Epidemiologic Studies of *Cyclospora cayetanensis* in Guatemala

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In 1996 and 1997, cyclosporiasis outbreaks in North America were linked to eating Guatemalan raspberries. We conducted a study in health-care facilities and among raspberry farm workers, as well as a case-control study, to assess risk factors for the disease in Guatemala. From April 6, 1997, to March 19, 1998, 126 (2.3%) of 5,552 surveillance specimens tested positive for *Cyclospora*; prevalence peaked in June (6.7%). Infection was most common among children 1.5 to 9 years old and among persons with gastroenteritis. Among 182 raspberry farm workers and family members monitored from April 6 to May 29, six had *Cyclospora* infection. In the case-control analysis, 62 (91%) of 68 persons with *Cyclospora* infection reported drinking untreated water in the 2 weeks before illness, compared with 88 (73%) of 120 controls (odds ratio [OR] 3.8, 95% confidence interval [CI] 1.4, 10.8 by univariate analysis). Other risk factors included water source, type of sewage drainage, ownership of chickens or other fowl, and contact with soil (among children younger than 2 years).

Before 1995, the coccidian parasite *Cyclospora cayetanensis* was primarily reported as a cause of gastroenteritis among children living in poor sanitary conditions (1,2) and adults from industrialized countries who lived or traveled in developing countries (3-5). From 1990 to 1995, three small *Cyclospora* outbreaks were reported in North America, at least one of them epidemiologically associated with drinking water (6-8). However, in the spring and early summer of 1996, the largest ever reported outbreak of cyclosporiasis, affecting more than 1,400 persons in North America (9), was associated with eating fresh raspberries; trace-back data indicated that the source of the berries was Guatemala (9). A year later, despite improvements in water quality and sanitary practices instituted on berry farms after the 1996 outbreak, Guatemalan raspberries were again implicated in cyclosporiasis outbreaks (10).

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The source of contamination of the implicated raspberries has not been established. Most likely, fecal contamination of water used for spraying fungicides and other substances directly on fruit was the source (9). Another possibility is that berries were contaminated with sporulated organisms during hand-sorting and packing; however, because *Cyclospora* may require 1 to 2 weeks in the environment to become infectious (2), direct fecal contamination from the hands of an infected worker is less likely. The possibility that berries might have been contaminated by bird droppings before being picked has also been raised (11), although no natural (12) or experimental infection with *C. cayetanensis* has been confirmed in any animal (M. Eberhard, pers. comm.). Studies of *Cyclospora* have been limited by this lack of an animal model; by dependence on microscopy for detection; by the lack of sensitivity of the available methods in water, fruit, and other environmental specimens; and by inability to culture the organism.

Cyclospora in Guatemala was first reported in 1994 (13). Since then, the organism has been reported among AIDS patients (14) and in several case series in hospital outpatient departments in Guatemala City (15-17). However, diagnosis of *Cyclospora* infection is not part of routine stool examination in health-care facilities in Guatemala.

In 1997, we initiated a study whose objectives were to provide the first comprehensive description of the epidemiology of *Cyclospora* in Guatemala and explore potential environmental sources of contamination. The study included surveillance among patients in outpatient departments and among raspberry farm workers, a case-control study to identify risk factors for *Cyclospora* infection, and screening of surface water specimens for fecal contamination and for *Cyclospora* oocysts.

Methods

Outpatient Surveillance

In two government health centers (both in the Department of Guatemala) and three hospital outpatient departments (two in the Department of Guatemala and one in the Department of Sacatepequez), we interviewed outpatients whose health-care providers had ordered stool specimens for bacterial pathogen testing or screening for helminth eggs. In each center, we screened up to 25 specimens submitted each day. The study was explained to potential participants and written consent was requested. For each person who agreed to participate, part of the stool specimen was collected, and information was recorded about age, sex, and current diarrhea (defined as 3 or more loose or liquid stools in 24 hours) or other gastrointestinal symptoms. For data analysis, we defined gastroenteritis as diarrhea, other gastrointestinal symptoms, or both. Persons with symptomatic *Cyclospora* infection were treated with trimethoprim-sulfamethoxazole unless they had a history of allergy to sulfa drugs. Asymptomatic persons were not treated, as the risk for side effects was thought greater than the benefits of treating a self-limited asymptomatic infection.

