

INTESTINAL PARASITES INFECTIONS IN HOSPITALIZED AIDS PATIENTS IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

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Summary:

To determine the prevalence and the species spectrum of intestinal parasites (IP) involved in hospitalized AIDS patients, a prospective observational and cross-sectional study was carried out in the four main hospitals in Kinshasa, Democratic Republic of the Congo. From November 2006 through September 2007, a single stool sample was collected from 175 hospitalized AIDS patients older than 15 years. Parasites were detected by light microscopy, including Ziehl-Neelsen, Fungi-Fluor, modified trichrome stains, and by immunofluorescence antibody tests and PCR for species diagnosis of microsporidia. At baseline, 19 patients (10.8 %) were under antiretroviral therapy and 156 (89.2 %) were eligible for ART. The main diagnosis for justifying hospitalization was intestinal infection associated with diarrhea in 87 out of 175 (49.7 %). 47 out of 175 (26.9 %) were found to harbor an IP, and 27 out of 175 (15.4 %) were infected with at least one opportunistic IP (OIP). Prevalence rate for OIP were 9.7 %, 5.1 %, 1.7 % and 0.6 % for *Cryptosporidium* sp., *Enterocytozoon bienewisi*, *Isospora belli* and *Encephalitozoon intestinalis* respectively. Considering patients with diarrhea only, prevalence rate were 12.6 %, 4.6 %, 3.4 % and 1.1 % respectively. The other IP observed were *Entamoeba histolytica/Entamoeba dispar* in nine cases (5.1 %), *Ascaris lumbricoides* in seven cases (4.0 %), *Giardia intestinalis* in three cases (1.7 %), hookworm in two cases (1.1 %) and *Trichiuris trichiura*, *Enterobius vermicularis*, *Schistosoma mansoni* in one patient each (0.6 %). No significant relationship was established between any individual IP and diarrhea. These results underline the importance of OIP in symptomatic AIDS patients regardless of diarrhea at the time of the hospitalisation, and showed that routine microscopic examination using stains designed for *Cryptosporidium* spp. or the microsporidia should be considered due to the absence of clinical markers.

KEY WORDS : AIDS, microsporidia, cryptosporidia, intestinal parasite, epidemiology, diagnosis, Central Africa.

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Résumé : INFECTIONS PARASITAIRES INTESTINALES DE PATIENTS ATTEINTS DE SIDA, HOSPITALISÉS À KINSHASA, RÉPUBLIQUE DÉMOCRATIQUE DU CONGO

Afin de déterminer la prévalence et le spectre étiologique des infections parasitaires (IP) du tube digestif chez les sujets atteints de SIDA hospitalisés à Kinshasa, République Démocratique du Congo (RDC), une étude prospective, transversale et observationnelle a été réalisée dans les quatre principaux hôpitaux de la ville. De novembre 2006 à septembre 2007, des échantillons de selles de 175 patients hospitalisés, âgés de plus de 15 ans et au stade de SIDA de l'infection par le VIH, ont été collectés. Les parasites ont été détectés en microscopie optique par un examen direct, une technique de concentration (méthode de Ritchie), une coloration de Ziehl-Neelsen modifiée, une coloration par le trichrome et une coloration par le Fungi Fluor. Le diagnostic d'espèce des microsporidies a été complété par une technique d'immunofluorescence utilisant des anticorps monoclonaux et par PCR. Au moment du diagnostic, 19 patients (10,8 %) étaient sous traitement antirétroviral et 156 (89,2 %) étaient éligibles pour l'initiation du traitement antirétroviral. Le principal diagnostic ayant motivé l'hospitalisation a été la diarrhée infectieuse pour 87 patients (49,7 %). 47 patients (26,9 %) étaient porteurs d'au moins un parasite intestinal (PI) et 27 (15,4 %) étaient infectés par au moins un parasite intestinal opportuniste (PIO). Les taux de prévalence des PIO étaient de 9,7 %, 5,1 %, 1,7 % et 0,6 % pour respectivement *Cryptosporidium* sp., *Enterocytozoon bienewisi*, *Isospora belli* et *Encephalitozoon intestinalis*. Si l'on considère les 87 patients diarrhéiques séparément, les taux de prévalences étaient alors de 12,6 %, 4,6 %, 3,4 % et 1,1 %. Les autres PI observés étaient *Entamoeba histolytica/Entamoeba dispar* dans neuf cas (5,1 %), *Ascaris lumbricoides* dans sept cas (4,0 %), *Giardia intestinalis* dans trois cas (1,7 %), des ankylostomides dans deux cas (1,1 %), et *Trichiuris trichiura*, *Enterobius vermicularis* et *Schistosoma mansoni* chez un patient chacun (0,6 %). Aucune association statistiquement significative avec la diarrhée n'a été retrouvée pour l'ensemble de ces parasites. Ces résultats montrent l'importance des PIO des patients symptomatiques hospitalisés au stade de SIDA de l'infection par le VIH en RDC, indépendamment de la présence d'une diarrhée au moment de l'hospitalisation. Du fait de l'absence de marqueur clinique spécifique, un diagnostic systématique de ces agents pathogènes avec les techniques appropriées est impératif chez ces patients particulièrement fragilisés.

