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SHORT COMMUNICATION

Genetic Diversity of Porcine Circovirus Subtypes from Aborted Sow Fetuses in Vietnam

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Abstract

Porcine circovirus type 3 (PCV3) is an emerging circovirus that is highly distributed among swine worldwide and associated with porcine dermatitis and nephropathy syndrome, reproductive failure, and multisystemic inflammation. Here, we investigated and characterized PCV3 from aborted fetuses in Vietnam. We found that the whole genomes of PCV3 collected in these Vietnamese pig farms share 98.4–99.45% sequence identity with reference PCV3 sequences. Several distinct mutation were identified in both the Rep protein and Cap protein of these strains. These strains were clustered into two distinct subtypes (3a1 and 3b). This study contributes to a better understanding of the molecular characteristics and genetic diversity of PCV3 in Vietnam.

Introduction

Porcine circovirus 3 (PCV3) is an emerging circovirus detected in swine that suffers from porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, and multisystemic inflammation [1–4]. It is one of three members of the *Circovirus* genus to infect swine and its genome is a circular ssDNA containing approximately 2000 bases with three identified ORFs [2]. ORF1 is on the positive strand and encodes Rep (protein involved in viral replication which may be 296–297 amino acids long), while ORF2 is located on the complementary negative strand and codes for the capsid protein (Cap) [2, 3]. Genotyping of PCV3 has previously identified three subtypes: PCV3a, 3b, and 3c [5]. In addition, subtype PCV3a has been further classified into three subclades (PCV3a1, PCV3a2, and PCV3a3) based on the genetic diversity of the cap protein [6].

PCV3 was detected in both healthy animals and animals with different diseases. Animals may be infected with PCV3 alone or co-infected with PCV3 and other viruses [7]. Recent studies have identified cases of co-infection of PCV3 and other viruses, such as PCV2 and Porcine reproductive and respiratory syndrome virus (PRRSV) [8]. PCV3 is a prominent pathogen in swines and has been found to exhibit greater pathogenicity in piglets, relative to PCV2 [9, 10].

Although PCV3 was discovered only recently, it has been shown to be circulating in swine populations worldwide. In 2015, PCV3 was first identified in the USA [2] and then in China [4, 6], Poland [11], South Korea [1], the United Kingdom [12], Brazil [13], Thailand [14], Germany [15], Denmark [16], Spain [16], Italy [16], and Vietnam [17]. Recently, it was reported that swine populations in northern Vietnam were infected with PCV3 (these belong to the PCV3a subtype). However, there is currently no available information on the prevalence of PCV3 in swine populations in the southern provinces of Vietnam. Thus, this study identified and characterized two PCV3 subtypes from cases of reproductive failure associated with PCV3 infection in South Vietnam.

Materials and Methods

Samples of fetal tissue (heart, lung, and liver) were submitted to Viet Han Veterinary Diagnosis Laboratory, Nong Lam University from sow farms in the Vietnamese

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provinces of Dong Nai, Binh Duong, and Lam Dong. Samples were delivered to the laboratory by cooled transport (4 °C). A part of the samples was processed for RNA isolation within 24 h and the remains were stored at − 20 °C. Three grams of sample (heart, lung, and liver) from the fetus were collected and homogenized in a 15 ml Falcon with 2 ml PBS using a SCILOGEX D160 Homogenizer. Then, the tissue homogenate was centrifuged at 4000 rpm for 10 min at 4 °C. One ml of the supernatant was used for nucleic acid isolation. Viral nucleic acid from tissues was extracted using the GeneJET Viral DNA/RNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. PCR was used to identify potential cases of infection with PRRSV, Parvovirus, PCV2, and PCV3 in specimens. We searched for PRRSV using PCR with primers PRRS-P71 and PRRS-P72 [18]. Parvovirus was detected with RT-PCR using primers PPV P1 and PPV P6 [19]. PCR was performed to search for PCV2 with primers CF8 and CR8 [20]. The PCV3 genome from PCV3-positive samples was amplified by PCR using two primer pairs, including PCV3-74 F, PCV3-1144 R, PCV3-genome-2-F, and PCV3-genome-2-R [15, 21]. The primer sequences and the product size are listed in table S1. PCR reaction contained 12.5 µl of 2X GoTaq G2 Green Master Mix, 5 µl cDNA, 1 µl of 10 µM Forward primer, 1 µl of 10 µM Reverse primer, and 5.5 ul of Nuclease-free water in a total reaction volume of 25 µL. The PCR products were then separated using 1.2% agarose gel electrophoresis. After purifying from the gel using GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Fisher Scientific, USA) as per the manufacturer's instructions, the products were directly cloned into a pGEM-T Easy vector (Promega, USA). The recombined vectors were transformed and amplified in *Escherichia coli* DH5α (Takara, Japan) for sequencing. ClustalW was used to align all sequences. Phylogenetic analysis was conducted using Maximum Likelihood in the software MEGA (version 10.1) [22].

