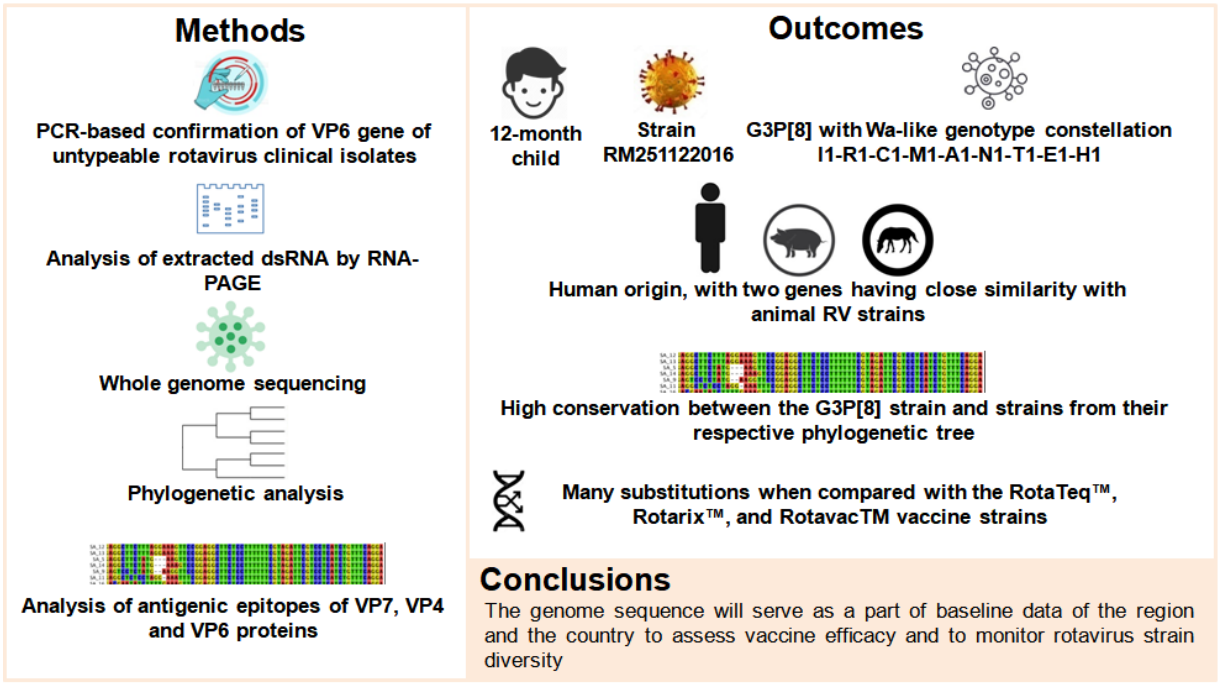
CHAPTER 5

CHARACTERIZATION OF UNUSUAL OR UNTYPEABLE ROTAVIRUS ISOLATES BY ELECTROPHEROTYPING AND WHOLE GENOME SEQUENCING

Graphical Abstract



5.1. Introduction

The genome of rotavirus is made up of 11-segments of double-stranded RNA (dsRNA) of differing lengths [1]. In epidemiological studies of rotaviruses, the most commonly and widely used classification approach is the binomial system called G-P genotyping, based on the genes encoding the two outer layer capsid proteins, VP7 and VP4 [2,3]. The segmented nature of the viral genome is the main factor for reassortment events, a mechanism of genetic evolution of RVs, between different genotype strains when co-infect the same host. A new classification approach mentioned in Chapter 2, based on the 11 segments assigns a specific genotype to each genome segments complying to the established nucleotide percent cut-off values and gives better understanding of how the reassortants evolves and inter-species transmission of the virus. So far, two major (Wa-

like and DS-1-like) and one minor (AU-1-like) based on the genes excluding VP7 and VP4 and reassortants between these genotype constellations circulate worldwide among humans [4].

We conducted a rotavirus surveillance study in Imphal, during December 2015 to March 2019 in children hospitalized with acute gastroenteritis in Imphal and identified several unusual rotavirus isolates based on separation of 11-dsRNA genome. A 12-month-old child presented with diarrhoea, mild fever, and vomiting was tested negative for RVA antigen using the Premier™ Rotaclone® ELISA kit (Meridian Bioscience Inc., USA). However, the Rota+Adeno+Astro+Noro EIA Combo Card kit (CertestBiotec, S.L., Spain) revealed a faint band and showed an unusual migration pattern of 11-dsRNA upon polyacrylamide gel electrophoresis. A viral metagenomics approach identified a Wa-like genotype G3P[8] strain named RVA/Human-wt/IND/RM25112/2016. Further we performed sequence analysis to identify amino acid variations in the antigenic epitopes of VP7, VP4 and VP6 proteins of RM251122016 strain, vaccine strains and other strains from the NCBI database.

5.2. Materials and methods

5.2.1. Electropherotyping (E-types) using Native-PAGE

A hospital-based surveillance study for RVA was conducted during December 2015 to March 2019. Those clinical stool samples which were positive by ELISA but untypeable by existing G-P primers were subjected for cDNA synthesis followed by PCR amplification of VP6 gene. If VP6 was not amplified, RNA was extracted using another method, cDNA was synthesized using random priming, and the VP6 gene PCR was repeated. If VP6 could not be amplified once again, VP6 primer-specific priming was carried out. Those samples which were negative for VP6 gene were considered as false positive ELISA. VP6 positive stool samples were subjected for G (VP7) and P (VP4) specific PCR followed by sequencing. Then, some of the untypeable isolates were selected for whole genome sequencing (WGS) using the procedure given in Figure 5.1.

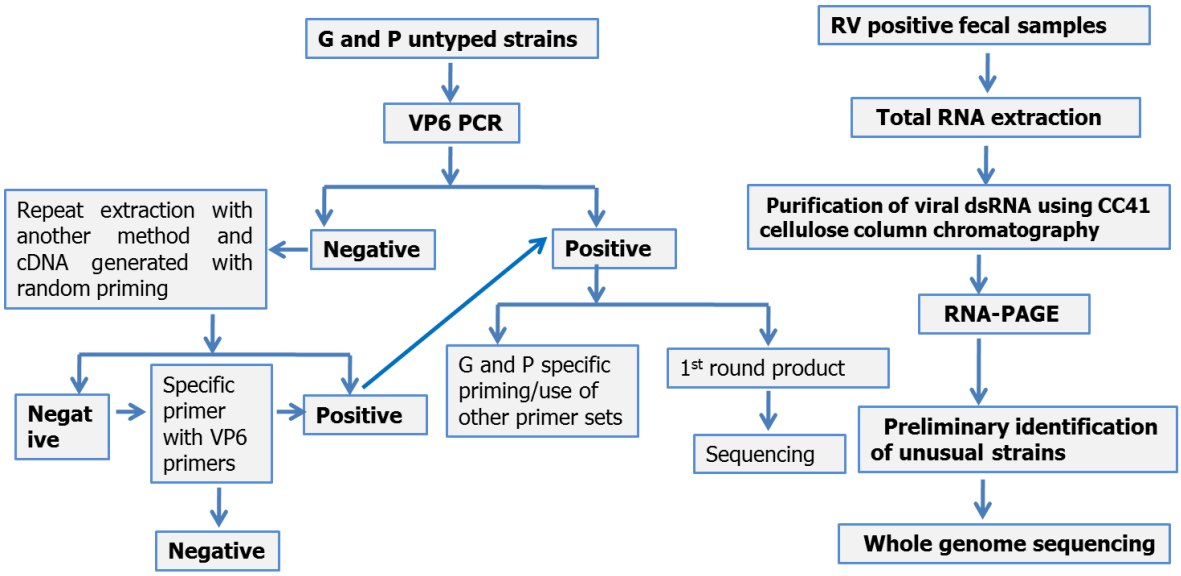


Figure 5.1. Laboratory strategy used to identify unusual and untypeable rotavirus strains isolated from the diarrheic children of clinical stool specimens. The 20% stool suspensions were centrifuged for 12 min at 14,500 rpm and filtered using a 0.22-micron syringe filter. Subsequently, total RNA was extracted from the filtrate in two vials by TRIzol method (Invitrogen™; Cat No.15596018). The rotaviral dsRNA was purified using CC41 cellulose column chromatography. The migration patterns of 11-dsRNA segments of rotavirus genome were analyzed on 14% polyacrylamide gel stained with silver nitrate. Rotavirus isolates are classified into short, moderate, and long electropherotypes (E-type) based on migration of gene segment 11. Generally, RVAs with long E-type belong to the subgroup II with serotypes 1, 3, 4 and 9 and short E-type belong to the subgroup I with serotype 2 [15]. Based on E-type, some of the unusual isolates have been selected for whole genome sequencing using next generation sequencing (NGS) approach (Figure 5.2).

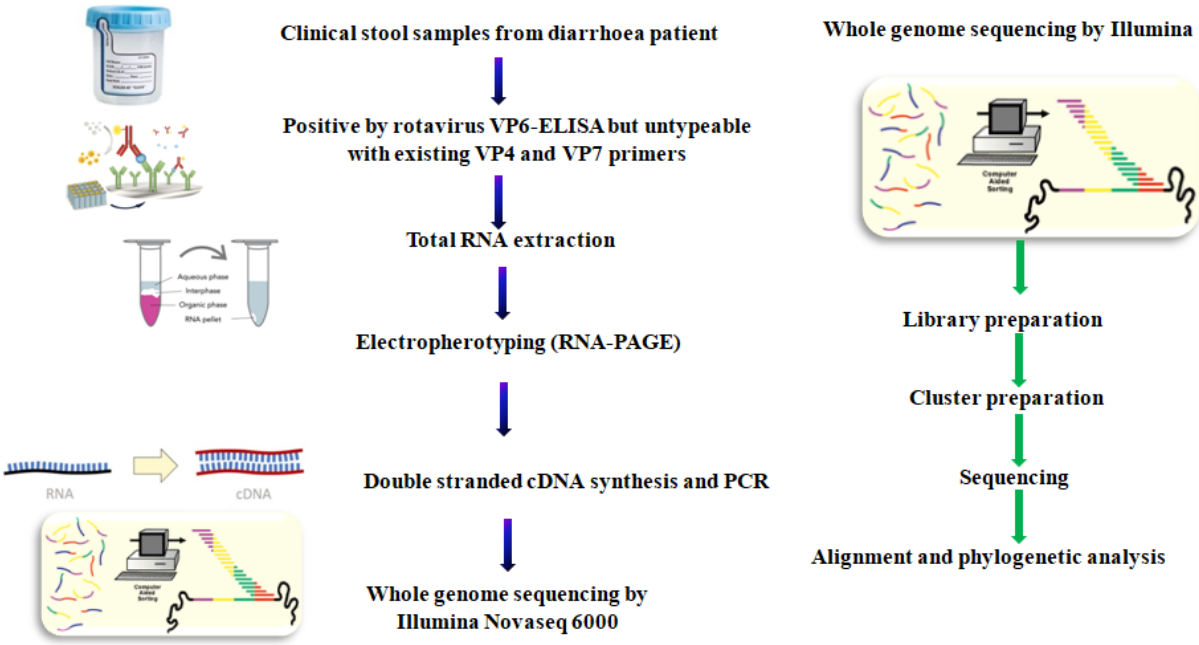


Figure 5.2: Workflow for preparation of rotavirus samples for whole genome sequencing by Illumina sequencing platform.

5.2.2. Detection of group B and C rotaviruses

Total RNA extraction was carried out from all group A rotavirus ELISA negative samples. The presence of 11-dsRNA was analyzed by RNA-PAGE. Further confirmation for the detection of group B and C rotaviruses was done by PCR with gene-specific primers given in Table 5.1.

Table 5.1: Assembly and genotyping details of the RVA RM251122016 genome

No	Primer Name	Sequence 5'->3'	Position (nt)	Target gene	Product length (bp)	Gene size (bp)	Reference
1	ADG8-1F	CTATTCAGTGTGTCGTGAGAGG	18-39	NSP2	489	1059	[40]
2	ADG8-2R	CGTGGCTTTGGAAAATTCTTG	486-506				
3	GrB_VP7_25F	CTTCTCGTCCTTGCTGCTG	25-43	VP7	790	1062	[41]
4	GrB_VP7_814R	GGGTTTTTACAGCTTCGGC	796-814				
5	GrB_VP4_13F	GCTATGTTGACGTATTTACG	13-33	VP4	1166	2362	
6	GrB_VP4_1178R	GTATAACCAGAAGCGTCCAC	1159-1178				
7	GrC_BMJ-107_VP7_FP	TGTTTGGAGATGTGATGA	546–563	VP7	518	1062	[42]
8	GrC_BMJ-13_VP7_RP	AGCCACATGATCTTGTTT	1046-1063				
9	GpC_BMJ-145_VP6_FP	AGTCCGTTCTATGTGATTC	1014-1032	VP6	318	1356	
10	GpC_BMJ-144_VP6_RP	CCTTCTGGGGATCATCCAT	1313-1331				
11	GCVP4-2FP	GTAAGGACTCATTGTGGCAAGA	820-843	VP4	465	2362	[43]
12	GCVP4-12RP	CATAAACAAGTTGCAACCTTGATG	1252-1275				

5.2.3. Clinical details of RVA isolate (RM251122016) and sample preparation for whole genome sequencing

Stool sample (RM251122016) collected from a 12-month-old male child presented with acute gastroenteritis was admitted in the Department of Paediatrics, RIMS, Imphal, India. The child was admitted in December 2016 due to mild fever, diarrhoea, and vomiting. The child had no history of vaccination and hailed from a rural area of Imphal East district, Manipur. The presence of RVA antigen was detected using Meridian Premier™ Rotaclone® kit (Meridian Bioscience Inc. USA) and a rapid immunochromatographic test, Rota+Adeno+Astro+Noro EIA Combo Card kit (CertestBiotec, S.L. Spain). Viral dsRNA extraction and cDNA synthesis was performed using the protocol described in Chapter 3.

