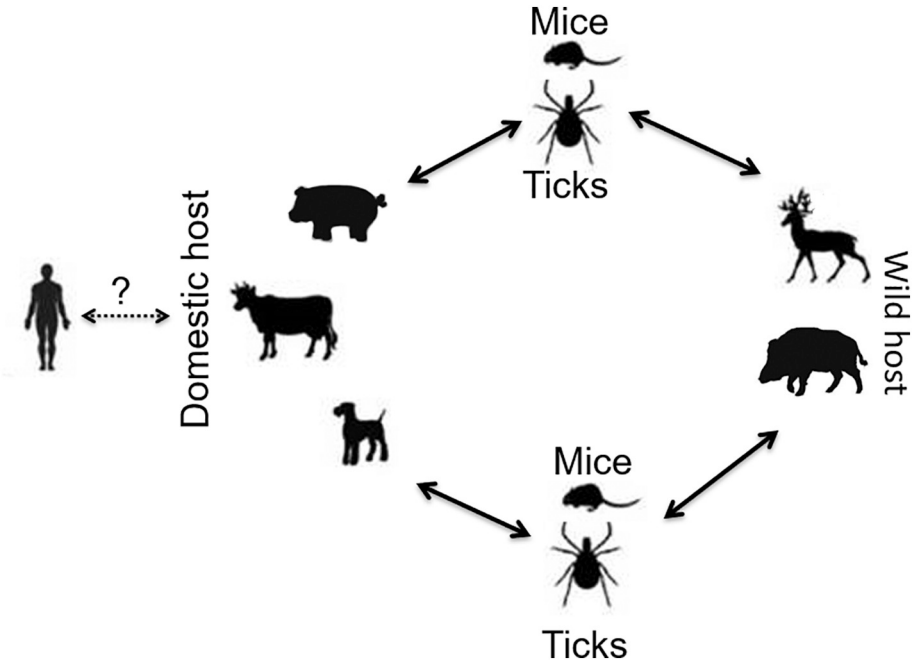

Fig. 2. Predicted life-cycle of PCV3. PCV3 has been detected in domestic hosts (pig, cattle and dog) and wild animals (wild boar and deer). Furthermore, mice and ticks have also been found to be infected with PCV3. These results suggest that PCV3 possesses cross-species transmission abilities and has an unexpectedly broad range and circulation among animals, which may serve as potential reservoirs for PCV3 and pose a threat to the swine industry or even to humans.

4. Pathogenesis

PCV3 was first detected in sows and aborted fetuses associated with PDNS-like clinical signs and reproductive failure (Palinski et al., 2016) and with cardiac and multiorgan inflammation (Phan et al., 2016). Typical PDNS lesions observed in sows, including necrotizing vasculitis, glomerulonephritis, granulomatous lymphadenitis, and bronchointerstitial pneumonia, were analyzed and both PCV3 DNA and antigen were detected in the PDNS-like tissues (Palinski et al., 2016). In cardiac and multiorgan inflammation cases, microscopic lesions were found to mainly appear as nonsuppurative myocarditis and/or cardiac arteriolitis (Phan et al., 2016). Furthermore, hepatic pathology, including necrotizing vasculitis and granulomatous lymphadenitis, have been observed in sows infected with PCV3 (Jiang et al., 2019a; Zhang et al., 2018a). Recently, Jiang et al. reported typical clinical signs of PDNS-like disease in piglets inoculated with PCV3 infectious DNA clones or

Table 1
Coinfection of PCV3 with other pathogens.

