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REGULAR ARTICLES

Molecular detection of porcine parvovirus 1–associated reproductive failure in southern India

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Abstract

This study used 56 aborted and stillborn fetuses from organized swine farms in Tamil Nadu and Kerala, southern states of India. All samples were screened by using a PCR assay that targets the *NS1* gene for PPV. Furthermore, the PCR positive samples were subjected to amplification of the VP2 gene of PPV1 with designed primers and sequenced for further study. The PCR screening of 56 samples found that 14.3% ($n=8$) were positive for PPV genome. According to *VP2* gene–based PCR for PPV1, 897 bp specific amplicons were detected in all eight of the samples. Two of the eight positive samples (L17 and T5) were sequenced and annotated randomly. The BLAST analysis of contig sequence INDTNCHN-T5 revealed 100% sequence homology with Chinese PPV1genome, whereas sequence from INDTNCHN-L17 revealed 99.43% sequence homology with Spain, Chinese, and German. PPV1 sequences and both the sequences INDTNCHN-T5 and INDTNCHN-L17 were submitted to the GenBank under the accession numbers MW822566 and MW822567 respectively. A phylogenetic analysis of the sequences in this study revealed specific grouping along with PPV1 strains in cluster E. Amino acid analysis of both isolated sequences in addition to the reference sequence from PPV1 showed variations in position 215 (I to T) in both the isolates, variation at position 228 (Q to E) in T5 isolate and variations at position 59 (L to M) and 314 (K to E) in L17 isolate. This study represents the first report of PPV1 cluster E in Tamil Nadu, southern India.

Keywords Reproductive failure · Porcine parvovirus 1 · Molecular detection · Characterization

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Abbreviations

PPV	Porcine parvovirus
ORF	Open reading frame
MEGA	Molecular evolutionary genetics analysis
SMEDI	Stillbirth mummification embryonic death and infertility
NCBI	National Center for Biotechnology Information
BLAST	Basic local alignment search tool
PCR	Polymerase chain reaction
MLT	Maximum likelihood method

Introduction

Porcine Parvovirus 1 (PPV1) is one of the prime causative agents associated with SMEDI (stillbirth, mummification, embryonic death, and infertility) syndrome, which causes marked loss to the swine industry worldwide (Mengeling et al. 2000). PPV was first isolated as a cell culture

contaminant from a porcine primary cell culture which was used for propagation of classical swine fever virus in Germany during the early 1960s (Mayr and Mahnel 1964). The incidence of PPV associated with abortions in swine was first described by Carwright and Huck (1967). Parvoviruses are small, non-enveloped, single-stranded, and negative-sense DNA virus that belongs to the family *Parvoviridae* (Molitor et al. 1984). Apart from classical PPV1, six novel porcine parvoviruses (PPV2–PPV7) were described in the past two decades (Palinski et al. 2016). As per International Committee on Taxonomy of Viruses (ICTV) classification, PPV1 belongs to the genus *Protoparvovirus*, whereas novel parvoviruses PPV2-PPV3, PPV4–PPV6, and PPV7 belong to the genera *Tetraparvovirus, Copiparvovirus*, and *Chapparvovirus* respectively (Xing et al. 2018). Currently, PPV1 is a well-evidenced and documented pathogen associated with reproductive failure in many swine producing countries (Zhang et al. 2010). All the newer PPV genotypes were detected in China, the USA, and Poland (McKillen et al. 2007). Incidence of PPV2, PPV3, and PPV4 is well documented in Hungary, Romania, Thailand, Japan, and South Africa (Cadar et al. 2013; Cságola et al. 2012; Saekhow and Ikeda 2015; Saekhow et al. 2016; Afolabi et al. 2018). PPV6 was reported to be co-infected with PCV2, PRRS viruses, etc. and causes abortion in pregnant sows (Schirtzinger et al. 2015). PPV7 was first discovered in 2016 from adult pigs in the USA and subsequently in Poland and Korea (Ouh et al. 2018). Unlike PPV1, the importance of novel porcine parvoviruses on swine health is poorly understood and these novel viruses were detected in healthy as well as suspected swine populations (Boisvert et al. 2010). There is a paucity of information on host spectrum and pathogenic potential of novel PPV2-7 genotypes (Xiao et al. 2013).