5.2.4. Whole genome sequencing (WGS) and phylogenetic analysis

For whole genome sequencing, the cDNA was fragmented, and library was prepared. Final libraries were quantified using Qubit 4.0 fluorometer (Thermofisher #Q33238) using DNA HS assay kit (Thermofisher #Q32851) following manufacturer's protocol. Paired-end (2x150 bp) sequencing was done using NOVASEQ 6000 (Illumina). Raw data assessment was performed using FastQC v.0.11.9 (default parameters). The Processed reads were submitted to Genome Detective [5] and CCMetagen [6] online server to know the taxonomic assignment of the sequences. Later, cleaned reads were aligned to all the strains of the rotavirus using Bowtie2 v2.4.2 [7] to know the alignment statistics. Using CCMetagen, the rotavirus specific reads were extracted and assembled with Megahit v1.2.9 [8]. Assembled reads were submitted to the ViPR (Virus Pathogen Database and Analysis Resource) online tool [9] for genotypic determination (Figure 5.3). The CCMetagen protocol, after quality trimming, yielded 8.7 million reads and eleven near-complete segments of genome of an RVA strain were recovered as shown in Table 5.2.

This strain was typed as G3P[8] with a human Wa-like genotype backbone; I1-R1-C1-M1-A1-N1-T1-E1-H1, which has common ancestor with porcine strains [10]. The gene segments showed similarity in the range of 96.93 % to 99.49 % with known RVA strains in the respective phylogenetic tree (Table 5.2). In terms of protein sequence, particularly for VP7 and VP4, 2.76% (9/326) and 4.10% (26/634) of the amino acid residues, respectively, were different from the RotaTeq G3P[8] vaccine strain. Phylogenetic analysis of VP7 and VP4 and the remaining nine genes were conducted in MEGA X (www.megasoftwares.com) [11] using maximum likelihood method and general time reversible model. The assembled genomes have been deposited in GenBank under the accession numbers OL906383 to OL906393. The raw reads have been deposited under SRA accession number SRX14381993 (https://www.ncbi.nlm.nih.gov/sra/SRR18240550).

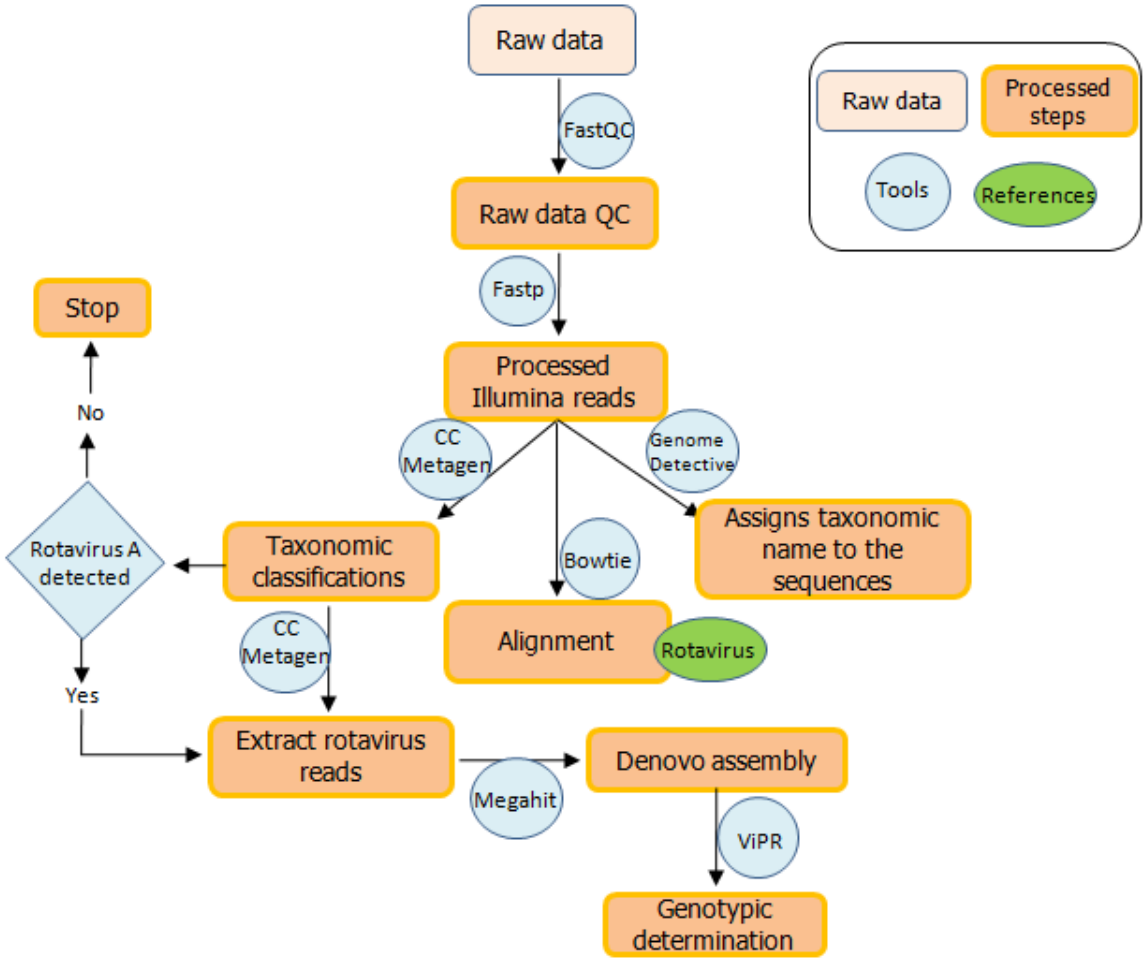


Figure 5.3: Workflow for analysis of rotavirus sequence using the raw sequence reads from Illumina Novaseq 6000.

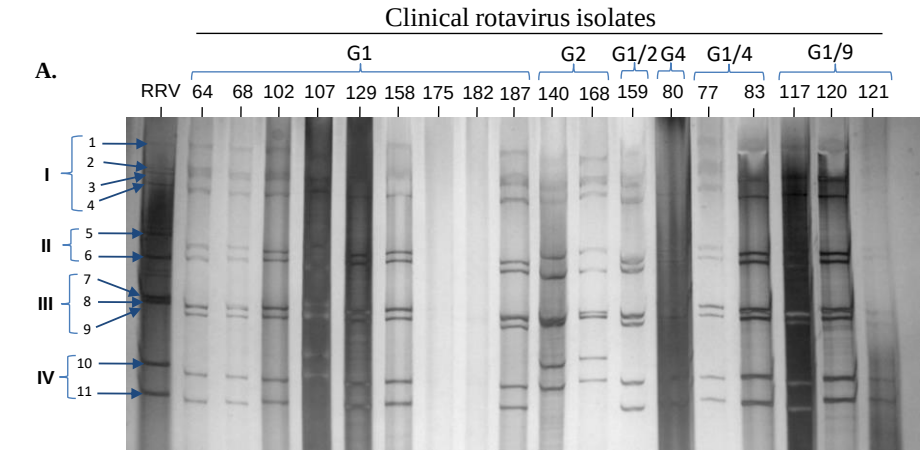
Table 5.2: Assembly and genotyping details of the RVA RM251122016 genome

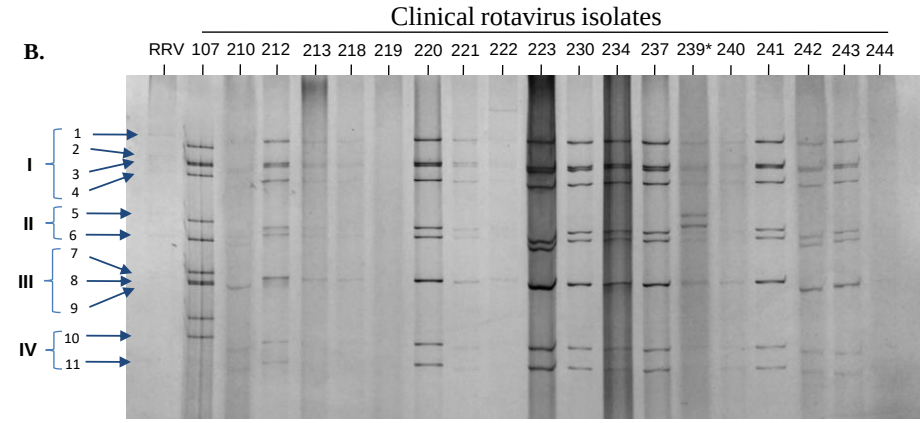
Segment no.	Segment length (bp)	Mean Coverage	Read Count	GC content (%)	ORFa length (bp) (Protein product)	Genotype	Closest strain in GenBank	Accession no. GenBank	Similarity (%)
1 (VP1)	3299	871	6,270	31%	955 (VP1)	R1	GU199492.1	OL906393	98.34
2 (VP2)	2915	1,179	7,358	31%	894 (VP2)	C1	DQ146661.1	OL906392	98.35
3 (VP3)	2737	1,068	6,369	29%	835 (VP3)	M1	DQ146651.1	OL906391	98.05
4 (VP4)	2443	1,103	6,666	31%	775 (VP4)	P[8]	DQ146652.1	OL906390	97.12
5 (NSP1)	1673	927	3,533	29%	497 (NSP1)	A1	EF560708.1	OL906389	98.08
6 (VP6)	1438	1,799	6,405	36%	397 (VP6)	I1	DQ146642.1	OL906388	98.83
7 (NSP3)	1205	1,055	2,223	32%	310 (NSP3)	T1	DQ146646.1	OL906387	97.32
8 (NSP2)	1201	650	1,763	33%	317 (NSP2)	N1	DQ146656.1	OL906386	98.32
9 (VP7)	1189	971	2,247	34%	326 (VP7)	G3	EF672602.1	OL906385	96.93
10 (NSP4)	922	951	2,017	38%	175 (NSP4)	E1	DQ146658.1	OL906384	98.86
11 (NSP5/6)	742	838	1,375	39%	197 (NSP5) 90 (NSP6)	H1	DQ146681.1	OL906383	99.49

5.3. Results and discussion

5.3.1. Electropherotyping of ELISA positive and negative stool specimens

The simian rhesus rotavirus (RRV), which is long E-type, was used as the reference strain for the electropherotyping of 327 RVA positive fecal samples (samples are found positive by ELISA and CerTest) Figure 5.4A & 5.4B. Of the 327 isolates, 254 showed characteristic RVA migration pattern, with long and short E-types detected in 206 and 48 isolates, respectively. The remaining rotavirus positive isolates did not show any visible RNA migration profile. This could be due to less viral particles in the fecal specimen and with increased concentration they might show a clear migration pattern on the gel. E-typing has been performed for 144 RVA negative samples and sample number 111 exhibited typical group B RNA migration pattern (Figure 5.4C).





CerTest for other enteric viruses	Isolate Number	Rotavirus	Adenovirus	Astrovirus	Norovirus
	140	+	-	+	-
	168	+	-	-	-
	107	+	-	-	-
	222	+	-	-	-
	239	+	-	-	-

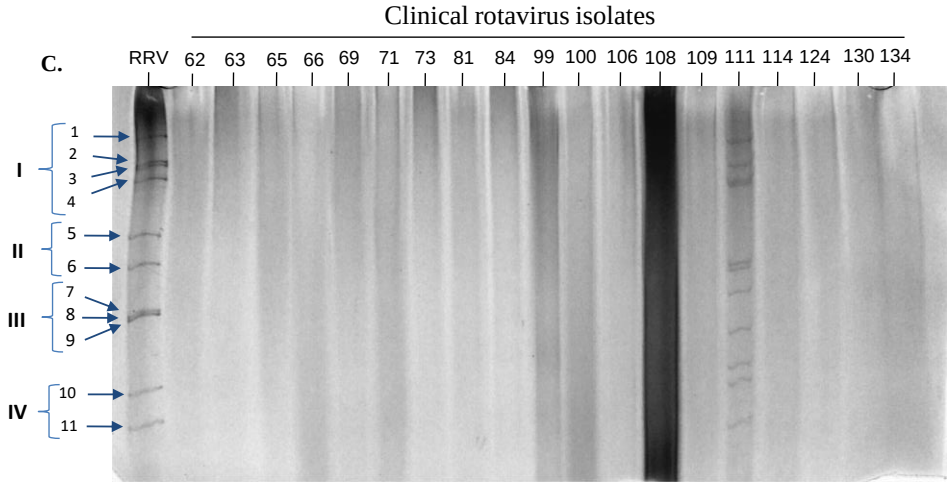


Figure 5.4: **Electropherogram of group A rotavirus from diarrheic children. (**A-C) Representative polyacrylamide gel electrophoresis showing the migration patterns of 11-segments of dsRNA.

5.3.2. Detection of group B and group C rotaviruses by PCR

After E-typing, the rotavirus group A ELISA negative specimens were subjected for total RNA extraction followed by PCR with gene-specific primers (Table 5.1) to confirm the presence or absence of group B and C rotavirus. As shown in Figure 5.5, specimen number 111, which showed typical group B RNA migration pattern revealed the presence of NSP2, VP4 and VP7 genes of group B rotavirus.

