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Background: Rotavirus as a causative agent of childhood diarrhea is known to cause serious illness among children less than 5 years of age. This study examined the epidemiology of rotavirus disease burden and diversity of G and P genotypes of rotavirus in Nepal.

Methods: Stool samples were tested for rotavirus by Enzyme Immuno Assay and Group A rotaviruses were detected by using both ELISA and RT-PCR in 2718 samples between 2009 and 2011.

Results: Rotavirus was more frequently detected among inpatients (28.5%) than outpatients (15.2%). Over the three-year study period, 653 (24.4%) cases were positive for rotavirus by ELISA. Genotyping by RT-PCR was done on 638 samples. The most prevalent genotype was G12P[6] (60.4%). Mixed infections were not uncommon (14% in 2009, 29% in 2010 an 7% in 2011). However, 41 were partially typed and 23 were completely untyped over the study period.

Conclusions: This study highlights the rotavirus disease burden and diversity of rotavirus strains circulating in Nepal. Continued sentinel surveillance will provide useful information to policy makers with regard to rotavirus vaccine introduction.

Keywords: Human rotavirus, prevalence, genotype, RT-PCR

Introduction

Worldwide, rotavirus is the most common cause of severe gastroenteritis among infants and young children.1 While diarrhea is the second most common cause of fatal childhood illness, about 1.34 million deaths occur worldwide among children aged less than 5 years due to rotavirus.3,4 Though the incidence of rotavirus infection among children in developed and developing countries is similar; outcomes vary widely with 82% of fatalities estimated to occur in developing countries.5 Each year, rotavirus causes approximately 111 million episodes of acute gastroenteritis requiring home care, 25 million clinic visits, and 2 million hospitalizations in the U.S. and Europe.3

Rotavirus belongs to the Reoviridae virus family and the virion comprises of three concentric protein layers. The outer capsid consists of two proteins, VP7 and VP4 that are used to classify rotavirus strains into G (glycoprotein) and P (protease sensitive)
genotypes, respectively.²⁻⁸ Of the 12 G and 15 P genotypes known to infect humans, genotypes G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] cause over 90% of rotavirus disease worldwide.⁹

Two effective rotavirus vaccines, a single-strain attenuated human rotavirus vaccine (Rotarix, GlaxoSmithKline Biological) and a multi-strain bovine-human reassortant vaccine (RotaTeq, Merck and Company) are now available. WHO recommends the inclusion of rotavirus vaccines in all national routine immunization schedules. In countries where diarrheal deaths account for ≥10% of mortality among children <5 years of age, the introduction of rotavirus vaccine is strongly recommended.¹⁰ Efficacy of these vaccines has ranged from 80% to 98% in industrialized countries, including Latin America, and 39% to 77% in developing countries, such as Africa and Asia.³,⁴

The present study summarizes the findings from three years of hospital-based rotavirus sentinel surveillance in Nepal and the epidemiology of rotavirus gastroenteritis and characterizes circulating rotavirus strains among children aged less than five years in Nepal.

Material and methods

Clinical methods

Children presenting to the pediatric emergency unit of Kanti Children’s hospital were screened for diarrhea by the treating medical officer or nurse. Children aged less than 60 months with acute diarrhea and from whom stool sample could be recovered within 24 hours of hospitalization were recruited. Ethical approval was obtained from the Institutional Review Board (IRB), Institute of Medicine, Tribhuvan University, Kathmandu, Nepal. The study was explained to the caregivers and written informed consent obtained.

Study setting, sample and data collection: This study was conducted between January 2009 to December 2011 in Tribhuvan University, Institute of Medicine, Public Health Research Laboratory, Maharajgunj, Kathmandu, Nepal.

Case enrollment was done on the basis of inclusion and exclusion criteria among children visiting Kanti Children’s hospital with symptoms of acute watery diarrhea, fever, vomiting, and abdominal pain. All children less than five years of age who were admitted for treatment of acute gastroenteritis diarrhoea and/or vomiting with or without intake of medications were included. Diarrhoea was defined by the occurrence of three or more liquid stools in a 24-hour period and presence of diarrhea at the time of clinical presentation with admission to the inpatient diarrheal treatment unit. The exclusion criteria were as follows: Hospital-acquired diarrhea, which was defined as onset of diarrhea more than 48 hours after hospitalization; bloody diarrhea; and chronic and/or persistent diarrhea, which was defined as diarrhea that lasted for more than two weeks.

