A multicenter prospective hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children less than five years of age in India

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ABSTRACT

Background: Rotavirus is the leading cause of severe, dehydrating diarrhea in children aged <5 years globally, with an estimated 25 million outpatient visits and 2 million hospitalizations attributable to rotavirus infections each year. The aim of this hospital-based surveillance was to summarize the local epidemiological and virological features of rotavirus and to estimate the disease burden in the population under surveillance in India.

Methods: During the 16 months surveillance period from April 2011 through July 2012, a total of 4711 children under the age of 5 years were admitted with acute diarrhea at 12 medical centers across medical schools throughout India. Stool samples were randomly collected from 2051 (43.5%) subjects and were analyzed for rotavirus positivity using commercial enzyme immunoassay kit (Premier Rotacne Qualitative Elisa) at the respective study centers. Rotavirus positive samples were genotyped for VP7 and VP4 by reverse-transcription polymerase chain reaction (RT-PCR) at a central laboratory.

Results: During the study period, maximum number of rotavirus related hospitalizations were reported from December 2011 through February 2012. Out of the 2051 stool samples tested for rotavirus, overall 541 (26.4%) samples were positive for rotavirus VP6 antigen in stool. The highest positivity was observed in the month of December, 2011 (52.5%) and lowest in the month of May, 2011 (10.3%). We found that majority of the rotavirus positive cases (69.7%) were in children <24 months of age. The most common genotypes reported were G1 (38%), G2 (18%), G9 (18%), G12 (9%) and mixed strains (17%).

Conclusions: The results of this study confirm the significant burden of acute rotavirus gastroenteritis as a cause of hospitalizations in five children in India.

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1. Introduction

Rotavirus infection, mostly caused by Group A viruses, is prevalent in human populations worldwide. Although the virus infects older individuals, the disease can be severe in immunologically naive infants and young children. The burden of severe rotavirus illness and deaths falls heavily upon children in low and middle-income countries: more than 80% of rotavirus-related deaths are estimated to occur in lower income countries of Asia and sub-Saharan Africa [1].

India has an especially large population at risk of clinically significant rotavirus gastroenteritis (GE); of the 1.2 billion people, 11% are <5 years old. Worldwide in 2008, diarrhea attributable to rotavirus infection resulted in 453,000 deaths (95% CI 420,000–494,000) in children younger than 5 years representing 37% of deaths attributable to diarrhea and 5% of all deaths in children younger than 5 years. Five countries accounted for more than half of all deaths attributable to rotavirus infection: Democratic Republic of the Congo, Ethiopia, India, Nigeria, and Pakistan with India alone accounting for 22% of deaths (98,621 deaths) [2].

Typical clinical signs of infection include fever, projectile vomiting, and profuse watery diarrhea, which may significantly dehydrate the infected child. Moderate to severe dehydration in young children is more often associated with rotavirus infection than other enteropathogens. There are no specific medications for rotavirus GE, but rehydration with oral rehydration salts (ORS) has long been a standard therapy for acute infantile diarrhea. Severe dehydration can be life threatening and requires treatment in a clinic or hospital where the child can receive intravenous (IV) fluids and appropriate case management.

The purpose of this observational study was to carry out a hospital-based surveillance of rotavirus gastroenteritis in children ≤59 months of age and develop estimates of disease burden in the population under surveillance.

2. Methods

2.1. Study centers and duration

A prospective hospital-based surveillance was conducted at 12 medical centers attached to Medical Schools across India. From North India subjects were enrolled from Dayanand Medical College & Hospital, Ludhiana; Chhatrapati Shahji Maharaj Medical University, Lucknow; Kalawati Saran Children Hospital, New Delhi; Post Graduate Institute of Medical Education and Research, Chandigarh and Sawai Man Singh Medical College, Jaipur. From South India, Gandhi Medical College, Hyderabad; JSS Medical College, Mysore; Kempegowda Institute of Medical Sciences, Bangalore and Kasturba Medical College & Hospital, Manipal were involved. From Western India, Goa Medical College, Goa recruited subjects. From Eastern part of India subjects were enrolled from Institute of Child Health, Kolkata and Kalinga Institute of Medical Sciences, Bhubaneswar (Fig. 1).