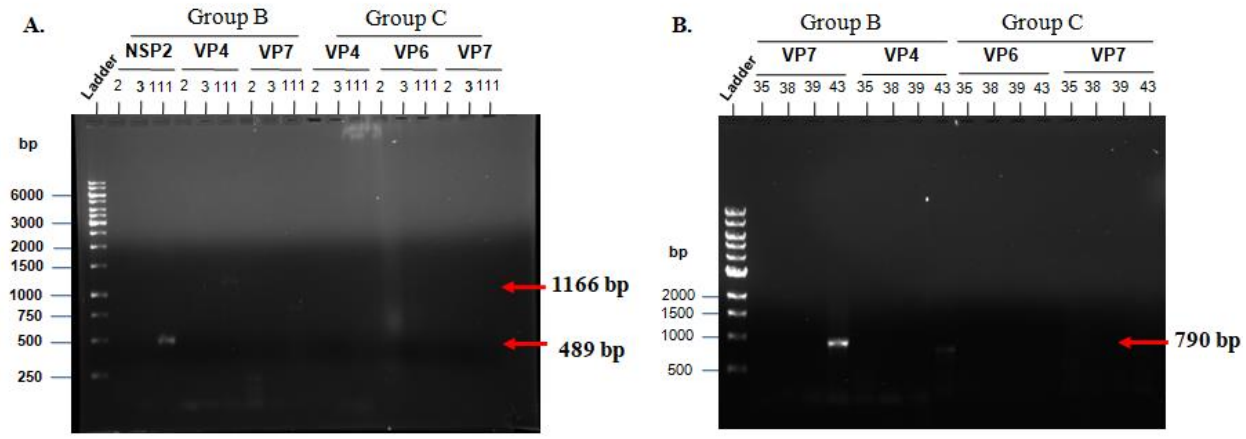


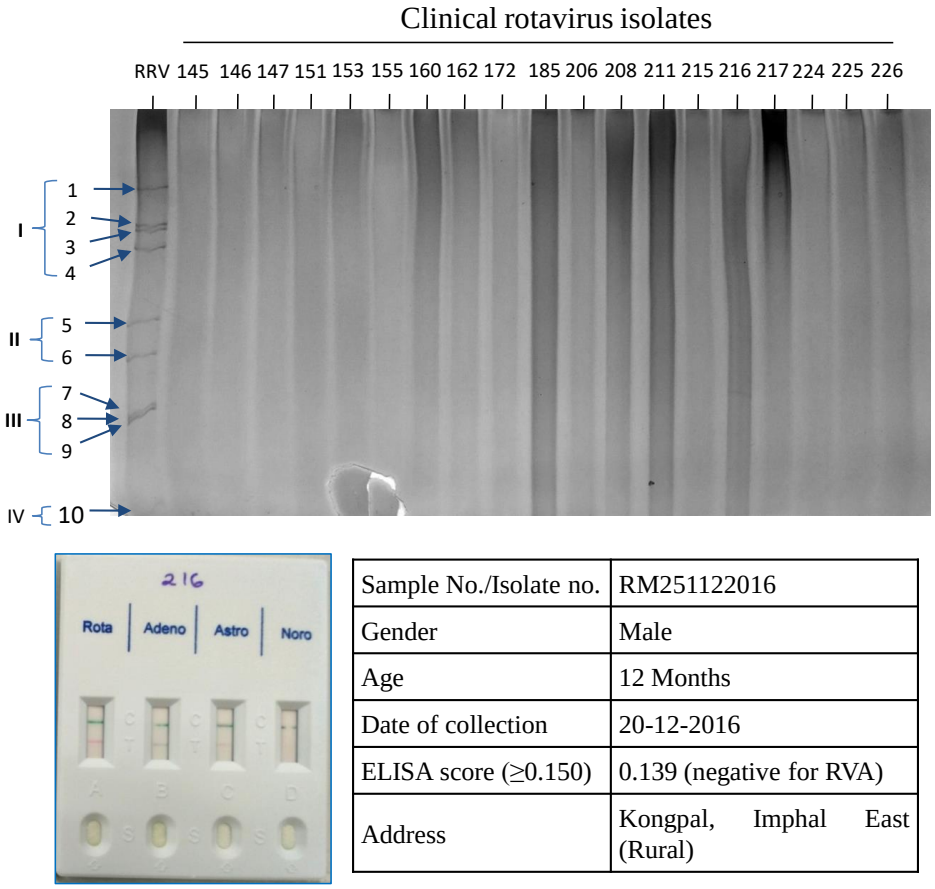
Figure 5.5: Detection of Group B and C rotaviruses in VP6-ELISA negative specimens by PCR. Group B {VP7 (790 bp), VP4 (1166 bp) and NSP2 (489 bp)} and Group C rotavirus genes {VP7 (518 bp), VP6 (318 bp) and VP4 (465 bp)}.

5.3.3. Whole genome sequencing

Clinical details of RVA isolate (RM251122016), CerTest result and RNA-PAGE image of RM251122016 are given in Figure 5.6. According to ViPR, a server for classification, rotavirus strain RM251122016 was identified as G3P[8] strain with a human Wa-like genotype backbone; I1-R1-C1-M1-A1-N1-T1-E1-H1, with VP7 and VP1 genes having close similarity with porcine and equine RV strains.

From de novo assembly and subsequent mapping to reference strains, full-length genome of strain RM251122016 was obtained. The nucleotide sequence length of segment 1 to 11 were 3299, 2915, 2737, 2443, 1673, 1438, 1205, 1201, 1189, 922, 742 bp, and their corresponding open reading frames (ORFs) were 2865, 2682, 2505, 2325, 1491, 1191, 930, 951, 978, 525, 591 bp, respectively. The nucleotide sequences were deposited in GenBank under accession numbers OL906383 to OL906393 for VP1-6 and NSP1-5

(Table 5.2). Circos plot analysis of rotavirus whole genome data of RM251122016 was made and given in Figure 5.7.



Sample No./Isolate no.	RM251122016
Gender	Male
Age	12 Months
Date of collection	20-12-2016
ELISA score (≥0.150)	0.139 (negative for RVA)
Address	Kongpal, Imphal East (Rural)

Figure 5.6: Analysis of dsRNA extracted from premier rotaclone VP6-ELISA negative RVA isolate (RM251122016). Inset table shows clinical details of RVA isolate (RM251122016). Whole genome sequencing was performed using Illumina.

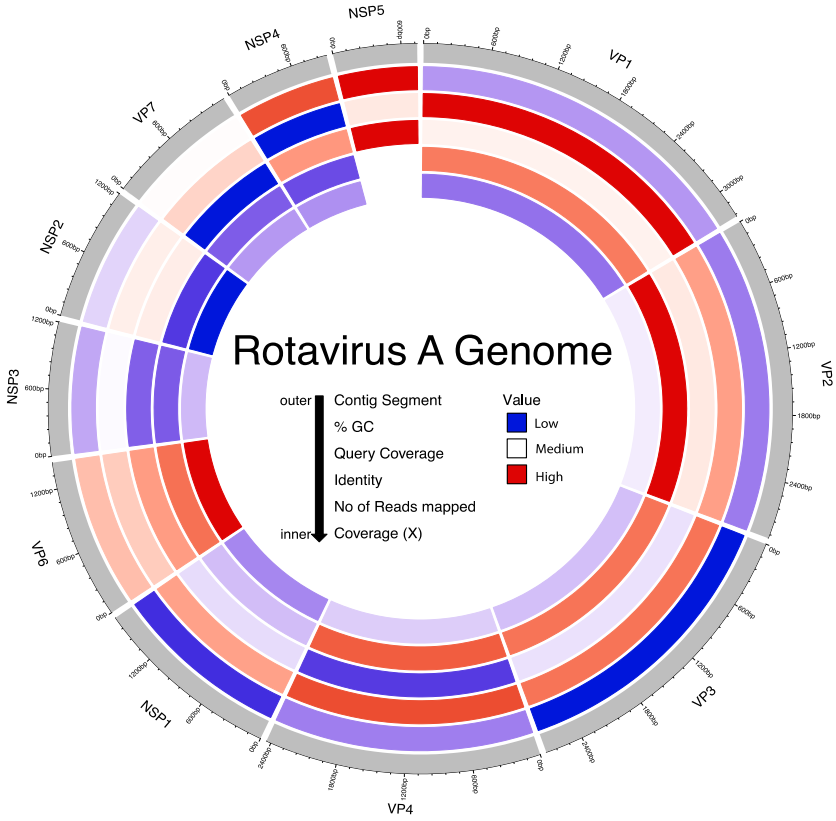


Figure 5.7: Circos plot of rotavirus whole genome data of RM251122016. Outermost circle represents contigs with their corresponding length in base pair and identified genes. From outer to innermost circles depict GC percentage, query coverage, identity, number of mapped reads and coverage (X), respectively. Values for each of the features are plotted as ranges from red (high values) to blue (lower values).

5.3.4. Genetic distances

The distance matrices showed that the genes of the isolate RM251122016 were closely related to human strains (range: 96.93- 99.49% nucleotide identity), and that the identity with Rotateq G3 strain, Rotateq VP4 gene of P[8] genotype, and Rotarix VP4 gene was in the range of 62.7- 83.6%, 88.9%, and 86.7%, respectively (P[8]) (Table 5.2).

5.3.5. Phylogenetic analyses

The phylogeny of the gene segments of RM251122016 isolate showed that VP7, VP6, VP4, VP3, VP2 and NSP5 have one common related strain which is human strain KCH1187 (G3-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1). VP7, VP4, VP3, NSP2 and NSP5 genes were related to another common strain, Tokyo17-21 (G3-P[8]-I1-R1-C1-M1-A1-

N1-T1-E1-H1), while VP3, NSP1, NSP2 and NSP5 genes with strain ME659 (G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) and VP4, VP3, VP2 and NSP2 with strain NGR_Ref (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) (Figure 5.8). All the VPs and NSPs genes were related to human RVs with VP1 showing close similarity with porcine and equine RVs and VP7 also revealed similarity with a bovine RV strain. The VP1 gene occupied the top position in a cluster containing human and porcine strains (Figure 5.8D), while VP7 gene shared mix clade with a bovine rotavirus strain RVA/Bf212/COVASU/Parbhani/2017 (Figure 5.8A).

The G-genotype of strain RM251122016 is G3 and shares 96.93% nucleotide sequence identity with its closest strain in Genbank, RVA/Human-tc/USA/P/1974/G3P1A[8] and 83.6% with Rotateq G3 strain, RotaTeq-WI78-8 (Table 5.2). The VP7 tree (Figure. 5.8A) showed a very strict clustering (nucleotide identity 99.23-99.71%) with strains KCH1187, Tokyo17-21, and B3605 detected in 2019, 2017, and 2017, respectively. The strain KCH1187 was identified in stool specimens from a vaccinated 11-month-old child having diarrhoea from Kenya in 2019. The sequencing report showed the viral genome has a Wa-like genomic backbone [12]. The Tokyo17-21 strain is G3P[8] with Wa-like genotype constellation identified in stool specimens from diarrheic child at the Tokyo Metropolitan Institute of Public Health in 2017 [13]. While, the strain, B3605, was isolated from an adult in Bangkok in 2017, during a surveillance study from July 2016 to December 2019 [14]. The VP7 gene occupies a mixed clade with bovine strain, RVA/Bf212/COVASU/Parbhani/2017 VP7, isolated from a buffalo fecal specimen from India in 2017 (Genebank MK043950).

The P-genotype of strain RM251122016 is P8 and shares 97.12% sequence identity with strain Dhaka25, 88.9% with Rotateq VP4 (P[8]) and 86.7% with Rotarix VP4 sequence (P[8]) (Table 5.2). The VP4 tree showed that the strain RM251122016 was clustered in the same clade with P[8] sequences of the strains UFS-NGS-MRC-DPRU8033 and RVA-U14 detected in humans. The human strains included in the phylogenetic tree showed P[8] genotypes (Figure 5.8B). The strain UFS-NGS-MRC-DPRU8033 showed G1P[8] genotype with Wa-like genotype constellation and it was identified from a child hospitalized with acute gastroenteritis in Rwanda in 2015 [15]. While, the strain RVA-U14, was G1P[8] genotype, isolated from a 28 weeks old child with acute gastroenteritis

in Japan in 2015. This strain was later used for developing rotavirus vaccine candidates using reverse genetics approach [16].

VP6 gene of RM251122016 strain has I1 genotype and shares 98.83% sequence identity with strain B4633 from Belgium (Table 5.2). The VP6 tree showed that the sequence was closely correlated with VP6 sequences of strains NGR_Ref, RVA-U14, STM457 and CMC_00048 detected in human strains between 2010 to 2019 (Figure 5.8C). The strain NGR_Ref was isolated from a 26-month girl child in Nigeria in 2017 and was G1P[8] genotype with a Wa-like genotype constellation [17]. While the strain STM457 showed typical G1P[8] and Wa-like backbone and it was isolated from a paediatric patient admitted due to acute gastroenteritis in Surabaya, Indonesia in 2018 [18]. Whereas, the strain CM_C00048 was identified from 605 days old female child, in Vellore in 2013 and it was G9P[8] genotype with Wa-like genotype constellation.

The genotype of VP3 gene of the strain in this study is M1 and shares 98.05% sequence identity with strain Dhaka25 (Table 5.2). The VP3 tree showed that the strain in this study was closely correlated with VP3 sequences of strains RVA/Human-wt/USA/2014741183/2014/G1P8 detected in 2014 and NGR_Ref strain (Figure 5.8F). The strain RVA/Human-wt/USA/2014741183/2014/G1P8, with a Wa-like genotype backbone, was identified from diarrhoea stool of a child in Houston, USA in rotavirus surveillance by New Vaccine Surveillance Network (NVSN) [19]. The genotype VP2 of the strain in this study is C1 and shares 98.35% sequence identity with strain Dhaka12 (Table 5.2). The VP2 tree showed that the strain in this study was closely related with VP2 sequences of strains, DBM2017-014, KLF1003, and KLF2470 detected in humans (Figure 5.8E). The strain DBM2017-014 was isolated from a nearly three-year-old male child suffering from diarrhoea, in Bangkok, Thailand in the year 2017. The strain showed Wa-like genotype backbone and long electropherotype [20]. Both the strains KLF1003 and KLF2470 showed G3P[8] genotype with Wa-like genotype constellation and identified from diarrheic children of Kenya [21].

