INTESTINAL PROTOZOAL INFESTATION PROFILE IN PERSISTENT DIARRHEA IN CHILDREN BELOW AGE 5 YEARS IN WESTERN NEPAL

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Abstract. Unlike acute diarrhea, the role of pathogens in persistent diarrhea in children in Nepal is unclear. Protozoal parasites are suspected to be a major cause. The study was carried out to find the association between protozoal agents and persistent diarrhea in children below age 5 years from western Nepal. Stool samples were collected from 253 children with persistent diarrhea, from 155 children with acute diarrhea (disease controls) and from 100 healthy children from the community (normal controls). Of 253 children with persistent diarrhea, 90 (35.5%) had protozoal infections, 63 (24.9%) helminthic infections, 32 (12.6%) had bacterial infections and 16 had mixed infections. *Giardia lamblia* was the most prevalent (67.7%), followed by *Entamaeba histolytica* (27.7%). HIV infection and severe malnutrition were associated with *Cyclospora cayetanensis* and *Cryptosporidium* spp causing persistent diarrhea. We conclude that stool microscopy should be routinely performed in children with persistent diarrhea since protozoal infections can be cured with effective treatment and control can be achieved by proper health education.

INTRODUCTION

Diarrhea is a major health problem in developing countries. Most acute episodes of diarrhea are due to infection of the bowel; they have an acute onset and last between two and seven days. A proportion of acute cases, about 1 in 10, become persistent, lasting more than 2 weeks, requiring medication in addition to oral rehydration therapy. Globally, an estimated 1.8 billion episodes of diarrhea occur each year leading to the death of 3 million children below age 5 years (WHO, 2002). In Nepal, the estimated number of diarrheal episodes in children below age 5 years was 11,171,000 in the year 1994 (4th after India, Bangladesh and Indonesia) and the morbidity rate was 3.3/1,000 children below 5 years of age (3rd after Bhutan and Bangladesh) (Park, 2002). The number of children below 5 years of age (total population = 3,533,962) affected with diarrheal disease in Nepal in the years 2001/2002 was 625,150 (incidence = 177/1,000) with the total number of deaths being 136 (case fatality rate = 0.2/1,000) (Department of Health Services, 2002). In western Nepal, total diarrheal episodes were 111,681/year (2001/2002) with 30 reported cases of deaths (case fatality rate = 0.1/1,000) in children below 5 years of age (Department of Health Services, 2002).

The pathogens involved in persistent diarrhea in Nepal have not previously been reported. Viruses are not commonly isolated from stools. Enterotoxigenic (ETEC) and enteropathogenic (EPEC) *Escherichia coli*, *Aeromonas*, *Campylobacter* and *Shigella* species are not usually associated with persistent diarrhea (WHO, 1998). Enteroadherent *E. coli* (EAEC) was isolated more frequently in the early phase of persistent diarrhea in India,

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Bangladesh and Mexico (Bhan *et al*, 1989; Baqui *et al*, 1992; Henry *et al*, 1992). Protozoal agents, such as *Giardia lamblia*, *Cryptosporidium* spp, *Entamoeba histolytica* and other intestinal protozoal parasites have been associated with persistent diarrhea (Chavalittamrong and Jirapinyo, 1984; De Silva *et al*, 1994; Bhandari *et al*, 1999; Park, 2002).

Socio-economic and cultural factors and lack of adequate basic sanitation have caused the children of Nepal to be vulnerable to intestinal parasitic infections. There has been no descriptive study in western Nepal to discover the protozoal profile in children with persistent diarrhea. This hospital-based study was conducted to determine the association between protozoal agents and persistent diarrhea in children below 5 years of age in this region.

MATERIALS AND METHODS

Diarrhea is defined as the passage of 3 or more liquid stools in a 24-hour period. Persistent diarrhea is defined as diarrhea lasting ≥14 days and acute diarrhea as diarrhea <14 days duration.

The study was carried out at the Manipal Teaching Hospital, Pokhara from April 1998 to March 2004. A total of 759 (253 x 3) stool samples were collected from 253 children under age 5 years (M=147, F=106) who were either admitted to the hospital or visited the OPD with persistent diarrhea. All stool samples were collected in waterproof screw capped plastic containers and processed immediately. Wet and iodine preparations were examined for trophozoites and cysts of protozoal parasites and eggs of helminths per standard methodology. Smears were stained by modified Ziehl-Neelsen staining procedure and observed for oocysts *of Cyclospora cayetanensis* and *Cryptosporidium* spp. The morphology of oocysts *of Cyclospora cayetanensis* and *Cryptosporidium* spp were compared with positive controls (reference slides from CDC, Atlanta). The samples were also processed for identification of suspected bacterial pathogens biochemically and serologically (in the cases of ETEC and EPEC) according to the recommended procedure (WHO, 1987).

