

Enteropathogenic microorganisms in children

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Introduction: Diarrhoeal disease is a leading cause of mortality and morbidity in the developing world. It is one of the most important causes of death in childhood and is still a considerable public health problem in developing countries especially among less than five years old.

Materials and Methods: Stool samples from children attending both ORT centre and OPD of Kanti Children's Hospital below 10 years of age were collected and simultaneously data on predisposing factors associated with gastroenteritis along with acute diarrhoea were collected according to the questionnaire designated. The collected stool samples were brought immediately to the laboratory. Upon arrival the stool samples were processed according to the standard laboratory methods.

Result: The distribution of enteropathogens showed that *rotavirus* was found highest constituting 27% of the total cases. Among parasites, protozoan (12.2%) dominated over helminthes (3.0%). Among the protozoa, *Entamoeba histolytica* (7.0%) was major causative agent of dysentery followed by *Giardia lamblia* (3.8%), *Cyclospora cayetanensis* (1.2%), and *Entamoeba coli* (0.3%). Among the helminthes, *Ascaris lumbricoices* was the major pathogen constituting 1.3% followed by *Trichuris trichiura* (0.8%), *Hookworm* (0.5%) and *Hymenolepis nana* (0.3%) of the total cases. Similarly, among bacteria *Shigella* spp. was highest of the total cases. *Shigella dysenteriae* in 2.0% (12 out of total 607); followed by *Shigella flexneri* in 1.0% and *Shigella boydii* in 0.3%. In case of *Vibrio cholera* it constitutes about 2.0% and *Salmonella* spp. in 1.3% of total cases. The study shows that the prevalence of *Salmonella Paratyphi A* in 0.3% (2 out of 607) followed by *Salmonella Paratyphi B* in 0.3% and *Salmonella Typhi* in 0.6%.

Conclusion: It can be concluded that lots of enteropathogens were involved in children diarrhoea. Among them rotavirus were predominant followed by parasites and bacteria. Children under 2 years were more affected age groups.

Key words: Diarrhoea, Enteropathogens, Rotavirus, Bacteria and Parasites

Introduction

Diarrhoea is one of the most important causes of death in childhood and is still a considerable public health problem in developing countries especially among less than five years old⁸. Diarrhoeal disease causes an estimated 1.7 million

death per year, 90% of these occurs in children, the vast majority in developing countries where many people lack access to safe drinking water³. The disease remains one of the largest health problems in many parts of the world. The disease is often mild and self-limiting but, particularly in the elderly and young children, the symptoms may be very

severe. 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 mortality².

Diarrhoea is an important cause of under nutrition. The disease also represents an economic burden for the developing countries. The infectious agents that cause diarrhoea usually spread by the fecal-oral route, which includes the ingestion of faecally contaminated water or food, person to person transmission and direct contact with infected faeces.

Distinct seasonal pattern occurs in many geographical areas. In temperate climates bacterial tends to occur more frequently during the warm season, whereas, viral diarrhoea, particularly disease caused by rotavirus, peak during the winter. In tropical areas, rotavirus diarrhoea tends to occurs throughout the year, increasing in frequency in drier, cooler month, during the warmer and rainy season. The incidence of persistent diarrhoea follows the same seasonal pattern as acute watery diarrhoea. Across the world, rotavirus is thought to be responsible for more than 125 million cases of diarrhoea each year in children and infants. Rotavirus is responsible for the deaths of as many as 600000 children each year⁴. The parasitic infestation is a common cause of morbidity and mortality in pediatric population in tropical countries. The prevalence of intestinal parasites in children varies in different regions of the world. It is particularly high in poor and developing countries due to use of contaminated drinking water, inadequate sanitary conditions and poor personal hygiene²⁴.

Many studies have focused on either bacterial or viral etiologies of diarrhea^{22,23,24}. The present study, comprising examinations for bacteria, virus and parasites therefore is aimed to reveal the present status of children that affected with diarrhoeal disease and also to isolate and identify the possible enteric organisms from their stool samples. This study also allows tracking of current levels and trends in diarrhoeal incidence and mortality and provides the basis for future projection and evaluations of different control strategies.