Raspberry Farm Cohorts

On three raspberry farms (two in the Department of Guatemala and one in the Department of Chimaltenango), cohorts of workers and their family members were recruited in early April 1997. All participating farms, except one, had been implicated in the 1996 outbreak and were still exporting raspberries. The farms included at least one from each of the two main areas where raspberries are cultivated. The study was described in detail to participants, and written consent was solicited. A stool specimen was collected every other week from workers (less regularly from family members) and each time a participant reported having gastroenteritis. Persons found to have *Cyclospora* infection were treated with trimethoprim-sulfamethoxazole unless they had a history of allergy to sulfa drugs; they were monitored until their stool specimens were negative. Because of concerns about potential contamination of berries, all workers and their family members with *Cyclospora* infection, whether symptomatic or asymptomatic, were treated. Surveillance was intended to continue throughout the spring and fall berry harvest seasons.

Case-Control Study

From May 7 to September 3, 1997, we recruited participants for the case-control study. Because surveillance on raspberry farms ended earlier than anticipated, only four case-control participants were from the farms; all others were recruited from health-care facilities. All persons with symptomatic *Cyclospora* infection detected through the surveillance system were sought in their homes; however, some could not be located because of incorrect addresses. The purpose of the visit was to offer 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; however, family members were not included in the case-control study, and no rigorous attempt was made to include all family members.

We recruited controls from persons who had a stool specimen screened in any of the surveillance system's health centers or hospitals 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. We chose the controls to permit a final age distribution similar to that seen for *Cyclospora* cases in the surveillance system and a roughly equal distribution by surveillance site; individual cases and controls were not matched. Thus, each week we rotated the surveillance site from which we recruited controls, and we attempted to enroll 10 controls per week: one to two children younger than 1.5 years of age, four children 1.5 to 4 years of age, three 5 to 14 years of age, and one to two persons older than 14 years of age.

We administered a questionnaire that focused on potential risk factors for *Cyclospora*, including drinking water source and handling, household sanitation, presence of animals, socioeconomic variables, and consumption of specific fresh fruits, vegetables, and herbs. Several key variables were defined: drinking untreated water was defined as having drunk other than commercially bottled water that had not been boiled, chlorinated, or filtered in the household; water source was divided into high risk (public standpipe, well, spring, or water bought from a vendor) and low risk (in-house piped municipal water or commercially bottled water). Among children <5 years of age, we defined breastfed as having been breastfed at all, whether exclusively or partially, at the time the child provided the specimen.

Laboratory Methods

Stool specimens were processed by using a standard formalin-ethyl acetate concentration procedure and screened by two methods: light-microscopy examination of a modified acid-fast stained smear and UV epifluorescence examination of a wet mount (18). To confirm the identity of the parasite as *C. cayetanensis*, three positive specimens stored in 2.5% dichromate at ambient temperature (approximately 23°C) were examined at regular intervals over a 2-week period, starting from the time the specimen was provided (18). We observed characteristic sporulation in all three.

Analysis of Water Samples

From May 13 to July 17, 1997, we collected a weekly 10-liter water sample from each of three rivers (Villa Lobos, Los Verdes, and Guacalate). Two sampling sites were located in the Department of Guatemala and one in the Department of Sacatepequez; these rivers all contain raw sewage outfalls. Standard methods were used to quantify the level of fecal contamination with *Escherichia coli* (19). The specimens were concentrated by the flocculation method developed for detection of *Cryptosporidium* in water (20), with the following modifications for logistical reasons: the specimens were left to flocculate overnight; the centrifugation speed was 600 g, the maximum for our centrifuge, rather than 3,000 g; and we used only one washing with Tween. The concentrated sediments were examined by bright-field and UV epifluorescence microscopy for *Cyclospora* oocysts.

Data Analysis

All data were entered in Epi-Info 6.04b. Surveillance data were analyzed in Epi-Info and in Excel spreadsheets. The case-control data were analyzed in SAS 6.12. Potential risk factors were assessed in a series of univariate analyses; unless otherwise specified, the significance of differences was tested by Mantel-Haenszel chi-square test. We evaluated potential interactions among variables through a series of stratified analyses before constructing models that included interaction terms. Factors that were significant at $p<0.10$ level were then examined in stepwise regression models. The final multivariable model was chosen from alternative models resulting from the stepwise regressions, including interaction terms when indicated.

