First report of detection and molecular characterization of porcine parvovirus in domestic and wild pigs in Kerala, India

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Abstract Porcine parvovirus (PPV) is a DNA virus of the genus *Parvovirus* of family *Parvoviridae*. It is the causative agent of many disease problems in pigs such as maternal reproductive failure, stillbirth, mummification, embryonic or fetal death, infertility, abortion and neonatal death. A study was conducted to assess the incidence of the virus in pigs in Kerala State in South India. A total of 38 samples were collected from domestic and wild pigs from different districts of the State. Polymerase chain reaction targeting a 265 bp fragment of the NS1 gene of the virus was carried out. Of the samples tested, 2 (5.26 %) were found to be positive for PPV virus genome, one of which was from a wild pig. One of the positive samples was sequenced and the nucleotide sequence obtained was compared with other sequences of PPV from India and abroad. The results revealed that the sequence had very close similarity to PPV sequences previously reported from India and to that of Chinese isolates. This is the first report of the existence of PPV in domestic and wild pigs in Kerala, India. The study highlights the need to test for the presence of PPV in addition to other infectious agents in diagnosis of cases of reproductive disorders in pigs.

Keywords PPV · Pigs · NS1 · Phylogeny · Kerala · India

Porcine parvovirus (PPV) is a DNA virus of the genus *Parvovirus* of subfamily *Parvovirinae* of family *Parvoviridae*. It has a linear single stranded genome of approximately 4.5–5.5 kb with two major open reading frames (ORFs). The ORF located in the 3' half of the genome encodes the non-structural (NS) proteins and another ORF towards the 5' half encodes the structural proteins. The genome has terminal palindromic sequences that enable each end to form hairpin or other complex base paired structures required for virus replication [6]. Even though all PPV isolates are antigenically related and belonging to a single serotype, isolates vary in virulence [2]. The occurrence of PPV in pigs was first reported in 1967 [1] and the virus is now considered to be endemic in most areas of the world and can be found in all pig herd categories. It has been identified as the main agent causing stillbirth, mummification, embryonic death and infertility (SMEDI) in swine [7]. Detection of the virus in pigs is usually carried out by employing the polymerase chain reaction (PCR) and several PCR protocols for detection of the virus have been reported [11, 12]. Phylogenetic analysis of PPV is commonly carried out on the basis of VP2 and NS1 gene sequences [3, 9].

The presence of PPV, alone or in association with porcine circovirus 2 (PCV2), in swine population in India was reported for the first time in 2010 [10]. Besides this, reports of the occurrence of the disease in the country are scarce. There has been an increase in incidence of abortions and reproductive problems in sows in the South Indian state of Kerala. Hence a pilot study was conducted to check for the presence of PPV in the pig population of the State and if detected, to characterize the virus by gene sequencing.

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A total of 38 samples were collected from pigs during the period from September, 2014 to April, 2016. The samples consisted of tissue samples from domestic, and wild pigs from the northern and central districts of Kerala viz. Kannur, Wayanad, Kozhikode, Thrissur and Palakkad. Of the total samples collected, 32 samples were from domestic pigs and 6 from wild boar.

Total DNA was extracted from the samples by using the DNeasy® Blood and Tissue Kit (Qiagen, Germany) as per the manufacturer's instructions. The samples were tested for the presence of PPV genome by employing PCR targeting the NS1 gene of the virus [12]. The primer sequences were PPV FP 5' AGTTAGAATAGGATGCGAGGAA 3' and PPV RP 5' AGAGTCTGTTGGTGTATTTATTGG 3' corresponding to nucleotide positions 1761–1782 and 2026–2002 respectively of NADL-2 strain of PPV (GenBank Accession number NC001718). The 25 µl PCR reaction mix consisted of 12.5 µl of EmeraldAmp® GT PCR Mastermix (TaKaRa Bio, USA), 1 µl each of 10 pmol PPV forward and reverse primers, 3 µl of DNA and rest nuclease free water (NFW). The cycling conditions for amplification of PPV were 95 °C for 2 min (initial denaturation), 35 cycles of 95 °C for 15 s (denaturation), 53 °C for 30 s (annealing) and 72 °C for 30 s (polymerization) followed by single cycle at 72 °C for min. (final extension). After the reaction, the PCR products were electrophoresed in 1.5 % agarose gels prepared in 0.5X TBE buffer and containing ethidium bromide at a final concentration of 0.5 µg/ml. The gels were visualized in a gel documentation system and the image captured.

