Multiyear Prospective Study of Intestinal Parasitism in a Cohort of Peace Corps Volunteers in Guatemala

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We conducted a prospective, longitudinal study in a cohort of 36 Peace Corps volunteers (PCVs) in Guatemala to study the incidence and natural history of intestinal parasitic infections during the PCVs' >2-year overseas stay. PCVs collected stool specimens at least monthly and when ill with gastrointestinal symptoms. Of the 1,168 specimens tested, 453 (38.8%) were positive for at least one parasite and 48 (4.1%) were positive for a pathogenic parasite. A median interval of 187 days (range, 14 to 752 days) elapsed before the first documented parasitic infection, and the median intervals from arrival until subsequent infections (e.g., second or third) were >300 days. The PCVs had 116 episodes of infection with 11 parasites, including up to 4 episodes per PCV with specific nonpathogens and *Blastocystis hominis*. The incidence, in episodes per 100 person-years, was highest for *B. hominis* (65), followed by *Entamoeba coli* (31), *Cryptosporidium parvum* (17), and *Entamoeba hartmanni* (17). The PCVs' *B. hominis* episodes lasted 6,809 person-days (28.7% of the 23,689 person-days in the study), the *E. coli* episodes lasted 2,055 person-days (8.7%), and each of the other types of episodes lasted <2% of the person-days in the study. Gastrointestinal symptoms were somewhat more common and more persistent, but not significantly so, in association with pathogen episodes than with *B. hominis* and nonpathogen episodes. Although infections with pathogenic parasites could account for only a minority of the PCVs' diarrheal episodes, the continued acquisition of parasitic infections throughout the PCVs' >2-year stay in Guatemala suggests that PCVs repeatedly had fecal exposures and thus were at risk for infections with both parasitic and nonparasitic pathogens throughout their overseas service.

The most common medical disorder among travelers from developed countries to developing countries is diarrheal illness (28, 29), which also is the most common reason that Peace Corps volunteers (PCVs) seek medical care (4, 7). Various bacterial enteropathogens, such as enterotoxigenic *Escherichia coli*, are the most commonly identified etiologic agents of travelers' diarrhea (5, 18, 21). However, surveillance data from 1990 suggested that intestinal parasitism was prevalent among PCVs and that infection with *Entamoeba histolytica* was particularly common among PCVs in Guatemala (7, 10). In that context, we conducted studies among PCVs in Guatemala to identify risk factors for diarrheal illness in general and to determine how common various parasitic infections are in this setting. We first conducted a clinic-based, case-control study, which included 48 case (diarrheal) episodes, 26 control episodes, and 115 stool specimens obtained during these episodes (10). Six (12.5%) of the case episodes could be accounted for by protozoal pathogens, specifically, *Cyclospora cayetanensis* (three episodes), *Cryptosporidium parvum* (one), *Giardia lamblia* (one), and *E. histolytica-Entamoeba dispar* (one). Infection with *Blastocystis hominis* was equally prevalent among case episodes (31%) and control episodes (32%).

Next, we conducted a prospective, longitudinal study in which a cohort of 36 newly arrived PCVs recorded daily dietary and symptom data and provided at least monthly stool speci-

mens throughout their >2-year stay in Guatemala, even when they were asymptomatic (11). Data for 23,689 person-days and for 1,168 stool specimens were collected. Our findings concerning risk factors for diarrheal illness have already been published (11). Here we present our analyses of the stool data concerning intestinal parasitism. Although we predicted that parasites would account for a minority of the PCVs' diarrheal episodes, we were interested in studying the incidence and natural history of infection with both pathogenic and nonpathogenic parasites.

MATERIALS AND METHODS

General. In October 1991, we recruited participants among PCVs en route to Guatemala. The study was approved by the institutional review board of the Centers for Disease Control and Prevention (CDC), and participants provided informed consent. Participants contributed person-days to the study from their arrival in Guatemala until they completed Peace Corps service or withdrew from the study. PCVs were asked to provide daily exposure and symptom data, irrespective of health status, on a structured log, which had one row per day of the month and columns for placing check marks by specific symptoms and exposures (11). PCVs also recorded their medications.

Stool and serum specimens. We asked PCVs to provide a baseline stool specimen in October 1991, before they left for Guatemala; at least one stool per month thereafter; a median of three at midservice; and a median of three at close of service. We encouraged PCVs to collect additional specimens when they had gastrointestinal (GI) symptoms, irrespective of whether the PCV was evaluated in the Peace Corps clinic in Guatemala City. The monthly specimens and those collected when PCVs were symptomatic are not distinguished in the analyses because stools could be of both types and PCVs varied in their thresholds for collecting nonroutine specimens and for being evaluated by medical staff. According to usual practice and separate from the study protocol, specimens collected from PCVs evaluated by medical staff because of GI symptoms also were examined by local Guatemalan laboratories. Whenever possible, the results of this testing were obtained, but details about testing methods were not.

