PARASITIC EPIDEMIOLOGICAL STUDIES OF *CYCLOSPORA CAYETANENSIS* IN NEPAL

Jeevan Bahadur Sherchand[1] and John H Cross[2]

[1]Tribhuvan University Teaching Hospital, Health Research Laboratory and Infectious and Tropical Disease Research and Prevention Center, Kathmandu, Nepal;
[2]Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

Abstract. Prior to 1995, the coccidian parasite *Cyclospora cayetanensis* was reported as a cause of gastroenteritis among children living in poor sanitary conditions and adults from industrialized countries who lived or traveled in developing countries. In 1999 and 2001, *Cyclospora* infection in Nepal was linked to the consumption of undercooked, contaminated green leafy vegetables and exposure to contaminated water. Some domestic animals were also found passing *Cyclospora*-like oocysts. To extend our understanding of the parasitosis, we conducted a surveillance in health-care facilities and studied vegetable farmers, as well as carrying out a case-control study. This was done to assess risk factors for the disease in Nepal. From September 2, 2002 to August 30, 2003, 137 (6.4%) of 2,138 surveillance specimens tested positive for *Cyclospora*; prevalence peaked in June with 36/137 (26.3%). Infection was most common in children 2-9 years of age and in adults with gastroenteritis. Among 176 vegetable farmers and family members monitored from June 25 to August 30, 18 had *Cyclospora* infection. In the case-control analysis, 61 (95.3%) of 64 persons with *Cyclospora* infection reported drinking unsafe water within 12 days before illness, compared with 64 (74.4%) of 86 controls [Odds ratio (OR) 7.0, at 95% confidence intervals (CI)]. Other risk factors included water sources, type of sewage drainage, domestic animals or chickens in the household, and contact with soil. In addition, we observed a pattern of clinical and epidemiological finding associated with the detection of cyclosporiasis.

INTRODUCTION

Cyclospora cayetanensis, a coccidian parasite of human pathogen (Ortega *et al,* 1993; Chiodini, 1994), causes explosive watery diarrhea that can persist for several weeks (Soave, 1996; Cross and Sherchand, 1997). The mechanisms of transmission of *Cyclospora* have not been determined, but a marked seasonality has been observed in endemic areas around the world, including Nepal (Cross *et al*, 1997; Sherchand, 2001). Although *C. cayetanensis* has been reported from all areas of the world, the biology of the organism and the means of transmission remain an enigma. Susceptible humans are suspected to be infected by ingesting sporulated oocysts. Water is probably an important vehicle, either drinking parasite-contaminated water directly or indirectly when water is used to grow plant foods. Water has been implicated in outbreaks in the United States, (Huang *et al,* 1995) and in Nepal (Rabold *et al,* 1994; Sherchand and Cross, 1999, 2001). Food-borne transmission is also suspected, with reports of oocysts in washing of leafy vegetables in Peru (Ortega, 1997) and Nepal (Sherchand *et al,* 1999; Sherchand and Cross, 2002). Patients suffer from a chronic watery diarrhea, fatigue, nausea, vomiting, abdominal cramps, anorexia, weight loss and myalgia. However, what is responsible for the pathogenesis is unknown.

Our study, between 1995 and 2002 in Nepal, has identified that possible sources of infection with *Cyclospora* are consumption of raw or undercooked, contaminated, green leafy vegetables, and exposure to contaminated water. Some domestic animals, such as chickens, dogs, monkeys and rats, were also found passing *Cyclospora*-like oocysts (Sherchand and Cross, 2001, 2002). To extend our knowledge of the parasitosis, the present studies were carried out from September 2, 2002 to August 30, 2003, to determine the prevalence of *Cyclospora* infection from different areas of healthcare institutions and community vegetable farmers, as well as a case-control study, to assess possible risk factors for cyclosporiasis in Nepal. In addition, animal specimens, water, vegetables and fruits were also collected and examined to determine the possible sources of infection.