The 16 months surveillance study was conducted from April 2011 through July 2012.

2.2. Inclusion criteria

Children ≤59 months of age presenting with severe acute gastroenteritis (defined by the passage of ≥3 loose stools with or without vomiting during the preceding 24 h period) and requiring hospitalization for at least 6 h were eligible for this study. An approved informed consent statement for obtaining stool samples was then read and signed by the parents/legally acceptable representatives of the subject, investigator and, when required, a witness. Upon obtaining consent, subjects were included in the study and their stool sample was obtained. Children older than 60 months, and those younger than 60 months but not requiring hospitalization for at least 6 h or whose parents did not consent for stool sampling were not included in the study.

2.3. Clinical assessment

Various parameters considered for clinical assessment of diarrhoeal severity were: time of onset, duration and maximum number of episodes of diarrhea and vomiting, intensity of fever and dehydration. These parameters were recorded in a Case Report Form. Severity of diarrhea was assessed using the Vesikari scoring system. As per the Vesikari Score Grading, a grade of 0–5 was considered as mild, 6–10 as moderate, 11–15 as severe and more than and equal to 16 as very severe [3].

2.4. Stool specimen collection

Approximately 5 ml of stool sample was collected in stool containers from the consenting subjects either on the day of presentation to hospital or within 48 h of hospital admission so as to avoid observing hospital-acquired infections. All the stool specimens were stored in a freezer at −20°C until testing and sufficient care was taken to avoid freeze–thaw cycles.

2.5. Detection of rotavirus

All the collected stools samples were tested for rotavirus VP6 antigen using a commercial enzyme immunoassay kit (Premier Rota clone Qualitative ELA, Meridian Bioscience Inc., Cincinnati, USA) at the respective study centers, in duplicates and with appropriate controls. All the rotavirus VP6 antigen positive stool samples were sent for genotyping from the study centers to the Central Laboratory at Department of Gastrointestinal Sciences, Christian Medical College, Vellore under required controlled conditions.

2.6. Strain surveillance and characterization

Genotyping of all rotavirus positive stool samples was conducted at the Central Laboratory in Vellore. Genotyping was performed by using Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Rotaviruses were classified into G- and P-types based on the variability in the genes encoding the two outer capsid proteins, VP7 and VP4, respectively. Viral RNA was extracted from stool specimens and reverse transcribed using random primers to
generate complementary DNA (cDNA). The cDNA was used as template for genotyping in hemi-nested multiplex PCRs for VP7 and VP4 genes using published oligonucleotide primers and protocols. The primers were designed to amplify common rotavirus G- and P-types as well as genotypes that are more common in India.

RNA extraction and reverse transcription RNA extraction was carried out using the instruction in the Qiagen stool minikit. With eluted RNA, cDNA is generated by reverse transcription using 400 U of Moloney murine leukemia virus reverse transcriptase (M-MLV) reverse transcriptase in the presence of random primers (hexamers; Pd(N6)) at 37 °C for 1 h. In each extraction, a rotavirus positive stool sample as positive control and DEPC treated water as negative control were included. The cDNA was used as a template for G- and P-typing PCRs. Five microlitres of cDNA was used in amplification reactions for the first round VP7 and VP4 gene products in 50 μl reactions and 1 μl of this amplified product serves as template for the 2nd round multiplex PCR.

For VP7 genotyping, the first round PCR primers VP7-F and VP7-R amplified an 881 bp region of the VP7 gene. The nested multiplex PCR incorporated the reverse primer (VP7-R) and the primers specific for amplification of genotypes G1, G2, G3, G4, G8, G9, G10 and G12. Primers Con2 and Con3 were used in the first round PCR to amplify an 876 bp fragment of the VP4 gene. The second round PCR included the consensus primer Con3 and primers specific for genotypes P[4], P[6], P[8], P[9], P[10] and P[11].

The genotypes were identified based on the PCR amplicon size on gel electrophoresis. PCR amplicons were resolved in 2% agarose gels stained with ethidium bromide (0.5 mg/ml) in Tris–Boric acid–EDTA (TBE) buffer at constant voltage. Images were photographed under UV light using a gel documentation system.

