Original Article

Epidemiology and Genotyping of Rotavirus Gastroenteritis in Children <5 Years in Sikkim, North East India

Shrijana Gurung, Ananya Chatterjee[1], Ruth Yonzan[2], Agniva Majumdar[1], Tashi Pegey Chhophel, Ekta Tewari, Dhruva Kumar Sharma[3], Hasina Banu[4], Mamta Chawla Sarkar[1], Shanta Dutta[1]

Departments of Virology and [2]Paediatrics, Sir Thutob Namgyal Memorial Hospital, Gangtok, Sikkim, [3]Department of Pharmacology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, [1]Regional Viral Research and Diagnostic Laboratory, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, [4]Department of Microbiology, IPGMER-SSKM Hospital, Kolkata, West Bengal, India

Abstract

Introduction: Rotavirus is a frequent cause of gastroenteritis in young children. It is seasonal in many countries but occurs year-round in India.Since the launch of the Rotavirus Vaccine (RVV) morbidity and mortality in children have greatly decreased. This study was carried out prior to the inclusion of RVV in the state vaccination schedule in 2019. The objective of the study was to estimate the burden of Rota Virus Gastroeneteritis in kids under 5 years of age, attending a government tertiary care hospital in the state and to identify the common circulating genotypes of Rotavirus. **Methods:** Stool samples from children with more than three episodes of loose stool that were negative for bacterial enteric pathogens were tested by Enzyme Linked Immunosorbent Assay (ELISA) for Rotavirus antigen. Positive stool samples were transported to the Regional Viral Research and Diagnostic Laboratory at National Institute for Cholera and Enteric Diseases for genotyping. **Results:** 200 stool samples were screened and 40 samples (20%) were positive for Rotavirus antigen by ELISA. G3P [8] - 33% (9/27), followed by G1P[8] –15%(4/27) and G2P4 – 11%(3/27) were the most common genotypes. **Conclusion:** Rotavirus is a significant cause of gastroenteritis in children under five years of age in the Indian state of Sikkim.

Keywords: Acute gastroenteritis, children, diarrhea, rotavirus, Sikkim

Introduction

Acute diarrhea remains a significant burden, especially in low- and middle-income countries, and is an important public health concern. Rotavirus (RV) is the most common virus causing acute gastroenteritis (AGE) in children <5 years across the globe[1] followed by norovirus and adenovirus.[2] Bacterial and Parasitic agents like Shigella, Vibrio, enterotoxigenic and enteropathogenic *Escherichia coli*, Giardia, Cryptosporidium, Enteamobea histolytica are common causes of acute diarrhea in children < 5 years but the morbidity and mortality is much lower when compared to RV.[2,3] The Reoviridae family of viruses includes the RV, a nonenveloped double-stranded segmented RNA virus.[1] Based on the antigenic variations of VP6, there are 10 different RV species that are categorized as A to J.[4-6] RV species A is the most common causative organism of infections in children, and it is classified into genotypes based on RNA segments 7 and 4 encoding VP7 and VP4, respectively. Subtypes cleaved by glycoprotein are termed G types (VP7) there are six G types G1–G4, G9, and G12 and protease cleaved are termed P types (VP4) and there are three P types, P[4], P[6], and P[8], predominate. The most prevalent circulating strains are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] account for >90% of all RV infections in the world.[4,5]

The majority of RV-related mortality have been recorded from low- and middle-income countries,[6,7] and it is a prominent

Address for correspondence: Dr. Shrijana Gurung, Department of Virology, Sir Thutob Namgyal Memorial Hospital, Gangtok - 737 101, Sikkim, India.
E-mail: doctorsiru@gmail.com

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viral etiological agent for AGE in children under 5 years old globally.[1] Prior to 2008, RV gastroenteritis (RVGE) is thought to have been the cause of 453,000 pediatric deaths worldwide.[8] An RV infection is thought to have caused 258 million episodes of diarrhea and 1,537,000 hospitalizations worldwide in 2016.[9] India is responsible for 22% of all RV deaths worldwide.[8] RV was projected to be the cause of 11.37 million incidents of AGE in children under the age of 5 each year in India between 2011 and 2013.[10]