MOTS CLÉS : SIDA, microsporidies, cryptosporidies, parasite intestinal, épidémiologie, diagnostic, Afrique Centrale.

INTRODUCTION

Democratic Republic of the Congo (DRC), located in the Central African region, is the third largest country on the African continent. With a population of more than seven million inhabitants, the capital Kinshasa (a city-province), located along the south bank of the Congo River, is also the third largest city in the whole continent after Lagos and Cairo (capitals of Nigeria and Egypt, respectively). AIDS pandemic is one of the major causes of death and morbidity in DRC. The HIV sera prevalence rate, according to the "Programme National Multisectoriel de Lutte contre le SIDA" (PNMLS) was estimated to be 4.04 % for the whole country in 2006 (UNGASS 2007 report, available at: http://data.unaids.org/pub/Report/2008/democratic_republic_of_congo_2008_country_progress_report_fr.pdf). However, due to successive political crises and war areas in different part of the country, great variations exist between provinces and even cities. The HIV positive rate were 3 %, 10 % and 27 % for Kinshasa, Matadi, a western port city, and Kalemie another port city in the south-east, respectively (from UNDP, United Nations Development Program available at: <http://www.undp.org/cd/News.aspx?newsID=64>).

Since 2005, together with the improvement of the political situation, antiretroviral therapy (ART) became available and free for DRC citizens. This represents around 300,000 subjects for the whole country, and 13,000 for Kinshasa City alone (from PNMLS, 2007). It is of course a great advance in the fight against the AIDS pandemic, but it also raises many problems to reach the ultimate goal of giving an appropriate therapy for all. In Kinshasa, like elsewhere in Africa, this appears to be insufficient for many patients who died shortly after the diagnosis of AIDS (Konate *et al.*, 1998; Lawn *et al.*, 1897; Macpherson *et al.*, 2008; Zachariah *et al.*, 2006; Mzileni *et al.*, 2008; Dillingham *et al.*, 2009). Since the presence of diarrhea, found in nearly 50 % of the patients at the time of the AIDS diagnosis, is associated with a great mortality rate, we can estimate that a part of them died because of intestinal parasites infections (Sewankambo *et al.*, 2000). It is to underline that most often in such a situation, the ART cannot be initiated in good conditions because of severe weight loss and wasting syndrome. Considering that worrying situation the World Health Organization (WHO) had recently defines diarrhea wasting syndrome, along with positive HIV serology test, to be an AIDS-defining illness (Anonymous, 2006).

