Virus Genes
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SHORT REPORT

Identification and whole-genome characterization of a novel Porcine Circovirus 3 subtype b strain from swine populations in Vietnam

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Abstract

Porcine circovirus 3 (PCV3) is a novel circovirus detected in pigs suffering from porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, and multisystemic infection. In this study, we identified PCV3 infection in aborted fetuses and reported the full-length genome sequence of a PCV3 strain identified from southern Vietnam. The complete genome of this PCV3 strain is 2000 nucleotides in length. We found that it shares 98.5–99.25% sequence identity with other reference sequences and that it clusters with the PCV3b subtype. Several specific mutated sites were found to be unique to this Vietnamese PCV3b strain, including I14M in the Rep protein and K139R, I150F, and P169T in the Cap protein. The sequence data that have been made publically available as part of this study will help investigators to better understand the molecular characteristics, genetic diversity, and evolutionary history of PCV3. Careful and in-depth investigations into the epidemiology, pathogenicity, and the evolution of this novel virus is a matter of urgent economic and agricultural interest in Vietnam.

Keywords PCV3 genotype · Porcine circovirus type 3 · Circovirus · Whole genome sequence · Abortion

Porcine circovirus 3 (PCV3) is an emerging circovirus detected in pig herds from many countries, including the USA [1], China [2], Poland [3], South Korea [4], the UK [5], Brazil [6], Thailand [7], Germany [8], Denmark [9], Spain [9], and Italy [9]. It is one of three members of the Circovirus genus, and its genome is a circular ssDNA containing approximately 2000 nucleotides with three identified ORFs [1]. ORF1 is located on the positive strand and encodes a protein (with 296–297 amino acids) that is involved in viral replication (Rep). ORF2 is located on the negative strand and codes for the capsid protein (Cap) [1, 10]. Genotyping of PCV3 reveals that it clusters into three subtypes: PCV3a, 3b, and 3c [8, 11, 12]. Here, we studied the PCV3 strain that circulates in a swine population in Vietnam, and comprehensively characterize and report its complete genome for the first time.

In late 2018, one sample from each of 13 farms was collected throughout three provinces (Dong Nai, Binh Duong, and Lam Dong) in the South of Vietnam. A PCV3 strain (NNH/DN8/2018) was identified from the lung of an aborted fetus collected from a swine-breeding farm in the Dong Nai province in southern Vietnam. Viral genetic material from lung samples was extracted using the GeneJET Viral DNA/RNA Purification Kit (Thermo, USA) according to the manufacturer's instructions. We tested for potentially related pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), Parvovirus, PCV2, and PCV3. RT-PCR was used to detect PRRSV using primers PRRS-P71 (GCTGTTAAACAGGGAGTGG) and PRRS-P72 (CGCCCTAATTGAATAGGTGAC) [13]. We searched for Parvovirus using PCR with primers PPV P1 (ATACAA

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Table 1 Nucleotide and amino acid sequence identities (%) between the genotypes in this study and those of reference sequences

Genbank accession no	Country	Isolated year	Identity (%)				
			Full genome	ORF1 (Rep gene)		ORF2 (Cap gene)	
				nt	aa	nt	aa
KT869077	USA	2015	98.70	99.10	99.32	97.67	97.20
KX458235	USA	2015	98.65	98.76	98.98	97.98	98.60
KX778720	USA	2015	98.50	98.99	99.32	97.51	97.67
KX966193	USA	2016	98.95	98.99	99.66	98.75	98.60
KY075986	China	2016	98.80	98.99	99.32	98.13	97.20
KY354038	China	2016	98.70	99.10	99.66	97.51	97.67
KY418606	China	2016	98.90	98.87	99.66	98.60	98.13
KY421347	China	2016	98.50	98.54	98.31	97.67	96.74
KY421348	China	2016	99.05	99.21	99.32	98.60	98.60
KY778776	China	2016	98.65	99.10	99.32	97.82	97.20
KY865242	China	2016	98.50	98.99	99.32	97.36	97.67
KY865243	China	2016	98.85	99.43	99.66	98.13	98.13
KY996337	Korea	2016	98.70	99.21	99.66	97.67	98.13
KY996342	Korea	2016	98.95	99.10	98.98	98.44	98.13
KY996345	Korea	2016	98.60	98.65	99.32	98.29	98.60
MF079253	Brazil	2016	98.65	98.76	98.98	97.98	97.20
MF079254	Brazil	2016	99.10	99.32	99.66	98.75	98.60
MF162298	Italy	2017	99.20	99.21	99.32	98.91	98.60
MF589106	China	2016	99.10	99.10	99.32	98.75	98.60
MF589107	China	2017	98.85	98.99	98.65	98.13	98.13
MF589652	Thailand	2016	99.25	99.43	99.66	98.75	98.60
MH367847	China	2017	98.65	98.76	98.98	97.82	98.13
MH603562	USA	2017	98.85	99.21	99.32	97.82	97.20
NC031753	USA	2015	98.70	99.10	99.32	97.67	97.20