Sampling: A total of 2718 stool samples were collected. Specimens were stored at −70°C until analysed using ELISA and molecular methods. All case-based data and clinical information was recorded on a standardized questionnaire and was analyzed using EPI-Info software.

Measurement variables: Data were collected using a pre-coded data collection tool. Variables included child’s age, sex, symptoms, and duration of symptoms, water source, and home hand washing practices, diarrhea contacts, housing facilities, education
and occupation of the caregiver, temperature of the child, hydration status, and rotavirus results.

**Rotavirus detection:** The specimens were tested by a solid-phase sandwich-type enzyme immunoassay method (Rotavirus Ag ELISA, Pro Spect, USA).

**Rotavirus Genotyping**
Detection of rotaviruses in clinical specimens and determination of the G-type and P-type was accomplished by extraction of the viral RNA from fecal specimens and analysis by reverse-transcription polymerase chain reaction (RT-PCR) with primers specific for the VP7 genes of G serotypes 1, 2, 3, 4, 8, 9, 10, and 12 and VP4 genes of P serotypes 4, 6, 8, 9, 10 and 11. Rotavirus genotyping was done in collaboration with Christian Medical College, Vellore – Rotavirus Regional Reference Laboratory and WHO Collaborating Centre, India. A total of 638 samples were rotavirus positive [2009 (n=221), 2010 (n=151), and 2011 (n= 266)] and were analyzed by RT-PCR method described by Gouvea et al\textsuperscript{12} and Gunasena et al.\textsuperscript{13}

**Results**

**Epidemiology of Rotavirus**
From January 2009 to December 2011, 2718 cases meeting the enrollment criteria were evaluated for rotavirus. Of these 653 (24.0%) cases were positive for rotavirus by EIA, among the positive cases, 638 samples were processed for genotyping by RT-PCR.

**Clinical Presentation among Hospitalized Children**
Abdominal pain was the most common symptom among the 2718 children with diarrhea. (See Table 1). Approximately 25% of cases with each of the evaluated symptoms were found to be positive for rotavirus. The degree of dehydration among rotavirus cases was more likely to be moderate to severe rather than mild.

**Distribution of rotavirus by EIA**
Rotavirus was detected by EIA among 25.4% (230/906) in 2009, among 23.9% (195/815) in 2010 and among 22.7% (228/997) in 2011. The predominant strain was G12P [6] during all three years of the study period, (45.7% in

<table>
<thead>
<tr>
<th>Table 1: Clinical presentation in hospitalized patients</th>
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<tbody>
<tr>
<td><strong>Symptoms</strong></td>
</tr>
<tr>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td><strong>Degree of dehydration</strong></td>
</tr>
<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Severe</td>
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</table>
2009; 28.5% in 2010, and 30.1% in 2011). Previously unidentified strains were also detected, such as G9P [6] was identified in 2010 (6%) and G12P [4] was first identified in 2011 (1.1%). There were a number of diarrheal cases with mixed infections (14.0% in 2009 and 29.8% in 2010 and 6.7% in 2011). Forty-one samples were partially typed and 23 were completely untyped over the three-year period.

**Month wise distribution of rotavirus positive in hospitalized children**

Rotavirus among hospitalized children showed a seasonal distribution with the highest percent of diarrhea cases caused by rotavirus during the winter months from December to March. The month with the highest percentage of rotavirus positive cases was January (38.5%) in 2009, February (53.4%) in 2010, and April (34.9%) in 2011.

**Age and year-wise distribution of rotavirus positive cases in hospitalized children**

Hospitalization and enrollment of children 0 to 11 months was more common than among older aged children. In 2009, rotavirus was most commonly identified (28.1%) among children 0 to 11 months, whereas the rates of rotavirus as the cause of diarrhea among hospitalized children was more common among children 12–23 months of age in 2009 (35.5%) and in 2011 (26.7%).

