

# Epidemiology, clinical, immune, and molecular profiles of microsporidiosis and cryptosporidiosis among HIV/AIDS patients

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**Background:** The objective of this study was to determine the prevalence of intestinal parasites, with special emphasis on microsporidia and *Cryptosporidium*, as well as their association with human immunodeficiency virus (HIV) symptoms, risk factors, and other digestive parasites. We also wish to determine the molecular biology definitions of the species and genotypes of microsporidia and *Cryptosporidium* in HIV patients.

**Methods:** In this cross-sectional study, carried out in Kinshasa, Democratic Republic of the Congo, stool samples were collected from 242 HIV patients (87 men and 155 women) with referred symptoms and risk factors for opportunistic intestinal parasites. The analysis of feces specimen were performed using Ziehl–Neelsen stainings, real-time polymerase chain reaction (PCR), immunofluorescence indirect monoclonal antibody, nested PCR-restriction fragment length polymorphism, and PCR amplification and sequencing. Odds ratio (OR) and 95% confidence intervals were used to quantify the risk.

**Results:** Of the 242 HIV patients, 7.8%, 0.4%, 5.4%, 0.4%, 2%, 10.6%, and 2.8% had *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Cryptosporidium* spp., *Isospora belli*, pathogenic intestinal protozoa, nonpathogenic intestinal protozoa, and helminths, respectively. We found five genotypes of *E. bieneusi*: two older, NIA1 and D, and three new, KIN1, KIN2, and KIN3. Only 0.4% and 1.6% had *Cryptosporidium parvum* and *Cryptosporidium hominis*, respectively. Of the patients, 36.4%, 34.3%, 31%, and 39% had asthenia, diarrhea, a CD4 count of <100 cells/mm<sup>3</sup>, and no antiretroviral therapy (ART), respectively. The majority of those with opportunistic intestinal parasites and *C. hominis*, and all with *C. parvum* and new *E. bieneusi* genotypes, had diarrhea, low CD4+ counts of <100 cells/mm<sup>3</sup>, and no ART. There was a significant association between *Entamoeba coli*, Kaposi sarcoma, herpes zoster, chronic diarrhea, and asthenia, and the presence of 28 cases with opportunistic intestinal parasites. Rural areas, public toilets, and exposure to farm pigs were the univariate risk factors present in the 28 cases with opportunistic intestinal parasites. In logistic regression analysis, a CD4 count of <100 cells/mm<sup>3</sup> (OR = 4.60; 95% CI 1.70–12.20; *P* = 0.002), no ART (OR = 5.00; 95% CI 1.90–13.20; *P* < 0.001), and exposure to surface water (OR = 2.90; 95% CI 1.01–8.40; *P* = 0.048) were identified as the significant and independent determinants for the presence of opportunistic intestinal parasites.

**Conclusion:** *E. bieneusi* and *Cryptosporidium* are becoming more prevalent in Kinshasa, Congo. Based on the findings, we recommend epidemiology surveillance and prevention by means of hygiene, the emphasis of sensitive PCR methods, and treating opportunistic intestinal parasites that may be acquired through fecal–oral transmission, surface water, normal immunity, rural area-based person–person and animal–human infection, and transmission of HIV. Therapy, including ART and treatment with fumagillin, is needed.

**Keywords:** diarrhea, *Enterocytozoon bieneusi*, *Cryptosporidium hominis*, *Cryptosporidium parvum*, risk factors, Africans

## Introduction

Enteric opportunistic parasites are associated with HIV/AIDS, chronic diarrhea, education, occupation, residence in a slum, exposure to pets and animals, antiretroviral therapy (ART), use of public toilets, water, and practicing unsafe homosexual activity.<sup>1–20</sup> The literature indicates that the prevalence of intestinal colonization due to microsporidia and *Cryptosporidium* are significantly higher among HIV-infected individuals with chronic diarrhea and CD4 lymphocyte counts of <200 cells/mm<sup>3</sup>.<sup>4,7,8,11,13,15,17,20</sup>

