Persistence of G10P[11] neonatal rotavirus infections in southern India

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Abstract

Background: Neonatal rotavirus infections are predominantly caused by distinct genotypes restricted to this age-group and are mostly asymptomatic.

Method: Stool samples from neonates admitted for >48 h in neonatal intensive care units (NICUs) in Vellore (2014–2015) and Chennai (2015–2016) in southern India, and from neonates born at hospitals in Vellore but not admitted to NICUs (2015–2016) were tested for rotavirus by ELISA and genotyped by hemi-nested RT-PCR.

Results: Of 791 neonates, 150 and 336 were recruited from Vellore and Chennai NICUs, and 305 were born in five hospitals in Vellore. Positivity rates in the three settings were 49.3% (74/150), 29.5% (99/336) and 54% (164/305), respectively. G10P[11] was the commonly identified genotype in 87.8% (65/74), 94.9% (94/99) and 98.2% (161/164) of the neonates in

Vellore and Chennai NICUs, and those born at Vellore hospitals, respectively. Neonates delivered by lower segment cesarian section (LSCS) at Vellore hospitals, not admitted to NICUs, had a significantly higher odds of acquiring rotavirus infection compared to those delivered vaginally [$p = 0.002$, OR = 2.4 (1.4–4.3)].

Conclusions: This report demonstrates the persistence of G10P[11] strain in Vellore and Chennai, indicating widespread neonatal G10P[11] strain in southern India and their persistence over two decades, leading to interesting questions about strain stability.

Keywords

Rotavirus infection; Neonate; Nursery; G10P[11] strain

1. Background

Rotavirus infections in early infancy have been reported from both the developing and developed world [1]. These infections in young infants can be both symptomatic and asymptomatic, with a large proportion of infants experiencing rotavirus infections at a much earlier age in low-income settings [2]. Previous reports from Vellore, south India, described 56% of infants experiencing a rotavirus infection before they reach six months of age [3]. Neonatal rotavirus infections have been reported from neonatal nurseries and intensive-care units, as well as from the community [4–6].

There are 36 G and 51 P types of group A rotaviruses associated with human infections [7]. The most common genotypes associated with moderate to severe acute rotavirus infection in infants are G1P[8], G2P [4], G3P[8], G4P[8], G9P[8] and G12P[8], although temporal and spatial genotype distributions vary [8]. Rotavirus genotypes specifically associated with neonatal infection are highly restricted to this age-group and rarely seen in older infants [9]. Different genotypes have been reported in neonatal infections worldwide (Table 1). Neonatal rotavirus infections are predominantly asymptomatic, although their association with gastrointestinal symptoms including necrotizing enterocolitis have been reported [10].

Previously published reports from Vellore, south India, showed the predominance of the G10P[11] genotype in contrast to the G9P[11] genotype reported from Delhi, north India, or the G3P[6] from Australia [4,11,12]. Prior studies in neonates from Vellore showed human milk oligosaccharides (HMO) increased the infectivity of the G10P[11] strain, by conferring structural stability to the strain, compared to the G1P[8] and G2P[4] genotypes, and potentially driving the persistence of the G10P[11] strains [13].

This study reports the prevalence of rotavirus infection and the strain type distribution in neonates in hospitals in Vellore and Chennai, pooling data from three studies.

2. Methods

Neonates from three studies were included. The first study enrolled neonates admitted for >48 h to the neonatal intensive-care unit (NICU) at Christian Medical College (CMC), Vellore during 2014–2015, for a study on the impact of HMO, breast milk and infant gut microbiome on neonatal rotavirus infections [13]. The second study included neonates

enrolled between 2015 and 2016 from the NICU of Sri Ramachandra Medical College (SRMC), a tertiary care hospital in Chennai city, the state capital of Tamil Nadu, located ~130 km from Vellore. The third study included new-borns recruited in the Rotavirus Vaccine Immunogenicity (RoVI) study cohort between 2015 and 2016 from hospitals in Vellore namely, CMC, Vellore; the Low Cost-Effective Care Unit (LCECU); the Community Health and Development hospital (CHAD); the Government Vellore Medical College and hospital (GVMCH), and three Urban Health Centres (UHC) [14]. A small number of infants were born at private hospitals. Only healthy infants not requiring hospitalization for >48 h were included in the RoVI cohort. Written informed consent was obtained from the parents of the neonates.

