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Emergence of novel Porcine circovirus 2 genotypes in Southern India

Parthiban S[1] | **Ramesh A**[1] | **Anbu Kumar Karuppannan**[1] | **Dhinakar Raj G**[2] | **Johnson Rajeswar J**[3] | **Hemalatha S**[4] | **Jaisree S**[5] | **Senthilkumar K**[6] | **Balasubramanyam D**[6] | **Parthiban M**[7] | **Sarathchandra Ghadevaru**[8]

[1] Vaccine Research Centre-Viral vaccines, CAHS, MMC, Chennai, Tamil Nadu, India

[2] Director, Centre for Animal Health Studies, MMC, Chennai, Tamil Nadu, India

[3] Professor & Head, Dept. of VMC, VCRI, Tirunelveli, Tamil Nadu, India

[4] Professor, Dept of VPP, MVC, Chennai, Tamil Nadu, India

[5] Assistant Professor, Central University Laboratory, CAHS, MMC, Chennai, Tamil Nadu, India

[6] Post Graduate Research Institute in Animal Sciences, Kattupakkam, Tamil Nadu, India

[7] Professor & Head, Dept. of ABT, MVC, Chennai, Tamil Nadu, India

[8] Dean, Faculty of Basic Sciences, MVC campus, Chennai, Tamil Nadu, India

Correspondence
S. Parthiban, Assistant Professor, Department of Animal Biotechnology, Faculty of Basic Sciences, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007.
Email: parthis17@gmail.com

Abstract

A total of 200 samples from Porcine circovirus 2 suspected (n = 112) and healthy (n = 88) swine populations collected from different districts of Tamil Nadu, south India were used in this study. The samples comprising of serum (n = 124), swabs from natural orifices (n = 52), and postmortem tissues (n = 24). All the samples were processed and subjected to the screening and detection of the PCV2 genome by a specific PCR assay. PCV2 genomes from positive samples were further subjected to genotyping with specifically designed primers for the full-length amplification of the *ORF2* gene which codes for capsid protein (Cp) and serves as an epidemiological marker. Randomly, 13 amplified *ORF2* genes were sequenced and the aligned sequences were subjected to signature motif analysis and phylogeny in MEGA X. The molecular prevalence of PCV2 infection in Tamil Nadu is 10.5% (n = 21). Signature motif and phylogenetic studies of 13 samples revealed 38.5% (n = 5) presence of each PCV2b intermediate 1(IM1) and PCV2b genotypes, followed by 15.4% (n = 2) PCV2d-2 and 7.7% (n = 1) PCV2d genotypes. The PCV2b-IM1 genotype has a 99.43% sequence homology with Vietnam isolate (JX506730). PCV2b genotypes showed 99.72% sequence identity with Chinese isolate (KX068219). PCV2d-2 genotypes reported in this study have 100% sequence identity with Taiwan isolate (MF169721). PCV2d genotype showed 97.87% sequence identity with Thailand isolate (MF314293). Amino acid analysis of all the 13 full-length ORF2 gene sequences revealed specific mutations in the immune reactive domains of A, B, C, and D. Capsid protein of three PCV2b and five PCV2b IM1 isolates had extra amino acid residue lysine (K) at 234 position of ORF2 similar to PCV2d. For the first time in South India, PCV2b IM1 and PCV2d-2 genotypes are reported. This study evidences the genetic shifts of PCV2 isolates in India and it is analogous to that of global genotypic shift.

KEYWORDS
genotypic shift, genotyping, novel genotypes, PCV2, Tamil Nadu

Abbreviations: BLAST, Basic Local Alignment Search Tool; Cp, Capsid protein; MEGA, Molecular Evolutionary Genetics Analysis; MLT, Maximum Likelihood method; NCBI, National Center for Biotechnology Information; NJ, Neighbour-joining.; ORF, Open reading frame; PCR, Polymerase chain reaction; PCV, Porcine circovirus; PCVADs, Porcine circovirus associated diseases; PDNS, Porcine dermatitis and nephropathy syndrome; PMWS, Postweaning multisystemic wasting syndrome; PRDC, Porcine respiratory disease complex; UPGMA, Unweighted pair group method with arithmetic mean