The PPV1 genome encodes for two open reading frames (ORFs), *ORF1* found at the 5′ end of genome, and codes for three non-structural (NS) proteins (NS1, 2, and 3) and plays a vital role in viral replication. The *ORF2* gene which is located at the 3′end of the viral genome codes for three structural capsid viral proteins (VP1, 2, and 3). The larger VP1 and smaller VP2 are translated from a nested set of coding sequences and VP2 is produced by splicing from VP1, differing only in their amino terminus. There are 729 amino acid residues in VP1, of which 120 amino acids at amino-terminal sites are unique to VP1. VP3 is a post-translational modification product of VP2. These structural proteins help the virus to recognize and bind to the host cell nucleus and establish infection (Bergeron et al. 1996; Simpson et al. 2002). This is one of the mutating DNA viruses with $3–5 \times 10^{-4}$ and 1×10^{-5} mutations/site/year were observed in *VP* genes and *NS* genes respectively. *NS1* gene was highly conserved among different PPV genotypes, whereas the VP2 gene was not much conserved. VP2 region is the major protective antigen and contains major antigenic domains that elicit neutralizing antibodies (Truyen and Streck 2012; Mengeling 2006; Martínez e al. 1992). Classical porcine parvovirus 1 has been the most studied virus around the globe as an important cause of porcine fetal death. PPV1 infection during the first 70 days of gestation can lead to reproductive failure and infection in post 70 days of gestation with immune-competent fetuses, develop an antibody response, and usually survive the infection. Two weeks after the initial infection of the dam, the virus spreads vertically to the fetus and establishes infection (Mengeling et al. 1980). It is one of the sturdiest virus found in the swine community it even withstands temperature of 90 °C. The virus particles resist common disinfectants like 70% ethanol, 0.05% quaternary ammonium compounds, low concentrations of sodium hypochlorite, and 0.2% peracetic acid. Affected animal sheds viruses in the environment through their natural orifices and the virus remains intact in farm premises and tools for months together and favors natural horizontal transmission of virus (Truyen and Streck 2012). At present, abortion and stillborn conditions in swine populations are alarming in south India, but in Tamil Nadu as of date, there is no documented evidence on PPV incidence. As per literature survey in southern India, there is only one PPV molecular incidence report documented from Kerala (Aishwarya et al. 2016). PPV molecular characterization and phylogenetic studies are scarce in southern states of India; hence, this study has been undertaken.

Materials and methods

Collection and processing of samples

A total of 56 tissue samples (pool of visceral organs, lymphoid organs, and placental tissues) were aseptically collected from aborted/mummified/dead fetuses of swine (Fig. 1) from different parts of Tamil Nadu and Kerala during the period 2020–2021. Collected tissues were triturated in a pestle and mortar with sterile sand in phosphate-buffered saline (PBS). Homogenized tissues were clarified at 6000 rpm for 15 min at room temperature to sediment all tissue debris. Supernatants were subjected to DNA extraction using DNeasy Blood and Tissue Kit (Qiagen, Germany) following the standard manufacturer's protocol and extracted nucleic acid was used as a template for screening PPV genome.

Molecular screening of PPV nucleic acid

Fifty six tissue samples were subjected to PPV detection using forward and reverse primer sequences 5′AGT

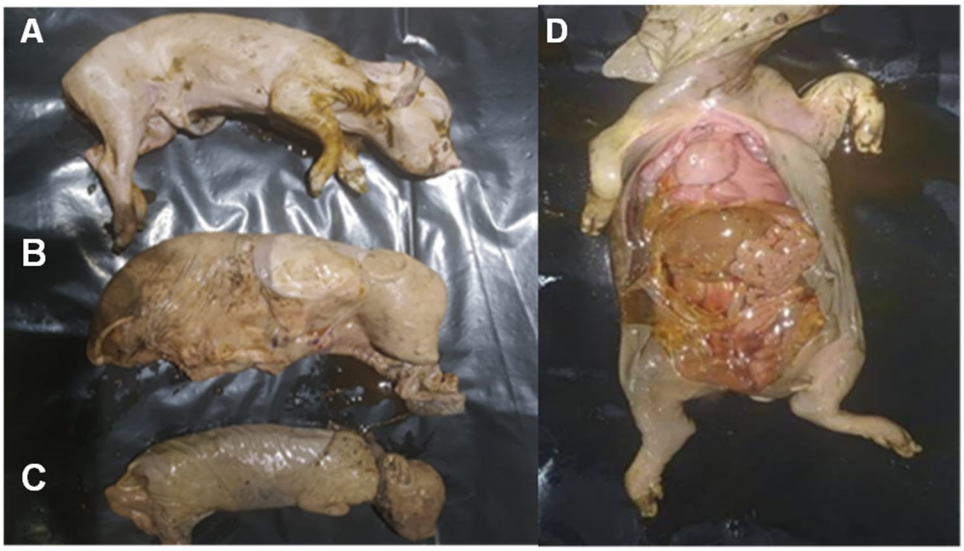

Fig. 1 Aborted, stillborn and mummified fetuses of porcine. **A**. Aborted dead fetus; **B**. Stillborn fetus covered with fetal membrane; **C**. Mummified fetus; **D**. Postmortem examination of stillborn fetus

TAGAATAGGATGCGAGGAA3′and 5′GAGTCTGTT GGTGTATTTATTGG 3′ respectively that bind to the 1761–1782 and 2002–2025 nucleotide positions of *NS1* gene in PPV and generate a specific product of 265 bp (Xu et al. 2012). PCR reaction mixture includes 12.5 µl of 2 × GoTaq Green master mix (Promega, USA), 1 µl of each forward and reverse primer (with 10 pmol/µl concentration), and 4 µl of the template DNA and nuclease-free water was added to make up the 25 µl reaction volume. PCR cycling condition included initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C and final extension step at 72 °C for 10 min. PCR products were analyzed by gel electrophoresis and documented. Initial screenings of samples were done by *NS1* gene–specific PCR and positive samples were further confirmed by amplifying the *VP2* gene.

