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Hospital-Based Study in Children with Rotavirus Gastroenteritis and Other Enteropathogens

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ABSTRACT

Background: Rotavirus is the most common cause of life threatening gastroenteritis in infants and young children in the world. The objective of the study is to find out current trends and incidents of rotavirus, including other enteropathogens related with children diarrhoea and lastly identify the most common rotavirus serotypes that circulate in Nepal.

Methods: A total of 1721 stool samples from less than 5 years of children were collected. Rotavirus in the stool samples were detected by Enzyme Immuno Assay (EIA) and strains were genotyped by Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Bacteria and parasites were detected by following standard microbiological procedures.

Results: In between 2009 to 2010, of the total 1721, the prevalence of rotavirus was 24.7%. Of them, 906 (52.6%) were collected in the year 2009 and 815(47.5%) in the year 2010. Rotavirus was frequently detected in inpatients (31.6%) than outpatient (16.8%). Rotavirus detection was higher in female (26.4%) than male (23.7%). The prevalence was seen higher in age group 0-23 months in both years. Among six different bacterial isolates, Escherichia coli was most frequently isolated (6.5%). Similarly, Giardia lamblia (1.3%) was most common among six different parasites detected. A total of rotavirus positive 425 stool samples were detected over 2 years (2009-230, and 2010-195), G12P6 was the predominant strain circulating in both (45% in 2009 and 28% in 2010) years. G9P6 emerged in 2010 (6%). There were significant numbers of mixed infections (14.0% in 2009 and 29.8% in 2010). Thirty five samples were partially typed and 15 were completely untyped over the two year period.

Conclusions: The study helps comprehend the prevalence of rotavirus along with other intestinal pathogens including bacteria and parasites. Major genotypes of rotavirus are also introduced in the study

Keywords: enteropathogens; gastroenteritis; genotyping; rotavirus.

INTRODUCTION

Diarrhoea, despite being a disease that is easy to cure, is still a global cause of death for a million children every year. Rotavirus is the single most important etiological agent causing severe diarrhoea in infants and young children in both developing and developed countries[3-6] under 5 years.[7] Acute diarrhoea is a common but potentially serious illness. A child on an average suffers, 10 to 15 episodes of diarrhoea in the first five years of their lives. Rotavirus infection is the most common cause of diarrhoea in infants and young children. The symptoms may range from non-severe illness, to hospitalization and death.[8] Each year rotavirus causes an estimated 111 million episodes of diarrhoea requiring only home care, 2 million hospitalizations and 400,000 deaths in children under 5 years; 82% of which occurs in children in the poorest countries.[9]

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Rotavirus has 2 outer capsid proteins, the glycoprotein VP7 (G) and the protease-susceptible hemagglutinin VP4 (P).[10] Both VP4 and VP7 are important targets for vaccine development, because these proteins independently elicit protective neutralizing antibodies. The 4G serotypes accountable for most cases of rotavirus gastroenteritis worldwide are G1, G2, G3, and G4. Studies indicate the serotype G9 becoming more prevalent during the past decade. The most common P serotype associated with these strains is P1 (genotype 8). Epidemiological studies of rotavirus infection have shown that there is great diversity of rotavirus strains circulating throughout the world.[11-13]

Though the study purpose is to find out the prevalence of rotavirus in children below 5 years of age,all the samples were processed to determine the prevalence of parasites and bacteria. Many species of protozoan parasites live in the gastrointestinal tract, infecting some 3.5 billion individual's worldwide.[14] Four species are of particular importance: *Entamoebahistolytica*, *Giardialamblia*, *Cyclosporacayetanensis* and *Cryptosporidiumparvum*.[9] Infections by most of these organisms can be asymptomatic, or can be treated with rehydration solutions, particularly in the case of viruses and some bacteria.[15] Diarrhoea can be caused by infections with bacteria, such as *Salmonella*, *Shigella*, or certain types of diarrhoeagenic *Escherichiacoli*. At least 25 different bacteria and protozoa can cause an identical clinical syndrome of gastroenteritis, over 75% of gastroenteritis cases are caused by viruses.[14]

METHODS

The study was carried out in Tribhuvan University Teaching Hospital (TUTH), Institute of Medicine, Public Health Research Laboratory, Maharajgunj, Kathmandu. Cases were identified every morning and the mother or care taker of the child gave their consent through questionnaires regarding their demographic, medical history, and socioeconomic data before a stool specimen was obtained (before two days of admission). The study received ethical approval letter from Institutional Review Board (IRB), TUTH, Institute of Medicine, Kathmandu, Nepal. 1,721 stool samples were collected in a clean and sterile screw capped container from January 2009 to December 2010 of children under five years age. The collected stool samples were then immediately brought to the laboratory by maintaining cold chain and were processed according to standard laboratory methods.

