First Detection of Rotavirus C in Asymptomatic Pigs from India with Unexpected Dominance and Characterization of the Structural and Nonstructural Genes

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Abstract

Epidemiological and molecular investigation was conducted on Rotavirus C (RVC), the viral agent documented with rising prevalence rate, disease severity and cross species transmission and large outbreak potential. Fecal specimens of pigs collected from two cities of Maharashtra state, India tested for RVC showed 20.1% detection rate with majority below 3 months of age. No significant difference in detection rates was observed in the specimens collected in 2009 from Northern and in 2013 from Western parts of Maharashtra. The phylogenetic analyses showed presence of the I7 and I10 genotypes of the VP6 gene and representative strains with G1 and E5 genotypes of the VP7 and NSP4 gene respectively. Full genome characterization of a single strain showed presence of G1, P1, I7, R1, C1, M3, A1, N5, T5, E5, H1 genotypes of the VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 genes respectively. This is the first evidence of detection of porcine RVC in asymptomatic pigs in India as well as with highest detection rate reported in asymptomatic pigs till date globally. Identification of porcine RVC at two time intervals and two different parts of Maharashtra state indicates the possibility of continuous circulation of RVC in pig population through asymptomatic infections.

Introduction

Rotavirus, a member of the family *Reoviridae*, is the most important viral pathogen that causes gastroenteritis in humans and young animals. Rotavirus is classified into 10 species (A–J) based on the VP6 gene with I and J being the new candidate group [1–3]. The most common rotavirus groups that infect humans and pigs are Rotavirus A, B and C (RVA, RVB and RVC) with RVC being the second most common after RVA and with a very distinctive epidemiology [4]. RVCs are well-known cause of gastroenteritis in nursing, weaning and post weaning pigs and have been associated with large diarrheal outbreaks [5, 6, 7]. Globally, circulation of RVC has been documented for many decades in developed countries with prevalence rate of 4–62.2% in diarrheic and non-diarrheic pigs [7–14]. The need of studies on porcine RVC strains circulating in different geographical regions has been highlighted owing to high genetic diversity as compared to those of RVC strains of other host species (Human, Bovine, and Canine) [15]. The availability of molecular data on more number of porcine RVC strains would be important to understand the evolutionary relationship of the internal gene segments [16]. Due to major focus on RVA and unavailability of the commercial kits, scanty studies on RVC were available from Asian countries namely from Japan, South Korea, China and India [17–21]. A single study on porcine RVC strains circulating in pigs of north eastern, northern and southern parts of India has been documented and none from Western India [21]. On this background, present study reports about the porcine RVC strains circulating in Maharashtra state of Western India and their molecular and phylogenetic analysis.

Materials And Methods

A total of 149 retrospective fecal specimens collected from Yorkshire (white) breed of pigs asymptomatic for diarrheal disease were included in this study. Ninety four specimens were collected in the month of March 2009 from pigs aged between 1 day to 12 months and with male to female ratio of 1.5 (Table 1) from Nagpur city, Maharashtra state, Western India. Remaining 55 fecal specimens were collected from Shirwal, Satara district of Maharashtra state, between June and December 2013. The age group of pigs ranged between 2 to 18 months with male to female ratio of 0.8. All these specimens were tested previously for RVA and stored at -80°C [22, Unpublished data]. The specimens were tested for RVC using the methodology as reported earlier [23]. In brief, SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase kit (Invitrogen, USA) was used for RVC detection using indigenously designed VP6 gene-specific primers pairs (VP6-