Stool samples were also collected from 155 children under 5 years of age (M=87, F=68) who had visited or were admitted to the hospital with acute diarrhea (disease control), and from 100 healthy children below 5 years of age (M=55, F=45) from the community (normal controls) with no prior diarrheal illnesses. The controls were matched with the cases for nutritional status category (weight for age ≤90% or >90% according to Gomez's international classification).

To study the seasonal pattern of diarrhea, each year was divided into 4 seasons: January-March (Spring), April-June (Summer), July-September (Monsoon) and October-December (Winter).

Chi-square and Student's *t*-test were used to determine statistical significance.

RESULTS

The baseline characteristics of the cases and controls were similar (Table 1). The dominant social class within the analyzed group corresponded to the low-medium category, with an income of 32,001-62,000 Nepali rupees per annum (US$430-830/yr) and an incomplete secondary level as the maximum education achieved in at least one of the parents. The basic sanitary level of the families was poor. The children did not grow up with the habit of washing hands before eating food and used utensils that were not cleaned properly. Most children consumed unboiled or unfiltered water outside the house. The practice of using open-air toilets and open-air water sources was prevalent in society. However, no significant association between sex and nature of diarrheal episodes was found.

Table 1
Baseline comparison of cases and controls.

Basic information	Persistent diarrhea cases (n = 204)	Acute diarrhea cases (Disease control) (n =155)	Without GI involvement (normal control) (n =100)
Mean age in months (-3SD)	31.3 (8.9)	31.0 (8.5)	33.5 (8.5)
Males (%)	58.1	55	55
Mean weight for age[a] (-3SD)	70.4 (9.9)	71.3 (10.0)	74.7 (10.2)
Breast fed	56.1	70	75.5
Literate parents (%)	36.3	36.1	36.2
Sanitary training in family	low	low	low
Income category[b]	low	low	low-medium
Using tap water	47%	49%	53%
Using river/lake water	23%	24%	20%
Regular sewage disposal	46%	49%	48%
Using house/closed toilets	45%	42%	44%
Using open air toilets	55%	58%	56%
Take foods outside house	66%	70%	41%

[a] Gomez's international classification

[b] Low (≤32,000 Nepali rupees, US$430/yr); Low-medium (32,001-62,000 Nepali rupees, US$430-830/yr); Upper-medium (62,001- 99,200 Nepali rupees, US$830-1,330/yr); and High (99,201- 137,600 Nepali rupees, US$1,330-1,850/yr).

Of 759 stool samples, 90 (35.5%) were positive for protozoal cysts, trophozoites or oocysts, 63 (24.9%) for helminthic infections and 32 (12.6%) for bacterial infections (Table 2). In 68 samples (26.8%), no causative agents were found. We did not look for viral agents. Mixed infections were seen in 16 samples (2 samples with two types of protozoa, 6 samples with protozoa and helminthes, 3 samples with more than one type of helminth, and in 5 samples there was a mixed infection with protozoa and bacteria).

G. lamblia was the most prevalent protozoal infection in children below 5 years with persistent diarrhea (67.7%, 61/90), followed by *E. histolytica* (27.7%, 25/90). Four samples, 2 each, were positive for *Cryptosporidium* spp and *C. cayetanensis*. *Ascaris lumbricoides* was the commonest helminth (39.6%, 25/63) identified, followed by *Trichuris trichiura* (28.5%, 18/63), hookworm (26.9%, 17/63) and *Strongyloides stercoralis* (4.7%, 3/63). The distribution of 32 bacterial isolates recovered from the cases was: 24 (56.2%) EAEC, 6 (18.6%) EPEC, 3 (9.3%) ETEC, 3 (9.3%) *Campylobacter* spp, 1 (3.1%) *Salmonella* spp and 1 (3.1%) *Shigella* spp.

