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BRIEF REPORT

# Genetic diversity in the capsid protein gene of porcine circovirus type 3 in Vietnam from 2018 to 2019

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## Abstract

Porcine circovirus type 3 (PCV3) was first detected in 2016 and has been reported in many pig-producing countries around the world, including Vietnam. PCV3 has been found in complex cases with multiple clinical syndromes in swine. In this study, we investigated the genetic diversity of PCV3 strains circulating in Vietnam. A total of 249 samples were collected from swine farms located in eight provinces of Vietnam, and 11.65% (29/249) of these samples were found to contain PCV3. The ORF2 genes from the 29 PCV3-positive samples were amplified, purified, and sequenced. Phylogenetic analysis showed that 23 of these strains belonged to the PCV3b subtype, while the remaining six strains belonged to subtype c and subtype a (a-1 and a-2). Analysis of the ORF2 genes indicated that the 29 PCV3 strains had high sequence identity (96.90-100% at the genomic level and 96.19-100% at the amino acid level). Fifteen amino acid substitutions were found in predicted B-cell epitopes in the capsid proteins of the Vietnamese PCV3 strains.

Porcine circovirus 3 (PCV3) was first reported in cases of porcine dermatitis, nephropathy syndrome, reproductive failure [1], and cardiac and multisystemic inflammation [2]. PCV3 was the only pathogen detected, and other general pig pathogens, such as porcine circovirus type 2 (PCV2), porcine pseudorabies virus (PRV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV), porcine rotavirus (RV), and classical swine fever virus (CSFV) were not detected. Subsequently, the involvement of PCV3 in diseases of pigs was found to be complex, with multiple clinical syndromes at different ages, including porcine dermatitis and nephropathy syndrome (PNDS) [2], reproductive problems [2–4], cardiac and multisystemic inflammation [2], porcine respiratory disease complex (PRDC) [5], and congenital tremors in neonatal pigs [6]. Accumulating evidence supports the assumption that coinfections with other pathogens might be associated with increased pathogenicity of PCV3 in pigs [1, 7], but although there have been several experimental studies, the role of PCV3 in pathogenesis is still uncertain [8].

PCV3 is circulating in many countries, including in the United States, China, Italy, Korea, Germany, Denmark, Spain, and Vietnam [3–6, 9, 10]. Vietnam is one of the top 10 pig-producing countries in the world. With swine producers already experiencing difficulties in coping with PCV2, there is great concern about the recent appearance of PCV3. Previously, we reported coinfection of PCV3 with pathogens that are possibly involved in PRDC, including PRRSV, PCV2, CSFV, and PRV [11, 12]. In 2016-2017, PCV3 strains from northern Vietnam were separated into

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PCV3a and PCV3b subtypes, with PCV3a-1 being the most prevalent [10]. In this study, to obtain an overview of the PCV3 situation in Vietnam, samples were collected from areas with a high density of swine production, and genetic analysis was performed.

A total of 249 samples were obtained during the period of 2018-2019 from different farms located in central and southern Vietnam. We collected 154 samples from 12- to 24-week-old pigs with typical manifestations of PRDC, 42 samples from sows, and 53 from aborted fetuses. Since PCV3 can be detected in different types of tissues [3], many types of samples were collected, including lymph nodes, lung, kidney, and/or serum from sows and fattening pigs and heart, liver, or lung from fetuses. All of the samples from each individual were pooled for virus detection by PCR. The nucleic acid in the samples was isolated using a Promega Wizard™ Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. PCR was performed to confirm the presence of PCV3, using primers and thermal cycling conditions reported previously [6].

To obtain the complete ORF2 sequence of PCV3, PCR products were analyzed by agarose gel electrophoresis and sequenced using primers specific for PCV3 ORF2 (R, 5'-CATCCATAATGGGATACCAC-3'; F, 5'-TCACTTAGA GAACGGACTTGTAACG-3'). The full-length nucleotide sequences of ORF2 obtained from Vietnamese PCV3 strains in this study were aligned using the CLUSTAL W package. Subsequently, a phylogenetic tree was generated in MEGA X, using the maximum-likelihood method with 1000 bootstrap replicates [13]. The amino acid sequences of all PCV3 capsid proteins were aligned using MegAlign software (DNASTAR, Inc. Madison, WI, USA) and visualized using Jalview 2.11.0 (www.jalview.org).