MJF3:ATHAGACCAGCDGCGYATGAC and VP6-MJR1: AGCCACATAGTTCACATTTTCATC). Initially, 5µl of RNA was denatured at 97°C for 5 min and was rapidly chilled on ice for 5 minutes followed by addition of master mix for RT-PCR. The cycling conditions were initial reverse transcription step at 45°C for 30 min followed by 45 cycles of amplification (1 min at 94°C, 1 min at 55°C and 1 min at 68°C) and a final extension of 7 min at 68°C in a thermal cycler. The amplified PCR products were analyzed using agarose gel electrophoresis and visualized under UV transilluminator. All RT-PCR positive products were excised from the gel and purified using QIA quick gel extraction kit (QIAGEN, Hilden, Germany). The nucleotide sequencing of the purified PCR products was carried out using the Big Dye Terminator cycle sequencing kit v 3.1 (Applied Biosystems, Foster city, CA) followed by purification using DyeEx 2.0 Spin Kit, (Qiagen, USA). The purified DNA was sequenced in ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, USA). BLAST (www.ncbi.nlm.nih.gov/blast) tool in NCBI database was used to determine the nucleotide identity of the sequences. Phylogenetic tree was constructed by using Maximum Likelihood method with 1000 bootstrap support by selecting the best suited model for each gene using molecular evolutionary genomic analyzer software MEGA 6.0 [24]. The reference strains included in the study for phylogenetic analysis were isolated from diverse host species namely porcine, human, bovine and canine representing all genotypes of the RVC and were retrieved from GenBank.

Table 1

Age and gender wise distribution of the Rotavirus C (RVC) positivity in fecal specimens of pigs collected from farms in Nagpur and Shirwal, Maharashtra state of India

Feature		Nagpur Farm (n=94)	Shirwal Farm (n=55)	Total (n=149)
		Number of RVC positive/ Total number of specimens (%)	Number of RVC positive/ Total number of specimens (%)	Number of RVC positive/ Total number of specimens (%)
Age group (months)	0-6	15/73 (20.5)	4/20 (20)	19/93 (20.4)
	6-12	1/21 (4.7)	5/20(25)	6/41 (14.6)
	12-18	-	5/15(33.3)	5/15(33.3)
Gender	Male	9/56 (16.0)	6/26 (23.0)	15/82 (18.3)
	Female	7/38 (18.4)	8/29 (27.6)	15/ 67(22.3)

The attempt to characterize full genome of RVC strains was carried out on number of available specimens. Molecular characterization of the few RVC positive strains was possible for the VP7 and NSP4 genes and single strain was characterized for all 11 genes using corresponding gene specific primers [Table 2] [23, 25, 26]. The GenBank accession numbers of the NSP4 (n=9), VP7 (n=3) and VP6 (n=27) gene of the RVC positive strains are OK491932 to OK491940, OK491941 to OK491943 and OK491945 to OK491970, OK562700 respectively. The nucleotide sequences of the remaining genes of single RVC strain are submitted in GenBank with accession numbers from OK398108 (NSP1), OK398109 (NSP2), OK398110 (NSP3), OK398111 (NSP5), VP1 (OL452030-OL452033), VP2 (OL452029), OK491944 (VP3) and OK398112 (VP4). The nucleotide sequence obtained for the VP6 gene of three strains (NIV142148/Shirwal/2013, 174bp; NIV142092/Shirwal2013, 180bp; NIV094286/Nagpur /2009, 162 bp) could not be submitted in the GenBank due to its < 200bp size.

