CYCLOSPORA CAYETANENSI S INFECTIONS AMONG DIARRHEAL PATIENTS FROM VENEZUELA

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Abstract. The prevalence and importance of Cyclospora cayetanensis as an enteropathogen among 71 patients (22–45 years old) with acquired immunodeficiency syndrome (AIDS) and 132 children with diarrhea (0–12 years old) from Venezuela was assessed retrospectively. Two to three stool samples from each patient attending our parasitology laboratory for parasitologic and medical assistance were examined. For identification of the coccidium, modified Ziehl-Neelsen carbolfuchsin staining of formalin-ether stool concentrates was used, and for other intestinal parasites, iron-hematoxylin-stained smears and formalin-ether concentrates were examined. Cyclospora oocysts were found in seven (9.8%) of 71 AIDS patients and seven (5.3%) of 132 children with diarrhea. Other pathogenic parasites were present in most of the patients (9 of 14, 64.3%) shedding oocysts. Cyclosporiasis predominated in children 2–5 years of age with respect to those ≤ one year of age (P < 0.05). The findings suggest that C. cayetanensis is common in diarrheal patients from Venezuela. However, the role of the parasite as the causal agent of diarrhea in these patients is uncertain.

INTRODUCTION

In recent years, the parasitic apicomplexan protozoan Cyclospora cayetanensis has emerged as an important human pathogen that causes enteric disease with prolonged diarrhea in both immunocompromised and immunocompetent hosts. In the latter, diarrhea is usually prolonged, but self-limited. In immunocompromised individuals, it may be prolonged and severe.1

Cyclosporiasis has a worldwide distribution. It has been reported with increasing frequency in North, Central, and South America, the Caribbean, Southeast Asia, Nepal, India, Bangladesh, Africa, Australia, England, Canada, and eastern Europe.1 In industrialized countries, the infection has been traditionally associated with diarrhea in travelers to endemic countries. However, in the 1990s, several waterborne and foodborne outbreaks were reported from North America and Canada.2,3 The infection occurs in persons of all ages, with higher prevalences in children and in tropical areas where infection rates as high as 20% have been observed.3,4

Limited information is available on the infection in patients infected with the human immunodeficiency virus; infection rates of 0.1% in developed countries5 and less than 1% to 11% in developing countries have been reported.1,8,9 The low rates of infection in industrialized and some developing countries have been related to the common use of trimethoprim-sulfamethoxazole (TMP-SMX) for prophylaxis against opportunistic infections in these patients.1,10 In Venezuela, the extent of the infection and its effects on human health is unknown. There is no information on the occurrence of Cyclospora among immunocompromised or immunocompetent diarrheal individuals.

The Parasitology Section of our institution is a laboratory for research on intestinal parasites and diarrheal diseases; it has served many immunocompetent and immunocompromised patients for whom formalin-ether concentrations10 and iron-hematoxylin-stained fecal smears11 are routinely made. Since 1991, routine modified acid-fast staining of formalin-ether concentrates12 of all specimens was implemented to screen for Cryptosporidium parvum. Studies on the prevalence of this coccidium in patients with acquired immunodeficiency syndrome (AIDS) and in children with diarrhea studied from 1990 to 1993 have been reported.13,14 In subsequent years, C. cayetanensis has emerged as another human pathogen. Therefore, to assess the prevalence and importance of this parasite in diarrheal patients, a retrospective study was designed involving both immunocompromised and immunocompetent patients attending our laboratory over a two-year period.

MATERIALS AND METHODS

Epidemiologic and clinical results presented here are based on a retrospective study of stained fecal smears and clinical records from 203 patients with acute or chronic gastrointestinal symptoms referred to us from January 1992 to February 1994 for parasitologic and medical assistance. This study was approved by the Technical Council of the Instituto de Investigaciones Clínicas, and adult patients and children’s parents gave fully informed consent for participation in our research studies at the time they attended the laboratory.