In the Democratic Republic of the Congo (DRC), our country, the role of emerging pathogens such as *Enterocytozoon bieneusi* and *Cryptosporidium* spp. in HIV-infected patients, with and without diarrhea, has been identified using standard parasitological methods, such as light microscopy, Ziehl–Neelsen staining, the Fungi-Fluor® Kit for Fungal Detection, modified trichrome tests, and traditional polymerase chain reaction (PCR).<sup>21,22</sup> However, the lack of sensitive and specific real-time PCR, nested PCR, and PCR-restriction fragment length polymorphism (RFLP) in the DRC has resulted in a lack of epidemiologic, clinical, and immune data related to intestinal parasites in HIV patients. Therefore, the objective of the present study was to determine the prevalence of intestinal opportunistic parasites, with special emphasis on microsporidia and *Cryptosporidium*, as well their association with HIV symptoms, risk factors, and other digestive parasites, and the molecular biology definition of the species and the genotypes of microsporidia and *Cryptosporidium* in HIV patients. Humanitarian collaboration with French hospitals (Saint-Louis, Pitié Salpêtrière, and INSERM UMR-S 945, Paris) has helped us to perform molecular biology in this work.

## Materials and methods

### Study design

This descriptive and observational cross-sectional study took place between December 2009 and January 2012.

### Ethical considerations

The institutional review boards and the Ethics Committee of the University of Kinshasa Faculty of Medicine approved the protocol of the study, which was conducted in compliance with the principles of the Helsinki Declaration II. The aim and the procedures of the study were explained to the participants and each participant (or a designated literate substitute, when necessary) signed an informed consent form.

## Study setting

We randomly selected four main hospitals in Kinshasa, DRC: the Cliniques Universitaires de Kinshasa, which is a teaching hospital in the southwestern part of the city of Kinshasa, the General Referral Hospital of Kinshasa in central Kinshasa, the General Referral Hospital of Kintambo in the northeastern part of Kinshasa, and the Military Referral Hospital of Camp Kokolo in the western part of Kinshasa.

## Patients and clinical specimens

We included 242 consecutive patients infected with HIV/AIDS. Epidemiologic, clinical, immune and laboratory data were collected from all participants.

## Epidemiology data

Each participant was interviewed using a standardized, structured questionnaire to collect data regarding the prevalence of intestinal parasites (pooled, nonopportunistic, and opportunistic) and sociodemographic, environmental, and risk factors. The sociodemographic data were defined by gender, age, and socioeconomic status (low vs high by composite variables for education, occupation, and income). The environment was defined by the residence: rural, semirural, and urban areas. The risk factors for microsporidiosis and cryptosporidiosis included person–person infection by persons with chronic diarrhea via public toilets, ART, homosexual activities, through water storage, water pipes, swimming, and surface water; consumption of high-risk foods (fresh raw fruits and vegetables and food from street vendors); and zoonotic transmission routes (presence of specific domestic farm and pet animals in the home, such as dogs, pigs, goats, and chickens, and contact with droppings from these animals). We collected the risk-factor data prior to the interview and collection of the laboratory data.

## Clinical spectrum

Medical students collected data regarding symptoms of the presence of HIV clinical manifestations, such as Kaposi sarcoma, herpes zoster, candidiasis, asthenia, and chronic diarrhea. We defined diarrhea as an average of >3 loose or liquid stools within a 24-hour period.

As defined by the World Health Organization,<sup>23</sup> the four clinical stages of HIV/AIDS comprise stages 1–2 for pre-AIDS and stages 3–4 for AIDS.

## Parasitological diagnosis

### Samples and staining

Stool samples (one for each patient) were studied using optical microscopy (direct examination and specific stainings).

Stools were collected in a vial containing phosphate-buffered saline and stored in a refrigerator at 4°C before handling. Microscopic analysis of feces and the direct concentration method according to the protocol of Ritchie<sup>24</sup> were performed in the laboratory of the Department of Parasitology, University Clinics of Kinshasa. Ziehl–Neelsen staining (modified Henricksen–Pohlenz) was used for detecting *Cryptosporidium* sp.<sup>25</sup>

## Species identification

### Indirect immunofluorescence

We used indirect immunofluorescence with monoclonal antibodies, which is an effective method for the diagnosing of microsporidiosis, for identification of *E. bieneusi* and *Encephalitozoon intestinalis*.<sup>26</sup>

## Molecular methods

### Extraction of DNA products

DNA extraction was performed using a QIAmp DNA kit (QIAGEN, Courtaboeuf, France) in 50 µL of phosphate-buffered saline and divided into an aliquot (1 Eppendorf® tube) and then stored at –20°C prior to analysis, according to the manufacturer's instructions.