The genotype VP1 of the strain in this study is R1 and shares 98.34% sequence identity with strain KTM368 (Table 5.2). The VP1 tree showed that the strain in this study was closely related with VP1 sequences of human strains, KLF0899, B08299, and TK2620, detected in 2018, 2008 and 2008, respectively, and porcine strains PRG9121 and PRG942 and distantly with RVA/Horse-tc/GBR/H-1/1975/G5P9[7]. KLF0899 is a strain

identified from diarrhoea stool of a child from Kenya in 2018, and it was G3P[8] with Wa-like genome backbone [21]. The strain B08299 was isolated from a 4-month-old boy suffering from acute gastroenteritis in Mumbai, India. The genotype of the strain was G11P[25], a rare RVA, and revealed interspecies transmission and reassortment with human rotavirus strains, contains VP1, VP3 and VP6 genes that have evolved from rotaviruses of unknown origin [22]. While the strain TK2620 was isolated from an adult patient with diarrhoea in Nepal in 2008. The VP2 and NSP1 genes were of human RVA origin, rest were either porcine-like or unique [23].

The porcine strains PRG9121 and PRG942 with G9P[7] and G9P[23] genotypes, respectively were isolated from pigs in 2006 from South Korea. The strains possessed intra-genotype reassorted segments among porcine RVA strains in the region [24]. Whereas the equine strain RVA/Horse-tc/GBR/H-1/1975/G5P9[7] was identified in United Kingdom and phylogenetically, VP1 and NSP5 genes were probably descended from porcine or Wa-like human RVs [25] (Figure 5.8D).

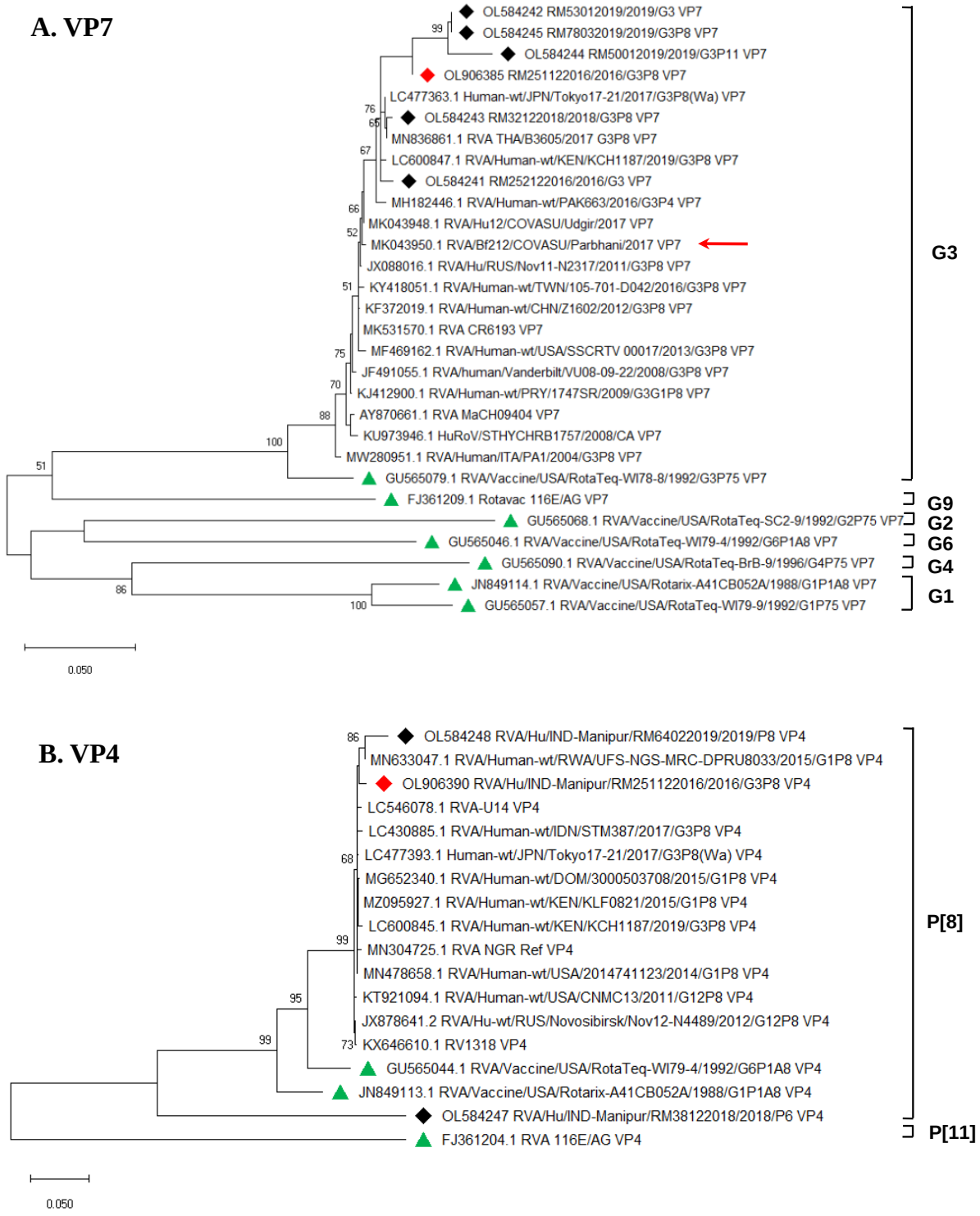
The genotype NSP1 of the strain in this study is A1 and shares 98.08% sequence identity with strain RVA/Human-wt/BGD/Dhaka6/2001/G11P[25] (Table 5.2). The NSP1 tree showed that the strain in this study was closely correlated with NSP1 sequences of strains HJM1646, ME659 and CMC_00044 detected in human strains in 2017, 2014 and 2012, respectively (Figure 5.8G). The strain HJM1646 with G12[P8] and Wa-like backbone was identified from fecal sample in Mozambique in 2017. While, the strain ME659 from Messina Italy, possessed a diverse and complex genetic sub-constellation, G12P[8] and Wa-like genotype backbone. It was reported that possibly it was a result of multiple reassortment events with other RVA strains in the same period [26]. The other similar strain, CMC_00044, was isolated in 2012 from an Indian female child who was 487 days old and had the G12P[8] genotype.

The NSP2 gene identified in this study has the genotype N1 and has 98.32% sequence identity with strain Dhaka25 (Table 5.1). The NSP2 tree revealed a strong correlation between the NSP2 sequence and the NSP2 sequence detected in human strains STM453, Tokyo17-21, STM430, and HJM1646. (Figure 5.8H). The strains STM430 with the G12[8] genotype and STM453 with the G1P[8] genotype both contained the Wa-like backbone were isolated from paediatric patients in Indonesia [27]. The genotype NSP3 of the strain in this study is T1 and shares 97.32% sequence identity with strain B4633

(Table 5.2). The NSP3 tree showed that the strain in this study was closely correlated with NSP3 sequences of strains KN105, SSCRTV_00027, E2484, VU11-12-60 and BCH 7221 detected in human strains in 2013, 2013, 2011, 2012, and 2016, respectively (Figure 5.8I).

The strain KN105 with genotype G3P[8] and Wa-like backbone was isolated from stool specimen of a child aged < 5 years who were hospitalized for acute gastroenteritis in Japan [28]. Further the strain SSCRTV_00027 was identified from a male child aged 3 years in Memphis, USA. NSP3 genotype was T1 but G genotype was unknown (GXP[8]). In contrast, strain E2484, a Wa-like human RVA, was identified in a 1-year-old male newborn in Wuhan, China. This strain was most likely the product of reassortment with a porcine-like VP7 that was acquired [29]. The next strain VU11-12-60 with genotype G3P[8] was identified from a male child aged 37 months in Davidson County in USA [30]. Additionally, a study conducted in India from January 2014 to December 2016 identified that the strain BCH 7221 possesses genotype G3P[8] and a Wa-like genotype constellation [31].

The genotype of NSP4 gene of the strain in this study is E1 and shares 98.86 % sequence identity with strain Dhaka25 (Table 5.2). The NSP4 tree showed that the strain in this study was closely correlated with NSP4 sequences of strains NUROT-1 and 27/Hn154/COVASU/Pligli detected in human strains in 2016 and 2017 (Figure 5.8J). The strain NUROT-1 having genotype G3P[8] was isolated from faeces in India in 2016. While the strain Hn154 NSP4 has E1 genotype, identified from stool specimen in India. The genotype NSP5 of the strain in this study is H1 and shares 99.49% sequence identity with strain Matlab13 (Table 5.2). The NSP5 tree showed that the strain in this study was clustered with NSP5 sequences of strains STM457 and STM453 detected in human strains in 2018 (Figure 5.8K).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OL584245 RM78032019/2019/G3P8 VP7
OL584244 RM50012019/2019/G3P11 VP7
OL906385 RM251122016/2016/G3P8 VP7
LC477363.1 Human-wt/JPN/Tokyo17-21/2017/G3P8(Wa) VP7
OL584243 RM32122018/2018/G3P8 VP7
MN836861.1 RVA THA/B3605/2017 G3P8 VP7
LC600847.1 RVA/Human-wt/KEN/KCH1187/2019/G3P8 VP7
OL584241 RM252122016/2016/G3 VP7
MH182446.1 RVA/Human-wt/PAK663/2016/G3P4 VP7
MK043948.1 RVA/Hu12/COVASU/Udgir/2017 VP7
MK043950.1 RVA/Bf212/COVASU/Parbhani/2017 VP7
JX088016.1 RVA/Hu/RUS/Nov11-N2317/2011/G3P8 VP7
KY418051.1 RVA/Human-wt/TWN/105-701-D042/2016/G3P8 VP7
KF372019.1 RVA/Human-wt/CHN/Z1602/2012/G3P8 VP7
MK531570.1 RVA CR6193 VP7
MF469162.1 RVA/Human-wt/USA/SSCRTV 00017/2013/G3P8 VP7
JF491055.1 RVA/human/Vanderbilt/VU08-09-22/2008/G3P8 VP7
KJ412900.1 RVA/Human-wt/PRY/1747SR/2009/G3G1P8 VP7
AY870661.1 RVA MaCH09404 VP7
KU973946.1 HuRoV/STHYCHRB1757/2008/CA VP7
MW280951.1 RVA/Human/ITA/PA1/2004/G3P8 VP7
GU565079.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75 VP7
FJ361209.1 Rotavac 116E/AG VP7
GU565068.1 RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P75 VP7
GU565046.1 RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A8 VP7
GU565090.1 RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P75 VP7
JN849114.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 VP7
GU565057.1 RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P75 VP7
G3
G9
G2
G6
G4
G1
0.050
B. VP4
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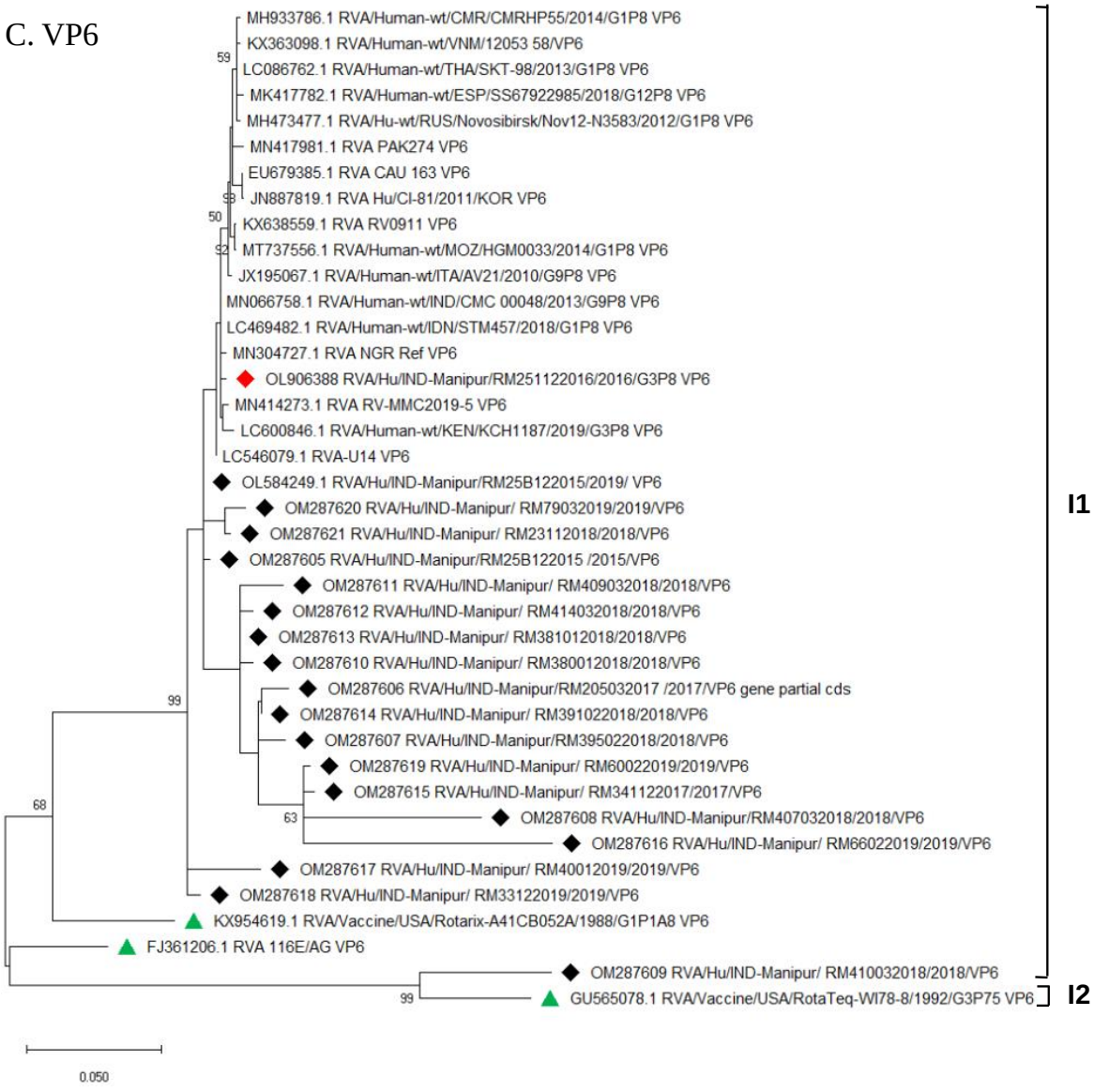
MN633047.1 RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8033/2015/G1P8 VP4
OL906390 RVA/Hu/IND-Manipur/RM251122016/2016/G3P8 VP4
LC546078.1 RVA-U14 VP4
LC430885.1 RVA/Human-wt/IDN/STM387/2017/G3P8 VP4
LC477393.1 Human-wt/JPN/Tokyo17-21/2017/G3P8(Wa) VP4
MG652340.1 RVA/Human-wt/DOM/3000503708/2015/G1P8 VP4
MZ095927.1 RVA/Human-wt/KEN/KLF0821/2015/G1P8 VP4
LC600845.1 RVA/Human-wt/KEN/KCH1187/2019/G3P8 VP4
MN304725.1 RVA NGR Ref VP4
MN478658.1 RVA/Human-wt/USA/2014741123/2014/G1P8 VP4
KT921094.1 RVA/Human-wt/USA/CNMC13/2011/G12P8 VP4
JX878641.2 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N4489/2012/G12P8 VP4
KX646610.1 RV1318 VP4
GU565044.1 RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A8 VP4
JN849113.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 VP4
OL584247 RVA/Hu/IND-Manipur/RM38122018/2018/P6 VP4
FJ361204.1 RVA 116E/AG VP4
P[8]
P[11]
0.050

