PARASITES DETECTED FROM DIARRHEAL STOOL SAMPLES COLLECTED IN NEPAL

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Abstract. Intestinal parasites were investigated in 396 diarrheal stool samples collected from individuals aged 1 to 68 years (males: 239 and females: 157) in Nepal. Samples were collected at different medical centers located in Kathmandu and from two public schools in a village setting in Kathmandu Valley and outside, during October 1999 to January 2001. The stool samples were mixed with 2% dichromate solution and transported to Japan for investigations. Parasites were detected by employing the formal-ether sedimentation technique. Of a total of 396 fecal samples investigated, 193 (49%) were positive for some kind of parasite. Altogether, 15 species of parasites were detected. *Giardia intestinalis* topped the list of protozoa, whereas *Trichuris trichiura* was the most frequently detected among helminth parasites. Of the 193 positive samples, 109 (56%) had single parasite infections, whereas 84 (43%) had multiple infections with a maximum of five species. Of the total positive, 45 (23%) had both protozoa and helminths whereas 37 (19%) had only protozoa. Females (52%) and children (15 years and under) (52%) had a marginally higher prevalence compared with males (46%) and adults (45%), respectively ($p>0.05$). Samples collected from two public schools in a village setting inside Kathmandu Valley and outside had a significantly higher positive rate compared with those observed in individuals visiting different medical centers in the city and suburban areas in Kathmandu ($p<0.05$).

INTRODUCTION

Diarrheal disease is a common illness that affects millions of people worldwide and attack rates range from 2 to 12 or more illnesses per person per year (Guerrant *et al*, 1990). It is common, particularly in developing countries, and continues to be a major cause of morbidity and mortality contributing to dehydration, malnutrition and other risks. It is associated with fecal contamination of the environment, lack of potable water (Adhikari *et al*, 1986; Guerrant *et al*, 1990; Rai *et al*, 2000b; 2001a; Ono *et al*, 2001), education, and housing in the midst of poverty. Diarrheal diseases mainly affect young children and are attributed to long-term cognitive deficits (Niehaus *et al*, 2002). Deaths due to diarrheal diseases account for an estimated 12,600 child deaths each day in Asia, Africa, and Latin America (Guerrant *et al*, 1990).

Diarrhea is produced by a variety of etiological agents. Of them, intestinal parasitic infection alone contributes to a great extent in the cause of diarrhea. Parasites and diarrhea are more frequent in poor populations (Khan *et al*, 1990) and in immunocompromised individuals (Ferreira and Borges, 2002). Intestinal parasites, particularly the protozoa, are more likely to infect individuals with impaired cellular immunity, such as those with hematological neoplasias, renal and heart transplant recipients, patients receiving high doses of corticosteroids, and patients with AIDS (Ferreira and Borges, 2002). Diarrhea is a common clinical manifestation of HIV infection regardless of

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whether or not patients have AIDS (Joshi *et al*, 2002). Enteric protozoa are also associated with traveler's diarrhea (Okhuysen, 2001). In the case of helminth parasites, there is little agreement on which worm definitely causes diarrhea (Genta, 1993).

Nepal, situated in the lap of the Himalayas, is a small and impoverished land-locked country located in South Asia. About 70% of health problems and causes of death in Nepal are due to infectious diseases (Rai *et al*, 2001a). Of these, diarrheal disease alone constitutes one of the major causes of morbidity and mortality (Bista, 2001; Rai *et al*, 2001a; 2003). Intestinal parasitosis alone is one of the most common public health problems in Nepal (Estevez *et al*, 1983; Rai and Gurung, 1986; Rai *et al*, 1995; 1998; 2000a; 2001a,b; 2002; Ishiyama *et al*, 2001) and is often associated with diarrhea and malnutrition (Sherchand *et al*, 1996; NMSS, 1998; Rai *et al*, 1998; 2000a; 2003; Ono *et al*, 2001). The reported prevalence rates of enteric parasitosis in Nepal vary from considerably low (Rai *et al*, 2002) to nearly one hundred percent (Estevez *et al*, 1983; Rai and Gurung, 1986) and have been associated with contamination of soil (Rai *et al*, 2000b) and water (Adhikari *et al*, 1986; Ono *et al*, 2001). Emerging diarrheagenic enteric parasites have also been reported recently (Gianotti, 1990; Hoge *et al*, 1995; Sherchand and Shrestha, 1996; Ono *et al*, 2001). In view of a high prevalence of enteric parasitosis and diarrheal diseases in Nepal, this study was conducted to elucidate the association of parasites with diarrhea among the Nepalese. This paper reports on the prevalence of intestinal parasites detected from diarrheal stool samples collected in Kathmandu, Nepal, from 1999 to 2001.