Table 2
List of primers used in the study

Sr. no.	Primer	Position		Sequence (5'-3')	Base pairs	Study Reference
		Start	End			
1	PRVC-NSP1-FB3	1	19	GGCTTTAAAAAAGAGCTGG	19	Soma et al. 2013
2	PRVC-NSP1-RB	1269	1247	GCCACATAGAGTTTAGTCTGTTG	23	
3	PRVC NSP1-69F	69	93	CATAYAGAGAGATGCTYTAYTGGT	24	Present study
4	PRVC NSP1-820R	820	795	GACATCTTGARTGNGCATARTARAA	25	
5	PRVC-NSP2-FC1	2	19	TCCCAGCTGATCACTGGT	18	Soma et al. 2013
6	PRVC-NSP2-RB2	1037	1019	AGCCACATATGCACAGCTG	19	
7	PRVC-NSP2-FS1	597	614	ATCGCGATCAGACTGCTT	18	
8	PRVC-NSP2-RS1	262	240	CCAACAAATCCGCAACTCTAC	21	
9	PRVC-NSP3-FS2	1	22	GGCTTTTAAAAGCACTTGCTGG	22	Present study
10	PRVC-NSP3-RS2	1285	1264	AGTGCTCCGTATATTCTGGACT	22	
11	PRVC NSP3-167F	167	192	TATATAATGCATGTRTWGGRAGAGC	25	
12	PRVC NSP3-893R	893	873	CCRTCATCRAARCACCACCA	20	
13	NSP4-J1F	1	23	GGCTTTAAAWTTTTTCAGATCACT	23	Joshi et.al 2017
14	NSP4-613R	613	591	AGCCMCATGAATTTTTTCATATC	22	
15	PRVC VP1-10F	10	35	TCCACAGTCGTAGATGGGGACTACG	25	Present study
16	PRVC VP1-1772R	1772	1746	GCTGTTGCACCATATTGAATAGCTTT	26	
17	PRVC-VP1-FC2	1572	1592	CACAATGGGATGCTTCTCAAC	21	Soma et al. 2013
18	PRVC-VP1-RB2	3308	3289	GCCACAATATGCAATCCACT	20	
19	PRVC VP1-2029F	2029	2052	GGATGTGAAATAGCAAARAGATA	23	Present study
20	PRVC VP1-2708R	2708	2685	GGCATYGTRTTRTTTTCTTCTTT	23	
21	PRVC VP2-41F	41	63	GATCAATCGAGGACAAATCGTC	22	Soma et al. 2013
22	PRVC VP2-1707R	1707	1684	CATRTTCATRACTATRCATGCTGA	24	
23	PRVC-VP2-FC3	1250	1271	GAATCGCTAGTTGCAATGCAGT	22	
24	PRVC-VP2-RB3	2736	2717	AGCCACAAAGTTTGAGGTCG	20	

Sr. no.	Primer	Position		Sequence (5'–3')	Base pairs	Study Reference
		Start	End			
25	PRVC VP2-1621F	1621	1654	TCAAAYAGRACYGCYCAAATAGC	23	Present study
26	PRVC VP2-2108R	2108	2085	ATACGTTCTGAYGTTTTYTCTAT	23	
27	PRVC-VP3-FS2	919	940	CCGTCATGTACGATAATTGGAG	22	Soma et al. 2013
28	PRVC-VP3-RS2	2166	2147	AGCCACATCATACGGTAGAG	20	
29	PRVC-VP4-1F	1	18	GATCRATGGCRTCYTCAC	18	Present study
30	PRVC-VP4-1219R	1219	1197	CCTGATGAATGTAATCCWGGAT	22	
31	PRVC-VP4-1074F	1047	1066	GATTATTGGGACGATTCAG	19	
32	PRVC-VP4-2246R	2246	2226	AGCCACATTTCAAGCTGGTC	20	
33	VP6-J1F	1	27	GGCTTTAAAAATCTCATTCACAATGG	27	Joshi et.al 2019
34	VP6-MJF3	898	918	ATHAGACCAGCDGCIATGAC	20	Present study
35	VP6-MJR1	1361	1339	AGCCACATAGTTCACATTTTCATC	22	Joshi et.al 2017
36	VP7-J1F	1	21	GGCATTTAAAAAAGAAGAAGCTGT	21	Joshi et.al 2019
37	VP7-677R	677	653	ATTCCATTACTTGTAGTATCTAATG	24	
38	VP7-J1063R	1063	1042	AGCCACATGATCTTGTTTACG	21	
39	NSP5- J548F	548	568	GATGAAGATATTGAGGGATG	20	Joshi et.al 2019
40	PRVC-NSP5-R	696	678	CAATGCCGATTCCAGTCA	17	Present study
41	PRVC-NSP5-RS2	695	679	TCAATGCCGATTCCAGT	17	Soma et al. 2013

Results

Out of 149 fecal specimens, RVC was detected in 20.1% (n=30) using the VP6 gene based RT-PCR. The detection rate was 17% (16/94) and 25.4% (14/55) at Nagpur and Shirwal farm site respectively. No significant difference in percent RVC positivity was observed among these two study sites ($p>0.05$). Ninety three (62.4%) specimens were collected from pigs below 6 months of age. Comparison of the proportion of RVC positives among different age groups (0-6 month, 6-12 month, 12-18 month) showed no statistical significant difference ($p >0.05$ for each) (Table 1). Similarly distribution of percent RVC positivity in male and female pigs showed no significant difference ($p >0.05$ for each) (Table 1).

