A Study of Risk Factors Associated With the Prevalence of *Cryptosporidium* in Villages Around Lake Atitlan, Guatemala

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***Cryptosporidium parvum* is an endemic, zoonotic coccidian parasitosis that is highly prevalent in third-world countries where waterborne fecal contamination of food and drink or person-to-person contact with oocysts are the most common methods of transmission of the enteric protozoan. This type of transmission of the parasite made the villages around Lake Atitlan, Guatemala a unique site to compare environmental risk factors with the level of *Cryptosporidium* infections in the local residents. The study was carried out in two villages, San Antonio Palopo and Santa Catarina Palopo, located in the highlands above the shores of the lake. Smears from stool specimens of patients with gastroenteritis were processed using Kinyoun's modified acid-fast stain and observed with light microscopy. Of the 100 residents examined from the two villages, 32% had *Cryptosporidium* infections. Female children had the highest prevalence of infection (44% in San Antonio Palopo and 46% in Santa Catarina Palopo, $p<0.05$), and they also had significantly higher infection rates than males, 50% vs. 17%, respectively. The prevalence rate was not influenced by the season of the year or by the location of the residents. We found differences in prevalence rates due to age and gender, and we suggest that the high infection rates of specific groups are associated with their exposure to the contaminated water supply from Lake Atitlan.**

Key Words: *Cryptosporidium*, disease transmission, Guatemala, risk factors, lake-water.

Intestinal cryptosporidiosis is a cause of diarrheal disease, worldwide, with 10 species of *Cryptosporidium* infecting more than 150 species of mammals [1,2]. The 1993 outbreak of cryptosporidiosis in Milwaukee, Wisconsin, attributed to contaminated city water, produced an estimated 403,000 infections [3]. This endemic, zoonotic coccidian parasitosis is highly prevalent in third-world countries that have waterborne fecal contamination of food and drink with oocysts and where there is direct infection by person-to-person contact as the common method of transmission of this enteric protozoan [4].

Diarrhea, following infection with *Cryptosporidium*, is most common in infants and children in developing countries, in immunosuppressed hosts and in people with deficient nutrition [5-7]. Cryptosporidiosis is a serious disease in these countries, because it increases health problems associated with poverty and malnutrition. Epidemiological changes due to biological differences and sanitation practices of individuals in this population would have an overall effect on disease prevalence. In an attempt to address this point, we examined the risk factors involved in the prevalence of cryptosporidiosis in villages around Lake Atitlan, Guatemala.

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Materials and Methods

Study population

The study was carried out in San Antonio Palopo and Santa Catarina Palopo in the highlands of central Guatemala. The two villages are located 12 miles from each other on the shores of Lake Atitlan, a large, fresh-water reservoir with streams running into it but without water leaving it, making it a confined water source for the local inhabitants. Data were obtained during two January and June visits in 2001 and in 2002.

Children between the ages of 2 and 13, with symptoms of abdominal pain and acute, watery, diarrhea, were selected for the study. The history of infection with *Cryptosporidium*, the immune status, prior or current pathogen infections, and the degree of malnutrition of the patients in our study were not known. Therefore, other unidentified pathogens and conditions could have had an affect on our results of risk factors associated with children having abdominal pain and diarrhea and harboring *Cryptosporidium.*

Of the 100 children in the study, 58 were male and 42 were female. San Antonio Palopo had 21 males and 25 females and Santa Catarina Palopo had 37 males and 17 female participants in the study. The Institutional Review Board of Nova Southeastern University approved the study and informed consent was obtained from subjects, parents or guardians.

Sample collection and laboratory analysis

Children with symptoms of diarrhea and abdominal pain were identified in the towns of San Antonio Palopo and Santa Catarina Palopo. Fecal samples were collected from patients, and smears were prepared on glass slides by mixing with PVA, followed by drying at room temperature. Dried specimens were transported to a laboratory at NSU and *Cryptosporidium* oocysts were identified after staining with Kinyoun's modified acid-fast stain [8]. A sample was considered negative for *Cryptosporidium* if oocysts were not detected after 20 minutes of scanning using oil immersion (1000x) light microscopy.