C. VP6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MK417782.1 RVA/Human-wt/ESP/SS67922985/2018/G12P8 VP6
MH473477.1 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N3583/2012/G1P8 VP6
MN417981.1 RVA PAK274 VP6
EU679385.1 RVA CAU 163 VP6
JN887819.1 RVA Hu/CI-81/2011/KOR VP6
KX638559.1 RVA RV0911 VP6
MT737556.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P8 VP6
JX195067.1 RVA/Human-wt/ITA/AV21/2010/G9P8 VP6
MN066758.1 RVA/Human-wt/IND/CMC 00048/2013/G9P8 VP6
LC469482.1 RVA/Human-wt/IDN/STM457/2018/G1P8 VP6
MN304727.1 RVA NGR Ref VP6
OL906388 RVA/Hu/IND-Manipur/RM251122016/2016/G3P8 VP6
MN414273.1 RVA RV-MMC2019-5 VP6
LC600846.1 RVA/Human-wt/KEN/KCH1187/2019/G3P8 VP6
LC546079.1 RVA-U14 VP6
OL584249.1 RVA/Hu/IND-Manipur/RM25B122015/2019/ VP6
OM287620 RVA/Hu/IND-Manipur/ RM79032019/2019/VP6
OM287621 RVA/Hu/IND-Manipur/ RM23112018/2018/VP6
OM287605 RVA/Hu/IND-Manipur/RM25B122015 /2015/VP6
OM287611 RVA/Hu/IND-Manipur/ RM409032018/2018/VP6
OM287612 RVA/Hu/IND-Manipur/ RM414032018/2018/VP6
OM287613 RVA/Hu/IND-Manipur/ RM381012018/2018/VP6
OM287610 RVA/Hu/IND-Manipur/ RM380012018/2018/VP6
OM287606 RVA/Hu/IND-Manipur/RM205032017 /2017/VP6 gene partial cds
OM287614 RVA/Hu/IND-Manipur/ RM391022018/2018/VP6
OM287607 RVA/Hu/IND-Manipur/RM395022018/2018/VP6
OM287619 RVA/Hu/IND-Manipur/ RM60022019/2019/VP6
OM287615 RVA/Hu/IND-Manipur/ RM341122017/2017/VP6
OM287608 RVA/Hu/IND-Manipur/RM407032018/2018/VP6
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OM287617 RVA/Hu/IND-Manipur/ RM40012019/2019/VP6
OM287618 RVA/Hu/IND-Manipur/ RM33122019/2019/VP6
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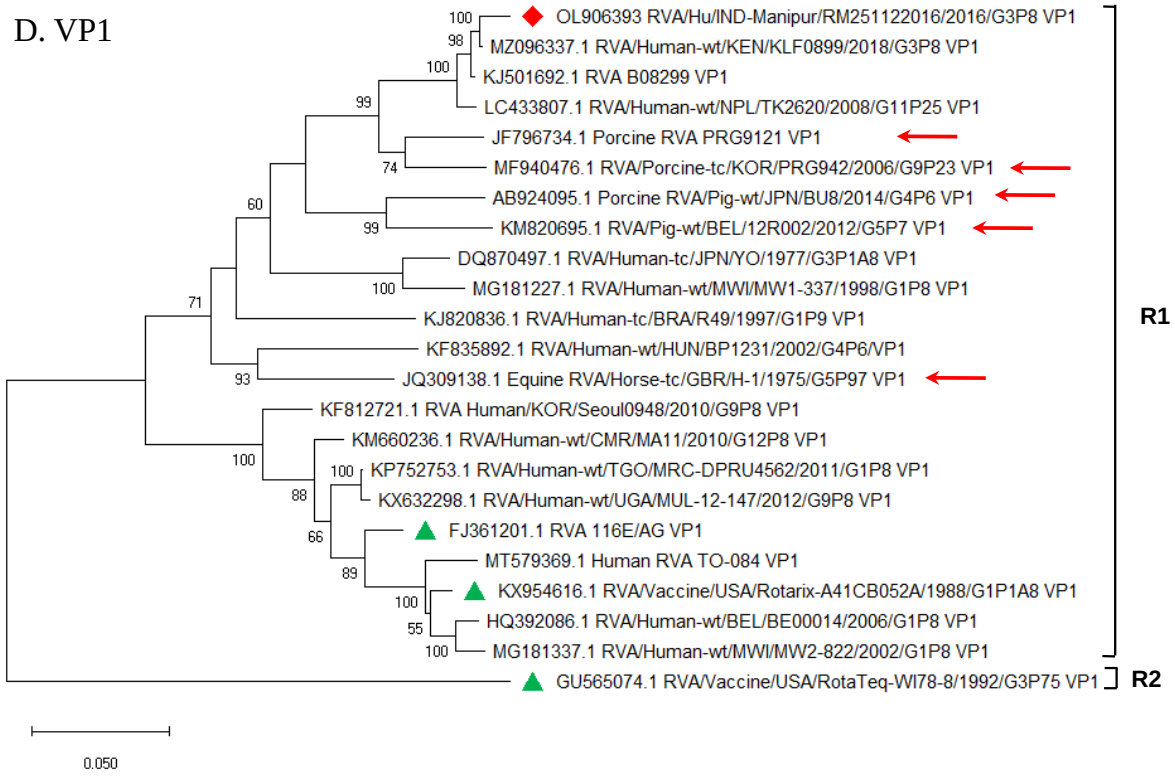
FJ361206.1 RVA 116E/AG VP6
OM287609 RVA/Hu/IND-Manipur/ RM410032018/2018/VP6
GU565078.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75 VP6
59
50
93
92
99
68
63
99
I1
I2
0.050

D. VP1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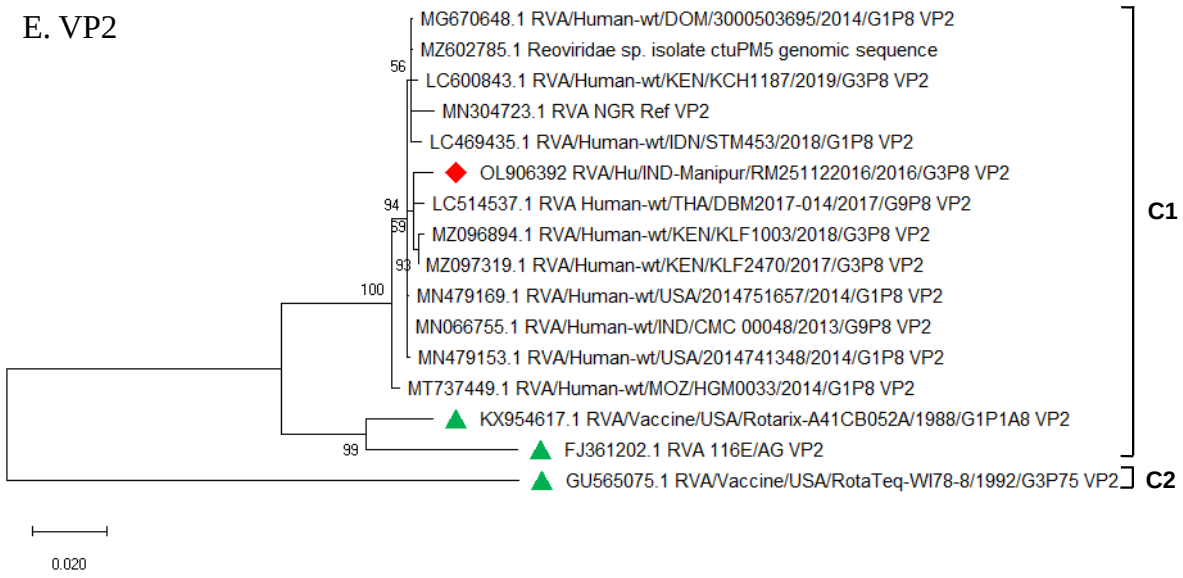
LC433807.1 RVA/Human-wt/NPL/TK2620/2008/G11P25 VP1
JF796734.1 Porcine RVA PRG9121 VP1
MF940476.1 RVA/Porcine-tc/KOR/PRG942/2006/G9P23 VP1
AB924095.1 Porcine RVA/Pig-wt/JPN/BU8/2014/G4P6 VP1
KM820695.1 RVA/Pig-wt/BEL/12R002/2012/G5P7 VP1
DQ870497.1 RVA/Human-tc/JPN/YO/1977/G3P1A8 VP1
MG181227.1 RVA/Human-wt/MWI/MW1-337/1998/G1P8 VP1
KJ820836.1 RVA/Human-tc/BRA/R49/1997/G1P9 VP1
KF835892.1 RVA/Human-wt/HUN/BP1231/2002/G4P6/VP1
JQ309138.1 Equine RVA/Horse-tc/GBR/H-1/1975/G5P97 VP1
KF812721.1 RVA Human/KOR/Seoul0948/2010/G9P8 VP1
KM660236.1 RVA/Human-wt/CMR/MA11/2010/G12P8 VP1
KP752753.1 RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P8 VP1
KX632298.1 RVA/Human-wt/UGA/MUL-12-147/2012/G9P8 VP1
FJ361201.1 RVA 116E/AG VP1
MT579369.1 Human RVA TO-084 VP1
KX954616.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 VP1
HQ392086.1 RVA/Human-wt/BEL/BE00014/2006/G1P8 VP1
MG181337.1 RVA/Human-wt/MWI/MW2-822/2002/G1P8 VP1
GU565074.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75 VP1
R1
R2
0.050

E. VP2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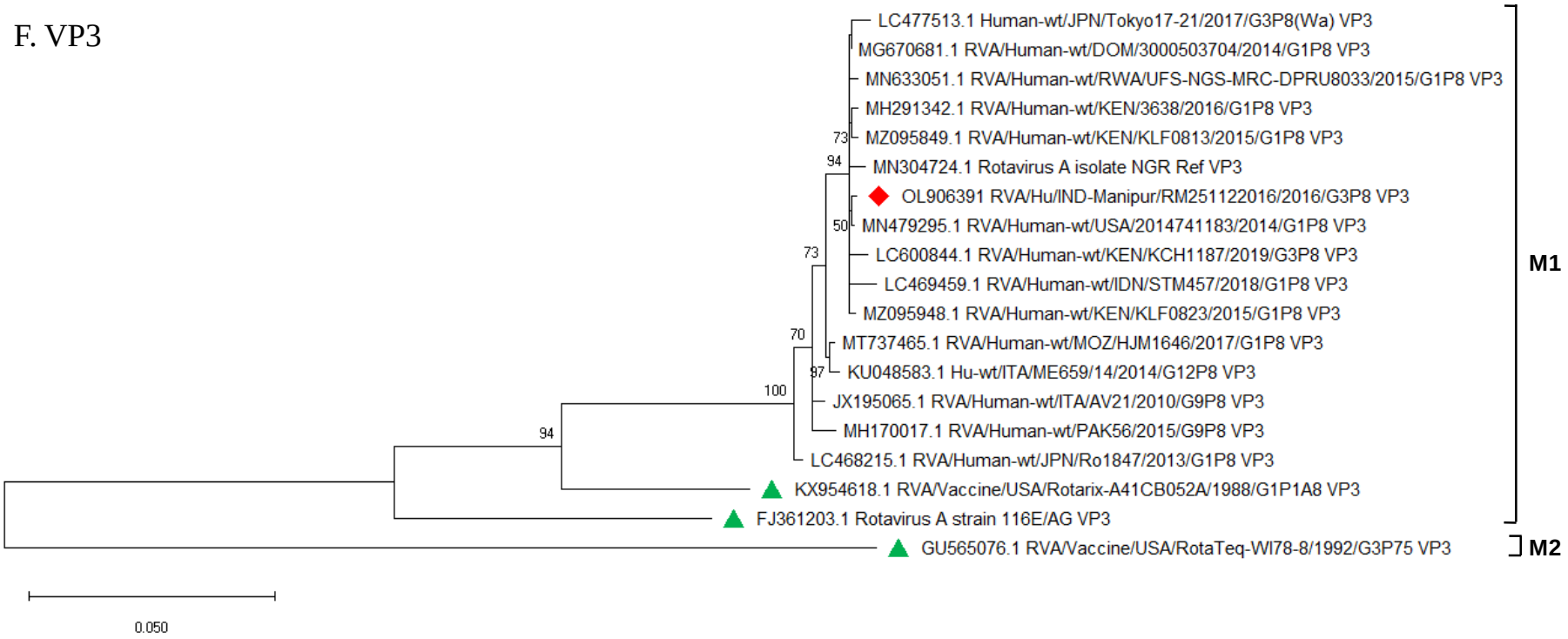
LC469435.1 RVA/Human-wt/IDN/STM453/2018/G1P8 VP2
OL906392 RVA/Hu/IND-Manipur/RM251122016/2016/G3P8 VP2
LC514537.1 RVA Human-wt/THA/DBM2017-014/2017/G9P8 VP2
MZ096894.1 RVA/Human-wt/KEN/KLF1003/2018/G3P8 VP2
MZ097319.1 RVA/Human-wt/KEN/KLF2470/2017/G3P8 VP2
MN479169.1 RVA/Human-wt/USA/2014751657/2014/G1P8 VP2
MN066755.1 RVA/Human-wt/IND/CMC 00048/2013/G9P8 VP2
MN479153.1 RVA/Human-wt/USA/2014741348/2014/G1P8 VP2
MT737449.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P8 VP2
KX954617.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 VP2
FJ361202.1 RVA 116E/AG VP2
GU565075.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75 VP2
C1
C2
0.020