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Each study-related stool specimen was preserved in two vials: one with 10% formalin and one with polyvinyl alcohol (Para-Pak Stool System; Meridian Diagnostics, Inc., Cincinnati, Ohio). PCVs kept stool kits in their homes and periodically obtained more from the clinic. Staff at CDC's field station in Guatemala examined the specimens for ova and parasites, and staff at CDC in Atlanta, Ga., reexamined all positive specimens and 10% of the negative specimens. Permanent slides of specimens fixed in polyvinyl alcohol were stained with trichrome (19) and examined by light microscopy for protozoa. Formalin-fixed specimens were concentrated by the formalin-ethyl acetate sedimentation technique (19) and examined unstained (a 22-mm-square-coverslip area) and after staining with the Kinyoun carbol-fuchsin modified acid-fast procedure (2). Two hundred oil immersion fields (100 $\times$ objective) were examined on stained slides. The parasite density per 10 oil immersion fields or a 22-mm-square-coverslip area was classified as rare (1 parasite), few (2 parasites), moderate (3 to 9 parasites), or many (≥ 10 parasites). *E. histolytica* and *E. dispar* were not differentiated. The specimens were also examined with a direct fluorescent-antibody assay for *C. parvum* and *G. lamblia* (Merifluor *Cryptosporidium/Giardia*; Meridian Diagnostics, Inc.) (8); most of this testing was done by a U.S. commercial laboratory, and the rest was done by CDC in Atlanta.

We also requested baseline, midservice, and close-of-service serum specimens. Staff at CDC in Atlanta tested the specimens with enzyme immunoassays for antibodies to *E. histolytica* (Alexon-Trend, Ramsey, Minn.) (M. Wilson, P. M. Schantz, and D. A. Ware, Abstr. 44th Annu. Meet. Am. Soc. Trop. Med. Hyg., abstr. 289, 1995) and *C. parvum* (22). For the latter testing, which was done with an investigational assay, a positive result was defined as seroconversion or a twofold or greater rise in antibody titer to the 17- or 27-kDa sporozoite surface antigen.

Definitions and statistical methods. An episode of infection with a specific parasite was defined as the occurrence of at least one stool positive for that parasite, irrespective of the findings for other parasites. Successive episodes with the same parasite had to be separated by at least four consecutive stools that were negative for that parasite and at least 30 days between the last stool documented to be positive in one episode and the first stool in the next episode. Because of these criteria, we assumed that successive episodes were statistically independent. Although the true dates of acquisition and clearance of infection were unknown, the duration of an episode was defined as the number of days from the first through the last positive stool within the episode.

In addition to episodes with individual parasites, we defined composite episodes for infections with the nonpathogens as a group and the pathogens *C. parvum* and *G. lamblia* as a pair. These episodes were defined as described above except that specimens within an episode could be positive for any of the parasites of interest and stools between episodes had to be negative for all parasites of interest.

To assess whether the presence of a specific parasite was associated with GI symptoms, we checked whether infected PCVs had recorded any GI symptoms (i.e., loose or watery stools [LWS], nausea, vomiting, and abdominal cramps) on their symptom logs anytime during the 29-day window period that surrounded detection of the parasite (i.e., the 2 weeks before the first day of the episode, the first day of the episode, and 2 weeks after the first day of the episode). We checked for symptoms in a large window period because asymptomatic PCVs typically collected specimens monthly and positive specimens could have reflected incubating disease or prolonged shedding during convalescence. If a PCV had ≥ 3 LWS on any day during the window period, we determined whether GI symptoms persisted. Specifically, we identified the first day with ≥ 3 LWS and then determined whether, in the next 13 days, the PCV had at least 4 days with ≥ 2 LWS or at least one other GI symptom. We excluded episodes from these analyses if the PCV had not completed a log for the period of interest. To reduce the likelihood that symptoms were attributable to bacterial infection, we excluded episodes if the PCV had noted bloody stools or a temperature of $\geq 38.9^\circ\text{C}$ or if study staff or a local laboratory had found leukocytes, erythrocytes, or bacterial pathogens in any stool collected during the window period. For some analyses, we excluded episodes if the PCV was coinfecting during the window period with particular parasites (see below).

We also determined whether the GI symptoms in the window period met the clinical criteria for diarrheal episodes, as defined in our risk factor investigation (11). Specifically, a PCV was defined as having a diarrheal episode if criteria for one or more of the following components of the multicomponent definition were met: (i) three or more LWS and at least one other symptom (i.e., nausea, vomiting, abdominal cramps, visible blood in stool, or self-reported fever) during the same 24-h period, (ii) two LWS and at least two other symptoms during the same 24-h period, or (iii) six or more LWS during a 24- to 72-h period. Successive diarrheal episodes had to be separated by at least 7 days with at most one LWS and no other symptoms.

We compared proportions with the uncorrected chi-square test or, if indicated, the Fisher exact test. We used the Poisson distribution to compute 95% confidence limits for incidence data. Statistical significance was set at $P < 0.05$.

