

## *Cyclospora cayetanensis* in a Pediatric Hospital in Morelia, México

Guadalupe E. Orozco-Mosqueda\*, Orlando A. Martínez-Loya, and Ynes R. Ortega

Hospital Infantil de Morelia Eva Samano de López Mateos, Servicios de Salud de Michoacán, Michoacán, Mexico; Center for Food Safety, University of Georgia, Griffin, Georgia

**Abstract.** *Cyclospora cayetanensis*, a coccidian parasite, can cause gastrointestinal illness in humans and is characterized by watery and persistent diarrhea and abdominal pain. Cyclosporiasis has been associated with traveler's diarrhea. The infection is acquired through food and waterborne transmission, particularly by consumption of contaminated fresh fruits and vegetables. In the present study, stool samples from 8,877 children were examined for ova and parasites at the Pediatric Hospital of Morelia in Michoacán, Mexico, during 2000–2009. Sixty children (0.67%) had *Cyclospora* in their stools. Diarrhea (45.8%), abdominal pain (39.6%), and vomiting (18.8%) were the most frequent symptoms of cases with cyclosporiasis. Most of the cases (93.3%) were observed during June–August, the rainy season. In 45 children, *Cyclospora* was the only parasitic pathogen detected (75%); 15 children were co-infected with commensal, pathogenic, or both groups of parasites. Our findings suggest that *C. cayetanensis* is endemic to Michoacán and shows characteristically temporal patterns.

### INTRODUCTION

*Cyclospora cayetanensis* is a coccidian parasite<sup>1</sup> that causes watery and persistent diarrhea in immunocompetent and immunocompromised persons.<sup>2</sup> It has been associated with traveler's diarrhea<sup>3</sup> and with foodborne and waterborne outbreaks of cyclosporiasis. The most widely known waterborne outbreak occurred in 1994 in Nepal where 12 of 14 soldiers and dependents developed diarrheal illness. Contaminated chlorinated water was believed to be the most likely source of infection.<sup>4</sup> Cyclosporiasis can also be acquired by the ingestion of contaminated uncooked fruits and vegetables,<sup>5–7</sup> particularly lettuce,<sup>8</sup> basil,<sup>9,10</sup> snow peas,<sup>11</sup> and berries.<sup>12–14</sup>

*Cyclospora* infections have been described in tropical and subtropical areas, and are considered endemic to certain regions in developing countries, such as Peru, Guatemala, Haiti, and Nepal.<sup>7,15–19</sup> A few reports have described cyclosporiasis in travelers returning from Mexico<sup>20,21</sup> or in Mexican nationals without prior travel history.<sup>7,22–24</sup> Trace-back investigations of an outbreak of *Cyclospora* infections in the United States were linked to consumption of basil grown in Mexico.<sup>10</sup>

This study reports the frequency, distribution, clinical presentation, and seasonality of *Cyclospora* alone and or in the presence of other parasites in children seeking medical attention at the Pediatric Hospital of Morelia in Michoacán, Mexico.

### MATERIALS AND METHODS

Children ≤ 15 years of age seeking medical attention at the Pediatric Hospital submitted stools samples for standard ova and parasite examination. Stool samples were examined by direct smear, and by concentration (Faust and Ritchie standard methods). Positive fecal smears were stained by using the Kinyoun and Gomori trichrome techniques. Confirmation of *Cyclospora* oocysts included morphometric characteristics and successful sporulation (i.e., identification of two sporocysts within the oocyst) in 2.5% potassium dichromate at 20 ± 2°C and by polymerase chain reaction (PCR),<sup>25</sup> followed by sequencing. All routine copro-parasitologic tests and clinical

evaluations were performed at the Microbiology and Parasitology Research Laboratory of the Pediatric Hospital of Morelia during January 2000–December 2009. Necessary ethics approvals were obtained from the Institutional Review Board at the Hospital. De-identified samples were submitted for confirmation (sporulation, autofluorescence, and PCR) to the University of Georgia.

