THE CONTRASTING EPIDEMIOLOGY OF CYCLOSPORA AND CRYPTOSPORIDIUM AMONG OUTPATIENTS IN GUATEMALA

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Abstract. We compared epidemiologic characteristics of Cryptosporidium and Cyclospora in surveillance data from outpatient departments in Guatemala. Routinely-submitted stool specimens were screened by microscopy. Age, sex, and symptom data were collected. Cyclospora was detected in 117 (2.1%) and Cryptosporidium in 67 (1.2%) of 5,520 specimens. The prevalence of Cyclospora peaked in the warmer months, while Cryptosporidium was most common in the rainy season. Both affected children more than adults, but Cryptosporidium affected children at a younger age than Cyclospora (median age 2 years versus 5 years; P < 0.001). Cyclospora showed a stronger association with diarrhea than Cryptosporidium, even when data were stratified by age. These contrasts may reflect differences in the relative importance of transmission modes, the frequency of exposure, and the development of immunity.

INTRODUCTION

The coccidian parasites Cyclospora cayetanensis and Cryptosporidium parvum have often been linked. The original study in which C. cayetanensis was identified as a coccidian was initially designed to study Cryptosporidium and C. cayetanensis was nicknamed “big Crypto” when it was seen on modified acid-fast stained smears.1 The same modified acid-fast and hot-safranin staining techniques can be used to detect both organisms.2 The two parasites cause prolonged, often intermittent diarrhea, both affect pediatric age groups in many developing countries, and both may lead to severe diarrhea in immunocompromised hosts.1–3 However, C. cayetanensis is genetically more closely related to Eimeria than to Cryptosporidium,5,6 and the two organisms have salient biologic differences. While Cryptosporidium is infectious at the time of excretion and can be transmitted directly from person to person and by fecally-contaminated fomites, C. cayetanensis is thought to require a period of days to weeks in the environment to sporulate into the infectious form.1 This characteristic makes direct person-to-person spread of C. cayetanensis unlikely. Water has been implicated as a likely vehicle for both infections.7–9 Cryptosporidium infects a wide range of mammalian hosts and can be a zoonotic infection,11 while C. cayetanensis has never been convincingly demonstrated to infect a non-human host.12,13 Because the two parasites differ biologically, it is not unexpected that epidemiologic differences should exist. This study presents the first systematic comparison of the epidemiologic characteristics of the two organisms.

MATERIALS AND METHODS

The surveillance system methods were described in a previous publication.7 The current analysis used surveillance data from two government health centers and three hospital outpatient departments, from which routinely-collected stool specimens were screened for Cryptosporidium and Cyclospora. We examined both diarrheal and non-diarrheal specimens from each participating health-care facility. Many of the specimens from subjects without diarrhea had been submitted for parasite screening as a requirement for public school or employment. All surveillance sites were in urban or periurban areas within 50 kilometers of Guatemala City in the moderate highlands. The populations served by these facilities were predominantly poor or working-class. The study protocol was reviewed and approved by the Centers for Disease Control and Prevention (CDC) institutional review board, as well as by local review committees as required in Guatemala, and all participants gave written informed consent before participation.

Laboratory methods. Stool specimens were processed using a standard formalin-ethyl acetate concentration procedure and screened by two methods, light-microscopic examination of a modified acid-fast stained smear, and ultraviolet epifluorescence examination of a wet mount.2 We categorized the number of parasites in each stool specimen: “many” was equivalent to 10 or more oocysts, “moderate” to 3 to 9 oocysts, and “few” to 2 or fewer oocysts per 10 oil immersion (1,000x) fields. During the last 6 months of surveillance, all specimens positive for Cryptosporidium by acid-fast examination were confirmed by direct immunofluorescence assay (IFA) using the Cryptosporidium-specific OW50 monoclonal antibody.14,15 To confirm the identity of Cyclospora cayetanensis, three positive specimens stored in 2.5% dichromate at ambient temperature (approximately 23°C) were examined at regular intervals over a period of 2 weeks starting from the time of excretion.7 We observed characteristic sporulation in all three.

Data analysis. All data were entered in Epi-Info 6.04b (CDC, Atlanta, GA, 1997). Analysis of surveillance data was conducted in Epi-Info and figures were prepared in Microsoft Excel (Microsoft Corporation, Redmond, WA, 1997). Unless otherwise specified, the significance of differences was tested by Mantel-Haenszel chi-square test.