Results

Hospital and Health-Center Surveillance

From April 6, 1997, to March 19, 1998, we screened 5,552 specimens from health-care facilities. Overall, *Cyclospora* was detected in 126 (2.3%) specimens. The rate of *Cyclospora* detected began to rise (3.8%) in May and peaked at 6.7% in June (Figure). This period corresponds to the first 2 months of the rainy season in Guatemala City. The prevalence remained above 3% in July and August and fell to undetectable levels after mid-November. In January and February, *Cyclospora* was detected in fewer than 1% of specimens.

Cyclospora infection was more frequent among children and among persons with gastroenteritis (Table 1). The prevalence of *Cyclospora* was 3.5% in specimens from children 1.5 to 4 years of age and 3.8% among children 5 to 9 years of age, irrespective of symptom status. Children in these age groups were five times more likely than adults screened in the surveillance system to have *Cyclospora* infection. Persons with gastroenteritis were two to three times more likely than asymptomatic persons of the same age to have *Cyclospora*.

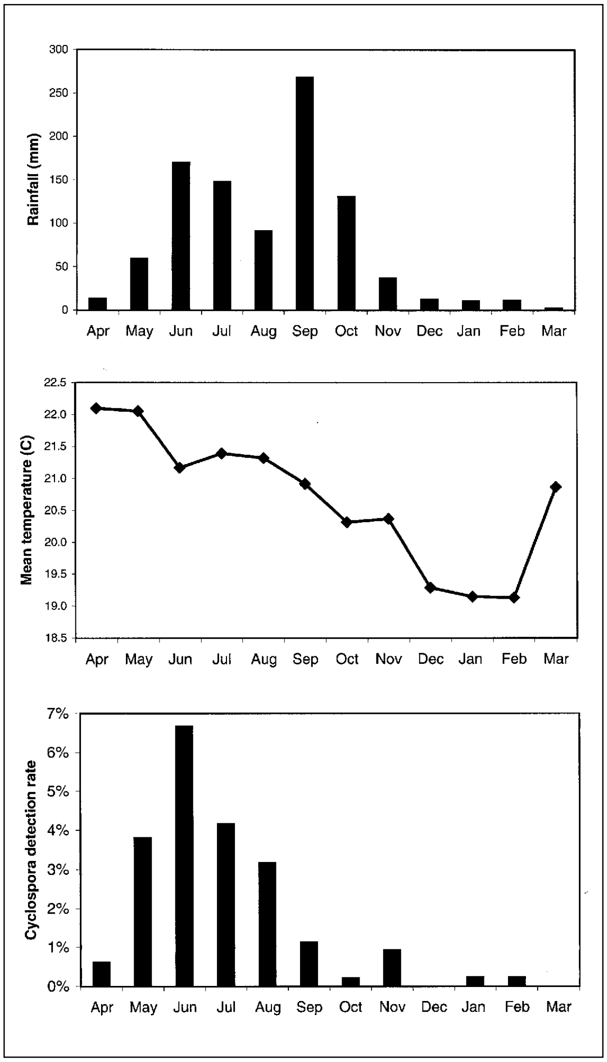

Figure. Surveillance for *Cyclospora cayetanensis* infection in stool specimens from three hospital outpatient departments and two health centers in Guatemala, April 6, 1997, to March 19, 1998. From the bottom, the three graphs demonstrate the *Cyclospora* detection rate, mean temperature in centigrade, and rainfall in mm by month. Median number of specimens per month 444 (324-638).

Cyclospora infection was relatively uncommon among children <18 months of age (1.0%). A substantial proportion of gastroenteritis cases during the early rainy season was associated with *Cyclospora* infection: from May 1 to June 30, 30 (19%) of 160 children 1.5 to 9 years old with gastroenteritis had *Cyclospora* detected in their stool. The overall rate of infection did not differ significantly by sex (44 [2.1%] of 2,067 male versus 57 [1.7%] of 3,266 female patients [p = 0.32]).

Although we did not collect data concerning HIV status, one hospital submitted specimens for screening from a clinic that served the HIV-infected population in Guatemala City. Of 32 specimens from adult patients attending this clinic, 9 (28%) were positive for *Cyclospora*, a much higher rate than that detected among other adults at the same hospital (2 [0.4%] of 448; RR 87, 95% CI 16.2, 847). Seasonality among these patients was the same as that observed for other participants in the surveillance system: eight of the nine *Cyclospora* detections occurred during May to August. These patients were not included in the case-control study.

