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Dissection of the Possible Routes on Porcine Circoviruses Infecting Human

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Abstract: In 10 years recently whether porcine circoviruses infect human is in dispute. Previous studies reported DNAs of porcine circovirus type 1 and porcine circovirus type 2 were detected in pigs, cattle, pepsin and rodents. However, the latest published article in the well-known journals also showed that porcine circovirus type 2 existed in the human stools, human vaccines and beef. This review dissected of the possible routes on porcine circoviruses infecting human such as foodborne infection, vaccination infection, xenotransplantation infection, airborne infection and vector infection based on some published literatures in order to cause some concerns in public health and cross-species transmission. Moreover, to better understand phylogenetic analysis of porcine circoviruses isolated from different origins (swine, bovine, human and pepsin), researchers constructed the phylogenetic tree based on its complete genome. This study systematically reviewed the possible routes on porcine circoviruses infecting human.

Key words: Human, swine, porcine circoviruses, routes of transmission, public health, cross-species transmission

INTRODUCTION

Porcine Circoviruses (PCV) belonged to a member of the genus *Circovirus* of the family Circoviridae which was considered as one of the smallest discovered animal viruses up to now. According to its pathogenicity and genotype, PCVs had two genotypes, PCV1 and PCV2. The former did not cause swine diseases however, the latter was associated with several swine diseases clinically, such as Postweaning Multisystemic Wasting Syndrome (PMWS), Porcine Dermatitis and Nephropathy Syndrome (PDNS), Porcine Respiratory Disease Complex (PRDC), reproductive failure (Finsterbusch and Mankertz, 2009; Ramamoorthy and Meng, 2009).

PCV1 possesses a kind of genome structure and the size of its genome is 1,759 bp however, PCV2 has three kinds of genome structure and five genotypes (PCV2a-PCV2e) and the size of its genome is 1767 or 1768 bp (Segales *et al*., 2008; Wang *et al*., 2009). There are some obvious differences on the position and sequence of capsid gene (*ORF2*) (Fig. 1). PCVs was widespread around the world, especially PCV2, it caused enormous economic losses for the world's swine industry. There is a controversy on whether PCVs infects humans (Allan *et al*., 2000; Bernstein *et al*., 2003; Hattermann *et al*., 2004a; Tischer *et al*., 1995). However, recently the lastest literature reported PCV2 was detected in the stools of patients suffering with diarrhea and healthy persons from Minnesota, USA (Li *et al*., 2010).

It further suggested that PCVs could infect human beings. Therefore, the present study mainly reviewed the possible routes of human infected by PCVs in order to highlight the public health and cross-species transmission.

FOODBORNE INFECTION

Ingestion or contact fresh pork products: The previous studies showed that swine (*Susscrota domestica* and *Sus scrofa*) was the only host of PCV1 and PCV2 (Finsterbusch and Mankertz, 2009; Ramamoorthy and Meng, 2009). So, the main route of human infected with porcine circoviruses was that people ingest fresh or unbaked pork infected by PCVs. Hattermann *et al*. (2004b) made a number of infection studies on human cell lines with PCV1 and PCV2. They found that PCV1 persisted in most cell lines (including 293 cell, RH cell, Chang liver cell, Hep2 cell without causing.

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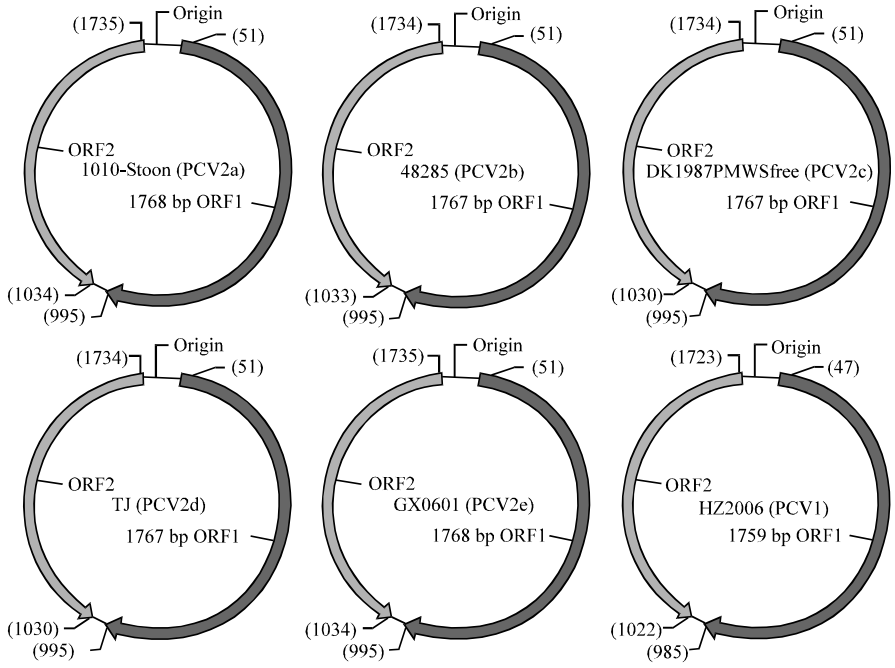