G. lamblia trophozoites were detected in a significantly higher proportion of persistent diarrheal cases (9.8%) than acute diarrheal and non-diarrheal controls (0% and 2%, respectively; $p<0.001$) (Table 3). Similarly, *E. histolytica* trophozoites were detected in a significantly higher proportion of persistent diarrheal cases (6.3%) than acute diarrheal and non-diarrheal controls (1% each). *Cyclospora cayetanensis* (0.7%) was isolated from only HIV-positive children, whereas in the case of *Cryptosporidium* spp (0.7%), one was isolated from an HIV-positive child and another from a

Table 2
Enteropathogens associated with persistent diarrhea in children (n =253) less than 5 years.

Category	Pathogens	Total number	Percentage
Protozoa	*Giardia lamblia*	61	67.7
(n = 90)	*E. histolytica*	25	27.7
	Cryptosporidium sp	2	2.2
	Cyclospora cayetanensis	2	2.2
Helminthes	*Ascaris lumbricoides*	25	39.6
(n =63)	*Trichuris trichiura*	18	28.5
	Hookworm	17	26.9
	Strongyloides stercoralis	3	4.7
Bacteria	EAEC	18	56.2
(n= 32)	EPEC	6	18.6
	ETEC	3	9.3
	Campylobacter sp	3	9.3
	Salmonella sp	1	3.1
	Shigella sp	1	3.1
Unknown	(? Viruses and others)	68	-

Table 3
Prevalence of intestinal protozoal parasites in cases (n = 253) and controls (n = 100 each).

Categories (Total protozoal parasites detected)	EH			GL			CRYP	CYC
Persistent diarrhea		25			61		02	02
(n = 90)	T	C	B	T	C	B	(0.70%)	(0.70%)
	10	09	06	15	36	10		
	T (Total) = 16 (6.3%)			T (Total) = 25 (9.8%)				
Acute diarrhea		11			04			
(n = 15)	01	10	0	00	04	00		
	T (Total) = 01 (1%)			T (Total) = 00 (0%)				
No diarrhea		16			8			
(n = 24)	01	15	00	02	06	00		
	T (Total) = 01 (1%)			T (Total) = 02 (2%)				

EH = *Entamoeba histolytica*; GL = *Giardia lamblia*; CRYP = *Cryptosporidium* sp; CYC = *Cyclospora cayetanensis*; T = Trophozoite; C = Cyst; B = Both trophozoite and cyst

child suffering from protein energy malnutrition. All 4 children had persistent diarrhea.

In none of the 21 (8.3%) breast-fed children aged 0-5 months were protozoal infections detected. In most of these cases, the etiological agent remained unknown. The rates of intestinal protozoal infections in age groups 6-11, 12-23, 24-35, and 36-60 months were 13.8, 19.3, 25.2, and 33.2%, respectively. In each age group, the rate of infection was significantly higher than the previous age group. Acute infection (increased numbers of trophozoites) with both *G. lamblia* and *E. histolytica* also increased with age.

Diarrheal episodes were encountered mainly in July-September (monsoon) (44.6%), followed by April-June (34.7%), corresponding with the increased infection rate and the number of trophozoites of both *G. lamblia* and *E. histolytica*. In comparison, diarrheal cases were significantly lower in January-March (Spring) (9%) and October-December (Winter) (11.4%) compared to that of Summer and Monsoon seasons.

DISCUSSION

The majority (33%) of the global deaths of all types of illnesses are as a result of infectious and parasitic diseases (WHO, 1998). In Southeast Asia, diarrheal diseases constitute the largest single cause of death in children below age 5 years. Although most of the deaths are due to acute diarrhea, persistent diarrhea may account for a large proportion of all deaths. WHO and UNICEF estimated that in 1991 persistent diarrhea accounted for only 10% of diarrheal episodes, but as many as 35% of diarrheal deaths in children under 5 years of age. Evidence from studies in Bangladesh, India, Peru and Brazil indicates that approximately 45% (range 23% to 62%) of diarrhea-associated deaths were due to persistent diarrhea (Dialogue on Diarrhoea and ADDR, 1992). Amebiasis and giardiasis follow an endemic pattern and focal epidemics occur when large groups of children are exposed to common sources in water-borne and food-borne epidemics (Piya *et al*, 2001).

In the present study, the protozoal infection rate was higher than the bacterial and helminthic infection rates in cases of persistent diarrhea. The children probably acquired the infection from school or at home, which relates to poor quality drinking water and poor personal hygiene. Contamination of drinking water with sewage due to close running of water pipes and sewage lines and consumption of green leafy vegetables have already been reported as predisposing factors for diarrhea in Nepal (Sherchand *et al,* 1999; Shrestha *et al,* 2001).