Results and Discussion

In this study, we investigated the potential pathogens that infect lung, liver, and heart tissues in aborted fetuses collected from sow farms in southern Vietnam. These were characterized by a greater incidence and degree of fetal abnormalities, such as increases in cases of fetal mummification and death per sow, and the incidence of abortion from sows in the farms also increased. The veterinarian initially evaluated the cause of fertility disorders to be infection with Porcine Parvovirus (PPV), but molecular tests came back negative. Instead, the extensive diagnosis in these mummified, dead, and aborted fetuses all tested positive for infection with PCV3. We found that two samples collected from Lam Dong and Binh Duong provinces were positive with PCV3 only, while the specimen from Dong Nai province was positive for both PCV3 and PCV2 (Table 1). Our findings are consistent with studies indicating that PCV3 may infect alone or co-infect with other viruses, such as PCV2 and PRRSV [7, 8]. These data indicate that PCV3 may have correlation with the abortion of swine in Vietnam.

The genome sequences of PCV3 strains from the provinces of Binh Duong (NNH/BD/2018), Lam Dong (NNH/LD/2018), and Dong Nai (NNH/DN4/2018) are available

Table 1 Epidemiological data on PCV3-positive farms and samples

No	Sample name	GenBank	Date of collection	Geographic origin	Origin and type of sample	PCV3	PCV2	Parvovirus	PRRSV
1	NNH/DN4/2018	MT847022	Nov, 2018	Dong Nai	Aborted fetuses: Heart	+	+	−	Not test
2	NNH/BD/2018	MT847024	Oct, 2018	Binh Duong	Aborted fetuses: Lung	+	−	−	Not test
3	NNH/LD/2019	MT847031	Oct, 2019	Lam Dong	Aborted fetuses: Liver, Lung, Heart	+	−	−	−

Table 2 Nucleotide and amino acid sequence identities (%) within and between genotypes/proteomes in this study and reference sequences

Genotypes		Identity (%)	Among collected strains	Reference strains
PCV3a	Full-length	nt	98.70–99.30	98.85–99.45
	ORF1 (Rep gene)	nt	99.10–99.66	96.40–99.88
		aa	98.65–98.98	94.61–99.66
	ORF2 (Cap gene)	nt	97.36–98.75	98.10–99.20
		aa	96.74–98.13	97.67–98.60
PCV3b	Full-length	nt	98.70–99.30	98.4–99.20
	ORF1 (Rep gene)	nt	99.10–99.66	96.07–99.55
		aa	98.65–98.98	93.93–99.66
	ORF2 (Cap gene)	nt	97.36–98.75	96.80–99.06
		aa	96.74–98.13	96.27–99.06

Reference strains include KT869077, KX458235, KX778720, KX898030, KX966193, KY075986, KY075987, KY075988, KY075989, KY075990, KY075991, KY354038, KY354039, KY418606, KY421347, KY421348, KY778776, KY778777, KY865242, KY865243, KY996337, KY996338, KY996339, KY996340, KY996341, KY996342, KY996343, KY996344, KY996345, MF079253, MF079254, MF162298, MF162299, MF589103, MF589104, MF589105, MF589106, MF611878, NC_31753, and MF611876

Rep Replication, *Cap* Capsid, *aa* amino acid, *nt* nucleotide

in the GenBank database under the accession numbers MT847024, MT847031, and MT847022, respectively. As with other PCV3 strains, the full genome sequence of PCV3 in this study was 2000 nucleotides in length [2]. It contains two ORFs (encoding Rep and Cap proteins) orientated in opposite directions [2]. Among these samples taken in Vietnam, no insertions or deletions were found in PVC3 when aligning to reference genomes. The multiple sequence alignment of these sequences showed that the nucleotide identity of the complete genome of PCV3 in Vietnam and other reference sequences of PCV3 was 98.4–99.45%. The complete genomes of PCV3 within these Vietnamese strains were found to share 98.70–99.30% sequence identity with the reference genome (Table 2). ORF1 contained 891 bp coding for 296 amino acids of the Rep protein. ORF1 within PCV3 genomes in Vietnam were found to share 99.10–99.66% and 98.65–98.98% nucleotide and amino acid identity with each other, respectively. They shared 96.07–99.88% and 93.93–99.66% nucleotide and amino acid identity with reference strains, respectively. The ORF2 contained 645 bp coding for 214 amino acids of the Cap protein and shared 97.36–98.75% and 96.74–98.13% nucleotide and amino acid identity with each other, respectively. It was found to share 96.80–99.20% and 96.27–99.06% nucleotide and amino acid identity with reference genomes, respectively (Table 2).

