Am. J. Trop. Med. Hyg., 97(3), 2017, pp. 777–781
doi:10.4269/ajtmh.16-0900

Intestinal Parasitic Infections in Human Immunodeficiency Virus-Infected and Noninfected Persons in a High Human Immunodeficiency Virus Prevalence Region of Cameroon

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Abstract. The problem of intestinal parasitic infection in human immunodeficiency virus (HIV)–infected people requires careful consideration in the developing world where poor nutrition is associated with poor hygiene and several coinfecting diseases. Studies have addressed this issue in Cameroon, especially in the low HIV prevalence area. The current study was conducted to determine the prevalence of intestinal parasitosis in people living with HIV (PLHIV) in Adamaoua and to identify associated risk factors. Stool and blood specimens from study participants were screened for intestinal parasites and anti-HIV antibodies, respectively. Of 235 participants, 68 (28.9%) were HIV positive, 38 of them on antiretroviral treatment (ART). The overall prevalence of intestinal parasites was 32.3%. Of 68 PLHIV, 32.3% (22/68) were infected with intestinal parasites, compared with 32.3% (54/167) of the HIV-negative patients. Univariate analysis showed no difference between the prevalence of intestinal parasites among PLHIV and HIV-negative patients ($P = 0.69$). ART was not associated with the prevalence of intestinal parasites. Multivariate analysis showed that the quality of water and the personal hygiene were the major risk factors associated to intestinal parasitosis. The level of education was associated with HIV serostatus: the higher the level of education, the lower the risk of being infected with HIV ($P = 0.00$). PLHIV and the general population should be screened routinely for intestinal parasites and treated if infected.

INTRODUCTION

Human immunodeficiency virus (HIV)-associated immunodeficiency often results in the appearance of opportunistic infections. These opportunistic infections may be bacterial, viral, or parasitic and are associated with increased morbidity and mortality.[1,2] Of these infections, copro-parasites are the leading cause of severe, chronic diarrhea.[3] About 60% of the world population is infected with intestinal parasites. They have significant morbidity.[4] The prevalence of these infections is remarkably high in sub-Saharan Africa where HIV is concentrated. In Cameroon, the prevalence of intestinal parasites have been shown to vary by study site and study years: 33% in 2006 in Yaounde,[5] 27.8% in 2012 in Douala,[6] and 14.6% in 2013 in Dschang.[7] Malnutrition prevailing in sub-Saharan Africa promote the spread of parasitic infections.[8] Digestive disorders in individuals with HIV are extremely common and 90% of patients frequently consult for gastrointestinal disorders during the course of the disease.[9] In people living with HIV (PLHIV), diarrhea caused by these parasites are responsible for electrolyte disorders and malabsorption of nutrients and oral drugs. These factors may result in anorexia, weight loss, and a general deterioration of the patient's health.[1] A study carried out in Calabar in Nigeria within a population of PLHIV on antiretroviral treatment (ART) showed a prevalence of 29% of copro-parasites with a prevalence of 12% among HIV negative.[10] Similar studies have been done in Cameroon in the Center region, in the West, and in the Littoral. No such study has been performed in the northern part of Cameroon. The goals for this study were to determine the prevalence rates for intestinal parasites in PLHIV in the Adamaoua Region of Cameroon, and associated risk factors.

MATERIALS AND METHODS

Study area and population. The study was conducted in the Regional Hospital of Adamaoua in Ngaoundere and other health facilities, where during the period from 2004 and 2011, HIV has decreased from 6.9% to 5.1%, but still high compared with the national prevalence (4.3%).[11] Adamaoua is one of the 10 regions of Cameroon. It borders Nigeria in the west and the Central African Republic in the east. Its capital is Ngaoundere. This mountainous area marks the border between the forest in the south and the savannas of the north. The land is poor and sparsely populated. The main economic activity is cattle breeding (zebus).

Islam is the main religion. The Fulani form the main ethnic group of this region, but there are minorities like Tikar and Gbaya, as well as other smaller ethnic groups. The majority of participants recruited in this study live in Ngaoundere, an urban setting, some in peri-urban setting (Meiganga, Ngaoundal, and Tignere) and the rest live in rural setting (Dang, Bawa, beka, and Tamounagui).

Process of the survey. Patients consulting at the hospitals were requested to participate in the study. After obtaining informed consent, a questionnaire was administered to each participant by the nurse who had been specifically trained for this task. At least one stool sample and one blood sample were collected from each of the participants and used for intestinal parasites and HIV testing, respectively. Only one stool sample was analyzed because individuals often only provided one of the three requested stool samples. Since there was a possibility of HIV misdiagnosis, HIV testing was performed for all our study participants according to the

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Cameroon national HIV testing algorithm. None of the participants were taking antiparasitic medications.

Laboratory procedures. The blood samples of all participants were screened for anti-HIV antibodies using Determine HIV 1/2 HIV rapid test (Alere, Chiba, Japan). Reactive samples were subjected to confirmation using Oraquick rapid test (OraSure Technologies, Thailand).