Raspberry Farm Cohort

The raspberry farm cohort comprised 164 workers and 18 family members of workers who submitted specimens from April 6 to May 29, 1997. All 176 workers on the three farms agreed to participate; however, not all submitted specimens. We collected 269 specimens from workers and 31 from their family members, a median of one specimen per person (1 to 4). Six of 182 persons had an episode of infection with *Cyclospora*. Of the six infections, two occurred in family members, ages 1 and 10 years; both had gastroenteritis. The other four *Cyclospora* infections occurred in farm workers; all but one were asymptomatic. On May 29, 1997, the Guatemalan Berry Exporters Commission required us to suspend surveillance.

Case-Control Study

During the case-control study period, we recruited 69 persons with *Cyclospora* infection and 125 controls. Five controls were excluded because they had gastrointestinal symptoms when their specimens were submitted, leaving 69 cases and 120 controls for the analysis. Of 69 cases, 33 (48%) were in patients < 5 years of age and 26 (38%) were in male patients; among 120 controls, 56 (47%) were <5 years of age and 56 (47%) were male (p = 0.23).

In addition to specimens from the case-control study, we screened stool specimens from 182 family members belonging to 56 families of case-patients. Of these, 14 persons from 11 families tested positive for *Cyclospora*. The detection rate was highest among children 1.5 to 9 years (10 [11.6%] of 86). There were no cases among 14 family members <18 months of age and few among adults >14 years of age (2 [4%] of 57).

Table 1. Prevalence of *Cyclospora* in specimens from outpatients attending three hospitals and two health centers. April 6, 1997, to March 19, 1998

	Prevalence by age group		Prevalence by presence of symptoms		
Age (yrs)	n[a]/N[b] (%)	RR (95% CI)[c]	n/N (%) with gastroenteritis	n/N (%) without gastroenteritis	RR (95% CI)[d]
<1.5	10/844 (1.2)	1.7 (0.8, 3.8)	7/502 (1.4)	3/330 (0.9)	1.5 (0.4, 5.9)
1.5-4	41/1,160 (3.5)	5.1 (2.8, 9.1)	31/583 (5.3)	10/567 (1.8)	3.0 (1.5, 6.1)
5-9	31/813 (3.8)	5.5 (3.0, 10.1)	22/451 (4.9)	8/354 (2.3)	2.2 (1.0, 4.8)
10-14	4/328 (1.2)	1.8 (0.6, 5.2)	2/147 (1.4)	2/176 (1.1)	1.2 (0.2, 8.4)
>14	15/2150 (0.7)	Referent	8/958 (0.8)	7/1,181 (0.6)	1.4 (0.5, 3.9)
All ages	101/5,295[e] (1.9)	--	70/2,641[e] (2.7)	30/2,609[e] (1.1)	2.3 (1.5, 3.5)

[a]n represents the number positive for *Cyclospora*.
[b]N represents the number of specimens in each category.
[c]Each age group compared with patients >14 years of age.
[d]Specimens associated with gastroenteritis versus those without gastroenteritis.
[e]Apparent inconsistencies in denominator data are due to the following missing data and exclusions. Data for 32 patients of the clinic serving the HIV-infected population were excluded. Age and symptom data were missing for 187 specimens; symptom data only were missing for 45 specimens.

The rate among family members ages 1.5 to 9 years did not differ from that among children of the same age in surveillance data from the same period (64 [9%] of 714; p = 0.42).

Data were available on characteristics of illness in 62 patients in the case-control study (Table 2). The median duration of illness before diagnosis was 15 days in those <5 years of age and 10 days in those ≥5 years of age. Young children also tended to have a higher median number of stools per day than older patients and were significantly more likely to have fever and mucoid stools. Of the 62 patients, 40 (65%) reported predominantly watery or liquid stools, but 33 (53%) reported that diarrhea was intermittent. Of the 62 for whom data were available, 37 (60%) had been treated with an antibiotic, most often metronidazole, before *Cyclospora* was diagnosed.

