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Infection and Diarrhea Caused by *Cryptosporidium* sp. among Guatemalan Infants

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During July 1985 to June 1986, fecal excretion of *Cryptosporidium* oocysts was determined prospectively in a cohort of 130 infants, aged 0 to 11 months, living in a marginal urban area of Guatemala City, Guatemala. A total of 1,280 stool specimens were examined; 158 of them were collected during episodes of diarrhea, and 1,122 were collected during symptom-free periods, every 2 to 3 weeks, from every child. Of the children, 20 (15.4%) excreted *Cryptosporidium* oocysts during the observation period. Of the diarrheal episodes, 13 (8.3%) were associated with *Cryptosporidium* sp. Of the control specimens, seven (0.6%) were positive for oocysts. Most of the infections were documented during the months of February to May, at the end of the dry season. *Cryptosporidium* infections are very common among Guatemalan infants and are an important cause of diarrhea and weight loss. The introduction of liquid or solid foods in the diets of the children, the presence of domestic animals (dogs, cats, or poultry), and the absence of toilet facilities in the house seem to be important risk factors for infection; also, deficient nutritional status may predispose the infected child to *Cryptosporidium*-associated illness.

Cryptosporidium sp. is a parasite which has recently been recognized as an important diarrhea-causing agent among humans (4). Although the first cases reported in the literature were mostly in individuals with immune system defects (14, 22), it is now clear that otherwise healthy subjects may suffer from diarrhea after an infection by the *Cryptosporidium* protozoan (3, 8, 11). The prevalence of *Cryptosporidium* infection seems to be higher among children than among adults (1), and, in children, it appears that the younger ones have a higher risk of *Cryptosporidium*-associated diarrhea (7). In South Africa and Liberia, for instance, the prevalence rates of *Cryptosporidium* infection in infants with gastroenteritis were reported to be 14.9% in two studies, with a sharp decrease in the rate for older age groups (7, 20). On the other hand, children living in marginal urban areas may be more likely to develop diarrhea caused by *Cryptosporidium* sp. than those from rural environments are (7, 12, 21).

The present study was undertaken with the purpose of determining the diarrhea-causing role of *Cryptosporidium* sp. among children aged 0 to 11 months living in marginal urban areas of Guatemala, a developing country in Central America.

MATERIALS AND METHODS

Study population and field procedures. The study was carried out in Colonia El Limòn, a marginal urban area of Guatemala City. At the beginning of the study, July 1985, there were 7,308 inhabitants in the locality, grouped in 1,156 families. Of these, 130 families with children under 1 year of age were randomly selected and enrolled in the program which ended in June 1986. In our sample, 37% of the fathers and 95% of the mothers did not have any formal education. Of the families, 50% earned less than 135 quetzales ($50) per month. Although 80% had indoor water pipes and 97% had flush toilets, running water was available to them for only about one-fourth of the year, during the rainy season. During the shortage period, privately owned and fire department tank trucks provide water, which is stored primarily in metal barrels. Other information which we collected included the presence of domestic animals and number of people living in the household.

After parental written informed consent was given, children aged 30 to 270 days, one per family, were enrolled in the study and followed for periods varying from three to nine months, up to their first birthdays. Of the infants, 66 were males and 64 were females. Field personnel visited the homes of the participants once a week to inquire about the presence of diarrhea in the children and to obtain information on their feeding modes. Four feeding categories were established as follows: exclusive breast-feeding; modified exclusive breast-feeding, which included clear liquids in addition to breast milk; partial breast-feeding, which included gruels or solids and breast milk; and no breast-feeding. Weights and lengths of the children were determined every 2 weeks. When a case of diarrhea was detected, the initial date of the episode was recorded and a fecal sample was collected for microbiological studies, which included the detection of helminths, protozoans, bacteria, and rotaviruses. Sick children were visited every other day after detection of the episode, which was considered to have ended when symptoms were absent for 72 h. At this time, the child was weighed again. Additionally, fecal material was collected routinely from each child every 2 to 3 weeks to detect the excretion of *Cryptosporidium* oocysts during diarrhea-free periods. Daily rainfall and maximum and minimum ambient temperatures in the community were monitored locally and recorded by our field personnel.