Phylogenetic analysis based on the complete genome of PCV3 was performed to investigate the evolutionary relationships with other PCV3 subtypes. Two PCV3 strains collected from Dong Nai and Binh Duong provinces were classified as being of the PCV3b subtype, and a PCV3 collected from Lam Dong was grouped into the PCV3 subtype a1 (Fig. 1). Nucleotide/amino acid sequence comparisons involving the completed genomes (ORF1 and ORF2) of the PCV3a and PCV3b strains from this study are given in Table 2.

We found three mutated sites located in the Rep protein of these Vietnamese strains. G172R was found in NNH/BD/2019. F211S and L221P were identified in NNH/DN/2019 and NNH/LD/2019, respectively. These variants do not appear in any of the reference strains (Fig. 2). Also,

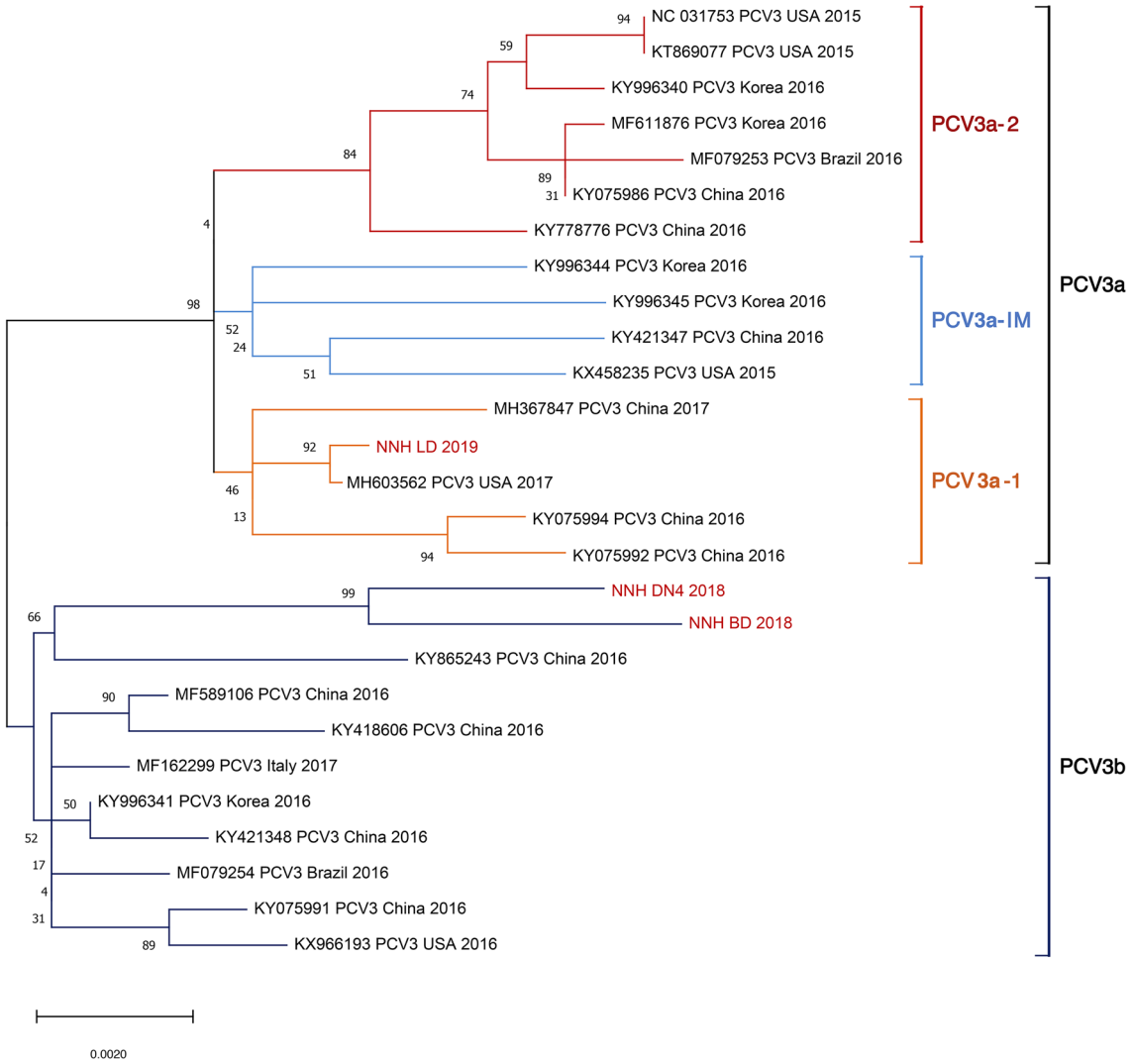

Fig. 1 Phylogenetic analyses of the complete genome sequences of PCV3 in southern provinces of Vietnam. Evolutionary tree analysis was conducted using MEGA v10.1. The tree was built using Maximum Likelihood and the Kimura 2-parameter model with 1000 bootstraps replicates. The tree is drawn with branch lengths measured in the number of substitutions per site. The scale bar represents a genetic distance of 0.002 of nucleotide substitutions per nucleotide. Red bold names indicate the strains detected in this study (Color figure online)