RESULTS

From April 6, 1997 to March 19, 1998, we screened 5,520 specimens from health care facilities. Cyclospora was detected in 117 specimens (2.1%), while Cryptosporidium was found in 67 (1.2%). Two specimens were positive for both organisms; both were from children younger than 1 year. For both organisms, the majority of detections had 2 or fewer
oocysts per 10 oil immersion fields (few oocysts) and very few specimens had 10 or more oocysts per 10 oil immersion fields (many oocysts). This pattern was more marked for *Cyclospora* (5%, 12%, and 83%; many, moderate, and few oocysts, respectively) than for *Cryptosporidium* (13%, 17%, and 70%, respectively) (*P = 0.03* for comparison of the distributions by parasite). Children younger than 2 years were more likely to have *Cryptosporidium* detected in large or moderate numbers than subjects 2 years or older (14 of 30 versus 7 of 34; *P = 0.03*). A similar trend for *Cyclospora* among children younger than 5 years compared with subjects 5 or older did not reach statistical significance (10 of 51 versus 5 of 50; *P = 0.18*).

The *Cyclospora* detection rate was markedly seasonal, occurring predominantly between May and August with a sharp peak in June; the monthly detection rate varied from 0% in December and March to 6.2% in June (Figure 1). *Cryptosporidium* showed a less pronounced seasonal pattern with a range of 0.2% in January to 2.5% in September. The peak of *Cyclospora* detections occurred as the seasonal rains were just beginning, when the mean temperature was descending from its yearly high, while *Cryptosporidium* occurrence coincided with the months of highest rainfall. The seasonal patterns were similar to those seen in the complete data set when the data were stratified by age and by the presence of diarrhea.

While both organisms were most prevalent in pediatric age groups, *Cryptosporidium*-positive specimens were from younger children than *Cyclospora*-positive specimens (Table 1). The median age of persons with *Cryptosporidium* was 2 years (range 0.3–52) compared with 5 years (0.6–75) for *Cyclospora* (*P < 0.001* by Wilcoxon 2-sample test). For all specimens, regardless of symptoms, the highest *Cryptosporidium* prevalence was seen among children 1 to 2 years of age, while *Cyclospora* was most frequent among children 3 to 9 years, followed by those 1 to 2 years (Figure 2). For *Cyclospora*, detections were rare among infants, and the prevalence among persons 60 years and older was higher than that among younger adults (*P = 0.09* by 2-tailed Fisher exact test for adults 60 years or older compared to those aged 20–59 years).

In our surveillance data, *Cyclospora* was more strongly associated with diarrhea than *Cryptosporidium* (Table 1 and Figure 3). For *Cyclospora*, the association was strongest among children 2 to 4 years (Prevalence ratio [PR] 6.9; 95% CI, 3.4–13.9; *P < 0.0001*). For children 5 to 9 years, the association did not quite reach statistical significance (PR 2.1; 95% CI 0.9–4.8; *P = 0.09* by Fisher exact test). Specimens with many *Cyclospora* oocysts were significantly more likely to be associated with diarrheal illness than those with

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**TABLE 1**

Prevalence of *Cyclospora* and *Cryptosporidium* detection by age group and symptom status among outpatients attending three hospitals and two health centers in Guatemala City.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>With diarrhea</th>
<th>Without diarrhea</th>
<th>With diarrhea</th>
<th>Without diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>3/284 (1.1)</td>
<td>12/695 (1.7)</td>
<td>9/284 (3.2)</td>
<td>17/695 (2.4)</td>
</tr>
<tr>
<td>2–4</td>
<td>23/240 (9.6)†</td>
<td>11/782 (1.4)†</td>
<td>7/240 (2.9)</td>
<td>14/782 (1.8)</td>
</tr>
<tr>
<td>5–9</td>
<td>7/99 (7.1)‡</td>
<td>24/713 (3.4)‡</td>
<td>1/99 (1.0)</td>
<td>2/713 (0.3)</td>
</tr>
<tr>
<td>10–19</td>
<td>1/74 (1.4)</td>
<td>5/519 (1.0)</td>
<td>0/74 (0)</td>
<td>4/519 (0.8)</td>
</tr>
<tr>
<td>20–29</td>
<td>0/84 (0)</td>
<td>3/474 (0.6)</td>
<td>2/84 (2.4)</td>
<td>2/474 (0.4)</td>
</tr>
<tr>
<td>30–39</td>
<td>1/90 (1.1)</td>
<td>2/404 (0.5)</td>
<td>0/91 (0)</td>
<td>0/404 (0)</td>
</tr>
<tr>
<td>40–49</td>
<td>0/77 (0)</td>
<td>1/284 (0.4)</td>
<td>1/77 (1.3)</td>
<td>0/284 (0)</td>
</tr>
<tr>
<td>50–59</td>
<td>0/39 (0)</td>
<td>2/173 (1.2)</td>
<td>0/39 (0)</td>
<td>1/173 (0.6)</td>
</tr>
<tr>
<td>≥60</td>
<td>2/50 (4.0)</td>
<td>2/205 (1.0)</td>
<td>0/50 (0)</td>
<td>0/205 (0)</td>
</tr>
<tr>
<td>All ages</td>
<td>37/1,038 (3.6)†</td>
<td>62/4,249 (1.5)†</td>
<td>20/1,038 (1.9)§</td>
<td>40/4,249 (0.9)§</td>
</tr>
</tbody>
</table>