Statistical analysis

Infected and non-infected patients were identified, and the number of people infected within age groups, gender and villages was used to determine the prevalence of *Cryptosporidium*. Comparisons of the prevalence rates of infection between age groups, gender and villages were made using McNemar's test [9]. Differences between groups were considered significant if $p < 0.05$.

Results

All three groups of children in the study had high infection rates (16-44%) for cryptosporidiosis, and there were no significant differences in the prevalence rates between younger and older age groups (Table 1). In Santa Catarina Palopo, female children in the 2 to 5 year age group had a higher prevalence of infection and had significantly overall higher infection rates than did males. Males in the 2 to 5 year age group in Santa Catarina Palopo had lower prevalence rates for *Cryptosporidium* than the older males. However, significant differences ($p<0.05$) in the presence of *Cryptosporidium* were not found between age groups or genders in San Antonio Palopo.

Total prevalence rates for *Cryptosporidium* infections were high for both San Antonio Palopo and Santa Catarina Palopo, with an overall infection rate for both towns of 32% (Table 2). Prevalence rates for males were lower than those for females when the data from both villages were combined. Overall, males in the 2 to 5 year age group had a lower prevalence rate for cryptosporidiosis than did older male children.

The prevalence of infection recorded for January, in the dry season, was 29% and it was35% for June, during the wet season (Table 3). Males, in the 2 to 5 year age group, exhibited significantly lower prevalence rates than did females, for both January and June. Males, between the ages of 2 and 10, had lower prevalence rates than did females in January. There was no significant difference ($p<0.05$) in the overall numbers of infected children due to the weather conditions.

Table 1. Comparison of prevalence rates of *Cryptosporidium* between male and female children in villages around Lake Atitlan[a,b]

	San Antonio Palopo Proportion (% Infected)			Santa Catarina Palopo Proportion (% Infected)		
Age group	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
2-5	2/10 (20)	4/9 (44)	6/19 (32)	3/19 (16)[c]	5/9 (56)[d]	8/28 (29)
6-9	2/7 (29)	4/9 (44)	6/16 (38)	4/11 (36)	1/3 (33)	5/14 (36)
10-13	1/4 (25)	2/7 (29)	3/11 (27)	2/7 (29)	2/5 (40)	4/12 (33)
All ages	5/21 (24)	10/25 (40)	15/46 (33)	9/37 (24)	8/17 (47)[d]	17/54 (31)

[a]Stool samples were analyzed using Kinyoun's modified acid-fast stain.
[b]Prevalence rates of infected males and females did not differ between villages.
[c]Males 2 to 5 years old had significantly lower prevalence rates ($p<0.05$) than males in other age groups in the same village.
[d]Females had significantly higher prevalence rates than males ($p<0.05$) in the same village.

Table 2. Prevalence rates of *Cryptosporidium* in males and females within age groups for both San Antonio Palopo and Santa Catarina Palopo[a]

	Proportion (% Infected)		
Age group	**Males**	**Females**	**Total**
2-5	5/29 (17)b,c	9/18 (50)	14/47 (30)
6-9	6/18 (33)	5/12 (42)	11/30 (37)
10-13	3/11 (27)	4/12 (30)	7/23 (30)
All ages	14/58 (24)b	18/42 (40)	32/100 (32)

[a]Stool samples were analyzed using Kinyoun's modified acid-fast stain.
[b]Prevalence rates of males were significantly less than those of females ($p<0.05$) within age groups.
[c]Males in the 2-5 age group had a lower prevalence rate ($p<0.05$) than males in the other age groups.

Discussion

The prevalence of cryptosporidiosis in children from Latin American countries varies from 2% to 31% [10], suggesting a high level of infection in Guatemalan villages and was the basis for this study. The demonstration of *Cryptosporidium* in children in villages around Lake Atitlan provided an opportunity to compare infection rates between geographically isolated villages that shared the same sources of infection by having the same flora, fauna, water source and life style risk factors. It was uncertain how often *Cryptosporidium* infections were acquired in these villages. The local environmental distribution of *Cryptosporidium* was dependent upon human, agricultural and wildlife sources and the significance of each individual source with regard to the presence of oocysts in the environment was unknown. Bovine reservoirs were considered as sources of human cryptosporidiosis, as previously demonstrated by Awad-El-Kariem [11,12]. There were few animal reservoirs of *Cryptosporidium* around the lake and they did not seem to have an effect on the prevalence rates of human infection, or if they did, the effect was hidden by the shared use of water.