Twenty-nine out of 249 pigs from eight central and southern provinces of Vietnam were positive for PCV3 by PCR (Supplementary Table S1). We found that the positive rates of PCV3 in fetuses and sows in abortion cases were 22.64% and 14.29%, respectively, while that of pigs in PRDC cases was 7.14%. The virus prevalence at the herd level on the surveyed farms was about 11.65%, which was higher than that reported previously in northern Vietnam (4.44%) [10]. Higher PCV3-positive rates in several countries have been reported. PCV3 was detected on 12 of 14 farms (86%) in Poland [14], 53 of 73 farms (73%) in South Korea [9], and 24 of 35 farms (69%) in China [3]. Factors that potentially influence the prevalence of PCV3 include biosafety, biosecurity, management of pig flow, and environmental conditions, as well as individual factors such as existing herd immunity, the presence of microorganisms, and infection with immunosuppressive pathogens [14]. Since we also used tissues as the main sources of our specimens, the sampling technique does not seem to be the reason for the differences in positivity. Previous studies showed that herd size, the geographical location of the farm or piglet-producing unit, PCV2 infection, and vaccination status had no effect on PCV3 prevalence [3, 14]. In this study, PCV3 was detected in samples collected in eight provinces, and this, together with previous reports, indicates that PCV3 has spread widely in Vietnam.

Phylogenetic analysis based on the ORF2 sequences of the 29 strains from this study and 23 reference sequences showed that the Vietnamese PCV3 isolates grouped into three different subtypes (a, b, and c) (Fig. 1). More than 79% (23/29) of isolates in this study belonged to subtype b. In a previous study, two out of three strains from three southern provinces were found to belong to subtype PCV3b [15]. In another study, PCV3b was found in lung tissue of aborted fetuses from a swine-breeding farm in southern Vietnam [16]. These results suggest that PCV3b is the most prevalent subtype in central and southern Vietnam. Meanwhile, the five isolates in group a of this study clustered into two subtypes (a-1 and a-2), and the remaining strain belonged to subtype c (Fig. 1). This analysis showed the diversity of the PCV3 in central and southern Vietnam. In another study, five strains from northern Vietnam were found to belong to subtype PCV3a-1 [10]. Here, we report the first identification of PCV3 strains belonging to subtypes c and a-2 in Vietnam.

The ORF2 nucleotide sequences of the 29 PCV3 isolates in the study were analyzed and compared to those of reference strains. The nucleotide and amino acid sequence identity among the PCV3 isolates from Vietnam ranged from 96.90% to 100% and from 96.19% to 100%, respectively. Similar observations were reported in northern Vietnam [10]. Within subtype PCV3b, the 23 strains examined in this study exhibited 97.21-100% and 96.67-100% sequence identity at the nucleotide and amino acid level, respectively. They also shared a high level of nucleotide and amino acid sequence identity (97.52-99.53% and 97.64-100%, respectively) with reference PCV3 strains isolated in China (MN788140, KY421348, MF460446), Korea (KY996339), Italy (MF162299), and Brazil (MF079254). A similar degree of genetic similarity of a PCV3b strain to reference strains was also noted in our previous study [16]. In addition, the PCV3a strains in the study also showed a high degree of nucleotide and amino acid sequence similarity to each other (98.29-100% and 97.64-100% identity, respectively) and to reference isolates (97.67-99.84% and 97.16-100%) (Supplementary Table S2). The PCV3c strain from this study was very similar to the reference isolates MN510467, MT312226, and MG934301 (99.22-99.38% nucleotide and 99.06-99.53% amino acid sequence identity, respectively). This is consistent with our previous results [15].

We identified 15 amino acid substitutions in the Cap proteins of the Vietnamese strains in this study (Fig. 2). Three strains of subtype PCV3a-1 contained the substitutions R10K, F104Y, and S156T, which were in the predicted epitopes A, D, and E, respectively [17]. The mutation F104Y

