RESEARCH ARTICLE
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Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605

Detection and vp6 gene based molecular characterization of rotaviruses of pigs in Kerala*

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Citation: Logeshwaran, G., Ravishankar, C., Nandhakumar, D., Sebastian, S. R., Sumod, K., Jayakrishnan, T. R., Ravindran, R. and John, K. 2020. Detection and VP6 gene based molecular characterisation of rotaviruses of pigs in Kerala. *J. Vet. Anim. Sci.* **51**(1): 25-30.

Received : 18.06.2019 *Accepted : 30.07.2019* *Published : 01-01-2020*

Abstract

Rotaviral enteritis is a common condition observed in farm animals especially piglets and calves. Though the presence of porcine rotaviruses (PRV) have been reported in pigs in Kerala, no study has been conducted to characterize them. This paper reports the finding of a study conducted to detect and characterize PRV based on VP6 gene. A total of 87 samples collected from cases of piglet diarrhoea were subjected to VP6 gene based reverse transcriptase polymerase chain reaction (RT-PCR) and five (5.74 per cent) was found to be positive. All the positive samples were from Palakkad district. On analysis of the nucleotide sequence it was observed that the viruses belonged to inner capsid type I5 and I14 indicating diversity in the PRV prevalent in Kerala.

Key words: *Rotavirus, pigs, reverse tran-scriptase polymerase chain reaction, VP6 gene*

Rotaviruses are a diverse group of viruses which are found virtually in all species and are responsible for gastroenteritis or diarrhea. They are classified under the *Rotavirus* genus of *Reoviridae* family. They have double stranded segmented genomes and a triple-shelled capsid. The complete genome of rotaviruses contains11 segments, which encodes for six structural and six non-structural proteins (Estes, 2001). The two outer capsid proteins are VP4 (encoded by segment 4) and VP7 (encoded by segment 9). The VP6 (encoded by segment 6) forms the middle layer capsid, which interacts with the core protein VP2 and the outer capsid proteins VP4 and VP7 (Mathieu *et al.*, 2001). Rotaviruses are classified into ten groups (A to J) (Crawford *et al*., 2017) and subgroups (SG) based on the antigenic epitopes present on VP6 gene (Estes, 2001). In India, there

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have been reports of detection of rotaviruses in different animals (Ghosh *et al*., 2006 and 2007; Malik *et al*., 2013). Since rotaviruses have a segmented genome, there are chances for assortment between rotaviruses from different species. In Kerala, the presence of rotaviruses has been detected in pigs (Iyyappan, 2015) and calves (Ambily, 2007). However, there is no information about the lineage of the viruses present in these animals in Kerala. This paper report the findings of a study undertaken to detect and haracterize the porcine rotaviruses (PRV) in Kerala based on nucleotide sequence of VP6 gene.

Materials and methods

Sample

A total of 87 diarrhoeic faecal samples, collected in virus transport medium from piglets reared in organized farms in Wayanad, Palakkad, Thrissur and Ernakulam districts of Kerala during the period January 2018 to April 2019 constituted the sample for the study. The samples were transported on ice and stored at -20°C after proper labeling, till further processing.