The incidence of *G. lamblia* in persistent diarrhea (9.8%) in the present study was more than that reported for Bangladesh (Baqui *et al*, 1992; Henry *et al*, 1992) and Brazil (Schnack *et al*, 2003), but less than India (Bhandari *et al*, 1999; Kaur *et al*, 2002) and Peru (Lanata *et al*, 1992). *G. lamblia* was not responsible for acute diarrhea in the present study, although a high incidence (13%) has been documented previously for other parts of Nepal (Hoge *et al*, 1995). The prevalence of giardiasis was more in malnourished children in Gambia compared to healthy controls (Sullivan *et al*, 1990), but such an association could not be established in our study since all the cases and controls were undernourished (Grade II malnutrition).

A significantly higher proportion of those with persistent diarrhea were infected with *E. histolytica* compared to the acute diarrheal (6.3% *vs* 1%) and non-diarrheal (6.3% *vs* 1%) controls. A similar high association has been reported for Bangkok (6.8%) (Chavalittamrong *et al*, 1984), Central Africa (12.8%) (Molbak *et al*, 1994), India (14%) (Kaur *et al*, 2002), and Brazil (56.4%) (Schnack *et al*, 2003). *E. histolytica* is not always reported as a cause of persistent diarrhea in children (Bhan *et al*, 1989; Baqui *et al*, 1992; Henry *et al*, 1992; Bhandari *et al*, 1999; Kaur *et al*, 2002) or acute diarrhea (Hoge *et al,* 1995).

Cryptosporidium spp and *C. cayetanensis* were associated with persistent diarrhea in our study in immunocompromized children only, although infection of immunocompetent hosts has been described (Shlim *et al,* 1991). *Cryptosporidium* spp itself is known to cause persistent diarrhea in children (Baqui *et al*, 1992; Molbak *et al*, 1994) and these pathogens have been reported to have a general prevalence rate of 0.9% in Africa, especially in Morocco, South Africa and Egypt (Asfaw and Goitom, 2000). *Cyclospora cayetanensis* has

been reported to have a prevalence rate of 29.8% in stool samples from the general population (Sherchand *et al,* 1999), but 5% in children below 5 years of age with acute diarrhea in Nepal (Hoge *et al,* 1995).

Breast-fed children are generally known to be more resistant to protozoal infections, though isolated cases are not infrequent (Molbak *et al*, 1994). It is noteworthy in this context to state that there is evidence of increased risk for *G. lamblia* and *Cryptosporidium* spp infections in water storage dams and reclamation plants (Menge *et al*, 2001). In Pokhara, there are dams on the rivers (Seti, Kalindi and Gondoki) flowing through the region and people use the stored river water for household purposes. Heavy rainfall, low temperature and ambient humidity in Summer and Monsoon seasons create a favorable environment for the acquisition and dissemination of intestinal protozoal parasitic infections.

As giardiasis and amebiasis were observed to be associated with persistent diarrheal episodes in children below 5 years, stool microscopy should be routinely performed in these patients, and effective treatment should be instituted upon the isolation of *E. histolytica* or *G. lamblia* trophozoites. Education regarding basic sanitation and improvement of family hygiene will help in controlling the infection.

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REFERENCES

Asfaw ST, Goitom L. Malnutrition and enteric parasitoses among under- five children in Aynalem village, Tigray. *Ethiop J Health Dev* 2000; 14: 67-75.

Baqui AH, Sack RB, Black RE, *et al*. Enteropathogens associated with acute and persistent diarrhea in Bangladesh children under five years of age. *J Infect Dis* 1992; 166: 792-6.

Bhan MK, Bhandari N, Sazawal S, *et al*. Descriptive epidemiology of persistent diarrhea among young children in rural northern India. *Bull WHO* 1989; 67: 281-8.

Bhandari N, Bahl R, Dua T, Kumar R, Srivastava R. Role of protozoa as risk factors for persistent diarrhea. *Ind J Pediatr* 1999; 66: 21-6.

Chavalittamrong B, Jirapinyo P. Intestinal parasites in pediatric patients with diarrheal diseases in Bangkok. *Southeast Asian J Trop Med Public Health* 1984; 15: 385-8.