1 | INTRODUCTION

The swine husbandry has a high potential for its expansion in India to meet the growing demand for food security. As per the 18th (2007), 19th (2012), and 20th (2019) Livestock census of India, the swine population is 11.13, 10.29, and 9.06 million, respectively. The population statistics of swine in India clearly show a steady decline in the swine population over the past 12-year period of time. The numbers of infectious diseases affecting the swine population impair health status and productivity; most of these diseases are emerging and remerging in nature. Porcine circoviruses (PCVs) are the smallest known vertebrate virus ranging 17–20 nm in size belonging to the genus *Circovirus* of the family *Circoviridae*. PCVs are non-enveloped single-stranded covalently closed circular DNA viruses with icosahedral nucleocapsid (Hamel et al., 1998). *Porcine circovirus 1* (PCV1) was originally discovered in 1974 as a cell culture contaminant and is non-pathogenic in nature (Tischer et al., 1982). In 1996, a distinct virus was recovered from post-weaning multisystemic wasting syndrome (PMWS) primarily affecting 6– to 15-week-old piglets and it is genetically and antigenically distinct from PCV1 named as *Porcine circovirus 2* (PCV2) (Ellis et al., 1998). PCV2 is ubiquitous in nature and most of the swine get exposed to it during their lifetime and results in subclinical and clinical infections. PMWS, porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), reproductive failure, enteric, necrotizing lymphadenitis, exudative dermatitis and congenital tremor are the globally reported clinical forms of PCV2 infection (Allan & Ellis, 2000; Clark, 1996; Harding, 2004; Kim et al., 2003; O'Connor et al., 2001; Opriessnig et al., 2007; Stevenson et al., 2001). Porcine circovirus associated diseases (PCVADs) is the term commonly used to denote all the clinical conditions associated with PCV2 infection (Opriessnig et al., 2007).

The genome of PCV1 and PCV2 contains 1759 and 1767–1768 nucleotides, respectively. PCV2 genes are arranged in 11 putative open reading frames (ORFs), but well-understood protein expressions are demonstrated for ORFs 1 to 6 alone. ORF1 codes for the *rep* gene and located on the positive strand in clockwise orientation encodes two non-structural replication proteins Rep and Rep′ 314 and 178 amino acids in length, respectively, involved in viral genome replication. ORF2 codes for *cap* gene and located on the negative (complementary) strand in counterclockwise direction encodes only structural capsid protein (Cp) with 702–705 bp in length coding for 233–234 amino acids and it is the most variable region in the PCV2 genome. Specific ligands in Cp bind to the target host cells through heparin sulphate and chondroitin sulphate B glycosaminoglycan receptors (Olvera et al., 2007). Signature motifs in Cp are the major epitopic determinants that elicit host immune response and serves as a molecular marker for PCV2 genotyping (Cheung, 2012). ORF3 is located on the complementary strand in counterclockwise orientation completely overlapping the ORF1 widely implicated in apoptosis, and it encodes a non-structural protein of 105 amino acids in length. ORF3-deficient PCV2 mutant was shown to be non-virulent in pigs compared to wild-type PVC2 (Karuppannan et al., 2009). ORF4 encodes a newly discovered protein with a role in suppressing caspase activity and regulating CD4+ and CD8+ T lymphocytes. ORFs 5 and 6 codes for non-structural proteins not essential for viral replication are involved in transcription of viral/host proteins for survival and immune evasion (Choi et al., 2018; He et al., 2013).

Molecular characterization of PCV2 isolates reveals eight distinct genotypes namely, PCV2a, PCV2b, PCV2c, PCV2d, PCV2e, PCV2f, PCV2g, and PCV2h as per modern genotyping methodology (Franzo & Segales 2018). PCV2a, PCV2b, and PCV2d are the most reported genotypes throughout the world and are shown to have five (2A–E), three (1A–C), and two (2d-1 and 2d-2) sub-genotypes, respectively (Xiao et al., 2015). PCV2c has been reported in Denmark and Brazil (Franzo et al, 2015) while PCV2e has been detected from China, Thailand, USA and Mexico (Davies et al., 2016). PCV2 is a highly mutating DNA virus with a mutation rate of 10^{-3} substitution/site/year that constantly generates novel genotypes or quasi-species by undergoing point mutations and genetic re-arrangement in natural and vaccine-induced immune responses to adapt and evade the immune system (Correa-Fiz et al., 2020). Since its first report, the predominant genotype was PCV2a; during 2003, the global genetic shift from PCV2a to PCV2b was reported in many geographical regions and PCV2b was the predominant genotype associated with many clinical and subclinical infections (Cortey et al., 2011; Franzo et al, 2015; Wang et al., 2020). From 2010 onwards, most of the global reports revealed genotype shift from PCV2b to PCV2d (Xiao et al., 2015, Mukherjee et al., 2018; Yao et al., 2019). These genotypic shifting associated with increased virulence and vaccine failures has raised the global alarm for PCV2 infections (Karuppannan & Opriessnig, 2017).

