INTRODUCTION

Diarrheal diseases are the most common illness affecting millions of people worldwide with an attack rate that ranges from two to twelve or more episodes of diarrhea per person per year (Guerrant et al., 1990). It is endemic in developing countries, and constitutes a major cause of morbidity and mortality attributed to dehydration, malnutrition and other risks. Diarrheal diseases are associated with fecal contamination of the environment, lack of potable water, poor education and housing, and poverty affecting mainly young children. Diarrheal diseases account for an estimated 12,600 child deaths each day in Asia, Africa and Latin America (Guerrant et al., 1990).

Various etiological agents, including enteric parasites cause diarrhea. Enteric parasites, particularly the protozoa, mainly infect individuals with impaired cellular immunity such as those with hematological neoplasias, renal and heart transplant recipients, patients receiving high dose corticosteroids, and patients with AIDS. Recently, several new diarrheagenic protozoa have been reported including in Nepal (Hoge et al., 1993; Sherchand et al., 1996). Enteric protozoan parasites in contribute significantly to the cause of traveler’s diarrhea, which affects 20 to 50% of people from developed countries visiting developing countries (Lima, 2001).

C. cayetanensis, previously referred to as a cyanobacterium-like body, is an emerging diarrheagenic protozoan parasite. The first human infection was reported in 1979 in a man in Papua New Guinea (Ashford, 1979). Since then, diarrheal illness associated with this parasite has been reported in several countries of the world.
in the feces of both immunocompetent and immunocompromised patients. Cyclosporiasis is characterized by mild to severe nausea, anorexia, abdominal cramping and watery diarrhea lasting for an average of three to six weeks in different study populations with longer duration (three months) in immunocompromised patients (Looney, 1998; Ortega et al., 1998). The infections are effectively treated with a seven-day-course of trimethoprim-sulfamethoxazole. Cyclospora infections are transmitted through ingestion of oocysts via contaminated food and water. Recently, there have been several water-borne (Looney, 1998) and food-borne outbreaks (Fleming et al., 1998) including one that occurred after a wedding reception. Most of the outbreaks which occurred in the USA were associated with the ingestion of desert containing berries imported from Latin American countries. Furthermore, the outbreak in Germany was associated with the mixed lettuce imported from Italy (Doeller et al., 2002).

Both Nepal and Lao People’s Democratic Republic (PDR) are developing and landlocked countries located in South and Southeast Asia, respectively. Nepal being situated at the foot of Himalayas, is a place of tourist attraction. Both enteric parasites and diarrhea are prevalent in Nepal (Rai et al., 2003). Protozoan parasites, including emerging parasites, contribute significantly to the cause of diarrhea. The first case of *C. cayetanensis* infection from Nepal was reported in 1993 among travelers and foreign residents (Hoge et al., 1993). Since then, several reports have listed *C. cayetanensis* as a cause of diarrhea (Sherchand et al., 1996). The largest number of cases and the first clinical description of cyclosporiasis came from Nepal in foreign travelers and expatriates who had prolonged diarrhea (Conner, 1997), and in an outbreak that occurred due to the contamination of chlorinated drinking water (Rabold et al., 1994). A few reports are available regarding Cyclospora infections among indigenous people, showing an infection rate ranged from zero to 29.8% (Sherchand et al., 1999). Intestinal parasitic infections are highly prevalent in Lao PDR but Cyclospora infection has not been reported (Vannachone et al., 1998). In view of the situation, this study was conducted on diarrheal fecal samples collected at different health care centers in Kathmandu Valley and public schoolchildren in Nepal and in Vientiane City, Lao PDR (Fig 1).

**MATERIALS AND METHODS**

The present study evaluated 2,083 diarrheal fecal samples collected in Nepal (n=1,397) and Lao PDR (n=686). Samples in Nepal were collected each month, from October 1999 to August 2002, whereas samples in Lao PDR were collected from February 2002 to June 2003.

Of the 1,397 samples collected in Kathmandu, Nepal, 1,326 samples were collected in the northern area [Maharajgunj: Birendra Police Hospital (n=569), Kanti Children’s Hospital (n=326)]; the central area [Putali Sadak: Shi-Gan Path Lab (n=205)]; the eastern area (Boudha: Boudha branch of Shi-Gan Path Lab (n=180), and Jorpati: Nepal Medical College Teaching Hospital (n=46)]; and the northeastern area [Bansbari: public school children (n=71)] in Kathmandu city.

The samples in Lao PDR were collected at the Friendship Hospital (n=471), Mahosot Hospital (n=169), Setthathyrath Hospital (n=14), Military Hospital (n=6) in Vientiane, and outside (two remote districts)(n=26).

About 5-8 ml of stool samples were transferred into a screw capped glass bottle (20 ml capacity), thoroughly mixed with an equal volume of 2% potassium dichromate solution and sealed with vinyl tape. Age and sex of each subject, and date of sample collection and area were noted. Samples were then stored at 4-10°C and subsequently transported to Japan each season for analysis.

