Enteropathogens Associated Diarrhea in Hospitalized Patients of Children's Hospital, Kathmandu

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Abstract

Introduction Diarrheal disease caused by bacteria, parasites or viruses continues to be an important cause of morbidity and mortality among young children in developing countries. Methods currently used in public health laboratories do not allow for the identification of Rotavirus, *Cyclospora* and pathogenic *E.coli* infection though they represent as an etiology in large proportion of patients with diarrhea, the possibility exists that a portion of the undiagnosed illness may be attributable to one or more of the above enteropathogens. In a view to determine the causative agents of diarrhoea, the current study described the various enteropathogens associated with diarrhoea in hospitalized children.

Objectives The purpose of this study is to reveal the bacterial, viral and parasitic agents associated with diarrhoea in hospitalized children in Kathmandu.

Methods Stool samples were collected from children under 11 years of age who developed diarrhoea and were admitted to Kanti Children's Hospital between May to October 2005 and investigated in Tribhuvan University, Institute of Medicine, Health Research Laboratory; by using both the combination of microbiological and immunological tools (EIA for Rotavirus detection, standard parasitological procedure for *Cyclospora* and other intestinal parasites, and selective culture method and serotyping were used to differentiate the species of bacteria).

Results A total of 440 diarrhoeal stool samples were collected and 285 (64.8%) enteropathogens were identified. The highest infection was due to intestinal parasites 104/285 (36.5%) followed by Rotavirus 92/285 (32.3%); pathogenic bacteria 57/285 (20%) and *Cyclospora* 32/285 (11.2%). Among the pathogenic bacteria (20%) isolated, the predominant bacteria were *Shigella species* (36.8%); *Vibrio species* (26.3%); *Escherichia coli* (22.8%) and *Salmonella species* (14.03%) respectively.

Conclusion Various enteropthogens responsible for diarrhoea especially Rotavirus, different pathogenic bacteria and *Cyclospora* infection, which are not examined routinely in public health laboratories, were found in significant proportion as a cause of diarrhoeal illness in children. The infection was highest in children under 2 years of age and occured in rainy season.

Key words Diarrhea, Rotavirus, Enteropathogens, Cyclospora

Introduction

Diarrheal disease caused by bacteria, parasites or viruses continues to be an important cause of morbidity and mortality among young children in developing countries[1]. Cumulative data from epidemiological studies show that approximately 20–50 percent of cases are attributable to known bacterial or parasitic pathogens, which suggests that viruses may be responsible for the remainder[2]. In fact, viral gastroenteritis has been found to be the second most common viral clinical entity in developed countries, following closely behind viral upper respiratory tract illness[3-4].

Immense micro floras are implicated in diarrhoeal illness[5-7]. Some of these, including *Salmonella, Shigella* and *Vibrio spp*. are well recognized enteric pathogens. Rotavirus, *Campylobacter spp*. and eterotoxigenic *E. coli* (ETEC) also have been

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identified as important causes. Recently, certain strains of *Aeromonas hydrophila* and *Plesiomonas shigelloides* have been associated causally by some investigators[8-10]. The coccidian parasite *Cyclospora cayetanensis* is a newly recognized enteric pathogen causing prolonged diarrhea in humans[11-12]. It has been implicated as an important cause of diarrhoeal illness in the context of Nepal[13-16]. Rotaviruses are reported to be the most common cause of severe childhood diarrhea worldwide[17]. Illness associated with Rotavirus has also been observed in older children and adults. Rotaviral gastroenteritis may result in mortality for populations at risk such as infants and the elderly[18]. Rotavirus was found in 25-40 percent of children with diarrhea in urban Kathmandu valleys of Nepal[19-20].

In Nepal however, the major causative agents; Rotavirus, *Cyclospora* and different species of pathogenic bacteria are not routinely examined though they are important causes of diarrhea in children. Therefore, this study is carried out to reveal the etiology of diarrhoeal illness in high risk population group especially young children less than 11 years of age using such unique tools.