PCV2 was considered an emerging threat to the swine population of India in the recent past. In 2006, the first PCV2 infection was detected from cases of PMWS (Kumar et al., 2006). In Tamil Nadu, the incidence of PCV2 infection was first reported during 2012 from an organized piggery unit, associated with stillborn and weak piglets with the involvement of PCV2b genotype (Karuppannan et al., 2016). Anoopraj et al. (2015) investigated PCV2 genotype circulating in the Northern part of India based on ORF2 gene and complete genome and found the incidence of PCV2a–2D, PCV2b–1C, PCV2d genotypes and more interestingly two recombinant strains (PCV2Izn-89-13 and PCV2Izn-218-13) with the inter-genotypic recombination between PCV2a–2C and PCV2b–1C genotypes. Continuous emergence of PCV2 genotypes have been documented worldwide, but in the southern part of India, circulating PCV2 genotypes are yet to be explored. To understand the current molecular epidemiology of PCV2 genotypes in southern India, the present study of molecular genotyping of PCV2 is undertaken.

2 | MATERIALS AND METHODS

2.1 | Collection and processing of samples

A total of 200 samples from PCV2 suspected (n = 112) and healthy (n = 88) swine populations raised in organized and unorganized farms of different geographical regions of Tamil Nadu, India, were used in this study. These samples comprised serum (n = 124), swabs from natural orifices (n = 52), and postmortem tissues (n = 24). The samples were collected following standard protocol without causing any stress to the animals. The collected samples were immediately transported to the laboratory under refrigeration condition and processed as per the standard virological procedures and stored at −40°C until analyzed. The study was approved by the Institutional Biosafety Committee (IBSC) with the approval number Lr.No. 0023/DFBS/3/IBSC/2020) and followed all the environmental biosafety measures.

2.2 | Genomic DNA extraction and screening for PCV2 by specific Polymerase chain reaction assay

Genomic DNA was extracted from all the 200 samples using DNeasy Blood & Tissue Kit (Qiagen, Germany) following the standard manufacturer's instructions and used as a template for PCV2 screening. Molecular screening of PCV2 was performed as previously described (Larochelle et al., 1999) and positive sample produced specific band size of 264 bp. Polymerase chain reaction (PCR) reactions were performed in 25 μL reaction volume containing 2x Master Mix RED (Ampliqon, Denmark) with 10 pmol/μL of each primer. PCR condition includes initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 65°C for 1 min, 72°C for 1 min, and final extension step at 72°C for 10 min. Samples found positive in this screening PCR were further selected for full length PCV2 ORF2 amplification PCR.

2.3 | PCR amplification and sequencing of full-length ORF2 genomes

Twenty PCV2 published sequences from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) were retrieved and aligned in clustalW for locating consensus region. Primer pair was designed in the consensus region using PCV2 reference strain (AF027217) in Primer 3 tool to amplify full-length *ORF2* gene of PCV2. The designed forward and reverse primer sequences are 5′CCATGCCCTGAATTTCCATA3′ and 5′ ACAGCGCACTTCTTTCGTTT3′, respectively, and bind to the 960–976 and 1761-1742 nucleotide positions of PCV2 and generate a specific product of 802 bp covering the full-length *ORF2* gene of PCV2. The specificity of the designed PCR primers was confirmed by the National Center for Biotechnology Information—Basic Local Alignment Search (NCBI-BLAST) tool and sequence analysis of amplified targets. The PCR reaction was performed in 50 μL reaction volume containing 2x PCR red dye master mix (Ampliqon, Denmark) and 10 pmols/μL of each primer (Eurofins Genomics of India Pvt. Ltd). The PCR reaction condition consists of 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, 57°C for 45 s, 72°C for 45 s and the final extension at 72°C for 10 min and infinitive hold at 4°C. All the PCV2 positive field samples were further subjected to full-length ORF2 amplification using this designed primer. The samples which yield specific product in this PCR assay were randomly selected and sequenced by Sanger sequencing technology using 3730xl applied biosystems (ABI 3730xl) 96-capillary DNA analyzer at a commercial DNA sequencing facility (Eurofins Genomics of India Pvt. Ltd, Bangalore and Bioserve Biotechnologies (India) Pvt Ltd, Hyderabad).

2.4 | Genetic characterization of sequenced PCV2 capsid genomes

The specificity and homology of the sequences obtained in this study were studied in the NCBI-BLAST search. The contig sequence is aligned in BioEdit7.2, SnapGene tools. The aligned contigs and their deduced amino acid sequences were further subjected to the signature motif analysis (Table 3) along with representative major PCV2 genotypes. Conserved nucleotide signature motifs and their corresponding deduced amino acid sequences are the tenets used for the PCV2 genotype identification (Thangthamniyom et al., 2017; Xiao et al., 2015).