Table 2. Characteristics of illness reported by 62 persons with *Cyclospora* infection who participated in the case-control study, Guatemala, May 7- September 3, 1997

	Age group of participants		
Characteristic	<5 years (n = 32)	≥5 years (n = 30)	p
Days of illness	15 (3-76)[a]	10 (2-90)[a]	0.05[b]
Stools per day	5 (1-10)[a]	3 (1-20)[a]	0.04[b]
Stool consistency			ns[c]
Liquid/watery	22 (69)	18 (60)	
Semisolid	10 (31)	6 (20)	
Solid	0 (0)	4 (13)	
Blood in stool	7 (22)	1 (3)	0.05
Mucus in stool	29 (91)	11 (37)	<0.001
Fever	24 (75)	9 (30)	<0.001
Vomiting	16 (50)	8 (27)	ns
Abdominal pain	10 (31)	13 (43)	ns
Anorexia	8 (25)	7 (23)	ns
Headache	2 (6)	7 (23)	ns
Treated before visiting health-care facility with			
Any antibiotic	22 (69)	15 (50)	ns
Metronidazole	11 (34)	9 (30)	ns
Cotrimoxazole	1 (3)	4 (13)	ns
Mebendazole or albendazole	7 (22)	2 (7)	ns

[a]Median (range).
[b]By Wilcoxon 2-sample test.
[c]ns = not significant.

A number of variables were associated with risk for *Cyclospora* infection in univariate analyses; all were related to water, sanitation, or presence of animals in the household (Table 3). Persons with *Cyclospora* infection were significantly more likely than controls to report having drunk untreated water in the 2 weeks before illness, having obtained drinking water from a high-risk source, or having swum in or drunk 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 elevated risk for infection. Persons with *Cyclospora* infection were twice as likely as controls to own dogs, chickens, or other fowl; other animals such as cats and pigs were not associated with increased risk for infection. We asked about eating 16 kinds of fresh, uncooked produce in the 2 weeks before illness, including raspberries, blackberries, lettuce, cabbage, mint, and cilantro; none was associated with an elevated risk for *Cyclospora* infection.

Among children <5 years of age, maternal education was protective: 10 of 33 children with

Table 3. Univariate analysis of factors associated with risk for *Cyclospora* infection among 69 cases and 120 controls in Guatemala

Characteristic	Proportion (n[a]/N[b] [%]) with characteristic Cases	Controls	OR (95% CI)
High-risk water source[c]	18/69 (26)	15/120 (13)	2.5 (1.1, 5.9)
Drank untreated water[d,e]	62/68 (91)	88/120 (73)	3.8 (1.4, 10.8)
Drank river or spring water[e]	12/68 (18)	8/120 (7)	3.0 (1.1, 8.9)
Swam in river or spring[e]	10/68 (15)	6/120 (5)	3.3 (1.0, 11.5)
Contact with soil[e]	54/69 (78)	73/120 (61)	2.3 (1.1, 4.9)
Septic tank vs. municipal drainage	33/69 (49)	39/120 (33)	2.0 (1.0, 3.8)
Any animals in household	53/69 (77)	74/120 (62)	2.1 (1.0, 4.3)
Dog	38/69 (55)	47/120 (39)	1.9 (1.0, 3.7)
Chickens	35/69 (51)	40/120 (33)	2.1 (1.1, 4.0)
Other poultry or birds	22/69 (32)	20/120 (17)	2.3 (1.1, 5.0)
Any poultry or birds	40/69 (58)	49/120 (41)	2.0 (1.1, 3.6)

[a]n represents the number positive for *Cyclospora*.
[b]N represents the number of specimens in each category.
[c]High-risk water sources defined as public standpipe, well, spring, water truck. Low-risk defined as municipal water piped into house or commercial bottled water.
[d]Untreated water defined as water that was not commercially bottled and had not been boiled, chlorinated, or filtered before drinking.
[e]In the last 2 weeks.

cyclosporiasis (compared with 31 of 55 controls) had mothers with 6 or more years of education (OR 0.34, 95% CI 0.12, 0.92). Breastfeeding also had a protective effect, although not of statistical importance: 3 of 33 case patients were breastfed, compared with 13 of 52 controls (OR 0.30, 95% CI 0.05, 1.25).