Information regarding the infant's gender, maternal age, gestational age at birth, birth weight and mode of delivery were collected. Maternal age was classified as young and older mothers using the median cut-off value. Infants born at >37 weeks were classified as term born. Birth weight was classified as normal if the birth weight was 2.5 kg. Stool samples were collected approximately within the first four weeks of life from neonates admitted at NICUs of SRMC and CMC, whereas for the RoVI cohort, stool samples were collected at weeks one, four and six of age to determine the persistence of rotavirus infection (Fig. 1). All the above categorical variables were expressed as percentages. Chi-square test for proportions was used to compare the exposure variables (infant's gender, maternal age, gestational age at birth, birth weight and mode of delivery) with neonatal rotavirus infection. A p-value <0.05 was considered as significant. Statistical analysis was carried out using Stata 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Stool samples collected from the NICUs were tested for the presence of rotavirus antigen using a commercially available ELISA kit (Premier™ Rotaclone®, Meridian Biosciences). Samples showing an OD value of 0.150 were reported as positive for rotavirus antigen. Stool samples collected in the RoVI cohort were tested for rotavirus antigen using an in-house validated ELISA [15]. ELISA positive samples were genotyped using a hemi-nested multiplex Polymerase Chain Reaction (PCR).

Nucleic acid was extracted from the samples using the QIAamp Viral RNA Mini Kit. Complementary DNA was synthesized using random primers, Pd(N)6 hexamers (Pharmacia Biotech), and genotyped as previously described using oligonucleotide primers to detect VP7 genotypes G1, G2, G3, G4, G8, G9, G10, and G12 and VP4 genotypes P[4], P[6], P [8], P[9], P[10], and P[11] [16]. Alternate primer sets and protocols were used if genotyping failed with the standard primer sets mentioned [17,18].

After the selection of 29 PCR confirmed G10P[11] samples, by random sampling (15 from the RoVI cohort and 14 from the NICU at CMC), partial sequencing of the VP4 (876 bp) and VP7 (881 bp) encoding genes was performed. The first-round amplicons from the genotyping PCR were purified using ExoSAP-IT reagent® (Affymetrix) and sequenced using Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems) as per the manufacturers' instructions. The sequences were resolved using an automated sequencer (Genetic Analyzer 3130, Applied Biosystems) and the forward and reverse sequences

were assembled using Sequencher V5.4.6 software (Genecodes Corporation) [19]. Multiple sequence alignment was performed using MAFFT V7.380 (Multiple Alignment using Fast Fourier Transform) software and edited using the Aliview software [20,21]. Phylogenetic analysis was performed using MEGA V11 software and phylogenetic trees were constructed using the General time-reversible substitution model with 1000 bootstrap values [22]. The trees were visualized and edited using Figtree V1.4.3 software [23]. The sequences were submitted to GenBank and assigned accession numbers MN968974-MN969031.

The studies were approved by the Institutional Review Board of CMC, Vellore for the Vellore site, and the Institutional Ethics Committee of SRMC, Chennai for the study at SRMC.

3. Results

Overall, 791 neonates were included in this study: 150 and 336 neonates admitted for >48 h at the NICU at CMC and SRMC, respectively, and 305 neonates from the RoVI study (Table 2).

Neonates included from the NICU at CMC, Vellore, were born at a mean gestational age of 32 weeks (SD 3.1; range 24 - 40 weeks), majority were born preterm (91.3%, 137/150) and 92.7% (139/150) were <2.5 kg (range 0.6 – 3.3 kg) (Table 2). Sixty-one percent of the babies (91/150) were born by LSCS (lower segment cesarian section) with 55.3% (83/150) being male babies. Feed intolerance was the most common diagnosis on admission (49/150, 32.7%). The other associated symptoms were vomiting, and vomiting with abdominal distension. One neonate was diagnosed with necrotizing enterocolitis. More than half of the neonates (88/150, 58.7%) did not have any associated gastrointestinal symptoms.

Of the 336 neonates included from NICU-SRMC, Chennai, 55.7% (187/336) were males, born at a mean gestational age of 33 weeks (SD 3.6; range 25 – 40 weeks), majority being preterm (287/336, 85.4%) and <2.5 kg (73.2%, 246/336) (range 0.6 – 4.4 kg). The majority were born by LSCS (259/336, 77.1%). The mean maternal age was 27 years (SD 4.7; range 18 – 46 years).

The 305 neonates from the RoVI cohort were born at a mean gestational age of 38 weeks (SD 1.2; range 34 – 42 weeks), majority were males (155/305, 50.8%), term-born (257/305, 90.5%) and >2.5 kg (269/305, 88.2%) (range 1.8 – 4.2 kg) (Table 2). The majority were born by normal vaginal delivery (208/305, 68.2%) and the mean maternal age was 23 years (SD 3.72; range 17 – 39 years).