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

TTCTATTTCATGGG) and PPV P6 (TATGTTCTGGTC TTTCCTCG) [14, 15]. We searched for PCV2 using PCR with primers CF8 (TAGGTTAGGGCTGTGGCCTT) and CR8 (CCGCACCTTCGGATATACTG). The genome of this PCV3 strain was then amplified by PCR using two primer pairs, including PCV3-74 F (CACCGTGTGAGT GGATATAC), PCV3-1144 R (CACCCCAACGCAATA ATTGTA) and PCV3-genome-2-F (TTGCACTTGTGT ACAATTATTGCG), and PCV3-genome-2-R (ATCTTC AGGACACTCGTAGCACCAC) [8, 11]. The PCR products were sequenced after being directly cloned into a pGEM-T Easy vector (Promega, USA). Sequence alignments were performed by Muscle. Phylogenetic analysis was conducted using Neighbor-Joining trees with the Maximum Composite Likelihood (ML) model in the software tool MEGA (version 10.1) [16, 17]. Bootstrap values were calculated using 1000 simulations [18]. The amino acid sequences of all PCV3 capsid proteins and replication proteins were aligned using the MegAlign software package, and visualization was perfomed using Jalview 2.11.0.

We harvested lung tissue of aborted fetuses from a swine-breeding farms in southern Vietnam and found that all 13 samples were positive for PCV3 and negative for Parvovirus. Four samples were positive for PCV2, and four samples were positive with PRRSV. Our results are consistent with what has been found in previous studies, wherein investigators document that PCV3 may infect alone or co-infect with other viruses, such as PCV2, PRRSV, among others [19, 20]. These findings suggest that PCV3 may play an etiologic role in the abortion of swine in Vietnam.

The complete genome of the PCV3 NNH/DN7/2018 strain was sequenced, and it is available in the GenBank database under the accession number MT847026. The full genome sequence of the PCV3 NNH/DN7/2018 strain was 2000 bases in length, with two ORFs encoding rep and cap proteins that are positioned in opposite directions [1]. Relative to current reference genomes, no insertions or deletions were found in the PVC3 NNH/DN7/2018 strain. The complete genome of the PCV3 NNH/DN7/2018 strain was found to share 98.5–99.25% sequence identity with other reference