Sample collection and laboratory procedures. Fecal samples were collected in paper cans at the homes of the participants. The specimens were placed in Styrofoam boxes with ice cans and, within 30 min, suspensions were prepared in buffered Formalin, polyvinyl alcohol, selenite broth, phosphate-buffered saline, veal infusion broth, and Cary-Blair medium. The stool suspensions were transported to the central laboratories of the Institute 2 to 6 h after collection. For the identification of *Cryptosporidium* sp., the Formalin-

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TABLE 1. Characteristics of children who excreted *Cryptosporidium* oocysts

Condition and no. of infants[a]	Sex[b]	Age (days)	Breast-feeding[c]	Episode duration (days)[d]	Other pathogens
With diarrhea					
19	M	317	P	?	
35	F	275	P	6	
83	M	290	P	?	
88	M	240	A	8	
96	M	113	A	65	
		125	A		
		147	A		Adherent *E. coli*, *Shigella sonnei*
102	F	167	P	17	
		177	P		
114	F	113	P	18	*Campylobacter jejuni*, *Shigella flexneri*
116	F	143	P	11	
121	M	105	P	7	
134	M	43	M	6	
142	M	95	A	8	*Campylobacter jejuni*
145	M	86	P	26	Toxigenic *E. coli*
		100	P		
147	M	63	P	41	*Campylobacter jejuni*
Without diarrhea					
33	F	125	P	NA	
42	F	283	P	NA	
60	M	190	P	NA	
95	F	166	A	NA	
104	M	71	P	NA	
117	M	140	A	NA	
137	F	107	P	NA	Adherent *E. coli*

[a] In infants with diarrhea, for age, $\bar{x} \pm$ the standard deviation was 158 ± 92 days and for episode duration, $\bar{x} \pm$ the standard deviation was 19 ± 18 days. In infants without diarrhea, for age, $\bar{x} \pm$ the standard deviation was 155 ± 69 days.

[b] Abbreviations: M, male; F, female.

[c] Abbreviations: M, modified; P, partial; A, absent.

[d] Symbols and abbreviations: ?, unknown; NA, not applicable.

preserved stools were treated with 10% KOH, vortexed, rinsed with 10% Formalin, and centrifuged. Using the uppermost layer of the sediment (6), two smears were prepared from each specimen. After ethanol fixation, the preparations were covered with 1% safranine, heated with an open flame for 2 min, and rinsed with tap water. Methylene blue (1%) was then added for 30 s (D. Baxby and N. Blundell, Lancet **ii**:1149, 1983). A sample was considered negative for *Cryptosporidium* sp. if oocysts were not detected in 500 oil immersion fields. Formalin-preserved stools were also used for the detection of protozoan cysts and helminths; protozoan trophozoites were sought in polyvinyl alcohol-treated, trichromic-stained material. Rotaviruses were detected by enzyme-linked immunosorbent assay (23) in the veal infusion broth. Pathogenic bacteria were isolated in Butzler-Virion, salmonella-shigella, and MacConkey agars streaked from the Cary-Blair transport medium. Suckling mice (15) and mouse adrenal tumor cells (17) were used to identify toxigenic *Escherichia coli*, whereas adherent *E. coli* was detected in HEp-2 monolayers (2), and *Campylobacter jejuni* was recognized by its morphologic and biochemical characteristics (10).

Statistical analysis. Possible risk factors for either infection or diarrhea were analyzed by comparisons of rates and proportions (5). When appropriate, relative risks were calculated (18). Z score was used to express the nutritional status of the children (9). This score represents the difference in standard deviations from the mean of the U.S. National Center for Health Statistics reference curve.

RESULTS

Cryptosporidium oocysts were excreted by 20 of the 130 children studied (15.4%) (Table 1). Of the 20 *Cryptosporidium*-positive children, 18 were detected between February and May, the end of the dry season (Fig. 1). None of the 20 infants was exclusively breast-fed when infected. In terms of child-weeks of observation, children who were receiving complementary foods (solids or gruels) had an infection rate of 2.34 (17 of 725); those who received clear fluids and breast milk had an infection rate of 2.38 (3 of 126), and the exclusively breast-fed children were not infected (0 of 139). Among 77 children in families with domestic animals (cats, dogs, or poultry), *Cryptosporidium* sp. was identified in 14 children (18.2%), whereas among 52 children in families without animals, 5 children (9.6%) were infected (Table 2). The coccidian was detected in 7 (0.6%) of the 1,122 samples obtained from children when they were healthy and during 13 (8.3%) of the 158 episodes of diarrhea. *Cryptosporidium* sp. was the only pathogen identified in 9 of the 158 (5.7%) diarrhea cases. The mean age of the children at the time of infection was 157 days, with no statistical differences between those who developed diarrhea (158 days) and those who were symptom-free (155 days).

The proportion of *Cryptosporidium* infections that were associated with diarrhea was 65% (13 of 20), inducing episodes that lasted from 6 to 65 days (mean, 19.4 days; standard deviation, 18.5 days), with 45% of them lasting more than 2 weeks. There was no association of illness duration with age. Sex and age did not seem to be associated with the development of diarrhea once infection had occurred. Nutritional status, as determined by weight for length, before infection may be an important factor; 4 of 4 (100%) of the children with a Z score below −0.5 developed diarrhea, whereas only 9 of 16 (56.3%) of those with adequate weight for length developed it (Table 2).