PCR amplification and sequencing of VP2 gene from PPV1 genome

To amplify the consensus region of *VP2* gene in PPV1, a primer pair was designed using complete *VP2* gene sequences retrieved from established PPV1 isolate (AY502115) in Primer 3 plus online tool (https://www.bioinformatics.nl/cgi-bin/primer3plus). The designed forward and reverse primer sequence are 5′GGGGTT GGTGTGTCTACAGG3′ and 5′ TCCTACCTGAGCTGG CCTAA3′ respectively and bind to the 2921–2940 and 3798–3817 nucleotide positions of *VP2* gene region in PPV1 and generates a specific product of 897 bp. The specificity of the designed primers was confirmed by the primer BLAST (Basic Local Alignment Search Tool) and sequencing of amplified specific PCR product. PCR reaction conditions and annealing temperatures were optimized in a gradient thermal cycler (Eppendorf Master Cycler, Germany). The optimized PCR reaction condition includes initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 60 s, annealing at 56 °C for 60 s, extension at 72 °C for 90 s, and the final extension at 72 °C for 10 min. PCR reaction mixture includes 12.5 ml of 2 × GoTaq Green master mix (Promega, USA), 10 pmols of each PPV1 forward and reverse primer custom synthesized (Eurofins Genomics of India Pvt. Ltd, Bangalore), and 2 µl of DNA template, and finally, nuclease-free water was added to make up the 25 µl reaction. PCR positive samples were randomly selected and sequenced at a commercial DNA sequencing facility (M/s.Eurofins Genomics of India Pvt. Ltd, Bangalore).

Molecular characterization of PPV1

The nucleotide sequences obtained from the commercial sequencing facility were aligned and annotated in SnapGene tool using PPV reference sequences. The aligned contigs were subjected to NCBI-BLAST search for similarity analysis. The contig sequences were further subjected for phylogenetic studies along with reference and established PPV1 isolates in Molecular Evolutionary Genetics analysis X (MEGA X) tool using maximum likelihood method (MLT) with Tamura-Nei model with 1000 bootstrap replicates to find evolutionary relatedness (Kumar et al. 2018). Deduced amino acid sequences of the isolates were further analyzed

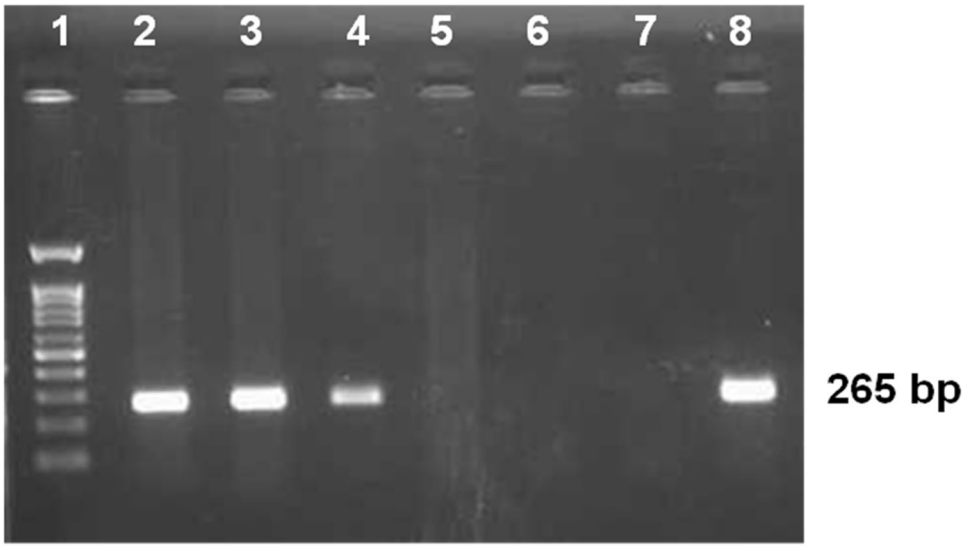

Fig. 2 Genome based Screening of PPV infections by PCR assay targeting *NS1* gene. Lane 1- 100 bp ladder, Lane 2 to 6—field samples Lane 7- Non-template control, Lane 8- known PPV positive DNA The specific PCR product 265 bp is labeled separately

for amino acid variations in the VP2 gene. The isolates in this study were characterized based on BLAST homology, phylogenic studies, and deduced amino acid sequence composition.