RNA extraction and complementary DNA (cDNA) synthesis

Total RNA was extracted from a 10 per cent faecal suspension using TRIzol reagent (Thermo Scientific, USA) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from the RNA using random hexamers utilizing RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, USA). Briefly, to a PCR tube kept on ice, added 8 µL of total RNA extracted from stool samples, 1 µL (0.2 µg/µL) of random hexamer primer and 3 µL of nuclease free water (NFW) to make the volume up to 12 µL. The mixture was incubated at 90°C for 5 min to denature the double stranded RNA and snap chilled on ice. To the above mixture, 4 µL of 5X reaction buffer, 1 µL (20 U/µL) Ribolock RNase inhibitor, 2 µL of 10 mM dNTP mix and 1 µL (200U/µL) RevertAid H minus M-MuLV reverse transcriptase were added and the mixture was incubated at 25°C for 5 min followed by 60 min at 42°C. The reaction was terminated by heating at 70°C for 5 min.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Reverse transcriptase polymerase chain reaction (RT-PCR) for detection of Group A rotavirus in the sample was performed employing VP6 gene specific primers as described by Song *et al*. (2006) with slight modifications. Briefly, the reaction was carried out in a volume of 25 µL containing 12.5 µL EmeraldAmp GT PCR master mix (2X) (Takara, Japan), 1µL each of respective primers (10 pmol), 2µL of cDNA, and 8.5µL of NFW.The PCR condition was standardized as 95°C for 3 min (initial denaturation), 34 cycles of 95°C for 30 sec (denaturation), 53°C for 1 min (annealing), and 72°C for 30 sec (extension). Final extension was carried out at 72°C for 5 min. Complementary DNA prepared from Rotasiil vaccine (Serum Institute of India, Pune, India) was kept as the positive control. No template control (NTC) which does not contain template was also kept.

The PCR products were resolved electrophoretically on a 1.25 per cent agarose gel in 0.5X Tris borate EDTA (TBE) buffer containing ethidium bromide and were visualized in a gel documentation system under UV illumination to identify the size of the amplicon.

Phylogenetic Analysis

Representative amplicons obtained were sequenced at M/s AgriGenome Labs Private Limited, Kakkanad, Cochin, Kerala. The chromatograms obtained after sequencing were analyzed by using Chromas software (http://www.technelysium.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).

For phylogenetic analysis, nucleotide sequences of PRV from other parts of India and from other countries were downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/). Phylogenetic analysis of the nucleotide sequences was carried out using MEGA7 software. Using Clustal W program of MEGA7

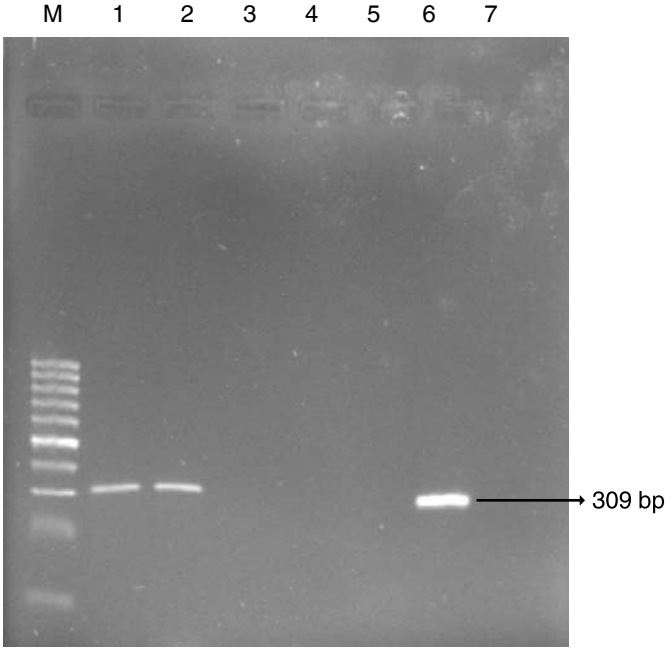
alignment of the downloaded sequences were carried out. The evolutionary history was inferred by using Maximum Likelihood method. The bootstrap consensus tree inferred from 100 replicates was taken to represent the evolutionary history of the sequences analyzed.

Results and Discussion

Of the 87 diarrhoeic faecal samples tested by VP6 gene based RT-PCR, 5 (5.74 per cent) were found to be positive as evidenced by a 309 bp amplicon (Figure 1). Specific amplicon of 309 bp was also obtained in the positive control also and no amplicons were detected in the NTC. All the positive samples were from Palakkad district. Iyyappan (2015), employed VP7 gene based RT-PCR, reported the detection of rotaviruses in 24 out of 67 (35.82 per cent) diarrhoeic feaecal samples from pigs in Thrissur district. However, in the present study, none of the samples collected from Thrissur were found to be positive for PRV. The reason for the low percentage positivity observed be due to the fact that the diarrhoea observed could have been due to etiologies other than rotavirus. In this study, VP6 based RT-PCR was employed for detection of PRV. Reverse transcriptase polymerase chain reaction based on VP6 gene of the virus has been used by other researchers also for the detection of PRV (Song *et al*., 2006; Ghosh *et al*., 2007). Besides VP6 gene based RT-PCR, VP7, VP4 and NSP2 based RT-PCR has also been used by various researchers for detection of rotavirus infections (Gentsch *et al*., 1992; Dubal *et al*., 2013; Lahon *et al*., 2014).