Materials and Methods

The study was carried out in Tribhuvan University, Institute of Medicine, Health Research Laboratory, Maharajgunj, Kathmandu, Nepal during October 2006 to September 2007. Stool samples from children attending both ORT centre and

OPD of Kanti Children's Hospital below 10 years of age were collected who passed watery or loose stools with or without mucus or blood, with or without vomiting; dehydration and abdominal pain were taken in this study.

Descriptive statistics was used to analyze the data to show association between enteropathogens infection and predisposing factors by using chi-square test and other relevant statistical tools. Clinical data from each patient were collected by using a questionnaire and statistical analysis was performed with MS Excel and SPSS 11.5.

Microscopic examination was the part of the study and carried out for the detection of oocyst, cyst, trophozoites, of protozoa and the detection of larva or eggs of helminthes. The detection was carried out at low power (10x) followed by high power (40x) of the microscopes, the suspected and possible parasite was observed under microscope by wet mount and iodine staining with special preference for *C. cayetanensis*. The *C. cayetanensis* thus observed was confirmed by modified Zeihl Neelson staining.

Salmonella-Shigella agar (Hi-media) and MacConkey agar (Oxoid, England) were used for isolation of *Salmonella* spp. and *Shigella* spp. and Thiosulphate Citrate Bile Sucrose Salt (Hi-media) agar was used for isolation of *Vibrio* spp. Alkaline peptone water was used for enrichment of *V. cholera* and Selenite-F broth was used for enrichment of *Salmonella* and *Shigella* spp.

Enzyme-linked immunosorbent assay was carried out for rotavirus specific antigens detection by using Rota Clone EIA kits (Meridian Bioscience Inc.) using a commercial kit according to the manufacturer's instructions.

Results

Out of 607 cases, the enteropathogens were observed 296 (48.8%) including bacteria, parasites and viruses. The prevalence of enteropathogens showed highest in males 61.5% i.e. 202 out of 296 than females 28.7% i.e. 94 out of 296, which was found statistically not significant ($P > 0.05$).

On the basis of patient's information, clinical symptoms and other characteristics are depicted in table 1. However majority of the patients had watery diarrhoea (60.9%) severe abdominal pain nausea and vomiting. Majority of mothers were illiterate (65.6%) and 67.9% patients had informed that they do not wash their hand before meal. The study showed most of the patients (54.5%) drank raw water and only 14.5 % had drank boil water.

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Table 1: Clinical symptoms and other characteristics

Clinical symptoms	Total no.
Nausea/vomiting	323 (53.2%)
Abdominal pain/vomiting	241 (39.7%)
Fever/abdominal pain/vomiting	43 (7.1%)
Watery	370 (60.9%)
Watery with mucus	213 (35.1%)
Mucus with blood	12 (1.9%)
Rice watery	12 (1.9%)
Severe	323 (53.2%)
Moderate	246 (40.5%)
Mild	38 (6.3%)
Mother's education	
Illiterate	398 (65.6%)
Literate	209 (34.4%)
Habit of washing hand before meal	
Yes	195 (32.1%)
No	412 (67.9%)
Water treatment	
Boil	88 (14.5%)
Filter	188 (31%)
Raw	331 (54.5%)

Distribution of enteropathogens

The distribution of enteropathogens (Figure 1) showed that rotavirus was found highest constituting 27% followed by parasites 15.2% and bacteria 6.6% of the total cases.

Age wise distribution of enteropathogens

The distribution of different enteropathogens among various age groups indicated that the highest number of enteropathogens was found in age group 0-2 yrs (55%) i.e.163 out of 296. Age wise distribution showed that the infection rate was found to be highest in age group 0-2 yrs holding 68.3% i.e.112 out of 164, 41.3% and 32.5% for rotavirus, parasites and bacteria respectively.

