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NOTES

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Cyclospora cayetanensis in Three Populations at Risk in Guatemala

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In 1996 and 1997, outbreaks of *Cyclospora cayetanensis* in North America were linked to Guatemalan raspberries. From April 1999 to April 2000, we undertook a survey for *C. cayetanensis* in raspberry farm workers, malnourished children, and human immunodeficiency virus and AIDS patients in Guatemala. Stool samples were analyzed using ethylacetate-formalin concentration, wet preparation, modified acid-fast staining method, and epifluorescence. Oocysts were found in 1.5% of the subjects, none of whom were raspberry farm workers.

Cyclospora cayetanensis is an emerging coccidian parasite whose life cycle, reservoir hosts, and prevalence among the human population have not been systematically studied (15). It was first described by Ashford in 1979 (1) and classified by Ortega et al. in 1994 (16). This parasite may cause gastroenteritis and produces symptoms that include protracted diarrhea, weight loss, and fatigue which can last from 1 to 5 weeks (3, 11, 14). The infectious diarrhea responds to antibiotic treatment with trimethoprim-sulfamethoxazole (14). The possibility of reservoir hosts has been considered, but so far, confirmed natural infection in animals other than humans has not been documented (7, 17).

Most of the currently available information has been derived mainly from studies on international travelers, expatriates, or at-risk groups, primarily children and human immunodeficiency virus (HIV) or AIDS patients (9, 12–14). The information available thus far includes two studies from Peru (14, 15), two from Nepal (10), and one from Guatemala (2). One of the studies from Peru reported a prevalence of 1.6% among children under 8 years of age (14) while Bern et al. reported an overall prevalence of 2.3% among children and adults from Guatemala (2). That study from Guatemala included data and surveillance collected for a short period of time, among people from different locations, with the main group being from outpatient clinics at city hospitals and health centers (2). There is no information available, however, describing the true prevalence of infection by this parasite in a natural population setting.

From 1996 to 1998, several outbreaks of diarrhea in the United States and Canada caused by *C. cayetanensis* were associated with the consumption of Guatemalan raspberries, although no *C. cayetanensis* was ever observed or detected from those raspberries (4, 5, 8). Based on this epidemiological association, a subsequent import ban of Guatemalan raspberries into the United States during and after the spring of 1998 resulted in huge economic losses and unemployment in Guatemala. These consequences and the continuing questions concerning the mode of contamination of the raspberries, the distribution and natural reservoir hosts of *C. cayetanensis* have further emphasized the need to establish the prevalence of this parasite among segments of the Guatemalan population.

In this study, fecal samples were obtained from 474 subjects of both sexes from April 1999 to April 2000. Two hundred six (43.5%) samples were from raspberry farm workers (mean age, 29 years; range, 15 to 61 years), 111 (23.4%) samples were from malnourished children (with moderate or severe malnutrition) (mean age, 11 months; range, 1 month to 4 years) from Hospital General San Juan de Dios or Colonia infantil de San Juan Sacatepéquez, and 157 (33.1%) samples were from HIV or AIDS outpatients (mean age, 32 years; range, 1 to 67 years) from Roosevelt Hospital Infectious Diseases' Clinic or Hogar San José. Informed and voluntary consent was obtained from all HIV or AIDS patients and from the parent or adult responsible for each malnourished child. Only one stool sample from each subject in these two populations was analyzed. The raspberry farm workers were informed and voluntarily submitted a fecal sample as part of a routine health analysis. All of the workers had to submit a fecal sample for *C. cayetanensis* analysis at the beginning of the harvest season; afterwards, 10% of the workers were chosen at random every month until the end of raspberry season, which lasted for 5 months.

