STUDY ON *CYCLOSPORA CAYETANENSIS* ASSOCIATED WITH DIARRHEAL DISEASE IN NEPAL AND LAO PDR

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Abstract. A study of diarrheal diseases associated with *Cyclospora cayetanensis* was conducted in Nepal and Lao PDR. A total of 2,083 samples were included in this study. Samples in Nepal were collected from October 1999 to August 2002 whereas samples in Lao PDR were collected from February 2002 to June 2003. *C. cayetanensis* was detected by direct microscopy using ultraviolet and differential interference contrast microscopy. The overall positive rate in Nepal was 9.2% (128/1,397). A higher positive rate was observed in children aged 10 years and under (11.1%) and was lowest in the age group of 51-60 years (3.1%). A significantly higher positive rate was observed in the summer (rainy season) (12.6%) with the lowest prevalence in the spring (dry season) (1.8%) ($p<0.05$). The positive rate was closely associated with rainfall (ml/month). Interestingly, only one of the total 686 samples (0.1%) from Lao PDR was found to be positive for *Cyclospora* oocysts.

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

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in the feces of both immunocompetent and immunocompromized 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 immunocompromized 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 4-10ºC 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 (oocysts/whole field). The findings were stratified by the age and sex of the subjects, sample collection area (centers), months, seasons (autumn: September-November, winter: December-February, spring: March-May, and summer: June-August), years and rainfall precipitation for the whole study period.

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 mm^2). 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 1–Country map and sampling point in Nepal and Lao PDR.

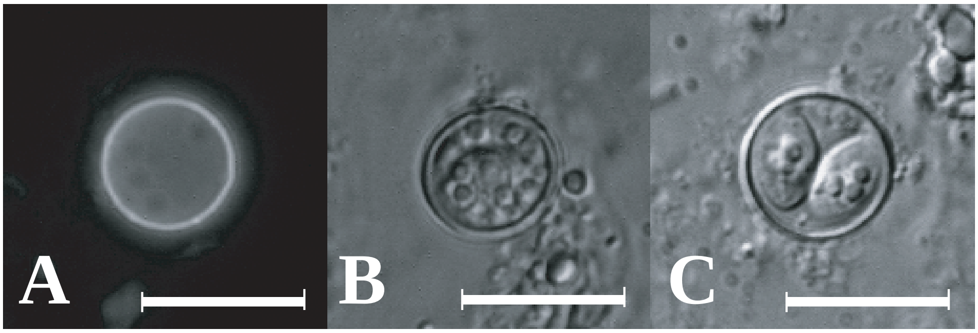

Fig 2–*Cyclospora* oocysts detected in fecal samples from Nepal. Fluorescence microscopy observation (A), DIC microscopy showing of immatured oocyst (B), and matured oocyst (C) (scale: 10 μm).

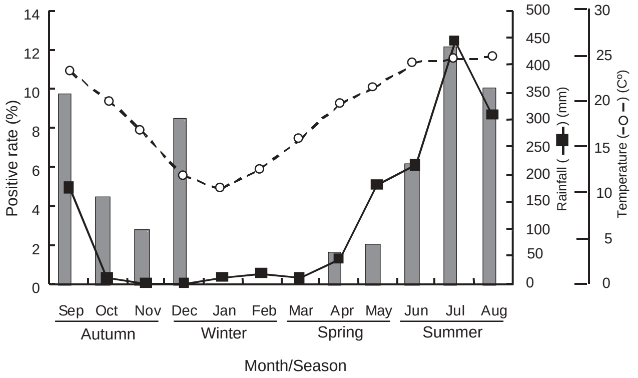

Fig 3–Relationship between positive rate of *C. cayetanensis* and rainfall/temperature in the three investigation periods during 1999-2002.

sons ($p<0.05$). This was followed by autumn (6.8%, 18/265) and winter (2.4%, 2/85) and the, lowest being in spring (1.8%, 4/221). Month-wise, a peak-positive rate was observed in the month of July followed by August but this was not true for all three years. The samples were positive for *Cyclospora* mainly during the rainy and warm seasons (Fig 3).

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.

Table 1
Positive rates of *C. cayetanensis* in diarrheal human fecal samples collected in Nepal.

Sex	Number of Sample	Number of Positive	%	p-value
Male	883	79	8.9^{a}	>0.05^{a,b}
Female	514	49	9.5^{b}	
Total	1,397	128	9.2	

Table 2
Positive rates of *C. cayetanensis* among different age groups in Kathmandu, Nepal.

Age group	Number of Sample	Number of Positive	%	p-value
0-10	520	58	11.1^{a}	
11-20	244	22	9.1^{b}	>0.05^{a,b}
21-30	353	33	9.3^{c}	>0.05^{a,c}
31-40	140	9	6.4^{d}	>0.05^{a,d}
41-50	61	3	4.9^{e}	>0.05^{a,e}
51-60	32	1	3.1^{f}	>0.05^{a,f}
61-81	47	2	4.3^{g}	>0.05^{a,g}
Total	1,397	128	9.2	

Table 3
Positive rates of *C. cayetanensis* in individual from different locations in Kathmandu, Nepal.

Location^{a}	Number of Sample	Number of Positive	%
Maharajgunj	895	78	8.7
Putali Sadak	205	16	7.8
Boudha	180	17	9.4
Bansbari	71	9	12.7
Jorpati	46	8	17.4

^{a}Maharajgunj: northern part of Kathmandu Valley, Putali Sadak: central part, Boudha and Jorpati: eastern part, Bansbari: northeastern part, schoolchildren.

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, leading to environmental contamination. 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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