Correspondence: Jeevan Bahadur Sherchand, Tribhuvan University Teaching Hospital, Health Research Laboratory and Infectious and Tropical Disease Research and Prevention Center, PO Box: 10404, Kathmandu, Nepal.
Tel: 977-1-4264115
E-mail: itdrc@healthnet.org.np

MATERIALS AND METHODS

Surveillance health care institution

In four outpatient clinics and hospitals of

Kathmandu, Kavre and Rupendehi districts, were included for study. Stool specimens were collected from those patients whose healthcare providers had ordered stool for parasitic and bacteriological examinations. In each center, we screened stool samples and the information about age, sex, current diarrhea status (defined as 3 or more loose or liquid stools in 24 hours) or other gastrointestinal and other clinical symptoms, were recorded.

Vegetable farmers cohort study

On the basis of verbal consent, 176 vegetable farmers and their family members were included in the study between June 25 to August 30, 2003. Before collection of stool specimens, the aim of the study was described in detail to the participants. Early-morning specimens were collected every week and each time a participant reported clinical symptoms of gastroenteritis was recorded. Participants found to have *Cyclospora* infection were treated with trimethoprim-sulfamethaxole and were monitored until their stool specimens were negative. If the participants were infected with helminthic parasites, they were given a single dose of albendazole.

Case-control study

From June 25 to August 30, 2003, we recruited participants from health-care institutions for the case-control study. All persons with symptomatic *Cyclospora* infection detected through the surveillance were sought in their homes; however, some could not be located because of incorrect addresses. The aim of the visit was to distribute antibiotic treatment; at the same time, we solicited participation in the case-control study. We also requested stool specimens from family members of case patients. For controls, we included those persons who had a stool specimen screened in the surveillance health care institutions during the period in which we were enrolling cases. Participants were eligible to be controls if they reported no gastrointestinal symptoms and had negative stool results. A questionnaire was administered to focus on potential risk factors for *Cyclospora*, including drinking-water source and handling, household sanitation, presence of animals, socioeconomic variables and consumption of vegetables and local fruits.

Laboratory methods

Stool specimens were processed by standard formalin-ethyl acetate concentration method and examined by three methods: direct light-microscopy examination, stool smear modified acid-fast stain and UV epifluorescence examination of a wet mount (Eberhard *et al,* 1999). To confirm the identity of the parasite as *Cyclospora cayetanensis,* the positive samples were stored in 2.5% dichromate solution at room temperature (22˚ to 25˚C) and were examined at regular intervals over a 2-week period, starting from the time the sample was collected. We observed microscopically sporulation characteristics of all positive samples.

Samples of water and green leafy vegetable

During sampling collections in the different areas, samples from irrigation canal, sewage water, different sources of public drinking water and green leafy vegetables were collected to determine contamination with *Cyclospora* oocysts and to investigate possible sources of infection. The leaves were washed in distilled water, the washings and samples of water were centrifuged and the sediments were examined microscopically. Excess amounts of sediment were resuspended in 2.5% potassium dichromate solution, and the sporulation recovery was noted.

RESULTS

Health care institution surveillance

A total of 2,138 stool samples was examined during the period September 2, 2002 to August 30, 2003, from healthcare institutions in different parts of Nepal (Fig 1). Oocysts of *Cyclospora cayetanensis* were found in 137 (6.4%) specimens, and the prevalence rate peaked in June, at 26.3% (Fig 2). In the winter season, December, January, and February, *Cyclospora* infection was detected in less than 1% of specimens. *Cyclospora* infection was more frequent among children and among persons with gastroenteritis (Table 1).

Age and sex-wise distribution of *Cyclospora* infection

A total of 2,138 stool samples was collected from 974 males and 1,164 females, ranging in age from 2 to 70 years of age (Table 1). Of the 137 positive patients, 59 (43.1%) were male and 78 (56.9%) were female. Although we did not collect data concerning TB and HIV status, one clinic submitted 42 specimens from HIV-infected patients, and 6 (14.3%) were positive for *Cyclospora.* Among 68 TB infected patients, 5 (7.3%) were *Cyclospora*-positive.

Vegetable farmer cohort study

The vegetable farm cohort study consisted of 176 farmers and family members who submitted stool specimens from June 25 to August 30, 2003. Eighteen (10.2%) were *Cyclospora*-positive, among whom 5 occurred in family members aged 2 to 10 years; all

Fig 1- Map of Nepal showing location of study areas.