Table 1 Details of tissue samples screened for PPV genome

S. no	Sample source (District)	Location, and type of farm	Farm size	Vaccination history	State	Year of sample	No of samples collected	No of samples positive by PCR	Percent positivity
1	Chennai	Madhavaram--Organized	120	Vaccinated only for CSFV Non vaccinated for PPV & PCV2	Tamil Nadu	2021	17	3	17.64
		Guindy-unorganized	30	Non vaccinated for CSFV, PPV & PCV2		2021	3	0	-
2	Chengalpattu	Kattupakkam-Organized	320	Vaccinated for CSFV & PCV2 Non vaccinated for PPV		2021	16	2	12.5
		Chengalpattu-Unorganized	40	Vaccinated for CSFV Non vaccinated for PPV& PCV2		2020	3	0	-
3	Tirunelveli	Tiruvelveli-Organized	40	Vaccinated for CSFV Non vaccinated for PPV& PCV2		2020	2	0	-
4	Vellore	Katpadi—Unorganized	20	Non vaccinated for CSFV, PPV & PCV2		2021	01	0	-
5	Pookod	Lakkidi, -Organized	200	Vaccinated for CSFV Non vaccinated for PPV& PCV2	Kerala	2020	14	3	21.4
Total							**56**	**8**	**14.3**

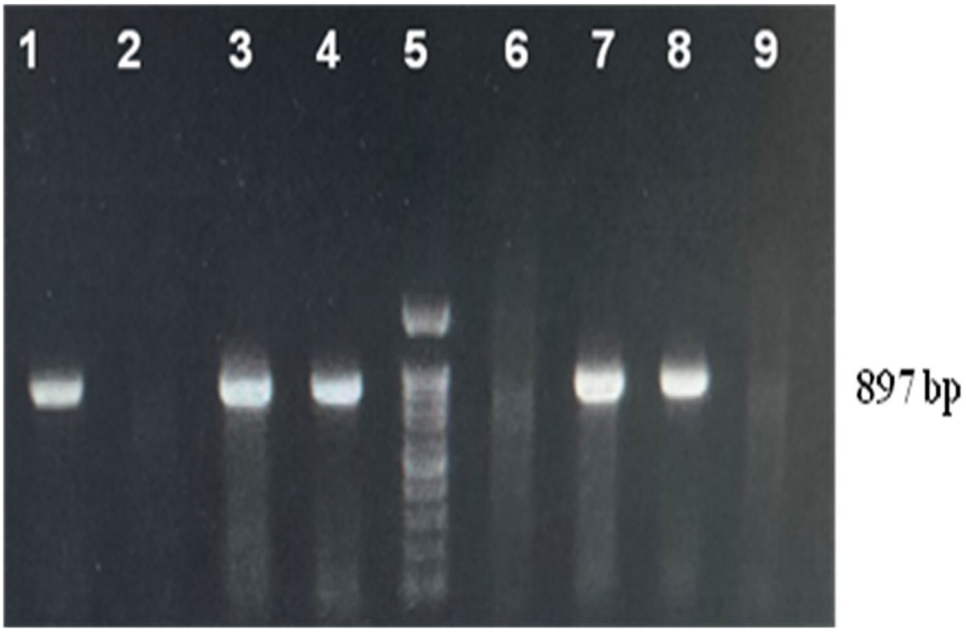

Fig. 3 Amplification of partial *VP2* gene of PPV1 using designed primer in this study. Lane 1, 2, 3, 4, 6, and 7- field samples, Lane 5 is 100 bp ladder, Lane 8—known positive DNA, Lane 9- Non-template control,. The amplified product is 897 bp and labeled separately

Results

Molecular detection of PPV

PCR-based screening for PPV genome in 56 field samples revealed, 14.3% ($n=8$) positivity by producing an amplicon of 265 bp in *NS1 gene*–based PCR assay specific to PPV (Fig. 2). The details of PPV screening results are displayed in Table 1.

Molecular characterization of PPV1

All the PPV positive samples ($n=8$) yielded a specific amplicon of 897 bp in the *VP2* gene–based PCR assay (Fig. 3). Two positive samples (T5 and L17) were randomly sequenced at a commercial DNA sequencing facility (M/s. Eurofins Genomics of India Pvt. Ltd, Bangalore) and assembled. Annotation of INDTNCHN-T5 and INDTNCHN-L17 of our local PPV sequences revealed 872 and 860 nts in length encoding 291 and 286 amino acids respectively. Both these sequences INDTNCHN-T5 and INDTNCHN-L17 were submitted to the GenBank under the accession numbers MW822566 and MW822567 respectively. Phylogenetic analysis of two *VP2* sequences (T5 and L17) obtained in this study along with forty-two established PPV1 and one PPV2 out-group sequence (Table 2) by maximum likelihood (ML) method in Molecular Evolutionary Genetics Analysis-X (MEGA-X) tool revealed specific grouping along with cluster E strains within PPV1 sequences (Fig. 4). NCBI-BLAST analysis of contig sequence from sample INDTNCHNT5 revealed 100% sequence homology with Chinese PPV1genome (MH183297), whereas sequence from sample INDTNCHNL17 revealed 99.43% sequence homology with Spain (MH558678), Chinese (MH183297), and German (JN400516) PPV1 sequences. Deduced amino acid sequence analysis of VP2 coding region of isolates obtained in this study with reference PPV1 amino acid residues revealed variations at position 215 (I to T) in both the isolates, variation at position 228 (Q to E) in T5 isolate and variations at position 59 (L to M) and 314 (K to E) in L17 isolate (Fig. 5).