2.7. Statistical analysis

Diarrheal hospital log book, case report forms and genotype result reports were used to generate data for statistical analysis. All logs and forms were scrutinized for completeness, the data entered into Excel 2012 (Microsoft, Redmond, WA, USA) and cleaned. Analysis was performed using QuickCalcs, version 5 (GraphPad Software Inc., La Jolla, CA, USA). Tests of proportion, Chi-squared tests were applied and a P value <0.05 was considered to be statistically significant.

2.8. Ethics

The study was conducted according to The Code of Ethics of the World Medical Association (Declaration of Helsinki), GCP guidelines issued by the Central Drug Standards and Control Organisation, India and the ethical guidelines by Indian council of Medical Research. Independent Ethics Committee/Institutional Review Board clearance was obtained before initiation of the study at each study center. The study was formally registered under the Clinical Trials Registry – India with a registration number of CTRI/2012/03/002475.

3. Results

3.1. Gender and age

During the 16-month surveillance period from April 2011 through July 2012, a total of 4711 children aged <5 years were admitted to the various medical centers with diarrhea and a diagnosis of acute gastro-enteritis as per the case definition. Out of the 4711 cases, 702 (14.90%) were in the age group 0–5 months, 1319 (27.99%) in the age group 6–11 months, 1559 (33.09%) in the age group 12–23 months and 1131 (24%) in the age group 24–59 months.

Of the 4711 admissions, stool samples were collected from 2051 consenting (43.5%) subjects and analyzed for VP6 rotavirus antigen in stool using the commercial enzyme immunoassay kit (Premier Rota clone Qualitative EIA) at respective study sites. Out of the 2051 stool samples, overall 541 samples were positive for rotavirus VP6 antigen, representing 26.4% of subjects hospitalized due to acute gastroenteritis. The rate of rotavirus positive stool samples ranged from as high as 52.5% recorded in December 2011 to as low as 10.3% recorded in May 2011. The highest percentages of cases positive for rotavirus occurred in the age groups 12–23 months and 6–11 months at all sites (32.75% and 27.9%, respectively). Of all children with rotavirus positive diarrhea, 18.84% were aged less than 6 months. Children less than 2 years of age represented 82% of the total disease burden. The mean age in months (± standard deviation) of rotavirus infected hospitalized children (15.19 ± 4.08) was lower when compared to the mean age of rotavirus uninfected hospitalized children (17.00 ± 4.26) which is a statistical significant difference (P value <0.01).

In addition to the reported 16 months data, data were analyzed separately for 12 months from August 2011 to July 2012 for overall rotavirus positive diarrhea during one complete calendar year. During this calendar year, out of 3917 severe diarrheal admission, stool samples were collected from 1868 consenting (47.7%) subjects and analyzed for VP6 rotavirus antigen in stool using the commercial enzyme immunoassay kit (Premier Rota clone Qualitative EIA) at respective study sites. Out of the 1868 stool samples, overall 516 samples were positive for rotavirus VP6 antigen, representing 27.62% of subjects hospitalized due to acute gastroenteritis.

Out of the 2051 cases who provided stool samples for the study, 63.18% subjects were males. However rotavirus positivity showed no significant difference between male and female subjects (26.5% among males and 26.1% among females) (Table 1).

Table 1: Distribution of rotavirus positive diarrhea by age, gender and season.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Stool samples tested</th>
<th>Rotavirus positive n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>345</td>
<td>65 (18.8)</td>
</tr>
<tr>
<td>6–11</td>
<td>620</td>
<td>173 (27.9)</td>
</tr>
<tr>
<td>12–23</td>
<td>629</td>
<td>206 (32.8)</td>
</tr>
<tr>
<td>24–59</td>
<td>457</td>
<td>97 (21.2)</td>
</tr>
<tr>
<td>Total</td>
<td>2051</td>
<td>541 (26.4)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1296</td>
<td>344 (26.5)</td>
</tr>
<tr>
<td>Female</td>
<td>755</td>
<td>197 (26.1)</td>
</tr>
<tr>
<td>Total</td>
<td>2051</td>
<td>541 (26.4)</td>
</tr>
</tbody>
</table>

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Out of the 2051 cases who provided stool samples for the study, 63.18% subjects were males. However rotavirus positivity showed no significant difference between male and female subjects (26.5% among males and 26.1% among females) (Table 1).

