SHORT COMMUNICATION
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.3.840-844

Detection of porcine parvovirus in domestic pigs in North Kerala#

P. Jishnu Haridas[1], Chintu Ravishankar[2*], K. Sumod[3], R. Rajasekhar[3], R. Anoopraj[4], G.S. Anjitha[1], S. Shashank[1] and Koshy John[5]
Department of Veterinary Microbiology
College of Veterinary and Animal Sciences, Pookode, Wayanad-673576
Kerala Veterinary and Animal Sciences University
Kerala, India

Citation: Haridas, P.J., Ravishankar, C., Sumod, K., Rajasekhar, R., Anoopraj, R., Anjitha, G.S., Shashank, S. and John, K. 2023. Detection of porcine parvovirus in domestic pigs in North Kerala. *J. Vet. Anim. Sci.* **54**(3):840-844
DOI: https://doi.org/10.51966/jvas.2023.54.3.840-844

Received: 30.11.2022 *Accepted: 14.03.2023* *Published: 30.09.2023*

Abstract

During the period from 2019 to 2021, a total of 45 tissue samples were collected from pigs in Kerala and tested for the presence of porcine parvovirus (PPV) by polymerase chain reaction (PCR) targeting NS1 gene of the virus. Of the samples tested, 4 (8.89 per cent) were found to be positive which was higher than the positivity reported for the virus in Kerala in 2016. Sequence analysis of the amplicons revealed a high degree of similarity to PPV sequences previously reported from India. Biosecurity measures should be adopted to control the spread of viral diseases.

Keywords: *Porcine parvovirus, PCR, NS1 gene*

Pigs are prolific breeders and have one of the highest feed conversion efficiencies making pig rearing lucrative. The second-most popular meat consumed worldwide is pork (Shahbandeh, 2022). The occurrence of infectious diseases can significantly lower the profitability of pig farming. Since pigs are prolific breeders, diseases causing reproductive dysfunction can have an adverse impact on the economics of pig production. Porcine parvovirus (PPV) (currently known as *Ungulate protoparvovirus 1*) is a single-stranded DNA virus classified under the family *Parvoviridae* (ICTV, 2021). The virus is one of the causative agents of stillbirth, mummification, embryonic death and infertility (SMEDI) syndrome, the other viral agents being porcine teschovirus and reproductive and

#*Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Kerala*

1. *MVSc Scholar*
2. *Professor*
3. *Assistant Professor*
4. *Assistant Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651*
5. *Professor and Head*

**Corresponding author: chintu@kvasu.ac.in, Ph. 9447716620*

respiratory syndrome virus (PRRSV) (Ehrenfeld *et al*., 2010). The most prominent and only clinical indication of PPV infection that has been well-established is maternal reproductive failure. In Kerala, there have been reports of reproductive dysfunction in pigs. Hence a study was undertaken to detect the presence of PPV in cases of reproductive dysfunction in pigs in Kerala.

A total of 45 tissue samples (tonsil, mesenteric lymph node, lung and spleen) were collected from pigs during the period from 2019 to 2021. The pigs were from farms with a history of reproductive disorders. From the tissues, total DNA was extracted by Qiagen DNeasy Blood and Tissue Kit as per the manufacturer's protocol. The presence of PPV genome in the clinical samples was detected by polymerase chain reaction (PCR) using specific primers for *NS1* of the virus as described by Xu *et al*. (2012) with slight modifications. The primer sequences used were PPV FP 5' AGTTAGAATAGGATGCGAGGAA3' 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 reaction mixture of the PCR comprised of 12.5 µL 2X EmeraldAmp GT PCR master mix (2X) (Takara), 1 µL each of forward and reverse primers (10 pmol), 2 µL of DNA and the NFW to make up the volume. The cycling conditions were 95°C for 2 min (initial denaturation), 35 cycles of 95° C for 15 sec (denaturation), 54°C for 30 sec (annealing) and 72°C for 30 sec (polymerisation) followed by a single cycle at 72°C for 2 min (final extension). A no-template control, which did not contain the sample DNA, was also kept. The PCR products were resolved in agarose gels containing ethidium bromide and viewed using a gel documentation system. Specific amplicons were purified using Gene JET Gel Extraction Kit (Thermo Fisher Scientific, USA) and sent to M/s AgriGenome Lab Private Limited, Cochin, India for sequencing. BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST) of the sequence data obtained was done to confirm the presence of porcine parvovirus.

Out of the 45 samples tested, four (8.89 per cent) were positive. Three positive samples were obtained from Wayanad while one positive was from Kozhikode district (Table 1). Specific amplicons of 265 bp were observed in the positive samples (Fig.1). This is higher than the percentage positivity reported by Aishwarya *et al*. (2016) wherein two out of the 38 samples (5.26 per cent) tested in Kerala were positive. This indicates that the virus is slowly spreading among pigs in the State. Reports on the detection of PPV in India are scarce and reports are mainly on the basis of detection of antibodies against the virus. In Punjab, seroprevalence ranging from 39.10 to 41.1 per cent has been reported and in the north-eastern states, a lesser prevalence ranging from 1.24 to 10.15 per cent have been reported (Kaur *et al*., 2016; Pegu *et al*., 2017; Deka *et al*., 2021).