### RESULTS

Ninety percent of the 8,877 stool samples from children seeking medical attention were collected at the outpatient clinic and the rest from hospitalized patients. Sixty cases (0.67%) of *C. cayetanensis* infections were detected. *Cyclospora* was most frequently identified in boys of school age (Table 1). Data for symptoms were available from 48 cases with cyclosporiasis (Table 2). Six children (10%) were admitted to the hospital but for complications not related to *Cyclospora* infection: three had neoplastic conditions, one had sepsis, and two had pre-existing rheumatoid diseases. Nine of 17 children with cyclosporiasis who had differential cell blood counts results (53%) also had eosinophilia (4–8%).

Infection with *Cyclospora* in the absence of other parasites was identified in 75% of the cases; 13% were co-infected with commensal parasites and 10% were infected with pathogenic parasites (Table 3). *Blastocystis* was the parasite most frequently identified in fecal samples containing *Cyclospora* oocysts. *Hymenolepis nana* and *Trichuris trichiura* were the only helminth eggs found in samples positive for *Cyclospora*.

Cases of *Cyclospora* infections were consistently identified in the rainy season (June–August) in all years during 2000–2009 (Figure 1). Ambient temperatures ranged from a daily minimum of 12°C to a daily maximum of 27–29°C during the months of high incidence of infections.

### DISCUSSION

This study identified *Cyclospora* in 0.67% of 8,877 children seeking medical assistance at the pediatric hospital of Morelia in Michoacán, Mexico. In Cuba, *Cyclospora* was identified in 0.2% of 7,956 fecal samples collected for 18 months at a pediatric hospital<sup>26</sup> and, in a two-year study at a pediatric hospital in Peru, less than 1% of 381 in-patient children with

\*Address correspondence to Guadalupe E. Orozco-Mosqueda, Hospital Infantil de Morelia, Eva Samano de Lopez Mateos, Justo Mendoza S/N, Colonia Centro, CP 58000, Morelia, Michoacan, Mexico. E-mail: erendira4@hotmail.com

TABLE 1

*Cyclospora* distribution in children by age and sex, Pediatric Hospital of Morelia, Michoacán, Mexico, 2000–2009

Age (years)	Female, no. (%)	Male, no. (%)	Total, no. (%)
> 1–2	5 (8.3)	10 (16.7)	15 (25)
3–5	6 (10.0)	12 (20.0)	18 (30)
6–11	9 (15.0)	13 (21.7)	22 (36.7)
12–15	2 (3.3)	3 (5.0)	5 (8.3)
Total	22 (36.6)	38 (63.4)	60 (100.0)

diarrhea and moderate to severe dehydration had *Cyclospora* infection.<sup>27</sup> In Nigeria, *Cyclospora* was identified in 11 (0.99%) of 1,109 stool samples analyzed at a laboratory hospital (mean  $\pm$  SD age of persons = 30  $\pm$  13 years). Of the positive cases, four were seropositive for human immunodeficiency virus.<sup>28</sup> In Turkey, 7% of the children visiting the Kars Maternal and Children's Hospital had *Cyclospora* infection.<sup>29</sup> In a hospital in Brazil, the prevalence of *Cyclospora* cases was 0.3%; 71.4% were females and case-patients had a median age of 34 years.<sup>30</sup> In Nepal, 6,562 stools samples from five medical units were examined for *Cyclospora*. These units included a pediatric hospital, a rural health center, a health clinic (expatriate clientele), and a tropical disease research center. Overall, *Cyclospora* was identified in 29.7% of 2,123 cases during 1995–1998 and 22.2% of 4,439 during 1999–2000. In the pediatric hospital, 30.3% cases during 1995–1998 and 25.7% during 1999–2000 had *Cyclospora* infection.<sup>19</sup>

The clinical presentation most frequently associated with cyclosporiasis includes diarrhea, weight loss, abdominal distension, nausea, vomiting, abdominal pain, flatulence, and fever.<sup>3,31,32</sup> In the present study, diarrhea was most commonly reported, which is consistent with other studies worldwide.<sup>16,19,26,31–33</sup>

Eosinophilia (4–8%) was observed in 53% of 17 cases of cyclosporiasis in our study. This condition has not been reported previously, although in histopathologic studies, eosinophils and lymphocytic infiltration have been observed in duodenal biopsy specimens of patients with cyclosporiasis.<sup>34,35</sup> Immunodeficiency does not seem to be a factor for high inci-