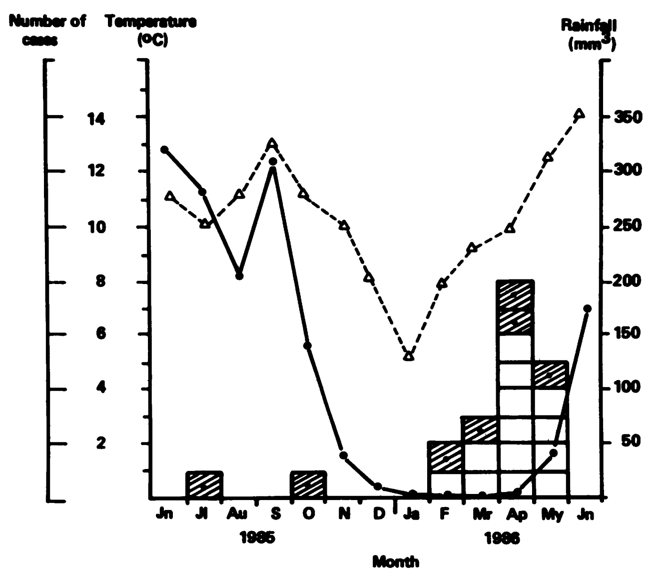

FIG. 1. Time of infection (□, symptomatic; ▨, asymptomatic) by *Cryptosporidium* sp. in relation to monthly rainfall (●) and minimum ambient temperature (△).

TABLE 2. Possible factors associated with infection and diarrhea due to *Cryptosporidium* sp.

Condition and factor	Categories	Relative risk (confidence interval)
Infection		
Family income (quetzales)[a]	≤200/≥200	0.73
No. of people living in the house	≥7/3–6	0.74
No. of people sharing a room with the child	≥4/1–3	1.13
Water source	Public/piped	1.00
Excreta disposal	Pit/toilet	1.66 (0.60–5.78)
Domestic animals in the house	Yes/no	1.89 (0.69–5.81)
Consumption of solids and/or gruels	Yes/no	2.07 (0.58–9.50)
Breast-feeding	Exclusive/other	6.74
Diarrhea		
Age (mo)	0–2/≥3	0.91
Nutritional status (Z score)		
Wt for age	≤−1/>−1	1.16 (0.47–2.17)
Wt for length	≤−0.5/>0.5	1.78 (0.62–1.87)

[a] 2.70 quetzales equals $1.

Cryptosporidium-associated diarrhea induced a marked deterioration of the nutritional status of the children. The changes in Z score of weight for age of the 11 cases in which the exact duration of the episode was recorded are depicted in Fig. 2. The average fall in Z score during the first 6 to 11 days of illness was 0.064 (±0.04) standard deviation per day (P = 0.01).

DISCUSSION

High rates of excretion of *Cryptosporidium* oocysts among infants with diarrhea have been reported from Liberia and Central and South Africa (1, 7, 20). In Bangladesh, Costa Rica, and Thailand, the reported excretion rates among preschoolers is somewhat lower, 3.2 to 7.6% (12, 19; D. N. Taylor and P. Echeverria, Lancet i:320, 1986). Besides the suggestion that *Cryptosporidium* infections are more common among younger children, there are indications that children living in urban slum areas may be more likely to develop diarrhea caused by *Cryptosporidium* sp. than are those living in rural communities (21).

In the present study, we have determined that the excretion of *Cryptosporidium* oocysts is very common among infants of marginal areas of Guatemala City. In this population, 15.4% of the children aged 0 to 11 months shed the protozoan in their feces at a mean age of 157 days, or 5 months. The prevalence rate is very similar to that found in urban Liberian children aged 6 to 11 months and to that reported from South Africa for infants treated for diarrhea at a local hospital (7, 20), demonstrating the importance of *Cryptosporidium* sp. as an enteric pathogen, specially in underprivileged populations.