Table 2: Age wise distribution of enteropathogens

Age group	Rotavirus +ve (%)	Parasite +ve (%)	Bacteria +ve (%)	Total no.
0-2 yrs	112 (68.3%)	38 (41.3%)	13 (32.5%)	163 (55%)
2-4 yrs	26 (15.9%)	18 (19.6%)	8 (20.0%)	52 (17.6%)
4-6 yrs	15 (9.1%)	15 (16.3%)	5 (12.5%)	35 (11.8%)
6-8 yrs	7 (4.3 %)	12 (13.0%)	4 (10.0%)	23 (7.7%)
8-10 yrs	4 (2.4 %)	9 (9.8%)	10 (25.0%)	23 (7.7%)
Total	164 (61.4%)	92 (34.5%)	40 (15.0%)	296

Number of cases of enteropathogens according to hospital admission

The enteropathogens were found significantly higher in ORT centre constituting 186 (63%) than OPD 110 (36.0%) ($P<0.05$). The figure 2 showed Rotavirus and bacteria were found highest in hospital admitted patient where as parasites in hospital outpatients.

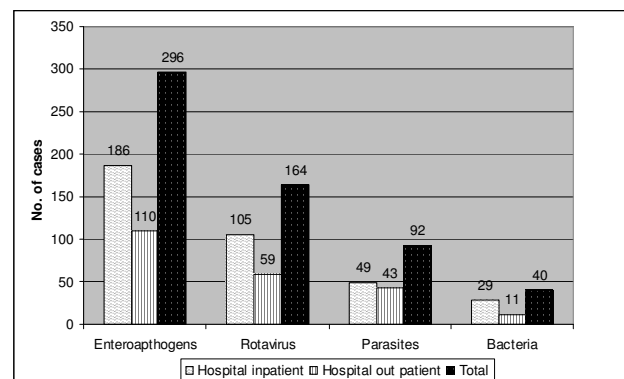


Fig. 2: Number of cases of enteropathogens according to hospital admission

Month and seasonal distribution of enteropathogens

Rotavirus was the most frequently isolated pathogen and was found highest in the season winter i.e. 45.1% (74/164 cases) of the month of January, December and February 52%, 41% and 40% as shown in figure 4. Similarly, parasites were found to be highest in summer season of the month June, July and August holding 43 cases out of 92. Bacterial

pathogens were also found to be highest in the month June, July and August constituting 24 case of the total 40 cases (Figure 3).

Fig. 3: Month and seasonal distribution of enteropathogens

Discussion and Conclusion

Diarrhoeal disease occupied the second place among the top ten diseases in Nepal⁶. This disease was directly related to the quality of water. However it varied according to seasons. The factors responsible for contaminating drinking water at source points in Nepal included the lack of protection and proper treatment of water, leakage in pipe distribution system, intermittent supply of water, poor drainage system and poor environment surroundings of water sources^{7,10,25}. Therefore, the identification of etiological agents is extremely important which helps in precise diagnosis and proper management of treatment procedures.

Diarrhoeal disease caused by bacteria, parasites and viruses continues to be an important cause of morbidity and mortality among young children in developing countries. Out of 607 cases, the prevalence of either of enteropathogens was observed 296 (48.8%) including bacteria, parasites and viruses and the finding was in accordance with **MC Georges**²⁰. The enteropathogens was found to be highest in males 68.2% i.e. 202 out of 296 than females 31.8% i.e. 94 out of 296, which was found statistically not significant ($P>0.05$).

In this study, the frequency of distribution of enteropathogens was found to be highest in hospital admitted patients. Out of total cases, 320 (52.7%) were found to be of ORT ward and 287 (47.3%) were found to be of OPD. Similarly, the enteropathogens were found significantly higher in ORT centre constituting 186 (63%) than OPD 110 (36.0%) ($p<0.05$).

The prevalence of enteropathogens was found highest in

the summer season of month July. This may be due to favorable condition for proliferation of pathogenic organisms especially parasites and bacteria during summer season. Among the different age groups the prevalence of enteropathogens were found to be highest in age group 0-2 years (55.0%) i.e. 163 out of 296. The prevalence of enteropathogens was significantly higher in children with illiterate mother constituting 61% than literate mother constituting 39% at 5% level of significance. This may suggests that it was the better hygiene knowledge and practice of literate mothers that reduces the risk of childhood diarrhoea than illiterate mother. Literate mothers were more likely to give correct care to their children when they have diarrhoea and also more likely to seek medical care for a child with diarrhoea. The enteropathogens were found significantly higher in children who didn't wash hand before meal 72% (213 out of 296) than wash hand before meal 28% (83 out of 296) at 5% level of significance. In this study, the enteropathogens were highest in raw water user 53.0% (157 out of 296) followed by filtered water 34.8% and boiled water 12.8% users. This study showed that the boiled water was more appropriate for drinking purpose than raw and filtered water. Since, diarrhoeal disease represents the paradigm for water-borne disease, so selecting the best available water, and then providing the barriers of storage, filtration and disinfection, are demonstrably highly effective in prevention.

