

## PREVALENCE AND PATHOGENIC ROLE OF *CYCLOSPORA CAYETANENSIS* IN A VENEZUELAN COMMUNITY

LEONOR CHACIN-BONILLA, MARGOT MEJIA DE YOUNG, AND JESUS ESTEVEZ  
*Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela*

**Abstract.** The prevalence and pathogenic role of *Cyclospora cayetanensis* among 212 subjects (age range = two months to 70 years) in an impoverished community in Venezuela were assessed retrospectively. For identification of the coccidium, modified Ziehl-Neelsen carbolfuchsin staining of formalin-ether concentrates was used. For other pathogenic parasites, iron-hematoxylin-stained smears and formalin-ether concentrates were examined. *Cyclospora* infections were identified in 13 (6.1%) subjects with a high percentage of asymptomatic carriers (11 of 13, 84.6%). Only two (15.4%) infants had diarrhea and the coccidium as the single detectable pathogenic parasite. The findings suggest that *Cyclospora* infections are relatively common and often asymptomatic in this region.

### INTRODUCTION

*Cyclospora cayetanensis* is an emerging cause of gastroenteritis in humans worldwide. A strong association of this parasite with diarrhea in patients with acquired immunodeficiency syndrome (AIDS), travelers, and pediatric groups has been found in health center populations.<sup>1</sup>

In developing countries, cyclosporiasis and associated symptoms occur more often in children.<sup>1–3</sup> Infection rates as high as 20% have been reported<sup>4</sup> and asymptomatic infections have been documented.<sup>4,5</sup> In Venezuela, the prevalence and significance of the coccidium as a causal agent of enteric disease is largely undefined. In individuals with diarrhea, infection rates of 5.3% in children and 9.8% in patients with AIDS have been reported.<sup>6</sup> The majority of epidemiologic studies on *Cyclospora* has involved highly selective populations, and there are virtually no community-based reports<sup>1</sup> with the exception of two studies from Peru<sup>5</sup> and Haiti.<sup>4</sup> However, the former was a prospective study limited to children less than 2.5 years of age and the latter involved only groups of mothers and children. Two reports from Guatemala based mainly on health care populations also included data and surveillance collected in cohorts of raspberry farm workers.<sup>2,7,8</sup> Thus, the epidemiologic features of the parasite are unknown and investigations based in whole populations are needed.

To assess the prevalence and pathogenic role of this coccidium in Venezuela, a retrospective study was designed involving a community previously evaluated by our group for other intestinal parasites.<sup>9</sup>

### MATERIALS AND METHODS

Clinical and epidemiologic data presented here are based on a retrospective study of clinical records and stained fecal smears from a representative sample selected house-to-house of 212 subjects from an impoverished suburban community in the Zulia State of Venezuela.<sup>9</sup> This project was reviewed and approved by the Instituto de Investigaciones Clínicas and informed consent was obtained from householders for participation in our research studies at the time of the intestinal parasite survey.

The study settlement was San Luis, which is located in the coastal lowlands near Lake Maracaibo in the Zulia State of northwestern Venezuela. The mean annual temperature, relative humidity, and amount of rainfall were 28°C, 78%, and 747 mm, respectively. The sanitary and socioeconomic stan-

dards were low. Thirty-eight percent of the individuals were unemployed and 40% were illiterate. Sixty-five percent of the housing units had indoor plumbing and 35% had piped water. Fifty-six percent had flush toilets and 3.7% had latrines. Forty percent of the individuals defecated in the backyards of the houses. Overflowing sewage and piles of rubbish were frequently observed. Households often had one or two dormitories for six or more occupants, providing the opportunity for frequent contact among residents. Domestic animals such as cats, dogs, pigs, and poultry were common.

Fifty-four families were selected by random sampling and then visited to ensure understanding of the aim of the study and acceptability and cooperation of the householders. Data pertaining to family identification, socioeconomic conditions, and gastrointestinal symptoms at the time of the survey and during the previous month were recorded. Single fecal specimens were collected from 212 individuals (age range = two months to 70 years) who provided representative coverage of the whole population, which was approximately 2,000 inhabitants. Specimens were obtained and processed within two hours after collection.

Data on age, sex, and gastrointestinal complaints were reviewed in the clinical evaluation records of this population. For identification of *Cyclospora* oocysts, modified Ziehl-Neelsen carbolfuchsin staining of formalin-ether concentrates were used.<sup>10</sup> The stained smears were examined under dry magnification (400×) and an ocular micrometer was used to measure the size of the oocysts. For other pathogenic parasites, parasitologic records of iron-hematoxylin-stained fecal smears<sup>11</sup> and formalin-ether concentrate examinations<sup>12</sup> were also reviewed. For data analysis, the chi-square and Fisher's exact tests were used. Results were considered significant if the *P* value was less than 0.05.

### RESULTS

Infection with *Cyclospora* was identified in 6.1% (13 of 212) of the subjects. The oocysts appeared as spherical structures 8–10 μm in diameter with variable acid-fast staining, as previously described.<sup>5</sup> Some stained dark red and had a variable number of dark inclusion bodies; others stained pink or remained unstained.

