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PREVALENCE OF INTESTINAL PARASITES INCLUDING MICROSPORIDIA IN HUMAN IMMUNODEFICIENCY VIRUS–INFECTED ADULTS IN CAMEROON: A CROSS-SECTIONAL STUDY

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Abstract. To assess the prevalence of intestinal parasites in a cohort of human immunodeficiency virus (HIV)–infected adults in Cameroon, a cross-sectional study was conducted. Detection of parasites was performed in 181 stool samples from 154 HIV-infected patients with a mean CD4 cell count of 238 cells/mm^3. Only 35 patients (22%) were receiving antiretroviral therapy at the time of stool sampling, and 46 (29%) had diarrhea. Opportunistic protozoa were found in 15 patients (9.7%), 8 of whom (53%) had diarrhea. *Enterocytozoon bieneusi* was found in eight patients, *C. parvum* in six patients, and *Isospora belli* in three patients. All *E. bieneusi* isolates tested belonged to the same genotype. The prevalence of opportunistic protozoa among patients with CD4 cell counts less than 50/mm^3 was 32%.

INTRODUCTION

The prevalence of intestinal pathogens among human immunodeficiency virus (HIV)–infected individuals has dramatically decreased in countries where antiretroviral agents are widely available.[1,2] However, in most African countries, few patients have access to antiretroviral therapy. Intestinal pathogens still represent a frequent cause of diarrhea, weight loss, and wasting. Prevalence rates of intestinal pathogens, including opportunistic protozoa, in Africa vary from study to study depending on the diagnostic technique used and the study population.[3–23] The diagnosis of some of these pathogens is difficult and requires special staining methods[24,25] and trained personnel. However, application of techniques such as the polymerase chain reaction (PCR) for the detection of microsporidia in stools could increase the diagnostic sensitivity.[26] To assess the prevalence of intestinal parasites in Cameroon, we performed a cross-sectional study in HIV-infected adults followed in an urban center with a special focus on opportunistic protozoa.

PATIENTS AND METHODS

This cross-sectional study was performed at the Yaounde Military Hospital in Cameroon between January 2001 and July 2003. The Cameroon National Ethics Committee reviewed and approved the study. Adult patients ≥ 18 years of age previously enrolled in the Projet Prevention du SIDA au Cameroun set up in 1997 in Yaounde were asked to volunteer for this study and to provide at least one stool sample for detection of ova and parasites, regardless of the presence of diarrhea, during their scheduled visit in the cohort. For each patient, data regarding age, sex, use of antiretroviral agents, and CD4 cell counts were recorded. Diarrhea was defined by laboratory staff on the basis of the appearance of stools. No bacterial cultures of stools were prepared.

For the detection of parasites, fresh stool samples were separated into three samples. The first sample was analyzed by light microscopy in the laboratory of parasitology in Yaounde for the detection of helminths and protozoa by use of both direct and formalin/ether (10%) concentration procedures. The Baermann technique, a larval concentration technique and an alternative to the charcoal culture, was also performed for the detection of *Strongyloides stercoralis* larvae. This sample was also analyzed by modified trichrome (Meridian, Cincinnati, OH) and fluorochrome staining methods for detection of microsporidial spores,[24,25] and by modified Ziehl-Neelsen staining for the detection of *Cryptosporidium sp*. and *Isospora belli*.[27]

The second sample (in 10% formalin) as well as stained slides, were shipped to France for confirmation of the results obtained in Yaounde.

The third sample was diluted in potassium bichromate and used for the detection of microsporidia by a PCR. The DNA was extracted as previously described.[26] Polymerase chain reaction amplification of the internal transcribed spacer of the *E. bieneusi* rRNA gene was performed as described by Velasquez and others.[28] Genotyping of *E. bieneusi* strains isolated from stool specimens was performed by digestion of the amplification products with *Nla* III and *Fnu* 4HI endonucleases (New England Biolabs, Beverly, MA) as described by Liguory and others.[29]

RESULTS

One hundred eighty-one stool samples from 154 patients were obtained during the study period. Only 57% (154 of 270) of the patients in the cohort agreed to participate in the study. The mean age of the patients was 36 years (range = 23–58), 63% were women, and their mean CD4 cell count was 238 cells/mm^3. Twenty-two patients (14%) were severely immunosuppressed and had less than 50 CD4 cells/mm^3. Only 35 patients (22%) were receiving antiretroviral therapy at the time of the study. Forty-six patients (29%) had diarrhea.

Intestinal parasites were found in 51 (33%) of the 154 patients studied. Among the 85 parasites identified, 42 were pathogenic. Helminths were identified in 19 patients, of whom 5 had *S. stercoralis* larvae. Non-opportunistic protozoa, mainly non-pathologic ameba, were identified in 28 patients. Seven patients had *Entamoeba histolytica*/*E. dispar* and one

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patient had *Giardia intestinalis*. *Cyclospora cayetanensis* was not detected in any of the 181 stool samples examined.