Several pathogens have been implicated with acute and chronic diarrhea in HIV disease in Africa. The most commonly reported include enteroaggregative *Escherichia coli*, *Shigella* spp., Rotavirus and intestinal parasites (IP), mainly *Enterocytozoon bienersi*, *Cryptosporidium*

spp., *Isospora belli*, and *Giardia intestinalis* (Gassama *et al.*, 2001). *Cryptosporidium* spp., *Ent. bienersi*, but also *G. intestinalis* are ubiquitous enteric protozoan pathogens that infect humans, domestic animals and wildlife worldwide. Both pathogens are significant causes of diarrhea and nutritional disorders in institutional and community settings. Since they are also significant waterborne pathogens, all diseases have a common link with poverty. In DRC, because of the lack of appropriate methods and trained technicians, IP prevalence rates were unknown before 2007 in HIV/AIDS patients. That situation was mostly the result of the complete disorganization of the sanitary system during the 1998-2004 war. In a recent article, we reported preliminary results, obtained in 50 hospitalized AIDS patients in Kinshasa, that confirmed the presence of these species, and especially *Ent bienersi* (first identification of this species locally) in DRC (Wumba *et al.*, 2007). In the present work, we carried out a larger prospective observational and cross-sectional study to determine the species spectrum, clinical presentation and prevalence of IP involved in AIDS patients hospitalized in the four main hospitals in Kinshasa.

METHODS

STUDY POPULATION AND ENROLLMENT

This study was designed to be cross-sectional with optional follow-up. Its main objective was to determine the prevalence of IP in AIDS patients at the moment of their hospitalization. Participants were recruited, between November 2006 and September 2007, from the four main hospitals in Kinshasa (DRC), the *Cliniques Universitaires de Kinshasa* (CUK) the main Teaching Hospital in DRC, two general hospitals, the *Hôpital Général de Kinshasa* and the *Hôpital Général de Kintambo*, and the *Centre Médical Monkole*, a specialized medical support centre for persons affected with HIV/AIDS. The criteria for inclusion in the study were documented HIV infection, presence of at least one AIDS-defining event including diarrhea wasting syndrome, age > 15 years and the ability to provide informed consent. Each participant was interviewed using a standardized and structured questionnaire to collect demographic and clinical data and asked to provide at least one stool specimen, irrespective of symptoms, for detection of parasites. Diarrhea was defined on the basis of an average number of > 3 loose or liquid stools within a 24h period.

ETHICAL CONSIDERATIONS

The institutional review boards, the Kinshasa School of Medicine Ethics and Scientific Committee, approved the

protocol and the study was done in compliance with the Helsinki declaration II. The aim and procedures of the study were explained, and an informed consent sheet was signed by each participant or a designated literate substitute when necessary.

LABORATORY METHODS

Biological analysis were performed in the Parasitology unit of the CUK in DRC for all the microscopic analysis and in the Parasitology-Myecology unit of the *Groupe Hospitalier Universitaire de la Pitié-Salpêtrière* (GHUPS) in Paris, France, for an external quality control and molecular biology analysis. At recruitment sites, stool samples were collected in plastic fecal collectors, immediately added with 10 % buffered Formalin as a preservative and stored at room temperature. Every day except Sunday, stools were brought back to the CUK Parasitology unit laboratory and investigated for helminthes and unicellular parasites. The stool specimens were concentrated with the modified Ritchie formalin-ether method procedure (MRC) followed by microscopic examination (Ritchie *et al.*, 1948). *Cryptosporidium* but also *Isoospora* and *Cyclospora* species were detected using the modified Ziehl-Neelsen stain (MZNS) (Henriksen *et al.*, 1981). Microsporidia were detected using both Weber's modified trichrome stain (MTS), and a Fungi-Fluor stain (FFS) (van Gool *et al.*, 1994; Weber *et al.*, 1992). Microsporidia species identification was performed using both an immunofluorescence antibody test using monoclonal antibodies (IFAT-Mab) directed against *Ent. bienewsi* and *Encephalitozoon intestinalis* and specific PCR's (Thellier *et al.*, 2001; Accoceberry *et al.*, 1999; Alfa Cisse *et al.* 2002; Breton *et al.*, 2007).