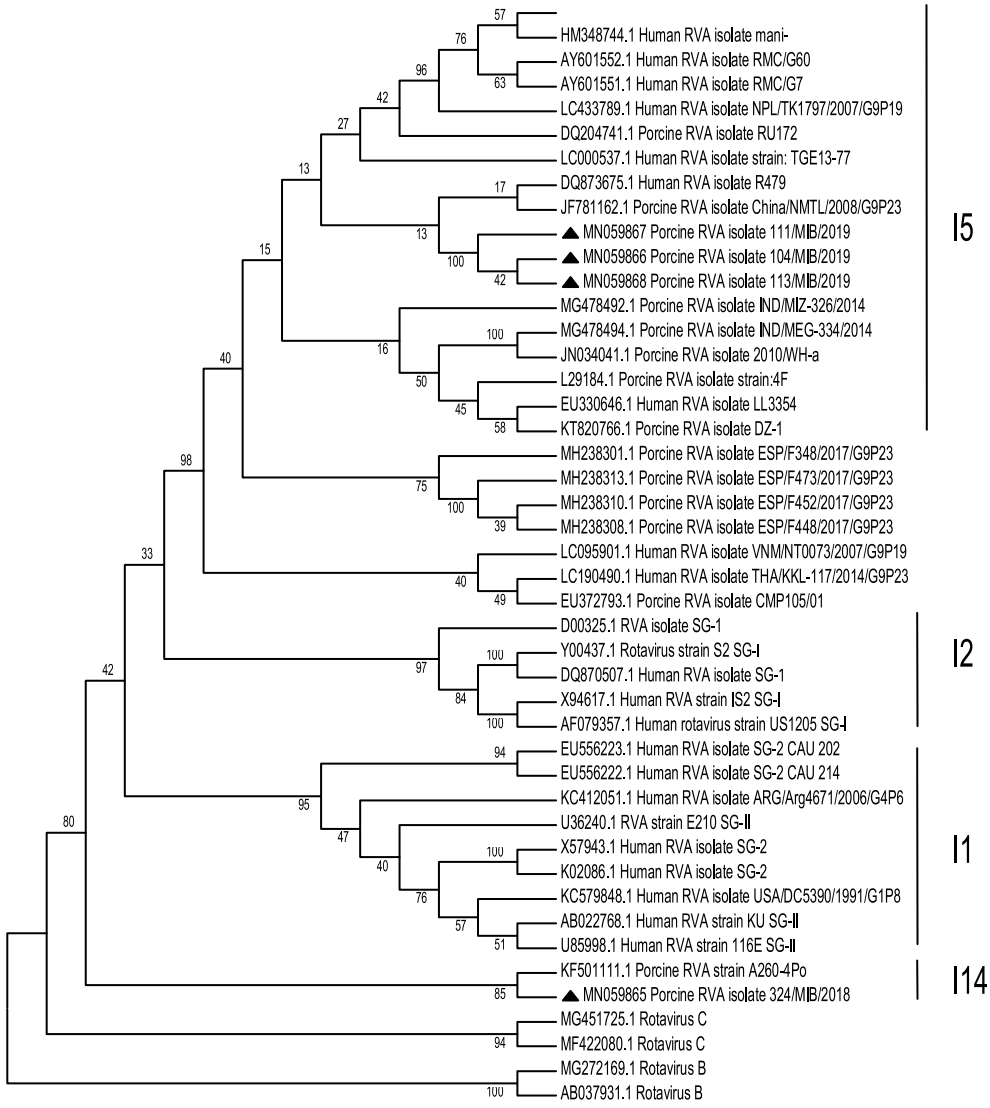
Amplicons obtained from four positive samples were sent to M/s AgriGenome Labs Private Limited, Kakkanad, Cochin, Kerala for sequencing. The samples sequenced were 324/MIB/2018, 104/MIB/2019, 111/MIB/2019 and 113/MIB/2019 and the sequences obtained have been assigned GenBank Accession numbers MN059865, MN059866, MN059867 and MN059868 respectively.