MATERIALS AND METHODS

Study population

The patients who submitted diarrheal stool samples to laboratories in Medical Centers of Kathmandu Valley, namely, Birendra Police Hospital (BPH; Maharajgunj), Kanti Children's Hospital (KCH; Maharajgunj), Shi-Gan Path Lab (Shi-Gan PL; Putali Sadak and its Boudha branch), and Nepal Medical College Teaching Hospital (NMCTH; Jorpati) for routine and direct microscopic examination were included in this study. Also included, were children from two public schools in a village setting, inside and outside of Kathmandu Valley, who submitted diarrheal stool samples during the school health check program.

Sample collection and preservation

After routine and direct examination, about five to eight milliliters of stool sample was transferred into a screw capped glass bottle (20 ml capacity), thoroughly mixed with an equal volume of 2% dichromate solution, and sealed with vinyl tape. The age and sex of subjects were noted. Samples were then transported to Japan for further analysis. These samples were collected from October 1999 to January 2001.

Detection of parasites

Parasites were detected by a concentration method employing the formal-ether sedimentation technique. Findings of routine and direct microscopic examinations done in Nepal were not considered in this study.

Data analysis

Findings were aggregated against the age and sex of the subjects, and sample collection areas. Statistical analysis was done by the chi-square test.

RESULTS

In the present study, of a total of 396 diarrheal stool samples analyzed for the presence of intestinal parasites, 193 (49%) showed some kind of intestinal parasite (Table 1). Females showed a marginally higher prevalence (52%) compared with males (46%) ($p>0.05$). Similarly, a marginally higher prevalence was observed in children (15 years and under) (52%) than in adults (45%) ($p>0.05$). Of the samples collected in different areas, a significantly higher prevalence (91%) was found in public school children living in rural settings inside and outside of Kathmandu Valley compared with those observed in Kathmandu city and suburban areas, namely, BPH, KCH, Shi-Gan PL, and Boudha branch of Shi-Gan PL/NMCTH (Table 2).

Altogether 15 species of parasites (eight pro-

Table 1
Prevalence of intestinal parasites in diarrheal stool samples in Nepal.

		Total n	Pos n	%	p-value
Sex	Male	239	111	46	>0.05
	Female	157	82	52	
Age-group	Children[a]	209	108	52	>0.05
	Adults	187	85	45	
Total		396	193	49	

[a]15 years and under.

Table 2
Prevalence of intestinal parasites in diarrheal fecal samples collected at different places in Nepal.

Areas	Total n	Pos n	%	p-value
Maharajgunj[1]	132	62	47	<0.05
Maharajgunj[2]	85	32	38	
Putalisadak[3]	56	23	41	
Boudha/Jorpati[4]	70	28	40	
School children[5]	53	48	91	
Total	396	193	49	

[1]Birendra Police Hospital (BPH) serving police personnel and their families; [2]Kanti Children's Hospital (KCH); [3]Shi-Gan Path Lab (Shi-Gan PL), Putalisadak; [4]Boudha branch of Shi-Gan PL/Nepal Medical College Teaching Hospital (NMCTH); and [5]Public school children in a village setting inside and outside of Kathmandu Valley.

Table 3
Parasite species detected from 221 parasite positive diarrheal stool samples collected in Nepal.

Parasite species	Total (n)	Percentage
Protozoa:		
Giardia intestinalis	40	10
Cyclospora cayetanensis	22	6
Entamoeba coli	20	5
Entamoeba sp	6	2
Cryptosporidium parvum	1	0
Endolimax nana	1	0
Iodamoeba butschilii	1	0
Isospora belli	1	0
Helminths:		
Trichuris trichiura	104	26
Hookworm	54	14
Ascaris lumbricoides	48	12
Vampirolepis nana	7	2
Clonorchis or *Opisthorchis*	5	1
Enterobius vermicularis	2	1
Hymenolepis diminuta	1	0

tozoa and seven helminths) were detected (Table 3). Among the protozoan parasites, *Giardia intestinalis* was the most common, followed by *Cyclospora cayetanensis, Entamoeba coli* and others. In the case of helminth parasites, *Trichuris trichiura* was detected most frequently followed by hookworm, *Ascaris lumbricoides* and others. Of the positive samples, 43% (84/193) had multiple (two or more species) parasitic infections with a maximum of five species. Twenty-three per cent (45/193) had both protozoan and helminthic infections, whereas 19% (37/193) had only protozoan infections. Eggs of very uncommon parasites such as *Hymenolepis diminuta* and *Clonorchis* (or *Opisthorchis*) were also recovered.