Discussion

PPV1 is the well-documented viral pathogen associated with reproductive failure in most swine-producing countries. The detection of PPV by PCR-based molecular technique is highly specific and sensitive in comparison to hemagglutination or immunofluorescence assays (Soares et al. 1999). *NS1* gene–specific PCR assay is the best suitable approach for molecular screening of PPV infections (Xu et al. 2012). *NS1* gene–based PPV1 surveillance in this study revealed 14.3% positivity which is lower when compared to global prevalence status. The global prevalence of PPV1 varies from 25.8 to 71.88% (Opriessnig et al. 2014). The most popular swine-producing country, China, reported PPV positivity of 55.40% ($n=241$) among 435 samples screened (Li et al. 2021). Mengeling et al. (1991) screened 302 dead fetuses in the USA and documented 35% ($n=105$) positivity of PPV1 genome. At the same time, Argentina documented 13% ($n=17$) PPV1 positivity out of 131 fetal tissues screened

Table 2 List of PPV genome genomes used in Phylogentic analysis

S. no	Accession number	Country	Year	Virus type
1	NC001718	REF-USA	2000	PPV1Cluster A
2	AY459350	China	2003	**PPV1**
3	AY502115	China	2003	**PPV1**
4	AY686602	China	2004	PPV1
5	JQ686669	China	2012	PPV1
6	MH447542	Republic of Korea	2018	PPV1
7	MH447544	Republic of Korea	2018	PPV1
8	MH447546	Republic of Korea	2018	PPV1
9	MH447550	Republic of Korea	2018	PPV1
10	MN627433	Hungary	2020	PPV1
11	KC296746	Germany	2012	PPV1
12	JQ249927	Romania	2011	PPV1
13	MK035431	India	2019	PPV1VP2
14	LC041171	India	2018	PPV1VP2
15	LC041168	India	2018	PPVVP2
16	LC041164	India	2018	PPVVP2
17	LC041170	India	2018	PPVVP2
18	MN182479	Argentina	2019	PPVVP2
19	MK776768	India	2019	PPVVp2
20	JX495963	India	2012	PPVVP2
21	MH183297	China	2019	PPV1
22	MH558678	Italy	2018	PPV1
23	MK092381	China	2020	PPV1
24	FJ853421	China	2009	PPV1
25	KC296744	Germany	2013	PPV1
26	AY684868	Germany	2006	PPV1
27	MK092382	China	2020	PPV1
28	KP245938	China	2015	PPV1
29	JX568154	Brazil	2012	PPV
30	MK378222	China	2017	PPV2 VP2
31	AY583318	China	2004	PPV1Cluster A
32	DQ675456	China	2006	PPV1Cluster B
33	AY390557	South Korea	2003	PPV1Cluster B
34	GQ884040	Europe	2005	PPV1Cluster C
35	U44978	USA	1985	PPV1Kresse Cluster C
36	EU790642	China	2004	PPV1Cluster D
37	HM627653	China	2009	PPV1Cluster D
38	HM627652	China	2010	PPV1Cluster E
39	JN400516	Germany	2009	PPV1Cluster E
40	GQ884042	Europe	2006	PPV1Cluster F
41	GQ884047	USA	2006	PPV1Cluster F
42	JN872448	China	2010	PPV1Cluster G
43	AY684869	Germany	2002	PPV1Cluster G

which clearly evidences variability of PPV prevalence across countries (Serena et al. 2019). But in India, during 2010, Uttar Pradesh where PPV1 was first reported had 7.14% positivity out of 70 tissue samples screened (Sharma and Saikumar 2010). Furthermore, serological testing by Kaur et al. (2016) from 90 serum samples from Punjab reported 41.1% ($n=37$) positivity for PPV antibodies. A molecular detection study by Pegu et al. (2017) evidenced 14.8% ($n=8$) of PPV1 positivity out of 54 porcine tissue samples screened which supports the genome-based PPV1 screening in this present study. In southern India, Aishwarya et al. (2016) reported PPV1 positivity of 5.26% ($n=2$) out of 38

