

Cyclosporiasis: Clinical and Histopathologic Correlates

Bradley A. Connor, Jason Reidy, and Rosemary Soave

From the New York Hospital–Cornell Medical Center and Beth Israel Medical Center, New York, New York

Although the histopathologic changes associated with *Cyclospora cayetanensis* infection have been previously described, the histopathology and the appearance of various life cycle stages have not been correlated with severity, stage, and duration of clinical disease. We report a prospective clinical investigation of disease characteristics and histopathologic findings in three otherwise healthy, immunocompetent patients with symptomatic *C. cayetanensis* infection, the duration of which ranged from 6 to 60 days. Varying degrees of gross and microscopic gastrointestinal inflammation were seen before treatment. An electron-dense phospholipid membrane/myelin-like material was variably present both before and after treatment. The greatest amount of myelin-like material was seen in the patient with prolonged disease. The results of our study suggest that inflammatory changes associated with *C. cayetanensis* infection may persist beyond parasite eradication. It is intriguing to speculate that the myelin-like material is a marker for persistent inflammation, but further study and confirmation are needed.

Cyclospora cayetanensis is a coccidian parasite that is associated with a syndrome of gastrointestinal illness and extreme fatigue. The parasite's life cycle and the mechanisms by which it interacts with human host target cells to cause disease are poorly understood. Early descriptions of the clinical illness associated with this organism suggest it is an upper gastrointestinal pathogen. Impairment of D-xylose absorption, documented in some *C. cayetanensis*-infected patients, implies involvement of the proximal small bowel [1]. Light microscopic examination of small intestinal tissue samples obtained in a case-control endoscopic study during a seasonal outbreak of *C. cayetanensis* infection in Nepal revealed striking histologic changes: acute and chronic inflammation, disruption of surface epithelium, villous atrophy, and crypt hyperplasia [2]. Although oocysts were detected in duodenal aspirates from two patients, they were not seen in tissue samples examined by electron microscopy.

C. cayetanensis infections have been detected in many parts of the developing world. Predictable seasonal outbreaks in Nepal have provided the opportunity for study of the clinical illness and its epidemiology and for confirming that cotrimoxazole is an effective treatment [3, 4]. The few cases that were reported from the United States and other developed countries before 1995 mostly occurred in returning travelers. Whether this reflected a low incidence of infection or under-recognition

in developed countries of a newly described organism is unclear.

In the spring of 1995, a small outbreak of community-acquired infection was described in residents of New York and Florida [5]. In 1996, a large outbreak of cyclosporiasis occurred in North America, affecting >1,400 persons in 20 states and two Canadian provinces [6]. A total of 161 laboratory-confirmed cases of *C. cayetanensis* infection were identified in New York City between May and August 1996. This provided an opportunity to further evaluate the clinical illness, endoscopic features, and histopathology of *C. cayetanensis* infection and compare the findings with those obtained previously in Nepal.

Materials and Methods

Parasitologic examination of stool and duodenal aspirate. Stool samples and duodenal aspirates were concentrated by centrifugation. Iodine-stained wet mounts and stained smears were examined for ova and parasites. Special stains were used to detect microsporidia and coccidian organisms. The presence of *C. cayetanensis* was confirmed by the finding of 8- to 10- μ m spherical organisms by means of light microscopic examination of modified acid-fast stained smears. All stool specimens were cultured for bacterial enteric pathogens.

Endoscopic procedure. Upper gastrointestinal endoscopy was done by the same gastroenterologist. Patients were asked to fast overnight. Topical anesthesia was achieved by use of a dyclonine gargle before each procedure. A fiberoptic video gastroscope (EVIS 100; Olympus, Lake Success, NY) was used for endoscopic assessment of the esophagus, stomach, and duodenum. Duodenal fluid was aspirated into sterile specimen traps by sterile saline irrigation of the duodenal bulb and descending duodenum. At least two forceps biopsy specimens of the distal duodenum were obtained in each case. Biopsy specimens were preserved in 10% neutral buffered formalin.