The phylogenetic analysis of the nucleotide sequence data obtained for the VP6 gene showed presence two genotypes of the VP6 gene namely I7 and I10. The phylogenetic tree of the representative study strains is shown in figure 1. The study strains grouped with GenBank strains of I7 genotype with 86% bootstrap support and were closely related to Indian strain (Accession number KX374482) and Japanese strain (AB889506) with percent nucleotide identity (PNI) values ranged between 96.6- 97.8% and 89.6- 90.9% respectively. PNI values within the study strain was 98.3% -100% and with human, bovine, canine and ferret strains was 83.7-84.8%, 84.4% - 85.4%, 82.1%-82.8% and 79.1%- 81.1 respectively. Three study strains grouped with GenBank Indian (KX374480) and Korean strain (KJ814478) of I10 genotype with PNI of 95.5- 98.5% and 92.8- 93.6% respectively. PNI values within the study strains was 96.2-100% and those between study strains and human, bovine, canine and ferret strains were 83.4-83.8%, 85.7%, 81.3- 82.3% and 79.8-80.8% respectively. With other porcine genotype strains it ranged between 79.6-88.3%.

A total of three RVC positive specimens were amplified by using the VP7 gene specific primers. Phylogenetic analysis classified them into G1 genotype with 94% bootstrap support. The PNI values within the study strain were between 99.4 - 100% whereas those with reference strains of G1 genotype were 85.5 - 90.8%. PNI between study strains and human, bovine canine and porcine strains other than G1 genotype was 82.8 - 83.1%, 69.5- 69.9%, 74.1- 74.6%, 71.3-84.7% respectively.

A total of nine RVC positive specimens were amplified using the NSP4 gene specific primers reported earlier for the characterization human RVC strains (Table 2). Further, phylogenetic analysis of the nucleotide sequences of the nine study strains classified them in to E5 genotype (Figure 3). PNI within these study strains was 97-100%. Identity of these strains with GenBank E5 genotypes strains and Indian strain was 85.3- 87.3% and 96.7% - 97.5% whereas identity with human, bovine and canine species was 64.5%, 64.8- 65.7% and 74.5-75.1% respectively. Among these nine RVC strains two were with I10 genotype for the VP6 gene (NIV94306, NIV94340).

Successful characterization of all 11 genes was possible only for single strain (NIV094290/Nagpur /2009) using different primer pairs (Table 2). The nucleotide sequence data for 81.8% of genome (14504 bp) was obtained with more than 70% sequence data for each gene with exception of the VP3 (53.3%) and NSP5 genes (19.2%) (Table 3). The PNI values and phylogenetic analysis classified RVC strain as G1, P1, I7, R1, C1, M3, A1, N5, T5, E5, H1 genotype of the VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 gene respectively (Table 3, Figure 4a-4h). Study strain showed highest PNI values with other Indian strains for the VP6 and NSP4 gene, with Japanese strains for the VP7, VP2, NSP1, NSP2, NSP3 and NSP5 genes and with Belgium, China and USA strains for the VP1, VP3 and VP4 genes respectively (Table 3). Comparative analysis of the PNI values of the study strain with other genotype strains showed occurrence of higher PNI values as compared to cut off values [20] exceptionally by I13 genotype strain (KF036287) for the VP6 ; R2 genotype strain (HQ185652) for the VP1; M1 genotype strain (M74219) for the VP3 ; E1 genotype strain (LC122630) for the NSP4; H4 genotype canine strain (KP988023) for the NSP5 gene (Table 3). The study strain classified as A1 genotype (NSP1 gene) during phylogenetic analysis showed PNI values between 82.6 and 83.2 with other A1 genotype strains. These PNI values were lower than the recommended cut off value of 84% for NSP1 gene.