Rotavirus infection was determined by using EIA (ProSpecTM) for the detection of rotavirus antigen in human fecal samples (Oxoid Ltd. RG24 BPW, UK), according to the instructions of the manufacturer. All EIA positive samples were subsequently confirmed by RT-PCR and the samples were frozen at -70°C for genotyping. In the study, we have sent all frozen rotavirus positive stool samples to WHO collaborating Centre of Christian Medical College, Vellore, for genotyping.

For the bacteriological samples and its identification, the stool samples were immediately cultured on the surface of MacConkey agar (HiMedia Pvt. Ltd., India) for the selection of *Escherichia coli* isolates, Selenite F broth and Alkaline peptone water were used for enrichment of *Salmonella* and *Shigella* spp. and *Vibrio* spp. respectively. *Salmonella, Shigella* and *Vibrio* spp. were subcultured in Salmonella-Shigella agar and Thiosulfate citrate bile salt sucrose agar respectively. The bacterial colonies grown were identified based on colony morphology, gram's staining, biochemical properties, and agglutination with specific anti sera.

For detection of parasitic infection, all stool samples were processed by using formalin acetate concentration method and examined microscopically by observing oocyst, cyst and trophozoites, of protozoa and larva or eggs of helminthes. A modified ZiehlNeelson staining procedure was used for detecting *Cryptosporidium* species and other coccidian oocysts, including *Cyclosporacayetanensis*, from the stool samples.

Analysis: Differences in proportion were assessed by Chi-square test. The values of variables were counted and summarized in tables of frequency. The difference was considered significant when p value was less than 0.05.

RESULTS

Among 1721 children attending Kanti Children's Hospital, 906 (52.64%) were collected in 2009 and 815(47.36%) in 2010. Of total cases 916 (53.22%) and 805 (46.78%) were inpatients and outpatients respectively. Among different enteropathogens involved in diarrhoeal diseases, rotavirus is a major diarrhoea causing agent (24.69%), followed by bacteria (15.10%), parasites (3.66%), others (0.63%) and mixed pathogens (1.51%). Among parasites, protozoan (52) dominated over helminthes (11). Among the protozoa, *Giardia lamblia*(1.33%) was the major cause of diarrhoea, followed by *Entamoebahistolytica* (0.75%), *Cyclosporacayetanensis* (0.63%), *Cryptosporidium parvum* (0.17%) and *Blastocystishominis* (0.11%). Similarly, among bacteria, *E. coli* (6.5%), was highest of the total cases followed by *Shigella*spp. (3.25%),*Campylobacter* spp. (1.8%), *Salmonella* spp. (2.03%), *Staphylococcus aureus* (0.75%) others (0.63%) and *Vibrio cholerae* (0.11%).

Table 1. Distribution of enteropathogens (N= 1721)

Pathogenic organisms	No of cases (%)
Rotavirus	425 (24.7%)
Bacteria	260 (15.1%)
*Escherichia coli**	112 (6.5%)
*Shigella*spp.	56 (3.3%)
Campylobacter spp.	31 (1.8%)
Vibrio cholerae	2 (0.11%)
Salmonella typhi	27 (1.6%)
*Salmonella*paratyphi A	8 (0.5%)
Staphylococcus aureus	13(0.7%)
Others:	11 (0.6%)
*(Enterobacter, Klebsiella, Aeromonas*and *Pseudomonas)*	
Parasites	**63 (3.6%)**
Giardia lamblia	23 (1.3%)
Entamoebahistolytica	13 (0.7%)
Cyclosporacyantensis	11 (0.6%)
Cryptosporidium parvum	3 (0.1%)
Balstocystishominis	2 (0.1%)
Helminthes	11 (0.6%)
Mixed infection	26 (1.5%)

***Escherichia coli*:** (EPEC=84; EAEC=17; ETEC= 11)

Clinical presentation in hospitalized patients

All children with diarrhoea share some important clinical symptoms, including abdominal pain, vomiting, dehydration, and watery stool. On the basis of clinical presentation, rotavirus positive cases were high in those who had nausea (27.95%) and vomiting (29.16%). Fever, vomiting, and dehydration were common symptoms in rotavirus infected children; severe dehydration occurred in 10.17% (40 out of 393) of the rotavirus-positive children.

Table 2. Clinical presentation in hospitalized patients

Symptoms	No. of cases	Rotavirus positive cases
Abdominal pain	1523	375 (24.6%)
Fever	864	221 (25.5%)
Nausea	887	248 (27.9%)
Vomiting	696	203 (29.2%)
Degree of dehydration		
Mild	234	91 (39.0%)
Moderate	470	123 (26.1%)
Severe	393	40 (10.1%)
Stool type		
Mucus	238	55 (23.1%)
Watery	911	350 (38.4%)
Watery + mucus	619	242 (39.1%)
Rehydration before consultation		
Intravenous	11	4 (36.4%)
Oral	891	345 (38.8%)
None	866	298 (34.4%)

Distribution of rotavirus

Rotavirus was identified in 290 (31.6%) of inpatients and 135 (16.8%) of outpatients. Number of males (1100) attending the hospital were higher than females (621). Of the total, 23.7% (261 out of 1100) were rotavirus positive for male and 26.4% (164 out of 621) were rotavirus positive among female.