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The study was approved by the New York Hospital–Cornell Medical Center Institutional Review Board, and participating patients gave signed informed consent.

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Reprints or correspondence: Dr. Bradley A. Connor, 50 East 69th Street, New York, New York 10021 (bconnor@pol.net).

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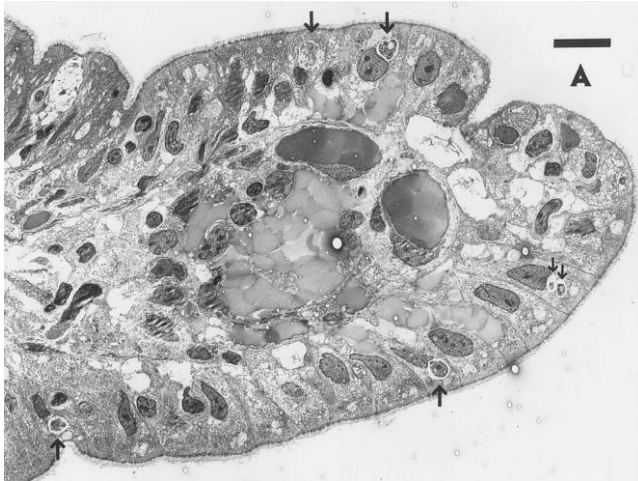
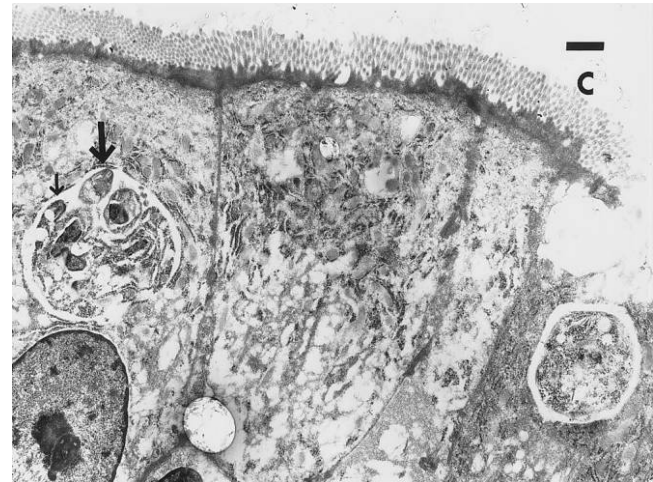


Figure 1. Intraepithelial organisms within parasitophorous vacuoles by electron microscopy of paraffin-processed tissue. *A*, Villous apex showing distribution of intraepithelial organisms (arrows) within parasitophorous vacuoles (original magnification, $\times 400$; bar = $10\ \mu\text{m}$). *B*, Multiple infection of an enterocyte with two schizonts containing numerous merozoites (at right) (original magnification, $\times 3,000$; bar = $1\ \mu\text{m}$). *C*, Budding of merozoites (ectomerogony) from meront cytoplasm (at left; see also figure 2). Meront (at right; trophozoite, uninucleate schizont) (original magnification, $\times 3,000$; bar = $1\ \mu\text{m}$).



Histopathologic examination of intestinal tissue specimens. Duodenal biopsy specimens were paraffin-processed, glutaraldehyde-fixed, serially sectioned, and examined by means of light and electron microscopy (EM).

Results

Patient 1

Case report. A 39-year-old man who lived in New York City developed nausea, abdominal cramping, diarrhea, and a fever of 38.3°C on 4 June 1996. Abdominal cramping and diarrhea persisted, and the next day he noted onset of extreme fatigue. Although initial improvement was noted after therapy with ciprofloxacin was begun, fatigue, abdominal cramping, and watery diarrhea with associated anorexia recurred within 24 hours. Loperamide had no effect on stool frequency.

