



Reservoirs of Porcine Circoviruses: A Mini Review

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Porcine circovirus (PCV) is one of the smallest known DNA viruses in mammals. At present, PCVs are divided into three species, PCV1, PCV2, and PCV3. PCV1 and PCV2 were found in the 1970s and the 1990s, respectively, whereas PCV3 was discovered recently in 2016. PCV1 does not cause diseases in pigs. However, PCV3, similar to PCV2, is reported to be associated with several swine diseases, including porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure. PCVs are very common in domestic pigs as well as wild boars. However, PCVs have been occasionally isolated from non-porcine animals, including ruminants (such as cattle, goats, wild chamois, and roe deers), rodents (such as NMRI mice, BALB/c mice, Black C57 mice, ICR mice, *Mus musculus*, and *Rattus rattus*), canines (such as dogs, minks, foxes, and raccoon dogs), insects (such as flies, mosquitoes, and ticks), and shellfish. Moreover, PCVs are frequently reported in biological products, including human vaccines, animal vaccines, porcine-derived commercial pepsin products, and many cell lines. PCVs are also abundant in the environment, including water samples and air samples. Interestingly, PCV1 and/or PCV2 antibody or antigen has also been detected in sera, stool samples and respiratory swab samples of human, revealing zoonotic potential of PCVs. Thus, PCVs inhabit many types of reservoirs. In this review, we summarize the reservoirs of PCVs, and this information would be helpful in understanding the natural circulating status and possible cross-species transmission of PCVs.

Keywords: porcine circovirus, PCV, PCV1, PCV2, PCV3, reservoirs

INTRODUCTION

Porcine circoviruses (PCVs) are members of the *Circovirus* genus of the *Circoviridae* family. Currently, there are three species in the genus, PCV type 1 (PCV1), PCV type 2 (PCV2), and PCV type 3 (PCV3), respectively. In 1974, PCV1 was discovered as a contaminant in porcine kidney cell lines (1). Subsequent studies have confirmed that PCV1 is apathogenic in pigs (2). In late 1990s, a new porcine disease, called post-weaning multisystemic wasting syndrome (PMWS), emerged in North America and Europe (3–6), and PCV2 was confirmed as the causal pathogen (7, 8). PCV2 has a global distribution and diverse genotypes (9, 10). In 2016, the third PCV, named PCV3, was discovered using high-throughput sequencing technology in U.S. swine herds suffering from porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure and other syndromes

(11, 12). A recent study suggested that PCV3 fulfilled Koch's postulates and could cause PDNS in piglets (13).

PCVs, especially PCV2 and PCV3, are very common in pigs, and can cause diverse clinical presentations including PMWS, PDNS, reproductive failure, interstitial pneumonia, and so on. Both, PCV2 and PCV3 have garnered immense interest in the world swine industry. Hitherto, most studies found in literature have focused on PCVs derived from swine sources. Occasionally, PCVs have also been isolated from non-porcine animals, biological products, and environmental samples.

PCVs IN NON-PORCINE ANIMALS

PCVs in Ruminants

In 1995, for the first time, Tischer et al. confirmed the presence of PCV1 antibodies in cattle in Germany using indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) (14). Later, a PCV2 nucleotide (1,768 bp) was identified in cattle with respiratory diseases and aborted bovine fetuses in Canada (15). In 2007, a new disease called hemorrhagic diathesis broke out in calves in Germany, and PCV2b (1,767 bp) was suggested as a potential causal pathogen (16, 17). In the United States and China, PCV2 was frequently detected using metagenomic sequencing method and special PCR method in beef from supermarkets, beef stalls, and goat samples (18–22). Interestingly, one previous study confirmed that calves were susceptible to PCV2 and presented lymph node swelling, reddening of oral and ocular mucosa, and diarrhea post inoculation (23).