Methods

The study was carried out between May to October 2005 and included 440 patients with acute diarrhea. From these hospitalized children, a total of 440 stool specimens collected and all stool specimens were cultured for bacterial investigation within 2 hours of collection on Salmonella-Shigella agar(HI- media, UK), Mac Conkey agar (Oxoid, England) and TCBS agar (HI-media.) plates and incubated overnight at 37°C. They were also cultured in Selenite F broth and peptone broth (HI-media) and incubated up to 4 hours. When there was growth in broth, an inoculum was sub cultured on SS agar and TCBS plates. After culture, the stool specimens were examined for parasites and Rotavirus antigen detection. Rota clone ELISA kits (Meridian Bioscience Inc.) were used to detect Rotavirus and sucrose floatation concentration methods were used for Cyclospora. They were then confirmed by modified Ziehl-Neelson's technique.

In addition, a structure questionnaire was filled out for each patient covering demographic information, area of residence, information about any symptoms associated with the disease (fever, vomiting, dehydration status), type and duration of diarrhea and type of treatment received—if any—in the hospital. Acuter diarrhea was defined as abnormal fecal discharge characterized by frequent—at least three times per day—liquid or semi-liquid loose stools, accompanied by symptoms such as nausea, vomiting, fever, dehydration and electrolyte loss.

Results

A total of 440 children with diarrhea were enrolled in which 248 (56.4%) were males and 192 (43.6%) were females. The clinical symptoms in diarrhoeal patients are illustrated in table 1.

Table 1: Clinical symptoms and other features of diarrhoel children

Clinical symptoms	No (%)
1. Diarrhoeal duration (N=440)	
3-7 days	201(45.7)
7-10 days	89 (20.5)
10—14 days	80 (18.2)
>15 days	70 (15.9)
2. Fever	197 (44.8)
3. Vomiting	410 (93.2)
4. Nausea	311 (70.7)
5. Abdominal pain	279 (63.4)
6. Dehydration (N=440)	
No	129 (29.3)
Mild to moderate	254 (57.7)
Severe	57 (13)

The distribution of enteropathogens in different age group is shown in table 2. The highest rate of *Cyclospora* (21%) and pathogenic bacterial infection; (19.4%), was found in age group 6-8 years while the lowest rate was (1.5 and 7.7%) found in 0-2 years age group. Rotavirus infection was predominant among 0-2 years of age and the lowest rate was between 6-8 years age group (Table 2).

Table 2: Age wise distribution of different enteropathogens

Age group	Total No	Cyclospora +ve(%)	Rotavirus+ve(%)	Bacteria +ve(%)	Other parasites(%)
0-2	195	3(1.5)	56(28.7)	15(7.7)	41(21.0)
3-5	103	9(8.7)	24(23.3)	19(18.5)	19(18.5)
6-8	62	13(21)	5(8.1)	12(19.4)	13(21)
9-11	80	7(8.8)	7(8.8)	11(13.8)	31(38.8)
Total	440	32(7.3)	92(20.9)	57(12.9)	104(23.6)

In the age group 0-2 years, 60.9 percent (56/92) cases were infected with Rotavirus, which is significantly different than other age group ($p<0.05$), Cyclospora was detected highest in age group 6-8 years; 13(40.6%) cases, pathogenic bacteria 33.3 percent (19/57) cases in 3-5 years age group. However, the overall distribution was predominant by Rotavirus, (table 2). In the study, a significant number of intestinal parasites other than *Cyclospora* were also detected as shown in table 3.

Table 3: Distribution of parasites other than *Cyclospora*

Parasites	No of Positive (%)
Ascaris lumbricoides	15(14.4)
Trichuris trichiura	11(10.6)
Hookworm	9(8.7)
Hymenolepis nana	11(10.6)
Entamoeba histolytica	28(26.9)
Entamoeba coli	1(1)
Giardia lamblia	21(20.2)
Balantidium coli	4(3.9)
Cryptosporidium parvum	4(3.9)
Total no. of parasites	104

Pathogenic bacteria were isolated in 57(12.9%) cases and their pattern of distribution is shown in figure 1 and table 4.