The patient had a 10-year history of irritable bowel syndrome characterized by intermittent episodes of chronic diarrhea. Radiography of the small bowel showed mild inflammatory changes in the distal ileum. His symptoms of irritable

bowel had been exacerbated by frequent work-related travel but resolved completely with careful attention to diet and avoidance of lactose-containing products. He had been symptom-free since mid-1995. He also had a history of recurrent prostatitis, most recently treated with trimethoprim-sulfamethoxazole in December 1995. In August 1993, a tubular adenoma with moderate- to high-grade dysplasia was removed at colonoscopy.

On presentation, he was afebrile, and results of an abdominal examination were normal. Upper gastrointestinal endoscopy was done on 10 June 1996. Submucosal hemorrhages of the gastric antrum with mild associated erythema and friability of the duodenal bulb and erythema of the postbulbar duodenum were noted. A tissue sample of the gastric antrum was negative for *Helicobacter pylori* by rapid urease test. A duodenal aspirate sample was negative for parasites, including *C. cayetanensis*. Mild nonspecific chronic inflammatory infiltrates in the lamina propria with largely preserved villous architecture were seen in duodenal tissue. Many *C. cayetanensis* oocysts but no other enteric pathogens were detected in stool. The patient began receiv-



Figure 2. Ectomerogony of a merozoite from a parental meront. Evident in the bud projecting into the parasitophorous vacuole are micronemes (M) and subpellicular microtubules (*arrows*) (original magnification, $\times 12,000$; bar = $1 \mu\text{m}$).

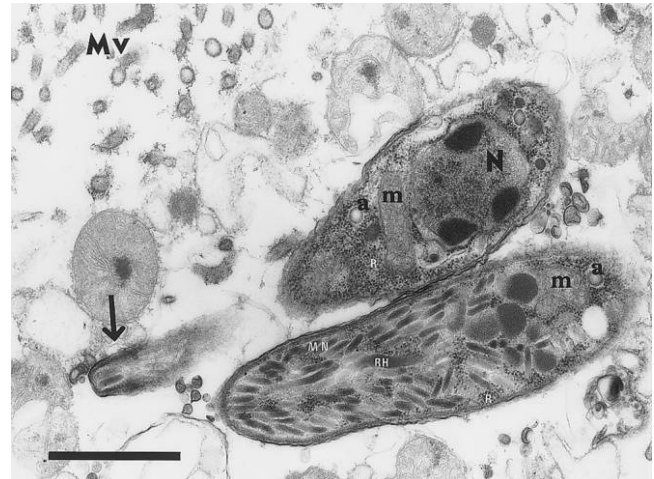


Figure 3. Mature luminal merozoites. Organisms are seen at discharge, surrounded by host-cell organelles, in proximity to the microvilli (Mv) of adjacent cells. Organelles evident in this plane include mitochondria (M), ribosomes (R), amylopectin granules (A), micronemes (MN), rhoptries (RH), a nucleus (N), and an apical complex (*arrow*) (original magnification, $\times 12,000$; bar = $1 \mu\text{m}$).

ing trimethoprim-sulfamethoxazole, double strength, b.i.d., on 11 June 1996. Three days later all of his symptoms resolved.

EM examination of endoscopically obtained biopsy specimens revealed pronounced enterocyte vacuolization and abundant intraepithelial reactive cells. Numerous intraenterocytic coccidial organisms within parasitophorous vacuoles were present, predominantly in apical areas (figure 1A). There was a decreasing density of organisms along the sides of the villi, and none were seen in the crypts. Single enterocytes were multiply infected (figure 1B).

Merogony/schizogony was evident. Uninucleate predivisive organisms within the host cell cytoplasm were roughly spherical and measured $3 \mu\text{m}$ in diameter (figure 1C). Cytoplasmic organelles seen at this phase included micronemes, subpellicular microtubules, and mitochondria.

Merozoite genesis appeared to have occurred via ectomerogony; daughter individuals were seen budding off of the larger parental cytoplasm (figure 2). Up to 11 individual merozoites were observed within a single schizont wall (with presumably at least one additional organism out of the section plane). It appears that merozoites underwent enlargement within the parasitophorous vacuole. Fusiform zoites measured $1 \times 3 \mu\text{m}$ when discharged into the duodenal lumen as the host cell ruptured (figure 3). Mature individual merozoites displayed a characteristic apical complex and possessed an array of 21 subpellicular microtubules. Also present were rhoptries, micronemes, amylopectin-like granules, a single nucleus, and mitochondria (figures 3, 4). Crystalline polar refractile bodies were not evident; also not seen in any developing individuals were the flagella or wall-forming bodies of micro- or macrogametogenesis.