The fact that our data do not suggest any association between incidence of *Cryptosporidium* infection and source of water may be due to the general unavailability of good-quality drinking water in the community. Since piped water is scarce, the inhabitants of Colonia El Limòn must obtain water by the barrel and store it for periods that may vary from 2 to 5 days. Furthermore, the majority of excretions of *Cryptosporidium* oocysts were detected during April and May, the warmer months at the end of the dry season, when the availability of water for personal hygiene is lowest in the community. The absence of rain also results in a massive accumulation of dust in the area, a fact that may facilitate respiratory *Cryptosporidium* infection, as suggested by Shahid and colleagues (19) in Bangladesh. In the infants, the oral route of infection, however, seems the most likely; all of the children excreting *Cryptosporidium* oocysts had received other liquids or foods in addition to breast milk or had been completely weaned, identical to conditions observed in Liberia and Costa Rica (7, 12, 21), where *Cryptosporidium* oocysts could not be found among the exclusively breast-fed children. Nevertheless, and as suggested by the observations of Høјlyng and colleagues (7) and Koch et al. (11), other members of the household may become infected by the respiratory route and, in turn, may be the source of person-to-person transmission to the infants. Domestic animals in the home environment may accentuate the level of intradomiciliary contamination for older people in the household; in our study, the direct contact of younger infants with home animals was minimal. A third factor suggesting a role of older members of the family in the natural history of infant *Cryptosporidium* infections is the higher incidence of the protozoan among those children of households with a pit latrine instead of a flush toilet. Nutritional status, as determined by weight for length, seems to be a determining factor for the outcome of infection, since all the children who were considered malnourished by this indicator and who became

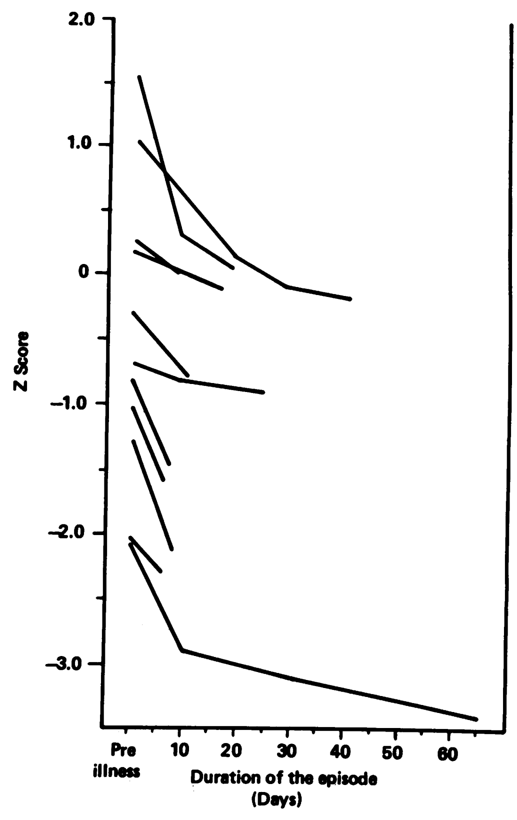

FIG. 2. Changes in nutritional status (Z score, weight for age) during episodes of diarrhea due to *Cryptosporidium* oocysts. Pre-illness weight was obtained 1 to 2 weeks before any disease occurred; final weight was obtained 3 days after cessation of symptoms. Each curve represents an infant. The mean slope of the curves was −0.0644 (P = 0.01) during the first 6 to 11 days of illness.

infected developed diarrhea, a finding that supports the studies from Costa Rica (21) and Central Africa (1).

The proportion of diarrhea episodes associated with *Cryptosporidium* sp. in this population, 8.3%, is lower than that reported from Asia, Africa, and Venezuela (1, 7, 13, 16, 20), but higher than that observed in other Central American countries (12, 21). Of the 13 *Cryptosporidium* infections, 9 (69%) were not associated with other potential enteropathogens. Additionally, in this study, *Cryptosporidium* oocysts were detected in sick subjects more often than were rotaviruses, *Shigella* spp., *Salmonella* spp., *Campylobacter jejuni*, and enteropathogenic *Escherichia coli* (data not presented), showing the importance of the parasite as a diarrhea-causing agent. Furthermore, 45% of the infections induced periods of disease that lasted more than 14 days, with two episodes lasting 41 and 65 days. Independent of its duration, *Cryptosporidium*-associated diarrhea had a dramatic effect on the nutritional status of the infants, especially during the first days of symptoms. These factors, high prevalence of infection, long duration of diarrheal episodes, and the nutritional consequences of illness, in association with the lack of appropriate curative measures, make *Cryptosporidium* sp. an important enteric pathogen in our population.

Finally, it is noteworthy that all the specimens from ill children in which *Cryptosporidium* oocytes were present had a high content of mucus. In some cases the mothers reported that their children were not passing stools but only mucus. In some communities, such as Colonia El Limòn, paramedical personnel tend to associate the presence of mucus in fecal material with intestinal infections due to invasive bacteria, predisposing them to inappropriate use of antibiotics in *Cryptosporidium*-associated diarrhea. Recognition of the prevalence of *Cryptosporidium* infection and better definition of the diarrheal syndrome associated with this parasite will be the basis of educating health personnel in more appropriate responses to such illness.

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