Pathogens	No. of samples	Coinfection rate (%)	Reference
PCV3 + PCV1	16	12.5	(Prinz et al., 2019)
	40	17.5	(Prinz et al., 2019)
PCV3 + PCV2	46	28.3	(Kim et al., 2017)
	150	2.0	(Chen et al., 2019b)
	132	39.39	(Zheng et al., 2017)
	159	1.26	(Chen et al., 2019a)
	340	27.6	(Zheng et al., 2018a)
	265	6.8	(Zhang et al., 2018b)
	105	15.2	(Ha et al., 2018)
	336	5.4	(Wang et al., 2019b)
	76	22.3	(Fu et al., 2018)
	67	17.9	(Saraiva et al., 2019)
	16	12.5	(Prinz et al., 2019)
	40	7.5	(Prinz et al., 2019)
	13	61.54	(Sukmak et al., 2019)
PCV3 + PCV1 + PCV2	16	6.25	(Prinz et al., 2019)
	40	2.5	(Prinz et al., 2019)
PCV3 + PRRSV	150	0.67	(Chen et al., 2019b)
	105	3.8	(Ha et al., 2018)
	13	61.54	(Sukmak et al., 2019)
PCV3 + PCV2 + PRRSV	150	0.67	(Chen et al., 2019b)
	159	0.63	(Chen et al., 2019a)
	13	30.76	(Sukmak et al., 2019)
PCV3 + PEDV	66	27.27	(Han et al., 2019)
PCV3 + PPV2	105	8.6	(Ha et al., 2018)
PCV3 + PPV6	105	20.0	(Ha et al., 2018)
PCV3 + PPV7	105	24.8	(Ha et al., 2018)
PCV3 + TTSuV1	132	83.3	(Zheng et al., 2018a)
	105	11.4	(Ha et al., 2018)
PCV3 + TTSuV2	132	71.2	(Zheng et al., 2018a)
	105	8.6	(Ha et al., 2018)
PCV3 + TTSuV1 + TTSuV2	132	50.0	(Zheng et al., 2018a)

PCV3 infectious DNA clones combined with immunostimulation; 40% of the inoculated animals died at a few days post inoculation (Jiang et al., 2019a). Clinical lesions and PCV3 antigen have been detected in the lungs, heart, kidneys, lymph nodes, spleen, liver, and the small intestine of inoculated piglets (Jiang et al., 2019a). The expression of interleukin-1 beta (IL-1β), IL-6, IL-23α, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and chemokine ligand 5 (CCL5) were found to be drastically enhanced in the infected piglets (Jiang et al., 2019a), indicating that proinflammatory cytokines and chemokines were significantly stimulated in the animals. These results demonstrate that both PCV3 virions and genomic DNA are infectious and pathogenic, and PDNS may be associated with PCV3 infection.

Moreover, although PCV3 is associated with clinical (different clinical syndromes) or subclinical (absence of symptoms) symptoms in domestic pigs (Chen et al., 2017; Collins et al., 2017; Franzo et al., 2018a; Kedkovid et al., 2018; Ku et al., 2017; Kwon et al., 2017; Liu et al., 2018; Vargas-Bermudez et al., 2019; Yuzhakov et al., 2018), the virus seems to be nonpathogenic in wild boars. One investigation conducted in Northern Italy showed that PCV3 is highly prevalent (30%) in 187 wild boars, and almost all sampled animals were in good health (Franzo et al., 2018b), suggesting that the wild boar is a reservoir for PCV3.

5. Detection methods

As PCV3 infection has been reported in many countries and coinfection of PCV3 with other pathogens is also prevalent in pig herds, it is necessary to develop rapid, specific and sensitive methods for virus detection or differentiation.

To detect PCV3 DNA in field samples, several diagnostic assays have been established, such as SYBR green-based real-time quantitative PCR (qPCR), TaqMan-based real-time PCR, recombinase polymerase amplification (rt-RPA), loop-mediated isothermal amplification (LAMP) and indirect enzyme-linked immunosorbent assays (ELISAs) (Chen et al., 2018; Deng et al., 2018; Park et al., 2018; Wang et al., 2017a; Wang et al., 2017b; Zheng et al., 2018b). Chen et al. developed a SYBR green-based real-time qPCR assay and used the assay to analyze 203 clinical samples from suckling piglets affected by congenital tremors (Chen et al., 2018). The results showed that the detection limit of PCV3 was 1.73×10^4 copies/μL and 1.73×10^2 copies/μL for conventional PCR and SYBR green-based real-time qPCR, respectively, suggesting that SYBR green-based real-time qPCR is more sensitive than conventional PCR (Chen et al., 2018).