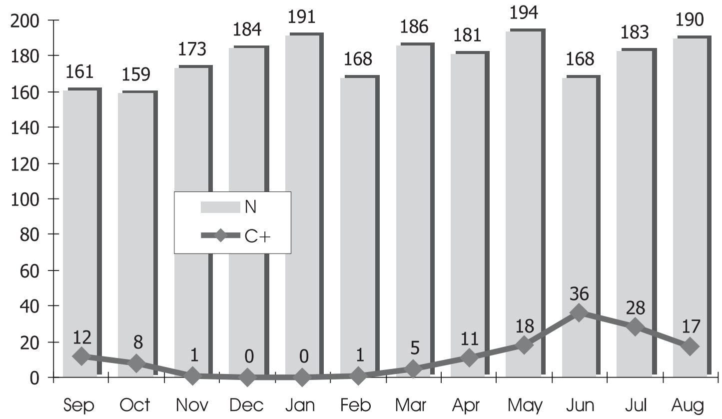

Fig 2- Surveillance of *Cyclospora cayetanensis* from four healthcare institutions, by month.

Table 1
Prevalence of *Cyclospora* in specimens from outpatients attending four health care institutions (September 2, 2002 to August 30, 2003).

Prevalence by age group		Prevalence by symptoms	
Age (yrs)	n+ve/NS (%)	n+ve/NS (%) gastroenteritis	n+ve/NS (%) no gastroenteritis
2-5	44/ 428 (10.3)	37/ 305 (12.1)	7/ 123 (5.7)
6-9	42/ 532 (7.9)	31/ 411 (7.5)	11/ 121 (9.1)
10-13	21/ 490 (4.3)	16/ 325 (5.0)	5/ 165 (3.0)
14-17	17/ 402 (4.2)	12/ 306 (4.0)	5/ 96 (5.2)
> 18	13/ 286 (4.5)	11/ 215 (5.1)	2/ 71 (2.8)
All ages	137/ 2,138 (6.4)	107/ 1,456 (7.3)	30/ 682 (4.4)

had diarrhea and gastroenteritis. Three farmers were asymptomatic and another 9 had abdominal pain and discomfort, whereas one farmer had blood in his stool. Other common intestinal parasites were found in these farmers and their family members; 41.5% were positive for hookworm, 35% for *Ascaris lumbricoides,* 55% for *Trichuris trichiura,* 40% for *Giardia lamblia,* 12.6% for *Entamoeba histolytica*, and 1.5% for *Cryptosporidium.* More than 40% had nonpathogenic protozoa, such as *Entamoeba coli, Iodamoeba butschlii.* No organisms were detected in only 8% of the samples. Of the 18 *Cyclospora*-positive stool samples, 32.5% were coinfected with *Giardia*, 5% with *Hymenolepis nana*, and 25% had more than one parasitic coinfection.

Case-control study

During the case control study, 64 persons were *Cyclospora* infections, and 86 controls. Of 64 cases, 38 (59.3%) were between the ages of 2-9 years, and 31 (48.4%) were males. Among the 86 controls, 48 (55.8%) were 2-9 years of age, and 51 (59.3%) were male (p >0.05). In addition to sample collections from the case-control study, we screened stool specimens from 169 family members belonging to 52 families of case patients. Of these, 38 persons from 34 families tested positive for *Cyclospora*. The detection rate was highest among children 2-9 years (21/74; 28.4%).

According to available data on the characteristics of illness in 64 patients in the case-control study (Table 2), the median duration of illness before diagnosis was 12 days in those 2-9 years of age and 10 days in those >9 years of age. Young children had a higher number of stools per day than older patients and were significantly more likely to have fever and mucoid stools. Of the 64 patients, 37 (57.8%) complained of watery or liquid stools, but only 5 (7.8%) reported normal (soft) defecation. Of the 64 for whom information was available, 27 (42.2%) had been treated with an antibiotic, most often metronidazole, cotrimoxazole, anti-helminthic drugs, such as mebendazole or albendazole, and occasionally herbal medicine, before *Cyclospora* was diagnosed.

Other risk factors associated with *Cyclospora* infection

Risk factors associated with *Cyclospora* infection from the case-control data analysis were related to

Table 2
Clinical characteristics of 64 persons with *Cyclospora* infection who participated in the case control study.