For comparative phylogenetic analysis, PCV2 sequences obtained in this study along with retrieved reference PCV2 ORF2 sequences from GenBank were aligned in clustalW and subjected to Molecular Evolutionary Genetics analysis X (MEGA X) tool for evolutionary analysis. The phylogenetic studies were undertaken by the Maximum Likelihood method (MLT) with the Tamura-Nei model with the highest log likelihood (Tamura & Nei, 1993), Neighbour-joining (NJ) method with a p-distance model (Saitou & Nei, 1987) and the unweighted pair group method with arithmetic mean (UPGMA) method (Sneath & Sokal, 1973) to infer evolutionary relatedness. All the three MLT, NJ and UPGMA methods of analyses were conducted in MEGA X using 1000 bootstrap replicates (Kumar et al., 2018). The isolates in this study were finally genotyped based on phylogenetic studies and signature motifs analysis.

3 | RESULTS

3.1 | Molecular detection of PCV2

Two hundred (200) samples from different regions of Tamil Nadu were subjected to molecular screening of PCV2 by specific PCR assay, out of which 21 samples were positive by showing a specific band size of 264 bp based on the stipulated target of the primer used (Figure 1). The overall positivity percentage is 10.5% with higher positivity in northern districts in comparison to the south districts of Tamil Nadu (Table 1).

3.2 | Genotyping and phylogenetic analyses of PCV2

All the 21 PCV2 positive samples yielded a specific amplicon of 802 bp in the full-length *ORF-2*-gene-based PCR assay (Figure 2). Randomly, 13 amplified *ORF2* genes were sequenced at commercial DNA sequencing facility (Eurofins Genomics of India Pvt. Ltd, Bangalore and Bioserve Biotechnologies (India) Pvt Ltd, Hyderabad). Assembly and

TABLE 1 District wise details of the clinical samples screened for PCV2

S.No	Name of the Districts with geographical location	No of samples collected	No of samples positive by PCR assay	Percent Positivity
1	Tirunelveli—South district	67	2	2.98
2	Kanchipuram—North district	47	7	14.89
3	Chennai—North district	35	10	28.57
4	Thanjavur—Central district	15	2	13.33
5	Tenkasi—South district	26	0	0
6	Kanyakumari—South district	10	0	0
	Total	**200**	**21**	**10.5**

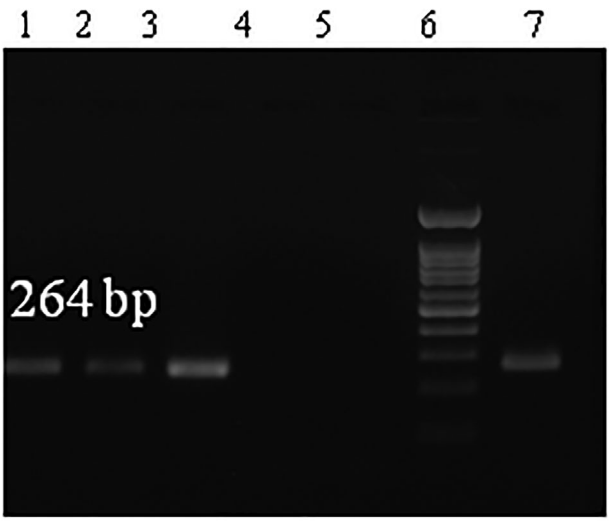


FIGURE 1 Genome based screening of PCV2 infections by specific PCR assay. Lane 1 to 4 includes field samples; lane 5 is non-template control; lane 6 is 100 bp ladder; lane 7 is known PCV2 positive DNA. The specific band size for PCV2 is 264 bp and labelled separately

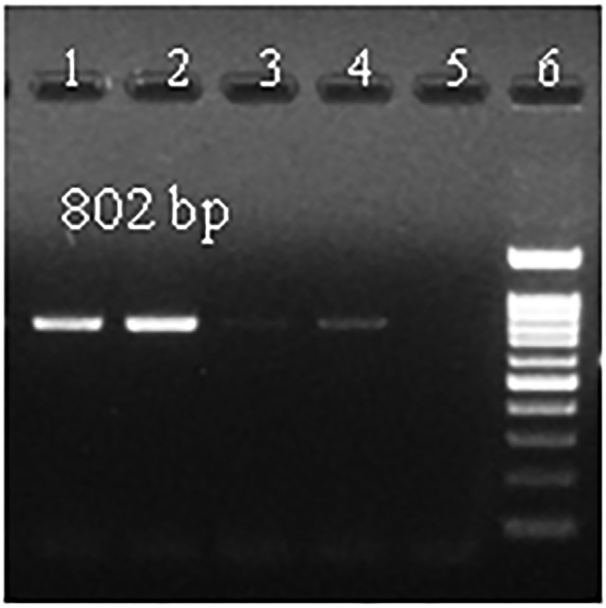


FIGURE 2 Amplification of the full length ORF2 gene using specifically designed PCV2 primer. Lane 1 to 3 includes field samples; lane 4 is known PCV2 positive DNA; lane 5 is non-template control; lane 6 is 100 bp ladder. The specific band for ORF2 genome is 802 bp and labelled separately

annotation of 13 sequences revealed 702 nts for 5 isolates and 705 nts for 8 isolates encoding 233 or 234 amino acids. All the 13 full length ORF2 sequences obtained in this study were submitted to the GenBank under the accession numbers MW125672 to MW125684.