TABLE 2

Clinical presentation of 48 of 60 children with *Cyclospora* infection seeking medical attention at the Pediatric Hospital of Morelia, Pediatric Hospital of Morelia, Michoacán, Mexico, 2000–2009

Symptom	No.	%
Asymptomatic	2	4.2
Diarrhea	22	45.8
Abdominal pain	19	39.6
Vomiting	9	18.8
Nausea	7	14.6
Abdominal discomfort	7	14.6
Headache	7	14.6
Gas	7	14.6
Hyporexia	5	10.4
Fever	4	8.3
Constipation	3	6.3
Anorexia	3	6.3
Malnutrition	1	2.1
Chills	1	2.1
Increased peristalsis	1	2.1
Anal pruritus	1	2.1
General malaise	1	2.1
Tenesmus	1	2.1
Postprandial fullness	1	2.1

TABLE 3

Co-infection platform of *Cyclospora* with other parasites, Pediatric Hospital of Morelia, Michoacán, Mexico, 2000–2009\*

Association	No. (%)
<i>Cyclospora</i> alone	45 (75.0)
Co-infections with commensal	1 (1.7)
<i>Endolimax nana</i>	1 (1.7)
Co-infections with pathogenic parasites	6 (10.0)
<i>Blastocystis</i> sp.	3 (5.0)
<i>Giardia lamblia</i>	2 (3.3)
<i>Trichuris trichiura</i> , <i>Hymenolepis nana</i>	1 (1.7)
Co-infections with commensal and pathogenic parasites	8 (13.3)
<i>E. coli</i> , <i>Blastocystis</i> sp.	4 (6.5)
<i>E. nana</i> , <i>Blastocystis</i> sp.	1 (1.7)
<i>E. coli</i> , <i>E. nana</i> , <i>Blastocystis</i> sp.	1 (1.7)
<i>G. lamblia</i> , <i>E. nana</i> , <i>Blastocystis</i> sp.	1 (1.7)
<i>E. coli</i> , <i>E. hartmanni</i> , <i>Blastocystis</i> sp., <i>H. nana</i>	1 (1.7)
Total	60 (100.0)

\*Bacteriologic testing was conducted for 16 children. *Escherichia coli* was identified in 12 children. No other bacterial enteric pathogens were isolated. Testing for enteroviruses was not conducted for these children.

dence of *Cyclospora* in children<sup>32,41</sup> and, in the present study, 90% of the infected children were immunocompetent.

In the present study, elementary school age children (6–11 years old) and younger children (3–5 years old) were most frequently infected with *Cyclospora*. In Guatemala, infection was most common among children 1.5–9 years old.<sup>16</sup> *Cyclospora* was more frequently identified in children 3–11 years old<sup>19</sup> in Nepal, and in an endemic community in Peru *Cyclospora* was present among children 1–9 years old.<sup>33</sup>

Of the 60 patients infected with *Cyclospora* identified in this study, 63.4% were male and 36.6% were female ( $P = 0.042$ ). In Cuba, of 20 reported patients with cyclosporiasis, 60% were male and 40% were female.<sup>26</sup> In Colombia, of 56 patients, 73.2% were female and 26.8% were male. However, these patients were associated with an outbreak of cyclosporiasis and presumably all were adults.<sup>5</sup> In Guatemala and Peru, the overall rate of infection in children was not significantly different by sex.<sup>16,33</sup>

In our study, 93.3% of the cases were reported during the months of June–August, which coincides with the rainy season in Morelia.<sup>36</sup> In other reported disease-endemic areas, *Cyclospora* infection was also present during the rainy season.<sup>37,38</sup> In Guatemala, infection is more frequently observed during the early rainy season (21–22°C),<sup>16</sup> whereas in Peru it occurs during the summer (16–19°C) in the coastal desert areas where precipitation is low.<sup>33</sup> In Haiti, prevalence is highest during January–April, which are the coolest (27–29°C) and driest months of the year.<sup>18</sup> In Katmandu, Nepal, the high infection season occurs during the summer and the beginning of the rainy season during months of June and July (average temperature = 19–29°C).<sup>19</sup>