A multicentric hospital study conducted in 2012–2013 found RV antigen in 26.4% of children with diarrhea,[5] and the National Rotavirus Surveillance Network (2012–2016) found a positivity of 36.3%[11] for RV in children under the age of 5 who have AGE. The prevalence of RV in these children has changed over time. The Indian Rotavirus Strain Surveillance Network (2005–2009) found a prevalence of 40%.[10] India is a vast country with mixed climatic subtypes and temperature because of which a difference in prevalence of RVGE among various states in India has been documented.[12] A difference in prevalence in various states and regions in India has been documented. In the north, the overall RV positive rate was 41.7% (663/1590), while in the south, it was 33.1% (1406/4244).[10] RV positivity in the Eastern region was 14.5% and was 53.5% in the West.[13]

The introduction of RV vaccine (RVV) in the national immunization program has been considered to be effective in reducing RVGE in children <5 years It has been suggested that the inclusion of the RVV in the national immunization program has helped reduce RVGE in children under the age of 5.[5] Given its efficacy in reducing RV-related morbidity and mortality, the World Health Organization has endorsed RVV to be incorporated into all national immunization programs.[1] On March 26, 2016, India was the first nation in South East Asia region to implement RVV.[14] Although it was readily available in the market in Sikkim and given as an optional vaccine to neonates, RVV was included in the State Immunization Schedule and provided free of cost to all children in the year 2020.

This study was conducted to estimate the burden of RVGE and the circulating genotype in Sikkim and associated demographics before the introduction of RVV into the State Immunization Program.

Methods

Ethics, inclusion criteria, and exclusion criteria

This was a hospital-based, descriptive, cross-sectional study, and it was cleared by the institutional ethics committee vide memo no. 10/IEC/STNM/18 dated April 21, 2018. Convenient samples from children <5 years attending the pediatric outpatient department at a tertiary care government hospital in Gangtok were collected over a period of 2 years from March 2018 till February 2020 till the desired number of samples was reached.

Inclusion criteria

All children with complaints of more than 3 episodes of loose stools were included in the study. Eligible patients were included in the study after obtaining informed consent from the parents/guardian.

Exclusion criteria

All children whose parents did not give their consent and those who were vaccinated with RVV were excluded from the study.

Sample size, collection, and screening

Assuming that 15% of the culture-negative stool samples turn out to test positive for RV, the study would require a sample size of 196 for estimating the expected proportion with 5% absolute precision and 95% confidence.

Stool samples were sent to bacteriology division within 2 h of collection and were screened for bacterial pathogens by culture on MacConkey and blood agar. All samples negative for enteric bacterial pathogens were screened for the group-specific RV VP6 antigen by solid-phase sandwich enzyme-linked immunosorbent assay (Premier Rotaclone, Meridian Bioscience, Inc., Cincinnati, Ohio). Positive samples were stored at −20°C and then transported to the Regional VRDL, National Institute of Cholera and Enteric Disease, Kolkata, for genotyping maintaining cold chain.

Rotavirus genotyping

The RV genotyping was carried out at the Regional VRDL of ICMR-NICED using standard reverse transcription polymerase chain reaction (PCR). Viral RNA extraction was performed from stool suspension followed by cDNA preparation by random priming. G (G1, G2, G3, G4, G8, G9, G10, G12) and P (P[4], P[6], P[8], P[9], P[10], P[11]) typing was performed using nested PCR reactions where the VP7 and VP4 regions were amplified, respectively, in step 1 and specific primers were employed in a multiplex PCR in step 2. The amplified products were visualized after separation by gel electrophoresis.

Data analysis

Data were analyzed using the Microsoft Excel 2007 for positive cases, demographic profile, symptoms, and seasonality.

Results

A total of 200 stool samples were screened for group-specific RV antigen. Out of them, 40 samples (20%) were positive for RV antigen. Positive samples were stored at −20°C. Among the 40 positive samples, 27 samples were sent to NICED, Kolkata, for further genotyping. The most common genotype was G3P [8] – 9/27, followed by G1P[8] – 4/27, G2P4 – 3/27, partially typable – 5/27, mixed partially typable – 04/27, and mixed G1P[8] and G1P[6] – 1/27 [Table 1].

Demographic profile

Twenty-five patients (62%) were between 0 and 12 months of age, 11 (27%) were between 13 and 24 months, 1 patient was between 25 and 36 months, and 3 patients were between 37 and months, as shown in Figure 1. The percentage of male

and female patients who tested positive was 55% (22/40) and 45% (18/40), respectively. Maximum number of patients were from east district 80% (32/40), the rest were from north district 10% (4/40), west district 8% (03/40), and south district 3% (1/40).