Table 3
Full genome characterization of the porcine rotavirus C strain

RNA segment (Length in base pairs)	Nucleotide sequence obtained in base pairs	Nucleotide sequence obtained (%)	Genotype identified (% nucleotide identity)	Closest to GenBank strain	Nucleotide identity with other genotype strains (%)	Cut-off values (%)
VP7 (1063)	962	90.5	G1 (86.5-93.3)	AB905212/ Porcine/Japan/2002	71.5 - 84.9	85
VP4 (2246)	2120	94.3	P1 (89.7)	M74218/Porcine /USA/1991/G1P1	71.8-82.1	85
VP6 (1352)	1294	95.7	I7 (89.8-98.6)	KX374482/Porcine/India/2016	81.9-87.7*	87
VP1 (3290)	2582	78.5	R1 (85.1-87.0)	KP982879/PorcineBelgium/2012, MK953076/Porcine /Belgium/2012	79.1-84.9*	84
VP2 (2736)	2622	95.8	C1 (85.4-88.5)	LC307103/Porcine /Japan/2002	79.3 – 84.6	85
VP3 (2145)	1144	53.3	M3 (87.8-89.9)	MK379292/Porcine /China/2017	77.9 - 85.7*	85
NSP1 (1235)	1199	97.1	A1 (82.6-83.2) **	LC306986/Porcine /Japan/2008	65.2- 81.1	84
NSP2 (995)	882	88.6	N5 (89.8-92.8)	LC306994/Porcine /Japan/2002	79.9- 86.7	87
NSP3 (1348)	1134	84.1	T5(86.9-88.4)	LC307027/Porcine/ Japan/2008	75.5 -77.8	85
NSP4 (613)	432	70.5	E5(86.7-96.7)	KY783642/Porcine-India/2015/	64.5-81.4*	81
NSP5 (693)	133	19.2	H1(87.3-91.2)	LC122599/Porcine/ Japan/2015	76.5-84.3*	80
Total (17716)	14504	81.8	-	-	-	-

Discussion

Disorganized means of pig feeding and farming, inadequate housing of herds, improper managements known to expose pigs to a number of infectious agents including gastrointestinal infection amongst which rotaviral infection are most common. Pig farming is measured as a spine for poor farmers belonging to rural areas in India and 40%

of it is restricted to north eastern states. Till date only a single study documented from India (north eastern, northern and southern parts) with 12% prevalence rate of RVC in symptomatic pigs [21]. In the present study fecal specimens of asymptomatic pigs (n=149) from Western India showed 20.1% detection rate. Majority of the RVC prevalence studies reported so far, were conducted in mixed population of diarrheic and non-diarrheic pigs with detection rates higher in diarrheic pigs [7, 17, 27]. The studies solely on asymptomatic pigs were documented from Ireland, Western Kenya and Uganda with 4.4 - 8.7% detection rates [9, 28]. Overall, this is the first study from India on recognition of the RVC in asymptomatic pigs as well as with highest detection rate reported in asymptomatic pigs till date globally. The high prevalence of RVC in symptomatic pigs has been allocated to high density of pig population in Tororo sub-country of Uganda [13]. Further, the high detection rate may possibly be due to use of sensitive diagnostic method. Comparative utility of RT-PCR assays needs to be examined from time to time due to the continuous accumulation of point mutations in rotavirus genome, reassortment events and possibility of cross species transmission leading to introduction of new genotypes and /or lineages [29]. Therefore, the nucleotide alignment of GenBank strains of porcine RVC (n=59), the one which was used for designing primers of the present study was also used for the analysis of primers reported by Amimo et.al (2017) and Collins et.al (2008). It should be noted that the forward primer (VP6F) reported by Amimo et.al (2017) (Figure 5) and Collins et.al (2008) (BMJ 44) showed multiple nucleotide substitutions at multiple sites indicating the necessity of appropriate modifications.