A representative PCR product was sequenced at M/s Scigenom Labs Private Limited, Kakkanad, Kochi, Kerala and the sequence obtained. The chromatograms obtained after sequencing were analyzed by using Chromas Lite v2.01 software (<http://www.techneleysium.com.au>) and sequence reading errors if any were edited. To confirm the identity of the obtained sequence, BLAST was performed within the non-redundant nucleotide database (<http://www.blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences of NS1 gene of PPV isolates from India and abroad that were available in GenBank were downloaded for phylogenetic analysis.

Phylogenetic and molecular evolutionary analyses of the sequences were carried out using MEGA v7 software [5]. A 265 bp fragment of the NS1 gene corresponding to positions 1761–2026 of NADL-2 strain was used for the analysis. The sequence obtained during this study and those downloaded from GenBank were aligned using the ClustalW program of MEGA v7 software. The sequences were then trimmed to correspond to the region given above. The evolutionary history was found out by inferred by the neighbor joining (NJ) method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the sequences analyzed. The

evolutionary distances used to infer the phylogenetic trees were computed using the Tamura 3-parameter method. The details of sequences employed for the phylogenetic analysis are given in Supplementary Table 1.

The PCR could detect PPV genome in 2 (5.26 %) of the 38 samples tested. The positive samples yielded specific amplicons of 265 bp on agarose gel electrophoresis. One of the samples was from domestic pig whereas the other was from a wild pig which was brought for post-mortem. The PCR product of sample from wild pig (301/MIB/PKD/2015) was sequenced and the sequence obtained was analyzed by BLAST. The sequence was closely related to PPV sequences from India and abroad (99–97 % nucleotide identities). The sequence was deposited in the GenBank under Accession number KX579944. The foreign isolate to which the Kerala isolate had similarity was from China.

On phylogenetic analysis it was observed that the sequence of Kerala isolate of PPV matched closely with the Chinese strain 16WS (KM268633) and to the Indian isolate P1 (KC479142) (Fig. 1). More close relationship was shown towards the Chinese strain than to the Indian strain. The sequence was divergent from the Indian isolate HSADL (KJ183033) as it was seen in a different cluster in the phylogenetic tree. Divergence from other Chinese isolates which were included in the analysis was also observed.

The most common viruses that bring about porcine reproductive failure are PPV and porcine reproductive and respiratory syndrome virus (PRRSV). Recurring estrus, abortion and delivery of mummified or still born foetuses are the important clinical signs of PPV infection (SMEDI—Stillbirth, Mummification, Embryonic Death and Infertility). While the virus can cause cute outbreaks when naïve gilts are introduced into infected herd, boars are affected little by PPV but act as non-infected carriers. Both horizontal and vertical transmission of PPV has been reported. Porcine parvovirus infection occur worldwide and prevalence rates vary. The disease is enzootic in Midwestern United States where swine production is common. It is usually seen in association with porcine circovirus 2 (PCV2) infection in pigs [7].

The most commonly used test to detect PPV in clinical samples is PCR and sensitive detection of the virus by employing the test have been reported [11, 12]. Phylogenetic analysis of porcine parvoviruses based on nucleotide sequence of the VP2 and NS1 has been carried out by many researchers for molecular epidemiological studies [3, 9]. Apart from data obtained from conventional sequencing, researchers are now resorting to metagenomics data to obtain sequences of novel parvoviruses in the pig population [8].