All specimens were processed by the formalin-ethyl acetate method (2). Briefly, a portion of the fecal sample was suspended in 10 ml of 10% formalin (Merck) in a 15-ml conical plastic tube (Falcon). Tubes were left standing at room temperature for 30 min, and then approximately 5 ml of ethyl

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TABLE 1. Distribution of parasites among the 474 subjects[a]

Parasite	Parasite prevalence in subjects							
	Raspberry farm workers (n = 206)		Malnourished children (n = 111)		HIV/AIDS outpatients (n = 157)		Total (n = 474)	
	n	%	n	%	n	%	n	%
Helminths								
Ascaris lumbricoides	65	31.6	3	2.7	2	1.3	70	14.8
Trichuris trichiura	52	25.2	2	1.8	3	1.9	57	12.0
Hymenolepis nana	10	4.9	0	0	1	0.6	11	2.3
Strongyloides stercoralis	3	1.4	0	0	4	2.6	7	1.5
Hookworm	9	4.4	2	1.8	1	0.6	12	2.5
Protozoa								
Entamoeba coli	64	31.0	0	0	29	18.5	93	19.6
Endolimax nana	28	13.6	0	0	35	22.3	63	13.3
Blastocystis hominis	38	18.5	4	3.6	12	7.6	54	11.4
Chilomastix mesnili	0	0	0	0	3	1.9	3	0.6
Giardia lamblia	10	4.9	0	0	18	11.5	28	5.9
Cyclospora cayetanensis	0	0	1	0.9	6	3.8	7	1.5
Cryptosporidium parvum	0	0	7	6.3	8	5.1	15	3.2

[a] Data were obtained from wet preparation examination only. No permanent stained smears were examined other than modified acid-fast stains for coccidia.

acetate (Merck) was added. The tubes were tightly closed, shaken for 30 s, and centrifuged for 5 min at 500 × *g*, and the top layer was removed while the supernatant was discarded the pellet was kept. Wet mounts were prepared to look for protozoa and/or helminths, and the remaining pellet was observed using epifluorescence at 450 nm. A smear was stained by a modified acid-fast staining method to look for *C. cayetanensis* and other coccidia (6).

C. cayetanensis was observed in samples of only 7 of 474 (1.5%) subjects, distributed as follows: 6 of the 157 HIV or AIDS patients (3.8%) and 1 of the 111 malnourished children (0.9%). No *C. cayetanensis* oocysts were observed in any of the samples from the raspberry farm workers. For the 474 subjects (Table 1), based on wet preparation only and modified acid-fast stains for coccidia, the most commonly observed parasites were *Entamoeba coli* (19.6%), *Ascaris lumbricoides* (14.8%), *Endolimax nana* (13.3%), *Trichuris trichiura* (12.0%), and *Blastocystis hominis* (11.4%). *Cryptosporidium parvum* was observed in samples from 15 (3.2%) subjects, of which seven (6.3%) were malnourished children and eight (5.1%) were HIV or AIDS patients. As soon as the results from stool analysis were obtained, they were referred to a physician so universal treatment with albendazole or an appropiate antiparasite drug could be established. For the raspberry farm workers, a campaign to eliminate parasites was suggested to the farms.

Whereas Bern et al. (2) reported an overall prevalence of 2.3% for *C. cayetanensis* from April 1997 to March 1998 with a peak in June 1997 of 6.7% and a specific prevalence of 3.3% among raspberry farm workers in Guatemala, our survey failed to detect oocysts among such workers, using a comparable sampling size and time span. Although the overall population size surveyed in our study was somewhat smaller than those in previously published reports, these negative albeit unexpected results are, nonetheless, highly significant. Prior to this study, it was assumed that at-risk patients and raspberry farm workers would represent subsets of the population in which *C. cayetanensis* infection rates would be high. In fact, our data reflected quite the opposite and suggested that *C. cayetanensis* is not as prevalent in Guatemala as previously reported by others. The contrast between the results of our study and those reported by Bern et al. (2) could potentially reflect a high seasonality of natural cyclosporiasis or the fact that a local outbreak was going on, but only at the time of surveillance (1997 to 1998). Unfortunately, this possibility could have been confirmed only if routine surveillance for the parasite had been implemented for at least two consecutive years. There may also be a need for improving methods of recovery from feces as well as food samples. These two studies collectively, however, do emphasize the need for further longitudinal epidemiological studies in which seasonality is an important parameter to be evaluated in order to properly establish the prevalence of this parasite in Guatemala, as well as the natural sources or routes of infection.

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