Fig. 1: Map of PCV1 and PCV2

any visible changes while PCV2-transfected cells (293 cell and Hep2 cell) showed an obvious Cytopathogenic Effect (CPE). Another study reported that there were ultrastructural alterations in human blood leukocytes ($CD4^+$, $CD8^+$, $CD14^+$, $CD19^+$ and $CD56^+$) induced by PCV1 infection (Arteaga-Troncoso *et al*., 2005). These results suggest that PCVs has the capability of infecting human cells and should be considered a potential risk of viral transmission during foodborne infection.

The latest report showed that out of 13 pork specimens purchased from different US stores, 7 of which clustered with PCV2, 1 with PCV1 and 1 highly divergent sequence (Li *et al*., 2010). In other words, PCVs positive rates were nearly 70% in stores. PCV1 and PCV2 had high infection rates in swine herds and often was digested from pork. Therefore, human should install different cutting boards used for different food (such as vegetables, fresh pork or undressed meat) in kitchen which might help us to reduce the PCVs transmission during foodborne infection.

Ingest undressed beef or fresh milk: Nayar *et al*. (1999) reported PCV2 was detected in cattle with respiratory syndrome and reproductive failure. That time they were temporarily named Bovine Circovirus (BCV) because they were isolated from cattle. Genome analysis showed that the size of its genome were 1768 bp (GenBank No.: AF109397 and NC_002068). Li *et al*. (2011) found that PCV2 existed in beef from Pakistan and Nigeria and the size of its genome were 1767 bp (GenBank No.: HQ738639-HQ738641). Genome homology analysis showed that they had 99% homologies with PCV2b. In fact human beings, especially those persons from Western countries had a kind of life habit about eating 70% cooked beef in real life.

Therefore, human could be easily infected by PCV2 when ingesting undressed or uncooked beef. Moreover, PCV2 might exist in fresh milk. At present, the commercial fresh milk was major sterilized through Pastometer's method in other words, the crude milk was heated to 62-63°C (lasting for 30 min) or 75-90°C (lasting for 15-16 sec) or higher temperatures (lasting for 2-3 sec) in the proceeding, the pathogenic microorganism were killed while the beneficial microbial population and nutrition were left. Regretfully, there was a kind of fact existing that people drank fresh milk directly in some remote countrysides or mountain areas which also might bring out the transmission of PCV2 from bovine to human.

Drink raw water: In recent years, there were more and more pig farms in China and even the world with the rapid development of animal husbandry. Although, some swine farms carried out harmless treatment of stools most farms, especially middle and small swine farms did not put safety disposal into practice. What is worse is that they discharged pig faeces to the fresh rivers. Now a days, the drinking water of human and animal was major from those

rivers. PCVs had strong resistivity to low pH, chloroform and high-temperature (70°C) in the surroundings (Finsterbusch and Mankertz, 2009). People and porcine might be infected by PCVs when we drink the water from those waterheads contaminated by pig faeces containing PCVs.

VACCINATION INFECTION

In the middle of 20th century, cell culture was used to the growth of viruses and it was realized that passage in cell culture was also a method of attenuation. Cell culture also permitted conscious selection of mutants by isolation of single clones and by incubation at temperatures below the normal temperature of the host. Therefore, since 1950, researchers saw the development of numerous attenuated virus vaccines including polio, measles, rubella, mumps and varicella (Table 1) (Plotkin, 2005).