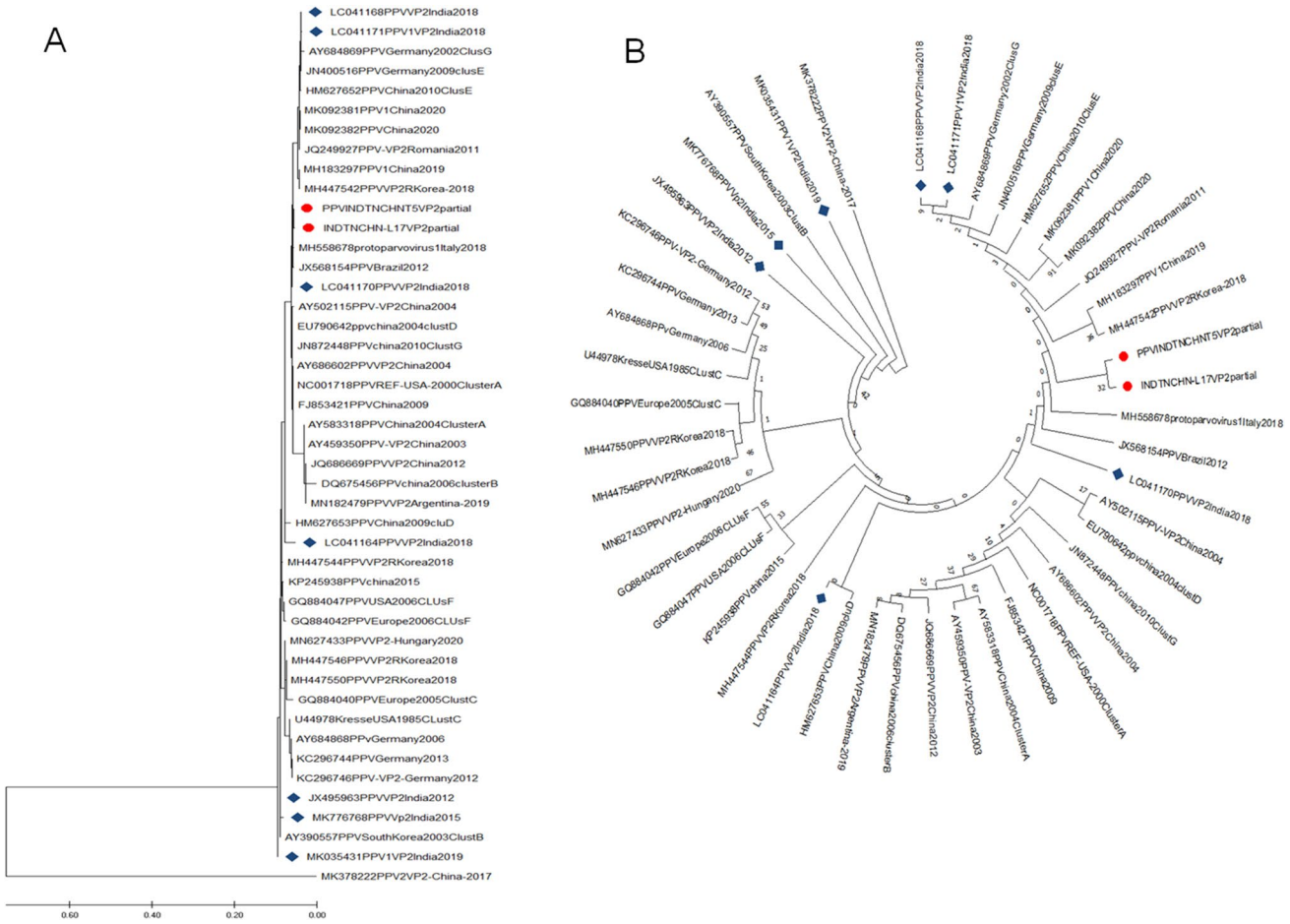

Fig. 4 Phylogenetic analysis PPV1 genome by Maximum Likelihood method based on p-distance model with 1000 bootstraps. **A** Traditional rectangular dendrogram. **B** Circular dendrogram. The phylogenetic tree was inferred based on alignment of VP2 protein and were midpoint rooted. The trees were drawn with two PPV1 sequences from this study (labeled by red colored circle) along with 42 established PPV1 sequences from the GenBank (including seven PPV sequences from India labeled in blue diamond) one PPV2 sequence as an out-group. The analyses were conducted in MEGA X with Bootstrap replicates of 1000

samples screened from Kerala, but this study included very less sample numbers and may not represent real PPV1 status in southern India. Most recent study by Bhattacharjee et al. (2021) from Northeastern states of India reported 36% ($n=18$) PPV1 positivity out of 50 samples screened which clearly evidences emergence of PPV1-associated infections in recent few years in India. The present study reveals that out of 56 samples screened, 14.3% were positive for PPV1-associated infection, whereas there may be many other etiological factors like porcine circovirus 2 and 3 (PCV2 & PCV3), classical swine fever virus (CSFV), and porcine reproductive and respiratory syndrome virus (PRRSV) and Brucellosis, etc., associated with reproductive failure and their prevalence various with different geographical locations which needs to be explored. Since reproductive failure is the major concern in swine husbandry which needs to be addressed immediately with differential diagnosis and risk-based surveillance in extensive population to find true prevalence status of PPV.