F. VP3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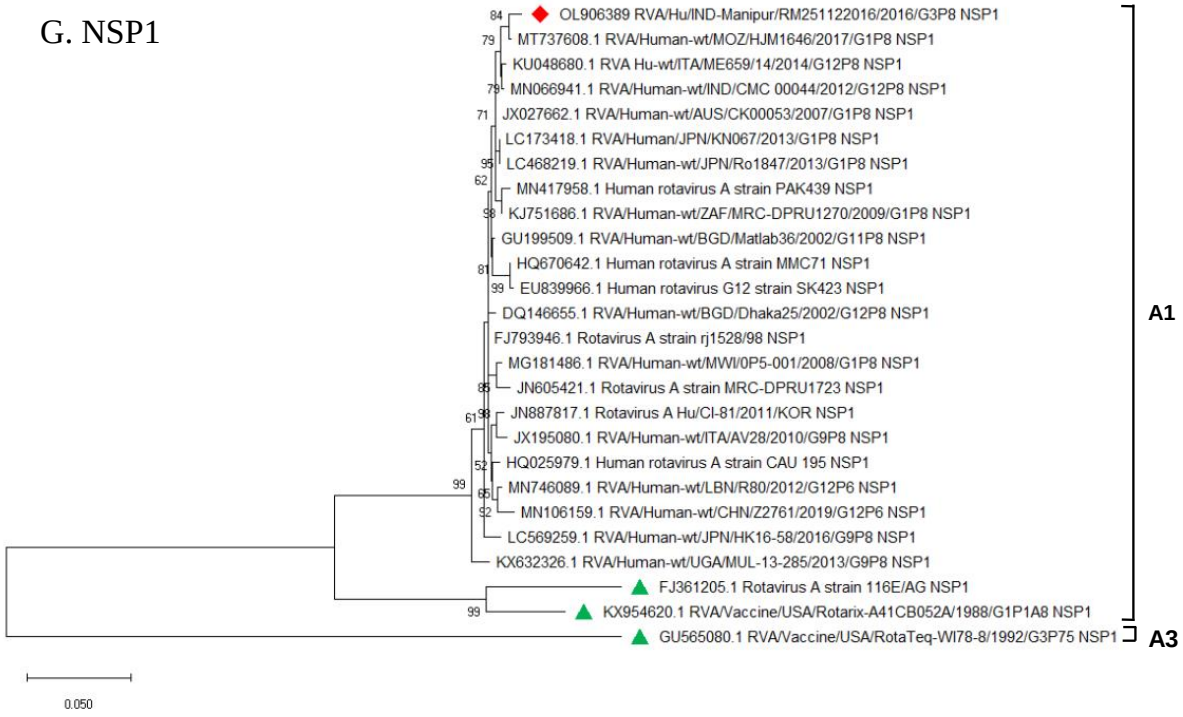
MH291342.1 RVA/Human-wt/KEN/3638/2016/G1P8 VP3
MZ095849.1 RVA/Human-wt/KEN/KLF0813/2015/G1P8 VP3
MN304724.1 Rotavirus A isolate NGR Ref VP3
OL906391 RVA/Hu/IND-Manipur/RM251122016/2016/G3P8 VP3
MN479295.1 RVA/Human-wt/USA/2014741183/2014/G1P8 VP3
LC600844.1 RVA/Human-wt/KEN/KCH1187/2019/G3P8 VP3
LC469459.1 RVA/Human-wt/IDN/STM457/2018/G1P8 VP3
MZ095948.1 RVA/Human-wt/KEN/KLF0823/2015/G1P8 VP3
MT737465.1 RVA/Human-wt/MOZ/HJM1646/2017/G1P8 VP3
KU048583.1 Hu-wt/ITA/ME659/14/2014/G12P8 VP3
JX195065.1 RVA/Human-wt/ITA/AV21/2010/G9P8 VP3
MH170017.1 RVA/Human-wt/PAK56/2015/G9P8 VP3
LC468215.1 RVA/Human-wt/JPN/Ro1847/2013/G1P8 VP3
KX954618.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 VP3
FJ361203.1 Rotavirus A strain 116E/AG VP3
GU565076.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75 VP3
73
94
50
73
70
97
100
94
M1
M2
0.050

G. NSP1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HQ670642.1 Human rotavirus A strain MMC71 NSP1
EU839966.1 Human rotavirus G12 strain SK423 NSP1
DQ146655.1 RVA/Human-wt/BGD/Dhaka25/2002/G12P8 NSP1
FJ793946.1 Rotavirus A strain rj1528/98 NSP1
MG181486.1 RVA/Human-wt/MWI/0P5-001/2008/G1P8 NSP1
JN605421.1 Rotavirus A strain MRC-DPRU1723 NSP1
JN887817.1 Rotavirus A Hu/CI-81/2011/KOR NSP1
JX195080.1 RVA/Human-wt/ITA/AV28/2010/G9P8 NSP1
HQ025979.1 Human rotavirus A strain CAU 195 NSP1
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FJ361205.1 Rotavirus A strain 116E/AG NSP1
KX954620.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 NSP1
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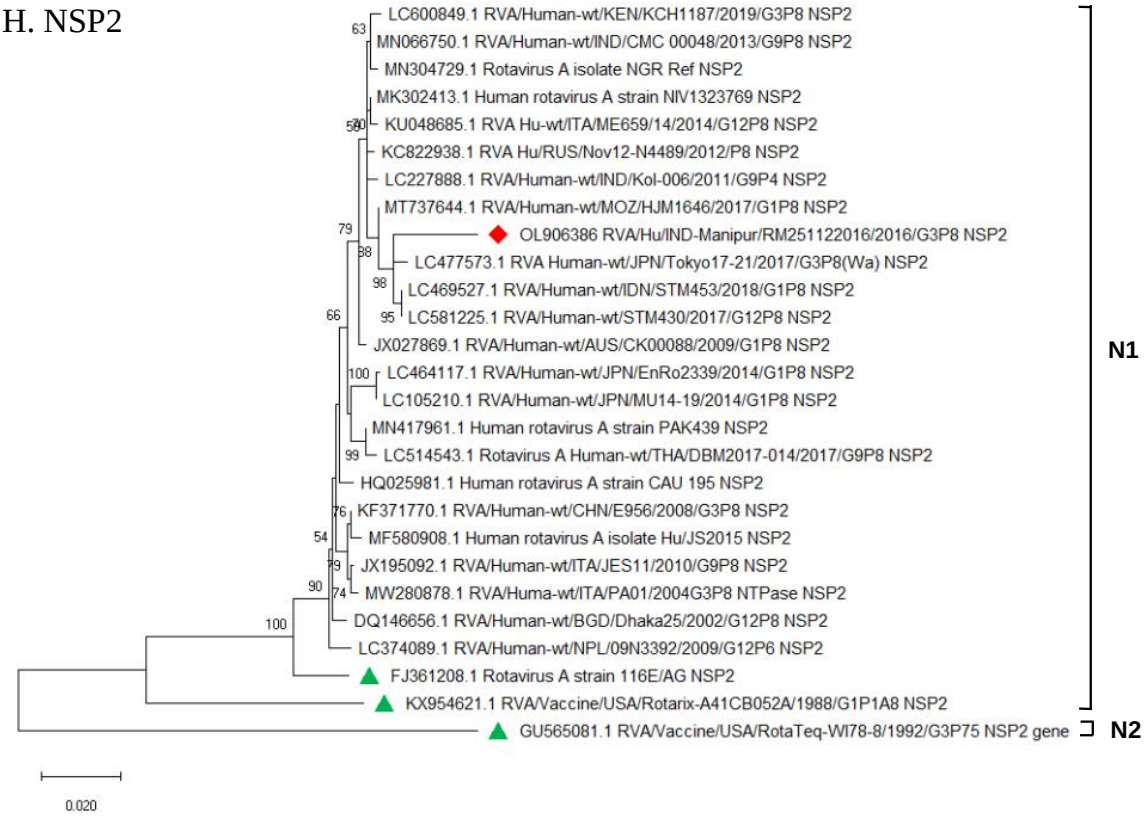
A1
A3
0.050

H. NSP2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KC822938.1 RVA Hu/RUS/Nov12-N4489/2012/P8 NSP2
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MT737644.1 RVA/Human-wt/MOZ/HJM1646/2017/G1P8 NSP2
OL906386 RVA/Hu/IND-Manipur/RM251122016/2016/G3P8 NSP2
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LC581225.1 RVA/Human-wt/STM430/2017/G12P8 NSP2
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LC514543.1 Rotavirus A Human-wt/THA/DBM2017-014/2017/G9P8 NSP2
HQ025981.1 Human rotavirus A strain CAU 195 NSP2
KF371770.1 RVA/Human-wt/CHN/E956/2008/G3P8 NSP2
MF580908.1 Human rotavirus A isolate Hu/JS2015 NSP2
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FJ361208.1 Rotavirus A strain 116E/AG NSP2
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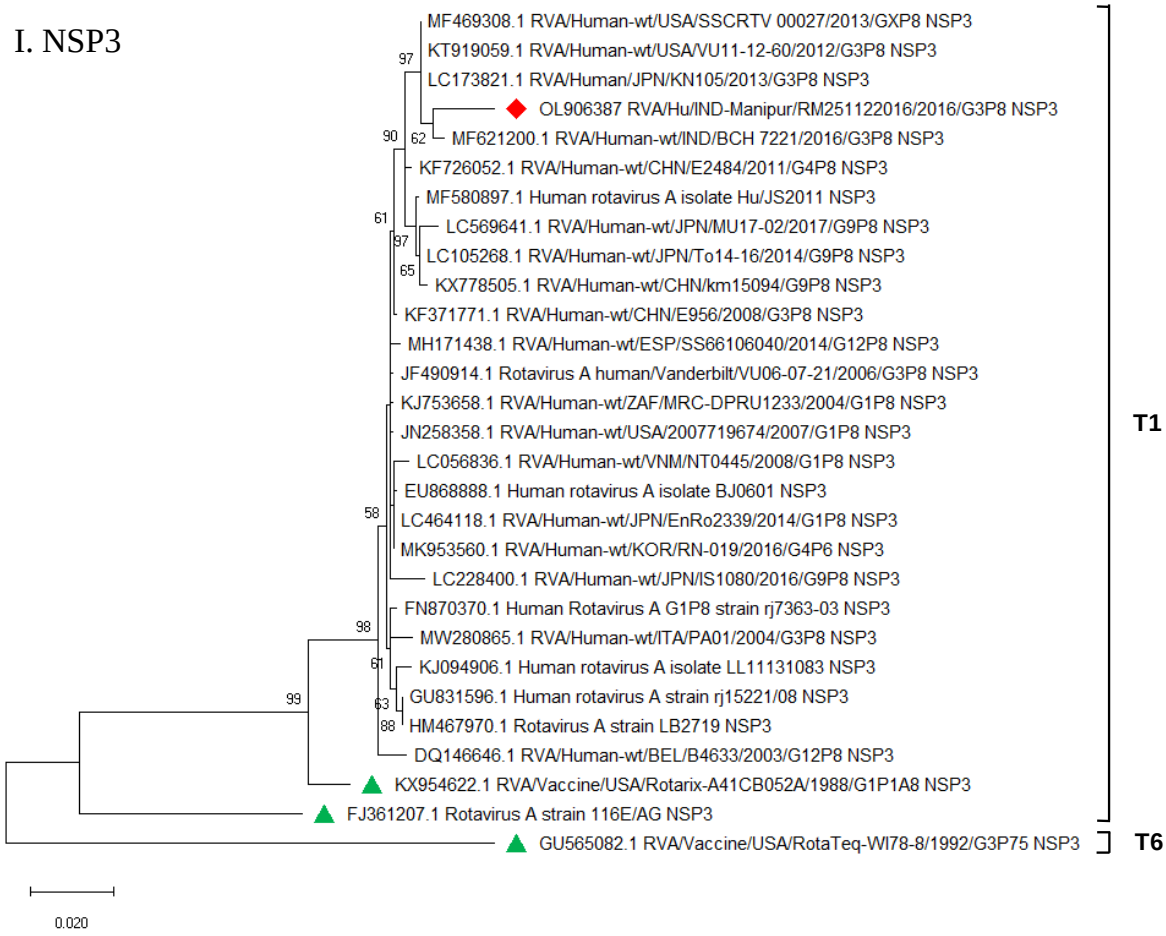
N1
N2
0.020