For all these techniques, an external Quality Control was performed in double-blinded conditions for all the positive samples and 20 % of randomly selected negatives samples in the laboratory of the GHUPS. The microsporidia microscopy positive specimens were also processed in Paris, for DNA extraction using the QIAamp® DNA Stool MiniKit (QIAGEN, Courtaboeuf, France) following manufacturer's instructions as described elsewhere (Alfa Cisse *et al.*, 2002; Breton *et al.*, 2007). The region of DNA amplified by PCR was that encoded by the small subunit of the rRNA of *Ent. bienewsi* and *Enc. intestinalis* (Coyle *et al.*, 1996). Common primers used to amplify both DNA species were INBI and PMP2 (Zhu *et al.*, 1993). Specific primers pairs were INBI/BIENE for *Ent. bienewsi* and INBI/EI500 for *Enc. intestinalis* DNA (Zhu *et al.*, 1993; Weiss *et al.*, 1994; Zhu *et al.*, 1993).

NUCLEOTIDE SEQUENCING OF THE ITS REGION OF THE *ENT. BIENEWSI* RRNA GENE

Enterocytozoon bienewsi genotypes were analyzed by nucleotide sequencing of the ITS region of the rRNA

gene. A PCR product of 508 bp, containing 122 bp of the small-subunit rRNA, 243 bp of the ITS region, and 143 bp of the large-subunit rRNA, was generated from *Ent. bienewsi* PCR samples using the primers MSP3 and MSP4B as previously described (Katzwinkel-Wladarsch *et al.*, 1996). PCR products were purified using the Concert Rapid PCR kit (GIBCO-BRL) and sequenced in both directions using the ABI Big Dye Terminator kit (v1.1) and an ABI 3100 automated sequencer (Applied Biosystems). Sequencing three PCR products from the same sample in both directions controlled the sequence accuracy.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

Nucleotide sequence of the ITS region of the rRNA gene of isolate RDC71 was deposited in GenBank under Accession Number FJ469914.

STATISTICAL ANALYSIS

Data were expressed as proportions (%) for categorical variables and means with standard deviations for continuous variables. Differences were compared by the Chi-square test for proportions and by the Student t-test for continuous variables with results considered statistically significant for $p < 0.05$. The kappa value was used to assess the concordance between the Congolese and the French laboratories.

RESULTS

Between November 2006 and September 2007, a total of 175 hospitalized AIDS patients older than 15 years, entered the study. Of these, 89 were women (sex ratio M/F = 0.97), and the median age was 41 ± 14 years, range (16-83 yrs). Men were 9 years older than women, 46 ± 15 years *vs* 37 ± 12 years ($p < 0.001$). A great part of the subjects, 65 of 175 (37.1 %) were unemployed with an equal proportion of men 34 (52.3 %) and women 31 (47.7 %). Similar proportions were home workers or civil servant, but it is to notice that all home workers were women, 35 of 175 (20.0 %), and all but one civil servant were men, 30 of 175 (17.1 %). Students were also represented, 25 of 175 (14.3 %) with a greatest proportion of women, 16 (64.0 %) than men, 9 (36.0 %).

At baseline, 19 of 175 patients (10.8 %) were under a first line ART while the other 156 (89.2 %) were eligible for ART, stage 3 or 4 of the WHO clinical staging 2007 revision (www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf). Twenty-nine of 175 (16.6 %) had a lymphocyte CD4⁺ T cells count when entering the study. The median CD4 count was 224 cells/ μ l, range (10-691). The main diagnosis for justifying hospitalization of patients was intestinal infection associated with diarrhea

in 87 of 175 (49.7 %) followed by severe oral candidiasis (31.4 %), herpes zoster (20.5 %), malaria (18.3 %) and severe cutaneous mycosis (14.9 %). In case of clinical diarrhea, consistencies of feces were all abnormal, from pasty to watery stools. Watery stools, similar to those encountered in choleric patients, were found in 52 of 87 (59.8 %).