Figure 1: Distribution of bacterial isolates

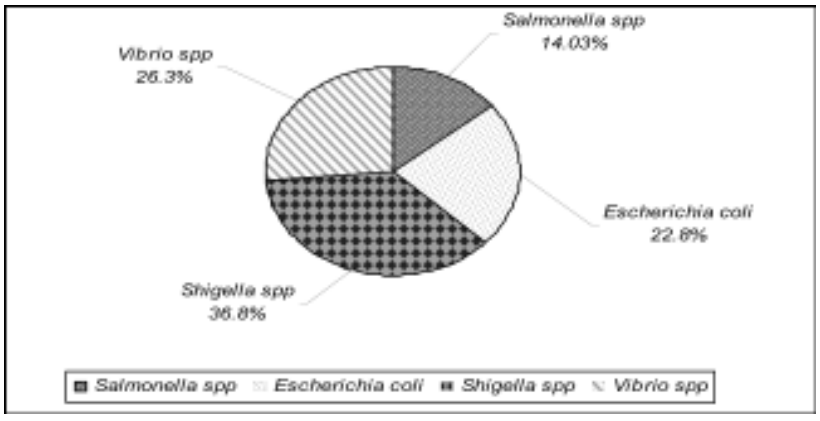


Table 4: Distribution of bacterial pathogens (N=57)

Type of organism	No of Organism
Vibrio Cholerae 01	15(26.3%)
Ogawa	12
Hikojima	2
Inaba	1
Escherichia coli	13(22.8%)
EPEC	8
ETEC	2
EHEC	2
EIEC	1
Salmonella spp	8(14.03%)
S. paratyphi A	3
S. paratyphi B	2
S. paratyphimurium	2
S. typhi	1
Shigella spp	21(36.8%)
S. dysenteriae	14
S. boydii	5
S. sonnei	2
S. flexneri	0

Of the total 440 sample examined, 92 (20.9%) cases were found Rotavirus positive (figure-2). The highest rate was aged 0-2 years found in the month of July (figure-3).

Figure 2: Age wise distribution of rotavirus infection

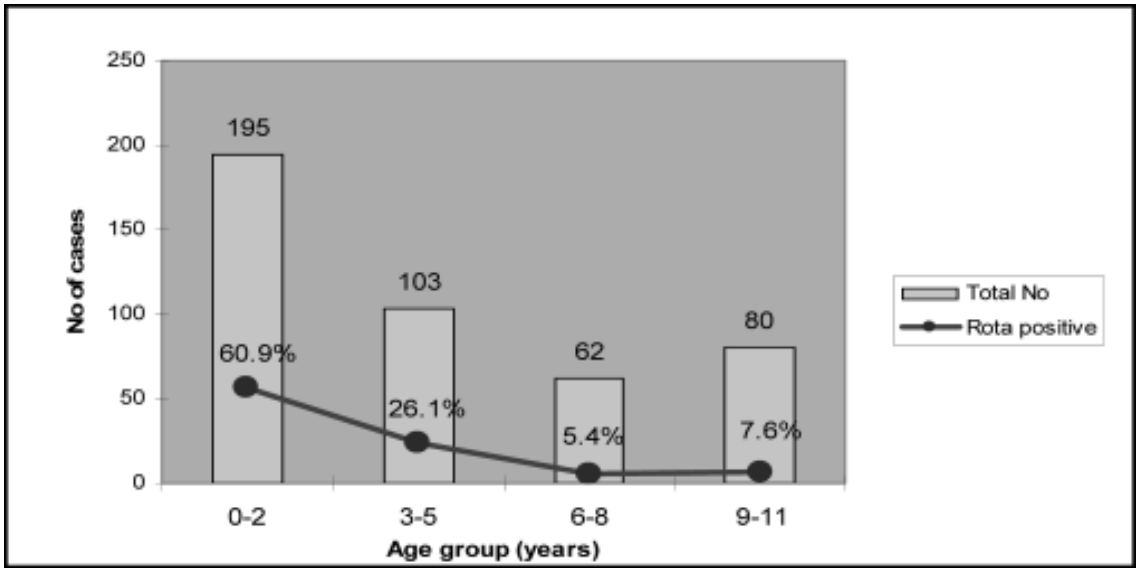
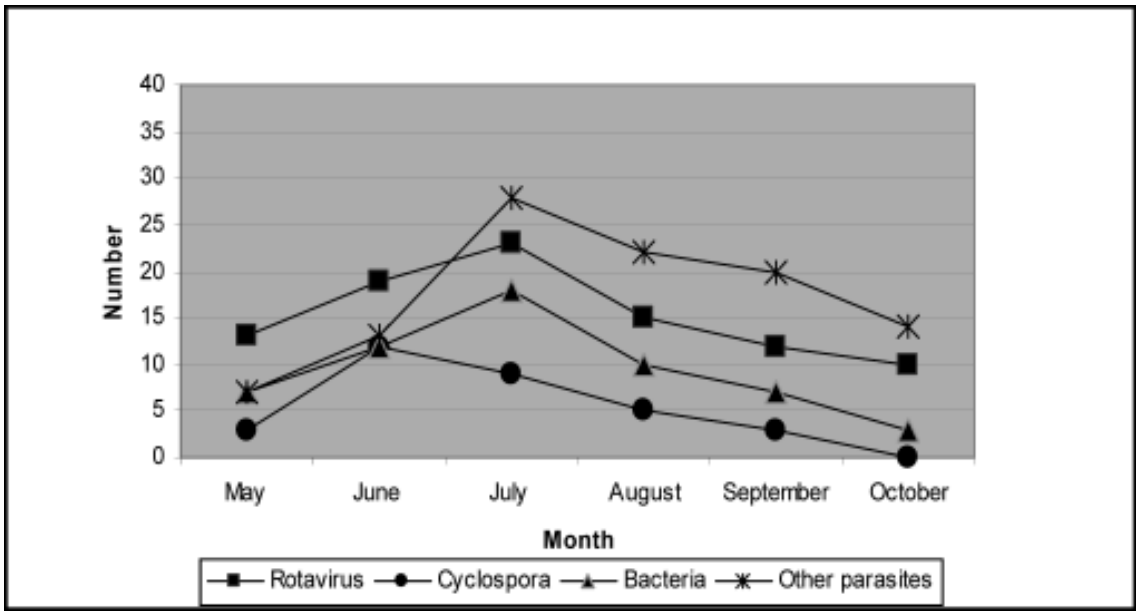