Cell culture provided a lot of conveniences for human, especially the development of vaccines however, it still had some shortcomings. These 2 years, some researchers found that there were the DNAs of PCV1 and PCV2 in orally administered rotavirus vaccine (Rotarix and RotaTeq) from GlaxoSmithKline (GSK) Co., Ltd. (Baylis *et al*., 2011; Ma *et al*., 2011; McClenahan *et al*., 2011; Victoria *et al*., 2010). Then, GSK detected their cell bank and seed viruses used to produce vaccines, the results showed that PCV1 existed all the time in Rotarix since from its research initial stage. US Food and Drug Administration (FDA) also made lots of studies from pre- to post-permission of the Rotarix and declared it had good safety records (McClenahan *et al*., 2011).

At present, the commercial zymines used to cell culture were major from swine origin, subsequently, those zymines containing PCVs were used to the development of numerous attenuated virus vaccines. Therefore, human might be infected by PCVs through the vaccination of contaminated vaccines containing PCVs.

Table 1: Live vaccines and their available time in the market

Design strategy	Vaccine	Years
Use of related animal virus	Smallpox	1798
Chemical attenuation	Rabies	1885
	Anthrax	1881
Passage *in vitro*	Bacillus Calmette-Guerin (BCG)	1927
	Yellow fever	1935
Cell culture passage	Plague	1908
	Oral Polio Vaccine (OPV)	1962
	Measles	1963
	Adenovirus	1971
	Japanese B encephalitis	1989
	Varicella	1995
	Rotavirus	2005
Cell culture passage	Rubella	1969
with cold adaptation	Live influenza	2003
Auxotrophy	Ty21a antityphoid vaccine	1983
Use of reassortants	Live influenza	2003
	Rotavirus bovine-human	2005

XENOTRANSPLANTATION INFECTION

Xenotransplantation included heterogeneous organ transplantation, heterogeneous cell transplantation and transplantation associated with heterogeneous products. In recent years, they had or should have been used to human. Some pathogens of swine origins might transmit to human through xenotransplantation which was considered as a great topic in the medical circles (Meng, 2003; Tucker *et al*., 2003).

In 2004, scientific researchers detected whether PCV1 and PCV2 existed in factor VIII, heparin, insulin and pepsin, their results suggested that a porcine-derived commercial pepsin product was contaminated with PCVs (Fenaux *et al*., 2004). Although, cell infection and animal experiments certified that contaminated PCVs had no infectivity and pathogenicity for cells and pigs in the future, researchers should pay great concern to xenotransplantation.

AIRBORNE INFECTION

At present, a series of literatures reported PCVs were detected in some animals including domestic pigs, wild pigs, mice and rat, etc. (Allan and Ellis, 2000; Lorincz *et al*., 2010; Nayar *et al*., 1999; Tischer *et al*., 1995). Verreault *et al*. (2010) got a conclusion that concentrations of airborne PCV2 of up to 10^7 genomes per cubic meter of air were detected which suggested PCV2 could become airborne in detectable concentrations in commercial swine confinement building environments. Therefore, those persons (especially front-line veterinarians and stock man) from pig farms contacting close with the above animals might be infected through inhaling aerosols containing PCVs virions.

VECTOR INFECTION

In addition, some researchers reported that DNAs of PCV2 were detectable in *Musca domestica* (house fly) and mosquitoes (Culex) on commercial pig farms (Blunt *et al*., 2011; Yang *et al*., 2011). Flies and mosquitoes were common vectors on animal habitations if we did not control them, some zoonosis (such as Japanese B encephalitis, Dengue fever and Malaria) could happen

during human populations. So, researchers should pay close attention to vector infection of PCV2 as well in spite of its unknown pathogenicity for human.

PHYLOGENETIC ANALYSIS BASED ON COMPLETE GENOME OF PCVs ISOLATED FROM SWINE AND OTHER ORIGINS

To better understand phylogenetic analysis of PCVs strains isolated from different origins, researchers obtained and selected some PCVs strains from swine (27 strains were isolated from Chinese swine herds suffering from PMWS and PDNS), bovine (5 strains), human (2 strains) and pepsin (2 strains) and constructed the phylogenetic tree (Fig. 2) using MAGE 4.0 Software.

Figure 2 shows that five PCV2 strains from bovine origin were divided into two different genotypes, PCV2a and PCV2b, respectively; two PCV2 strains from human origin were divided into two different genotypes, PCV2b and PCV2a, respectively; two PCVs strains from pepsin were divided into two different genotypes, PCV2a and PCV1, respectively however, 27 PCV2 strains from swine origin were divided into five different genotypes, PCV2a, PCV2b, PCV2d, PCV2e and PCV2f, respectively.