Of the 13 subjects shedding oocysts, 11(84.6%) were asymptomatic at the time of sampling or during the previous month and two (15.4%) had diarrhea that lasted from four to ten days by the time of the survey. Both patients were infants,

including one of five months of age, who had the coccidium as sole pathogenic parasite detected. Of the 13 *Cyclospora*-positive individuals, 11 (84.6%) were coinfecting with other pathogens, including helminths and other protozoa. Among the latter, *Giardia lamblia* was the most frequent (30.8%).

The distribution of *Cyclospora* infections among age groups showed a tendency for a higher occurrence in individuals 6–15 years of age, although no significant differences were found (Table 1).

## DISCUSSION

This is one of the first studies among indigenous persons from developing countries to assess the prevalence and pathogenic role of *Cyclospora*, and it appears to be the first community-based investigation involving an entire population. This report provides new data on the epidemiology of this parasite. Although underestimation of the prevalence of *Cyclospora* may result from the present data since single stool specimens were examined, it is likely that enhanced sensitivity can be obtained by detection of more low-intensity infections through the examination of concentrated and Ziehl-Neelsen-stained stool samples. Despite this limitation, the results suggest that the coccidium is common in this area. The infection rate of 6.1% observed is higher than those observed in at-risk populations of raspberry farm workers (0–3.3%) from Guatemala,<sup>2,7,8</sup> but lower than that reported from Haiti.<sup>4</sup> Comparison of our results with others based in health center populations<sup>1–3</sup> or concerned only with young children<sup>5</sup> is more difficult.

The age distribution of *Cyclospora* infections suggest that subjects are exposed to the parasite at a very early age and that infections tend to be more common in the 6–15-year-old children, although no significant differences were obtained among the age groups. This statistical result may be due to the small sample size of the infections since some reports have suggested that children less than nine years of age are more susceptible to infection.<sup>2,3</sup> However, these studies were based in health center populations and were thus skewed toward younger children who appear to be more susceptible to clinical manifestations in tropical countries.<sup>1–3</sup> The detection of *Cyclospora* in adolescents and adults in this impoverished area contrasts with the absence of the parasite in those  $\geq 11$  years of age in slums from Peru.<sup>13</sup>

Diarrhea was not a prominent feature of *Cyclospora* infection in this study. Only two (15.4%) infants had diarrheal illness that could be related to the parasite. However, other etiologic agents were not identified. The high proportion (84.6%) of asymptomatic infections was remarkable. How-

ever, since stool specimens were collected at one point in time and diarrheal symptoms were analyzed retrospectively, it is not known how many of these infections occurred during the incubation period prior to the developing of symptoms. Indeed, a study from Nepal showed one patient with histopathologic changes consistent with infection with *Cyclospora* who was asymptomatic, but developed diarrhea within five days of endoscopy.<sup>14</sup> Despite this limitation, the appreciable rates (72–94%) of asymptomatic infections, even in children less than three years old, which was documented in longitudinal studies of indigenous populations from Haiti<sup>4</sup> and Peru,<sup>5</sup> support our results. Thus, it appears that in developing countries, the situation at the general population level is quite different than that observed in health center populations in whom a strong association of the parasite with diarrhea has been recognized.<sup>1</sup>

In this study, the high rate of asymptomatic carriage of the coccidium suggests that it may not play a consistent pathogenic role in this population. Thus, the probability that individuals shedding oocysts could have developed diarrhea after the primary infection with the parasite should not be minimized. In tropical countries, asymptomatic excretion of intestinal pathogens is frequently observed.<sup>15</sup> Similarly, a high proportion (71.4%) of asymptomatic *Cryptosporidium* infections in the study community was previously reported.<sup>9</sup> The overwhelming parasitism also found in this area<sup>9</sup> reflects its unsanitary living conditions. In this kind of setting, continuous exposure to *Cyclospora* may be associated with protection against parasite-associated symptoms. In this study, the age distribution of diarrheal illness affecting only infants and the presence of asymptomatic infections in all the other age groups appear to suggest the development of partial immunity that protects from the pathogenic effects of the coccidium, but not from reinfections. Studies from Peruvian shanty towns suggest that immunity becomes complete by adolescence.<sup>13</sup>

In conclusion, our findings suggest that *Cyclospora* infections are relatively common and often asymptomatic in the general population of this Venezuelan region. However, further studies are needed to better characterize the epidemiology and significance of *C. cayetanensis* in this country.

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Authors' addresses: Leonor Chacin-Bonilla, Margot Mejia de Young, and Jesús Estévez, Instituto de Investigaciones Clínicas, Universidad del Zulia, Apartado Postal 1151, Maracaibo, Venezuela, Telephone: 58-261-759-7247, Fax: 58-261-783-5587, E-mail: ebonillaro@yahoo.com

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TABLE 1.

Age distribution of *Cyclospora cayetanensis* infections in 212 subjects from a Venezuelan community

Age (years)	No. tested	Infected	Symptomatic
		No. (%)	No. (%)
0–5	60	3 (5.0)	2 (66.6)
6–15	61	7 (11.5)	0 (0)
16–25	56	2 (3.6)	0 (0)
$\geq 26$	35	1 (3.3)	0 (0)
Total	212	13 (6.1)	2 (15.4)

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