RESULTS

General information about the PCVs. As previously described (11), 36 (55.4%) of the 65 eligible PCVs participated in the study. The participants had a median age of 24 years (range, 22 to 70), and 18 (50.0%) were women. Participants and nonparticipants were similar with respect to age, sex, and the ultimate duration of Peace Corps service (11). Most participants stayed in the study throughout their Peace Corps service, which began in late October 1991 and usually ended in late 1993 or early 1994. Eight left the Peace Corps after a median of 10 months, and two provided stool specimens somewhat erratically for >1 year and ultimately dropped out of the study before their Peace Corps service ended.

General information about the stool specimens. The 36 PCVs submitted 1,168 stool specimens, including 36 baseline stools, 74 midservice stools, 88 close-of-service stools, and 970 other stools (83.0%) (Table 1). The median number of specimens per PCV was 35 (range, 6 to 63). The compliance rate for submitting at least one specimen per month while enrolled in the study was 96%. Of the 1,168 specimens, 453 (38.8%) were positive for at least one parasite and 48 (4.1%) were positive for a pathogen (includes *E. histolytica*-*E. dispar*) (Table 1). Infections with 11 different parasites were documented, and up to five parasites were found per specimen. The proportions of specimens positive for the various protozoal and helminthic pathogens ranged from 0.2 to 1.5%, and those for the various nonpathogens ranged from 0.2 to 7.1% (Table 1); the proportion positive for *B. hominis*, which was placed in its own category because of uncertainty about its pathogenicity, was 29.9%.

All baseline specimens were negative, except those from four PCVs infected with *B. hominis* (Table 1). Three of these PCVs had subsequent episodes of infection with *B. hominis* and other parasites, whereas the other PCV (referred to here as PCV B) did not have any other documented parasitic infections. Not counting PCV B's baseline infection, 32 (88.9%) of the 36 PCVs became infected with at least one parasite while in Guatemala (median, two parasites; range, one to seven parasites). Nineteen PCVs (52.8% of 36) became infected with at least one pathogen (Table 1).

The proportions of the 36 PCVs who became infected with specific parasites were as follows, in descending order: *B. hominis*, 77.8% (excluding PCV B's infection); *Entamoeba coli*, 33.3%; *C. parvum*, 30.6%; *Endolimax nana*, 22.2%; *Entamoeba hartmanni*, 19.4%; *G. lamblia*, 16.7%; *Chilomastix mesnili*, 16.7%; *Ascaris lumbricoides*, 8.3%; *E. histolytica*-*E. dispar*, 5.6%; *Dientamoeba fragilis*, 5.6%; and *Iodamoeba bütschlii*, 5.6% (Table 1). Thus, *B. hominis* was the most commonly identified parasite, *C. parvum* was the most commonly identified pathogen, and *Ascaris* was the only identified helminth. The three successive evaluations at baseline, midservice, and close of service showed that the proportions of PCVs infected with *B. hominis* progressively increased from 11.1% (including PCV B) to 37.9% (including PCV B) to 53.1%, respectively, and the proportions of PCVs infected with *E. coli* increased from 0% to 6.9% to 12.5%, respectively (Table 1).

Parasitic infections were acquired slowly but steadily

TABLE 1. Parasites identified in stool specimens from PCVs in Guatemala^a

Parasite	No. (%) of PCVs infected with the parasite				No. of stool specimens (% of 1,168) positive for the parasite
	Baseline (36 PCVs)	Midservice (29 PCVs)	Close of service (32 PCVs)	Ever during study (36 PCVs)	
Any parasite	4 (11.1)	14 (48.3)	18 (56.3)	33 ^b (91.7)	453 (38.8)
Any pathogen ^{c,d,e}	0	4 (13.8)	2 (6.3)	19 (52.8)	48 (4.1)
Any nonpathogen ^c	0	3 (10.3)	6 (18.8)	18 (50.0)	147 (12.6)
Pathogenic protozoa					
<i>Cryptosporidium parvum</i>	0	4 (13.8)	0	11 (30.6)	18 ^f (1.5)
<i>Giardia lamblia</i>	0	0	0	6 (16.7)	10 ^g (0.9)
<i>Entamoeba histolytica</i> - <i>E. dispar</i> ^d	0	0	1 (3.1)	2 (5.6)	12 (1.0)
<i>Dientamoeba fragilis</i>	0	0	0	2 (5.6)	2 (0.2)
Nonpathogenic protozoa					
<i>Entamoeba coli</i>	0	2 (6.9)	4 (12.5)	12 (33.3)	83 (7.1)
<i>Endolimax nana</i>	0	0	0	8 (22.2)	17 (1.5)
<i>Entamoeba hartmanni</i>	0	0	0	7 (19.4)	31 (2.7)
<i>Chilomastix mesnili</i>	0	1 (3.4)	1 (3.1)	6 (16.7)	31 (2.7)
<i>Iodamoeba bütschlii</i>	0	0	0	2 (5.6)	2 (0.2)
Helminth, <i>Ascaris lumbricoides</i>	0	0	1 (3.1)	3 (8.3)	6 (0.5)
Other parasite, <i>Blastocystis hominis</i>	4 (11.1) ^{h,i}	11 (37.9) ⁱ	17 (53.1)	29 (80.6) ^{i,j}	349 (29.9) ^k

^a The baseline stool specimens ($n = 36$) were collected in October 1991, the midservice specimens ($n = 74$) were collected from 11 January 1993 through 5 April 1993, and the close-of-service specimens ($n = 88$) were collected from 11 July 1992 through 26 April 1994 but typically in late 1993 or early 1994. Thus, the first three columns of data have cross-sectional data, whereas the last two columns include data for the study as a whole.