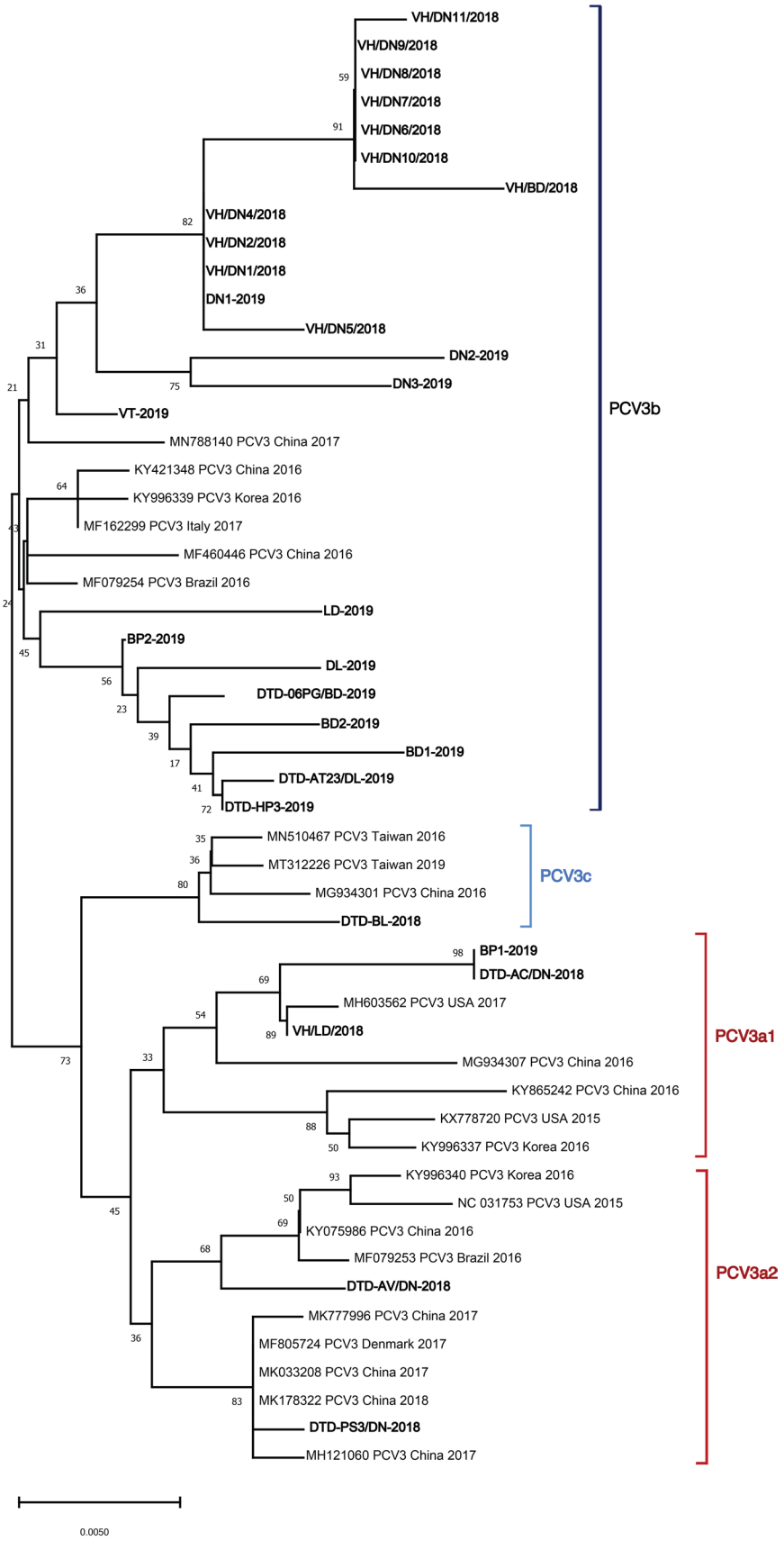


**Fig. 1** Phylogenetic tree based on ORF2 sequences of the isolates from this study and 23 reference sequences. The trees were constructed by the maximum-likelihood method in MEGA X with 1000 bootstrap replicates. The strains from this study are shown in bold.