Patient population. The patients came from different areas of Zulia state in western Venezuela. The majority (147 of 203, 72.4%) resided in poor sectors. Seventy-one patients with AIDS and chronic diarrhea, as defined by the Centers for Disease Control and Prevention, (Atlanta, GA)15 were referred to us by the Regional Clinical Immunology Unit of Maracaibo, the capital of the state. They were 22–45 years old; 56 were men and 15 women. Most of them (66 of 71) had no data on quantification of T helper and T suppressor lymphocytes. One hundred thirty-two patients with acute or chronic diarrhea were referred to us by several public hospitals and private clinics. They ranged in age from 0 to 12 years old; 76 were male and 56 were female.

Clinical evaluation. Data on age, sex, symptoms relating to enteric disease, and recent history of anti-infective drugs were reviewed in the clinical evaluation records supplied by physicians who referred the patients and by adult patients or children’s parents in the laboratory’s interviews. Diarrhea was defined as the presence of three or more stools a day that were looser than usual for a period of at least three days before examination. It was considered chronic, according to
the World Health Organization current definition, if its duration was ≥ 1 month.

**Stool examination.** Two to three stool specimens from each patient were examined. For identification of *Cyclospora* infections, modified Ziehl-Neelsen carbolfuchsin staining of 580 formalin-ether concentrates of 203 patients were used. An ocular micrometer was used to measurement the size of the oocysts. The stained smears were examined by light microscope under dry magnification (400×). *Cyclospora* oocysts were identified by their characteristic size, morphology, and variable acid-fast staining. To determine the size of these oocysts and to differentiate them from those of *Cryptosporidium parvum*, an ocular micrometer was used. Parasitologic records of iron-hematoxylin–stained fecal smears and formalin-ether concentrate examinations were also reviewed for other pathogenic parasites. Microsporidal, bacterial, and viral pathogens were not sought.

**Statistical analysis.** For statistical analysis, the chi-square test and Fisher’s exact test were used. Results were considered significant if *P* was less than 0.05.

**RESULTS**

Table 1 shows the results of stool examinations for enteropathogenic parasites. The overall prevalence of *C. cayetanensis* was 6.9% (14 of 203 individuals). The infection rates in AIDS patients (9.8%, 7 of 71) and children (5.3%, 7 of 132) were not significantly different. The oocysts were consistent in appearance with a previous description of the parasite. They appeared as spherical organisms, uniform in size, 8–10 μm in diameter, twice larger than those of *Cryptosporidium parvum* (4–6 μm). The oocyst wall occasionally had a wrinkled appearance, collapsed on the sides. Some organisms stained dark red and had a variable number of dark inclusion bodies; others stained pink or remained unstained, as clear spheres. In general, very few oocysts (less than 10 per coverslip) were observed, with the exception of samples from five AIDS patients that had 40–60 oocysts per coverslip.

Infection rates with one or more pathogenic parasite species in AIDS patients and diarrheal children were high (53.5% and 60.6%, respectively). In the former group, the most commonly found pathogen was *Cryptosporidium parvum* (35.2%); in the latter, *Giardia lamblia* was the most frequently observed protozoa. Intestinal coccidia, with the exception of *Isospora belli*, were detected in both groups but were more frequent in AIDS patients. *Cryptosporidium parvum* predominated (*P < 0.05*) in these patients. Characteristics of subjects who passed oocysts are shown in Table 2. The parasite was detected as sole pathogen in five (35.7%) of 14 patients. The remainder had coinfections. Those with AIDS had a history of chronic diarrhea, continuous or intermittent, for 1–18 months; some of them had an intense disease. In children, diarrhea varied from mild to moderate and ranged from seven to 43 days; three (42.8%) of seven had chronic diarrhea. Watery diarrhea was present in nine (64.3%) of 14 patients. The prevalence of symptoms in patients with or without cyclosporiasis did not differ significantly. A history of recent anti-infective drugs was detected in 10 of 203 (4.9%) patients. The use of antibiotics was significant (*P < 0.05*) in these patients. Characteristic of subjects with or without cyclosporiasis did not differ significantly. A history of recent anti-infective drugs was detected in 10 of 203 (4.9%) patients. The use of antibiotics was significant (*P < 0.05*) in these patients. Characteristic of subjects with or without cyclosporiasis did not differ significantly. A history of recent anti-infective drugs was detected in 10 of 203 (4.9%) patients. The use of antibiotics was significant (*P < 0.05*) in these patients. Characteristic of subjects with or without cyclosporiasis did not differ significantly. A history of recent anti-infective drugs was detected in 10 of 203 (4.9%) patients. The use of antibiotics was significant (*P < 0.05*) in these patients. Characteristic of subjects with or without cyclosporiasis did not differ significantly.
ezuela. This study provides new information on the epidemiology of the coccidium. The infection rate (9.8%) of *Cyclospora* observed in AIDS patients was relatively high, similar to that reported from Haiti (11%), and higher than those published in Peru (less than 1%), Tanzania (1%), Thailand (2.2%), Honduras (6.8%), Cuba (3%), and Egypt (4%).

