Intestinal Coinfection with *Enterocytozoon bieneusi* and *Cryptosporidium* in a Human Immunodeficiency Virus–Infected Child with Chronic Diarrhea

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The microsporidian *Enterocytozoon bieneusi* has been recognized as an important cause of chronic diarrhea in severely immunodeficient adults infected with human immunodeficiency virus (HIV). We report the first case of intestinal *E. bieneusi* infection in a child. The 9-year-old boy with connatal HIV infection presented with failure to thrive, chronic diarrhea, and intermittent abdominal pain. His CD4 lymphocyte count was 0.05×10^9/L and dropped to 0.01×10^9/L. No HIV-associated opportunistic infection other than oral hairy leukoplakia and oral candidiasis had been found before microsporidia were detected. Treatment of microsporidiosis with albendazole was of no benefit. During follow-up, the boy also developed intestinal cryptosporidiosis. Evaluation of chronic diarrhea in severely immunodeficient HIV-infected children should include examination for intestinal microsporidia. We recommend the use of a new coprodiagnostic technique that allows detection of microsporidial spores in stool specimens. Furthermore, consideration of dual or even multiple parasitic infections in the differential diagnosis of chronic diarrhea may have both important clinical and epidemiological implications.

The microsporidian protozoan organism *Enterocytozoon bieneusi*, first reported in 1985 [1, 2], has been recognized as a human immunodeficiency virus (HIV)–associated intestinal opportunistic pathogen [1–9]. Preliminary epidemiological studies have indicated that it may be present in 10%–30% of severely immunodeficient HIV-infected adults with chronic diarrhea [3–6, 9].

We report the first case in which an HIV-infected pediatric patient with chronic diarrhea was found to have *E. bieneusi* spores in stool specimens by light-microscopic and electron-microscopic examination. During follow-up, the child also developed intestinal cryptosporidiosis.

Case Report

The patient, a 9-year-old boy, was born in Switzerland to an HIV-seropositive Swiss mother with a history of intravenous drug use. Between the ages of 3 and 8 years, the boy lived with his mother in Ecuador before his return to Switzerland. His mother died of AIDS-related complications in February 1992. She did not have diarrhea. The boy developed normally and was healthy until summer 1991, when he experienced intermittent diarrhea, abdominal pain, and mild dry cough without fever.

In September 1991, he was referred to the University Children's Hospital in Zurich because of persistent gastrointestinal symptoms. He had three to six watery stools per day and intermittent nonspecific diffuse abdominal pain. His weight (19.3 kg) and height (118 cm) were below the third percentile. Physical examination was normal apart from oral hairy leukoplakia and oral candidiasis. He was found to be positive for antibody to HIV-1, and the results of HIV-1 culture also were positive. The hematocrit was 0.31, the leukocyte count was 4.6×10^9/L with a $CD4^+$ count of 0.05×10^9/L and a $CD8^+$ lymphocyte count of 2.03×10^9/L. Tests of functional immune status disclosed antibody deficiency and cellular unresponsiveness to recall antigens and mitogens. Results of alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, creatinine tests and urinalysis were normal. Repeated stool examinations for ova and parasites, including cryptosporidia, and viruses were negative, as were bacterial and mycobacterial stool cultures. The chest roentgenogram and ultrasonographs of the abdomen were normal.

Primary prophylaxis for *Pneumocystis carinii* pneumonia (trimethoprim-sulfamethoxazole, 36 mg/kg body weight per day) and monthly substitution with intravenous gammaglobulin (0.4 g/kg body weight) were initiated. Oral candidiasis was treated with fluconazole.

He attended school and was well except for persistent diarrhea, with three to six watery or soft stools per day. During the following 9 months, his $CD4^+$ lymphocyte count dropped to 0.01×10^9/L, and both his growth (+0.7 cm) and weight gain (+0.5 kg) were poor.

In June 1992 microsporidial spores were found by light-microscopic and electron-microscopic examination of stool

Received 18 December 1992; revised 10 March 1993.

Grant support: This work was supported in part by a grant from the Swiss National Program for AIDS Research (91-7066).

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Clinical Infectious Diseases 1993;17:480–3