In stepwise regression analyses, the variables that remained significant were drinking untreated water and, among young children, contact with soil (Table 4). Drinking untreated water was associated with a fourfold increase in risk. For contact with soil, there was a significant interaction with age, carrying a 20-fold increased risk for *Cyclospora* infection among children <2 years of age but not among those 2 years of age or older (p for interaction term = 0.03). The presence of chickens in the household was associated with a twofold increased risk but did not reach statistical significance in the multivariable model (p = 0.054). The presence of chickens, poultry, or other birds in the household could be substituted for chickens in the model with similar results (OR 1.7; p = 0.09). The results of the model were unchanged when data were adjusted for socioeconomic variables, including educational level (for adult participants and of mother for children <15 years of age), housing type, and ownership of items such as radio, television, bicycle, or motor vehicle.

Table 4. Multivariable logistic regression model of risk factors for *Cyclospora* infection among 68 cases and 120 controls

Characteristic	OR (95% CI)[a]	p
Drank untreated water	4.2 (1.4, 12.5)	0.009
Chickens	1.9 (1.0, 3.7)	0.054
Contact with soil among children <2 years old	19.8 (2.2, 182)	0.008
Contact with soil among persons ≥ 2 years old[b]	1.4 (0.6, 2.9)	ns[c]

[a]Model adjusted for age category (<2 years, ≥2 years).
[b]Odds ratio for persons 2 years or older calculated as (OR for persons <2) *(OR for interaction term for soil contact with age <2). p value for the interaction term = 0.03.
[c]ns = not significant.

River Water Analysis

All three rivers had evidence of heavy fecal contamination throughout the period of study, with mean *E. coli* counts of 4.7×10^5, 1.9×10^7, and 4.5×10^6 colonies per 100 cc in the Department of Guatemala sampling sites and the Department of Sacatepequez site, respectively. We detected both sporulated and unsporulated *Cyclospora* oocysts in two of the 30 specimens, one specimen from each of the two rivers in the Department of Guatemala; both positive specimens were collected on May 26, 1997. The number of oocysts seen was small (2 oocysts and 3 oocysts, respectively). However, calculating from the amount of sediment obtained in each specimen (40 and 20 ml), the amount of sediment examined (20 and 40 µl), and the recovery rate of oocysts (5% to 10%; estimated from studies of seeded specimens, M. Alvarez de Mejia, M.B. Lopez, unpub. results), we estimate that these findings may represent actual concentrations of 15,000 or more oocysts per 10-liter specimen.

Conclusions

This study advances our understanding both of the epidemiology of *C. cayetanensis* in a disease-endemic area and of the context in which repeated outbreaks of foodborne cyclosporiasis have occurred in North America. We now know that *Cyclospora* is commonly associated with pediatric gastroenteritis in Guatemala, as it is in Peru, Nepal, and other developing countries (1,2,5,21). Moreover, as in Peru and Nepal, the organism displays a marked seasonality. In Guatemala, the prevalence of *Cyclospora* in specimens submitted to health-care facilities increased around the time of the spring raspberry harvest in April-June (9). In June, we recorded weekly detection rates as high as 10%. In our surveillance data from May through June, 19% of cases of pediatric gastroenteritis were associated with *Cyclospora*. We were also able to detect oocysts in surface water specimens, despite the fact that the available methods for detection in water have very low sensitivity (22). By October, the detection rate in human surveillance specimens had dropped below 1%. These data suggest that the association of *Cyclospora* outbreaks with raspberries from the spring but not the fall harvest may be related to the inherent seasonality of the organism.

A major limitation of our study was our inability to test for other diarrheal pathogens. We cannot be certain that every gastroenteritis episode during which we detected *Cyclospora* was due to *Cyclospora*. However, in the aggregate data, we observed a consistent epidemiologic and clinical pattern associated with detections of *Cyclospora*.

Although 1 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 Guatemala City is similar to that in Kathmandu, another subtropical city at approximately the same altitude (1,500 m) above sea level (3,23). In Guatemala as in Kathmandu, the rainy season starts in May or June, with warmer temperatures in the preceding months; in both places, *Cyclospora* appears around this time. Nevertheless, the organism's seasonality cannot be explained by rainfall alone, since in Lima an equally marked pattern is seen in the near absence of rain (21). Several years of surveillance in multiple sites with varying climates, as well as a better understanding of the organism's biology in the environment, will be necessary to explain why *Cyclospora* infections apparently fluctuate with the seasons.