^b Of the 33 infected PCVs, 32 acquired new infections in Guatemala. One of the four PCVs without documented acquisition of infection while in Guatemala was in the Peace Corps for only 9 months; the other three PCVs were a 70-year-old woman, a 22-year-old woman, and a 23-year-old man.

^c Does not include *B. hominis*, a parasite of unclear pathogenicity.

^d *E. histolytica*-*E. dispar* was grouped with the pathogens because the pathogen *E. histolytica* was not differentiated from the nonpathogen *E. dispar*.

^e Four PCVs became infected with multiple pathogens: one PCV was infected with *G. lamblia*, *E. histolytica*-*E. dispar*, and *D. fragilis*; one was infected with *C. parvum* and *G. lamblia*; one was infected with *C. parvum* and *E. histolytica*-*E. dispar*; and one was infected with *D. fragilis* and *A. lumbricoides*.

^f All 18 stool specimens were positive by the immunofluorescence technique, and 3 specimens from two PCVs were also positive by acid-fast staining.

^g All 10 stool specimens were positive by the immunofluorescence technique, and 7 specimens from five PCVs were also positive by routine microscopic examination.

^h All four persons who had *B. hominis* in their baseline specimens had some subsequent stools positive for *B. hominis*, and all except one of these PCVs (PCV B) had subsequent episodes of *B. hominis* infection. Two of the four PCVs had traveled elsewhere in Latin America earlier in the year of enrollment.

ⁱ Includes PCV B.

^j Twenty-eight PCVs became infected in Guatemala.

^k Includes 22 positive specimens from PCV B and 6 positive specimens from three other PCVs who were infected at baseline and whose baseline episodes continued for various periods.

throughout the PCVs' overseas stay. The time from arrival in Guatemala until parasitic infections were documented is shown in box plots in Fig. 1 and 2. A median of 187 days (range, 14 to 752) elapsed before the first documented infection (Fig. 1), which was sixfold longer than the median time until the first diarrheal episode occurred (11). The median intervals from arrival until subsequent parasitic infections (e.g., second and third) were >300 days. For specific parasites, the median interval was 480 days (range, 322 to 767) for *C. parvum* versus 265 days (range, 36 to 615) for *G. lamblia* (Fig. 2). Overall, infection with the various parasites was not markedly seasonal (Fig. 3).

Episodes of infection. The PCVs had 116 episodes of parasitic infection, some of which overlapped because of coinfection with multiple parasites (Tables 2 and 3). The median number of episodes per PCV for the 32 infected PCVs was two (range, 1 to 12). The 18 women (50.0% of 36 PCVs) contributed 12,757 person-days to the study (53.9% of 23,689) and 69 episodes (59.5% of 116), and the 23 persons who were <30 years of age (63.9% of 36) contributed 15,976 person-days (67.4%) and 76 episodes (65.5%); the proportions were not significantly different. The incidence, in episodes per 100 person-years, was highest for *B. hominis* (65), followed by *E. coli*

(31), *C. parvum* (17), and *E. hartmanni* (17) (Table 2). Although no PCV had more than one documented episode of infection with the same pathogen, individual PCVs had up to four episodes with specific nonpathogens and *B. hominis*. On average, successive episodes were separated by >130 days and by >6 negative stools, which suggests that the episodes represented reinfections rather than relapses (Table 3).

The durations of the episodes were highly variable but show the potential for excretion of parasites, particularly nonpathogens and *B. hominis*, for hundreds of days. Twenty (47.6%) of the 42 *B. hominis* episodes and 6 (30.0%) of the 20 *E. coli* episodes, but none of the 11 *C. parvum* episodes, were documented to last >100 days (Table 2). Overall, the PCVs' *B. hominis* episodes lasted 6,809 person-days (28.7% of 23,689), the *E. coli* episodes lasted 2,055 person-days (8.7%), and each of the other types of episodes lasted <2% of the person-days in the study. For some episodes of infection with nonpathogens and *B. hominis*, coincidental antimicrobial therapy might have accounted for the fact that the episode stopped rather than continued indefinitely (Table 2).