Within the diarrhoea group, the highest prevalence was seen in children from 3 to 23 months of age, in both year and Hospital admitted and outpatient department. The distribution of rotavirus among various age groups indicated that the highest number was found in age group 3-23 months. Age wise distribution showed that the infection rate was found to be increasing, highest in age group 0-11 months holding 28.1%, followed by age 12-23 months 27.9% of the total cases. Whereas in 2009 it was highest in 0-11 months holding 28.3%. In 2010 it was found highest in age group 12-23 holding 35.5%. The prevalence rate was highest in age group 0-23 and decreasing after 23 months.

Table 3. Age distribution of rotavirus

Age (Months)	Total no. of cases	Total rotavirus (+)	Total cases HIP	Total RT (+)	Total cases HOP	Total (+) cases	Total cases 2009	rotavirus (+)	Total cases 2010	rotavirus (+)
0-11	956	269 (28.13%)	536	189 (35.26%)	420	80 (19.04%)	497	141 (28.37%)	459	128 (27.88%)
12-23	401	112 (27.9%)	225	77 (34.22%)	176	35 (19.88%)	224	58 (25.89%)	177	54 (30.50%)
24-59	364	44 (12.08%)	155	25 (16.12%)	209	19 (9.09%)	185	31 (16.75%)	179	13 (7.26%)
Total	1721	425 (24.69%)	916	291 (31.76%)	805	134 (16.64%)	906	230 (25.38%)	815	195(23.92%)

Note: HIP=Hospital inpatient; HOP=Hospital outpatient

Seasonal distribution of rotavirus

Though the infection occurred all year round, the trend of prevalence was higher during winters (Figure 1)The seasonal and monthly frequency of isolation of rotavirus in this study was found to be highest from December to March which did not differ substantially between two years of the study. A majority of positive cases occurred in January (38.5% in 2009) and February (53.4% in 2010) followed by March (38.3% in 2009 and 43.9% in 2010).

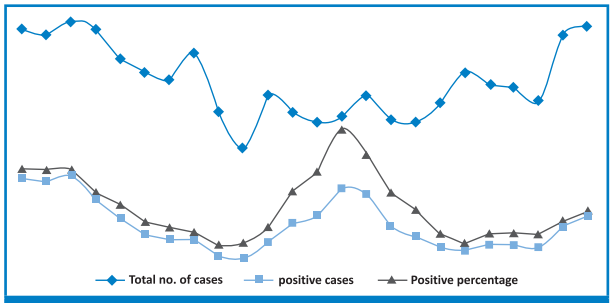

Figure 1. Month-wise distribution of rotavirus

Distribution of major combination of G and P types among rotavirus-positive specimens of hospitalized children

There are annual variations in the predominant G type. The data showed that G12 strains (both G12P6 and G12P8), were likely to have been introduced in or before 2008, but the G12P6 is the predominant strain (45.7% in 2009 and 28.5% in 2010) in both year though mixed type is slightly higher in year 2010 (29.8%).

Table 4. Distribution of major combination of G and P types among rotavirus-positive specimens of hospitalized children

NEPAL ROTAVIRUS GENOTYPING			
GENOTYPES	YEAR		
	2009	2010	Total
G1P8	6 (2.7%)	13 (8.6%)	19
G12P6	101(45.7%)	43 (28.5%)	144
G12P8	7 (3.2%)	4 (2.6%)	11
G2P4	32 (14.5%)	7 (4.6%)	39
G1P6	9 (4.1%)	3 (1.9%)	12
G9P8	3 (1.3%)	3 (1.9%)	6
G9P4	2 (0.9%)	3 (1.9%)	5
G9P6	0	10 (6.6%)	10
MIXED	31 (14.0%)	45 (29.8%)	76
PARTIALLY TYPED	16 (7.2%)	19 (12.6%)	35
UNTYPED	14 (6.3%)	1 (0.7%)	15
TOTAL	221	151	372

DISCUSSION

Diarrhoeal diseases can cause severe dehydration under 5 years, but can be prevented by oral rehydration therapy. However some regions of Nepal and other developing countries still can't offer the easily available and low cost oral rehydration. The disease is often mild and self-limiting but the symptoms may be very severe among the old and the young. Studies in developing countries have shown that children in the first 2 years of the life may have up to 10 episodes of diarrhoeal disease, often with significant mortality.[9]