Opportunistic protozoa including *C. parvum*, microsporidia, and *I. belli* (Table 1) were found in 15 patients (9.7%), 8 of whom (53%) had diarrhea. The median CD4 cell count of these patients with opportunistic protozoa was 72 cells/mm^3 (range = 4–246). All three patients with *I. belli* spores had diarrhea. *Cryptosporidium parvum* oocysts were found in six patients, and direct microscopy detected microsporidia spores in the stools of six patients. The PCR detected *E. bieneusi* DNA in the stools of two other patients in whom no spores could be seen by direct microscopy. Neither of these two patients had diarrhea. Four of the *E. bieneusi* strains isolated in this study showed genotype IV, which is a rare genotype in France.[29]

Overall, among the 22 patients in this study who had less than 50 CD4 cells/mm^3, 7 (32%) were infected with either *E. bieneusi* or *C. parvum*. Only three patients receiving antiretroviral therapy had opportunistic parasites identified in stools (*Microsporidia*, *Cryptosporidium*, and *I. belli*, respectively). However, the remaining 32 patients receiving antiretroviral therapy were free of intestinal parasites.

DISCUSSION

In this cross-sectional study of HIV-infected adults in Cameroon, the overall prevalence of intestinal parasites in stool samples was 33%. This prevalence is lower than those previously reported in other studies conducted in Africa.[12,19,30] This low prevalence could be related to the location in which our study was conducted, an urban center, and to the population studied, adults not selected for the presence of diarrhea. Only 29% of our patients had diarrhea at the time of stool examination. Furthermore, since no bacterial cultures of stools were prepared in this study, any association between parasites and diarrhea in this study should be made cautiously.

Various intestinal parasites were found in the stools of our patients. Pathogenic helminths were found in 12% of the patients, a prevalence similar to that reported in other studies of HIV-infected patients in Cameroon and Mali.[23,30] Only seven (4.5%) patients had *E. histolytica/E. dispar* cysts in their stools, a prevalence that was lower than the prevalence rate of 12% reported in Cameroon in patients with diarrhea.[23]

Our study focused mainly on opportunistic protozoa, a frequent cause of chronic diarrhea and wasting in HIV-infected patients in developing countries in Africa.[3–23,31] *Cryptosporidium parvum*, microsporidia, and *I. belli* accounted for the most of these opportunistic protozoa. Their diagnosis is difficult because of the special staining methods required. In this study, where only 22% of the patients were receiving antiretroviral therapy, the prevalence of opportunistic protozoa was 9.7%. However, among patients with less than 50 CD4 cells/mm^3, this prevalence increased to 32%. Previous studies in Africa have found prevalence rates ranging from 3.5% to 28%, but were conducted mainly in patients with diarrhea.[3–15]

Cryptosporidium parvum oocysts were found in 3.9% of the patients, half of whom had diarrhea. This was in the range of the prevalence of 8.7% reported in Côte d'Ivoire by Assoumou and others[20] among 217 patients who were not selected for diarrhea. However, higher prevalence rates up to 25% have been reported in other studies usually conducted in patients with diarrhea. The low prevalence reported in our study is not unexpected because the patient population was not selected on the basis of gastrointestinal symptoms.

Microsporidia, which were identified by PCR as *E. bieneusi*, were detected in eight patients. The prevalence reported in our study (5.2%) was lower than those reported in previous studies.[8,10,12,14–16,18,19,23] Less than half of our patients with microsporidia had diarrhea. This suggests that either a significant proportion of our patients were asymptomatic carriers of this opportunistic protozoa, a condition previously described in patients with high CD4 cell counts, or that they had other clinical symptoms frequently associated with microsporidia such as cachexia, weight loss, and malabsorption. Genotype IV of *E. bieneusi* identified in this study was rarely detected in a previous study in France, suggesting differences in *E. bieneusi* strain distribution.[29] Moreover, genotype IV corresponds to genotype K described by Dengjel and others[32] from a cat sample. Genotype K was found in only one human case in Europe,[33] but in 6 of 10 sequences from human cases in Uganda.[34] However, further studies that test a larger number of *E. bieneusi* strains from Africa are needed to confirm these findings.

In conclusion, in this cross-sectional study of 154 HIV-infected adults in Yaounde, the overall prevalence of intestinal parasites was 33%, and that of opportunistic protozoa was 9.7%. However, the prevalence of opportunistic protozoa increased to 32% in patients with less than 50 CD4 cells/mm^3. The results of our study should therefore prompt physicians caring for HIV-infected patients in Cameroon to request stool examination and specific tests for microsporidia, *Cryptosporidium*, and *Isospora*, especially in patients with low CD4 cell counts.

TABLE 1

Opportunistic protozoa (OP) identified in 154 human immunodeficiency virus–infected adults in Yaounde, Cameroon

	No. of patients (%)*	No. of patients (%) without diarrhea	No. of patients (%) with diarrhea
Total	154	108 (70)	46/154 (30)
Patients with OP	15 (9.7)	7/108 (6.4)	8/46 (17.3)
Cryptosporidia	6 (3.8)	3/108 (2.8)	3/46 (6.5)
Microsporidia	8 (5.2)	5/108 (4.6)	3/46 (6.5)
Isospora belli	3 (2)	0/108 (0)	3/46 (6.5)

* One patient can have more than one pathogen.

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