### Real-time PCR

We carried out a real-time PCR for samples at the Saint Louis Hospital Parasitology mycology service in Paris, France, using a TaqMan® 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) for identification of the two species (*E. bieneusi* and *E. encephalitozoon*).<sup>27</sup> The real-time PCR also served for identification of the genus *Cryptosporidium* for targeting a fragment of 145 bp 18 s rRNA gene, which is specific for three species (*Cryptosporidium parvum*, *Cryptosporidium hominis*, and *Cryptosporidium meleagridis*), according to the protocol of Fontaine et al.<sup>28,29</sup> To make the PCR more specific and sensitive, two primers and one probe were used for each species.

### *Cryptosporidium* spp. by nested PCR and PCR-RFLP

The nested PCR used two primer pairs amplifying a 179–271 bp 18 s rRNA gene fragment.<sup>30</sup> Restriction assays were performed in a 30 µL volume with two units of restriction enzyme and 5 µL of PCR product per reaction. Mixes were incubated in a heating block. Digestion products were visualized under ultraviolet light after 2% agarose gel electrophoresis and SYBR Green I staining. The enzymes used were TaqI (Roche-Boehringer, Mannheim, Germany) and AseI (New England Biolabs, Beverly, MA).<sup>30</sup>

## PCR amplification of *E. bieneusi*

The standard method for determining the genotype of *E. bieneusi* was based on the DNA sequence of the internal transcribed spacer (ITS) region of the rRNA gene. Genotypic analysis of *E. bieneusi* isolates was performed by sequencing the ITS portion of the rRNA gene. On the basis of these results, a phylogenetic interpretation regarding the sources and routes of transmission of the different groups of *E. bieneusi* genotypes was proposed.<sup>31</sup>

## Nucleotide sequencing of the ITS region of the *E. bieneusi* rRNA gene

*E. bieneusi* 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 19 samples, using the primers MSP-3 [5'-GGA ATT CAC ACC GCC CGT C (A/G)(C/T)TAT-3'] and MSP4B (5'-CCA AGC TTA TGC TTA AGT CCA GGG AG-3'), as described previously.<sup>32</sup> PCR products were purified using a Concert Rapid PCR kit (Gibco-BRL, Gel Company Inc, San Francisco, CA) and sequenced in both directions using an ABI Big Dye Terminator kit (v 1.1) and an ABI 3100 automated sequencer (Applied Biosystems). The sequence accuracy was controlled by sequencing two PCR products from the same sample.

## Phylogenetic analysis

The ITS sequences were obtained by comparing with those from previously published records in GenBank®<sup>33</sup> using BLAST analysis (National Center for Biotechnology Information, Bethesda, MD). Multiple alignments of our new ITS nucleotide sequences and approximately 250 sequences were retrieved from GenBank (available by January 2012).

## Nucleotide sequence accession numbers

Nucleotide sequences of the ITS region of the rRNA gene of one isolate for each genotype from DRC were deposited in the GenBank database. The accession numbers for the DRC isolates are JQ437573, JQ437574, and JQ437575 for the genotypes KIN1, KIN2, and KIN3, respectively.

## Statistical analysis

Data were analyzed using the proportions (%) for categorical variables and means with standard deviations for continuous variables. Differences were compared using the chi-square test for proportions and the Student's *t*-test for continuous variables.

Analysis of variance was used to compare means across more than two groups. Odds ratios (OR) were calculated with 95% confidence intervals (95% CI) using univariate analysis for potential risk factors (associated factors by contingency table) and logistic regression model (multivariate analysis) for independent determinants for opportunistic enteric parasites, after adjusting for confounding factors. A  $P$  value  $< 0.05$  was considered statistically significant. All analyses were performed using Stata<sup>®</sup> software (v 11; StataCorp, College Station, TX) and SPSS software for Windows (v 19; IBM Corp, Armonk, NY).