Clinical feature

The most common accompanying clinical feature among the children who tested positive was vomiting as seen in 24 patients (60%) followed by fever in 23 patients (58%) and cough in 9 patients (23%) [Table 2]. Only 4 patients (10%) were admitted with dehydration. All patients recovered with no complications. Positive cases were encountered throughout the year, as shown in [Figure 2] with the number of cases rising noticeably in the month of March.

Type of feeding

Information regarding the type of feeding before 6 months of age was recorded. Information was acquired only from 195 mothers. One hundred and twenty-two infants were given mixed feeding of breast milk, homemade complementary food, and infant formula powder. Seventy-three children were provided exclusive breast milk until 6 months of age [Table 3].

It was observed that positivity of RV disease was higher in infants who had mixed type of feeding $n = 30$ than compared to those who were exclusively breastfed $n = 7$. A significant association between mixed feeding and RVGE, $P = 0.015$, was seen when analyzed using Chi-square test for independence.

Discussion

According to the 2019 Global Burden of Disease survey, diarrheal disease was among the top three causes of morbidity and mortality in children,[15] and RV, one of its main causes. In their multicenter study conducted between April 2011 and July 2012 in 12 medical centers in India, Saluja *et al*. reported a prevalence of 26.4%.[5] Similarly, the National Rotavirus Surveillance Network study that involved 28 hospitals in India estimated a prevalence of 36.3% in India. The same study also provided region-wise prevalence which showed that the neighboring northeastern states of Assam and Nagaland had a prevalence of 38.4% and 40.3%, respectively.[4] Giri *et al*. observed an RVGE positivity of 35.5%,[11] but they did not include northeastern regions in this study. Raorane *et al*. in their analysis on genotypic determinants RVGE observed a positivity of 36% in the state of Meghalaya.[16] Omatola *et al*. in their systematic review and meta-analysis of viral gastroenteritis among African children have found that RV was the most common etiologic with a pooled prevalence of 31%.[17] The positivity of RVGE in our hospital was 20% (40/200) which was not as high as the rest of the country.

As was observed in all the surveillance studies conducted in India, RVGE was seen throughout the year with an increased prevalence between December and February.[4,5,10] Economic development has been considered as an important yardstick in predicting the seasonal occurrence of RVGE where low income countries like India have a less seasonal pattern.[18] This may be the reason for the year round disease transmission in India.

Table 1: Genotypes of rotavirus

Genotype	Number of samples (%)
G1P[6]	1 (3.7)
G1P[8]	4 (14.8)
G2P[4]	3 (11.1)
G3P[8]	9 (33.3)
Mixed - G1P[8], G1P[6]	1 (3.7)
Partially typable	5 (18.6)
Mixed partially typable	4 (14.8)

Table 2: Associated clinical symptoms

Age group (months)	Fever	Vomiting	Cough	Dehydration
0–12	12/25	15/25	5/25	1/25
13–24	7/11	5/11	2/11	3/11
25–36	1/1	1/1	1/1	0/1
37–48	3/3	3/3	1/3	0/3

Table 3: Type of feeding before 6 months of age and rotavirus positivity

Type of feeding	RV positive	RV negative
Mixed feeding (119/195)	30	92
Exclusive breastfeeding (72/195)	7	66