To help address the question of whether *B. hominis* is a pathogen, we determined whether GI symptoms occurred near the beginning of *B. hominis* episodes (i.e., in the 29-day win-

FIG. 1. Distributions (shown in box plots) of the number of days from arrival in Guatemala until infection with sequential parasites (e.g., the first parasite to infect the PCV, irrespective of which parasite it was) was documented. For each box plot, the number of PCVs is shown on the left and the number of PCVs (percentage of total on left) infected with pathogens is shown on the right. *E. histolytica*-*E. dispar* is classified as a pathogen, and *B. hominis* is not. On the x axis, both the number of days and the corresponding year are shown.

dow period centered around the first day of the episode) (Table 4). We compared the data for *B. hominis* with those for composite episodes that considered the nonpathogens as a group and the pathogens *C. parvum* and *G. lamblia* as a pair. Symptoms were comparably common near the beginning of *B. hominis* and nonpathogen episodes. Although the differences were not statistically significant (Table 4), symptoms were somewhat more common and more persistent in association with pathogen episodes. Likewise, in pathogen episodes, the first stool was somewhat more likely, although not significantly so, to be classified as loose or watery and to be in close proximity to a diarrheal episode. Overall, of the 208 (of 307) clinically defined diarrheal episodes for which at least one stool specimen was checked either during the episode or during the 15-day period centered around the first day of the episode, 11 episodes (5.3%) had a specimen that was positive for a protozoal pathogen.

Testing of stool specimens by local laboratories. Separate from the study protocol, 129 stool specimens were tested by local Guatemalan laboratories, typically during evaluations of GI illness. Portions of 126 specimens from 28 PCVs were

tested for parasites, and portions of 35 specimens from 22 PCVs were tested for bacteria. Leukocytes were noted in 20 specimens (15.5%), some of which also had blood or mucus. The testing for bacteria yielded one specimen positive for *Shigella sonnei*, one positive for a *Shigella* sp., and one reportedly positive for "pathogenic" *Escherichia coli*; all three specimens had many leukocytes.

The local testing for parasites could be compared with the testing done by study staff. (Of note, only the results from study staff were considered study results and included in the tables.) For *E. histolytica*-*E. dispar*, the local testing apparently yielded 11 more positive specimens from eight PCVs who were never identified by study staff as infected. For the eight specimens for which a specimen from the same or an adjoining day was examined by study staff (split specimens were examined for only two), local laboratories might have misidentified *B. hominis* for *E. histolytica*-*E. dispar* in five specimens and leukocytes for *E. histolytica*-*E. dispar* in a specimen from a PCV with shigellosis. Local testing yielded two additional specimens from two PCVs that reportedly were positive for *G. lamblia*. In specimens from adjoining days, study staff found *B. hominis* in

FIG. 2. Distributions (shown in box plots) of the number of days from arrival in Guatemala until infection with particular parasites was documented. The data are for each PCV's first episode of infection with the parasite. For each box plot, the number of PCVs is shown on the left. On the x axis, both the number of days and the corresponding year are shown. The box plots are ordered by ascending medians.

one PCV's stool and *C. mesnili* in the other's. Local testing apparently yielded three more specimens from three PCVs that were positive for *A. lumbricoides*. Study staff examined specimens from the same or an adjoining day for two of the three and did not find anything that might have been misidentified as *A. lumbricoides*.

Serologic testing. Two or three serum specimens from each of 26 PCVs were available for serologic testing. All specimens were negative for antibody to *E. histolytica*, including those from the two PCVs with stools positive for *E. histolytica-E. dispar*.

Five PCVs had evidence of *C. parvum* infection by both stool testing and serologic testing with an investigational enzyme immunoassay, six PCVs had evidence of *C. parvum* infection by serologic testing but not stool testing, and six PCVs had positive stool specimens but negative serologic results. Five of the last six discrepancies might be attributable to a delay of ≥ 11 months between the positive stool and the serum specimen.

DISCUSSION

We conducted a multiyear study of the incidence and natural history of intestinal parasitic infections in a cohort of 36 PCVs

in Guatemala. The study, which included 65 person-years of data, 1,168 stool specimens, and 116 episodes of infection with 11 parasites, was unusual in that it was prospective and longitudinal rather than cross-sectional or clinic based. We are not aware of any other study of intestinal parasitism in which a group of initially asymptomatic adults was monitored so closely, for so long, and with such a high compliance rate.

Our main conclusions are as follows. Intestinal parasitism, considering the parasites as a group, was common. New parasitic infections were gradually acquired throughout the PCVs' >2 -year stay in Guatemala and were not markedly seasonal. As expected, infections with pathogenic parasites could account for a minority of the PCVs' diarrheal episodes. *C. parvum* was the most commonly identified pathogen, *E. histolytica-E. dispar* was rarely found, and *A. lumbricoides* was the only identified helminth. *B. hominis* was by far the most commonly identified parasite, and in several respects, such as prolonged shedding, it behaved more like a nonpathogen than like a pathogen.

The continued acquisition of parasitic infections throughout the PCVs' >2 -year overseas stay indicates that the PCVs repeatedly had fecal exposures. Although many of the infections

FIG. 3. Month of onset of episodes of infection with various parasites. (A) Data for episodes of infection with *C. parvum* (black bars) and *G. lamblia* (white bars). (B) Data for nonpathogens. (C) Data for *B. hominis*. The stool specimens were collected from 22 October 1991 through 26 April 1994 (median, 1 December 1992). Thus, each month includes data for multiple years.

were with nonpathogens, the fecal exposures also placed the PCVs at risk for infections with pathogens, both parasitic and nonparasitic. Our previously published data about the PCVs' risk factors for diarrheal illness in general also led us to conclude that the PCVs repeatedly had potentially risky dietary exposures and that the risk for diarrheal episodes persisted, although it decreased, as the length of stay in Guatemala increased (11). We did not attempt to identify risk factors for infection with specific parasites because we identified insufficient numbers of evaluable episodes for meaningful multivariate analyses. In addition, given that routine specimens were collected monthly, we often were uncertain when the parasitic infections actually were acquired and therefore what the exposure period of interest was. We decided to focus instead on qualitative comparisons of the patterns of infection with indi-

vidual parasites and with the pathogens versus the nonpathogens.