In 75% of the cases of *Cyclospora* infections identified, it was the only pathogenic parasite associated with gastrointestinal illness. Of the 15 stool samples found to contain *Cyclospora* and another infectious agent, *Blastocystis* sp. was most frequent (73.3%), followed by *Entamoeba coli* (40%) and *Endolimax nana* (26.7%). Of the helminths, *Hymenolepis nana* (13.3%) was most frequent. In another study in a native community in Venezuela, co-infections with *Cyclospora* and *Entamoeba histolytica* were most common, but co-infections with *Blastocystis* were observed in only 10.5% of the cases.<sup>39</sup> Tests for the presence of bacterial and viral infections were not conducted

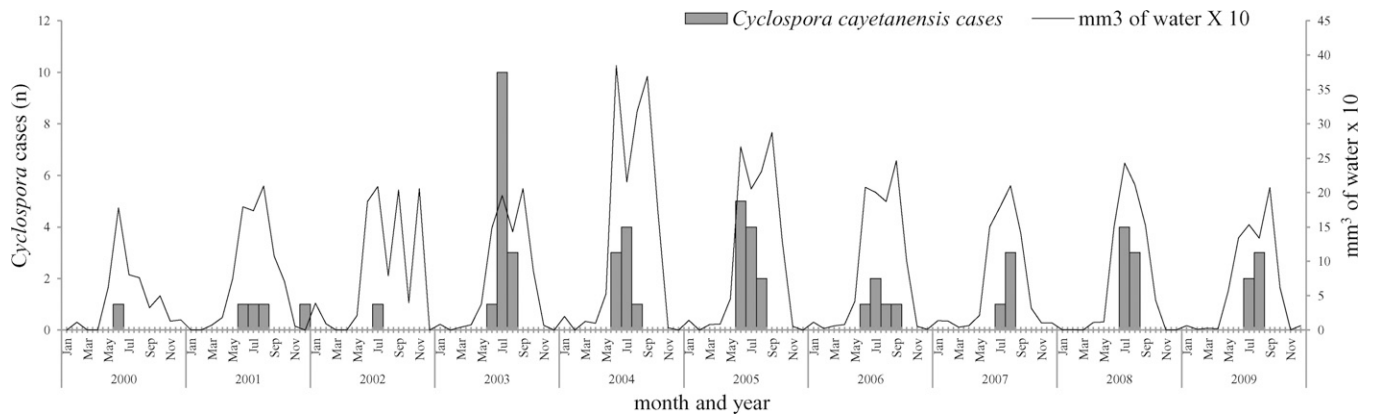


FIGURE 1. Seasonality of *Cyclospora* in children seeking medical attention at the Pediatric Hospital of Morelia, Michoacán, Mexico, 2000–2009.

in the present study, but in a limited number of samples, *Escherichia coli* was the most frequently identified bacterium.

Testing for *C. cayetanensis* in patients with gastrointestinal illness is not routinely conducted. Thus, adequate diagnosis and treatment are not always conducted promptly. In addition, the lack of epidemiologic information for *Cyclospora* makes it difficult to include its differential diagnosis in some locations. Daily and consistent oocyst excretion was not observed in 50% of the cases in our study, suggesting the need for more sensitive assays to diagnose *Cyclospora* infections. Molecular testing such as nested PCR may aid by increasing the sensitivity and specificity for detecting *Cyclospora*.<sup>40</sup>

In conclusion, *Cyclospora* was identified in children with symptoms of illness at the Pediatric Hospital of Morelia, Mexico during 2000–2009. Only 45.8% of the children had diarrhea, which suggested prior exposures. Further epidemiologic studies are needed to determine the prevalence of *Cyclospora* infection in Mexico and Central America, and if eosinophilia is a significant characteristic of cyclosporiasis.

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Authors' addresses: Guadalupe E. Orozco-Mosqueda and Orlando A. Martínez-Loya, Laboratorio de Investigación en Microbiología y Parasitología, Hospital Infantil de Morelia Eva Samano de Lopez Mateos, Justo Mendoza S/N Colonia Centro, CP 58000 Morelia, Michoacán, Mexico, E-mail: erendir4@hotmail.com. Ynes R. Ortega, Center for Food Safety, University of Georgia, Griffin, GA, E-mail: ortega@uga.edu

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