43 patients of the 175 (24.6 %) were found to harbor an IP, and 27 (15.4 %) were infected with at least one opportunistic intestinal parasite (OIP). The prevalence rates of OIPs' were 9.7 % (17/175) for *Cryptosporidium* sp., 5.1 % (9/175) for *Ent. bieneusi*, 1.7 % (3/175) for *I. belli* and 0.6 % (1/175) for both *Enc. intestinalis* and *Sarcocystis hominis*. The other IP observed were also protozoan parasites, *Entamoeba histolytica/Entamoeba dispar* in nine cases (5.1 %) and *G. intestinalis* in three cases (1.7 %) but also helminths such as *Ascaris lumbricoïdes* in seven cases (4.0 %), hookworm in two cases (1.1 %), and *Trichiuris trichiura*, *Enterobius vermicularis*, *Schistosoma mansoni* in one patient each (0.6 %).

Considering the 87 patients with diarrhea, 23 (26.4 %) harbored an IP and 16 (18.4 %) were infected with an OIP. The OIP prevalence rates were then 12.6 % (11/87) for *Cryptosporidium* spp., 4.6 % (4/87) for *Ent. bieneusi*,

3.4 % (3/87) for *I. belli*, 1.1 % (1/87) for *Enc. intestinalis* and 1.1 % (1/87) for *S. hominis*. The others IP observed in that situation were *E. histolytica/E. dispar* in four cases (4.6 %), *A. lumbricoïdes* in four cases (4.6 %) and *G. intestinalis* in two cases (2.3 %). The relationship between IP and diarrhea was not statistically significant for any parasite.

Concerning the techniques used for OIP identification in Kinshasa, as expected, only *I. belli* (the 3 cases) and the *S. hominis* case were detected by standard fecal examinations whereas *Cryptosporidium* spp and microsporidia species were only detected by dedicated techniques, MZNS and MTS/FFS respectively (Table I). Microsporidia species identification was performed using IFAT-MABs in all cases.

The concordance between Kinshasa and Paris laboratories results was measured for the 43 positive samples and 26 of the negative samples randomly selected. The kappa value was 1, a perfect correlation, for all techniques (MRC, MZNS, MTS, IFAT-MABs) except one since a false positive result was recorded for microsporidia using FFS in Paris (kappa value = 0.97, IC95% [0.91-1.00]).

The specific PCR for *Ent bieneusi* and *Enc intestinalis*, performed in Paris, was positive for only three out of

Patients n	Diarrhea yes	Diarrhea no	DE ⁺ & MRCM ⁺	MZNS [*]	FFS [*]	MTS [*]	IFAT-MAb-Eb/Ei [•]
12	7	5	-	Csp	-	-	-
7	2	5	-	-	Msp	Msp	Eb
1	1	-	-	-	Msp	Msp	Ei
1	1	-	Ib	-	-	-	-
5	1	4	Eh/Ed	-	-	-	-
5	3	2	Al	-	-	-	-
2	1	1	Gi	-	-	-	-
2	1	1	Cm	-	-	-	-
1	-	1	Ank	-	-	-	-
1	-	1	Al Ank	-	-	-	-
1	1	-	Eh/Ed	Csp	-	-	-
1	1	-	Ib Sh Gi	Csp	Msp	Msp	Eb
1	-	1	Eh/Ed	Csp	-	-	-
1	1	-	-	Csp	Msp	Msp	Eb
1	1	-	Eh/Ed Ev	-	-	-	-
1	1	-	Ib Sm	-	-	-	-
1	1	-	Eh/Ed Tt	-	-	-	-
1	1	-	Al	Csp	-	-	-
130	63	67	-	-	-	-	-
175	87	88	3 Ib; 1Sh	17 Csp	10 Msp		9 Eb + 1 Ei

⁺DE = Direct Examination; ^{*}MRCM = Modified Ritchie Concentration Method; ^{*}MZNS = Modified Ziehl Neelsen Stain; ^{*}FFS = Fungi-Fluor Stain; ^{*}MTS = Modified Trichrome Stain; [•]IFAT-MABEb/Ei = ImmunoFluorescence Antibody Test using monoclonal antibodies directed against *Ent. bieneusi* or *E. intestinalis*