C. parvum was the most commonly identified pathogen, in part because we examined stools with an immunofluorescence technique that is more sensitive than acid-fast staining (1). Had we not done immunofluorescence testing for either *C. parvum* or *G. lamblia*, the numbers of infected PCVs would have fallen from 11 (30.6%) to 2 (5.6%) for *C. parvum* and from 6 (16.7%) to 5 (13.9%) for *G. lamblia*. Thus, *G. lamblia* would have been the most commonly identified pathogen. If the results of the serologic testing for antibody to *C. parvum*, which was done with an investigational enzyme immunoassay for epidemiologic rather than clinical purposes, are added to the results of the stool testing, the number of infected PCVs rises from 11 (30.6%) to 17 (47.2%). Even more seropositive persons might have been identified had serum specimens been collected more frequently and therefore closer to episodes of infection. The *C. parvum* cases diagnosed by stool examination were not markedly seasonal, which is consistent with what was found in 1997 and 1998 in a study among Guatemalans in outpatient facilities (3).

Although *E. histolytica* infection purportedly was common among PCVs in Guatemala before we began our study (10), we documented only two episodes of infection with *E. histolytica*-*E. dispar*. None of the PCVs who were tested had developed detectable antiamebic antibody, which suggests that invasive amebiasis was uncommon. Although local Guatemalan laboratories identified *E. histolytica*-*E. dispar* more often than study staff did, this could reflect misidentification of other parasites, cells (e.g., leukocytes), or debris as *E. histolytica*, which is a notoriously common problem (16).

We did not identify any cases of cyclosporiasis in this study, although we identified three in our case-control study (10). We could have missed some infections with this and other parasites by testing insufficient numbers of specimens during some illness episodes (13); by collecting routine stool specimens monthly rather than more often, which would have been impractical; and by using techniques that by present standards are suboptimally sensitive for detection of particular parasites. For example, UV fluorescence microscopy is now known to be more sensitive than examination of acid-fast-stained slides for detection of *Cyclospora cayetanensis* (12). In addition, we did not use special stains for microsporidia or any molecular techniques for detection of parasites. Unfortunately, the present repertoire of well-evaluated assays for detection of serum antibodies to GI parasites is very limited.

Although we did not design our study to address the controversial question of whether *B. hominis* is a pathogen (9, 14, 15, 17, 20, 26, 27, 31, 32), we took advantage of the high incidence of *B. hominis* infection by comparing the patterns of acquisition and excretion of *B. hominis* with those of the known pathogens and nonpathogens. We were intrigued to note that, in several respects, *B. hominis* behaved more like a nonpathogen than a pathogen. First, infection was more common with *B. hominis* and with the nonpathogens as a group than with the pathogens as a group. Second, some PCVs had several episodes of infection with *B. hominis* and individual nonpathogens but not with any pathogen. Third, PCVs more commonly excreted *B. hominis* and nonpathogens for prolonged periods, sometimes hundreds of days. Lastly, PCVs were somewhat

TABLE 2. Incidence and duration of episodes of intestinal parasitic infection in PCVs in Guatemala^a

Parasite	No. of episodes (no. of PCVs)	Median no. of episodes per PCV (range) ^b	Incidence of episodes per 100 person-years (95% confidence limits)	No. (%) of episodes that lasted > 100 days ^c	Median duration (days) of episodes (range) ^d	Median period (days) from first to last positive stool (range) ^d	No. of episodes possibly affected by antimicrobial therapy/no. of evaluable episodes (%) ^e
Pathogenic protozoa							
<i>Cryptosporidium parvum</i>	11 (11)	1	17 (9, 29)	0		1 (1–15)	Not applicable
<i>Giardia lamblia</i>	6 (6)	1	9 (4, 19)	0		12 (1–65)	4/5 (80.0)
<i>Entamoeba histolytica-E. dispar</i>	2 (2)	1	3 (0.5, 10)	1 (50.0)		166 (24–308)	1/1 (100)
<i>Dientamoeba fragilis</i>	2 (2)	1	3 (0.5, 10)	0		1	0/2 (0)
Nonpathogenic protozoa							
<i>Entamoeba coli</i>	20 (12)	1 (1–4)	31 (19, 46)	6 (30.0)	41.5 (1–641)	109 (1–732)	5/15 (33.3)
<i>Endolimax nana</i>	9 (8)	1 (1–2)	14 (7, 25)	1 (11.1)	1 (1–104)	1 (1–307)	2/7 (28.6)
<i>Entamoeba hartmanni</i>	11 (7)	1 (1–3)	17 (9, 29)	2 (18.2)	1 (1–247)	124 (1–730)	5/10 (50.0)
<i>Chilomastix mesnili</i>	8 (6)	1 (1–3)	12 (6, 23)	1 (12.5)	17 (1–305)	17 (1–743)	3/6 (50.0)
<i>Iodamoeba bütschlii</i>	2 (2)	1	3 (0.5, 10)	0		1	1/2 (50.0)
Helminth, <i>Ascaris lumbricoides</i>	3 (3)	1	5 (1, 12)	0		3 (1–5)	2/2 (100)
Other parasite, <i>Blastocystis hominis</i> ^f	42 (28)	1 (1–4)	65 (47, 86)	20 (47.6)	88 (1–762) ^g	209 (1–783) ^g	13/24 (54.2)