On 6 September 1996, a follow-up stool examination yielded negative results for *C. cayetanensis* and all other enteric pathogens. Repeat upper gastrointestinal endoscopy was normal except for mild prepyloric antral erythema. Samples of duodenal aspirate were negative for parasites, including *C. cayetanensis*. Duodenal biopsies revealed nonspecific chronic inflammation of the lamina propria and intact villous architecture.

EM examination of specimens obtained at follow-up. The ultrastructure of duodenal tissue appeared normal. Focally, there was vacuolization and gapping of enterocytes, with infrequent appearance of an electron-dense phospholipid membrane/

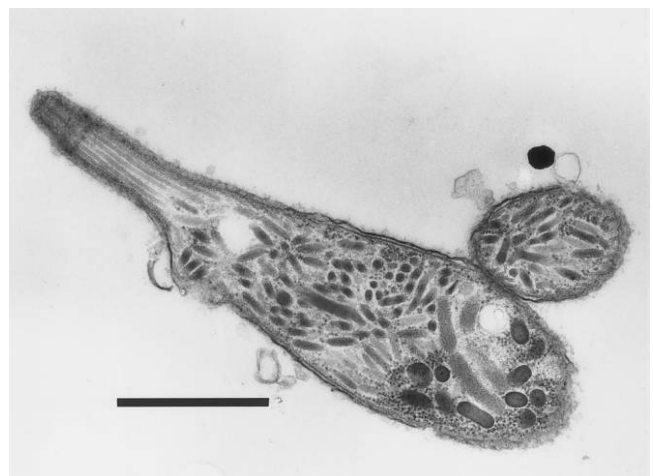


Figure 4. Merozoite with extended apical complex (original magnification, $\times 12,000$; bar = $1 \mu\text{m}$).

myelin-like material (MLM). There was slight increase in intraepithelial reactive cells, with mild focal evidence of chronic inflammation. No organisms were noted.

Patient 2

Case report. A 29-year-old man who lived in New York City developed abdominal cramping and diarrhea on 29 May 1996, 6 days after attending a wedding party. Initial signs and symptoms included fever, light-headedness, and diaphoresis, and subsequently abdominal cramping and diarrhea ensued, with six or seven loose watery bowel movements a day alternating with 1–2 days of normal bowel movements. Fatigue and anorexia were also present. He reported inability to eat complete meals and noted a 10-pound weight loss. When the patient became aware that numerous other guests at the wedding party had developed similar symptoms, he sought medical attention. A stool specimen obtained on 21 June 1996 was found to contain *C. cayetanensis* oocysts. No other enteric pathogens were noted. Upper gastrointestinal endoscopy done on 24 June 1996 revealed mild erythema of the gastric antrum and cardia and mild postbulbar duodenal erythema. A duodenal aspirate sample was negative for parasites, including *C. cayetanensis*. Duodenal biopsies revealed mild chronic inflammation in the lamina propria with intact villous architecture. He reported resolution of his symptoms within 3 days of beginning treatment with trimethoprim-sulfamethoxazole b.i.d. and has continued to feel well.

EM examination of endoscopically obtained specimens. After exhaustive examination of duodenal biopsy specimens, a single coccidian organism was identified. The individual organism was seen in the supranuclear cytoplasm and appeared to be a merozoite in cross-section with a diameter of 2.5 μm . Micronemes and subpellicular microtubules were evident in the organism's cytoplasm, and its surface contained MLM. No parasitophorous vacuole was seen, raising the possibility that this organism had just entered the host cell.