The severity of disease was higher in rotavirus infected children than the rotavirus uninfected children (Table 2). In spite of the duration of the hospital stay being similar for both rotavirus infected and rotavirus uninfected children, the infected children presented slightly more vomiting episodes.

3.2. Geographical distribution

Rotavirus antigen positivity in stools varied from region to region across India. The average rotavirus positivity reported from various regions was as follows: North India 20.9% (range across study period 0.0–53.3%), Eastern India 24.6% (range across study period 0.0–58.6%), South India 33.9% (range across study period 0.0–66.7%) and 29.9% (range across study period 0.0–53.1%) from Western part of India. The difference reported from the four regions is statistically significant having two-tailed P value of 0.0342 using the Chi-square test. No statistically significant differences were observed between regions by gender.
Table 2
Characteristics of rotavirus-infected and uninfected children hospitalized with acute gastroenteritis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rotavirus infected children (n = 541)</th>
<th>Rotavirus uninfected children (n = 1510)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male 344 (63.58%)</td>
<td>948 (62.78%)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Female 197 (36.42%)</td>
<td>562 (37.22%)</td>
<td>–</td>
</tr>
<tr>
<td>Age, mean months ± SD</td>
<td>15.19 ± 4.08</td>
<td>17.00 ± 4.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hospital stay, mean days ± SD</td>
<td>3.14 ± 1.58</td>
<td>2.97 ± 1.44</td>
<td>0.02</td>
</tr>
<tr>
<td>Vomiting (for &gt;3 days)</td>
<td>182 (33.64%)</td>
<td>492 (32.58%)</td>
<td>–</td>
</tr>
<tr>
<td>Episodes (for &gt;5 days)</td>
<td>161 (29.75%)</td>
<td>557 (36.89%)</td>
<td>–</td>
</tr>
<tr>
<td>Vesikari score, mean ± SD</td>
<td>14.42 ± 1.90</td>
<td>13.64 ± 2.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Disease severity by Vesikari score</td>
<td>Mild 6 (1.1%)</td>
<td>9 (0.59%)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Moderate 49 (9.06%)</td>
<td>183 (12.12%)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Severe 295 (54.53%)</td>
<td>748 (49.54%)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Very severe 191 (35.30%)</td>
<td>570 (37.75%)</td>
<td>–</td>
</tr>
</tbody>
</table>

3.3. Seasonality

Of the 4711 cases of acute severe gastroenteritis recorded, all study sites combined reported the highest number of cases in the month of May 2012 and the lowest number of cases in the month of April 2011 (Fig. 3). Northern, southern, eastern and western parts of India reported highest numbers of cases in the months of June 2012, July 2012, May 2012 and June 2012 respectively while they reported lowest number of cases in the months of March 2012, August 2011, April 2011 and November 2011, respectively.

A distinct seasonality of rotavirus positivity was observed in different parts of India with peak months of rotavirus hospitalization from December through February in north, east and western parts of India. In south India, rotavirus hospitalizations were observed throughout the year without any distinct seasonal peak (Fig. 2).