Characteristics of persons	Age group of participants: 2-9 years (n=38)	Age group of participants: > 9 years (n=26)	p-value
Days of illness	12 (3-65)	10 (2-75)	> 0.05
Stool per day	6 (1-10)	4 (1-15)	> 0.05
Stool consistency	N (%)	N (%)	
Liquid/ watery	25 (65.8)	12 (46.2)	
Semisolid	12 (31.6)	10 (38.5)	
Soft	1 (2.6)	4 (15.4)	
Blood in stool	11 (29.0)	2 (7.7)	> 0.05
Mucus in stool	28 (73.7)	8 (30.8)	< 0.005
Chinical symptoms			
Fever	25 (65.8)	7 (27.0)	< 0.005
Vomiting	15 (39.5)	6 (23.1)	
Abdominal pain	12 (31.6)	11 (42.3)	
Anorexia	9 (23.7)	7 (27.0)	
Headache	3 (8.0)	5 (19.2)	
Treatment information			
Had any antibiotics	13 (34.2)	14 (53.8)	
Metronidazole	8 (21.05)	12 (46.1)	
Cotrimoxazole	3 (8.0)	5 (19.2)	
Antihelminthic	8 (21.05)	12 (46.2)	
Herbal medicine	3 (8.0)	6 (23.1)	

sources of water, sanitation, and keeping domestic animals in the household (Table 3). Persons with *Cyclospora* infection were significantly more likely than controls to report drinking untreated water within the 12 days before illness, having obtained drinking water from an unsafe water source, or drinking water from a river or spring. In addition, having a septic tank rather than municipal sewage drainage, and having had direct contact with soil, were associated with an increased risk of infection. Persons with *Cyclospora* infection were twice as likely as controls to own dogs or chickens, while other animals, such as cats and pigs, were not associated with increased risk of infection. We asked about eating different fresh uncooked produce in the 12 days before illness, including lettuce, carrot, cabbage, radish, mint and bushberries; none was associated with an elevated risk of *Cyclospora* infection. However, examination of the washed solutions of green leafy vegetables, such as cabbage, lettuce, spinach and mustard leaves, found them to be contaminated with *Cyclospora* (Table 4).

Examination of vegetables for *Cyclospora*

Green vegetables collected from study areas and local vegetable markets consisted of cabbage, lettuce, cauliflower, spinach, green onion, radish, green leafy vegetables, mustard leaves and carrot; cabbage, lettuce, spinach and mustard leaves were found to be contaminated with *Cyclospora* (Table 4). *Cyclospora* was further confirmed by the development of 2 sporocysts, after 2 weeks' incubation in potassium dichromate solution.

Table 3
Characteristics of risk factors associated with *Cyclospora* infection, among 64 cases and 86 controls.

Characteristic	Proportion with characteristic [n/ N (%)]		
	Cases	Control	OR (95% CI)
Unsafe water source[a]	16/ 64 (25)	11/ 86 (12.8)	2.1
Drank untreated water[b]	61/ 64 (95.3)	64/ 86 (74.4)	7.0
Drank river or spring water	13/ 64 (20.3)	6/ 86 (7.0)	3.4
Contact with soil/ field	48/ 64 (75.0)	52/ 86 (60.5)	1.9
Dogs	29/ 64 (45.3)	28/ 86 (32.6)	1.7
Chickens	37/ 64 (57.8)	42/ 86 (48.8)	1.4
Any animals in household	44/ 64 (68.7)	54/ 86 (62.8)	1.3
Uncooked vegetables	23/ 64 (36.0)	26/ 86 (30.2)	1.2

n= number positive for *Cyclospora*; N= number of specimens
[a] Unsafe water source=public standpipe, well, spring, water truck. Little safe water as municipal water piped into house, or commercial bottled water.
[b] Drank untreated water=not commercially bottled and had not been boiled, chlorinated, or filtered before drinking.

Table 4
Study of *Cyclospora* oocyst contamination in vegetables, June to August 2003.