In 2011, a circovirus-like virus sequence (PorkNW2, GenBank accession number HQ738638) was discovered in both pork and beef samples from stores in San Francisco (18). In 2014, two similar sequences were re-detected in beef samples from the Sunset district of San Francisco (19). In fact, these circovirus-like virus sequences were significantly close to the aforementioned PCV3 in pigs (11, 12). Based on these research reports, it is speculated that PCV3 is also widespread in cattle. A recent epidemiological investigation from China confirmed our speculation. Their results showed that 74 out of 213 (34.7%) clinically healthy bovine samples from eight regions in the Shandong Province were positive for PCV3, and that the bovine-origin PCV3 genome sequences had a close relationship with porcine-origin PCV3 genome sequences (24). Surprisingly, PCV3 was also reported in the samples of wild chamois and roe deers (25). The data suggest that ruminants, especially cattle, are noteworthy reservoirs of PCVs. These epidemiological data bring to light two important scientific issues that warrant further research: (1) to determine whether PCV3 has a multi-host receptor and (2) to identify whether some cross-species transmission routes (such as direct contact, insect biting) might contribute to the circulation of PCV3 in pigs and ruminants.

PCVs in Rodents

Seroconversion of PCV1 antibody was detected in several murine species including NMRI, BALB/c, and Black C57 (14). Based on evidence from numerous studies, mice are often considered important animal models of PCV2 infection (26–35). However,

only a few studies have conformed PCV infection in field rodent samples. Since 2010, several research teams from Hungary, Brazil, Korea and China have provided molecular evidence of PCV2 in rodents circulating on swine farms (36–39). In the study from Brazil, PCV2 antigen was identified in the spleen, lungs and kidneys of rodent species *Mus musculus* and *Rattus rattus* using immunohistochemistry assay (37). In addition, rodent-origin PCV2 genome sequences had remarkable similarity with gene sequences of PCV2 isolated from pigs (37, 39). It is noteworthy that one recent study identified PCV3 in commercially sourced laboratory mice including BALB/c and ICR mice (40). This is in contrast with from the Hungarian study, which claims that rodents need certain contact with pigs, without which PCV2 is negative in rodents (36). Owing to limited information, we are currently unable to ascertain the origin of murine PCV3 in laboratory mice. Without significant clinical manifestation, rodents are more of a carrying reservoir and an animal model of PCVs. On farms, unconstrained rodents may promote the spread of PCVs.

PCVs in Canines

Fur animals have significant economic value. In recent years, cases of infections due to porcine pathogens (such as Hepatitis E virus, Pseudorabies virus) have progressively in minks and foxes (41–44). PCV2 was also reported in minks, foxes, and raccoon dogs with diarrhea or reproductive failure in China (45–47). Interestingly, the samples were tested negative for common pathogens (such as Mink enteritis virus, Canine distemper virus, Coronavirus, Rotavirus, or Astrovirus) of minks and foxes, indicating that PCV2 was also an important pathogen contributing to the clinical diseases of minks and foxes. Infection of PCV2 in fur animals may be caused by consumption of food containing porcine-origin products and possibly by cross-species transmission. These mechanisms could also explain PCV2 and PCV3 infection in dogs (48, 49).

PCVs in Insects

Generally speaking, PCV is not considered as an arbovirus. However, PCV2 has been often detected in *M. domestica* flies and culex mosquitoes in pig farms (50, 51). Surprisingly, PCV3 was first identified in ticks (*Ixodes ricinus*) collected from wild roe deers that were negative for PCV3, making the source of PCV3 ambiguous (25). PCV infection is postulated to occur when hosts get bitten by carrier flies, mosquitoes, and ticks.

PCVs in Mussels

Bivalve shellfishes (such as oysters, clams, and mussels) intake nutrition by filtering up to 4.8 L/h of surrounding water and simultaneously concentrate microorganisms that are present (52–54). Animal pathogens can contaminate the beds via runoff from fields fertilized using animal waste. One previous study reported high detection rate for PCV2 and *E. coli* (41%, 12/29 and 28%, 8/29) in blue mussels (*Mytilus edulis*) from Danish commercial harvesting areas, while common food-borne pathogens (such as hepatitis E virus, rotavirus, *Salmonella*) were absent. The geographic distribution of the PCV2-positive shellfish samples revealed that positive samples were localized to

Limfjord in the northern part of Jutland and a bay area in the south-western part of Jutland, Denmark (55). This suggested that these bay areas were contaminated by porcine waste, and PCV2 may be a specific indicator of porcine waste in shellfish.