## Results

### Patient characteristics

There were 242 participants in the study, 87 men (35.9%) and 155 women (64.1%), with a 2:1 women-to-men ratio. The mean age of the participants was  $39.2 \pm 11.8$  years (range, 15–73 years).

### Prevalence of gastrointestinal parasites

Table 1 summarizes the prevalence rates of pathogenic intestinal protozoa (2%), nonpathogenic intestinal protozoa (10.6%), and helminths (2.8%).

### Prevalence of microsporidia and *Cryptosporidium* spp.

The real-time PCR reported prevalence rates of 8.2% ( $n = 20$ ), 7.8% ( $n = 19$ ), 0.4% ( $n = 1$ ), and 5.4% ( $n = 13$ ) for all microsporidia, *E. bienewisi*, *E. intestinalis*, and *Cryptosporidium* spp. among 242 stool specimens, respectively.

### Analysis of *E. bienewisi* and *Cryptosporidium* spp. genotypes

Figure 1 shows isolates of five *E. bienewisi* strains yielded from the PCR product that were considered sufficient for the

nucleotide sequencing of the ITS region of the rRNA gene. We reported the five different genotypes for *E. bienewisi* (two previously called NIA1 and D and three new ones) in Table 2.

PCR-RFLP products had 0.4% ( $n = 1$ ) *C. parvum* and 1.6% ( $n = 4$ ) had *C. hominis* among all 242 stool samples.

### Clinical and immune profiles

Of the 242 participants, 88 (36.4%) had asthenia, 83 (34.3%) had diarrhea, and 75 (31%) had CD4 counts  $< 100$  cell/mm<sup>3</sup>.

There was a significant association between asthenia (53.6%,  $n = 15$ ;  $P = 0.04$ ), chronic diarrhea (57.1%,  $n = 16$ ;  $P = 0.007$ ), Kaposi sarcoma (75%,  $n = 21$ ;  $P < 0.0001$ ), herpes zoster (64.3%  $n = 18$ ;  $P < 0.001$ ), CD4 count  $< 100$  cells/mm<sup>3</sup> (53.6%,  $n = 15$ ;  $P = 0.006$ ), and the presence of opportunistic intestinal parasites (microsporidia + *Cryptosporidium*) in 28 patients. In addition, there was a significant relationship between digestive candidiasis, World Health Organization HIV stages, and the presence of microsporidia, *E. bienewisi*, and *Cryptosporidium* (results not shown).

Twelve (63.2%) of the 19 *E. bienewisi* cases had diarrhea; seven of the 13 *Cryptosporidium* spp. cases had chronic diarrhea; three of the *C. hominis* cases had chronic diarrhea, one *C. parvum* case had diarrhea, and one *C. hominis* case did not have chronic diarrhea among the five different species identified by PCR-RFLP. The patients with the three new genotypes designated by GenBank as KIN1, KIN2, and KIN3 suffered from chronic diarrhea. The patients with these new genotypes had CD4 levels of  $< 200$  cells/mm<sup>3</sup>.

### Epidemiological data

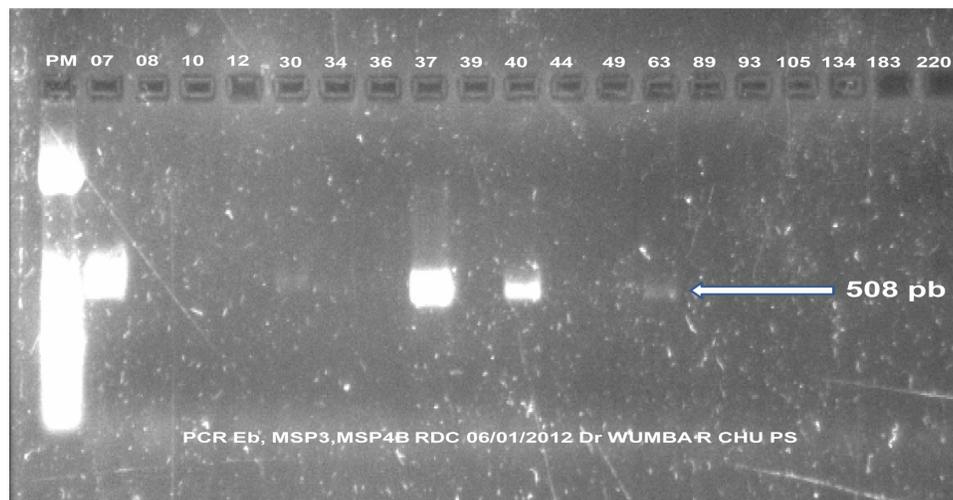
Homosexual activity was not reported by the study population. There was a significant and negative association between the presence of opportunistic intestinal parasites ( $n = 28$ ) and urbanization in the Kinshasa region (Figure 2).