On BLAST analysis, the nucleotide sequences generated in the study were found to have 98.50 to 99.50% similarity with isolates from Uttar Pradesh and 98.99% similarity with isolates from Madhya Pradesh (Table 2).

In farms, PPV spread horizontally between acutely infected and in-contact naive pigs by touch, swallowing or breathing virus-laden excretions and secretions. Oronasal and transplacental infections are, respectively, the most typical entry points for postnatal and prenatal pigs (Mengeling *et al*., 2000) and infected pigs presumably act as important PPV reservoirs (Brown, 1981; Mengeling, 1999). However, there is no proof to support often and continuous shedding of PPV and it is more likely that a contaminated environment served as the main virus reservoir (Mengeling and Paul, 1986).

Since infection with PPV does not produce any specific clinical signs in pigs other than decreased reproductive rates, diagnosis of the condition has to be carried out by molecular methods. However, in farms with a high incidence of reproductive disorders with SMEDI, porcine parvoviral infection could be suspected. Since SMEDI could be caused by other viruses such as PRRS and porcine teschoviruses, diagnosis by molecular techniques will reveal the specific cause of the disease. In order to prevent the

Table 1. Results and details of samples tested for PPV by PCR

Sl. No.	Sample No.	District	Result	GenBank Accession No.
1	247/MIB/2019	Wayanad	-	
2	278/MIB/2019	Wayanad	-	
3	286/MIB/2019	Wayanad	-	
4	301/MIB/2019	Kozhikode	-	
5	12/MIB/2020	Wayanad	-	
6	13/MIB/2020	Wayanad	-	
7	57/MIB/2020	Wayanad	-	
8	58/MIB/2020	Wayanad	-	
9	1/MIB/2021	Kozhikode	-	
10	5/MIB/2021	Wayanad	-	
11	16/MIB/2021	Wayanad	-	
12	17/MIB/2021	Kozhikode	-	
13	21/MIB/2021	Wayanad	-	
14	33/MIB/2021	Thrissur	-	
15	34/MIB/2021	Thrissur	-	
16	47/MIB/2021	Kozhikode	-	
17	78/MIB/2021	Kannur	-	
18	79/MIB/2021	Wayanad	-	
19	80/MIB/2021	Wayanad	-	
20	91/MIB/2021	Wayanad	-	
21	95/MIB/2021	Kozhikode	-	
22	111/MIB/2021	Wayanad	-	
23	122/MIB/2021	Wayanad	-	
24	127/MIB/2021	Wayanad	-	
25	129/MIB/2021	Wayanad	-	
26	132/MIB/2021	Wayanad	-	
27	148/MIB/2021	Wayanad	-	
28	157/MIB/2021	Wayanad	+	ON693274
29	159/MIB/2021	Wayanad	+	ON693275
30	168/MIB/2021	Kozhikode	+	ON693276
31	174/MIB/2021	Kozhikode	-	
32	181/MIB/2021	Kozhikode	-	
33	183/MIB/2021	Kozhikode	-	
34	189/MIB/2021	Wayanad	+	ON693277
35	190/MIB/2021	Wayanad	-	
36	192/MIB/2021	Wayanad	-	
37	194/MIB/2021	Wayanad	-	
38	197/MIB/2021	Wayanad	-	
39	200/MIB/2021	Wayanad	-	
40	204/MIB/2021	Wayanad	-	
41	214/MIB/2021	Wayanad	-	
42	229/MIB/2021	Wayanad	-	
43	248/MIB/2021	Wayanad	-	
44	260/MIB/2021	Wayanad	-	
45	261/MIB/2021	Wayanad	-	