I. NSP3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MF580897.1 Human rotavirus A isolate Hu/JS2011 NSP3
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LC105268.1 RVA/Human-wt/JPN/To14-16/2014/G9P8 NSP3
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MH171438.1 RVA/Human-wt/ESP/SS66106040/2014/G12P8 NSP3
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EU868888.1 Human rotavirus A isolate BJ0601 NSP3
LC464118.1 RVA/Human-wt/JPN/EnRo2339/2014/G1P8 NSP3
MK953560.1 RVA/Human-wt/KOR/RN-019/2016/G4P6 NSP3
LC228400.1 RVA/Human-wt/JPN/IS1080/2016/G9P8 NSP3
FN870370.1 Human Rotavirus A G1P8 strain rj7363-03 NSP3
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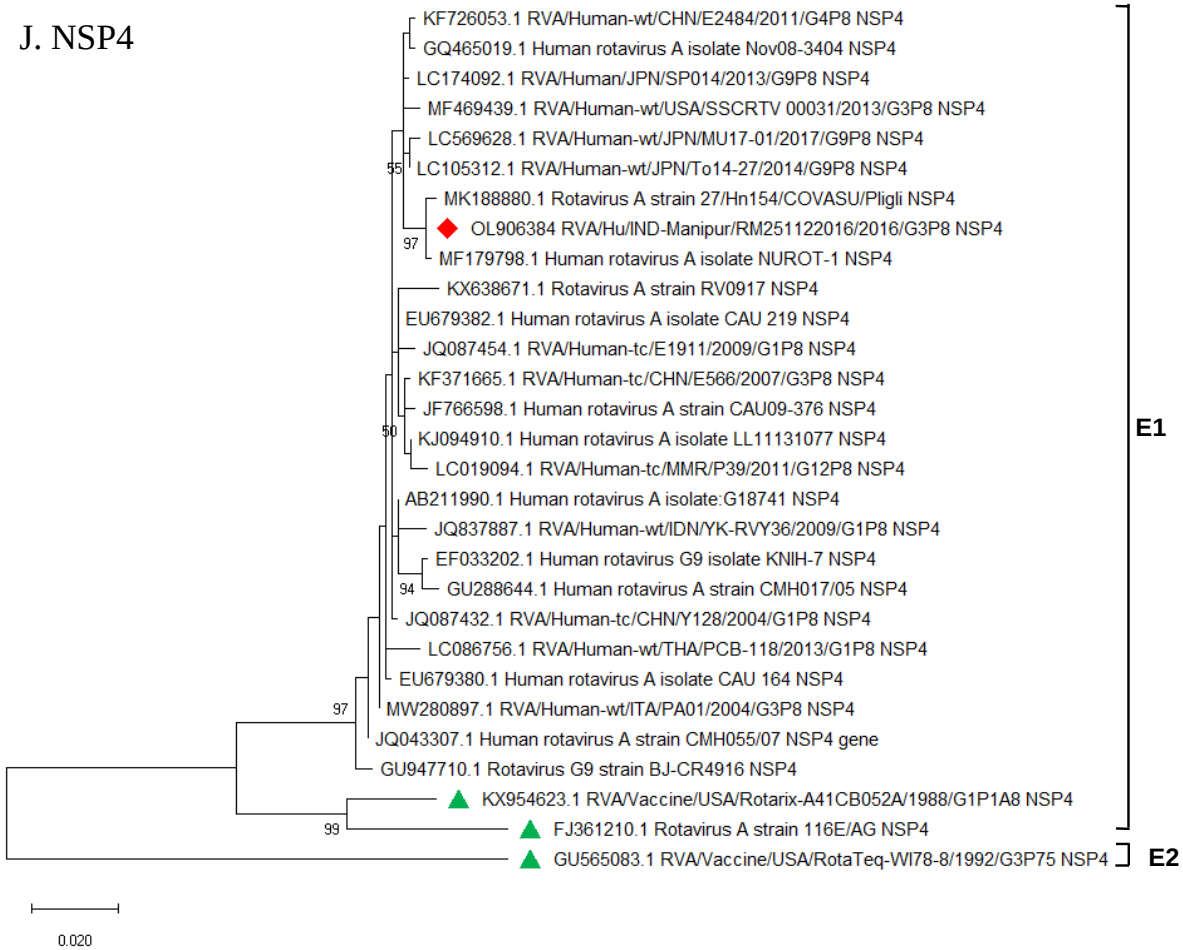
GU565082.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P75 NSP3
T1
T6
0.020

J. NSP4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LC105312.1 RVA/Human-wt/JPN/To14-27/2014/G9P8 NSP4
MK188880.1 Rotavirus A strain 27/Hn154/COVASU/Pligli NSP4
OL906384 RVA/Hu/IND-Manipur/RM251122016/2016/G3P8 NSP4
MF179798.1 Human rotavirus A isolate NUROT-1 NSP4
KX638671.1 Rotavirus A strain RV0917 NSP4
EU679382.1 Human rotavirus A isolate CAU 219 NSP4
JQ087454.1 RVA/Human-tc/E1911/2009/G1P8 NSP4
KF371665.1 RVA/Human-tc/CHN/E566/2007/G3P8 NSP4
JF766598.1 Human rotavirus A strain CAU09-376 NSP4
KJ094910.1 Human rotavirus A isolate LL11131077 NSP4
LC019094.1 RVA/Human-tc/MMR/P39/2011/G12P8 NSP4
AB211990.1 Human rotavirus A isolate:G18741 NSP4
JQ837887.1 RVA/Human-wt/IDN/YK-RVY36/2009/G1P8 NSP4
EF033202.1 Human rotavirus G9 isolate KNIH-7 NSP4
GU288644.1 Human rotavirus A strain CMH017/05 NSP4
JQ087432.1 RVA/Human-tc/CHN/Y128/2004/G1P8 NSP4
LC086756.1 RVA/Human-wt/THA/PCB-118/2013/G1P8 NSP4
EU679380.1 Human rotavirus A isolate CAU 164 NSP4
MW280897.1 RVA/Human-wt/ITA/PA01/2004/G3P8 NSP4
JQ043307.1 Human rotavirus A strain CMH055/07 NSP4 gene
GU947710.1 Rotavirus G9 strain BJ-CR4916 NSP4
KX954623.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8 NSP4
FJ361210.1 Rotavirus A strain 116E/AG NSP4
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E1
E2
0.020

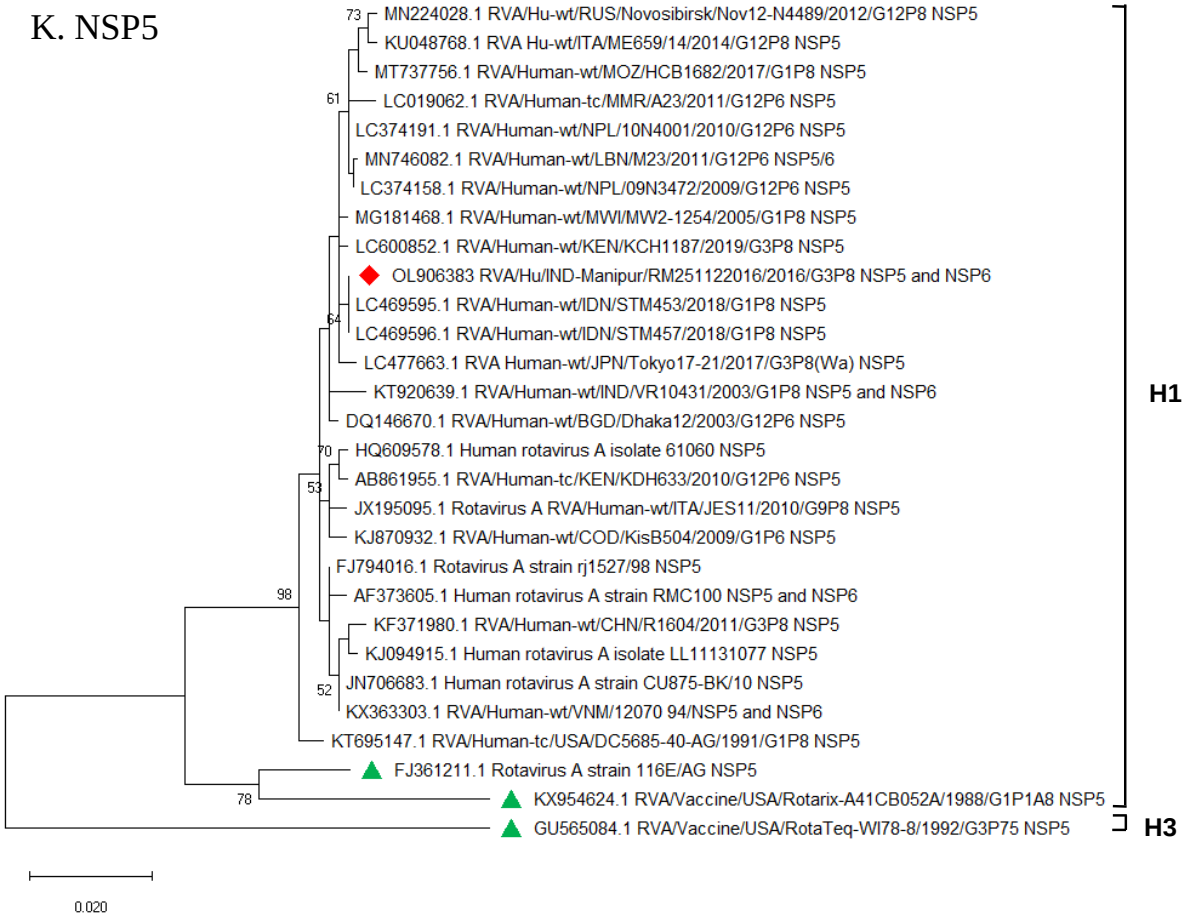

Figure 5.8: Phylogenetic analysis of 11-gene segments of RVA isolate RM251122016. The bootstrap consensus tree was inferred from 1000 replicates. (A) VP7, (B) VP4, (C) VP6, (D) VP1, (E) VP2, (F) VP3, (G) NSP1, (H) NSP2, (I) NSP3, (J) NSP4 and (K) NSP5. (Red arrow on the right side indicates animal rotaviruses).

5.3.6. Comparative sequence analysis of VP7, VP4 and VP6 genes of rotavirus strains from Manipur and vaccines strains

The deduced amino acid (aa) sequences of VP7, VP4 and VP6 of the isolate RM251122016 was compared with the vaccine strains (Rotarix™, RotaTeq™ and Rotavac^{TM}) and RV strains in the phylogenetic trees (Figure 5.8). In the VP7 antigenic regions (7-1a, 7-1b and 7-2) of the G3 strain, a single change L97S (7-1a) was observed when compared to human G3P[8] strain HuRoV/STHYCHRB1757/2008/CA, and another single aa change at residue A221D compared to human G3P[8] strains CR6193, Hu12/COVASU/Udgir/2017, RVA/Human-wt/USA/SSCRTV_00017/2013/G3P[8], Hu/RUS/Nov11-N2317/2011/G3P[8], RVA/Human-wt/CHN/Z1602/2012/G3P[8], RVA

human/Vanderbilt/VU08-09-22/2008/G3P[8], MaCH09404, RVA/Human/ITA/PA1/2004/G3P8, HuRoV/STHYCHRB1757/2008/CA, RVA/Human-wt/TWN/105-701-D042/2016/G3P8 and RVA/Human-wt/PRY/1747SR/2009/G3G1P[8] and same amino acid change with bovine strain Bf212/COVASU/Parbhani/2017 (Table 5.3). Interestingly, 25 residues of 29 in the VP7 antigenic region were found conserved between the G3 strain in this study and the G3 Rotateq™ strain component (Table 5.3). Further, the VP4 antigenic regions namely, VP8* (8-1, 8-2) and VP5* (51, 5-2, 5-3, 5-4 and 5-6) comparison was performed (Table 5.4). The strain identified in this study and the other circulating strains did not differ in any amino acid composition. However, amino acid changes was observed when compare with Rotateq™ and Rotarix™ vaccine sequences in the 8-1 antigenic region. The residues E150D and D196G were different from both the vaccine strains whereas the residue S190N was different from Rotarix™ (Table 5.4). Two amino acid differences were found at residues R383S and H386D of VP5* between RM251122016 strain and the Rotateq™ and Rotarix™ vaccine sequences (Table 5.4).

In the VP6 protein, four immunodominant regions, 32–64 (site I), 155–167 (site II), 208–274 (site III) and 380–397 (site IV) have been identified previously [34,35]. The analysis of the antigenic sites between the RM251122016 strain and the human strains in the phylogenetic tree showed VP6 was highly conserved. Only two strains, RVA/Human-wt/KEN/KCH1187/2019/G3P[8] and RVA/Human-wt/VNM/12053_58/VP6, showed one amino acid substitution at F248L at site III (Table 5.5). With the VP6 component of the RotaTeq-WI78-8/1992/G3P7[5], seven aa substitutions were found at position I39V and N60T (site I), V211I, V217A, Y248F and V252I (site III), and V396I (site IV). There are also two differences from the Rotarix™ strain (residues V56I and V252I) (Table 5.5).

Table 5.3. Surveillance of mutations in antigenic region of VP7 between RM251122016, RVA sequence in NCBI database and vaccine strain. Antigenic residues are divided into three epitopes (7-1a, 7-1b, and 7-2). Green colored residues are different from the most similar genotype in RotaTeq. Amino acid changes that have been shown to escape neutralization with monoclonal antibodies (Mcdonald SM 2009) are indicated with asteric.