* Values are the number positive/number tested (% positive).
† *P < 0.0001* by Mantel-Haenszel chi-square test for the comparison of prevalence among those with diarrhea.*
‡ *P = 0.09* by 2-tailed Fisher exact test.
§ *P < 0.01*
FIGURE 2. The prevalence of Cryptosporidium and Cyclospora by age group in years. Median number of specimens by age group = 369 (255–1,895).

FIGURE 3. The prevalence of Cryptosporidium and Cyclospora by age group in years with the presence or absence of diarrhea. The prevalence of Cyclospora was significantly higher among those with diarrhea compared with those without diarrhea for children in the 2-, 3- and 4-year-old age groups. Median number of specimens by age group: diarrheal / non-diarrheal = 96 (34–365) and 293 (207–1,561).

DISCUSSION

This study highlights the contrasts between Cyclospora and Cryptosporidium in Guatemala, including differences in seasonality, in the age groups most affected, and in the strength of their association with diarrhea. These contrasts provide clues to characteristics such as the importance of different transmission modes and development of immune protection.

The monthly patterns of occurrence we found are consistent with those described in other studies. While the pattern for Cryptosporidium in developing countries may be related to contamination of vulnerable drinking water sources by heavy seasonal rains, the case of Cyclospora appears to be less straightforward. In Lima, Peru, Cyclospora prevalence is markedly seasonal in the absence of rain, while in Haiti, Cyclospora occurrence coincides with the cooler part of the year, when temperatures are closer to those of temperate Guatemala City and Kathmandu in the warmest months of the year. Elucidating the reasons for the seasonality of Cyclospora, in particular, will require a more profound understanding of the biologic characteristics and environmental triggers that control its infectivity and transmission.

Differences in the age patterns of occurrence of the two organisms are striking. Cryptosporidium, in common with enteric pathogens such as rotavirus, affects children most in the first 2 years of life. By contrast, Cyclospora was found more frequently among somewhat-older children. It is likely that these age patterns reflect differences in modes of transmission. Both organisms are spread by contaminated water or food. Cryptosporidium, in addition, is easily spread person-to-person among infants and young children in crowded home or day-care environments. In contrast, because of its biologic characteristics, direct person-to-person spread of Cyclospora is unlikely. Infants have less exposure to contaminated water than older children as parents may take greater care to ensure they drink only safe water and infants are less likely to seek sources of water on their own. In our case-control study of risk factors for Cyclospora in Guatemala, infants were one-fifth as likely to have drunk untreated water as persons 1 year of age or older (P < 0.01 by 2-tailed Fisher exact test [Bern C, unpublished data]). They are also less likely to eat fresh, raw produce that may be a source of sporulated C. cayetanensis oocysts. These
behaviors may contribute to the differences in age-specific occurrence of the two parasites.

In our study, the association with diarrheal illness was stronger for Cyclospora than for Cryptosporidium. Several earlier studies have reported a much stronger association of Cryptosporidium with diarrheal illness than we found, but the differing study designs make interpretation difficult. Studies confirm that both organisms commonly cause infection in the absence of diarrhea. Our data suggest that, in the population we studied, Cyclospora may cause a somewhat more severe spectrum of disease than Cryptosporidium, leading over-representation in clinic attendees. In addition, we found that the larger the number of Cyclospora oocysts detected, the greater the likelihood of diarrhea, an association we did not find for Cryptosporidium.

The observation of the relationship between oocyst quantity in stool and the probability of diarrhea, together with the pattern of age-specific occurrence, suggest that in low-income Guatemalan communities, immunity to Cryptosporidium develops very early in life and is probably maintained at a high level by repeated subclinical exposure, possibly multiple times per year. Cohort studies in comparable communities confirm repeated Cryptosporidium infections in the first 3 years of life, many of them without diarrhea. In contrast, if the age patterns we observed reflect immune status, apparent immunity to Cyclospora appears late in childhood or in adolescence and may wane with age. Exposure to Cyclospora may be less frequent or the immune mechanisms may be less efficient than against Cryptosporidium. However, these findings remain more suggestive than conclusive. Our findings underscore the importance of conducting carefully-designed studies, including epidemiologic studies which directly address the questions of transmission modes and development of immune response and studies of the biology of the parasites which may elucidate the reasons for their seasonality.

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