Figure 3: Month wise distributions of enteropathogens



Discussion

Diarrhoeal diseases are the main social problems in Nepal as in other developing countries in tropical zones[21-24]. The present study which covered the peak diarrhoeal seasons of six months (from May to October 2005), was conducted to determine the prevalence of viral, parasitic and bacterial enteropathogens associated with diarrhea in hospitalized children under 11 years of age. Using a combination of traditional and molecular diagnostic techniques, 285 (64.8%) enteropathogens were detected in total of 440 patients with diarrhea studied.

Among the parasitic agents, *C. cayetanensis*, an emerging pathogen was detected in 32(7.3%) of the total cases, with highest infection rate in the age group 6-8 years. Initial studies indicated that it causes diarrhea mainly in immunocompromised patients and prolonged diarrhea in expatriate populations in certain developing countries including Nepal[25]. However, a subsequent study in Nepal suggested that it also causes diarrhea among the indigenous children[13]. In previous studies in Nepal, it was detected in 24.6 percent of children with prolonged diarrhea[15]. The highest infection observed at this age group may be due to the children of these age groups are most active and enthusiastic and they go outdoors most often for playing where they may encounter with soil and other inanimate objects that might have been contaminated with the organism. Similarly, these children are not well aware of their health and feeding habits which make them more susceptible to infection. The consumption of raw vegetables and untreated water, malnutrition and immunocompromisation also add to the infection.