	VP7 antigenic regions																														
	7-1a																	7-1b				7-2									
Strain	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
OL906385.1 RVA/Hu/IND-Manipur/RM251122016/2016/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
OL584241.1 RVA/Hu/IND-Manipur/RM252122016/2016/G3	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
OL584242.1 RVA/Hu/IND-Manipur/RM53012019/2019/G3	I	T	N	N	S	W	K	D	Q	D	A	V	D	N	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
OL584243.1 RVA/Hu/IND-Manipur/RM32122018/2018/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
OL584244.1 RVA/Hu/IND-Manipur/RM50012019/2019/G3P[11]	I	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	P	T	L	S	E	N	G
OL584245.1 RVA/Hu/IND-Manipur/RM78032019/2019/G3P[8]	I	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
LC477363.1 Human-wt/JPN/Tokyo17-21/2017/G3P[8](Wa)	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
LC600847.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
MH182446.1 RVA/Human-wt/PAK663/2016/G3P4	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
MN836861.1 THA/B3605/2017_G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	D	G
MK531570.1 CR6193	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
MK043948.1 Hu12/COVASU/Udgir/2017	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
MF469162.1 RVA/Human-wt/USA/SSCRTV_00017/2013/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
JX088016.1 Hu/RUS/Nov11-N2317/2011/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
KF372019.1 RVA/Human-wt/CHN/Z1602/2012/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
MK043950.1 Bf212/COVASU/Parbhani/2017	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
JF491055.1 RVA human/Vanderbilt/VU08-09-22/2008/G3P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
AY870661.1 MaCH09404	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
MW280951.1 RVA/Human/ITA/PA1/2004/G3P8	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
ANO46618.1 HuRoV/STHYCHRB1757/2008/CA	T	T	N	N	L	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
KY418051.1 RVA/Human-wt/TWN/105-701-D042/2016/G3P8	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
KJ412900.1 RVA/Human-wt/PRY/1747SR/2009/G3G1P[8]	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	T	N	N	N	K	D	A	T	L	S	E	A	G
RotaTeq-WI78-8/1992/G3P7[5] VP7	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	A	N	K	D	K	D	A	T	L	S	E	A	G
RotaTeq-WI79-9/1992/G1P7[5] VP7	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
RotaTeq-SC2-9/1992/G2P7[5] VP7	A	N	S	D	E	W	E	N	Q	D	T	M	N	K	Q	D	V	S	N	S	R	D	N	T	S	D	I	S	G
RotaTeq-BrB-9/1996/G4P7[5] VP7	S	T	S	T	E	W	K	D	Q	N	L	I	D	K	Q	D	T	A	D	T	R	A	S	G	E	S	T	S	G
RotaTeq-WI79-4/1992/G6P1A[8] VP7	V	N	A	T	E	W	K	D	Q	D	A	V	E	K	Q	N	P	D	N	A	K	D	S	T	Q	S	T	T	G
Rotarix-A41CB052A/1988/G1P1A[8] VP7	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
116E/AG VP7	I	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	N	S	T	L	S	E	N	G
	*	*	*	*	*	*	*	*	*					*	*	*		*	*	*		*		*	*	*	*	*	*

Table 5.4. Surveillance of mutations in antigenic region of VP4 between RM251122016, RVA sequence in NCBI database and vaccine strain. Antigenic residues are divided into four epitopes (8-1, 8-2, 8-3 and 8-4). Amino acid changes that have been shown to escape neutralization with monoclonal antibodies (Mcdonald SM 2009) are indicated with asteric.

Orange: Residues that are different from Rotarix, Green: Residues are different from the most similar genotype in RotaTeq. Blue: Residues different from both Rotarix and RotaTeq.

	VP8*																								
	8-1											8-2	8-3										8-4		
Strain	100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	116	125	131	132	133	135	87	88	89
OL906390.1 RVA/Hu/IND-Manipur/RM251122016/2016/G3P[8]	-	S	Q	D	S	N	A	N	L	N	G	E	R	-	-	-	-	-	-	-	-	-	-	-	-
OL584248.1 RVA/Hu/IND-Manipur/RM64022019/2019/P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
OL584247.1 RVA/Hu/IND-Manipur/RM38122018/2018/P[6]	D	S	S	E	Y	S	S	N	L	S	E	E	H	T	T	Q	S	T	E	N	N	N	I	N	Q
LC477393.1 Human-wt/JPN/Tokyo17-21/2017/G3P[8](Wa)	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
LC546078.1 RVA-U14 VP4	D	S	Q	D	S	N	A	N	L	N	D	E	R	N	P	V	D	N	R	N	D	D	N	T	N
MZ095927.1 RVA/Human-wt/KEN/KLF0821/2015/G1P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
MN478658.1 RVA/Human-wt/USA/2014741123/2014/G1P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
LC430885.1 RVA/Human-wt/IDN/STM387/2017/G3P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
JX878641.2 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N4489/2012/G12P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
KX646610.1 RV1318 VP4	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
KT921094.1 RVA/Human-wt/USA/CNMC13/2011/G12P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
MG652340.1 RVA/Human-wt/DOM/3000503708/2015/G1P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
LC600845.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
MN633047.1 RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8033/2015/G1P[8]	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
MN304725.1 NGR_Ref segment 4 VP4	D	S	Q	D	S	N	A	N	L	N	G	E	R	N	P	V	D	N	R	N	D	D	N	T	N
RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]	D	S	Q	E	S	N	A	N	L	N	D	E	R	N	P	V	D	N	R	N	D	D	N	T	N
RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]	D	S	Q	E	S	S	A	N	L	N	N	E	R	N	P	V	D	S	S	N	D	N	N	T	N
Rotavirus A strain 116E/AG segment 4 viral protein 4	T	S	A	A	V	T	F	N	P/V	P	N	S	Y	A	Q	T	S	T	D	N	S	S	S	N	D
	*	*	*	*	*	*			*			*	*		*		*			*	*	*	*	*	*

Table 5.5. Surveillance of mutations in antigenic region of VP6 between RM251122016, RVA sequence in NCBI database and vaccine strain. Antigenic residues are divided into four sites (Site I, II, III and IV). Orange: Residues that are different from Rotarix, Green: Residues are different from the most similar genotype in RotaTeq. Blue: Residues different from both Rotarix and RotaTeq.

Antigenic site I

Strain	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
OL906388.1 Rotavirus A strain RM251122016 segment 6	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MT737556.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P[8] segment 6	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MN417981.1 Human RVA strain PAK274 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MN414273.1 RVA strain RV-MMC2019-5 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MN304727.1 RVA isolate NGR_Ref segment 6 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MN066758.1 RVA/Human-wt/IND/CMC_00048/2013/G9P[8] segment 6	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MK417783.1 RVA/Human-wt/ESP/SS96099331/2018/G12P[8] VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MH933786.1 RVA/Human-wt/CMR/CMRHP55/2014/G1P8 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
MH473477.1 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N3583/2012/G1P[8] VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
LC600846.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8] VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
LC546079.1 RVA-U14 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
LC469482.1 RVA/Human-wt/IDN/STM457/2018/G1P[8] VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
LC086762.1 RVA/Human-wt/THA/SKT-98/2013/G1P[8] VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
KX363098.1 RVA/Human-wt/VNM/12053_58/VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
EU679385.1 Human RVA isolate CAU 163 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G
KX954619.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	V	R	N	W	T	F	D	F	G
GU565078.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5] VP6 gene	Q	Q	F	N	Q	M	I	I	T	M	N	G	N	E	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	N	F	D	F	G
FJ361206.1 Rotavirus A strain 116E/AG segment 6 VP6 gene	Q	Q	F	N	Q	M	I	V	T	M	N	G	N	D	F	Q	T	G	G	I	G	N	L	P	I	R	N	W	T	F	D	F	G

Antigenic site II

Strain	155	156	157	158	159	160	161	162	163	164	165	166	167
OL906388.1 Rotavirus A strain RM251122016 segment 6	P	N	I	F	P	Y	S	A	S	F	T	L	N
MT737556.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P[8] segment 6	P	N	I	F	P	Y	S	A	S	F	T	L	N
MN417981.1 Human RVA strain PAK274 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
MN414273.1 RVA strain RV-MMC2019-5 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
MN304727.1 RVA isolate NGR_Ref segment 6 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
MN066758.1 RVA/Human-wt/IND/CMC_00048/2013/G9P[8] segment 6	P	N	I	F	P	Y	S	A	S	F	T	L	N
MK417783.1 RVA/Human-wt/ESP/SS96099331/2018/G12P[8] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
MH933786.1 RVA/Human-wt/CMR/CMRHP55/2014/G1P8 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
MH473477.1 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N3583/2012/G1P[8] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
LC600846.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
LC546079.1 RVA-U14 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
LC469482.1 RVA/Human-wt/IDN/STM457/2018/G1P[8] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
LC086762.1 RVA/Human-wt/THA/SKT-98/2013/G1P[8] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
KX363098.1 RVA/Human-wt/VNM/12053_58/VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
EU679385.1 Human RVA isolate CAU 163 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
KX954619.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
GU565078.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5] VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N
FJ361206.1 Rotavirus A strain 116E/AG segment 6 VP6 gene	P	N	I	F	P	Y	S	A	S	F	T	L	N

Antigenic site IV

Strain	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397
OL906388.1 Rotavirus A strain RM251122016 segment 6	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MT737556.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P[8] segment 6	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MN417981.1 Human RVA strain PAK274 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MN414273.1 RVA strain RV-MMC2019-5 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MN304727.1 RVA isolate NGR_Ref segment 6 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MN066758.1 RVA/Human-wt/IND/CMC_00048/2013/G9P[8] segment 6	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MK417783.1 RVA/Human-wt/ESP/SS96099331/2018/G12P[8] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MH933786.1 RVA/Human-wt/CMR/CMRHP55/2014/G1P8 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
MH473477.1 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N3583/2012/G1P[8] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
LC600846.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
LC546079.1 RVA-U14 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
LC469482.1 RVA/Human-wt/IDN/STM457/2018/G1P[8] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
LC086762.1 RVA/Human-wt/THA/SKT-98/2013/G1P[8] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
KX363098.1 RVA/Human-wt/VNM/12053_58/VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
EU679385.1 Human RVA isolate CAU 163 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
KX954619.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K
GU565078.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5] VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	V	K
FJ361206.1 Rotavirus A strain 116E/AG segment 6 VP6 gene	D	N	L	Q	R	V	F	T	V	A	S	I	R	S	M	L	I	K

Antigenic site III

Strain	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
OL906388.1 Rotavirus A strain RM251122016 segment 6	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MT737556.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P[8] segment 6	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MN417981.1 Human RVA strain PAK274 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MN414273.1 RVA strain RV-MMC2019-5 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MN304727.1 RVA isolate NGR_Ref segment 6 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MN066758.1 RVA/Human-wt/IND/CMC_00048/2013/G9P[8] segment 6	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MK417783.1 RVA/Human-wt/ESP/SS96099331/2018/G12P[8] VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MH933786.1 RVA/Human-wt/CMR/CMRHP55/2014/G1P8 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
MH473477.1 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N3583/2012/G1P[8] VP6	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
LC600846.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8] VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
LC546079.1 RVA-U14 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
LC469482.1 RVA/Human-wt/IDN/STM457/2018/G1P[8] VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
LC086762.1 RVA/Human-wt/THA/SKT-98/2013/G1P[8] VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
KX363098.1 RVA/Human-wt/VNM/12053_58/VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
EU679385.1 Human RVA isolate CAU 163 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
KX954619.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] VP6	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
GU565078.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5] VP6 gene	F	E	H	V	V	Q	L	R	R	V	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S
FJ361206.1 Rotavirus A strain 116E/AG segment 6 VP6 gene	F	E	H	I	V	Q	L	R	R	A	L	T	T	A	T	I	T	L	L	P	D	A	E	R	F	S	F	P	R	V	I	N	S

Strain	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274
OL906388.1 Rotavirus A strain RM251122016 segment 6	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MT737556.1 RVA/Human-wt/MOZ/HGM0033/2014/G1P[8] segment 6	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MN417981.1 Human RVA strain PAK274 VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MN414273.1 RVA strain RV-MMC2019-5 VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MN304727.1 RVA isolate NGR_Ref segment 6 VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MN066758.1 RVA/Human-wt/IND/CMC_00048/2013/G9P[8] segment 6	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MK417783.1 RVA/Human-wt/ESP/SS96099331/2018/G12P[8] VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MH933786.1 RVA/Human-wt/CMR/CMRHP55/2014/G1P8 VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
MH473477.1 RVA/Hu-wt/RUS/Novosibirsk/Nov12-N3583/2012/G1P[8] VP6	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
LC600846.1 RVA/Human-wt/KEN/KCH1187/2019/G3P[8] VP6 gene	A	D	G	A	T	T	W	L	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
LC546079.1 RVA-U14 VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
LC469482.1 RVA/Human-wt/IDN/STM457/2018/G1P[8] VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
LC086762.1 RVA/Human-wt/THA/SKT-98/2013/G1P[8] VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
KX363098.1 RVA/Human-wt/VNM/12053_58/VP6 gene	A	D	G	A	T	T	W	L	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
EU679385.1 Human RVA isolate CAU 163 VP6 gene	A	D	G	A	T	T	W	F	F	N	P	I	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
KX954619.1 RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] VP6	A	D	G	A	T	T	W	F	F	N	P	V	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
GU565078.1 RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5] VP6 gene	A	D	G	A	T	T	W	Y	F	N	P	V	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q
FJ361206.1 Rotavirus A strain 116E/AG segment 6 VP6 gene	A	D	G	A	T	T	W	L	F	N	P	V	I	L	R	P	N	N	V	E	V	E	F	L	L	N	G	Q	I	I	N	T	Y	Q

5.4. Conclusions

The human rotavirus strain RM251122016 has two gene segments (VP1 and VP7) that are quite similar to animal rotaviruses. VP1 is related to porcine and equine rotaviruses, while VP7 is similar to bovine rotaviruses. This RVA genome sequence will serve as a part of baseline data of the country to assess vaccine efficacy and to monitor strain diversity. Continuous rotavirus surveillance is necessary to anticipate the emergence of rare strains and introduction of new strains into the human population as a result of interspecies transmission and to evaluate the vaccine effectiveness.

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5.6. Publication and manuscript under preparation for the Chapter:

1. Devi, Y. D., Dey, U., Kumar, A., Singh, C. S., & Namsa, N. D. (2022). Genome Sequence of a Wa-Like G3P[8] Rotavirus from a 12-Month-Old Child with Diarrhea in Manipur, India. Microbiology resource announcements, 11(8), e0125421. https://doi.org/10.1128/mra.01254-21.

2. Yengkhom Damayanti Devi, Upalabdha Dey, Aditya Kumar, Chongtham Singh and Nima D Namsa. Whole Genome Characterization of a G3P[8] rotavirus A strain isolated from a one year child in Manipur. (Manuscript drafted)