Rotavirus related hospitalizations were highest from October through March for all the regions (Table 3).
Table 3
Regional distribution of rotavirus positive diarrhea by age, gender and season.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>North</th>
<th></th>
<th></th>
<th>East</th>
<th></th>
<th></th>
<th>South</th>
<th></th>
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<th>West</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>164</td>
<td>27 (16.4)</td>
<td>22</td>
<td>59</td>
<td>9 (15.2)</td>
<td>22</td>
<td>9 (40.9)</td>
<td>100</td>
<td>20 (20.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6–11</td>
<td>225</td>
<td>52 (23.1)</td>
<td>163</td>
<td>39 (23.9)</td>
<td>57</td>
<td>18 (31.6)</td>
<td>175</td>
<td>64 (36.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12–23</td>
<td>175</td>
<td>48 (27.4)</td>
<td>184</td>
<td>57 (30.9)</td>
<td>127</td>
<td>50 (39.4)</td>
<td>143</td>
<td>51 (35.6)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>24–59</td>
<td>124</td>
<td>17 (13.7)</td>
<td>72</td>
<td>13 (18.0)</td>
<td>147</td>
<td>43 (29.2)</td>
<td>114</td>
<td>24 (21.0)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>688</td>
<td>144 (20.9)</td>
<td>478</td>
<td>118 (24.6)</td>
<td>353</td>
<td>120 (33.9)</td>
<td>532</td>
<td>159 (29.9)</td>
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Gender

<table>
<thead>
<tr>
<th>North</th>
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<th>East</th>
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<th>South</th>
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<th>West</th>
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<tbody>
<tr>
<td>Male</td>
<td>440</td>
<td>96 (21.8)</td>
<td>292</td>
<td>70 (23.9)</td>
<td>211</td>
<td>70 (33.2)</td>
<td>353</td>
<td>108 (30.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>248</td>
<td>48 (19.3)</td>
<td>186</td>
<td>48 (25.8)</td>
<td>142</td>
<td>50 (35.5)</td>
<td>179</td>
<td>51 (28.5)</td>
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<tr>
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<td>532</td>
<td>159 (29.9)</td>
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Admission month

<table>
<thead>
<tr>
<th>North</th>
<th></th>
<th></th>
<th></th>
<th>East</th>
<th></th>
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<th>South</th>
<th></th>
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<th>West</th>
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<tbody>
<tr>
<td>January–March</td>
<td>79</td>
<td>30 (37.9)</td>
<td>79</td>
<td>29 (36.7)</td>
<td>64</td>
<td>29 (45.3)</td>
<td>220</td>
<td>97 (44.1)</td>
<td></td>
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</tr>
<tr>
<td>April–June</td>
<td>350</td>
<td>64 (18.3)</td>
<td>160</td>
<td>19 (11.9)</td>
<td>162</td>
<td>44 (27.2)</td>
<td>175</td>
<td>22 (12.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July–September</td>
<td>171</td>
<td>22 (12.9)</td>
<td>162</td>
<td>34 (20.9)</td>
<td>89</td>
<td>28 (31.5)</td>
<td>85</td>
<td>14 (16.5)</td>
<td></td>
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</tr>
<tr>
<td>October–December</td>
<td>88</td>
<td>28 (31.8)</td>
<td>77</td>
<td>36 (46.8)</td>
<td>38</td>
<td>19 (50.0)</td>
<td>52</td>
<td>26 (50.0)</td>
<td></td>
<td></td>
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<td>532</td>
<td>159 (29.9)</td>
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</tbody>
</table>

3.4. Genotyping

Strain characterization by ELISA for all stool samples that tested positive for rotavirus VP6 antigen was carried out. Genotyping was performed at the Central Laboratory using reverse-transcription polymerase chain reaction (RT-PCR). The most dominant genotype was G1P8 (23.84%) followed by G2P4 (12.93%) and G9P4 (8.13%) (Fig. 4 and Table 4).

The age-specific analysis of genotyping revealed differences with increasing age: rotavirus infections due to G12P6, which were responsible for 7% of cases across all age groups, contributed toward 21% of burden in children less than 6 months. This decreased to 8% in the age group 6–11 months and around 2–3% in children older than 12 months of age. Across all age groups, mixed infections were responsible for nearly 25% of the positive cases (Fig. 5).

4. Discussion

This study used a standardized approach based on the generic protocol for hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children [4]. On an average rotavirus antigen was detected in 26.4% (ranging from as high as 52.5% to as low as 10.3%) of all diarrhea-related hospital admissions among children aged less than 5 years during 16 months study period. Overall 80% of rotavirus positive cases occurred among children less than 2 years old.

Taking into account one complete calendar year from August 2011 to July 2012, rotavirus antigen was detected in 27.6% (ranging...
from as high as 52.5% to as low as 15.3%) of all diarrhea related hospital admission among children aged less than 5 years.