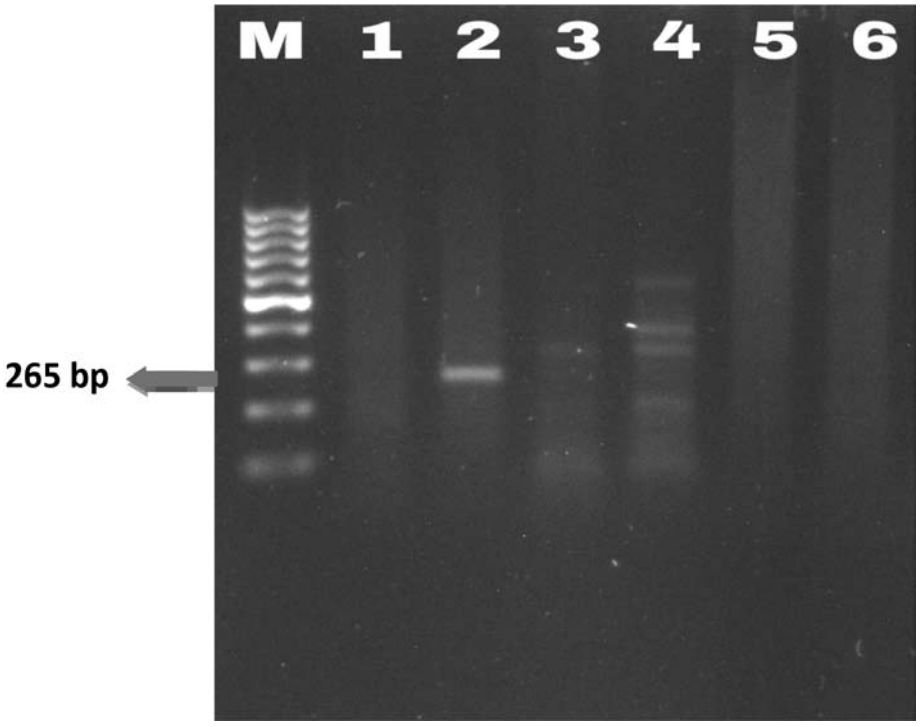

Fig. 1. Agarose gel showing 265 bp amplicons generated by targeting NS1 gene of PPV
Lane M - 100 bp ladder
Lane 2 - Clinical sample (positive)
Lane 1, 3, 4, 5, 6 – Clinical samples (negative)

Table 2. Nucleotide identity of NS1 gene sequences of PPV with other Indian PPV sequences

Sl. No.	Sample No.	Accession No. of closest matching Indian sequence	State	% identity
1	157/MIB/2021	KC479142	Uttar Pradesh	98.99
2	159/MIB/2021	KC479142	Uttar Pradesh	98.50
3	168/MIB/2021	KC479142	Uttar Pradesh	99.50
4	189/MIB/2021	KJ183033	Madhya Pradesh	98.99

spread of the virus, strict biosecurity measures must be implemented and periodic assessment of the prevalence of the disease condition has to be carried out.

Summary

The study revealed the prevalence of porcine parvovirus infection among pigs exhibiting reproductive dysfunction in Kerala. Porcine parvovirus was detected in 8.89 per cent of the samples tested and it was observed that the infection rate is higher than those reported previously. On sequence analysis, the viruses detected were similar to those detected in other parts of India. Strict biosecurity measures are required to prevent the spread of the virus among pigs in Kerala.

Acknowledgement

The authors thank the Dean, College of Veterinary and Animal Sciences, Pookode for providing the facilities for the conduct of the study.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Aishwarya, J., Ravishankar, C., Rajasekhar, R., Sumod, K., Bhaskar, N., Shaji, S., John, K. and Mini, M. 2016. First report of detection and molecular characterization of porcine parvovirus in domestic and wild pigs in Kerala, India. *Virus disease.* **27**: 311-314.

Brown, T.T. 1981. Laboratory evaluation of selected disinfectants as virucidal agents against porcine parvovirus, pseudorabies virus, and transmissible gastroenteritis virus. *Am. J. Vet. Res.* **42**: 1033-1036.

Deka, D., Barman, N.N., Deka, N., Batth, B.K., Singh, G., Singh, S., Agrawal, R.K. and Mukhopadhyay, C.S. 2021. Sero-epidemiology of porcine parvovirus, circovirus, and classical swine fever virus infections in India. *Trop. Anim. Hlth. Prod.* **53**: 1-12.

Ehrenfeld, E., Domingo E. and Roos, R.P. (1st Ed.). 2010. *The Picornaviruses*. ASM Press, Washington, DC., 200p.

International Committee on Taxonomy of Viruses (ICTV). 2021. Current ICTV taxonomy release. https://ictv.global/taxonomy [30 Nov 22].

Kaur, A., Mahajan, V., Leishangthem, G.D., Singh, N.D., Bhat, P., Banga, H.S. and Filia, G. 2016. Epidemiological and immunopathological studies on Porcine parvovirus infection in Punjab. *Vet. World*. **9**: 827.

Mengeling W.L. 1999. Porcine parvovirus. In*:* B. E Straw, S. D´Allaire, W. L. Mengeling, D. J. Taylor (ed.), *Diseases of Swine*. (8th Ed.). Ames. Iowa. Iowa State University Press. pp. 187-200.

Mengeling, W.L., Lager, K.M. and Vorwald, A.C. 2000. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. *Anim. Reprod. Sci.* **60**: 199-210.

Mengeling, W.L. and Paul, P.S. 1986. Interepizootic survival of porcine parvovirus. *J. Am. Vet. Med. Assoc.* **188**: 1293-1295.

Shahbandeh. 2022. Meat consumption worldwide 1990-2021, by type.https://www.statista.cm/statistics/274522/global-per-capita-consumption-of-meat/ [07 Mar 23]

Pegu, S.R., Sarma, D.K., Rajkhowa, S., Choudhury, M., Sarma, D. and Das, J.P. 2017.Molecular detection of porcine circovirus type 2 and porcine parvo virus in pigs having reproductive problems and histopathological studies in the tissue of aborted pig foetuses. *Indian J. Anim. Res*. **51**: 732-736.

Xu, X.G., Chen, G.D., Huang, Y., Ding, L., Li, Z.C., Chang, C.D., Wang, C.Y., Tong, D.W. and Liu, H.J. 2012. Development of multiplex PCR for simultaneous detection of six swine DNA and RNA viruses. *J. Virol. Methods.* **183**: 69-74. ■