The burden of opportunistic intestinal parasites decreased ( $P$  for trend = 0.023) with the highest, the intermediate, and the lowest rates in the rural, semiurban, and urban areas, respectively, in Kinshasa residences.

The other univariate risk factors for the presence of opportunistic intestinal parasites were a CD4 count  $< 100$  cells/mm<sup>3</sup>, no ART, exposure to farm pigs, drinking and recreational surface water, and public toilets (Table 3). The decreasing but significant power of the association (OR) was for exposure to pigs, ART, public toilets, surface water, and a CD4 count  $< 100$  cells/mm<sup>3</sup>. Three new genotypes KIN1, KIN2, and KIN3 were found from 5 species of *E. bienewisi*. From the four *Cryptosporidium* species, one was identified as *C. parvum* and three as *C. hominis*. The sociodemographic

**Table 1** Prevalence of other intestinal nonopportunistic parasites in human immunodeficiency virus/acquired immunodeficiency syndrome patients

Parasites	Total	%
<b>Pathogenic intestinal protozoa</b>		
<i>Entamoeba histolytica</i>	3	1.2
<i>Giardia lamblia</i>	2	0.8
<b>Nonpathogenic intestinal protozoa</b>		
<i>Entamoeba coli</i>	20	8.2
<i>Endolimax nana</i>	4	1.6
<i>Iodamoeba butschlii</i>	1	0.8
<b>Intestinal helminths</b>		
<i>Ascaris lumbricoides</i>	5	2
<i>Trichuris trichiura</i>	1	0.8



**Figure 1** Positive results from polymerase chain reaction (PCR)-nucleotide sequencing of the internal transcribed spacer region of the *Enterocytozoon bieneusi* (Eb) rRNA gene, samples 7, 30, 37, 40, and 63.

data and food exposure were not associated with the presence of opportunistic intestinal parasites (results not shown).

After adjusting for Kinshasa residence, public toilets, and exposure to farm pigs in the multivariate analysis, only a CD4 count < 100 cells/mm<sup>3</sup>, no ART, and exposure to surface water were identified as significant and independent determinants for the presence of opportunistic intestinal parasites (Table 4).

## Discussion

The present study reports the epidemiological, clinical, immune, and molecular profiles of opportunistic and non-opportunistic intestinal parasites in HIV-infected individuals in the DRC, Central Africa. Microsporidia and *Cryptosporidium* spp. were the important etiologic agents identified using both older and new laboratory methods for the diagnosis of parasitic diseases.<sup>34</sup>

In our African setting, the methods for the diagnosis of intestinal parasites in HIV/AIDS patients with chronic diarrhea have stagnated since 1981 until publication of our studies.<sup>21,22</sup>

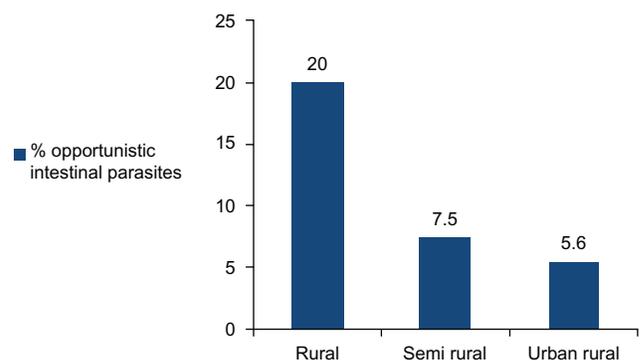
**Table 2** Different genotypes for *Enterocytozoon bieneusi* (Eb) identified by sequencing