Figure 1. Detection of VP6 gene of rotavirus of pigs by RT-PCR



Lane M	-	100 bp DNA ladder
Lane 1, 2	-	Positive samples
Lane 3, 4, 5	-	Negative samples
Lane 6	-	Positive control (Rotasiil vaccine)
Lane 7	-	Negative control

Figure 2. Maximum likelihood tree constructed using nucleotide sequences of VP6 gene of rotavirus



On BLAST analysis, it was observed that all the sequences were similar to rotavirus A VP6 gene sequences from pigs. The samples 104/MIB/2019, 111/MIB/2019 and 113/MIB/2019 showed the identity of 97.53%, 96.84% and 97.37%, respectively to porcine rotavirus A VP6 gene sequences. However, the sequence of sample 324/MIB/2018 showed a comparatively lesser degree of similarity (88.89%) to other published porcine rotavirus A sequences.

Phylogenetic analysis of PRV employing VP6 gene has been used by many researchers (Martel-Paradis *et al*., 2013; Lachapelle *et al*. 2014; Nyaga *et al.,* 2014) and is a useful tool to characterize rotaviruses based on sequence of VP6 gene. The inner capsids (I) genotypes of rotaviruses are based on sequences of the VP6 gene and the reported I genotypes of PRV are I1, I2 and I5 (Matthijnssens *et al*., 2008 and 2011).

On phylogenetic analysis, it was observed that samples 104/MIB/2019, 111/MIB/2019 and 113/MIB/2019 clustered together though one isolate (111/MIB/2019) was slightly divergent. The cluster had close relationship

with porcine rotavirus A (JF781162) and to a human RVA isolate R479 (DQ873675), both from China. The closest Indian isolates were from Mizoram (MG478492) and Meghalaya (MG478494) and both were isolated from pigs. All the three isolates belonged to inner capsid type I5. The sample 324/MIB/2018 was observed to form a separate cluster along with an isolate A260-4 isolated from pigs in Canada which had the inner capsid type I14 (Figure 2). Similar divergence of I14 type strains were also observed by Lachapelle *et al*. (2014) while analyzing the genetic diversity of PRV from Canada.

There are no reports of genetic analysis of PRV sequence from Kerala till date. In the present study, the nucleotide sequence from a portion of the VP6 gene of the virus was used for genetic analysis and it was observed that the PRV detected had similarity to other rotavirus sequences from India and abroad and that the viruses come under I5 and I14 inner capsid types indicating a genetic diversity among the prevalent viruses.

Acknowledgments

The study has been carried out under the Science Research Scheme (SRS) funded by Kerala State Council for Science Technology and Environment (KSCSTE), Pattom, Thiruvananthapuram, Kerala. The authors thank the Dean, College of Veterinary and Animal Sciences, Pookode for providing facilities for conduct of the study.

References

Ambily, R. 2007. Detection of rotavirus in the faeces of diarrhoeic calves by reverse transcriptase-polymerase chain reaction and silver staining. MVScthesis. Kerala Agricultural University, Thrissur. 74p.

Crawford, S.E., Ramani, S., Tate, J.E., Parashar, U.D., Svensson, L., Hagbom, M., Franco, M.A., Greenberg, H.B., O'Ryan, M., Kang, G. and Desselberger, U., 2017. Rotavirus infection.*Nat. Rev. Dis. Primers***3**:17083.