1058-4838/93/1702-0023$02.00

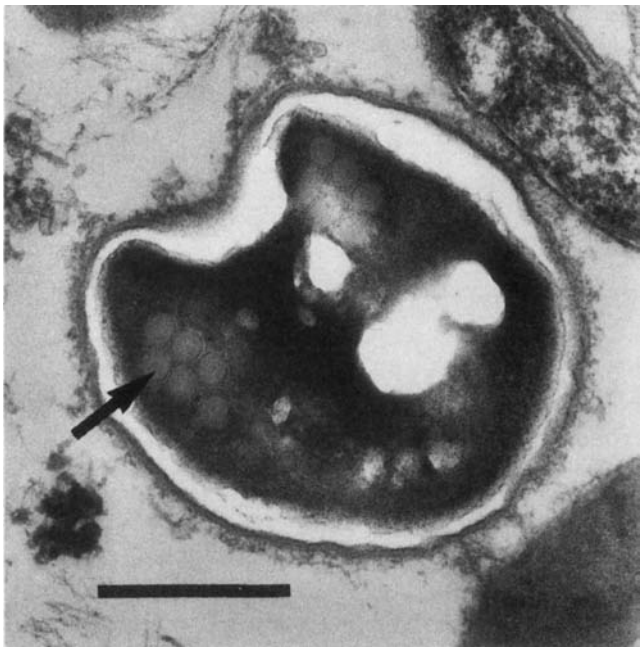

Figure 1. Transmission electron micrograph of an individual microsporidial spore from a stool specimen of an HIV-positive child with chronic diarrhea. The spore is characterized by polar tubes (arrow) and a spore wall consisting of a dense outer spore coat, an electron-lucent endospore layer, and a plasma membrane (bar = 0.5 μm).

specimens with use of a new, recently described coprodiagnostic technique [9] (figure 1). No other intestinal pathogens were detected.

In September 1992, he was hospitalized for 25 days because of severe wasting. He experienced up to 10 watery stools per day. His height was 121 cm, and his weight had dropped to 16.5 kg. His temperature was normal. Electrolyte values were as follows: sodium, 129 mmol/L (normal, 137–145); potassium, 1.3 mmol/L (normal, 4.0–5.2); chloride, 95 mmol/L (normal, 97–107); and calcium, 1.86 mmol/L (normal, 2.2–2.6). Levels of liver enzymes and creatinine were normal. Stool examination still revealed microsporidial spores. In addition, *Cryptosporidium* oocysts were found in stool specimens at this time. Because the persistent cough had become more severe and the chest roentgenogram showed small perihilar interstitial infiltrates, bronchoalveolar lavage was performed. No parasites, including cryptosporidia and microsporidia, or mycobacteria were found in the bronchoalveolar fluid, but bacterial cultures were positive for pneumococci. A 10-day course of amoxicillin clavulanate was given. Total parenteral nutrition was started. Albendazole (200 mg twice daily) was given for 4 weeks. Monthly intravenous therapy with gammaglobulin was continued. The grandmother caring for the boy refused antiretroviral therapy with zidovudine for the boy.

When the boy left the hospital, he had regained 2.7 kg and his weight was the same as it had been 1 year earlier (19.2 kg). His chest roentgenogram was normal. He still had six watery bowel movements per day and a persistent dry cough. Examination of 16 consecutive stool specimens (before, during, and after the 4-week course of albendazole), including a count of parasites in smears prepared with 20 μL of stool suspension, revealed persistent excretion of microsporidial spores (180–240 spores/10 oil-immersion fields at 1000× magnification) as well as cryptosporidial oocysts (2–7 oocysts/slide).

Detection of Microsporidial Spores in Stool Specimens

Microsporidial spores were found by light-microscopic examination (1000× magnification, oil immersion) of smears prepared from an unconcentrated suspension of stool in 10% formalin and stained with a chromotrope-based stain as previously described [9]. The microsporidial spores measured 0.9–1.2 × 1.5 μm and were ovoid and refractile; the spore wall stained bright pinkish-red. Most spores showed a distinct belt-like stripe stained pinkish-red that girded the spores diagonally or equatorially. The microsporidial species was identified by electron microscopical examination, which showed the typical characteristics of the *E. bieneusi* spore (figure 1) [3, 8, 9].

Detection of Cryptosporidial Oocysts in Stool Specimens

Stool samples were processed by an adaption of the formalin–ethyl acetate stool-concentration technique of Ritchie. The concentrates were examined for oocysts by the modified cold Kinyoun acid-fast staining technique and by an indirect immunofluorescence detection procedure according to the instructions of the manufacturer (Crypto-Cel, Cellabs, Brookvale, Australia).

Discussion

Diarrhea, weight loss, and failure to thrive are common in symptomatic HIV-infected children [10–15]. Nevertheless, epidemiological studies designed to determine the causes of gastrointestinal disease in HIV-infected children as well as the origin and transmission of intestinal pathogens are rare. In an American inner-city hospital, the incidence of diarrhea was 2.6–7 times higher in HIV-seropositive than in HIV-seronegative children [13]. Among 201 symptomatic HIV-infected children in central Africa, 97% presented with severe weight loss and 62% presented with diarrhea lasting >4 weeks [11]. Whereas the prevalence of *Cryptosporidium* species and *Isospora belli* in stool specimens of children in a tropical country was high (40% and 8%, respectively), among HIV-infected pediatric patients with diarrhea [12], a European study found rotavirus in 9 of 20 children, cryptosporidia in 2, and a bacterial pathogen in 1 [14]. The prevalence

Table 1. Microsporidiosis in children: summary of case reports.