Number	5	PCR RT	PCR MSP3/ MSP4B	Genotypes
07		Eb	Positive	KIN1 JQ437573 (new)
30		Eb	Positive	KIN2 JQ437574 (new)
37		Eb	Positive	KIN3 JQ437575 (new)
40		Eb	Positive	NIA1 (Esporn et al <sup>43</sup> ; Wumba et al <sup>22</sup> )
63		Eb	Positive	D (Breton et al <sup>31</sup> )

**Abbreviation:** PCR, polymerase chain reaction.

For many years, microscopy was the only method available for feces analysis. Further, indirect immunofluorescence with monoclonal antibodies was the only tool in the diagnosis of microsporidia before PCR began to be used in the DRC. Molecular-based approaches used in this study comprised extraction of DNA products: real-time PCR and nested PCR-RFLP. They were developed and applied for detecting microsporidia (*E. bieneusi* and *E. intestinalis*) and *Cryptosporidium* spp. in stool specimens from HIV-infected patients.

The burden of pathogenic intestinal protozoa (*Entamoeba histolytica/dispar* and *Giardia lamblia*), nonpathogenic intestinal protozoa (*Entamoeba coli*, *Endolimax nana*, and *Iodamoeba butschli*), intestinal helminths (*Ascaris lumbricoides* and *Trichuris trichiura*), and opportunistic intestinal parasites (microsporidia, *Cryptosporidium* spp., and *Isospora belli*) in this study was lower than the prevalence of enteric parasites among HIV-infected individuals worldwide.<sup>1-22</sup> The low prevalence of HIV/AIDS in the DRC<sup>35</sup> may explain the



**Figure 2** Relationship between the presence of opportunistic intestinal parasites and residence in the Kinshasa region.

**Table 3** Risk factors for *Enterocytozoon bieneusi* (Eb)/*Cryptosporidium* spp. (Csp) infections among human immunodeficiency virus/acquired immunodeficiency syndrome patients (univariate analysis)

Variables	Number tested	Number with Eb-Csp	OR	95% CI	P
CD4 count (cells/mm <sup>3</sup> ) < 100	75	15 (53.6)	2.49	66.05–166.81	0.006
No ART	95	20 (21.1)	4.70	1.90–11.40	0.0002
Domestic animals					
Farm pigs	8	5 (10.7)	4.99	1.10–22.61	0.02
Sanitary conditions					
Surface water	36	8 (28.6)	2.66	1.06–6.68	0.03
Public toilets	98	19 (67.9)	3.53	1.49–8.33	0.002

**Note:** Values in parenthesis indicate the percentage.

**Abbreviations:** ART, antiretroviral therapy; CI, confidence interval; OR, odds ratio.

present rates of microsporidia, *E. bieneusi*, *E. intestinalis*, and *Cryptosporidium* spp.

The very high prevalence of HIV/AIDS in countries of Southern Africa<sup>36,37</sup> and homosexual activity in Western developed countries<sup>38–40</sup> are reflected in the high prevalence (average 52%) of *Cryptosporidium* and microsporidia among HIV patients from Zimbabwe,<sup>3</sup> South Africa,<sup>15</sup> and Italy.<sup>16</sup>

The expansion of the use of PCR techniques with HIV/AIDS patients in this study led to the identification of genotypes in microsporidia and *Cryptosporidium* spp. Microsporidia are single-celled, obligate intracellular parasites that are now reclassified from fungi.<sup>41,42</sup> *E. bieneusi* was the most frequently used PCR-based method in this study. *E. bieneusi* is also the most common species reported to infect humans.<sup>42</sup>

In this study, the genotypes NIA1 and D of *E. bieneusi* were present in two patients. Espersen et al in 2007<sup>43</sup> and Wumba et al in 2010<sup>22</sup> reported the NIA1 genotype and Breton et al<sup>31</sup> reported the D genotype in 2007. Our study is the first to report three new genotypes of *E. bieneusi*, referred to as KIN1, KIN2, and KIN3. The tropical Kinshasa environment-based discoveries of the new genotypes were specific for chronic diarrhea, low CD4 levels, and no ART. The present positive association between opportunistic intestinal infection, including the highest proportion of *E. bieneusi* and diarrhea, may exhibit adaptation to individual cell types,<sup>38,44</sup> tropism of the epithelium of the small bowel and, the cryptic cells.<sup>44–46</sup> The significant association between

microsporidia and *Cryptosporidium* spp. and chronic diarrhea in HIV patients has now been reported in the literature.<sup>5,15</sup>