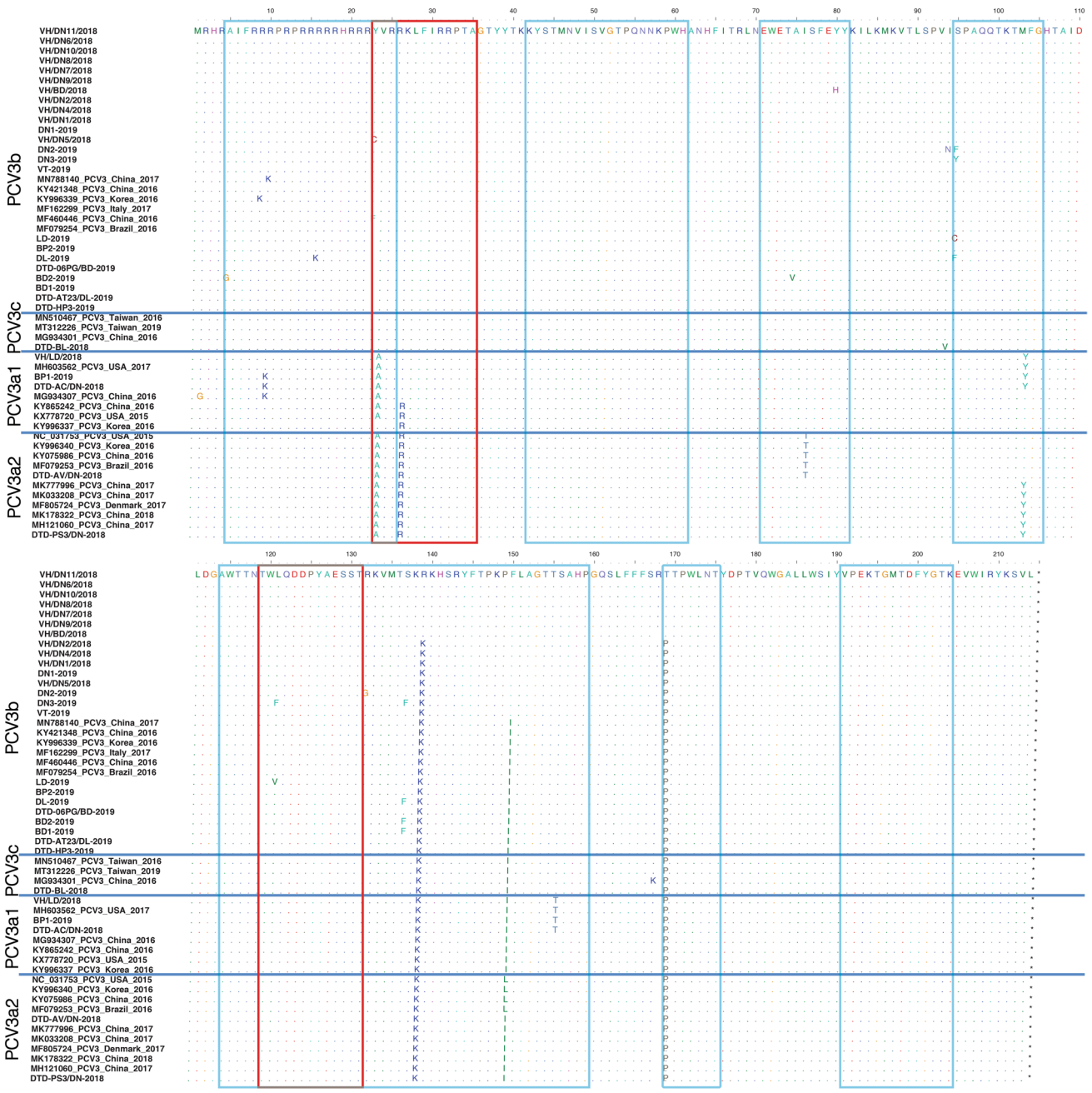


**Fig. 2** Mutations in the Cap protein of the PCV3 isolates from this study. The number line on the top row represents the positions of the amino acid residues. Red arrows indicate the amino acids that characterize the different subtypes of PCV3 found in this study. The green, red, blue, and purple boxes indicate the amino acid positions of B-cell epitopes in the NLS region [20], antibody recognition domains [18], B-cell epitopes, and T-cell epitopes [19], respectively. The grey areas represent *in silico*-predicted B-cell epitopes [17].

was also observed in the amino acid sequences of PCV3a-2 isolates, whereas the strain belonging to subtype c had only an I94V mutation in Cap. Meanwhile, the PCV3b isolates from this study contained 12 amino acid changes, including A5G (1/23), R16K (1/23), and Y23C (1/23) in the predicted epitope A; A75V (1/23) and Y80H (1/23) in the predicted epitope C; I94N (1/23) and S95F/C/Y (4/23) in the predicted epitope D; and L121F/V (2/23), R132G (1/23), S137F (4/23), K139R (7/23), and I150F (15/23) in the predicted epitope E [17]. In addition, two amino acid substitutions (Y23C and L121F/V) were identified in antibody recognition domains (amino acids 24-35 and 119-131) [18]. Six amino acid variations in predicted B-cell epitopes included amino acid positions 94, 95, 121, 132, 137, and 139, while

two amino acid changes (104 and 121) were found in T-cell epitopes [19]. Furthermore, there were two substitutions of amino acids 10 and 16 in B-cell epitopes of the PCV3 ??nuclear localization signal?? (NLS) region [20]. In our previous studies, there were only four amino acid changes in the Cap protein of PCV3 strains isolated in 2018, namely F104Y [15], K139R, I150F, and P169T [16]. The findings of the present study show that PCV3 seems to be gradually accumulating more mutations, which may pose potential challenges in disease control. PCV3 is distributed in the southern and central regions of Vietnam, and the prevalence of PCV3 at the herd level was determined to be 11.65%. Phylogenetic analysis of ORF2 sequences revealed that most of the PCV3 strains circulating in Vietnam belong to subgroup PCV3b, but some isolates were found to belong to subtypes c and a (PCV3a-1 and PCV3a-2). All PCV3 strains shared a high level of sequence similarity at the nucleotide and amino acid levels, but some amino acid substitutions were found in the Cap of PCV3 strains in the study.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05661-x.

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**Availability of data and material** The ORF2 sequences of the 29 PCV3 strains identified in this study have been deposited in the GenBank database under the accession numbers ON184728-56.

## Declarations

**Conflict of interest** The authors 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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