Whole genome, *VP2* and *NS1* gene–based sequencing, and phylogenetic analysis were followed in many countries for molecular epidemiology of PPV (Oh et al. 2017). Phylogenetic analysis of PPV1 isolates in the past 50 years revealed eight phylogenic clusters which are identified by alphabets serially from A to H. Furthermore, the protective effects of commercial vaccines against these new strains are not 100% effective indicating antigenic diversity (Streck et al. 2015). Soares et al. (2003) analyzed genetic variability of PPV1 field strains in Brazil from 1994 to 2000 using partial fragments of the *VP2* gene and found F and G clusters circulating in the field. Zimmermann et al. (2006) detected type C and D clusters of PPV1 associated

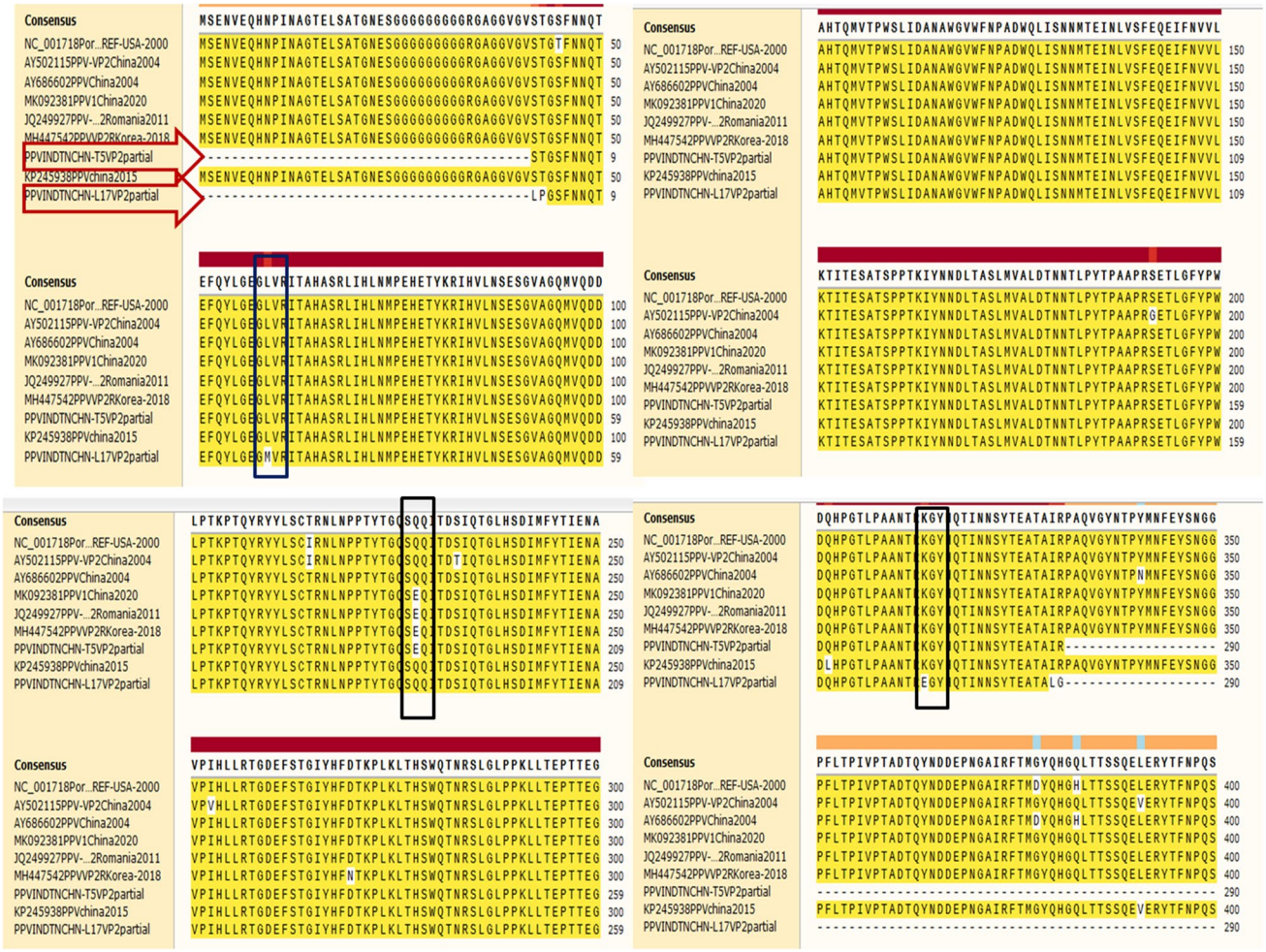

Fig. 5 Deduced amino acid sequences of partial *VP2* gene of PPV1. This alignment included deduced amino acid partial sequences of VP2 protein from two PPV1 sequences from this study (T5 and L17) and seven PPV1 sequences from different countries including one reference PPV1 sequences retrieved from GenBank. All the sequences were aligned in ClustalW and viewed in SanpGene alignment tool. The PPV1 sequences were demarcated by the red colored arrows and aminoacid variations sites were marked by rectangle shape balck box