Among the 150 neonates from the NICU at CMC, Vellore, 49.3% (74/150) were positive for rotavirus by stool ELISA (Table 3, Fig. 1). No significant predisposing factors were identified in neonates from this cohort (Table 4). Of the 74 rotavirus positive neonates, none had diarrhea. Feed intolerance (49/74, 66.2%) was the most common gastrointestinal symptom in these neonates. Of the 74 positive samples, 89.2% (66/74) were fully typed. G10P[11] was present in 87.8% (65/74), one sample was genotyped as G12P[11] and eight samples remained untyped.

Of the 336 neonates from NICU-SRMC, 29.5% (99/336) were positive for rotavirus by stool ELISA (Table 3, Fig. 1). No significant factors to acquire rotavirus infection were identified among these neonates. (Table 4). Ten neonates (2.9%, 10/99) had associated gastrointestinal symptoms. Of the 99 rotavirus positive samples, 94.9% (94/99) could be fully typed and were of the G10P[11] genotype, two stool samples were partially typed- G10P[UT] and three samples were untypable.

Of the 305 neonates from the RoVI study, 54% (164/305) were positive for rotavirus by ELISA during the first week of life (Table 3, Fig. 1). By week four and six, only two and one infant/s, respectively, were positive for rotavirus. Among the neonates born at different hospitals in Vellore, neonates born at the tertiary hospitals (CMC and GVMCH) had the highest rates of infection, 75% (52/70) and 78% (46/59), respectively. In the RoVI cohort, neonates born by LSCS had a significantly higher odds of acquiring a rotavirus infection when compared to those delivered vaginally [$p = 0.002$, OR = 2.4 (1.4–4.3)]. No other factors were found to predispose the neonates for a rotavirus infection (Table 4). All those with neonatal rotavirus infections in the RoVI study were asymptomatic. Of the 164 stool samples positive for rotavirus by ELISA, 98.1% (161/164) could be completely typed and belonged to the G10P[11] genotype, one sample was partially typed-G10 P[UT], one was a mixed infection (G1 + 10, P[1] and P[11]) and one sample remained untyped even after using alternate primer sets. At week four, two infants had G10P[11] infection and one infant at week six had a G10P[11] infection. The infants positive at week four of age were infected at week one of age as well. The one infant positive at week six of age was negative for rotavirus at week one and week four.

Phylogenetic analysis of the VP4 and VP7 regions carried out for the 29 randomly selected G10P[11] samples demonstrated a high degree of homology between the RoVI cohort strains and the strains isolated previously in Vellore (Fig. 2A and 2B).

4. Discussion

Linking data from the present study with published data, the persistence of the G10P[11] strain in neonates born in Vellore, both in the community healthcare settings and the tertiary hospital nursery over the last two decades is evident [4,24]. The inclusion of samples collected from a birth cohort allowed us to establish the short duration of this infection, as the infection cleared in the majority of the infants by four weeks of age.

This is the first report describing neonatal rotavirus infections in an NICU setting from Chennai. Rotavirus was identified in 29.5% of the neonates admitted with 2.9% having associated gastrointestinal symptoms. Studies from different parts of the world have reported gastrointestinal symptoms ranging from 13.3% in Korea to 30.4% in Greece, and as high as 43.9% in Vellore among neonates infected with rotavirus [4, 25,26]. It is interesting to note the difference in rotavirus infection rates between the NICU settings of Vellore and Chennai (49.3% and 29.5%, respectively), showing the difference in the burden of infection between different tertiary care settings. This could be attributed to a stricter time-bound collection of samples in the RoVI study, where we have shown a high positivity which falls precipitously after the first 4 weeks of life.

The neonates enrolled from the CMC and SRMC NICUs had no specific risk factors associated with rotavirus infection namely, gender, maternal age, gestational period, birth weight or mode of delivery. This is similar to a previous study conducted in the NICU setting of CMC, Vellore, where the above-mentioned factors showed no association with a neonate acquiring either a symptomatic or asymptomatic rotavirus infection [4]. Also, a similar study from Bangladesh did not demonstrate any significant factors predisposing neonates to acquire rotavirus infection [27]. However, in contrast, a study from Madrid showed that prematurity and lower birth weight predisposed neonates to rotavirus infection in a nursery setting [10]. The association between neonatal rotavirus infection and the mode delivery with reference to vaginal delivery and LSCS has not been clearly established. In a study from Australia, normal vaginal delivery with the early establishment of breastfeeding reduced the risk of acute gastroenteritis related hospitalization in young children, though this was not rotavirus specific [28]. In the RoVI cohort, neonates born by LSCS had a higher risk of neonatal rotavirus infection. Neonates born by LSCS in this setting are usually kept under observation in the nursery before being roomed-in with the mother. This suggests that even a short duration of stay by the new-borns in the nursery, who are naïve to rotavirus exposure, potentially increases the risk of acquiring a rotavirus infection. Previous studies from Vellore have documented the presence of G10P[11] rotavirus in the environmental swabs taken from the nurseries [4,29].

