

## *Cyclospora* Species as a Gastrointestinal Pathogen in Immunocompetent Hosts

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**Previous reports of diarrhea resulting from *Cyclospora* species have been linked to travelers and immunocompromised patients. We conducted a prospective study of 1,042 formalin-ethyl acetate fecal concentrates collected from patients with diarrhea. Between May and November 1993, we identified three patients for whom studies were positive for nonrefractile spherical organisms measuring 10  $\mu$ m in diameter and containing a cluster of refractile membrane-bound globules. The cysts exhibited variable acid fastness consistent with *Cyclospora* species. These three patients had no history of recent travel and presented with relapsing, watery, nonbloody diarrhea that lasted from 12 days to 8 weeks. No other parasitic or bacterial pathogens were identified in their stools. All three instances of diarrhea occurred in May or June. No common source of food or water was identified. None of these patients were immunosuppressed, and their diarrhea resolved spontaneously. We suggest that *Cyclospora* species should be considered in community-acquired diarrhea.**

*Cyclospora cayetanensis* is a newly recognized protozoal parasite. It has been implicated as the etiologic agent of prolonged watery diarrhea, fatigue, and anorexia in humans. The mechanism of transmission is presumed to be ingestion of contaminated water (1). First reported in the diarrheal stools of travelers to Mexico and Haiti (13), these *Cyclospora* organisms have since been identified in patients in the United States, Caribbean Islands, Central and South America, Southern and South East Asia, Africa, and Eastern Europe. In the United States, most reported cases of infection have been in travelers to tropical countries (5, 11) and in immunocompromised patients (4, 6, 12). The syndrome of watery diarrhea caused by *Cyclospora* spp. bears remarkable similarities to that caused by *Cryptosporidium* spp. Similarities include the high ratio of asymptomatic to symptomatic infection in Peruvian children documented by Ortega et al. (9). We describe a prospective study of patients with diarrhea in which three unrelated immunocompetent patients with no histories of recent foreign travel were found to have *C. cayetanensis* in their stools.

### CASE REPORTS

**Case 1.** In May 1993, a 63-year-old man was seen by his physician because of 2 weeks of intermittent watery diarrhea accompanied by anorexia, fatigue, mild abdominal cramps, and weight loss. This history was preceded by a few days of low-grade fever, nausea, and vomiting. Six days before his illness, the patient arrived in Massachusetts from his Florida home, where he had a septic system that was working well. He had no history of recent foreign travel and did not drink well water. Apart from mild asthma, he was otherwise healthy. He was afebrile, and physical examination revealed normal findings. A stool specimen was negative for bacterial agents of diarrhea and other parasites but contained many *Cyclospora* organisms. The diarrhea persisted for 8 weeks, leading to a 20-lb (1 lb = 0.5 kg) weight loss. After complete resolution of his symptoms, a stool specimen was negative for *Cyclospora* spp.

**Case 2.** In June 1993, a 34-year-old healthy woman experienced loose stools every time she ate, beginning 48 h after eating fried clams at a local restaurant. She complained of low-grade fever, anorexia, abdominal cramps, and weight loss. She had no exposure to well water and had not recently traveled overseas. Physical examination revealed normal findings, and a fecal smear for white blood cells was negative. No bacterial or routine parasitic pathogens other than *Cyclospora* species were found in her stool. The diarrhea lasted 10 to 12 days and resolved spontaneously.

**Case 3.** In late May 1993, a 33-year-old woman from New Hampshire experienced explosive diarrhea, with fatigue and epigastric pain. The patient had not recently traveled abroad but had lived in a house with well water as a drinking source. Heavy snow melts had led to the flooding of a surrounding river up to a neighbor's house where three otherwise healthy individuals subsequently experienced several weeks of watery diarrhea. About 1 week before the onset of her symptoms, she had filled her swimming pool with well water. Three of four adults living in the house subsequently had loose stools lasting 2 to 5 weeks, but two of them did not seek medical attention. The stool specimen was Guaiac negative but demonstrated *Cyclospora* organisms. Her illness resolved spontaneously after 5 weeks. A follow-up stool specimen was negative for *Cyclospora* spp.

### MATERIALS AND METHODS

**Specimen processing and examination.** The study was conducted between 8 May and 1 November 1993, with a population of both inpatients and outpatients. The 1,042 consecutive stool specimens submitted for routine ova and parasite examination were also examined for the presence of *C. cayetanensis* and *Cryptosporidium parvum*. The stool specimens were collected from patients with both acute and chronic diarrhea. Approximately 43% of the specimens were obtained from patients referred to our institution because of refractory symptoms. Another 57% were from patients seen by their primary care providers for acute symptoms of gastroenteritis. In this latter group, 10% were pediatric patients. Stools were received unpreserved, along with a portion preserved in polyvinyl alcohol (Meridian Diagnostics, Cincinnati, Ohio). The unpreserved stool samples were refrigerated at 4°C pending concentration. The formalin-ethyl acetate concentration was performed by using the Fecal Concentrator Kit (Remel, Lenexa, Kans.) within 24 h of specimen receipt. The concentration was achieved by centrifugation at 500  $\times$  g for 10 min. At the time of concentration, an aliquot of each unpreserved specimen was placed in a cryogenic vial (Vanguard International, Neptune, N.J.) and frozen at -70°C for further evaluation. A second

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FIG. 1. (a) Acid-fast oocyst of *Cyclospora* under oil immersion magnification ( $\times 1,000$ ). Scale, 1 unit = 1  $\mu\text{m}$ . (b) Mature *Cyclospora* oocyst contains two sporocysts.

permanent smear was prepared from the formalin-ethyl acetate concentrate and stained by a modified acid-fast procedure to identify the presence of coccidian protozoans (2). An additional smear from all positive samples was prepared from the concentrate and stained by using the Merifluor *Cryptosporidium/Giardia* Direct Immunofluorescence Assay (Meridian Diagnostics). Staining was performed according to the manufacturer's recommended procedure.