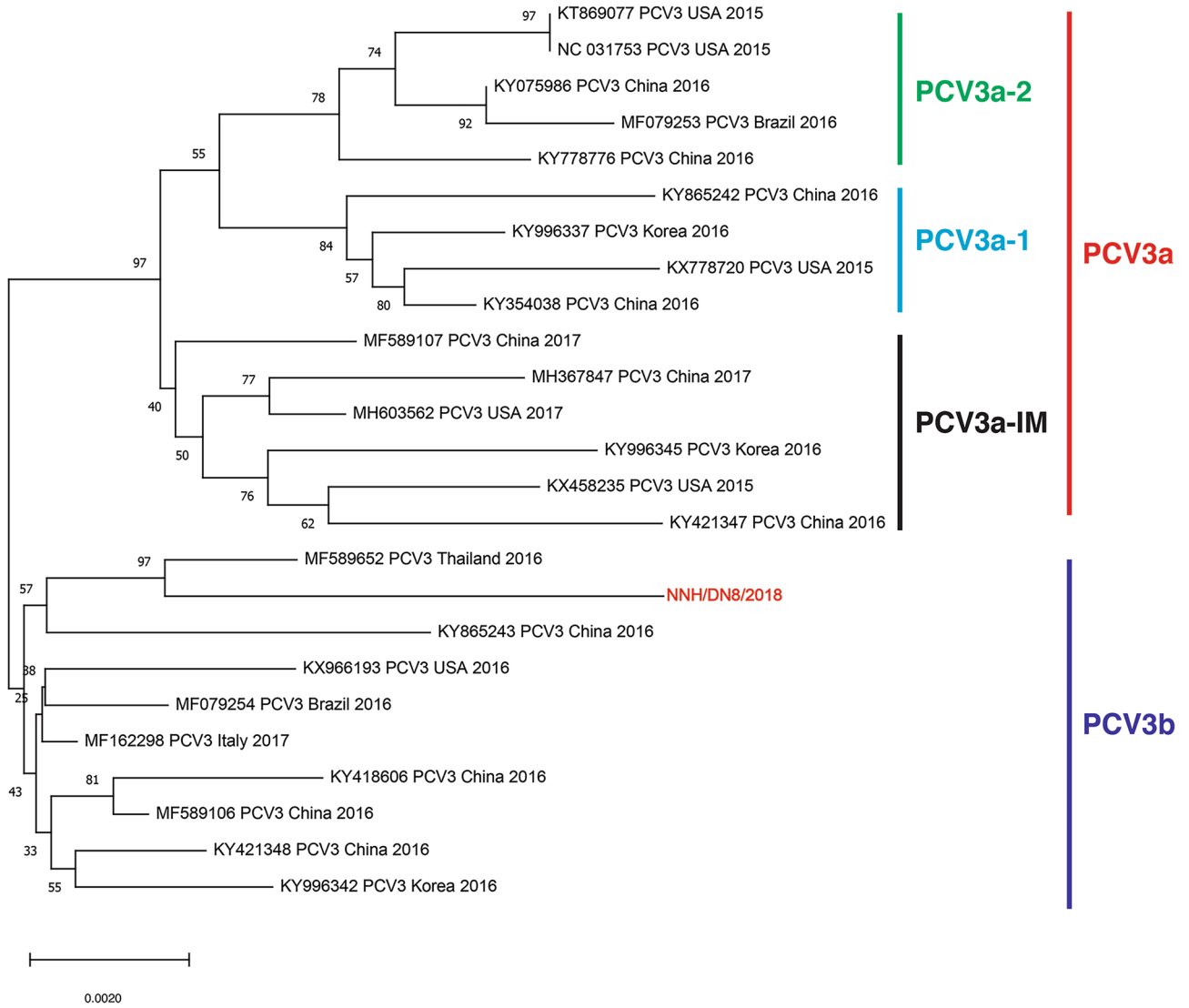

Fig. 1 Phylogenetic tree construction using the neighbor-joining method (based on the complete genome of PCV3). The neighbor-joining tree was constructed using the molecular evolutionary genetics analysis (MEGA) software version 10.1. Bootstrap values were obtained using 1000 resampled datasets of the data and are indicated on each node. The scale bar represents a genetic distance of 0.002 of nucleotide substitutions per nucleotide site. PCV3 strains obtained in the present study are displayed in red

sequences of PCV3 that have been circulating in the USA (strain NC_031753), Italy (strain MF162298), Brazil (strain MF079254), South Korea (strain KY996345), and China (strain KY865242) in recent years (Table 1). The ORF1 contained 891 nucleotides coding for 296 amino acids of the Rep protein and shared 98.54–99.43% and 98.31–99.66% nucleotide and amino acid identity with reference strains, respectively [1]. The ORF2 had 645 nucleotides encoding 214 amino acids of the capsid protein, and it was found to share 97.36–98.91% and 97.20–98.60% nucleotide and amino acid identity with reference genomes, respectively.

Phylogenetic analyses of the complete genome of PCV3 indicated that the PVC3 NNH/DN8/2018 strain clusters into the PCV3b subtype (Fig. 1). Furthermore, we identified one mutation (I14M) in the Rep protein of Vietnamese strains only (Fig. 2a). Three specific mutations (K139R, I150F, and P169T) were observed in the Cap protein of Vietnamese strains (Fig. 2b). Taken together, we found that the PCV3b subtype (along with its whole sequenced genome) currently circulating in Vietnam exhibits unique characteristics.