Phylogenetic analysis of thirteen full-length ORF2 sequences obtained in this study along with 33 published PCV2 ORF2 sequences and 1 PCV1 out-group sequence (Table 2) by MLT, NJ and UPGMA method in MEGA X yielded similar clustering pattern (Figure 3). Five PCV2 sequences (TNTHANF12, TNTHANF14, TNCHN56-4T, TNCHN56-F251, and TNCHN51-M694) in this study clustered along with PCV2b genotypes; five PCV2 sequences (TNCHN51-L3, TNCHN51-L4, TNCHN51-L5, TNCHN51-L6, and TNCHN51-L9) in this study formed a separate cluster along with intermediated PCV2b-IM1 strains, two sequences (TNCHN51-L8 and TNCHN56-Still3) clustered along with PCV2d-2 sequences and one sequence (TNCHN51-L7) clustered along with PCV2d genotype. Signature motif analysis of all the 13 PCV2 sequences obtained in this study along with 18 published PCV2 sequences revealed 38.5% (n = 5) of each PCV2b intermediate clade 1(IM1) and PCV2b genotypes, followed by 15.4% (n = 2) of PCV2d-2 genotype and 7.7% (n = 1) of PCV2d genotype (Figure 4). All the five PCV2b isolates in this study had three unique amino acid mutations at residues 57 (V→I), 63 (R→K), and 200 (T→I) in comparison to established PCV2b isolates. Besides, three PCV2b isolates (TNCHN56-4T, TNCHN56-F251, and TNCHN51-M694) had an additional amino acid residue lysine (K) at 234 position of ORF2. All the five PCV2b-IM1 clusters reported in this study had three unique amino acid substitution at residues 131(T→P), 190–191(TG/AG→SA), 232 (N/K→H) with extended amino acid lysine (K) at 234 position as like that of published PCV2b-IM1 genotypes. Two PCV2d-2 sequences reported in this study had two amino acid mutations at residues 105 (F→L) and 188 (Q→H) (Figure 3). The evolving PCV2b-IM1 genotypes in this study had more than 99.43% sequence homology with the Vietnam isolate (GenBank Accession Number. JX506730). All the

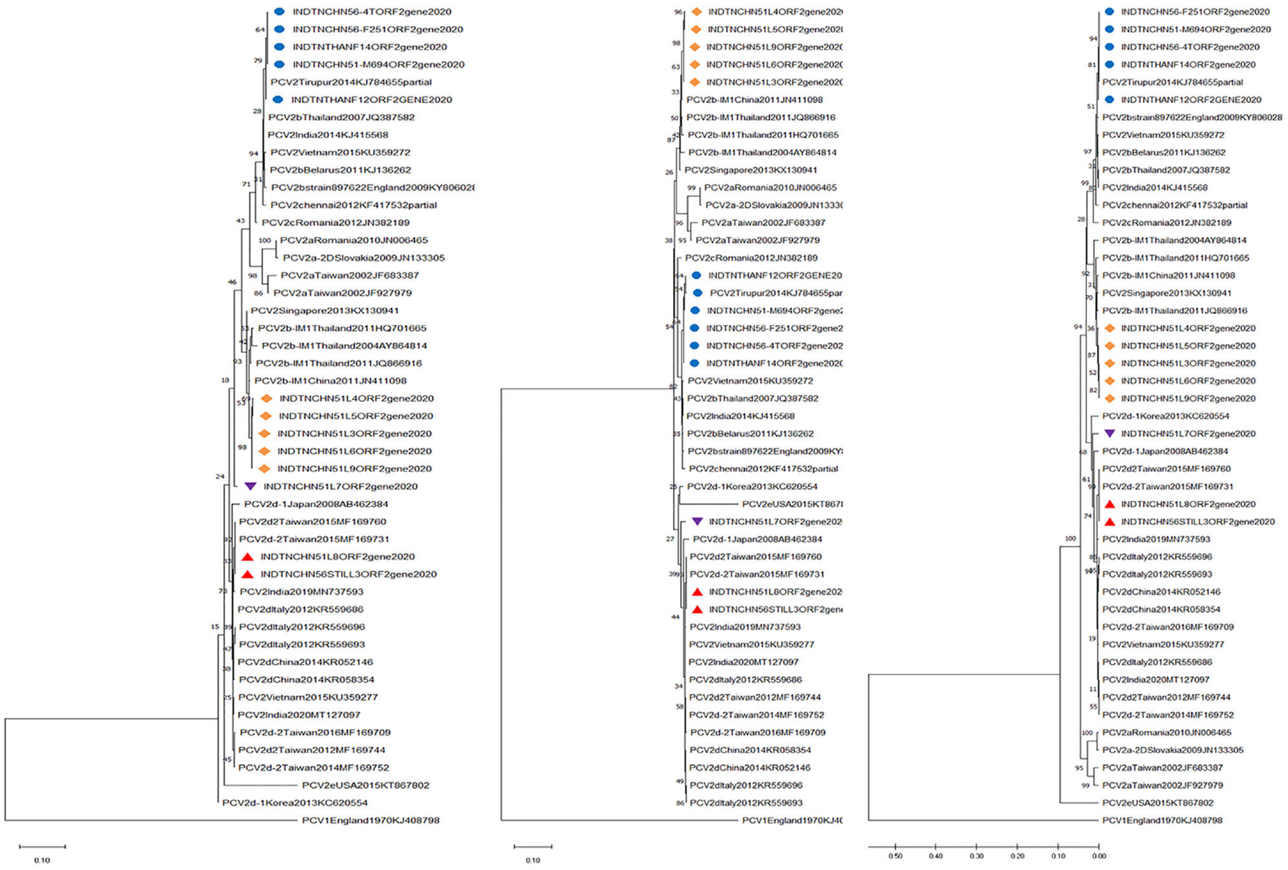
TABLE 2 List of reference PCV2 sequences retrieved from GenBank and used in phylogenetic analysis