A repeat stool examination on 19 July 1996 was negative for enteric pathogens, including *C. cayetanensis*. Results of upper gastrointestinal endoscopy on the same day were normal. A duodenal aspirate sample was negative for ova and parasites, including *C. cayetanensis*, and biopsies revealed normal duodenal mucosa.

EM examination of duodenal biopsy specimens obtained at the follow-up endoscopy revealed normal ultrastructure with only a mild increase in intraepithelial lymphocytes. MLM was not apparent.

Patient 3

Case report. A 52-year-old man, who was a career employee of UNICEF based in Kathmandu, Nepal, was seen on 20 June 1996 for evaluation of diarrhea of 8 weeks' duration. The case history actually began in June 1995, when a diagnosis of

C. cayetanensis infection was made at the CIWEC (Canadian International Water and Energy Associates) Clinic in Kathmandu after the patient presented with severe diarrhea. He was treated with cotrimoxazole for 1 week, with complete resolution of his symptoms and clearance of the organism. Three months later, he was treated with a sulfa-containing antibiotic for pharyngitis and developed a desquamating skin reaction. He was advised to avoid sulfa in the future on the basis of a presumed hypersensitivity. Because of his previous experience with *C. cayetanensis* and lack of treatment options, he became very careful with respect to food consumption and trained his kitchen staff in Nepal on proper preparation and thorough cooking of food. He avoided eating in restaurants and brought his own cooked food when he visited friends and associates in Kathmandu.

He visited New York in April 1996 and took great pleasure in consuming foods, such as fresh fruit and salads, that he normally avoided in Nepal. He developed abdominal cramping and diarrhea on the flight from New York to Sweden on 22 April 1996. A physician in Sweden could not make a specific diagnosis. When he returned to Kathmandu on 12 June 1996, abdominal cramping, diarrhea, and extreme fatigue persisted. A diagnosis of *C. cayetanensis* infection was made on 12 June 1996, and he was treated with paromomycin, 750 mg t.i.d. Despite transient improvement, symptoms recurred, and *C. cayetanensis* oocysts were detected in a fecal specimen on 15 June 1996. The patient returned to New York on 17 June 1996 with persistent fatigue and diarrhea; he also noted a weight loss of 5 kg since the onset of his symptoms. He completed a 1-week course of paromomycin on 19 June 1996.

He was afebrile with a normal abdominal examination. Examination of stool revealed a few *C. cayetanensis* oocysts but no other enteric pathogens. Upper gastrointestinal endoscopy revealed patchy erythema of the gastric antrum body, cardia, and fundus and friability of the duodenal bulb. Erythema and edema of the postbulbar duodenum were also noted. Numerous curved bacilli consistent with *H. pylori* were identified in the gastric antrum. Biopsies of the distal duodenum revealed congestion and edematous duodenal mucosa, with mild variation in villous shape. No microorganisms were seen in duodenal aspirate specimens.

EM examination of two duodenal tissue specimens. No parasitic organisms were seen by light microscopic examination of duodenal tissue. The prominent pathologic feature was the marked accumulation of electron-dense MLM (figure 5). Accumulations were seen primarily in the large number of suprabasal spaces separating many of the enterocytes; in many areas of the apices and sides of the villi, spaces with MLM were sufficiently numerous so as to alternate with the enterocytes. MLM was frequently seen wedged between the upper edges of the epithelial cells and was seen in the duodenal lumen as well. Lesser amounts of MLM were seen sub-basally in breaches of the basal membrane and within reactive cells. Marked vacuolization of the epithelium was noted in addition to the intercellular spacing. Aspects of chronic inflammation were apparent.