Fecal examination was done by direct microscopic method (18 x 18 mm area) employing a 10 µl fecal sample. The preparation was examined under a fluorescent microscope (200-1,000 magnification) by observing autofluorescence of Cyclospora oocysts. All autofluorescent positive oocyst-like structures were examined by differential interference contrast microscope. The number of oocysts were counted.
RESULTS

In Nepal, the overall C. cayetanensis oocyst positive rate was found to be 9.2% (128/1,397) with equal distribution in males (8.9%) and females (9.5%) (p>0.05) during the investigation period (Table 1). This was true for all age groups. The highest (11.1%) and lowest (3.1%) positive rates were observed in children aged 10 years and under, and in the age group of 51-60 years, respectively (Table 2). However, the differences were not significant (p>0.05). Enteric parasites other than Cyclospora, including Isospora belli and the eggs of Opisthorchis viverrini, were also detected.

The mean number of oocysts found was 28.8 (range 1 to 712) per wet mount (18 x 18 mm²). Fig 2 shows oocysts of C. cayetanensis detected in this study.

A significantly higher positive rate (12.6%, 104/826) was observed in summer (June-August), a warm and rainy season, compared with the positive rates found in the other three sea-
Fig 3—Relationship between positive rate of *C. cayetanensis* and rainfall/temperature in the three investigation periods during 1999-2002.

The present study revealed the highest positive rate in the eastern area (Jorpati (17.4%)) compared with schoolchildren (in the northeastern area) and patients submitting faecal samples in the eastern area (Boudha), the northern area (Maharajgunj), and the central area (Putali Sadak) (p<0.05) (Table 3). The second highest positive rate (12.7%) was observed in schoolchildren. However, the difference was not significant (p>0.05).

Only one of the total 686 samples (0.1%) from Lao PDR was found to be positive for Cyclospora oocysts. The patient was a 4-year-old boy.

**DISCUSSION**

In the present study, *C. cayetanensis* was detected in 9.2% of diarrheal human samples studied in Nepal. These findings were closer to those reported in children with diarrhea (5.3%) and in AIDS patients (9.8%) in Venezuela (Chacin-Bonilla et al, 2001) and in India (6.6%) (Deodhar et al, 2000). The present data, however, is not in agreement with the findings of both reports previously done in similar setting in Kathmandu, Nepal. Sherchand et al (1996) reported positive rates of 0.0% and 0.1% for Cyclospora in children and adults with abdominal discomfort, respectively. Subsequently, in 1999, they reported a very high positive rate
(29.8%) in samples collected in various health care facilities in Nepal (Sherchand et al, 1999).

Overall, no differences in positive rate was seen between the sexes at all age groups.

Cyclospora infections do not appear to induce immunity in humans, therefore, reinfection can occur at all ages. In endemic countries, for instance in Nepal, the annual attack rate for Cyclospora species has been reported to be as high as 40% (Sherchand et al, 1999). A higher positive rate was observed in children (10 years and under), which appears to be associated with their hygienic habits contributing to higher attack rates. Children and visitors from developed countries are more susceptible to diarrheal illness. Adults from developed countries, who moved to developing countries, such as Nepal, remain at high risk for diarrhea during their first two years of residence (Shlim et al, 1999).

The present study also showed marked seasonal variation of Cyclospora infection with a high incidence during summer (the peak was seen in the month of July). This has also been reported by other investigators from elsewhere (Connor, 1997) and from Nepal (Sherchand et al, 1999). During summer, other diarrheagenic organisms also become active, including cholera (Rai et al, 2003). This has been attributed to a poor sanitary system leading to fecal contamination of environment. The heavily polluted river and well water is used for irrigation purposes. Before bringing to market, vegetables grown in contaminated fields are dipped and rinsed in a polluted river, well or pond water for the purpose of cleaning. Even drinking water sources in the Kathmandu Valley are heavily contaminated with fecal matter (Adhikari et al, 1986). This is due to the influx of people in the valley, unplanned housing, poor sewerage systems, and rainfall causing street flooding and environmental contamination with fecal matter. Chlorination is virtually ineffective in Kathmandu probably due to the high level of contamination with organic matter. Oocysts of Cyclospora are resistant to chlorination compared with diarrheagenic bacteria, and can be present even in coliform-free water. It appears, therefore, that water and vegetables plays a major role in transmitting Cyclospora infection in Nepal as reported elsewhere. Oocysts of Cyclospora have also been detected in sewage water, vegetables, and feces of animals and birds (Sherchand et al, 1999) which may be an additional source of environmental contamination (Connor, 1997; Doeller et al, 2002).

The lowest positive rate was observed from January to March, the driest period of the year, indicating that oocysts cannot survive long in the environment during the dry season. Month-wise, the peak incidences were observed in the months of July and August. The infection rate correlated with temperature and especially with rainfall, where the correlation was remarkably high.

Higher positive rates were observed in schoolchildren and patients from surrounding Bansbari and Jorpati, indicating that Cyclospora was more common in village setting. Though the sample size was small, it was interesting to note that none of the samples collected in small hilly areas were positive. The present findings suggest the parasite was common in villages inside the Kathmandu Valley. This may correlate with the influx of people in the valley from elsewhere in Nepal resulting in slum conditions.

In contrast, only one of the 686 samples from the Lao PDR was found to be positive for C. cayetanensis oocysts. To the best of our knowledge, no cases of Cyclospora infection in Lao PDR have been reported (Phetsouvanh et al, 1999), this is the first case reported. This indicates that Cyclospora is not active in Lao PDR in spite of a high prevalence of other intestinal parasites (Vannachone et al, 1998). Because of the high morbidity and mortality of people with diarrheal diseases, a high priority should be placed on improving the sanitary and environmental conditions in Nepal.

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REFERENCES