Samples	June N/ +ve (%)	July N/ +ve (%)	August N/ +ve (%)
Cabbage	47/ 2 (4.2)	31/ 0	34/ 0
Lettuce	40/ 0	67/ 3 (4.5)	44/ 0
Cauliflower	21/ 0	30/ 0	41/ 0
Green onion	44/ 0	41/ 0	36/ 0
Radish	42/ 0	38/ 0	42/ 0
Spinach	38/ 3 (7.9)	42/ 0	41/ 0
Mustard leaves	54/ 0	56/ 2 (3.6)	55/ 0
Carrots	45/ 0	41/ 0	43/ 0

N= Number of different vegetable samples; +ve = Positive for *Cyclospora*; (%)= Percentage

Table 5
Parasitological study of *Cyclospora* from different sources of water, June to August 2003.

Sources of water	June +ve/ N (%)	July +ve/ N (%)	August +ve/ N (%)
Tap water	0/ 31	0/ 22	0/ 30
Tube-well	0/ 13	0/ 14	0/ 15
Pond	0/ 15	0/ 16	0/ 15
Sewage	2/ 12 (16.7)	3/ 14 (21.4)	0/ 12
Irrigation canal	0/ 11	1/ 11 (9.0)	0/ 10
River or spring water	0/ 3	0/ 4	0/4

Examination of water samples

Water samples were collected from different areas and studied for *Cyclospora*, as shown in Table 5. *Cyclospora* contamination was found in June and July in irrigation canal and sewage water. Several other parasites: *Giardia lamblia*, *Amoeba,* unidentified trophozoites, ova of *Ascaris lumbricoides*, larvae of helminths and many small insects and worms were detected. In 3 sources of water samples (pond, sewage and irrigation canal), *Cryptosporidium parvum* oocysts were also identified.

Examination of stool samples from different animals

Of a total 504 different animal samples examined, 2.2% animals were found to be positive for *Cyclospora* oocysts. Among these, chickens (4.2%), monkeys (5.7%), dogs (5.1%), and rats (2.9%) were found positive, as depicted in Table 6.

Table 6
Cyclospora oocysts examination in different animals (2003).

Animals	No. examined	Cyclospora-positive
Chicken	72	3 (4.2%)
Pig	30	0
Monkey	35	2 (5.7%)
Dog	78	4 (5.1%)
Cat	33	0
Cow	51	0
Buffalo	43	0
Goat	62	0
Rat	68	2 (2.9%)
Pigeon	32	0
Total	504	11 (2.2%)

DISCUSSION

Cyclospora cayetanensis is a coccidian parasite that infects humans and causes prolonged diarrhea in both immunocompetent and immunocompromized hosts. The mechanisms of pathogenesis and virulence factors of *Cyclospora cayetanensis* are yet to be defined, but tissue damage and jejunitis have been reported (Ortega, 1997). The present study advances our knowledge of the epidemiology of *Cyclospora cayetanensis* in Nepal, where there are repeated outbreaks of emerging diseases associated with gastroenteritis. Although *Cyclospora* infection has been reported from all parts of the world, most epidemiological information comes from studies in Nepal, Haiti, Peru, and the United States, where it is endemic (Ortega *et al,* 1993; Hoge *et al,* 1995; Zerpa *et al,* 1995; Cross and Sherchand, 1997; Sherchard *et al*, 1999; Sherchand and Cross, 2001, 2002). Cyclosporiasis appears to be seasonal, with peak incidence during the rainy seasons, from April to June in Peru, and May to September in Nepal (Hoge *et al,* 1993; Ortega *et al,* 1993; Cross and Sherchand, 1997; Sherchand *et al*, 1999; Sherchand and Cross, 2001). Although all age groups can acquire the disease, the highest attack rates occur among children older than 18 months (Hoge *et al,* 1995), whereas in our study the highest attack rates was found among children aged between 2-5 years. There is no apparent immunity to infection, and reinfection can occur at any ages (Connor *et al,* 1993).