This finding and the predominance of infection in children and its rarity in adults in developing countries suggest that this parasite is an important opportunistic pathogen in Venezuela. As in Haiti, this result might be due, at least in part, to the infrequent use of TMP-SMX in our patients; in Venezuela, the use of this drug combination for *P. carinii* or *Toxoplasma* prophylaxis is uncommon. The high prevalence (35.2%) of *Cryptosporidium parvum* in this study is in agreement with a regional report and studies from other countries showing that this parasite is predominant in diarrheal disease associated with AIDS. This fact and the relatively low rate of *Cyclospora* infection compared with that of *Cryptosporidium* in this study and that from Haiti, where the use of TMP-SMX prophylaxis is uncommon, might be explained in part by some differences in the mechanisms of transmission of both coccidia. Direct person-to-person transmission of *Cryptosporidium parvum* infection might be a factor. This mechanism is unlikely for *Cyclospora* since oocysts are shed unsporulated.

The prevalence (5.3%) of *Cyclospora* in pediatric patients was similar to that observed in Nepalese diarrheal children (5%). With respect to children studied in community-based surveys, our infection rate was lower than those reported from Peru (6–18%) and Haiti (15–20%), and similar to that reported from Guatemala (2.3–6.7%).

The role of *C. cayetanensis* in the pathogenesis of diarrhea in our patients is uncertain since the parasite was associated in most of the cases with other pathogenic parasites. In addition, *Microsporida*, bacteria, and viruses, which would likely account for the symptoms in some of the patients, were not sought. However, the literature suggests that prolonged watery diarrhea and weight loss are often found in those with *cyclosporiasis*, as in our patients. In addition, of four patients who had both symptoms, three had *Cyclospora* as the sole parasitic pathogen and one had an *Ascaris* infection, which has not been described as a cause of watery diarrhea. Thus, it seems likely that the coccidium was, at least, partially responsible for the illness in these patients, two of them were infants.

Most *Cyclospora* infections seem to result in a few oocysts being passed, at least in immunocompetent individuals. This study confirms this observation since the number of oocysts was typically low (less than 10 oocysts per coverslip) in almost all the positive cases even though they were patients with diarrhea. However, it should be noted that five patients with AIDS had 40–60 oocysts per coverslip. This result is in agreement with the histologic evidence that these patients appear to harbor more parasites than do the immunocompetent subjects.

The current data suggest that in this area cyclosporiasis is acquired early in life and is more frequent in children two or more years old, as previously reported. However, we must be cautious in extrapolating our results to the general population since our data were based on patients with diarrhea and are skewed towards symptomatic individuals.

*Cyclosporiasis* appears to have a marked seasonality. However, we did not observe any monthly variations in the occurrence of the infection. This result might be a reflection of the small sample size of the infected cases.

Our findings suggest that human infection with *Cyclospora* is endemic and widely distributed in Zulia state, since the positive cases came from different sectors of the state. Likewise, the infection probably occurs in the rest of the country and the absence of reported cases might be due to a lack of awareness of this parasite and the absence of routine laboratory screening for acid-fast organisms in stool samples.

In conclusion, *C. cayetanensis* is a common parasite in patients with diarrhea from Venezuela and appears to play a role in the etiology of diarrhea. However, there is a strong need for future research in the general population to clarify the epidemiology, the relative rates of symptomatic and asymptomatic infections, and the role of the parasite as an etiologic agent of disease to the community level. The present study, however, provides data on the epidemiology of *cyclosporiasis* in this country.

### References