Sl. No.	Accession No.	Genotype	Country	Year of submission
1.	JN006465	PCV2a	Romania	2010
2.	JF683387	PCV2a	Taiwan	2002
3.	JF927979	PCV2a	Taiwan	2002
4.	JN133305	PCV2a-2D	Slovakia	2009
5.	KJ136262	PCV2b	Belarus	2011
6.	JQ387582	PCV2b	Thailand	2007
7.	KR058354	PCV2d	China	2014
8.	KR052146	PCV2d	China	2014
9.	KR559696	PCV2d	Italy	2012
10.	KR559693	PCV2d	Italy	2012
11.	KR559686	PCV2d	Italy	2012
12.	MF169760	PCV2d-2	Taiwan	2015
13.	MF169744	PCV2d-2	Taiwan	2012
14.	KY806028	PCV2b	England	2009
15.	MF169752	PCV2d-2	Taiwan	2014
16.	MF169731	PCV2d-2	Taiwan	2015
17.	MF169709	PCV2d-2	Taiwan	2016
18.	KT867802	PCV2e	USA	2015
19.	JN382189	PCV2c	Romania	2012
20.	KJ784655	PCV2	India	2014
21.	KF417532	PCV2	India	2012
22.	AY864814	PCV2b-IM1	Thailand	2004
23.	JQ866916	PCV2b-IM1	Thailand	2011
24.	HQ701665	PCV2b-IM1	Thailand	2011
25.	JN411098	PCV2b-IM1	China	2011
26.	KU359277	PCV2	Vietnam	2015
27.	JQ387582	PCV2b	Thailand	2007
28.	AB462384	PCV2d-1	Japan	2008
29.	KU359272	PCV2	Vietnam	2015
30.	KX130941	PCV2	Singapore	2013
31.	KJ415568	PCV2	India	2014
32.	MN737593	PCV2	India	2019
33.	MT127097	PCV2	India	2020
34.	KJ408798	PCV1	England	1970

PCV2b genotypes in this study showed 99.72% sequence identity with Chinese isolate (KX068219). PCV2d-2 genotype reported in this study has 100% sequence identity with Taiwan isolate (MF169721). PCV2d genotype showed 97.87% sequence identity with Thailand isolate (MF314293).