The rotavirus was the most frequently isolated pathogen which was found to be 27.0%. The study showed that the highest rate of rotavirus infection was seen in hospitalized patients (64 %) than the patients who visited OPD (36 %). It was also known that rotavirus usually dominate in hospital based survey in children^{5,11,12}. The study conducted in Sentinel hospital in China during 1998 June 1999 found a total of 283 (95.6%) to be rotavirus infection in children under 5 years of age. The result was found to be higher than our findings.

Rotavirus infection is commonly prevalent in between age group 0-5 yrs^{11,13}. Similarly, this study showed that the infection rate was found to be higher in age group 0-2 yrs holding 30.3 % (112 out of 370). The finding was in accordance with other study^{13,15}. Rotavirus was a major cause of pediatric gastroenteritis and responsible for causing half of the cases to be suffered with acute diarrhoeal illness among hospitalized patients of 6-24 months of age¹⁴. Rotavirus was predominant in winter¹¹, it accounted for 45.1% in

winter season followed by spring season 24.7%, autumn season 24.1% and summer season 14.1% among total rotavirus positive cases.

The prevalence of parasitic infestation was found to be

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15.2% positive cases among the total cases. The finding was very low in accordance with others study²⁴. This might be due to the reason that many of the children received antibiotics prior to attending hospital and also during hospital stay. Therefore, there was a strong possibility that certain parasitic infection were missed during the study period. On the other hand, the government policy for antihelminthic program launched at community level, which may be another reason for lowest parasitic prevalence rate in this study. Apart from this, primary school children in selected districts are being provided with deworming tablets twice yearly by World Food Program, Plan Nepal and Save the Children (US). In this study the protozoan (12.2%) dominated over helminthes (3.0%). Of them *E. histolytica* (7.0%) was major causative agent of dysentery. WHO report has documented a high mortality from amebic dysentery world wide²⁴. The parasitic infestation showed higher in OPD (53.3%) than ORT (46.7%) among total positive cases of parasite. The infestation of parasites was highest in the season summer i.e. 43 cases (43/92).

Globally, *Salmonella* and *Shigella* remain the major contributors to acute enteric infections and diarrhoea¹. Bacillary dysentery or shigellosis is being a public health problem mainly in developing countries especially below 10 years. In this study, *Shigella* spp. showed highest bacterial etiological agent that causes diarrhoea in children. *Shigellae* are highly communicable enteric pathogens, as illustrated by the experimentally determined infectious dose of 10-100 organisms for North American adult volunteers¹⁷. Among total stool sample processed, 20 (3.3%) found to be *Shigella* spp. Shigellosis is a highly contagious disease of poor and crowded communities, with faeco-oral (hand-to-mouth) transmission, and an extremely low minimum infectious dose¹⁸. Among the bacterial isolates prevalence of *Shigella* spp. was highest in age group 0-2 yrs (50% i.e. 10/20). These age-specific trends suggested that significant risk factors for acquisition of *Shigella* infections include, weaning from breast milk and introduction of children into day care centers that have an inherent potential for faeco-oral transmission of intestinal bacteria. Secondary transmission of shigelloses can also occur at a rate exceeding 50% in households with young children¹⁹. Similarly, the prevalence of *Salmonella* spp. was high in age group 2-4 yrs (50% i.e. 4/8). The study reported that the outbreak of cholera generally occurred in Nepal at the end of June to September each year with peak period being June to August suggesting favorable condition for its proliferation⁹. In this study, *V. cholera* was found highest in July (7 cases) followed by June (3 cases) and August (2 cases) but there was no single cases found in September.

The diarrhoeal disease mainly concerned with impure water, low socio-economic state, poor sanitation coupled with low literacy rates of parents particularly the mothers were the main causes of this prevalent malady. So, proper health education system should be developed.

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