PCVs IN HUMAN SAMPLES

There has always been controversy regarding PCVs infecting human. At the outset, Tischer et al. confirmed the presence of PCV1 antibodies in human sera, and the IFA results showed a significantly higher number of positive sera (20%) in non-hospitalized “healthy” persons from the former German Democratic Republic than that (8.6%) in blood donors from Berlin-West (14). Interestingly, scientists from Northern Ireland, the United States, and Germany could not detect antibodies or antigens of PCV2 in human samples (56–59). However, in other studies, antibody of PCV1 or antigen of PCV2 was detected in human sera, digestive tract samples and respiratory tract samples (60–65). Although there is serological and molecular proof of PCV presence in humans, the detection rate is very low. Thus, the clinical significance of PCVs in human beings remains largely unknown.

PCVs IN BIOLOGICAL PRODUCTS

PCV1 was first confirmed as a contaminant in porcine kidney cell lines (1), following which, non-infectious PCV1 and PCV2 were detected in porcine-derived commercial pepsin products used for humans (66). Owing to its porcine origin, the detection of PCVs in pepsin and other porcine biological products is perfectly plausible. PCV1 was also reported in commercial veterinary vaccines against classic swine fever virus (CSFV), porcine parvovirus (PPV), and pseudorabies virus (PRV) (67, 68). However, in 2010, PCV1 was reported in human rotavirus vaccine (69), which caused a significant stir because of unknown clinical significance of PCVs in humans. Since that incident, the Food and Drug Administration (FDA) and several research institutions began investigating the source of the vaccine contamination and assessing the safety of the vaccine. Their results indicated that several cell lines, virus seeds and vaccines were contaminated by PCV1 and/or PCV2 (70–75). Porcine-derived commercial pepsin product used for cell culture and vaccine production was considered as the main source of vaccine contamination (76). Although the contaminated vaccines caused no clinical diseases in people, humans can be considered likely reservoirs for PCVs (63, 77). Thus, via human fecal matter, PCVs might have been introduced into environmental water or other places (64).

PCVs IN ENVIRONMENTAL SAMPLES

As we know, many viruses can spread through aerosols. Whether PCVs can spread via aerosol is little known. However, Canadian researchers found that high viral loads (up to 10^7 genomes per cubic meter of air) of PCV2 existed in swine confinement

buildings (78). Another study also identified the existence of PCV2 in bioaerosol samples from pig farms and abattoirs. In addition, PCV2 was detected in nasal washes of workers (4/78) from pig farms (65). This revealed that PCV2 was a potential airborne virus.

Intriguingly, PCV2 can be detected in various types of water samples from Brazil, including (a) water used for swine consumption, subjected to conventional treatment followed by chlorination; (b) tap water meant for human consumption, subjected to conventional treatment followed by chlorination; (c) surface water without treatment, used for swine consumption; (d) water from the Pinhal river, which crosses; (e) groundwater collected from a tap located at a school; and (f) water from the Jacutinga river, which is redirected and treated for domestic supply (79). This indicated that PCV2 was widely prevalent in the environment.

CONCLUSIONS AND DISCUSSION

In summary, from the current knowledge, we infer that PCVs have multiple reservoirs; they are not limited to the swine family, but have broad distributed in ruminants, rodents, canines, insects, and other species. Consequently, we deduce the occurrence of possible cross-species transmission (such as pigs to rodents, pigs to cattle, pigs to fur animals) of PCVs. In most cases of detection or infection in non-porcine animals, the main reason has been ingestion of porcine products or direct contact with pigs (36–39, 45, 47, 51). Cases of infection of PCVs in ruminants are not well-evaluated, because ruminants hardly ingest porcine products or have rare or no contact with pigs. Due to the wide distribution of PCVs in various types of water samples (79), there is a high risk of infection in ruminants via PCV-contaminated water. In fact, owing to their wide tissue tropism, PCVs could exist in almost all tissues of pigs and wild boars (80, 81). Furthermore, PCVs were frequently detected in fecal samples of non-porcine animals (45, 48, 62, 63). Discharge of PCV-positive fecal matter into water bodies increases the occurrence of PCV contamination. A comprehensive understanding of PCV reservoirs would be invaluable for implementing accurate measures to control the spread of PCVs.

AUTHOR CONTRIBUTIONS

S-LZ: conceptualization and writing—original draft preparation. S-SL, W-KW, D-HL, X-HW, QZ, Q-LC, and Y-WS: writing—review and editing. S-LZ and YX: supervision. S-LZ and W-KW: project administration. All of authors have approved this manuscript for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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