^a If a PCV was simultaneously infected with more than one parasite, each is listed separately.^b The numbers of PCVs who had more than one episode of infection with *B. hominis*, *E. coli*, *E. hartmanni*, *Endolimax nana*, and *C. mesnili* were nine, four, three, one, and one, respectively.^c Significantly more *B. hominis* episodes than *C. parvum* episodes lasted > 100 days ($P = 0.004$), whereas the proportions of *E. coli* and *C. parvum* episodes that lasted > 100 days were not significantly different ($P = 0.07$). The proportions of *B. hominis* and *E. coli* episodes that lasted > 30 days (64.3 and 55.0%, respectively) were significantly different than the proportion of *C. parvum* episodes that lasted > 30 days (0%) ($P < 0.001$ and $P = 0.008$, respectively).^d If no PCV had more than one episode of infection with the parasite, the duration of the episodes is provided only in the sixth column. If some PCVs had more than one episode, the duration is provided both for the individual episodes (fifth column), assuming that each represented reinfection, and for the entire period from the first to the last positive stool, irrespective of the number of negative stools separating positive stools (sixth column), assuming that apparent episodes actually represented intermittently detectable shedding. The durations of the episodes are minimums because stool specimens were not collected daily, some PCVs had positive stools when last tested (see footnote *e*), and some PCVs received antimicrobial therapy (see last column). Episodes that consisted of only one positive stool specimen were considered to have lasted 1 day.^e These data show whether antimicrobial therapy, which often was coincidental (e.g., it was given because of *Giardia* infection, but the PCV also happened to be infected with *B. hominis*), might account for apparent or real cessation of excretion and therefore the end of the episode. To attribute the end of an episode to antimicrobial therapy, at most one negative stool could have separated the last positive stool in the episode and use of the agent, and at least four consecutive negative stools must have followed use of the agent. For the pathogens, only antimicrobial agents known to be effective against the microbe were considered. For *B. hominis* and nonpathogens, any therapy that might have affected excretion was considered. Episodes near close of service in PCVs who were infected when last tested or who had not had four consecutive negative stools before leaving the study were considered nonevaluable because we could not determine whether the episode had ended. Given that not all evaluable episodes were treated, these data do not indicate the proportion of episodes that responded to therapy.^f Excludes PCV B, the one PCV infected with *B. hominis* at baseline who did not have subsequent episodes of *B. hominis* infection.^g Excludes all four PCVs infected with *B. hominis* at baseline.

TABLE 3. Characteristics of episodes of intestinal parasitic infection and of interepisode periods in PCVs in Guatemala^a

Parasite	No. of episodes (no. of PCVs)	Median no. of stools per episode (range) ^b		Median no. of days between episodes (range) ^c	Median no. of negative stools between episodes (range) ^d
		Positive stools	Negative stools		
Pathogenic protozoa					
<i>Cryptosporidium parvum</i>	11 (11)	1 (1–4)	0 (0)	NA ^e	NA
<i>Giardia lamblia</i>	6 (6)	1.5 (1–3)	0 (0–2)	NA	NA
<i>Entamoeba histolytica</i> - <i>E. dispar</i>	2 (2)	6 (2–10)	2.5 (0–5)	NA	NA
<i>Dientamoeba fragilis</i>	2 (2)	1 (1)	0 (0)	NA	NA
Nonpathogenic protozoa					
<i>Entamoeba coli</i>	20 (12)	2 (1–23)	0 (0–5)	134.5 (56–364)	7 (4–14)
<i>Endolimax nana</i>	9 (8)	1 (1–6)	0 (0–3)	202	10
<i>Entamoeba hartmanni</i>	11 (7)	1 (1–16)	0 (0–3)	213 (150–331)	13.5 (5–28)
<i>Chilomastix mesnili</i>	8 (6)	2.5 (1–15)	0 (0–6)	188.5 (133–244)	11 (6–16)
<i>Iodamoeba bütschlii</i>	2 (2)	1 (1)	0 (0)	NA	NA
Helminth, <i>Ascaris lumbricoides</i>	3 (3)	2 (1–3)	0 (0)	NA	NA
Other parasite, <i>Blastocystis hominis</i> ^f	42 (28)	5 (1–29)	1 (0–6)	157.5 (33–289)	6.5 (4–17)

^a If a PCV was simultaneously infected with more than one parasite, each is listed separately.^b For example, an episode with three positive stools, followed by two negative stools, followed by one positive stool, would be classified as having four positive and two negative stools.^c By definition, this interval had to be ≥ 30 days.^d By definition, the number of negative stools between episodes had to be ≥ 4 .^e NA, not applicable.^f Excludes PCV B, the one PCV infected with *B. hominis* at baseline who did not have subsequent episodes of *B. hominis* infection.

more apt, although not significantly so, to be symptomatic if infected with pathogens rather than *B. hominis* or nonpathogens. In our case-control study, *B. hominis* was equally prevalent during case and control episodes (31 to 32%) (10).