two specific mutations of the Cap protein (Y24C and P169T) were found in NNH/DN/2019 and NNH/BD/2019. They are unique to these Vietnamese strains. One mutation (F104Y) in the Cap protein was common between NNH/LD/2019 and MH603562 (Fig. 3). These two PCV3 strains were of subtype a1. PCV3 infections were reported in the northern part of Vietnam and those cases were found to be of the PCV3a subtype [17]. In the previous study, we reported the circulation of the PCV3b subtype in the southern provinces of Vietnam in particular (and in Vietnam in general) [23].

Conclusion

We found that the whole genomes of PCV3 collected in these Vietnamese pig farms shared 98.4–99.45% sequence identity with reference PCV3 sequences and clustered into two distinct subtypes (3a1 and 3b). Three variants (G172R, F211S, and L221P) were identified in the Rep protein and Y24C, P169T, and F104Y were identified in the Cap protein of these strains. In conclusion, our results indicate that PCV3 has emerged in swine herds in Vietnam and that this

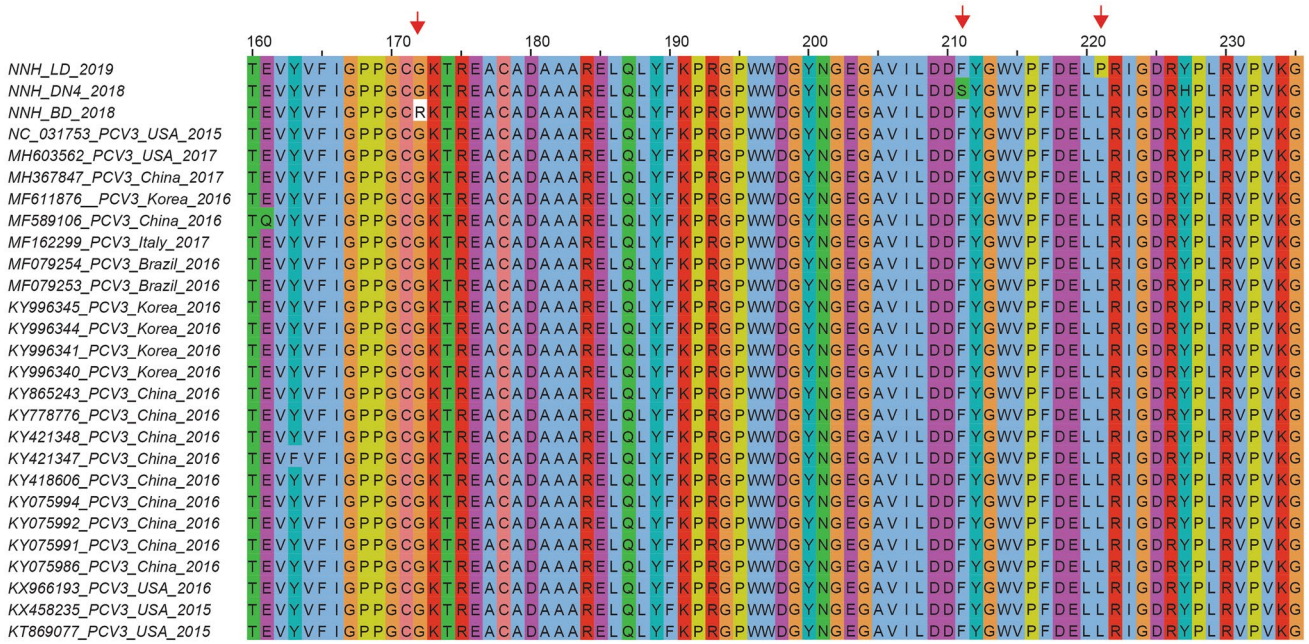

Fig. 2 Alignment of Rep protein sequences of the PCV3 strains of this study with reference sequences. The number line on the top row displays amino acid positions. Red arrows indicate the mutated sites of PCV3 strains in this study, relative to reference strains (Color figure online)