4 | DISCUSSION

PCV2 is an economically important emerging viral pathogen in the global swine community associated with multiple clinical and subclinical outcomes. The infected pigs may act as potential virus shedders and disseminate virus into the environment (Brunborg et al, 2004). Most of the PCV2 reported cases in India are from the northern and north-eastern parts, except for the few incidences reported from southern India. This study elucidates PCV2 genotypes circulating in the southern part of India with sizable samples. Molecular screening for PCV2 genome in this study revealed 10.5% positivity whereas 20.41% and 16.4% genomic positivity for PCV2 were reported from Meghalaya (Mukherjee et al., 2018) and Mizoram (Varte et al., 2018), respectively. The prevalence report of PCV2 in the southern part of India is limited in comparison to the northeastern part of India; this may be due to the



a. Maximum Likelihood method b. Neighbor-Joining method c. UPGMA method

FIGURE 3 Phylogenetic analysis based on ORF2 genome sequences using: (a) Maximum Likelihood method, (b) Neighbour-Joining method, and (c) UPGMA method. The trees were drawn with 13 PCV2 sequences from this study (indicated in coloured triangle, circles, rectangle and shapes) and 33 reference PCV2 sequences from the GenBank, including one PCV1 sequence as an out-group. The analyses were conducted in MEGA X with Bootstrap replicates of 1000

TABLE 3 Signature motifs on ORF2 of major PCV2 genotypes

PCV2 Genotype	Signature motif with amino acid positions in the capsid protein of PCV2	
	89–90	190–191
PCV2a	IS	SR
PCV2b	RS	AG or TG
PCV2b-IM1	LT	SA or TG
PCV2d	LT	TG or TA

Note: Alphabet is the specific code of particular amino acid; A, Alanine; G, Glycine; I, Isoleucine; L, Leucine; S, Serine; R, Arginine; T, Threonine.

lowest population density of swine with varying agro-climatic conditions in southern India. Signature motifs and phylogenetic analysis of 13 PCV2 ORF2 full-length sequences in this study revealed the presence of PCV2b, PCV2b-IM1, PCV2d, and PCV2d-2 genotypes circulating in the field. The prevalence of PCV2 infections in India is raising gradually with the involvement of PCV2a, PCV2b, PCV2d, and recombinant genotypes in the field (Anoopraj et al., 2015; Bhattacharjee et al 2015; Karuppannan et al., 2016, Pegu et al., 2017; Mukherjee et al., 2018). Thangthamniyom et al. (2017) studied the genetic diversity of PCV2 in Thailand using full-length ORF2 sequences. Their results revealed a high prevalence of PCV2d, PCV2b and PCV2b IM1 genotypes, thereby supporting findings from this study. Many reports documented that PCV2b-1C genotypes are now classified as either PCV2d or PCV2b-IM1 genotypes because the genetic cutoff value of this genotype is less than 0.035; this shows the close relationship between PCV2b, PCV2b-IM1 and PCV2d genotypes (Jantafong et al., 2011; Xiao et al., 2015). In the PCV2 evolutionary chain, PCV2d would have been originated from PCV2b and PCV2b-IM1 might be the intermediate cluster between PCV2b to PCV2d evolution process. Two PCV2d-2 genotypes reported in this study were obtained from the PCV2 vaccinated herd. In the recent past, PCV2d-2 is the most reported genotype in vaccinated herds of swine worldwide than PCV2b (Xiao et al., 2016). Vaccination-induced host immune responses may force the pathogen to alter its genetic composition.

Four antibody recognition domains have been recognized for PCV2 capsid protein namely A, B, C & D located at 51 to 84, 113 to 132, 169 to 207, and 228 to 233 positions, respectively (Khayat et al., 2011). Spe-