Eh/Ed: *Entamoeba histolytica* / *Entamoeba dispar*; Cm: *Chilomastix mesnili*; Al: *Ascaris lumbricoïdes*; Gi: *Giardia intestinalis*; Sm: *Schistosoma mansoni*; Ev: *Enterobius vermicularis*; Tt: *Trichuris trichiura*; Ank: *Ankystoma* sp.; Csp: *Cryptosporidium* sp.; Msp: microsporidia species; Eb: *Enterocytozoon bieneusi*; Ei: *Encephalitozoon intestinalis*; Ib: *Isospora belli*; Sh: *Sarcocystis hominis*

Table I. - Diagnosis of intestinal parasites in optic microscopy in 175 hospitalized AIDS patients in four hospitals in Kinshasa (DRC).

the ten samples positive by microscopic methods, because of PCR inhibition probably due to the formalin addition. Among these samples, only two ITS sequences could be obtained (samples RDC69 and RDC71) for genotyping. Both sequences were 100 % identical to genotype NIA1 (Esporn *et al.*, 2007).

DISCUSSION

Demographic and clinical data from this study were similar to those reported among African AIDS patients, in particular the predominant heterosexual transmission (sex ratio M/F = 0.97), and comparable occupational behavior (Same-Ekobo *et al.*, 1997). The fact that very few of those patients, 19 (10.8 %) and 29 (16.6 %) out of 175, were respectively under ART or with a previous result of CD4⁺ cell count at the moment of their hospitalization was not surprising since most of them were diagnosed AIDS on that occasion. The prevalence of OIP (15.4 %), as determined by the results of the screening, is globally on the range of results previously reported in other studies conducted in Africa on AIDS patients. In detail, the 9.7 % prevalence rate of *Cryptosporidium* sp. was slightly higher than the rates reported from four studies in Kenya, Zimbabwe, Zambia and Cameroun, similar to that reported in one study from Senegal, but lower than the 16–41 % prevalence rates reported from many sub-Saharan countries (Gassama *et al.*, 2001; Same Ekobo *et al.*, 1997; Adjei *et al.*, 2003; Fisseha *et al.*, 1998; Gatei *et al.*, 2006; Houpt *et al.*, 2005; Khumalo-Ngwenya *et al.*, 1994; Maiga *et al.*, 2002; Mengesha *et al.*, 1994; Mwachari *et al.*, 1998; Sarfati *et al.*, 2006; Simango *et al.*, 2004; Tadesse *et al.*, 2005; Tumwine *et al.*, 2005; Cegielski *et al.*, 1999; Dieng *et al.*, 1994). The difference is more contrasted with microsporidia (5.7 %), and *I. belli* (1.7 %) in comparison with the 9.4–31.3 % range for microsporidia and 4.4–15 % range for *I. belli* observed in many African countries (Gassama *et al.*, 2001; Khumalo-Ngwenya *et al.*, 1994; Tumwine *et al.*, 2005; Cegielski *et al.*, 1999; Dieng *et al.*, 1994; konate *et al.*, 2005; Lebbad *et al.*, 2001; Maiga *et al.*, 1997; Same - Ekobo *et al.*, 1997). However, recent studies in Central Africa (Cameroon and Gabon), using the same methods for diagnosis and without selection of patients based on clinical criteria, also reported low prevalence rates, lower than 6 % for microsporidia and lower than 3 % for *I. belli* (Breton *et al.*, 2007; Sarfati *et al.*, 2006). Multiple OIP infections were seen in five of the 27 patients with OIP (18.5 %) while co-infections with other parasites were present in four of 27 (14.8 %) (Table I). Concerning diarrhea, the 49.7 % prevalence found in this study is in the range of recent comparable studies conducted in Senegal (49.7 %) Malawi (48.8 %) and Ethiopia (45.2 %) (Gassama *et al.*, 2001; Ngoma *et al.*, 2008; Assefa *et al.*, 2009). In a recent study in Malawi,