DISCUSSION

Both intestinal parasites (Estevez *et al*, 1983; Rai and Gurung, 1986; Gianotti, 1990; Rai *et al*, 1995; 1998; 2000a; 2001a,b; 2002; Ishiyama *et al*, 2001) and diarrheal diseases (Rai *et al*, 2001a; 2003; Bista, 2001; Ono *et al*, 2001) are highly prevalent in Nepal. The present study, however, showed a relatively low prevalence of parasitosis (49%) among subjects with diarrhea. This can be due to the high prevalence of helminthiasis

among the general population compared with protozoan parasitosis (Rai and Gurung, 1986; Rai *et al*, 1995; 2001b; 2002) and parasitic diarrhea is mainly caused by protozoan parasites (Genta, 1993). However, the parasite load of 63% and 68% reported in children and adults with abdominal discomfort, respectively has been reported earlier (Sherchand *et al*, 1996).

No significant difference in the prevalence of enteric parasitosis was observed in males and females, which suggested that parasitic diarrheal diseases were independent of sex in Nepal. Ono *et al* (2001) also reported no significant difference in the prevalence of diarrheal diseases in the two sexes caused by different enteropathogens, bacteria, virus and parasites. Similar findings have also been reported in the general population and school children in Nepal (Rai *et al*, 1986; 1995; 2001b; 2002; Ishiyama *et al*, 2001). Similarly, no significant difference was observed in the prevalence of enteric parasites in children and adults. These findings indicated that both children and adults, irrespective of sex, were equally exposed to enteric parasites, particularly diarrheagenic protozoa. This can be attributed to unplanned urbanization, which results in poor sanitary and hygienic conditions, and contamination of drinking water with fecal matter in the Kathmandu Valley (Adhikari *et al*, 1986; Ono *et al*, 2001).

In this study, one to three species of potentially diarrheagenic enteric protozoan parasites were recovered in about half of the samples investigated. The present findings reveal that over one-third (42%) of the diarrheal diseases in Nepal are associated with enteric protozoan parasites.

The present study revealed a significantly high prevalence of enteric parasitosis in school children in the northern part of the Kathmandu Valley and outside compared with samples collected in other areas, including children attending Kanti Children's Hospital. This finding indicated that diarrheagenic parasites were more prevalent in suburban and village settings in the valley.

In this study, eight species of protozoa, most of them being potential and others opportunistic causes of diarrheal diseases, were detected. *G. intestinalis* topped the list of protozoan parasites and was in agreement with those reported earlier (Rai and Gurung, 1986; Rai *et al*, 1995; 2002; Ishiyama *et al*, 2001; Ono *et al*, 2001) followed by *Cyclospora cayetanensis*, *E. coli* and others. *Blastocystis hominis* reported earlier in people with abdominal complaints (Sherchand *et al*, 1996) and the general population (Gianotti, 1990; Rai *et al*, 2001b) was not detected in this study. This can be due to the autolysis of *B. hominis* cysts during the time lapsed between sample collection and examination.

Among the helminth parasites, *T. trichiura* was detected most frequently followed by hookworm and *A. lumbricoides*. This was in contrast to most findings reported earlier from Nepal in which *A. lumbricoides* topped the list (Rai and Gurung, 1986; Rai *et al*, 1995; 2000a; 2001b; 2002). However, this was in agreement with the findings of Ishiyama *et al* (2001). This might be, in part, due to an intake of antihelminthic drugs, usually albendazole that does not clear *T. trichiura* infection (Hanjeet and Mathias, 1991), at the beginning of diarrhea because most Nepalese believe that abdominal discomfort and/or diarrhea is caused by *Ascaris*, locally known as *Juka*. Interestingly, for the first time in Nepal, *Clonorchis (*or *Opisthorchis)* eggs were also detected in 1% of the subjects studied (details will be reported elsewhere).

The present study revealed that 42% of the diarrheal diseases in Nepal were associated with enteric protozoan parasites, as long as the samples were carefully examined using concentration techniques. Further, water supply systems need to be corrected and protected from fecal contamination because the water in the Kathmandu Valley has been found to contain various diarrheagenic agents, including protozoan parasites (Adhikari *et al*, 1986; Ono *et al*, 2001). Awareness programs are most essential because contaminated food and/or drinks transmits these diarrheagenic parasites. Since parasites and diarrhea are more frequent in poor populations (Khan *et al*, 1990) and in immunocompromised individuals (Ferreira and Borges, 2002), preventive measures and surveillance systems must be emphasized considering the fact that HIV infection in Nepal is rapidly spreading.

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