Among specimens collected from Nagpur farm, 19 were positive for RVA [22] and 6 showed dual infection with RVA and RVC. In Shirwal farm, single specimen showed dual infection with RVA and RVC (data not shown). It should be noted that in spite of dual infection of RVA and RVC in a total of seven pigs, diarrheal symptoms were not recorded in any of these animals. The possibility of collection of the samples from pigs recently recovered from diarrheal episodes was minimal as 63.3% (19/30) RVC positive specimens belong to pigs below 3 months of age and collection of specimens (for Shirwal site) was done over a six months period. No significant difference in RVC detection rates observed in the specimens collected in 2009 from Nagpur and in 2013 from Shirwal farm ($p>0.05$) which belong to Northern and Western parts of Maharashtra state respectively indicates the possibility of continuous circulation of RVC in pigs of Maharashtra state through asymptomatic infections.

Previously, highest RVC infection rate has been documented in post weaning pigs (22-55 days) and in older pigs (5–6 months) [11, 28]. In non diarrheic pigs from USA, 6.6% prevalence rate of RVC in weaning pigs was reported [27]. It has been reported that insufficient lactogenic protection provided by gilts plays a key role in the development and increase in clinical RVC disease [30]. Maternally derived circulating RVA specific antibodies have been reported to mediate high levels of passive protection against human RVA disease [31]. However, no such studies reported till date on passive immunity against RVC. Therefore, asymptomatic RVC infections observed in the present study point out the possibility of lactogenic protection of pigs and need of further serological studies to confirm the same.

Among the scanty data available on genetic characterization of RVC strains from Asian countries, circulation of the G1, G3, G5, G6, G7, G9, G12 and G13 genotypes of the VP7, P1, P4–P9 genotypes of the VP4, I1, I4, I6 and I7 genotypes of the VP6 and M1, M3, M6 genotypes of the VP3 gene have been reported [18 - 20, 32]. A single study from India reported circulation of the I2, E4-E8, H6 genotypes of the VP6, NSP4 and NSP5 genes respectively among diarrheic pigs [21]. In the present study two genotypes of the VP6 gene namely I7 and I10 along with G1, P1 and E5 genotypes of the VP7, VP4 and NSP4 genes were identified respectively. Till date, studies solely on asymptomatic pigs indicated dominance of the G1, occurrence of the G6 genotype in Ireland and G3P5 genotype in a single strain from Belgium [9, 33]. Genotyping data was not available for the studies on asymptomatic pigs reported from Western Kenya, eastern Uganda and China [17, 28]. Whole genome characterization of the RVC strains from asymptomatic pigs has so far been reported for a single Belgium strain with G3, P5, I11, R1, C1, A7, N8, T5, E5, H1

genotype of the VP7, VP4, VP6, VP1, VP2, NSP1, NSP2, NSP3, NSP4 and NSP5 gene respectively whereas the VP3 gene remained ungrouped [33]. Full genome characterization of a present study strain classified it as G1, P1, I7, R1, C1, M3, A1, N5, T5, E5, H1 genotype of the VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 gene respectively. High nucleotide similarity of the study strain with strains originated from different countries (except for the VP6 and NSP4 gene) and for majority genes with Japanese strains may be due to lack of genomic data from India (Table 3). The cut-off values of PNI for identification of different genotypes have been described earlier [20]. Using the same criterion, for few genes (VP6, VP1, VP3, NSP4 and NSP5) overlapping PNI values of the study strain with other genotype strains rather than the designated ones were observed exceptionally (Table 3). During NSP1 gene phylogenetic analysis study strain grouped with A1 genotype with 94% bootstrap support however, lower PNI values than the recommended cut off value of 84% were observed as compared to A1 genotype strains. Overall, more molecular data from India and different parts of the world is required for validation of the genotyping criteria suggested by Suzuki et.al (2017) as well as to elucidate the evolutionary relationship of the RVC strains.