Cyclospora has a number of other remarkable characteristics. It tends to cause prolonged diarrhea (2,3,24). Because all our patients were treated once the diagnosis was made, we could not estimate a total duration of illness; nevertheless, among children <5 years of age, the median length of illness before diagnosis was 15 days. Among children with diarrhea in developing countries, the proportion of persistent episodes (>14 days long) is 3% to 23% depending on the study cited. These episodes, however, carry a higher risk for malnutrition and death than shorter episodes (25-27). Perhaps because of the long duration of illness, persons with *Cyclospora* gastroenteritis are likely to receive various antibiotics before the correct diagnosis is made: in our study, 70% of children <5 years of age had received at least one antibiotic, and a third of all our patients had received metronidazole. Prompt diagnosis would avert such inappropriate use of antibiotics.

In North America, patients have been infected with *Cyclospora* through eating fresh raspberries, pesto dishes, and mesclun lettuce (9,28-30). In Guatemala, the main vehicle of infection appears to be untreated water. That water truly is an important vehicle for *Cyclospora* in Guatemala is underlined by the other water-related factors that were associated with infection. Our results support the findings in other studies that contaminated water is a likely source of infection (1,3,31). However, multiple routes of transmission for *Cyclospora* in Guatemala almost certainly exist. Among very young children, soil contact was a strong risk factor; an outbreak investigation in the United States also raised the possibility that soil might be a potential source of infective oocysts (6). 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 the postulated lack of direct person-to-person transmission.

The fact that the presence of chickens or other domestic fowl was a significant risk factor is intriguing but difficult to interpret. Scientists have failed to establish experimental *C. cayetanensis* infection in any bird; a limited survey of 110 wild birds captured in May 1997 in Guatemala did not demonstrate natural infection, although other coccidia were observed (M. Eberhard, pers. comm.). Although the association remains when the data are adjusted for socioeconomic status, we cannot rule out the possibility that ownership of fowl is a marker for some other unidentified factor. The role of birds in *Cyclospora* transmission merits further investigation.

C. cayetanensis is a pathogen commonly associated with pediatric gastroenteritis in Guatemala, especially from May through August. The seasonality of *Cyclospora* in Guatemala follows a pattern similar to that seen in Kathmandu. Our case-control analysis suggests that contaminated water and, for young children, soil, are likely vehicles of transmission. More sensitive diagnostic tools are urgently needed to help establish how fresh produce becomes contaminated.

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The study protocol was reviewed and approved by the Centers for Disease Control Institutional Review Board, as well as by local review committees required in Guatemala. In addition, for the part of the study conducted on raspberry farms, agreements were signed with the Guatemalan Berry Exporters Commission and the three participating farm owners regarding the terms of work, including human subjects protection and confidentiality.

Dr. Bern is a medical epidemiologist in the Division of Parasitic Diseases, CDC. Her research interests include the epidemiology of the enteric parasites *Cyclospora*, *Cryptosporidium*, and microsporidia.