Past studies of the effect of the weather on prevalence rates for cryptosporidiosis have had varying

Table 3. *Cryptosporidium* infections in children: comparison of prevalence rates of males and females within age groups by season for San Antonio Palopo and Santa Catarina Palopo[a]

	January			June		
	Proportion (% Infected)			Proportion (% Infected)		
Age group	Males	Females	Total	Males	Females	Total
2-5	3/17 (18)[b]	4/7 (57)	7/24 (29)	2/12 (17)[b]	5/11 (45)	7/23 (30)
6-9	2/8 (25)[b]	2/5 (40)	4/13 (31)	4/10 (40)	3/7 (43)	7/17 (41)
10-13	1/5 (20)	2/7 (29)	3/12 (25)	2/6 (33)	2/5 (40)	4/11 (36)
All ages	6/30 (20)[b]	8/19 (42)	14/49 (29)	8/28 (29)	10/23 (43)	18/51 (35)

[a]Stool samples were analyzed using Kinyoun's modified acid-fast stain.
[b]Males in January and June had significantly lower prevalence rates ($p<0.05$) than did females.

results. High levels of infection are found in the dry season in Lima, Peru, 33% [13], the United States, 22-27% [14] and in Guatemala City, 8.3% [5]. Other investigations found high prevalence rates during the wet seasons in the United States, 33-43 % [14], Mexico City, 11% [6], in England and Wales, 2-22% [15], and around Guatemala City, 1% [16].

In contrast, we found no significant differences in the levels of *Cryptosporidium* infections between seasons. The one consistent source of infection was the deep volcanic lake. The water level was constant and it was the main source for drinking, bathing and washing of household goods. It consisted of run-off water from the mountains around it, where the villages were located and where the villagers practiced very poor sanitation.

In our study, lake water was the most important method of *Cryptosporidium* transmission. Children had a tendency to be less aware of hygiene practices and played in dirt yards contaminated with fecal material. Chickens and dogs were commonly found roaming freely in each of the towns, resulting in widespread contamination of the soil with their feces. Cattle and other domestic animals were rarely seen, however, human feces were frequently found on the ground close to human dwellings.

A previous study of children with *Cryptosporidium* found that there were no significant differences in infection due to age or gender [17]. Our data, however, showed that males in the age group 2-5 years had fewer infections than did females (17% *vs.* 50%, respectively, $p<0.05$), suggesting that the male's exposure to *Cryptosporidium* was affected by life style risk factors that increased the exposure of the females to the untreated water. Male and female children of ages 2-5 were basically treated the same by their parents, making their risk factors very similar, and the reasons for the high level of infection in this age group of female children were not obvious. Most males over 10 years of age were up on the mountain sides working in the fields during the day while the female children helped their mother washing clothes in the lake, cleaning and cooking, thus having more exposure to untreated water, increasing their risk of infection. There are quite likely several unidentified routes of disease spread in these villages and further studies are required to determine transmission patterns.

In conclusion, we observed a large number of *Cryptosporidium* infections in children living in two villages around Lake Atitlan, Guatemala, and we postulate that the lake water was responsible for transmission of the disease. The finding that children between the ages of 2 and 10 were highly susceptible (30-37%) to infection with *Cryptosporidium*, and the high prevalence rate in females (50%), suggested a difference in exposure to infections from that of male (17%) children. There was an apparent consistency of infection during both the dry and wet seasons (significantly different infection rates were not

observed) corresponding to a constant exposure to the untreated water supply, coming mainly from the lake.

This study extends the knowledge of the characteristics of *Cryptosporidium* infections by demonstrating that a constant source of contaminated water, regardless of the time of season, results in high prevalence rates in children, and sanitation practices determine the acquisition of infections. Further studies of risk factors in the villages are needed to identify the transmission patterns of cryptosporidiosis.

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