RV: Rotavirus

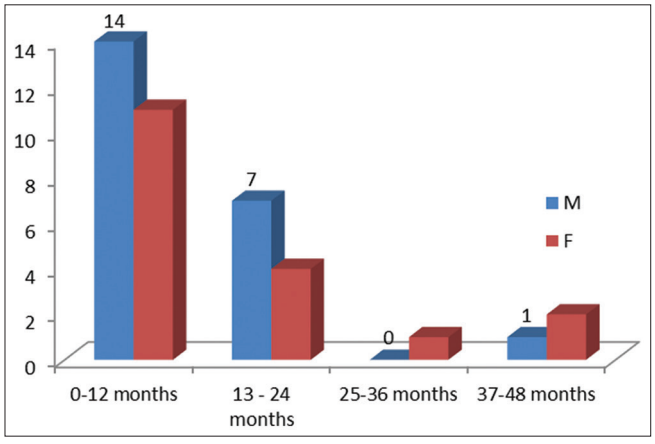

Figure 1: Age group of patients

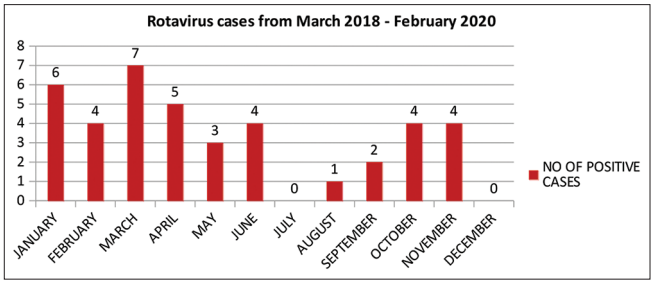

Figure 2: Seasonal variation of rotavirus gastroenteritis

In a research conducted in the state of Orissa in India to study the effect of seasons on RVGE, an increased number of RV diarrhea was encountered in the months of June and July and the authors are of the view that temperature, rainfall, and humidity influenced the prevalence of RVGE.[12] On the contrary, in a study conducted at Kolkata by Giri *et al*., RVGE was detected throughout the year with an increased number of cases during the winters.[11] RV remains in the environment due to the suitable weather conditions during winters, and it is known to survive in soil in areas of low humidity and rainfall.[18,19] Transmission is also facilitated by crowding of people indoors during winter.

In our study, RVGE was present throughout the year in 2018 and 2019 with a slight increase during the month of March. March in Sikkim is pleasant and cool with an average temperature of 13.7°C and has the lowest humidity of 77% and rainfall of 259 mm.[20]

The most common RV strains causing infection worldwide are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8].[21,22] G1P[8] was the most commonly isolated strain in 62.7% of hospitalized RVGE children in India[23] and 39% of outpatient and hospitalized patients in Africa.[17] G1P[8] and G1P[6] genotypes and G1P[8] and G1P[4] were the most common circulating strains in Goa and Meghalaya, respectively.[16] Unlike the other states where G1P[4] is most prevalent, G3P[8] was the most common circulating strain in Sikkim (33.3%). Mazid *et al*. observed that 56% of RV strains in Bangladesh were G3P[8][24] while Nanda and Choudhury in Eastern India recorded 41.30% of RV as G3P[8].[25] Omatela CA *et al*. reported a prevalence of 11.9% of G3P[8] genotype from Africa.

The majority of G3P[8] strains that are now in circulation worldwide are human variants with Wa-like (genotype 1) genomic constellations, but reassortant equine-like G3P[8] strains with DS-1 like (genotype 2) genomic backbone can spread easily among humans and have been reported from Australia,[26] the United States,[27] and Italy.[28] The G3P[8] strains are the result of reassortment between RV strains of human and animal origin,[29] thus warranting surveillance and testing for RV in view of newer variants that may cause vaccine breakthrough infections.

Thirty children with RVGE in our study had been given mixed-type feeding before 6 months of age, and seven children with RVGE were on exclusive breastfeeding. A significant correlation was observed between mixed feeding and RVGE, with $P = 0.015$. Early initiation and exclusive breastfeeding for the first 6 months of life have been known to protect an infant from enteric diseases including RV.[30] Numerous studies support this view and have put forward observations that constituents in breast milk like mucin inhibit replication of the virus, and the glycolipids prevent attachment of pathogens to the epithelium by camouflaging as pathogen receptors.[31] Moreover, breastfed infants are colonized with *Lactobacillus* and *Bifidobacterium* spp., thus limiting invasion of the intestine by RV and other enteric pathogens.[32] Hamer *et al*. observed that the risk of diarrhea increased with complementary feeding with an odds ratio of 1.27.[33] Inadequate milk production and local social customs and beliefs have been attributed to the early introduction of complementary food. Contaminated water, unsanitary conditions while preparing food, and inadequate washing of hands may all lead to an increased incidence of diarrheal disease in infants and small children.[33]

Conclusion

This was a single-center, hospital-based study and so the sample size was less. Nonetheless, this study highlights RV as a common causative organism causing childhood gastroenteritis in Sikkim, and there is a considerable burden of RVGE in the community with genotypes that are unlike the rest of the country. After the introduction of vaccine, RV surveillance has not been carried out.

RV has a segmented genome which are prone to genetic reassortment and rearrangements which may lead to the emergence of newer variants of the virus. Thus, continuous surveillance of RVGE and monitoring of the genotype of the circulating strains becomes imperative.

Research quality and ethics statement

The study was approved by the Institutional Ethics Committee (Sir Thutob Namgyal Memorial Hospital Institutional Ethics Committee/10/IEC/STNMH/18). The authors followed applicable EQUATOR Network guidelines during the conduct of this research project.

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Conflicts of interest

There are no conflicts of interest.

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