Cyclospora is an increasingly recognized cause of traveler's diarrhea, causing up to 11 to 20% of cases of diarrhea in studies of expatriates in Nepal (Shlim *et al,* 1991; Hoge *et al,* 1995). In the United States, the outbreak of diarrheal disease associated with *Cyclospora* in 21 medical residents in 1990 was epidemiologically linked to a contaminated water supply (Huang *et al,* 1995). Subsequently, more than 1,000 confirmed cases in the US and Canada were reported (CDC, 1996). In this study, oocysts of *Cyclospora* were found in irrigation canal and sewage water in June and July, during the high transmission period. *Cyclospora* infection occurs most commonly via contaminated water (Rabold *et al,* 1994; Huang *et al,* 1995) and the oocysts are resistant to chlorination and not readily detected by methods currently used to assure the safety of drinking water. *Cyclospora* has been detected in water samples, however, the available method for detection in water has very low sensitivity (Sturbaum *et al,* 1998). Contaminated food has long been proposed as a possible route for transmission of *Cyclospora* (Connor and Shlim, 1995). Vegetables, in particular, are suspicious since they are often ingested raw or undercooked. Vegetables are easily contaminated and provide organisms with an optimal environment for survival prior to host ingestion. It is believed that *Cyclospora* must sporulate for at least 7-10 days in the environment to be infectious. Fertilization of plants with human waste or indirectly via contaminated water used for crop irrigation and to freshen produce could lead to contamination of vegetables with *Cyclospora.* In this study, cabbage, lettuce, spinach and mustard leaves were found to be contaminated with *Cyclospora*, which confirmed that food-borne transmission is feasible. It remains to be determined if recovered oocysts are infectious. The source of vegetable contamination with oocysts is still unknown, but it may be due to fecally-contaminated water used on the vegetables from irrigation water or directly from food handlers. Moreover, in Nepal, vegetables coming to the markets are dipped and rinsed in the highly contaminated water of small ponds or rivers, to wash and clean them, but they actually become contaminated again. There are thousands of such instances of how food is rendered unsafe due to unhygienic conditions, handling and practices, and poor environment. In cities of Nepal, the water supply is contaminated through the seepage of sewage into water pipes. In rural areas, the source of water itself (wells, ponds, rivers etc) is polluted by waste disposal. One common food contamination problem is insects and rodents, and, as a result, food becomes unfit for human consumption.

The main limitation of this study was the inability to test for other diarrheal pathogens. We could not be certain that every gastroenteritis episode, during which we detected cyclosporiasis, was due to *Cyclospora.* However, in the aggregate data, we observed a consistent epidemiological and clinical pattern associated with detection of *Cyclospora.* Although one year of surveillance is insufficient to say with certainty what the pattern of *Cyclospora* infections will be from year to year, the data so far suggest that the seasonality of *Cyclospora* in Kathmandu City is similar to that in Guatemala, at approximately the same altitude (1,200-1,500m) above sea level (Hoge *et al,* 1993; Sherchand and Cross, 2002). In Nepal, a possible source of infection appears to be untreated water, that water truly is an important vehicle for *Cyclospora* in Nepal is underlined by the other water-related factors that were associated with infection. Our results support the findings of other studies, that contaminated water is a likely source of infection (Hoge *et al,* 1995; Sherchand and Cross, 2002). However, multiple routes of transmission for *Cyclospora* in Nepal almost certainly exist. Among very young children, soil contact was a strong risk factor; an outbreak investigation in Guatemala (1999) also raised the possibility that soil might be a potential source of infective oocysts (Bern *et al,* 1999). Family members of patients had a rate of infection similar to that of persons in the same age group screened in our surveillance system at the same time of year, a finding consistent with postulated lack of direct person-to-person transmission.

The fact that the presence of chickens or other domestic animals was a significant risk factor is intriguing, but difficult to interpret. Scientists have failed to establish experimental *Cyclospora* infection in any bird; a limited survey of more than 100 birds captured in June/July 2001 and 2002, in Nepal, did not demonstrate natural infection, although other coccidians have been observed (Eberhard *et al*, 1999).

Cyclospora is a pathogen commonly associated with pediatric gastroenteritis in Nepal, especially from June through August. The seasonality of *Cyclospora* in Kathmandu follows a pattern similar to that seen in Guatemala. Our study on case-control analysis suggests that contaminated water, and, for young children, soil, are likely vehicles of transmission. Although more studies are needed to clarify the direct link between *Cyclospora* infection and these sources, the results suggests that sewage water and green leafy vegetables are possible sources of infection in Nepal.

