

Short Report: Presence of *Enterocytozoon bienewsi* Associated with Intestinal Coccidia in Patients with Chronic Diarrhea Visiting an HIV Center in Haiti

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Abstract. This study investigated the presence of *Enterocytozoon bienewsi* as a possible cause of chronic diarrhea in Haitian patients attending the GHESKIO AIDS clinic in Port-au-Prince, Haiti. Coccidian oocysts were found by polymerase chain reaction (PCR) in the stools of 58/74 patients with chronic diarrhea and included the following agents: 45 (60%) *Cryptosporidium* spp., 27 (34%) *Cyclospora cayetanensis*, and 11 (15%) *Isospora belli*. Four patients (5.5%) were co-infected with *E. bienewsi* and one (1.4%) had *E. bienewsi* alone. The PCR-restriction fragment length polymorphism (RFLP) method made it possible to document the presence in human feces of *E. bienewsi* in Haiti. As in sub-Saharan Africa, the association of *E. bienewsi* with coccidian parasites found in Haitian patients with diarrhea is probably caused by the high level of fecal contamination of soils and surface waters usually associated with countries with low hygienic standards.

Chronic diarrhea accompanied by weight loss is a common and often debilitating manifestation of the human immunodeficiency virus (HIV) infection. *Enterocytozoon bienewsi* has been reported to be associated with chronic diarrhea and wasting in acquired immunodeficiency syndrome (AIDS) patients in Europe, the United States, South America, and Africa. However, intestinal microsporidiosis has also been incriminated as a cause of travelers' diarrhea in immunocompetent patients¹ and has been detected in immunocompetent inhabitants of sub-Saharan Africa. Despite the first description of *E. bienewsi* from a Haitian patient with AIDS living in France,² the presence of this intestinal parasite has only recently been established in Haiti. On three occasions in 2007, the first fresh stool specimens (up to 25 per month for a total of 74) of a total of 810 patients with chronic diarrhea attending an AIDS clinic in Port-au-Prince obtained during the months of March ($N = 25$), May ($N = 25$), and September 2007 ($N = 24$) were preserved with potassium dichromate and shipped to France. These specimens were evaluated by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) for the presence of *E. bienewsi* as a possible cause of chronic diarrhea in Haiti. All patients had a history of intermittent liquid or semi-liquid stools for >3 weeks.

Weber modified trichrome, fluorochrome staining (Uvibio), and indirect immunofluorescent antibody assays using anti-*E. bienewsi* and *Encephalitozoon intestinalis* monoclonal antibodies (Bordier Affinity Products, Crissier, Switzerland) were used for microscopic detection of microsporidium spores. The PCR-RFLP technique was used to identify *Cryptosporidium* spp.³ Genotyping of *E. bienewsi* strains isolated from stool specimens was performed by digestion of amplification products with *NlaIII* and *Fnu4HI* endonucleases (New England Biolabs, Beverly, MA).⁴

The 74 patients consisted of 30 males and 44 females (sex

ratio = 0.59). Their distribution by age and HIV serologic status was as follows: 13 children < 14 years of age (17.6%), of whom 6 were HIV positive; 60 adults 16–52 years of age (81.1%), all HIV positive; and 1 HIV-infected female of unknown age.

Of 58 patients with coccidian oocysts documented in their stools by PCR-RFLP, 45 (60%) were infected with *Cryptosporidium* spp., 27 (34%) with *Cyclospora cayetanensis*, and 11 (15%) with *Isospora belli*; 4 (5.5%) were co-infected with *E. bienewsi*, and 1 (1.4%) was infected with *E. bienewsi* alone (Table 1). Molecular genotyping of 50 strains of *Cryptosporidium* showed the following distribution in these patients: 31 *C. parvum* (42%), 17 *C. hominis* (23%), and 2 *C. felis* (2.7%); 5 adults and 1 12-month-old child (8%) had a co-infection with *C. parvum* and *C. homini*.

Enterocytozoon bienewsi has recently been found in Port-au-Prince in HIV-positive patients at GHESKIO by reverse transcriptase (RT)-PCR (R. Dillingham and J. Pape, personal communication). To our knowledge, Haiti is the second Caribbean country where this parasite has been detected in humans. In this study, this microsporidial fungus was found in patients living in both urban and rural areas. Among these patients, it was found twice in HIV-negative children and three times in HIV-positive adults, two of whom were strongly immunocompromised with low CD4 counts (< 50/100 μ L). All patients presented with chronic debilitating diarrhea. In Martinique, only four cases of microsporidiosis have been found in HIV-positive patients between January 1, 1997 and December 31, 1999, from 4,684 parasitologic stool examinations performed on 2,407 patients.⁵ In our small study, we detected five cases (6.6%) from 74 stool samples obtained during a 3-month period. However, Edouard and others⁵ did not state whether their four cases in Martinique were autochthonous or imported. Indeed, the high level of sanitation and good living conditions in Martinique exclude significant human fecal contamination of the environment.

The delay in report of *E. bienewsi* in humans in Haiti is because of the fact that the PCR-RFLP method was used in this study and that this technique has only been recently introduced at the GHESKIO center. It is important in Haiti to train laboratory technicians in biomolecular methods to iden-

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TABLE 1

Demographic characteristics of the five Haitian patients infected with *E. bienersi*, with or without concomitant intestinal coccidial infection

| Age | Sex | Residence | HIV | CD4 | Stool examination | | | |
|-----------|--------|--|-----|-----|-------------------|------------------|-------------------------|--------------------|
| | | | | | <i>C. hominis</i> | <i>C. parvum</i> | <i>Cy. cayetanensis</i> | <i>E. bienersi</i> |
| 10 months | Male | Village de Dieu, Bicentenaire (Port-au-Prince) | – | | | + | | + |
| 20 months | Female | Thiote (eastern-south province) | – | | + | | | + |
| 23 years | Female | Route de Frères (Port-au-Prince) | + | 281 | + | + | | + |
| 27 years | Male | Galette Dumay (western province) | + | 42 | | | + | + |
| 38 years | Male | Thor Lamentin (Port-au-Prince) | + | 19 | | | | + |

tify such pathogens, and it is essential to have reference laboratories with the capacity to conduct such techniques.

This small survey indicates that *E. bienersi* is frequently associated with coccidial parasites in patients with diarrhea in Haiti. These findings are similar to those reported from sub-Saharan Africa.^{6–10} It is the consequence of high fecal contamination of soils and surface waters in these tropical countries where poverty and promiscuousness are usual and hygienic standards are low.

Received April 15, 2008. Accepted for publication June 20, 2008.

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