Bacteria, parasites and viruses are the foremost cause of morbidity and mortality for children under 5 in developing countries like Nepal; sustained study regarding this etiological agent should be carried out. In the current study, enteropathogens were detected in 774/1721 (44.97%) and the result was similar with other studies.[14]

Of the total, the prevalence of rotavirus was found to be highest (24.69%).This was in accordance with the previous studies.[15-21] Investigations on diarrhoeal diseases in young children in the Gaza, Palestine and Saudi, demonstrated that *Salmonella* spp., *Cryptosporidium* spp., *Campylobacter* spp. and rotavirus were the major pathogens, and overcrowding was linked with an increased risk of diarrhoea.[22,27]

In this study, the frequency of distribution of rotavirus was found to be high in hospital admitted patients. The study showed that the highest rate of rotavirus infection was seen in hospitalized patients 31.65 % (290 out of 916) than the patients who visited OPD 16.77 % (135 out of 805).This was statistically significant ($p<0.05$) and in accordance with other studies.[17-21,23] Rotavirus was a major cause of pediatric gastroenteritis and responsible for acute diarrhoeal illness among hospitalized patients of 6-24 months of age.[13-15] Similarly, this study also showed that the infection rate was higher in age group 0-23 months.

G12P6 is the predominant strain (45.70% in 2009 and 28.47% in 2010) in both years though mixed type is slightly higher in year 2010 (29.80%). Similarly, Cameroon and Zimbabwe study, where G9P [8] and G12P [6], respectively, were the most frequently detected genotypes. Though this strain was not frequent in other countries, it is the major serogroup that circulates among children under 5 years in Nepal.[17-26]

Rotavirus infection occurred all year round but peaked during the fall and winter months.[17,19, 20] From December to March; a clear seasonal pattern was seen. A majority of positive cases occurred in January (38.46% in 2009) and February (53.44% in 2010) followed by March (38.29% in 2009 and 43.93% in 2010). The major symptoms of gastroenteritis are nausea, vomiting, diarrhoea, loss of appetite, fever, weakness, abdominal cramps, and

severe cases of the disease can lead to dehydration which in some cases is fatal.[14] In our study, vomiting, fever and dehydration were seen at prevalence of 29.16, 25.57 and 10.17%, respectively in the children infected with rotavirus whom were hospitalized. Oral rehydration and maintenance of proper fluid and electrolyte balance remain the primary treatment for children with rotavirus gastroenteritis.[10] In this study rotavirus was detected in 345 children treated with oral rehydration and 4 were treated with intravenous fluids whom were hospitalized. Of them 38.8% was rotavirus positive who were supposed to use oral rehydration therapy and 34.4% were rotavirus positive that had not used any rehydration therapy. This showed that oral rehydration does not significantly reduce the number of the organism but help in treatment by balancing electrolytes.

Bacteria that cause gastroenteritis include, but are not limited to Salmonella and Shigella (the two most common pathogens), *Campylobacter jejuni*, *Escherichiacoli*, *Clostridiumdifficile* and *Vibriocholerae*.[27] Bacillary dysentery or shigellosis is being a public health problem mainly in developing countries especially below 10 years. Studies have shown that one-third to one half of all diarrhoea-associated deaths among children occurred following episodes of persistent diarrhoea where dysentery accounts for 10% to 15% of diarrhoeal episodes in children under the age of 5, but up to 25% of diarrhoeal deaths.[28] In this study, diarrhoeagenic *Escherichia coli* (DEC) was the predominant enterpathogen. However, *Shigella*spp. and rotavirus were more predominant in the dry season than the rainy season and *Giardia lamblia* was more prevalent in the rainy season.[29-31] In this study, protozoan dominated over helminthes, among the protozoa, *Giardialamblia* (1.33%) was major causative agent of diarrhoea in Nepal followed by *Entamoebahistolytica* (0.75%) and *Cyclosporacyantensis* (0.63%), which was in consistent with other study.[31]

CONCLUSIONS

The study highlights the existence of significant diversity of rotavirus strains with unusual G and P combinations. High prevalence of group A rotavirus infection in children with diarrhoea and also determination of circulating rotavirus genotypes provides useful data for formulating new and more effective vaccines, especially for infants aged younger than five years of age. However, simultaneous infections of rotavirus, bacteria and parasites had no significant collaborative influence on clinical symptoms compared to the influences of rotavirus infection or bacterial infection. Such co-infections could cause difficulties for pediatricians and health care workers for diagnosis, treatment and prophylaxis of diarrhoea in children. Therefore, rotavirus surveillance study will help to inform decision as whether rotavirus vaccine should be considered for inclusion into Nepal National Immunization Program. Continue surveillance will remain important.

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CONFLICT OF INTEREST

We declare no conflict of interest for this article.

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