Intestinal parasites other than Cyclospora were found in 104 cases (23.6%). Among them protozoan dominated over helminthes. Among the helminthes, the highest prevalence was observed for *Ascaris lumbricoides* (14.4%) followed by *Trichuris trichiura* and *Hymenolepsis nana* (10.6%) and Hookworm (8.7%). Similarly, *Entamoeba histolytica* (26.9%) followed by *Giardia lamblia* (20.2%), *Balantidium coli* (3.9%) and *Entamoeba coli* (1%) were found respectively among the protozoan. Coccidian parasite, *Cryptosporidium parvum* was detected in 3.9 percent cases. Despite the government policy for antihelminthic program and health education program launched at community level, the intestinal parasite infestation still remains the major cause of diarrheal diseases in children. This can be taken as inadequate implementation of the program or can be said as the carelessness of people themselves as they take diarrheal disease as a minor disease, often being termed as social disease in many developing countries by different researchers. Coincidently, parasitic infestation peaks during the warmer and rainy season reflecting poor drainage and waste management and also as the favorable condition for the disease transmission.

Thirteen percent of bacterial pathogens were also found as significant cause of diarrhea in this study.

Among them, *Shigella spp* (21 cases, 36.8%) was most frequently detected followed by *Vibrio spp* (15 cases, 26.3%), *Escherichia coli* (13 cases, 22.8%) and *Salmonella spp* (8 cases, 14.03%). The age group 6-8 years was found highly infected (19.4%). The highest prevalence of bacterial infection found in this age group can be explained as the children at this age group are more actively involved in playing outdoors and careless about their feeding and drinking habits, lacking in proper knowledge of health education and proper personal hygiene, etc. and immunity to combat bacterial infection is also not well developed at this age. Moreover, the prevalence of bacterial pathogen reported herein could be affected by the fact that the reliable data on exclusion of self-treated individuals could not be made available.

Rotavirus was the most prevalent viral etiologic agent and was detected in 20.9 percent of the hospitalized children. Our findings were supported by Sherchand and Haruki[26], who reported that approximately 30.6 percent of infants and young children under 5 years of age with diarrhea from hospitals were infected with Rotavirus. The prevalence of Rotavirus in Nepal as estimated by present study is comparable to that reported for other developing and developed countries, which ranges from 30-50 percent[4]. The predominance of Rotavirus (60.9%) is clearly the reflection of age group 0-2 years. The finding is in accordance with similar results found by other researchers[19,21,27-31]. It appeared that infants below 4 months of age were initially protected to some extent by maternal antibodies against severe diarrhea due to Rotavirus, and they seem to have acquired adequate immunity between 12 and 16 months of age. The greater risks of infants and young children in the period between 6 to 12 months with declined levels of maternal antibodies to Rotavirus infection have been documented[32-33].

Seasonal variation of enteropathogen infection indicated that the infection rate was highest in peak rainy season (July and August). In particular, parasitic infection was found highest followed by Rotavirus, bacterial pathogen, and *C. cayetanensis*. This reveals that incidence of enteropathogen appears to be due to the heavy fecal contamination of drinking water, rapid and unplanned urbanization, improper disposal of wastes, poor sanitary facilities and health education, etc. In many parts of cities (Kathmandu, Lalitpur and Bhaktapur) in Kathmandu valley, drinking water supplies have been reported to be contaminated with sewage due to running of water pipe and sewage line closer together, water flowing intermittently causing negative pressure thus suckling fluids and air from leaky pipe lines[34]. In developing countries, contamination of water may occur at the source, but may also occur at the time of collection and storage at home[35].

Though Rotavirus infection peaks during winter seasons, this study showed the prevalence rate of Rotavirus infection as 20.9 percent during the warmer months like May to October which is in accordance with the study that identified Rotavirus more often in warmer months[36]. The seasonal nature of Rotavirus infection was not universal, and in countries within 10° of the equator, infection occurred year around[37]. Rotavirus is a disease of later warm and early cool season but seasonally may vary year to year[38].

Cyclospora infection was found highest in June which was in accordance with the findings of other researchers[16, 39]. The environmental conditions during this season are favorable for sporulation of cysts and thus help to maintain the chain of infection.

Often assignment of an etiologic agent role in specific cases of diarrhea was particularly difficult because more than one potential pathogens were isolated from the same stool specimen in 8.2 percent of cases studied. This could be the reflection of environmental contamination or it may also be that multiple pathogens act synergistically to produce diarrhea. This result suggests that the future research into the questions of quantitative association of microorganisms and diarrhoeal disease; synergism between pathogens and serological response to infections may be required to establish the etiology for some pathogens.

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