Studies from Delhi, India, reported a 46% reduction in subsequent rotavirus infections in infants with a previous neonatal rotavirus infection [5,30]. The data from Delhi lead to the subsequent development and licensure of the Rotavac® vaccine based on the neonatal strain G9P[11]. Clinical trials using a neonatal dosing regimen involving Rotavac® and RV3-BB, another vaccine developed from a neonatal strain G3P[6], are currently underway [31,32]. In contrast, previous studies in Vellore showed that neonatal rotavirus infection did not provide any subsequent protection from rotavirus diarrhea of any severity (6). Despite this, it will be important to analyze any potentially enhancing or inhibitory effect of the G10P[11] neonatal strain on vaccine take and vaccine-induced immunity. No positive samples were available for sequencing from the SRMC—Chennai site. The study was conducted independently to ascertain and document neonatal rotavirus infections for the first time from Chennai and sequence analysis was not a part of the protocol.

The sustained persistence of the G10P[11] neonatal strains in Vellore contrasts with the study findings from Delhi in northern India. Previous reports from Delhi showed the predominance of G9P[11] strain between 1986 and 1992 which was replaced by a second novel strain, G9P[6] in 1993 [33]. Between 2005–2006, G12P[6] strain was the dominant strain among neonates in nurseries in Delhi [34]. Reasons for the persistence of the G10P[11] rotavirus strain among neonates in Vellore are not fully understood but available data suggests host-virus co-evolution, the role of the HMOs in enhancing infectivity and the age-restricted tropism exhibited by the strain through the binding to H-antigen glycan precursors [13,35].

The establishment of rotavirus infection in the gut requires the VP8 domain of the VP4 spike protein to bind to specific glycans. The association of G10P[11] with neonatal infection has been explained by the unique specificity of the spike domain to host glycans that are

restricted to the neonatal period. Recent crystallographic studies have demonstrated that the bovine component of the strain P[11] VP8 domain binds specifically to type II glycans found within the bovine gut [35,36]. However, the strain binds to both type I and type II precursor glycans found abundantly in the early neonatal gut as well as the human breastmilk. The VP8 region is the least conserved region among all rotavirus structural proteins. It may gradually assimilate sequence changes, leading to zoonotic crossover to humans in the neonatal period. The presence of these glycans predominantly in the neonatal gut is known and the assessment of the effect of HMOs and receptor tropism of the neonatal strains should help elucidate the factors related to the persistence of the G10P[11] strains.

The strength of this study was that we were able to compare neonatal rotavirus infection in NICU settings of two tertiary care hospitals and in a community-based birth cohort. The addition of the community cohort is an important highlight showing a high burden of asymptomatic neonatal rotavirus infection in the community, with the persistence of G10P[11] strain in the NICU settings. A limitation of the study was the non-availability of data on the time interval between the delivery and initiation of breastfeeding in the new-borns. Further, no data was available on the duration of the stay at the hospital for individual mothers.

This study highlights the widespread nature and high transmissibility of the G10P[11] rotavirus genotype and its continued persistence among neonates in diverse health care settings across Vellore and Chennai. We show the persistence of the G10P[11] rotavirus strain over the last two decades in the nursery at Vellore. We note the virus was not restricted to only the large hospital nurseries but also widely prevalent in the community hospitals in Vellore. This data highlights the importance of surveillance in neonates for rotavirus infections, even without gastrointestinal symptoms, as these may impact subsequent rotavirus vaccine response.