Figure 2 also suggested that PCV2f was an emerging genotype in swine herds and was described for the first time. Moreover, interestingly to us, PCV2 strains from swine origin had more genotypes than those from other origins, the possible reasons were as follows:

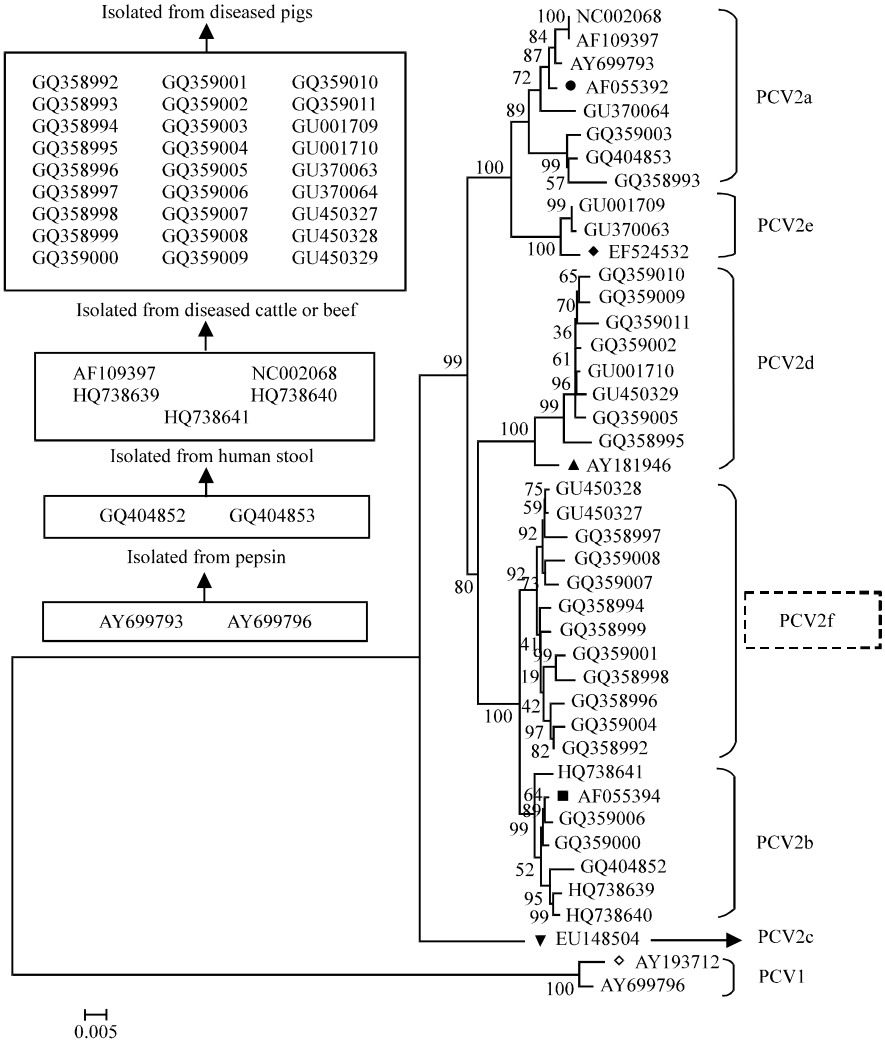

Fig. 2: Phylogenetic analysis of different PCVs isolates; PCV2a reference strain labeled with ●; PCV2b reference strain labeled with ■; PCV2c reference strain labeled with ▼; PCV2d reference strain labeled with ▲; PCV2e reference strain labeled with ◆; PCV1 reference strain labeled with ◊

- Human made more epidemiological studies in pigs than in other animals
- PCVs infecting other animals were all from swine origin

CONCLUSION

PCVs, especially PCV2, brought about more and more serious diseases (PCVAD) for swine herds, so its control also became more and more difficult for veterinarians. However, PCV1 and PCV2 was also detected in human stools, vaccines of human use, beef, pepsin and rodents. This study mainly reviewed the possible routes of human infected by PCVs which told us to pay more attention to PCVs in the aspect of sanitation and cross-species transmission. What about its pathogenicity for human? Whether horizontal transmission or vertical transmission caused by PCVs could occurred among human populations? Whether PCV2c-PCV2e or PCV2f existed in human? Whether pigs were infected through infected human again? In future, through more studies from scientific researchers, researchers should have gotten reasonable answers to those puzzled questions.

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