In this study, we first identified a PCV3b subtype circulating in Vietnam and reported its complete sequenced genome. Our findings, along with the sequence data that we have made available to the broader research community, may help to provide a better understanding of the molecular characteristics, epidemiology, and evolutionary history of PCV3 in Vietnam.

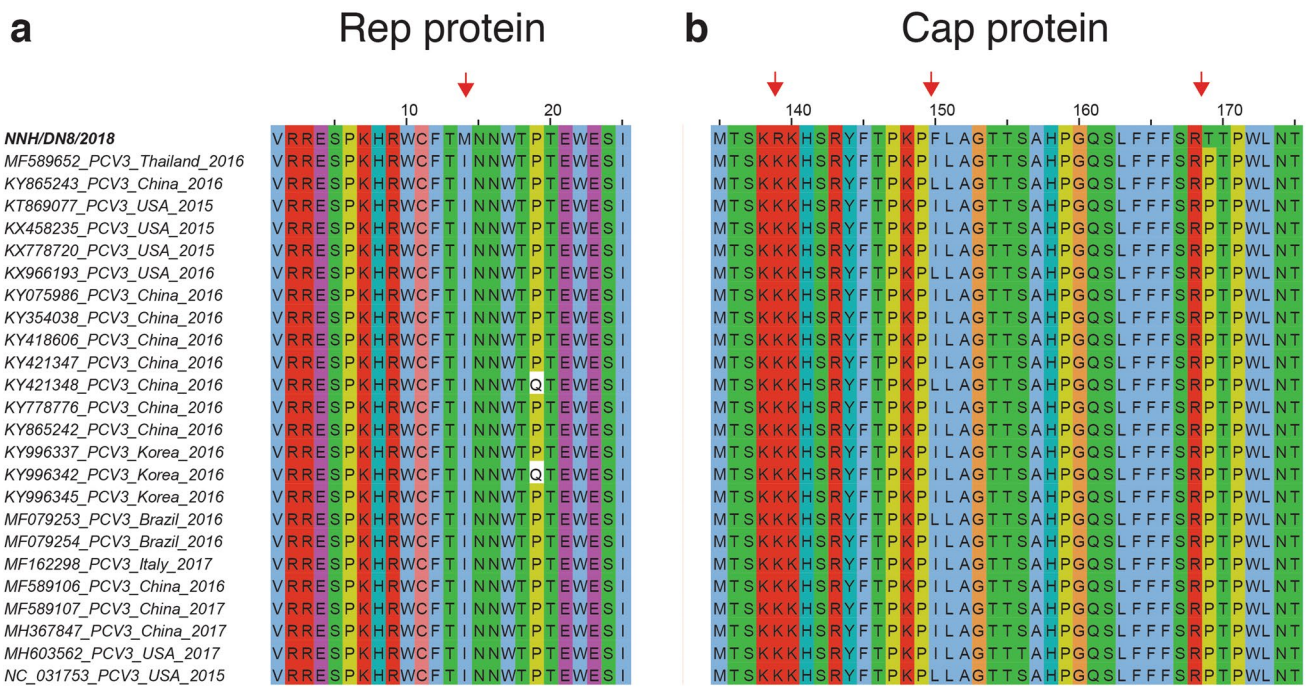

Fig. 2 Mutations within the Rep and Cap proteins of the PCV3 strains in this study. **a** Rep protein. **b** Cap protein. The number line on the top row represents the site of the amino acid residues. Red arrows indicate the mutations of PCV3 strains in this study, relative to reference strains

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Author contributions N.H.N, D.D.T and M.N.N. designed the study; T.Q.N and T.T.N performed experiments; N.H.N and M.N.N. analysed the data; N.H.N, D.D.T and M.N.N. wrote the manuscript.

Data availability The complete genome sequence of the PCV3 NNH/DN7/2018 strain has been deposited in GenBank under the accession number MT847026.

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 authors confirm that we have adhered to the ethical policies of the journal, as noted on the journal's author guidelines page. No ethical approval was required as this is a review article with no original research data.