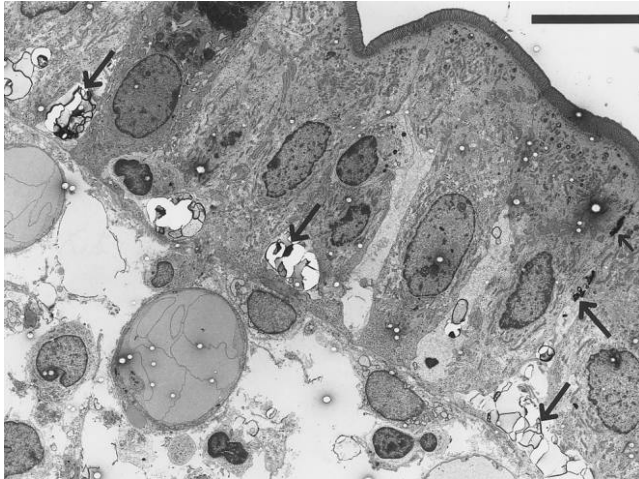


Figure 5. Myelin-like material: accumulations of myelin-like phospholipid membrane material (arrows) between bases and sides of enterocytes (original magnification, $\times 1,100$; bar = 10 μm).

Because of his allergy to sulfa and no proven alternative therapy for *C. cayetanensis*, no additional antibiotic therapy was offered. The patient returned to Sweden before his return to Kathmandu. Symptoms of abdominal cramping, diarrhea, and fatigue persisted, and he developed peptic symptoms with acid reflux and gnawing epigastric pain and was treated with a proton pump inhibitor and antibiotics for *H. pylori*. Intermittent diarrhea and fatigue persisted for several weeks after his return to Kathmandu.

Discussion

Infection with coccidia generally occurs when sporulated oocysts are ingested and excyst in the proximal small bowel. The sporozoites that are released invade the intestinal epithelial cells, where they develop into trophozoites and undergo schizogony, with a resultant merozoite-containing schizont. These merozoites are extruded and can invade other epithelial cells. In this way, a continued propagation of symptoms may occur as new merozoites may undergo further asexual cycles and/or develop into macro- or microgametes. When these latter forms fertilize, an oocyst results, which subsequently is passed in stool.

Our EM findings support the contention that sporulated *C. cayetanensis* organisms excyst in the small bowel and enter the epithelial cell. Only asexual phases of the *C. cayetanensis* life cycle were found in our study. All were found within a membrane-bound vacuole that separated the intracellular coccidia from the host cell cytoplasm, a parasitophorous vacuole. All observed organisms were comparable to the type I meront recently described for *C. cayetanensis* [7]. Because *C. cayetanensis*-infected patients excrete unsporulated oocysts in their stools, it is assumed that the organism completes the sexual phase of its life cycle within the human host. In fact, in a recent

study of 17 patients who underwent endoscopy, Ortega et al. [7] described the finding of gametocytes by both light and electron microscopy. However, the report does not provide information regarding the number of sexual forms found in any one specimen or the number of specimens from different patients found to have sexual forms. Furthermore, no details are provided as to when the sexual phases were seen with respect to illness onset (e.g., early, late, at any time). Before the report of Ortega et al. [7], various attempts to demonstrate sexual phases of *C. cayetanensis* had failed [8, 9]. Despite an exhaustive search, we were unable to identify sexual phases of the life cycle in our specimens. This may reflect a paucity of these organisms in the intestinal cells, perhaps owing to their presence in extraintestinal sites such as the liver or biliary tree (as is true for related coccidian organisms such as *Cryptosporidium*) [10, 11], or a short-lived presence such that the window of opportunity of capturing them is quite narrow.

Asexual phases of the *C. cayetanensis* organisms were seen within the enterocyte, most commonly midway between the nucleus and brush border and always closer to the villous surface. In our three patients, it appears that as clinical disease evolved, the organisms were eradicated, thus lending support to the self-limiting nature of this infection that was noted in studies in Nepal before identification of known efficacious therapy. Interestingly, the dense MLM appeared in the later stages of disease. The finding that one patient (patient 3) continued to be symptomatic 60 days after illness onset, despite the absence of parasites, suggests the presence of an ongoing inflammatory or immunologic process. Further support for this hypothesis is the fact that patient 3 had previously been infected with *C. cayetanensis* in 1995. The presence of large amounts of MLM suggests that it may be either a marker of or a contributor to an as-yet-uncharacterized process. In patient 2, who had had the illness for ~ 1 month, only one organism was seen and only after an exhaustive search. In addition, the MLM was identified (table 1).