References

1. Hoge CW, Echeverria P, Rajah R, Jacobs J, Malthouse S, Chapman E, 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.
2. 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.
3. Hoge CW, Shlim DR, Rajah R, Triplett J, Shear M, Rabold JG, et al. Epidemiology of diarrhoeal illness associated with coccidian-like organisms among travellers and foreign residents in Nepal. Lancet 1993;341:1175-9.
4. Soave R. *Cyclospora*: an overview. Clin Infect Dis 1996;23:429-37.
5. Soave R, Herwaldt BL, Relman DA. *Cyclospora*. In: Hughes JM, Conte JE, editors. Infectious disease clinics of North America. Vol 12, No. 1. Philadelphia: W.B. Saunders Company; 1998.
6. Koumans EHA, Katz DJ, Malecki JM, Kumar S, Wahlquist SP, Arrowood MJ, et al. An outbreak of cyclosporiasis in Florida 1995: a harbinger of multistate outbreaks in 1996 and 1997. Am J Trop Med Hyg 1998;59:235-42.
7. Huang P, Weber JT, Sosin DM, Griffin PM, Long EG, Murphy JJ, et al. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. Ann Intern Med 1995;123:409-14.
8. Carter R, Guido F, Jacquette G, Rapoport M. Outbreak of cyclosporiasis associated with drinking water. Proceeding of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1996 Sep 15-18; New Orleans, Louisiana. Washington: American Society for Microbiology, 1996.
9. Herwaldt B, Ackers ML, the Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med 1997;336:1548-56.
10. Herwaldt BL, Beach MJ, the Cyclospora Working Group. The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. Ann Intern Med 1999;130:210-20.
11. Osterholm MT. Cyclosporiasis and raspberries—lessons for the future [editorial]. N Engl J Med 1997;336:1597-8.
12. Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C, 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.
13. Pratdesaba RA, Velaquez T, Torres MF. Occurrence of *Isospora belli* and cyanobacterium-like bodies in Guatemala. Ann Trop Med Parasitol 1994;88:449-50.
14. Arathoon E, Velasquez T, Estrada y Martin RM, Mayorga R. *Cyclospora cayetanensis*, un nuevo patógeno causante de diarrea en pacientes infectados por el VIH. Revista del Colegio de Médicos y Cirujanos de Guatemala 1994;4:36-7.
15. Merida SC. Prevalencia de *Cyclospora* en niños menores de 60 meses. Doctor of Medicine Thesis, School of Medicine, Universidad Francisco Marroquin, Guatemala City; 1998, 59 pages.
16. Cuellar NS. Prevalencia de infecciones intestinales causadas por coccidios: *Cryptosporidium* spp, *Cyclospora cayetanensis*, e *Isospora belli* en pacientes con SIDA. Masters Thesis, Faculty of Laboratory Sciences and Pharmacy, Universidad de San Carlos de Guatemala, Guatemala City, 1997, 71 pages.

17. Alvarado KM. *Cyclospora cayetanensis* como agente causal de diarrea en pacientes de la consulta externa del Hospital General San Juan de Dios. Masters Thesis, Faculty of Laboratory Sciences and Pharmacy, Universidad de San Carlos de Guatemala, Guatemala City, 1997, 41 pages.
18. Eberhard ML, Pieniazek NJ, Arrowood MJ. Laboratory diagnosis of *Cyclospora* infections. Arch Pathol Lab Med 1997;121:792-7.
19. Environmental Protection Agency. Test Methods for *Escherichia coli* and enterococci in water by the membrane filter procedure. Manual EPA-600/4-85/076: Environmental Monitoring and Support Laboratory, 1985.
20. Vesey G, Slade JS, Byrne M, Shepherd K, Fricker CR. A new method for the concentration of *Cryptosporidium* oocysts from water. J Appl Bacteriol 1993;75:82-6.
21. Madico G, McDonald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. Clin Infect Dis 1997;24:977-81.
22. 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.
23. Connor BA, Shlim DR, Scholes JV, Rayburn JL, Reidy J, Rajah R. Pathologic changes in the small bowel in nine patients with diarrhea associated with a coccidia-like body. Ann Intern Med 1993;119:377-82.
24. Baqui AH, Sack RB, Black RE, Haider K, Hossain A, Alim ARMA, et al. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children under 5 years of age. J Infect Dis 1992;166:792-6.
25. Lima AAM, Fang G, Schorling JB, de Albuquerque L, McAuliffe JF, Mota S, et al. Persistent diarrhea in Northeast Brazil: etiologies and interactions with malnutrition. Acta Paediatr Supple 1992;381:39-44.
26. Black RE. Persistent diarrhea in children of developing countries. Pediatr Infect Dis J 1993;12:751-61.
27. Cruz JR, Bartlett AV, Mendez H, Sibrian R. Epidemiology of persistent diarrhea among Guatemalan rural children. Acta Paediatr Suppl 1992;381:22-6.
28. Centers for Disease Control and Prevention. Update: Outbreaks of cyclosporiasis—United States and Canada, 1997. MMWR Morb Mortal Wkly Rep 1997;46:521-2.
29. Centers for Disease Control and Prevention. Update: Outbreaks of cyclosporiasis—United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46:461-2.
30. Centers for Disease Control and Prevention. Outbreaks of cyclosporiasis—United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46:451-2.
31. Rabold JG, Hoge CW, Shlim DR, Kefford C, Rajah R, Echeverria P. *Cyclospora* outbreak associated with chlorinated drinking water [letter]. Lancet 1994;344:1360.