Microsporidial species	Child's age/sex	Clinical manifestation	Immune status	Detection of parasite	Year [reference]
Encephalitozoon cuniculi	9 y/M	Convulsions	Not known	CSF, urine	1959 [18]
E. cuniculi	2 y/M	Convulsions	Low CD4/CD8 ratio	Serum antibody, urine	1984 [21]
Nosema connori	4 mo/M	Disseminated infection	Thymic aplasia	Autopsy	1973 [19]
Microsporidium ceylonensis	11 y/M	Corneal ulcer	Not known	Histological examination	1973 [20]
Enterocytozoon bieneusi	9 y/M	Diarrhea, failure to thrive	Low CD4 count, HIV infection	Stool specimen	1993, present case

of microsporidiosis in pediatric patients remains to be investigated.

Preliminary epidemiological data have suggested that routine stool examination might detect a potentially treatable infection in ~50% of children with HIV-associated diarrhea [15]. It is not known whether an endoscopic examination would increase the diagnostic yield when a comprehensive examination of stool is negative. Until recently, detection of intestinal *E. bieneusi* infection has depended on the identification of organisms in small-bowel tissue obtained by endoscopic biopsy [3]. Because of the invasive nature of the endoscopic biopsy, it has not been widely used for pediatric patients with AIDS, thus hampering the ability to detect microsporidia. The most recently described, novel diagnostic technique serves as a practical, noninvasive means of detecting microsporidial spores in stool specimens [9].

Microsporidia are obligate intracellular spore-forming protozoa whose range of hosts includes most invertebrates and all classes of vertebrates [16]. The genera *Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, and *Nosema*, as well as microsporidian organisms not yet classified, have been associated with human disease primarily in immunocompromised individuals [16, 17]. More than 150 cases of microsporidiosis in adult patients with AIDS have been described, the majority due to *E. bieneusi*. Among persons not infected with HIV, eight cases of microsporidiosis have been reported, four of them in children [16, 18–21] (table 1).

E. bieneusi has been found in small-intestinal enterocytes, duodenal aspirates, and fecal specimens of only those HIV-infected patients who presented with chronic diarrhea [3, 5, 9]. Epidemiological data have established a causal relation between the presence of the parasite and chronic diarrhea [5, 9]. Furthermore, the parasite has been identified in the biliary tract of some patients with AIDS-related sclerosing cholangitis [22]. Of note, most recently *E. bieneusi* was found in the bronchoalveolar lavage fluid and bronchial epithelium of an HIV-infected patient with chronic cough and small posterobasal infiltrates on chest roentgenogram [23]. The origin of *E. bieneusi* and the mechanisms of its transmission are unknown. Because the organism appears to develop mainly in enterocytes of the small intestine, infection by ingestion of spores is plausible.

The 9-year-old severely immunodeficient boy reported here failed to thrive and had diarrhea that persisted for 16 months of follow-up. No intestinal pathogens, including cryptosporidia, were found in stool specimens until a novel coprodiagnostic technique for detection of *E. bieneusi* became available [9] and intestinal microsporidiosis was diagnosed. Our pediatric patient presented with intermittent nonspecific abdominal pain, a presentation contrasting with that seen in adults with *E. bieneusi* infection, who rarely have abdominal pain. It is not known whether abdominal pain in the boy was related to intestinal microsporidiosis. Intermittent abdominal pain may also be a manifestation of AIDS cholangiopathy, a condition that has been described in adults and in two children [24, 25]. In some patients, AIDS cholangiopathy and AIDS-related sclerosing cholangitis have been related to opportunistic pathogens such as *Cryptosporidium* species, cytomegalovirus, and—most recently—*E. bieneusi* [22, 24, 25]. The boy, however, had no chemical or ultrasonograpic signs of involvement of the biliary tract. After 1 year of follow-up, when gastrointestinal symptoms became most severe, a diagnosis of coinfection with microsporidia and cryptosporidia was made. Although we can not exclude the possibility that cryptosporidiosis was unrecognized before its definitive diagnosis, we feel that this diagnosis was not missed, because stool specimens were repeatedly examined carefully for cryptosporidial oocysts. Nevertheless, epidemiological as well as laboratory data have suggested that coprodiagnostic methods may fail to detect *Cryptosporidium* oocysts in stool specimens of infected patients [4, 6, 26, 27]. Both the child's increasing gastrointestinal symptoms and nonresponsiveness to albendazole therapy might be explained by the presence of *Cryptosporidium* infection.

Recent reports have suggested that treatment of microsporidiosis might result in clinical improvement. Preliminary reports of a good response rate among patients treated with metronidazole [5], however, could not be confirmed [28]. Treatment with albendazole led to a significant clinical improvement in some patients, although *E. bieneusi* was still present in biopsy specimens of the small intestine obtained after treatment [28]. In our patient, quantitative excretion of microsporidial spores did not change during a 4-week course of albendazole therapy.

Evaluation of severely immunodeficient HIV-infected children with chronic diarrhea should include examination for intestinal microsporidia. For such an examination, we recommend the use of the new coprodiagnostic technique [9] that permits detection of microsporidia spores in stool specimens. Furthermore, consideration of dual or even multiple parasitic infections in the differential diagnosis of chronic diarrhea may have both important clinical and epidemiological implications.

Acknowledgments

The authors are indebted to Ruth Keller, Herbert Kuster, and Professor T. Bächi, for technical help and to Sandra Opravil for editorial assistance.

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