Acknowledgements

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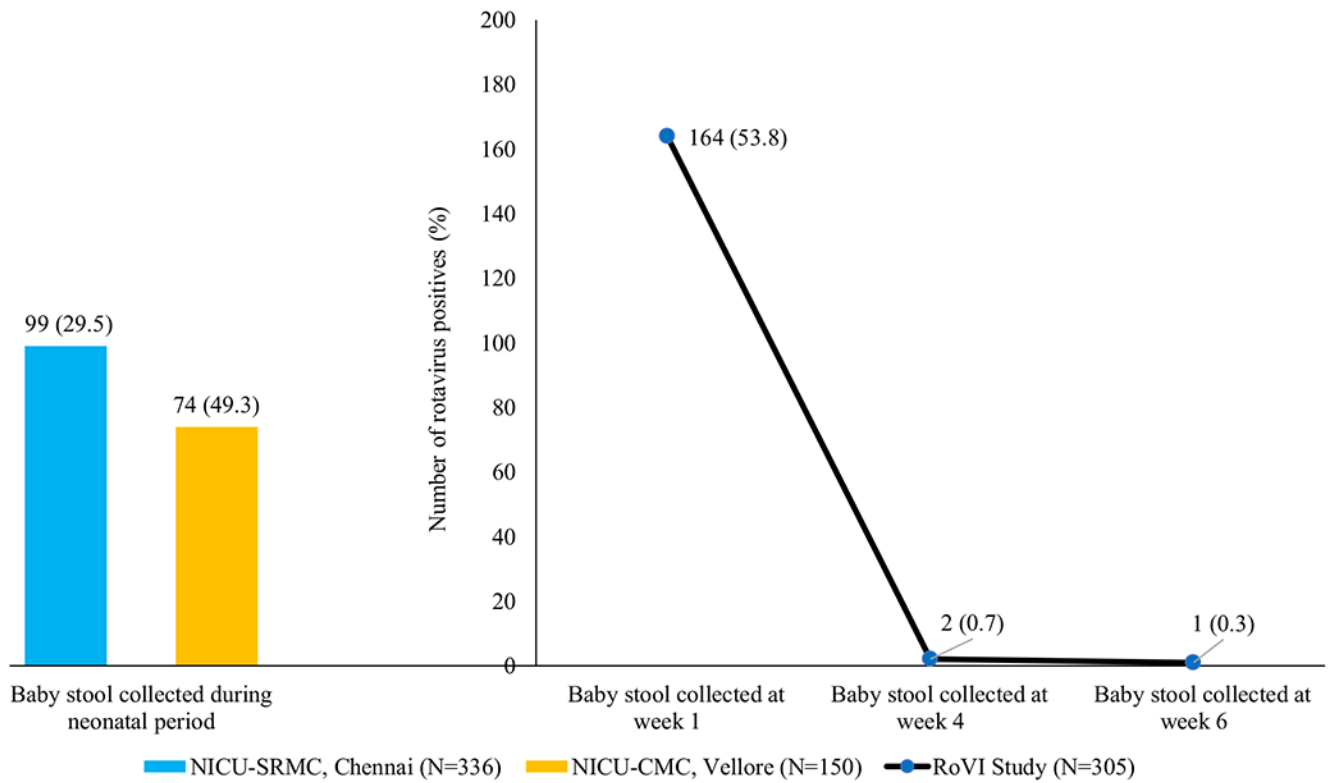
Fig. 1.
Proportion of infants with rotavirus infection (stool tested using ELISA) from the three study settings that is the Neonatal Intensive Care Units (NICUs) at Christian Medical College, Vellore (CMC) and Sri Ramachandra Medical College and Research Institute, Chennai (SRMC); and healthy infants from the community cohort of the Rotavirus Vaccine Immunogenicity (RoVI) study.

● Strains from RoVI cohort, ● Strains previously isolated from Vellore, ■ Rotavirus reference strains of human origin, ■ Reference strains of bovine origin
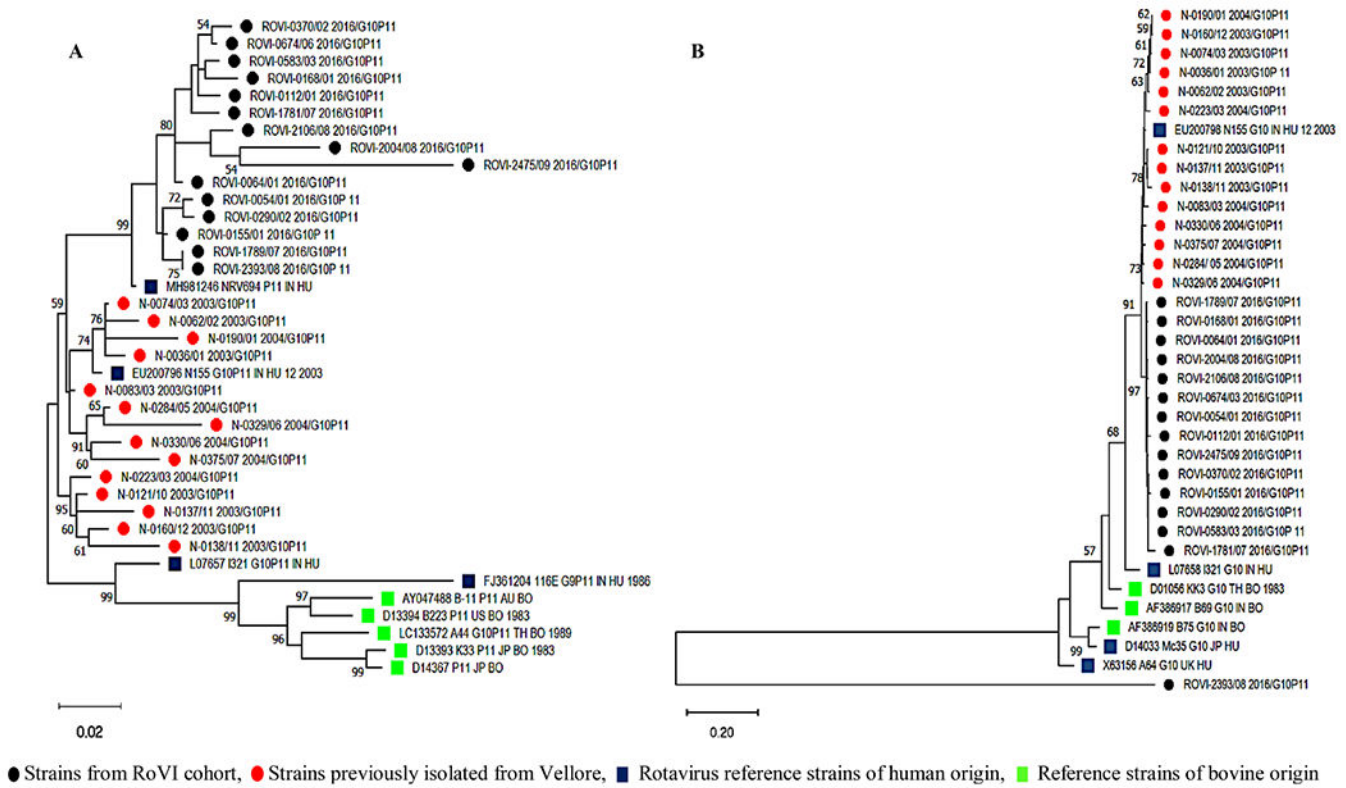
Fig. 2.
VP4 (A) and VP7 (B) sequence analysis showing the degree of homology of G10P[11] strains between RoVI cohort and isolates identified previously from Vellore.