References

1. Palinski R, Pineyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM (2017) A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol 91(1)
2. Shen H, Liu X, Zhang P, Wang L, Liu Y, Zhang L, Liang P, Song C (2018) Genome characterization of a porcine circovirus type 3 in South China. Transbound Emerg Dis 65(1):264–266
3. Stadejek T, Wozniak A, Milek D, Biernacka K (2017) First detection of porcine circovirus type 3 on commercial pig farms in Poland. Transbound Emerg Dis 64(5):1350–1353
4. Kwon T, Yoo SJ, Park CK, Lyoo YS (2017) Prevalence of novel porcine circovirus 3 in Korean pig populations. Vet Microbiol 207:178–180
5. Collins PJ, McKillen J, Allan G (2017) Porcine circovirus type 3 in the UK. Vet Rec 181(22):599
6. Tochetto C, Lima DA, Varela APM, Loiko MR, Paim WP, Scheffer CM, Herpich JI, Cerva C, Schmitd C, Cibulski SP, Santos AC, Mayer FQ, Roehe PM (2018) Full-genome sequence of porcine circovirus type 3 recovered from serum of sows with stillbirths in Brazil. Transbound Emerg Dis 65(1):5–9
7. Kedkovid R, Woonwong Y, Arunorat J, Sirisereewan C, Sangpratum N, Lumyai M, Kesdangsakonwut S, Teankum K, Jittimanee S, Thanawongnuwech R (2018) Porcine circovirus type 3 (PCV3) infection in grower pigs from a Thai farm suffering from porcine respiratory disease complex (PRDC). Vet Microbiol 215:71–76
8. Fux R, Sockler C, Link EK, Renken C, Krejci R, Sutter G, Ritzmann M, Eddicks M (2018) Full genome characterization of porcine circovirus type 3 isolates reveals the existence of two distinct groups of virus strains. Virol J 15(1):25
9. Franzo G, Legnardi M, Hjulsager CK, Klaumann F, Larsen LE, Segales J, Drigo M (2018) Full-genome sequencing of porcine circovirus 3 field strains from Denmark, Italy and Spain demonstrates a high within-Europe genetic heterogeneity. Transbound Emerg Dis 65(3):602–606
10. Phan TG, Giannitti F, Rossow S, Marthaler D, Knutson TP, Li L, Deng X, Resende T, Vannucci F, Delwart E (2016) Detection of

a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virol J 13(1):184
11. Ku X, Chen F, Li P, Wang Y, Yu X, Fan S, Qian P, Wu M, He Q (2017) Identification and genetic characterization of porcine circovirus type 3 in China. Transbound Emerg Dis 64(3):703–708
12. Ouyang T, Niu G, Liu X, Zhang X, Zhang Y, Ren L (2019) Recent progress on porcine circovirus type 3. Infect Genet Evol 73:227–233
13. Guarino H, Goyal SM, Murtaugh MP, Morrison RB, Kapur V (1999) Detection of porcine reproductive and respiratory syndrome virus by reverse transcription-polymerase chain reaction using different regions of the viral genome. J Vet Diagn Invest 11(1):27–33
14. Larochelle R, Antaya M, Morin M, Magar R (1999) Typing of porcine circovirus in clinical specimens by multiplex PCR. J Virol Methods 80(1):69–75
15. Oh WT, Kim RY, Nguyen VG, Chung HC, Park BK (2017) Perspectives on the evolution of porcine parvovirus. Viruses 9(8): 196
16. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
17. Stecher G, Tamura K, Kumar S (2020) Molecular evolutionary genetics analysis (MEGA) for macOS. Mol Biol Evol 37(4):1237–1239
18. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739
19. Chen N, Huang Y, Ye M, Li S, Xiao Y, Cui B, Zhu J (2019) Co-infection status of classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circoviruses (PCV2 and PCV3) in eight regions of China from 2016 to 2018. Infect Genet Evol 68:127–135
20. Klaumann F, Correa-Fiz F, Franzo G, Sibila M, Nunez JI, Segales J (2018) Current knowledge on porcine circovirus 3 (PCV-3): a novel virus with a yet unknown impact on the swine industry. Front Vet Sci 5:315