The above comparisons should be considered only qualitative and suggestive, in part because the factors that we could address in the context of our study do not reliably differentiate pathogens and nonpathogens. For example, although commensals probably are more apt than pathogens to be shed for

prolonged periods (6, 23–25, 33), pathogens can be excreted for weeks and sometimes months, and they sometimes cause asymptomatic infection and reinfection. Clearly, the durations of excretion we observed were partly dependent on our definition of an episode, which might not have always distinguished accurately between intermittent shedding and reinfection. Our symptom analyses were also complicated by the high background rate of GI symptoms, the need to exclude many infection episodes from the analyses because of documented or

TABLE 4. Association between intestinal parasitic infections and GI symptoms in PCVs in Guatemala^a

Type of episode (n)	No. (%) of episodes that were associated with:				
	GI symptoms	≥ 3 LWS	Persistence of symptoms	Proximity to a diarrheal episode	Stool specimen classified as loose or watery ^b
<i>B. hominis</i> episodes					
All evaluable episodes (28) ^c	18 (64.3)	12 (42.9)	5 (17.9)	9 (32.1)	4 (14.3)
Without coinfection with nonpathogens (22) ^d	14 (63.6)	10 (45.5)	4 (18.2)	7 (31.8)	4 (18.2)
Composite nonpathogen episodes					
All evaluable episodes (24) ^c	18 (75.0)	9 (37.5)	3 (12.5)	6 (25.0)	5 (20.8)
Without coinfection with <i>B. hominis</i> (13)	10 (76.9)	5 (38.5)	2 (15.4)	3 (23.1)	3 (23.1)
Composite <i>C. parvum</i> and <i>G. lamblia</i> episodes					
All evaluable episodes (10) ^c	9 (90.0)	6 (60.0)	3 (30.0)	5 (50.0)	4 (40.0)
Without coinfection with <i>B. hominis</i> or nonpathogens (5)	4 (80.0)	2 (40.0)	2 (40.0)	2 (40.0)	2 (40.0)

^a See Materials and Methods for information on which GI symptoms were counted; how diarrheal episodes, composite episodes with nonpathogens and pathogens, and coinfection were defined; and which episodes were considered evaluable. Composite episodes were created to increase the number of episodes per category that could be included in the analyses. None of the comparisons (e.g., the likelihood that episodes with *B. hominis* versus those with pathogens were associated with symptoms) were statistically significant. All *P* values were >0.20 , except the one for the comparison of the likelihood that the evaluable *B. hominis* episodes versus the evaluable pathogen episodes were associated with LWS, for which the *P* value was 0.17.^b Refers to the consistency of the first stool examined in the episode.^c Besides the exclusionary criteria in Materials and Methods, episodes were excluded if the PCV was coinfecting during the window period with a pathogenic parasite.^d The densities of *B. hominis* in the first stool in these 22 episodes were classified as many (8 episodes), moderate (6 episodes), few (4 episodes), and rare (4 episodes) (for details of the classification of densities, see Materials and Methods). Of the eight episodes with many *B. hominis* organisms, five episodes were associated with GI symptoms, and two of these episodes were associated with persistent symptoms.^e None of the PCVs who had these episodes were coinfecting during the window period with another pathogenic parasite.

possible coinfection with other microbes, and the possibility that what is now called *B. hominis* includes both pathogenic and nonpathogenic species or strains (9, 14). Although we could not resolve the long-standing controversy about the pathogenicity of *B. hominis*, we urge caution in attributing symptomatology to *B. hominis* infection. In situations such as ours, in which the incidence and prevalence of *B. hominis* infection are high, so is the probability of coincidentally finding *B. hominis* when symptomatic persons are evaluated.

Although we purposefully focused on parasitic infections, we had hoped to test some stool specimens for other enteropathogens as well. Unfortunately, having PCVs freeze or refrigerate fresh stool specimens in their homes for later testing for bacteria and viruses was impractical, and the aliquots of the specimens provided in clinic that we froze were ruined when the freezer malfunctioned. Therefore, the only testing for bacteria was done by local laboratories, and no specimens were tested for viruses. However, even in studies that include testing for all known enteropathogens, the etiologic agents for at least a substantial minority of episodes of travelers' diarrhea remain unidentified (5, 21, 30). Clearly, our understanding of the epidemiology and natural history of intestinal infection among travelers and expatriates will increase as the diagnostic tools improve.

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