In this study 38 samples were tested for PPV by PCR targeting NS1 gene of the virus. Of the samples tested, two

Fig. 1 Neighbour joining tree constructed using 265 nucleotides of NS1 region of PPV isolates. Legend for Fig. 1 *Filled triangle*: isolate obtained during this study. *Filled circles*: other Indian isolates. Boot strap replicates = 1000. Evolutionary distances between sequences were calculated by Tamura 3 parameter method. Branch length are given as 0.05 substitutions per position according to *scale bar* underneath the tree

were found positive by PCR with a percentage positivity of 5.26 %. The presence of the virus in pigs in Kerala has not been reported so far. In India, the presence of PPV in piglets died in the first week after the birth and from aborted foetuses has been reported [10]. In this study, one of the positive samples was collected from wild pigs. Antibodies against PPV have been detected in wild boar in many countries [3]. The circulation of this virus in wild boars should be viewed with great concern as it is a hurdle in control of these viruses in domestic pigs.

Reports of phylogenetic analysis of Indian isolates of PPV are scarce. Phylogenetic analysis of the sequence obtained during the study with other published Indian sequences of PPV indicated that they are closely related. However, the sequence of one of the published Indian sequences was more closely related to that obtained in this study and the other Indian sequence was slightly divergent and clustered in another branch in the phylogenetic tree. An interesting observation was that the sequence obtained in this study was more closely related to that of a Chinese isolate (16WS) than to Indian isolates.

The study could detect the presence of PPV in the pig population of Kerala. While classical swine fever, another devastating disease of pigs, was reported in the State

previously, PPV is being detected for the first time. The result of the study warrants the inclusion of PPV in the differential diagnosis of cases of pig mortality and reproductive dysfunction.

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References

1. Cartwright SF, Huck RA. Viruses isolated in association with herd infertility abortions and stillbirths in pigs. *Vet Rec.* 1967;81:196–7.
2. Choi CS, Molitor TW, Joo HS, Gunther R. Pathogenicity of a skin isolate of porcine parvovirus in swine fetuses. *Vet Microbiol.* 1987;15:19–29.
3. Hao X, Lu Z, Sun P, Fu Y, Cao Y, Li P, Bai X, Bao H, Xie B, Chen Y, Li D, Liu Z. Phylogenetic analysis of porcine parvoviruses from swine samples in China. *Virology.* 2011;8:320–7.
4. Jeoung H, Lim S, Kim J, Cho Y, Kim YK, Song J, Hyun B, An D. Serological prevalence of viral agents that induce reproductive failure in South Korean wild boar. *BMC Vet Res.* 2015;11:78–82.
5. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 2016;33:1870–4.

6. MacLachlan NJ, Dubovi EJ. Fenner's veterinary virology. 4th ed. Amsterdam: Elsevier Inc.; 2011. p. 353.
7. Mengeling WL, Ridpath JF, Vorwald AC. Size and antigenic comparison among the structural proteins of selected autonomous parvoviruses. *J Gen Virol*. 1988;69:825–7.
8. Palinski RM, Mitra N, Hause BM. Discovery of a novel Parvovirinae virus porcine parvovirus 7 by metagenomics sequencing of porcine rectal swabs. *Virus Genes*. 2016;52:564–7.
9. Shangjin C, Cortey M, Segalés J. Phylogeny and evolution of the NS1 and VP1/VP2 gene sequences from porcine parvovirus. *Virus Res*. 2009;40:209–15.
10. Sharma R, Saikumar G. Parvovirus and PCV2 in crossbred Indian pigs. *Trop Anim Health Prod*. 2010;42:515–22.
11. Soares RM, Durigon EL, Bersano JG, Richtzenhain LJ. Detection of porcine parvovirus DNA by the polymerase chain reaction assay using primers to the highly conserved non-structural protein gene NS-1. *J Virol Methods*. 1999;78:191–8.
12. Xu XG, Chen GD, Huang Y, Ding L, Li ZC, Chang CD, Wang CY, Tong DW, Liu HJ. Development of multiplex PCR for simultaneous detection of six swine DNA and RNA viruses. *J Virol Methods*. 2012;183:69–74.