Department of Health Services. Control of diarrheal diseases. Annual report. Child health, Chapter 2c. His Majesty's Government of Nepal, Ministry of Health, 2001/02: 44-53.

De Silva NR, De Silva HJ, Priyanka Jayapani VP. Intestinal parasitoses in the Kandy area, Sri Lanka. *Southeast Asian J Trop Med Public Health* 1994; 25: 469-73.

Dialogue on Diarrhoea online and the Applied Diarrhoeal disease research project (ADDR). Persistent Diarrhoea Clinical Update: a supplement to Issue no. 48 - March 1992. Cambridge, MA: Harvard Institute for International Development. [Cited 2006 Aug 20]. Available from: URL: http://rehydrate.org/dd/su48.htm

Henry FJ, Udoy AS, Wanky CA, Aziz K. Epidemiology of persistent diarrhea and etiologic agents in Mirzapur, Bangladesh. *Acta Paediatr* 1992; 81: 27-31.

Hoge CW, Echeverria P, Rajah R, *et al*. Prevalence of *Cyclospora* species and other enteric pathogens among children less than 5 years of age in Nepal. *J Clin Microbiol* 1995; 33: 3058-60.

Kaur R, Rawat D, Kakkar M, Uppal B, Sharma VK. Intestinal parasites in children with diarrhea in Delhi, India. *Southeast Asian J Trop Med Public Health* 2002; 33: 725-9.

Lanata CF, Black RE, Maurtua D, Gil A, Gabilondo A, Yi A. Etiologic agents in acute persistent diarrhea in children under three years of age in periurban Lima, Peru. *Acta Paediatr* 1992; 81 (suppl 381): 32-8.

Menge JG, Haarhoff J, König E, Mertens R, van der

Merwe B. Occurrence and removal of *Giardia* and *Cryptosporidium* at the Goreangab Reclamation Plant. *Water Sci Technol Water Suppl* 2001; 1: 97-106.

Molbak K, Wested N, Hojlyng N, *et al* .The etiology of early childhood diarrhea: A community trial from Guniea Bissau. *J Infect Dis* 1994; 169: 581-7.

Park K. Diarrhea. In: Park's textbook of preventive and social medicine. 17th ed. Jabalpur: Banarsidas Bhanot, 2002: 175-8.

Piya S, Adhikari S, Rajbhandari TP. A study on diarrhea in children in relation to behavioral and environmental factor. *J Sci Tech* 2001; 3: 85-8.

Schnack FJ, Fontana LLM, Marbosa PR, Silva LS, Baillargeon CM, Barichello T. Enteropathogens associated with diarrheal disease in infants (<5 years old) in a population sample in Greater Metropolitan Criciuma, Santa Catarina State, Brazil. *Cad Saude Publica* 2003; 19: 1205-8.

Sherchand JB, Cross JH, Jimba M, Sherchand S, Shrestha MP. Study of *Cyclospora cayetanensis* in healthcare facilities, sewage water and green leafy vegetables in Nepal. *Southeast Asia J Trop Med Public Health* 1999; 30: 58-63.

Shlim DR, Cohen MT, Eaton M, Rajah R, Long EG, Ungar BL. An alga-like organism associated with an outbreak of prolonged diarrhea among foreigners in Nepal. *Am J Trop Med Hyg* 1991; 45: 383-9.

Shrestha B. Intestinal parasitic infestation in healthy school children of Lalitpur district. *J Nepal Med Assoc* 2001; 41: 266-70.

Sullivan PB, Marsh MN, Philips MB, *et al*. Prevalence and treatment of giardiasis in chronic diarrhea and malnutrition. *Arch Dis Child* 1990; 65: 304-6.

World Health Organization. Health situation in the South-East Asia region 1998-2000. Geneva: World Health Organization, 2002.

World Health Organization. Programme for Control of Diarrheal Diseases. Manual for laboratory investigations of acute enteric infections. Geneva: World Health Organization. *CDD/83.3 rev 1.* 1987: 1-113.

World Health Organization. Diarrhoeal Disease Control Programme. Persistent diarrhea in children in developing countries: report of a WHO meeting. Geneva: World Health Organization. W*HO/CDD/88.27.* 1988: 14.

World Health Organization. The state of world health: In: The World Health Reports 1998. Life in the 21st century: a vision for all. Geneva: WHO, 1998: 57-8.