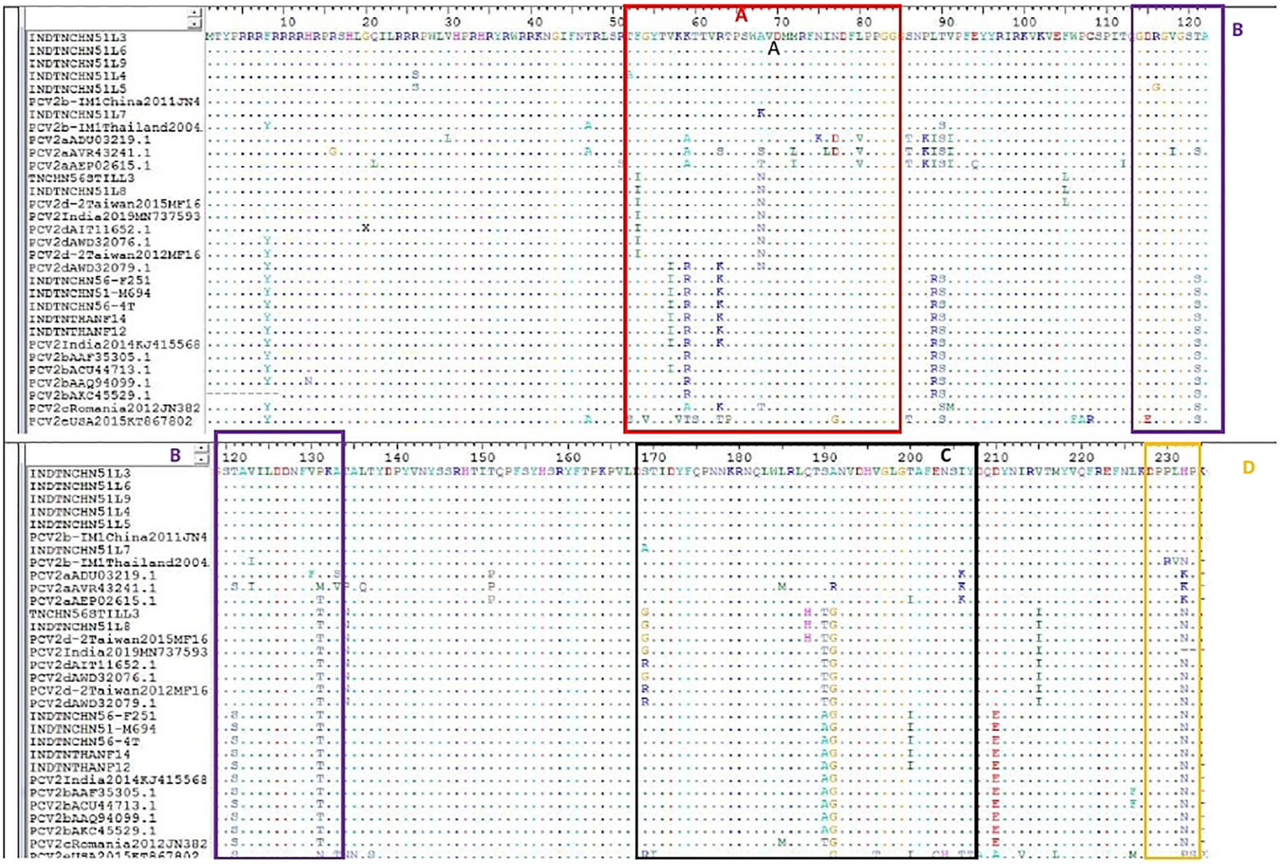


FIGURE 4 Alignment of deduced amino acid sequences of PCV2 ORF2 gene. This alignment included deduced amino acid sequences of ORF2 from 13 PCV2 sequences from this study and 18 PCV2 reference sequences of different genotypes from Genbank. All the sequences were aligned in ClustalW and viewed in BioEdit sequence alignment editor tool. Four antibody recognition domains namely A, B, C and D located at 51 to 84, 113 to 132, 169 to 207, and 228 to 233 positions, respectively, were demarcated by the coloured boxes, in red, purple, black and orange, respectively

cific mutations observed in this study are mostly at varying residues of immunoreactive domains A to D. Change in amino acid composition at these sites may alter virus characters like virulence, tissue tropism, and immune response (Saha et al., 2012). PCV2 genotypes are evolving through genetic recombination and point mutation and the evolution is as rapid as that of RNA viruses (Firth et al., 2009). Prevalence of PCV2b IM1, PCV2d, and PCV2d-2 genotypes in southern India indicates the PCV2 genotypic shift in circulating strains. Barman et al (2018) documented PCV-2d genotype associated field infections in northeastern states like Meghalaya, Mizoram, Assam, and Tripura and evidenced PCV2 genotype shifting in northern India. PCV2b-IM1, PCV2b, PCV2d-2 and PCV2d genotypes circulating in Tamil Nadu, a state in southern India also allied to that of prevailing global PCV2 genotypic shifting. The rapid evolution has increased the genetic variation of PCV2 and enables PCV2 to spread quickly worldwide. PCV2b-IM1, PCV2b, PCV2d-2 and PCV2d genotypes reported in this study shares sequence identity of more than 95% with Vietnam, Chinese, Taiwan and Thailand isolates, respectively. Similarly, PCV2b and PCV2d genotypes in Thailand are also closely related to the Vietnamese and Chinese isolates, respectively (Thangthamniyom et al., 2017). PCV2 genotypes circulating in field conditions are essentially to be monitored in order to elucidate the impact of this emerging pathogen in India in comparison to neighbouring countries. The movement of swine at international borders plays important role in virus transmission; hence, it is essential to monitor the distribution and genetic diversity of PCV2 in the swine community in the different geographical regions. The candidate vaccine based on prevailing genotype paves the way to combat this emerging pathogen.

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CONFLICT OF INTEREST

All the authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

AUTHOR CONTRIBUTION

The research was done by PS as a part of his Ph.D. research project. PS, RA, AK, DG and HS designed the study and supervised the research. PS, RA, AK and JJ worked and collaborated in the lab work. JS, SK, BD helped in sample collection and transport. PS, RA and AK compiled results as well as the manuscript. PM and GS critically reviewed the manuscript. All authors read and approved the final manuscript.

ORCID

Parthiban S https://orcid.org/0000-0002-9852-4245

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