with reproductive failure in Germany based on *VP2* gene characterization. Keeping above studies as reference, the PPV1 genome in the present study was also characterized based on *VP2* gene sequencing. All the eight clusters of PPV1 were associated with field infections. PPV1 clusters of A, B, and E have been reported from Austria, China, Romania, and Switzerland. Research findings from Europe and China revealed that clusters C, D, and F are the predominant clusters circulating in domestic swine (Streck et al. 2015). Each PPV1 clusters are subdivided into subgroups that include strains from both domestic and wild swine populations with varying virulence properties related to the amino acid composition of VP2 proteins (Cadar et al. 2012).

Varying amino acid substitutions have been observed in strains from several countries. Hot spots were found to be located on the capsid surface, and a surface profile distinct from the vaccine strains was observed. For PPV1, 12 linear epitopes have been proposed between amino acid positions 5–51, 85–101, 130–140, 154–167, 190–240, 260–314, 272–320, 378–458, 467–478, 502–514, 535–542, and 547–576 in the VP2 proteins numbered serially from 1 to 12, containing a huge potential role, as determined by B cell epitope prediction software. The virulent PPV1 field strains have 5 common amino acid substitutions at I-215-T, D-378-G, H-383-Q, S-436-P, and R-565-K when compared to non-virulent strains. Substitutions at 378, 383, and 436 amino acid positions of 8th B-cell epitope region determine the tissue tropism of PPV1 (Chung, et al. 2020).

Both the PPV1 (T5 and T7) sequences in this study were obtained from tissues of stillborn and mummified fetuses of swine and had amino acid substitutions at 215 positions similar to that of virulent PPV1 isolates. Additionally, one of the Tamil Nadu PPV1 sequences (T5) had

host immune evasion mutations at VP2 amino acid position 228-E as like that of German 27a (AY684871) virulent field isolate (Zeeuw et al. 2007). Based on the above said evidence, the two PPV1 sequences (T5 and L17) in this study are characterized as pathogenic strains. It is hypothesized that variations in the amino acid composition of capsid could be due to viral adaptation to host and or vaccinal immune response refereed as escape mutants (Cadar et al. 2012). Commercial PPV1 (whole virus inactivated) vaccines are derived from attenuated NADL2 strains used in many countries prevent only reproductive loss and do not eliminate virus infection and dissemination (Mengeling et al. 1980). The emergence of new clusters of PPV1 in the field with vast divergence from vaccine strain necessitates the development of an alternative vaccine development approach involving suitable candidate vaccine strain circulating in the field (Streck et al. 2015). Therefore, the emergence and spread of viruses with varying amino acid profiles require close surveillance. Molecular characterization and phylogenetic analysis of PPV in India are scarce. The molecular detection of the PPV genome in the swine population of Tamil Nadu has not been reported so far. This study documents incidence of PPV1 cluster E strains for the first time in Tamil Nadu. The PPV1 isolates in this study showed homology to China and European countries isolates and these findings are supported by Cadar et al. (2012), who found that PPV1 cluster E includes the highly virulent Kresse strain along with the challenge UK and Brazilian strains of PPV. Although it could be hypothesized that these phylogenetic clustering might be due to live pig import from China and European countries to India. Additionally, investigations in contaminated commercial biological products, as porcine cell lines associated vaccines, may elucidate the origin and route of transmission of PPV across the countries. This is the foremost molecular characterization report of PPV documented from Tamil Nadu. To determine the prevalence, transmission, molecular epidemiology, and impact of PPV in commercial swine husbandry, it is necessary to extend this study to larger populations. Currently, the PPV is a very less-explored pathogen with no complete scientific data available in Indian context; hence, there is no indigenous vaccine available to control this infection; in general, the current control measures are at primitive level by adopting general hygiene.

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Author contribution PS, SRKV, PM, RP, and RA designed the study. SRKV, PS, and TPS worked and collaborated in the lab work. SK, BD, HS, BR, and RC helped in the sample collection and shipment. SRKV, PS, and RP compiled results as well as the manuscript. PM and DRG critically reviewed the manuscript. All authors read and approved the final manuscript.

Data availability All the original data are available with corresponding author on request all the information will be shared.

Code availability Not applicable.

Declarations

Ethical approval Since the clinical samples were collected from aborted fetus without any invasive means hence this study doesn't require Institutional animal ethics committee approval.

Consent to participate Not applicable.

Consent for publication All the authors reviewed the paper and given their consent for publication.

Conflict of interest The authors declare no competing interests.

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