Further, the present study identified the potential (univariate) and independent risk factors of opportunistic intestinal parasites (microsporidia and *Cryptosporidium* spp.), confirming several studies in the literature.<sup>1–5,15–19,38</sup> The environmental persistence for the distribution of microsporidia and the geographic distribution of opportunistic intestinal parasites<sup>42</sup> are consistent with the present significant association between rural residences in the Kinshasa region of the DRC and the presence of opportunistic intestinal parasites. Exposure to surfaces (related to poverty in rural residences) was positively associated with opportunistic intestinal parasites.

This finding favors the detection of microsporidia spores in surface water.<sup>21,38,46–48</sup> Consistent with the study by Likatavicius and Van de Laar in the literature,<sup>38</sup> the present study found that exposure to farm pigs carries a potential risk of infection by opportunistic intestinal parasites. However, there is no formal proof of animal–human transmission of microsporidiosis,<sup>38</sup> as exposure to pets and other animals was not identified in multivariate analysis.

This study cannot rule out exposure to public toilets as a potential risk factor for opportunistic intestinal parasites.<sup>38</sup> There is an urgent need to eliminate microsporidia spores in the environment via fecal matter.<sup>21,38,46,49</sup> Absence of ART and immune dysfunction in the present HIV-infected patients were a hallmark of microsporidia and *Cryptosporidium* spp infection.<sup>21,38,49</sup> Conforming to

**Table 4** Independent determinants of opportunistic enteric parasites (multivariate analysis)

Independent variables	Nonstandardized		Wald chi-squares	Exp(B) OR (95% CI)	P
	B	SESE			
CD4, < 100 vs ≥ 100 cells/mm <sup>3</sup>	1.519	0.500	9.211	4.60 (1.70–12.20)	0.002
ART, no vs yes	1.613	0.490	10.831	5.00 (1.90–13.20)	<0.001
Surface water, yes vs no	1.075	0.543	3.918	2.90 (1.01–8.40)	0.048
Constant	–1.979	0.491	16.251		<0.0001

**Abbreviations:** ART, antiretroviral therapy; CI, confidence interval; OR, odds ratio.

results of different studies,<sup>21,50–55</sup> an advanced stage of immunodepression for a CD4 count of <100 cells/mm<sup>3</sup> in the present study increased fivefold the risk of opportunistic intestinal parasites.

These findings will have implications for prevention, diagnosis methods, clinical spectra, and treatment among individuals with HIV.<sup>20,38,42,56,57</sup> The prevention of microsporidia and *Cryptosporidium* spp. infection via individual and population-based prophylaxis should be established in terms of hygiene. Strict rules should be respected to avoid oro-fecal transmission, including hand washing, washing fresh vegetables, drinking boiled water, and limiting contact with animals susceptible to transmission of microsporidia and *Cryptosporidium*.<sup>38,47</sup> The Government of the DRC is encouraged to treat drinking water sources with chloride or ozone.<sup>47</sup> The diagnosis performance of opportunistic intestinal parasites might be established on the basis of the parasitological studies of feces.

The genus, genotypes, subgenotypes, and species could be identified by molecular methods in the DRC.

In the treatment of opportunistic intestinal parasites, the following medications are efficient: albendazole, benzimidazole derivatives, fumagillin, nitazoxanide, TNP-470, ovalicin, fluoroquinolones, antimetabolites, polyamine analogs, and interferon gamma.<sup>38</sup>

ART should be available for all Congolese HIV patients, as it reduces the viral load and improves CD4 counts, as well as reducing prevalence, morbidity, and mortality related to HIV infection.<sup>25,38,56</sup>

This cross-sectional study may be limited to some degree, as the progression of immune status is not precise and, despite the use of PCR methods only for single stool specimens, the prevalence rates of *E. bienersi* may be underestimated.

## Conclusion

The prevalence of *E. bienersi* and *Cryptosporidium* spp. is increasing in Kinshasa, DRC, and the findings of this study recommend epidemiological surveillance and prevention by hygiene. It is necessary to emphasize sensitive PCR methods and to treat microsporidia and *Cryptosporidium* spp.

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