**Criteria for identification of *C. cayetanensis*.** To establish the presence of *C. cayetanensis*, the organisms needed to be (i) nonrefractile spheres measuring 8 to 10  $\mu\text{m}$  in diameter and containing a cluster of refractile, membrane-bound globules on wet-mount examinations from the concentrated stool specimen and (ii) variably acid-fast spheres measuring 8 to 10  $\mu\text{m}$  in diameter when stained with modified kinyoun stain. The unpreserved frozen stool samples from patients fulfilling these criteria were placed in a 2.5% potassium dichromate solution. This suspension was incubated at room temperature in a petri dish and examined daily for up to 2 weeks for the presence of mature sporulated oocysts.

## RESULTS

During the study period, 1,042 stool specimens were evaluated. We identified five specimens from three patients whose stools contained *C. cayetanensis*. *C. parvum* was not detected in any of the specimens tested. The nonrefractile oocysts of *C. cayetanensis* measured 8 to 10  $\mu\text{m}$  in diameter and were consistent in appearance with previous descriptions of the coccidian (7, 9, 11, 12). They were easily seen under low, dry magnification ( $\times 100$ ) and high, dry magnification ( $\times 400$ ) in the wet mount from the formalin-ethyl acetate concentrate. Inclusions within the spheres appeared as refractile globules taking up most of the internal area. These inclusions appeared as a single globule or as a group of separate refractile globules. The organisms were consistently spherical, and no other shapes were seen.

The oocysts stained variably acid-fast in the smears made from the concentrated specimens (Fig. 1a). Organisms ranged from light pink to dark red, and some appeared mottled and unevenly stained. A small percentage of the oocysts did not stain but appeared as bubbly, clear, glassy, and refractile spheres. Modified acid-fast smears of specimens from the patients positive for *C. cayetanensis* were also made directly from the unpreserved stool. Oocysts stained more evenly and con-

sistently acid-fast positive, with fewer organisms staining acid-fast negative. The stainability of the organisms decreased with extended exposure to formalin. After several weeks in formalin, the oocysts were almost all acid-fast negative and appeared as clear, glassy spheres.

Oocysts did not stain with trichrome; they appeared as clear, glassy, and wrinkled spheres. In addition, oocysts in the concentrated specimens from the patients positive for *C. cayetanensis* did not react in the Merifluor *Cryptosporidium/Giardia* Direct Immunofluorescence Assay.

Sporulation of the oocysts confirmed the identification of the parasite as *C. cayetanensis* (Fig. 1b). The three patients were evaluated for the presence of other causative agents of diarrhea, including *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species. No other agents could be identified. Patients with specimens positive for the parasite were identified within a 2-week period between the last week of May and the first week of June.

## DISCUSSION

Our findings suggest that *Cyclospora* species should be considered possible etiologic agents for community-acquired diarrhea in the immunocompetent host. To date, only one other report (3) has described a case of *Cyclospora* diarrhea acquired in the community from exposure to contaminated sewage that had backed up into the patient's basement. Many questions remain about the epidemiology of this newly described organism. The acute clinical presentation of cryptosporidial enteritis and *Cyclospora* diarrhea are similar: frequent (often explosive), watery, nonbloody stools accompanied by crampy abdominal pain, nausea, and fatigue (14). Fever is usually absent. Unlike cryptosporidiosis in the normal host, which usually lasts about 10 to 14 days, the illness caused by *Cyclospora* species is often prolonged, with an average duration of 43 days in one report (9). It is important, therefore, for the clinical laboratory to differentiate between these two organisms, especially in light of

recent reports of the possible therapeutic value of trimethoprim-sulfamethoxazole in treating *Cyclospora* diarrhea (8, 10). There is currently no known effective treatment for cryptosporidiosis.

Laboratory identification of *Cyclospora* species is similar to that of *Cryptosporidium* species. The principal distinguishing feature between the two organisms is the size of the oocyst. The oocyst of *Cyclospora* measures 8 to 10  $\mu\text{m}$ . The relative size of the *Cyclospora* oocyst makes the parasite more easily identifiable than the smaller *Cryptosporidium* oocyst (4 to 6  $\mu\text{m}$ ) in wet-mount examination of a formalin-ethyl acetate concentrate.

Sporulation of the cyclosporan oocyst took 13 days with specimens collected from our patients. Previous reports have indicated formation of this sporocyst within 5 (9) to 7 (7) days. Sporulation was attempted on the fresh stool that had previously been frozen at  $-70^{\circ}\text{C}$ , which may have increased the time to sporulation.

After laboratorians have become familiar with this newly described pathogen, they should be able to recognize it readily in stool specimens submitted for routine parasitologic examination. The necessity for demonstrating sporulation to confirm the identification of the parasite is unclear. As with *Cryptosporidium* species, however, an acid-fast stain should be performed to verify the identification of *Cyclospora* species.

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