To obtain more evidence on the source of infection, specimens from rodents, birds, insects and domestic animals are needed for extensive study. Wider dissemination of skilled laboratory diagnosis is a prerequisite for a better understanding of the epidemiology of this infection and its association with disease. *In vitro* cultivation systems for drug screening and controlled trials of drug therapy are needed. Better knowledge of the behavior of *Cyclospora* in AIDS patients, along with other coccidia, *Cryptosporidium, Isospora*, and *Toxoplasma* need to be studied in the context of Nepal. In addition, more sensitive, inexpensive, reliable, diagnostic tools are urgently needed to help establish how fresh produce becomes contaminated.

ACKNOWLEDGEMENTS

The authors thanks the following members for their co-operation: Dr Dirgh Singh Bam, Dr Chandra Shakya, Dr Shushil Shakya, Ms Sarala Sherchand, Mr Govinda Gurung, Mr Ashok Chaudhary and all staff of Tribhuvan University Institute of Medicine and Infectious and Tropical Disease Research and Prevention Center/ Nepal.

REFERENCES

Bern C, Hernandez B, Lopez MB, *et al.* Epidemiologic studies of *Cyclospora cayetanensis* in Guatemala. *Emerg Infect Dis* 1999;5:766-74.

Centers for Disease Control and Prevention (CDC). Update: outbreaks of *Cyclospora cayetanensis* infection–United States and Canada, 1996. *MMWR Morb Mortal Wkly Rep* 1996;45:611-2.

Chiodini PL. A new parasite: human infection with *Cyclospora cayetanensis*. *Trans R Soc Trop Med Hyg* 1994;88:369-71.

Connor BA, Shlim DR, Scholes JV, Rayburn JL, Reidy J, Rajah R. Pathogenic changes in the small bowel in nine patients with diarrhoea associated with a coccidia-like body. *Ann Intern Med* 1993;119:377-82.

Connor BA, Shlim DR. Foodborne transmission of *Cyclospora*. *Lancet* 1995;346:1634.

Cross JH, Sherchand JB. *Cyclospora cayetanensis*: "Look and you will find". *Int Med Res J USA* 1997; 1:81-5.

Cross JH, Sherchand JB, Sharma P, Echeverria P. *Cyclosporiasis* at the Kanti Children's Hospital in Kathmandu, Nepal: a cursory survey. *J Trop Med Parasitol* 1997;20:30-2.

Eberhard ML, Nace EK, Freeman AR, *et al.* *Cyclospora cayetanensis* infections in Haiti: a common occurrence in the absence of watery diarrhea. *Am J Trop Med Hyg* 1999;60:584-6.

Hoge CW, Shlim DR, Ghimire M, *et al*. Placebo-controlled trial of co-trimoxazole for *Cyclospora* infections among travellers and foreign residents in Nepal. *Lancet* 1995;345:691-3.

Hoge CW, Shlim DR, Rajah R, *et al*. Epidemiology of diarrhoeal illness associated with coccidian-like organisms among travelers and foreign residents in Nepal. *Lancet* 1993;341:1175-9.

Huang P, Weber J, Sosin DM. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. *Ann Intern Med* 1995;123:409-14.

Ortega YR, Roxas CR, Gilman RH, *et al.* Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *Am J Trop Med Hyg* 1997;57:683-6.

Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. *Cyclospora* species- a new protozoan pathogen of humans. *N Engl J Med* 1993;328:1308-12.

Rabold JG, Hoge CW, Shlim DR, Kefford C, Rajah R, Echeverria P. *Cyclospora* outbreak associated with chlorinated drinking water. *Lancet* 1994;344:1360-1.

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

Sherchand JB, Cross JH. Emerging pathogen *Cyclospora cayetanensis* infection in Nepal. *Southeast Asian J Trop Med Public Health* 2001;32:143-50.

Sherchand JB, Cross JH. Studies on *Cyclospora cayetanensis* infection in Nepal. Vancouver, Canada: The 10th International Congress of Parasitology-ICOPA 10, August 4-9, 2002: 71-88.

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

Soave R. *Cyclospora*: an overview. *Clin Infect Dis* 1996;23:429-378.

Sturbaum GD, Ortega YR, Gilman RH, Sterling CR, Cabrera L, Klein DA. Detection of *Cyclospora cayetanensis* in wastewater, *Appl Environ Microbiol* 1998;64:2284-6.

Zerpa R, Uchima N, Huicho L. *Cyclospora cayetanensis* associated with watery diarrhoea in Peruvian patients. *J Trop Med Hyg* 1995;98:325-9.