Table 1

Neonatal rotavirus infections and strains reported from various countries between 1980 and 2018.

Country	Year	Prevalence n (%)	Predominant genotype	Method of detection
India (Bangalore) [6]	1988–1997	321/882 (39.3%)	G10P[11]	Serotyping (subgroup ELISA) and PAGE
South Africa [37]	1988–1989	Not reported	G4P[6]	cDNA hybridization probes
Egypt [38]	1992–1993	64/180 (35.6%)	Untypable[a]	Serotyping (subgroup ELISA)
India (Delhi) [11]	1993	38/169 (22%)	G9P[11]	Genotyping PCR
Bangladesh [27]	1995	61/146 (42%)	G4P[6]	Genotyping PCR
South Africa [39]	1997	80/114 (70%)	G4P[6]	PAGE, serotyping and genotyping PCR
Malawi [40]	1997–2000	35/142 (25%)	G8P[6]	PAGE and genotyping PCR
India (Vellore) [4]	2003–2006	619/1411 (43.9%)	G10P[11]	Genotyping PCR
India (Delhi) [34]	2005–2006	39/245 (16%)	G12P[6]	PAGE and genotyping PCR
Greece [25]	2009–2013	126/415 (30.4%)	G4P[8]	Genotyping PCR
India (Pune) [41]	2016–2018	151/621 (24.3%)[b]	G12P[11]	Genotyping PCR

[a]38.7% were untypable.

[b]Includes two symptomatic neonates.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PAGE, polyacrylamide gel electrophoresis; cDNA, complementary deoxyribonucleic acid; PCR, polymerase chain reaction.

Table 2

Baseline characteristics of the mother-infant pairs of the neonates from NICU-CMC, Vellore; NICU-SRMC and RoVI cohort.