**Distribution of major combination of G and P types of rotavirus in hospitalized children**

The most common circulating genotype in the population under surveillance was G12P [6] (45.7% in 2009, 28.5% in 2010 and 30% in 2011) over the three-year investigation, though the mixed type was slightly higher in 2010 (29.8%).

**Figure 1: Month-wise distribution of rotavirus from 2009 to 2011**
Discussion

Diarrhea continues to be a major public health problem and leading cause of death in developing countries. The present study is a continuation of a prospective study among children less than five years of age with diarrhea evaluated at Kanti Children’s Hospital, which is the only government-supported children’s hospital in Nepal. The majority of the children who tested positive for rotavirus antigen had clinical features of diarrhea, vomiting, and moderate to severe dehydration. Overall, the prevalence of rotavirus during the three-year study period was 24.0% which is similar to previously published studies from the same institution, but comparatively lower than evaluations at other study sites. Fever and vomiting were more often associated with rotavirus infection compared to other causes of diarrhea. Rotavirus was a major cause of pediatric gastroenteritis and was the underlying etiology of diarrhea for one-fourth of children 6 to 24 months of age hospitalized with an acute diarrheal illness.

The present study found a clear seasonal pattern of acute rotavirus gastroenteritis that peaked in winter, i.e., December to February (31.3%, 35.4%, and 30.6% in year 2009, 2010, and 2011, respectively) followed by early spring i.e. March to May, (31.3%, 32.9%, and 26.1% in 2009, 2010, and 2011, respectively) with only 15.3% in both summer (June to August) and autumn (September to November), which is in contrast to similar study conducted in Nepal 2006.

Figure 2: Age and year-wise distribution of rotavirus
Global epidemiologic surveys have identified G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] as the most common G/P genotype combinations associated with diarrhea in humans. However, recent studies in developing countries have shown increased identification of a high proportion of rotavirus strains with unusual G/P combinations, which may have implications for vaccine efficacy.\textsuperscript{24,25} The genotypic distribution pattern from this study identified G12P[6] as the predominant strain (35.1%), with G2P[4] strains as the second most common genotype identified (17.7%) over the three years study. G1P[8], which is the single strain present in the licensed Rotrix oral vaccine\textsuperscript{9} was the third most common strain identified among 8.3% of all specimens. This strain was uncommon in 2009 (2.7%). G1P[6] was the fourth most common type identified but was uncommon in 2010 (2.0%), but relatively more common in 2011 (10.2%). Thus, variations were found to occur in genotype distribution from year to year over the three-year study period. Results from other countries differ from ours.\textsuperscript{2,3,6,8,26} In this study, the strain G9P6 that occurred only in year 2010 (6.6%) and was the predominant strain in a study from India in 1996.\textsuperscript{27} The incidence of G9 with either P[8] or P[6] was also reported by Jain et al in 2001 to be 17% in stool samples collected from hospitals in Bhopal, New Delhi, Davengere, Lucknow, Nagpur and Shimla from 1996 to 1998.\textsuperscript{28}