A review of studies performed in India during 1990–2005 estimated that rotavirus disease accounted for 20.8% of all diarrhea related hospital admissions [5] whereas Kang et al. reported rotavirus positivity in India at 39% [6] which is higher than the average positivity reported in our study.

This study also documents the early incidence of rotavirus disease in India. The percentage of children with dehydrating gastroenteritis who were less than six months of age was as high as 12%. The youngest case recorded was one month old at the time of hospitalization. An earlier study from central India showed that rotavirus disease was more common during cooler months, with seasonal peaks matching the lowest temperatures [7]. In this study, a distinct winter peak was seen in the months of December to February during the total 16 months of surveillance across 12 sites in India, especially in northern India which has a distinct winter season from November to February. Interestingly, the sites in southern India did not demonstrate this trend as the area experiences the least annual variation in temperature of the four regions.

The worldwide emergence of the G12 strain in 2005 and its increasing incidence during the past two years parallels the emergence and subsequent spread of G9 strains that occurred approximately a decade ago. In the mid-1990s, G9P[6] and G9P[8] strains were reported in India, Japan, the United Kingdom, and the United States. Subsequently, G9P[8] spread globally, and it currently accounts for 4.1% of all rotavirus infections [8]. In our study, a higher percentage of G12 (17.74%) was observed especially in the Eastern part of India as compared to the rest of India. Various studies have found G12 strains in association with multiple VP4 types, namely P[4], P[6], P[8], and P[9], suggesting re-assortment among commonly circulating strains [9,10].

The increased reporting of infection with G12 strains may be associated with re-assortment, resulting in generation of a strain that is better adapted to replication in humans, similar to the events that preceded the spread of G9 strains in the past decade. The emergence of G12 strains highlights the need for a surveillance system to respond rapidly to changes in circulating virus and to ensure that vaccines remain effective against emerging strains.

Reported G12 cases from our study provided further evidence of the notion that G12 strains should no longer be considered as unusual or rare strains but that they exhibit a capacity to spread among children just like human rotavirus strains of other commonly seen G types.

In addition to the challenges posed by the emergence of new strains in the population under surveillance, we found high levels of circulation of unusual recombinant strains, such as G1P[4], G1P[6], G2P[6], G2P[8], G9P[4], and G9P[6] in different parts of the country. This indicates that there may be both regional and temporal variations in rotavirus strain predominance, which will be important to consider when assessing the impact of vaccination on rotavirus strains. Continued rotavirus strain surveillance after vaccine introduction will allow for epidemiologic assessment of vaccine effectiveness against the range of currently circulating strains and other potential strains that may be identified in the future. Both the number of re-assortant strains and the high proportion of mixed infections are indications of the variety of sources from which children are likely to acquire infections. Of rotavirus-positive specimens, some remained untypeable for both G type and P types. Possible explanations include too few virus particles with...
intact RNA in the stool specimens, the viruses not being recognized by the primer sets, and the viruses not belonging to genotypes included in the primer set. Since the study protocol was set up to capture acute gastroenteritis cases reporting to only one clinic in each of the study sites and there was no active effort to look for and log every case of diarrhea reporting to the hospital and attached health centers, there is a possibility that the estimation of the number of acute diarrhea cases in the study age group is lower than the actual number of cases. Additionally, this manuscript may have possibility of potential bias due to under reporting of severe rotavirus-positive diarrhea due to inclusion of two low rotavirus-positive seasons (April 2011–July 2011 and April 2012–July 2012) and only one high rotavirus positive season (August 2011–March 2012).

In summary, this study highlights the high prevalence of rotavirus gastroenteritis in India, the higher severity of rotavirus disease than that of other diarrheal diseases, and the circulation of a diverse range of rotavirus strains, including several uncommon and emerging strains like G9 & G12. This study report has generated geographically representative data to inform public health policy in India. With the prospect of rotavirus vaccine introduction in the Indian EPI Schedule in the near future, the importance of rigorous surveillance to monitor disease and strains before and after vaccine introduction cannot be overemphasized.

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