van Oosterhout *et al.*, report a 56 % person year incidence of digestive illness among 660 AIDS patients, 95 % before starting on ART (van Oosterhout *et al.*, 2005). But surprisingly, we found no association between the presence of IP in stools and diarrhea. In detail, 11 out of the 27 OIP infected patients, more than 40 %, were harboring OIP without digestive symptoms at admission. Those correspond to 6 of 17 (35.3 %) *Cryptosporidium* spp. and five of nine (55.6 %) *Ent. bieneusi* infections. In contrast, the three patients with *I. belli* infections were all diarrheic. The situation was not different considering the other IP since one out of three (33.3 %) patients infected with *G. intestinalis* and four out of nine (44.4 %) patients infected with *E. histolytica* / *E. dispar* were also non diarrheic. It is noteworthy that in this study diarrhea couldn't be linked to any intestinal parasite in more than 70 % of the cases. Unfortunately, diagnosis of bacteria and viruses couldn't be investigated. In comparison, Gassama *et al.* in Senegal found that the more frequent pathogens were enteroaggregative *E. coli* (19.6 %), microsporidia (9.4 %), *Cryptosporidium* sp. (8.2 %), rotavirus (8.2 %), *Shigella* sp. (7.6 %), *Candida albicans* (7.6 %) and *Isospora belli* (4.4 %) (Gassama *et al.*, 2001). One explanation could be a higher CD4 cell count level in our population study. Unfortunately we cannot cross that information since the CD4 cell count couldn't be performed in this study. Nevertheless, according to WHO clinical staging, our population study appears to be very immunocompromised since all our patients were classified stage 3 or 4. Two other explanations appears to be more convincing, firstly a defect of power in statistical analysis, because of a too small population sample, and secondly, the abilities of species genotypes of *Cryptosporidium* spp. or the microsporidia to induce or not a pathology in man. It is well known now that, even in very immunocompromised patients, the clinical forms of OIP are very diverse in man, varying from a moderate digestive embarrassment to a severe digestive pathology with weight loss and profound fatigue (Bern *et al.*, 2005; Cama *et al.*, 2007). The pathogenic capacity of *Ent. bieneusi* in particular has been questioned since some authors found no association between intensity of microsporidia infection and clinical symptoms (Claridge *et al.*, 1996; Rabaneck *et al.*, 1993). It has been recently reported in Peru that only some *Ent. bieneusi* or *C. parvum* or *C. hominis* genotypes presents a statistically significant association with diarrhea (Bern *et al.*, 2005; Cama *et al.*, 2007). In our study, because of prolonged conservation in formalin, samples containing *Ent. bieneusi* spores were positive in PCR only in three out of nine (33.3 %) and genotype available in only two. Those two genotypes obtained from patients without diarrhea were both of type NIA1 (Esporn *et al.*, 2007). Interestingly, that genotype, found previously in

Africa in human in Niamey (Niger), belongs to sub group 1c, and is very close to genotypes A (Peru1) and K (Peru2), one base pair difference, for which were found no association with chronic diarrhea in Bern *et al.* study. So these results highlight the need for systematic genotyping to clarify the relationship between OIP genotype and symptomatic infections.

Globally, and despite a lack of some information's in our study, such as lymphocytes CD4 cell count, our results confirm that parasite intestinal infections are a major concern for AIDS patients, especially when starting ART. A main result was the demonstration that in hospitalized symptomatic AIDS patients, intestinal parasitic infections can go unrecognized since up to 45 % of patients with intestinal parasite were non diarrheic. It might have long-lasting consequences not only for the individual AIDS patient who is undergoing ART, whose life is at risk, but also for the communities among whom uncontrolled pathogens can spread (crowded conditions or institutions such as hospitals or day care centers). Therefore, due to the absence of clinical markers, routine stool examination for intestinal parasite but also stains designed for *Cryptosporidium* spp. or the microsporidia diagnosis are of critical importance in AIDS patients, especially when undergoing ART.

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