n (%)	NICU-CMC, Vellore (N = 150)	NICU-SRMC, Chennai (N = 336)	RoVI cohort, Vellore (N = 305)						
			CMC (n = 70)	CHAD (n = 73)	LCECU (n = 28)	GVMCH (n = 59)	UHC (n = 62)	Private hospital/Others (n = 13)	RoVI cohort, Vellore - overall (N = 305)
Gender									
Male	83 (55.3)	187 (55.6)	34 (48.6)	45 (61.6)	13 (46.4)	26 (44.1)	30 (48.4)	7 (53.8)	155 (50.8)
Female	67 (44.7)	149 (44.4)	36 (51.4)	28 (38.4)	15 (53.6)	33 (55.9)	32 (51.6)	6 (46.2)	150 (49.2)
Maternal age [a]									
Young mother	NA	179 (53.3)	36 (51.4)	36 (49.3)	20 (71.4)	27 (45.8)	40 (64.5)	9 (69.2)	168 (55.1)
Older mother	NA	157 (46.7)	34 (48.6)	37 (50.7)	8 (28.6)	32 (54.2)	22 (35.5)	4 (30.8)	137 (44.9)
Gestational age (weeks) [b]									
37	137 (92)	287 (85.4)	4 (6)	6 (8.7)	3 (12)	6 (10.5)	8 (14.3)	0 (0)	27 (9.5)
>37	12 (8)	49 (14.6)	63 (94)	63 (91.3)	22 (88)	51 (89.5)	48 (85.7)	10 (100)	257 (90.5)
Birth weight (kg)									
2.5	139 (92.7)	246 (73.2)	5 (7.1)	5 (6.9)	3 (10.7)	10 (17)	11 (17.7)	2 (15.4)	36 (11.8)
2.5	11 (7.3)	90 (26.8)	65 (92.9)	68 (93.1)	25 (89.3)	49 (83)	51 (82.3)	11 (84.6)	269 (88.2)
Mode of delivery									
Normal vaginal	53 (35.3)	76 (22.6)	27 (38.5)	49 (67.1)	28 (100)	35 (59.3)	62 (100)	7 (53.8)	208 (68.2)
Suction	1 (0.7)	1 (0.3)	9 (12.9)	5 (6.9)	0 (0)	0 (0)	0 (0)	1 (7.7)	15 (4.9)
Forceps	3 (2)	0 (0)	9 (12.9)	3 (4.1)	0 (0)	0 (0)	0 (0)	0 (0)	12 (3.9)
LSCS	91 (60.7)	259 (77.1)	25 (35.7)	16 (21.9)	0 (0)	24 (40.7)	0 (0)	5 (38.5)	70 (23)
Assisted breech	2 (1.3)	-	-	-	-	-	-	-	-
Rotavirus Infection [c]									
Yes	74 (49.3)	99 (29.5)	52 (75.4)	51 (69.9)	4 (14.3)	46 (78)	5 (8)	6 (46.2)	164 (54)
No	76 (50.7)	237 (70.5)	17 (24.6)	22 (30.1)	24 (85.7)	13 (22)	57 (92)	7 (53.8)	140 (46)

[a] Young mothers were defined as <23 years and <27 years of age in the RoVI cohort and NICU-SRMC, Chennai study, respectively.

[b] Missing data for 1 subject in NICU-CMC and 21 subjects in the RoVI cohort.

[c] Unknown for one subject in the RoVI cohort.

Abbreviations: NICU, Neonatal Intensive Care Unit; CMC, Christian Medical College, Vellore; SRMC, Sri Ramachandra Medical College and Research Institute, Chennai; RoVI, Rotavirus Vaccine Immunogenicity study; CHAD, Community Health and Department, Vellore; LCECU, Low Cost Effective Care Unit, Vellore; GVMCH, Government Vellore Medical College and Hospital; UHC, Urban Health center; NA, Not Available; LSCS, Lower Segment Caesarean Section.

Table 3

Characteristics of neonates with rotavirus infection.

n (%)	NICU-CMC, Vellore (n = 74)	NICU-SRMC, Chennai (n = 99)	RoVI cohort, Vellore (n = 164)						RoVI cohort, Vellore, overall (n = 164)
			CMC (n = 52)	CHAD (n = 51)	LCECU (n = 4)	GVMCH (n = 46)	UHC (n = 5)	Private hospital/ Others (n = 6)	
Gender									
Female	34 (45.9)	41 (41.4)	27 (51.9)	22 (43.1)	2 (50)	26 (56.5)	2 (40)	2 (33.3)	81 (49.4)
Male	40 (54.1)	58 (58.6)	25 (48.1)	29 (56.9)	2 (50)	20 (43.5)	3 (60)	4 (66.7)	83 (50.6)
Maternal age [a]									
Young mother	NA	58 (58.6)	28 (53.9)	25 (49)	2 (50)	20 (43.5)	4 (80)	4 (66.7)	83 (50.6)
Older mother	NA	41 (41.4)	24 (46.1)	26 (51)	2 (50)	26 (56.5)	1 (20)	2 (33.3)	81 (49.4)
Gestational age (weeks) [b]									
37	68 (91.9)	85 (85.9)	3 (6)	5 (10.2)	1 (33.3)	5 (11.1)	2 (40)	0 (0)	16 (10.3)
>37	6 (8.1)	14 (14.1)	47 (94)	44 (89.8)	2 (66.7)	40 (88.9)	3 (60)	4 (100)	140 (89.7)
Birth weight (kg)									
2.5	68 (91.9)	70 (70.7)	5 (9.6)	4 (7.8)	1 (25)	7 (15.2)	1 (20)	0 (0)	18 (11)
2.5	6 (8.1)	29 (29.3)	47 (90.4)	47 (92.2)	3 (75)	39 (84.8)	4 (80)	6 (100)	146 (89)
Mode of delivery									
Normal vaginal	27 (36.5)	25 (25.3)	20 (38.5)	34 (66.7)	4 (100)	29 (63.0)	5 (100)	2 (33.3)	94 (57.3)
Suction	0 (0)	0 (0)	6 (11.5)	4 (7.8)	0 (0)	0 (0)	0 (0)	1 (16.7)	11 (6.7)
Forceps	3 (4)	0 (0)	8 (15.4)	2 (3.9)	0 (0)	0 (0)	0 (0)	0 (0)	10 (6.1)
LSCS	44 (59.5)	74 (74.7)	18 (34.6)	11 (21.6)	0 (0)	17 (37)	0 (0)	3 (50)	49 (29.9)