### Table 2: Distribution Rotavirus genotyping and combination of G and P types of rotavirus in hospitalized children (2009, 2010 and 2011)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1P[8]</td>
<td>6</td>
<td>13</td>
<td>34</td>
<td>53 (8.3%)</td>
</tr>
<tr>
<td>G12P[4]</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3 (0.5%)</td>
</tr>
<tr>
<td>G12P[6]</td>
<td>101</td>
<td>43</td>
<td>80</td>
<td>224 (35.1%)</td>
</tr>
<tr>
<td>G12P[8]</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>13 (2.0%)</td>
</tr>
<tr>
<td>G2P[4]</td>
<td>32</td>
<td>7</td>
<td>74</td>
<td>113 (17.7%)</td>
</tr>
<tr>
<td>G1P[4]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G1P[6]</td>
<td>9</td>
<td>3</td>
<td>27</td>
<td>39 (6.1%)</td>
</tr>
<tr>
<td>G2P[8]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G2P[6]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G3P[8]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G9P[8]</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>10 (1.6%)</td>
</tr>
<tr>
<td>G9P[4]</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>15 (2.4%)</td>
</tr>
<tr>
<td>G9P[6]</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10 (1.6%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>31</td>
<td>45</td>
<td>18</td>
<td>94 (14.7%)</td>
</tr>
<tr>
<td>Partially typed</td>
<td>16</td>
<td>19</td>
<td>6</td>
<td>41 (6.4%)</td>
</tr>
<tr>
<td>Untyped</td>
<td>14</td>
<td>1</td>
<td>8</td>
<td>23 (3.6%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>221</strong></td>
<td><strong>151</strong></td>
<td><strong>266</strong></td>
<td><strong>638</strong></td>
</tr>
</tbody>
</table>
G12 genotype was first detected in the Philippines,\(^6\) and appears to be more prevalent in recent years in developing countries.\(^{29}\) This study demonstrates that a variety of different rotavirus types circulate simultaneously in Nepal.

The observation of G and P strains identified in this study suggests the circulation of various rotavirus strains in Nepal and may help to characterize the antigenic variation of the rotavirus. In the present study, the third most frequent circulating genotypes in the population under surveillance were mixed genotypes. High mixed infection with different rotavirus strains may reflect frequent contamination of water sources with rotavirus strains that facilitate generation of novel rotavirus strains through a re-assortment process.\(^{29}\) Non-typeable rotavirus strains were present in 3.6% over the three-year study period. Partially typed virus strains were identified in 6.4% of children with acute diarrhea. The presence of mixed genotypes may indicate infection because of poor sanitation or transmission of recombinant strains transferred from animals to humans.\(^{30, 31}\)

Our study has some limitations. Firstly, in the laboratory, we did not examine for all pathogens but only for rotavirus and enteric adenoviruses in addition to pathogens for which routine screening is performed according to hospital protocols. Results of these investigations have provided evidence of the burden of disease associated with rotavirus, but did not determine an etiology for nearly half of all diarrheal cases. Additional examinations for noroviruses, sapoviruses, astroviruses, and a variety of enteric bacteria would be needed to complete this picture. Secondly, the sentinel-hospitals the only government children’s hospital in the country and may not be representative of admissions to smaller or private hospitals. It is possible that children with uncomplicated diarrhea are admitted less often to referral centers. Thirdly, this study was based on a single sentinel site representing a catchment population of about three million, which does not represent complete scenario of the nation to ward rotavirus disease burden in Nepal.

This year-round surveillance of rotavirus genotypes will contribute to identifying important and emerging strains of rotavirus among Nepalese children. Continuation and expansion of laboratory-based rotavirus sentinel surveillance in other areas of Nepal is likely to yield important information for improved public health control of rotavirus associated outbreaks, as well as aid in the tracking of changes in strains that cause serious diarrhea among infants and young children.

**Conclusions**

There is high disease burden of rotavirus gastroenteritis among children less than five years of age. Genotyping analysis highlights that the existence of significant diversity of rotavirus strains with unusual G and P combinations. Therefore, rotavirus sentinel surveillance and genotyping will provide important background information for decision-making about whether to introduce rotavirus vaccine into Nepal National Immunization Program.

**Recommendation**

In Nepal, we have no information about the medical and social costs either to parents, for treatment of their children when they are ill with rotavirus diarrhea, or to the health sector, for the direct medical care provided by the government. Such an analysis might help advance the case for a rotavirus vaccine and establish the economic value of routine immunization. In addition, we have no exact information about the cost of
vaccines currently licensing (Rota Teq; Merck and RotaRix; GlaxoSmithKline) and their effectiveness in developing Asian countries like Nepal. Hence, we recommend continuing the rotavirus surveillance in other sentinel site of Nepal to obtain more information and to represent national scenario of rotavirus disease burden estimation, which can strongly support-evidence based decision making on vaccine introduction, monitor serotype/genotype distribution, and allow evaluation of vaccine impact after vaccine introduction.

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Conflict of interest
None to declare

References


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