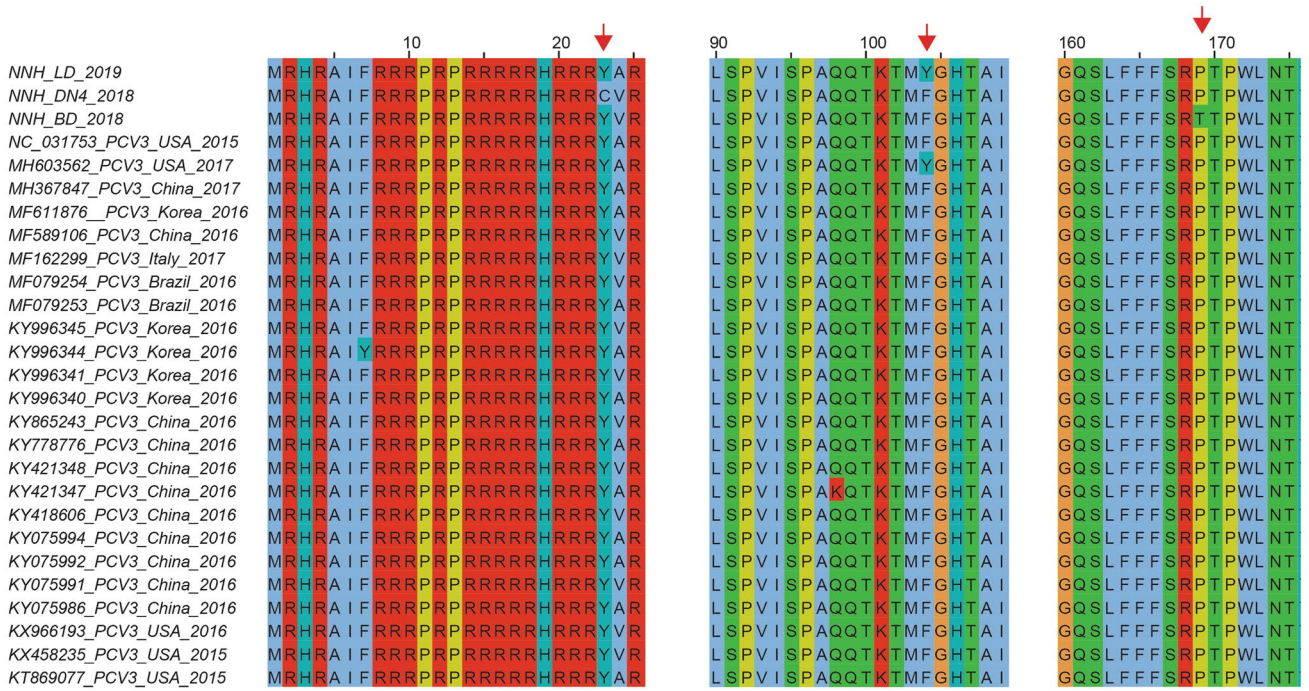

Fig. 3 Alignment of Cap protein sequences of the PCV3 strains in this study with reference sequences. The number line on the top row displays amino acid positions. Red arrows indicate the mutated sites of PCV3 strains in this study, relative to reference strains (Color figure online)

virus presents as the PCV3a1 and PCV3b subtypes. We hypothesize that infection with PCV3 may have associated with abortion in swine populations in Vietnam.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-021-02641-3.

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Author Contributions NHN, DDT, and MNN designed the study. TQN and TTN performed experiments. NHN and MNN analyzed the data. NHN, DDT, and MNN wrote the manuscript.

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Data Availability The complete genome sequences of the PCV3 strains identified in this study have been deposited in GenBank under the accession numbers MT847022, MT847024, and MT847031.

Declarations

Conflict of interest All authors have read the journal's policy on disclosures of potential conflicts of interest, and we declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval The study was conducted in compliance with the institutional rules for the care and use of laboratory animals and using a protocol approved by the Ministry of Agriculture and Rural Development (MARD) Vietnam (TCVN 8402:2010).

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