[a] Young mothers were defined as <23 years and <27 years of age in the RoVI cohort and NICU-SRMC, Chennai study, respectively.

[b] Missing data for 8 subjects in the RoVI cohort.

Abbreviations: NICU, Neonatal Intensive Care Unit; CMC, Christian Medical College, Vellore; SRMC, Sri Ramachandra Medical College and Research Institute, Chennai; RoVI, Rotavirus Vaccine Immunogenicity study; CHAD, Community Health and Department, Vellore; LCECU, Low Cost-Effective Care Unit, Vellore; GVMCH, Government Vellore Medical College and Hospital; UHC, Urban Health center; NA, Not Available; LSCS, Lower Segment Caesarean Section.

Table 4

Comparison of rotavirus infection in the three study settings.

Variable		NICU-CMC, Vellore (n = 150)				NICU-SRMC, Chennai (n = 336)				RoVI cohort, Vellore (n = 305)			
		RV Positive n (%)	RV Negative n (%)	p-value	OR (95% CI)	RV Positive n (%)	RV Negative n (%)	p-value	OR (95% CI)	RV Positive n (%)	RV Negative n (%)	p-value	OR (95% CI)
Gender	Male	40 (48.2)	43 (51.8)	0.76	0.9 (0.47, 1.71)	58 (31)	129 (69)	0.49	1.18 (0.74, 1.9)	83 (53.5)	72 (46.5)	0.94	0.98 (0.63, 1.54)
	Female	34 (50.7)	33 (49.3)			41 (41.4)	108 (45.6)			81 (54)	69 (46)		
Maternal age [a]	Young mother	NA	NA	NA	NA	58 (32.4)	121 (67.6)	0.21	1.36 (0.84, 2.18)	83 (49.4)	85 (50.6)	0.09	0.68 (0.43, 1.06)
	Older mother	NA	NA			41 (26.1)	116 (73.9)			81 (59.1)	56 (40.9)		
Gestational age (weeks) [b]	37	68 (49.6)	69 (50.4)	0.98	0.98 (0.3, 3.2)	85 (29.6)	202 (70.4)	0.88	1.05 (0.54, 2.06)	16 (59.3)	11 (40.7)	0.64	1.22 (0.54, 2.72)
	>37	6 (50)	6 (50)			14 (28.6)	35 (71.4)			140 (54.5)	117 (45.5)		
Birth weight (kg)	2.5	68 (48.9)	71 (51.1)	0.72	0.8 (0.23, 2.74)	70 (28.5)	176 (71.5)	0.50	0.84 (0.47, 1.41)	18 (50)	18 (50)	0.63	0.84 (0.42, 1.69)
	2.5	6 (54.5)	5 (45.5)			29 (32.2)	61 (67.8)			146 (54.3)	123 (45.7)		
Mode of delivery	LSCS	44 (46.8)	50 (53.2)	0.42	0.76 (0.39, 1.48)	74 (28.6)	185 (71.4)	0.51	0.83 (0.48, 1.44)	49 (70)	21 (30)	**0.002**	2.43 (1.37, 4.31)
	Vaginal	30 (53.6)	26 (46.4)			25 (32.5)	52 (67.5)			115 (48.9)	120 (51.1)		

[a] Young mothers were defined as <23 years and <27 years of age in the RoVI cohort and NICU-SRMC, Chennai study, respectively.

[b] Missing data for 1 subject in NICU-CMC and 21 subjects in the RoVI cohort.

Abbreviations: NICU, Neonatal Intensive Care Unit; CMC, Christian Medical College, Vellore; SRMC, Sri Ramachandra Medical College and Research Institute, Chennai; RoVI, Rotavirus Vaccine Immunogenicity study; RV, Rotavirus; NA, Not Available; LSCS, Lower Segment Caesarean Section.