Compared with SYBR green-based real-time qPCR and conventional PCR, LAMP is a simple, rapid, sensitive, specific, visible and cost-effective method. Two LAMP assays have been developed for PCV3 detection in the field (Park et al., 2018; Zheng et al., 2018b). The results showed that the LAMP can specifically detect PCV3 in clinical samples, and the assays were as sensitive as real-time qPCR assays, resulting in a better performance than that of conventional PCR (Park et al., 2018; Zheng et al., 2018b).

For the simultaneous detection of PCV3 and other agents, duplex or multiplex diagnostic assays are promising tools to simultaneously monitor swine viruses in the field. Han et al. developed a SYBR green I-based duplex real-time qPCR assay for the synchronous detection of PEDV and PCV3 (Han et al., 2019). The results showed that the assay can specifically detect PEDV and PCV3 in samples collected from piglets suffering from diarrhea, as the detection limits were 34.6 copies/μL and 61.2 copies/μL for PEDV and PCV3, respectively (Han et al., 2019). Kim et al. designed a multiplex real-time PCR assay to simultaneously detect PCV2 and PCV3 (Kim et al., 2017). The detection limit of the assay was below 50 copies of the target genes of PCV2 and PCV3 (Kim et al., 2017). A multiplex quantitative real-time PCR assay for the differentiation of PCV3 and PCV2 has recently been established by Wang et al., with amplification efficiencies of 98–99% for plasmids and 92–96% for field samples (Wang et al., 2019b). These assays can specifically detect the target viruses without cross-reacting with each other or with other viruses.

To detect PCV3 antigen, an ELISA was established by Deng et al., who used the ELISA to analyze 1688 serum samples collected from 2013 to 2017 (Deng et al., 2018). The results showed that the ELISA could detect PCV3 antigen in serum samples at a maximum dilution of 1:3200, without serological cross-reactions with other swine pathogens, such as PCV1, PCV2, PPV, PRRSV, pseudorabies virus and CSFV (Deng et al., 2018). These results demonstrate that the ELISA assay is sensitive and specific for PCV3 detection (Deng et al., 2018). Recently, a monoclonal antibody (mAb 1H1) specific for PCV3 Cap protein was developed in the same laboratory, and the monoclonal antibody was found to be effective against the Cap protein at a titer of 1/25,600 (Deng et al., 2018). Based on the monoclonal antibody, an immunochemistry staining method was established for the detection of PCV3 from clinical samples with high sensitivity and specificity (Deng et al., 2018). A capsid protein-based indirect ELISA was recently developed to detect PCV3 antibody (Zhang et al., 2019a). The results demonstrated that the indirect ELISA assay is as sensitive as that of qPCR assays, and PCV3 prevalence was found to be higher in sows with reproductive failure than in healthy sows (Zhang et al., 2019a). These assays are rapid and reliable, with high repeatability and reproducibility, for the quantitative detection of viruses in the field.

6. Conclusions and future perspectives

PCV3 is a pathogen associated with PDNS-like clinical disease, reproductive failure, and multisystemic inflammation. The virus spreads widely in almost all tissues of pig and wild boar in various countries, with a gradual increase of the infection. Furthermore, coinfection of PCV3 with other agents is common in pig herds. PCV3 can infect other animals, such as dogs, cattle and mice, and is closely related to bat circovirus; these animals may serve as a reservoir for PCV3 infection and circulation and pose a threat to the swine industry or even to humans. However, to date, the exact pathogenesis of PCV3 remains unknown, and effective methods for the prevention of the disease are inefficient, partly because the virus has yet to be successfully isolated and propagated *in vitro* (Ouyang et al., 2019; Zhang et al., 2018a). Thus, isolation of the virus and monitoring of the molecular epidemiology and genetic diversity of PCV3 in clinical samples is an urgent task, which will provide better insight for the elucidation and prevention of PCV3-related disease. Moreover, more studies need to focus on coinfections of PCV3 with other pathogens in domestic pigs and wild boar.

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Competing interests

The authors declare no conflict of interest.

Author contributions

Writing-original draft, T.O. and G.N.; writing-review and editing, X.L. and X.Z.; revising and supervising, Y.Z. and L.R.; and funding acquisition, L.R.

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