Dubal, Z.B., Bhilegaonkar, K.N., Barbuddhe, S.B., Kolhe, R.P., Kaur, S., Rawat, S., Nambiar, P. and Karunakaran, M., 2013. Prevalence and genotypic (G and P) determination of porcine group A rotaviruses from different regions of India. *Trop. Anim. Health Prod.* **45**(2):609-615.

Estes, M. K. 2001. Rotaviruses and their replication. In Fields, B. N., Knipe, D. N.,Howley, P. M.,Chanock, R. M.,Melnick, J. L., Monath, T. P.,Roizman B. and StrausS. E. (ed.)*Fields Virology*. (4th Ed). Vol. 2, Lippincott–Raven, New York pp. 1747–1785.

Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K. and Bhan, M.K., 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J. Clin. Microbiol.* **30**:1365-1373.

Ghosh, S., Varghese, V., Samajdar, S., Bhattacharya, S.K., Kobayashi, N., and Naik, T.N.2006. Molecular characterization of a porcine Group A rotavirus strain with G12 genotype specificity. *Arch. Virol.* **151**:1329–1344.

Ghosh, S., Varghese, V., Samajdar, S., Sinha, M., Kobayashi, N. and Naik, T.N., 2007. Molecular characterization of bovine group A rotavirus G3P [3] strains. *Arch. Virol.* **152**:1935-1940.

Iyyappan, R. 2015. Reverse trancriptase-polymerase chain reaction based detection of rotavirus and coronavirus in the faeces of diarrhoeic piglets. MVSc thesis. Kerala Veterinary and Animal Sciences University, Pookode.78p.

Lachapelle, V., Sohal, J.S., Lambert, M.C., Brassard, J., Fravalo, P., Letellier, A. and L'Homme, Y. 2014. Genetic diversity of group A rotavirus in swine in Canada. *Arch. Virol.* **159**:1771-1779.

Lahon, A., Ingle, V.C., Birade, H.S.,Raut, C.G. and Chitambar, S.D. 2014. Molecular characterization of group B rotavirus circulating in pigs from India: Identification of a strain bearing a novel VP7 genotype, G21. *Vet. Microbiol.* **174**:342-352.

Malik, Y.S., Kumar, N., Sharma, K., Haq, A.A., Kumar, A. and Prasad, M. 2013. Sequence and phylogenetic analysis of bovine rotavirus isolates (G6 genotypes) from India. *Adv. Anim. Vet. Sci.***1**:41-43.

Martel-Paradis, O., Laurin, M.A., Martella, V., Sohal, J.S. and L'Homme, Y. 2013. Full-length genome analysis of G2, G9 and G11 porcine group A rotaviruses. *Vet. Microbiol.***162**:94-102.

Mathieu, M., Petitpas, I., Navaza, J., Lepault, J., Kohli, E., Pothier, P., Prasad, B.V., Cohen, J. and Rey, F.A. 2001. Atomic structure of the major capsid protein of rotavirus: implications for the architecture of the virion. *EMBO J.***20**:1485-1497.

Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M.K., Gentsch, J.R., Iturriza-Gómara, M., Kirkwood, C.D., Martella, V. and Mertens, P.P. 2008. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Arch. Virol.***153**:1621-1629.

Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Bányai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R. and Iturriza-Gómara, M. 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch. Virol.***156**:1397-1413.

Nyaga, M.M., Esona, M.D., Jere, K.C., Peenze, I., Seheri, M.L. and Mphahlele, M.J. 2014. Genetic diversity of rotavirus genome segment 6 (encoding VP6) in Pretoria, South Africa. *Springer Plus.***3**:179-183.

Song, D.S., Kang, B.K., Oh, J.S., Ha, G.W., Yang, J.S., Moon, H.J., Jang, Y.S. and Park, B.K. 2006. Multiplex reverse transcription-PCR for rapid differential detection of porcine epidemic diarrhea virus, transmissible gastroenteritis virus, and porcine group A rotavirus. *J. Vet. Diagn. Invest*.**18**:278-281. ■