These findings led to a retrospective review of the EM material obtained from patients in the 1991 Nepal endoscopic study [2]. We confirmed the absence of parasites in those specimens. However, a consistent finding was the presence of MLM, which at the time had been dismissed as artifact. An exhaustive literature search and personal communication with several pathologists and parasitologists did not provide much enlightenment as to the nature and significance of the presence of MLM. In our experience, scarce to modest amounts of MLM are a common finding in association with upper gastrointestinal protozoal infections, including those due to *Cryptosporidium*, *Microsporidium*, *Isospora*, and *Giardia* species. The amount of MLM observed in specimens obtained in the 1991 Nepal study and in the study described herein was far greater than is commonly seen with other intestinal infections. One could speculate that it is a marker for cell injury. It has been demonstrated in other coccidia, notably *Isospora suis* [12, 13], that particular meront (asexual phase) subtypes retain motility and have the ability

Table 1. Cyclosporiasis: clinical and histopathological correlates in three patients in New York.

Finding	Patient 1		Patient 2		Patient 3
	6/10/1996	9/6/1996	6/24/1996	7/19/1996	6/21/1996
Clinical symptoms	+	–	+	–	++
Duration of symptoms before endoscopy	6 days	None	26 days	None	60 days
Duration of treatment					
Before endoscopy	Ciprofloxacin, 500 mg b.i.d. × 3 days; loperamide, 2 mg × 4 days	None	None	None	Paromomycin, 750 mg t.i.d. × 7 days
After endoscopy	TMP-SMZ b.i.d. × 7 days	None	TMP-SMZ b.i.d. × 7 days	None	None
Endoscopy					
Gross appearance					
Gastritis	++	+	+	–	+++
Bulbar duodenitis	++	–	–	–	++
Postbulbar duodenitis	++	–	–	–	++
Organisms in duodenal aspirate	–	–	–	–	–
Histopathology					
Light microscopy					
Acute inflammation	–	–	–	–	–
Chronic inflammation	+	+	+	–	–
Villous changes	–	–	–	–	+
Electron microscopy					
Enterocyte vacuolization	++++	+	–	–	++
Intraepithelial reactive cells	++++	+	–	–	+
Intraepithelial organisms	++++	–	+	–	–
Parasitophorous vacuoles	++++	–	–	–	–
Myelin-like material	–	+	++	–	++++

NOTE. Dates listed under patients are dates of endoscopic examination and are given as month/day/year. – = absent; + = present (number of pluses denotes degree).

to repeatedly penetrate additional host cells, even while in advanced states of development. Such a model offers a potential explanation for our subjects' protracted illnesses with only small numbers of asexual-phase organisms observed in intestinal locations. MLM may thus represent the ruptured membranes of host cells progressively abandoned by small numbers of highly pathogenic meront organisms. That significant amounts of MLM have not been reported elsewhere may indicate that such aggressive low-level asexual phases may be uncommon among the coccidia that infect human hosts. Alternatively, the presence of MLM might reflect ongoing inflammatory reactions or immunologic activation that were initially triggered by the pathogenic pathogens and are not being quenched. Further study of this phenomenon may help elucidate the pathogenesis of chronic cyclosporiasis that is seen in a small subset of infected patients who remain symptomatic despite parasite clearance.

In summary, we have described the clinical and histopathologic findings by light and electron microscopy in three immunocompetent patients with *C. cayetanensis* infection. Our study is somewhat limited by the small number of patients, necessitated by the invasive nature of the study procedure. It was hoped that a comprehensive study of a few patients would

provide the basis for optimally designed future studies. Our findings provide support for the clinical observation that symptoms usually resolve with parasite eradication. Our findings also suggest that inflammatory changes associated with *C. cayetanensis* infection may persist beyond parasite eradication. The presence of a myelin-like material as a marker for persistent inflammation is intriguing but needs further definition. Further histopathologic studies of *C. cayetanensis*-infected patients may provide valuable insights into the pathogenesis of this disease.

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