The main limitation of the current study is long term storage of the clinical specimens. Reduction in RVC detection rate possibly will be negligible as double stranded RNA are known to be more stable and reported to have wide applications for accurate assessment in environmental and biological systems [34]. Failure to characterize complete genome of the remaining study strains was mostly due to low viral load as the infection were subclinical as well as shortage in amount of few fecal specimens.

Apart from this the increase in disease severity as well as prevalence rate (from 10 to 46%) of RVC over the period has been reported [11, 35]. The rise in RVC infections in piglets was attributed to large scale RVA vaccination carried out to control and mitigate disease especially in developed countries [7, 11, 14]. Even though, host specific (bovine, canine, human, and porcine) nature of the RVC genotypes is known, (excluding the VP6 and VP3 gene in which human and porcine strains share evolutionary relationship) evidences of cross species transmission and reassortment events have been documented [15, 21, 36]. Identification of porcine RVC in bovine, bovine RVC in porcine, porcine RVC in human and human RVC in porcine species has been reported [21, 37- 40]. In addition, high RVC antibody prevalence documented in rural as compared to urban region was suspected to be due to close proximity of the humans with animals [38]. Therefore, RVC the viral agent with escalating prevalence rate and disease severity over the period needs to be monitored closely keeping in mind its cross species transmission and large outbreak potential.

Identification of porcine RVC in large number of asymptomatic pigs in the present study may possibly have significance in perpetuation of the virus in the environment. Molecular characterization studies on RVC strains isolated from age matched weaning and post weaning, diarrheic and non-diarrheic piglets/pigs from the same geographical regions will be useful to define the role of maternal immunity and genotypes of RVC in symptomatic or asymptomatic infections. The epidemiological and molecular studies need to be conducted to know exact disease burden and genomic diversity existing in RVC strains circulating in different parts of the world. The data obtained will be useful for development of more accurate diagnostic tools and for future vaccine candidate.

Declarations

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose

Authors contribution

Conceptualization, chief Investigation, analysis, manuscript write up, review and over all execution by Dr. Madhuri S. Joshi, conducting laboratory tests, analysis, compilation of data, manuscript write up by Shalu A. Arya, conducting laboratory tests, maintenance of demographic and clinical data by Manohar S. Shinde, key role in project management, supervision, manuscript writing, review & editing by Dr. Varanasi Gopalkrishna. *All authors read and approved the final manuscript.*

Data Availability

The datasets generated during the current study are available in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>).

Ethics approval

The study was approved by the institutional Animal Ethics committee on 3rd November 2014.

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Figures

Figure 1

Maximum likelihood tree of the partial nucleotide sequences of the VP6 (460bp) gene constructed in MEGA 6. Best fit model was T9+G with 1000 bootstrap replicates. The study strains are indicated with filled circle. The genotypes of the reference strains are indicated on right side (I 1–I 13).

Figure 2

Maximum likelihood tree of the partial (563bp) nucleotide sequences of the VP7 gene constructed in MEGA 6. Best fit model was T9+G+I with 1000 bootstrap replicates. The study strains are indicated with filled circle. The genotypes of reference strains are assigned on right side (G1–G18).

Figure 3

Maximum likelihood tree of the partial (458bp) nucleotide sequences of the NSP4 gene constructed in MEGA 6. Best fit model was T9+I with 1000 bootstrap replicates. The study strains are indicated with filled circle. The reference strains genotypes are assigned in right (E1–E5).

Figure 4

a-h Maximum Likelihood trees of the nucleotide sequences of the VP1, VP2, VP3, VP4, NSP1, NSP2, NSP3 and NSP5 gene segments are shown in figure. The study strain (NIV94290) is indicated with filled circle. Best fit model GTR+G+I with 1000 bootstrap replicates was used for all genes. The reference strains of different genotypes are shown with square bracket in right. Bars indicate nucleotide substitutions per site.

Figure 5

Determination of frequency of nucleotide substitutions in VP6 F primer published by Amimo [et.al](#) (2013) using GenBank nucleotide sequences of Porcine Rotavirus C strain (n=59)