Stool samples were analyzed by direct microscopy with physiological saline and iodine, and after concentration by the formalin ether method coupled with the Ziehl–Neelsen modified technique for the detection of *Cryptosporidium* sp. and *Isospora belli*.[12] Giemsa staining was also performed on the concentrated parasites for the identification of microsporidies.[13]

Statistical analysis. Data were registered in Microsoft Excel 2016 and analyzed with R 3.2.5 statistical software. χ^2 test (or Fisher's test when necessary) was used to compare the prevalence of intestinal parasites according to HIV status. Multivariate analysis was used to perform and visualize the profile of patients infected with protozoa and helminths. And then, multivariate logistic model was used to evaluate the risk of parasitic infections by HIV infection status, hygienic conditions, and other variables (marital status, age, sex, educational level, etc.).

Ethics statement. The study protocol was approved by the "Cameroon Bioethics Initiative" (CAMBIN) under the registration N° CBI/320/ERCC/CAMBIN. The confidentiality of each participant was respected.

Written informed consent was obtained prior to questionnaire administration and samples collection.

Patients newly diagnosed HIV positive were referred to HIV Treatment Center for counseling, care and management. Those diagnosed with intestinal parasites were offered appropriate medications.

RESULTS

Demographic characteristic of study population. The majority of participants (85.9%) recruited in this study live in Ngaoundere (202/235), 5.5% (13/235) in peri-urban setting (Meiganga, Ngaoundal, and Tignere) and the rest (8.5%; 20/235) live in rural area (Dang, Bawa, beka, and Tamounagui).

A total of 235 people 16 to 90 years of age (mean 31.6 ± 12) were recruited. The number of female participants was higher (158; 67.2%) than that of male participants (77; 32.8%); thus, a sex ratio F/M of 2.1. Complete data were collected from all participants who had provided stool and blood samples as well as answered the questionnaires (see Table 1).

HIV testing. Sixty-eight patients were HIV positive (28.9%) and 167 HIV negative (71.1%). Thirty-eight infected patients were on ART, whereas 30 were treatment naïve (see Table 1).

Parasitic infections. Fresh stool analyses showed prevalence of parasites of 14% (33/235). After concentrating the stools, a prevalence of 25.5% (60/235) was found. This prevalence rose to 32.3% (76/235) after specific histologic stains. No difference was observed in the prevalence of intestinal parasitic infection according to the HIV status. Also, there was no association between density of parasites and HIV status (Table 2).

The following parasites were identified in the study population: *Blastocystis hominis* (18.3%), *Entamoeba histolytica/dispar* (6.4%), *Entamoeba coli* (5.96%), *Endolimax nana* (3.8%), *Crystosporidium parvum* (3%), *Iodamoeba buetschlii* (2.1%), *Trichomonas intestinalis* (1.7%), *Giardia lamblia* (1.3%), *Embadomonas intestinalis* (0.4%), *Cyclospora cayetanensis* (0.4%), *Ascaris lumbricoides* (0.4%), and hookworm (0.4%). More specifically, *B. hominis* was the most frequently identified parasites equally in PLHIV (16.2%) and HIV-negative (19.2%) participants. Six cases of *C. parvum* were identified, three among PLHIV and three among HIV-negative participants. Two participants were infected with microsporidies, one PLHIV, and the other HIV negative. No case of *I. belli* was detected.

Multiparasitism. Multiple parasitism was rare in our population. In the HIV-infected group, 47 (69.1%) patients were not infected by intestinal parasites; one parasite was identified in 15 (22%) patients, two parasites in four (5.9%) patients, and three parasites in two (2.9%) patients.

Risk factors associated with intestinal parasite infection and with HIV infection. No association was found between the sex, the matrimonial status, the level of education, the HIV serostatus, and the presence of intestinal parasites. Furthermore, no statistical difference was found between HIV status and the parasites density and presence of multiples parasites as shown in Table 2. The only variables associated with prevalence of intestinal parasites were the quality of water (odds ratio [OR] = 2.58 [1.28–5.22]), the personal hygiene/hand washing (OR = 2.64 [1.37–5.15]), and the cleaning after defecation (OR = 2.60 [1.19–5.86]) as presented in Table 3. No association was found between the ART treatment and the density of the intestinal parasites ($P = 0.51$). On the other hand, HIV status was inversely associated with the educational level ($P < 0.05$); (see Table 4).

DISCUSSION

The primary goal of this study was to determine the frequency of HIV-intestinal parasites coinfection in the Adamaoua and to evaluate the risk factors associated with this coinfection.

The proportion of new HIV infections identified among the participants was 9.3%, which was higher than the 5.1% found during the demographic survey.[11] This may be due to the fact that we recruited the participants in the hospital, whereas the demographic survey recruited the participants from the community. Up to 44.1% of the HIV-positive patients were not yet on treatment, and this is not in agreement with the new 2015 World Health Organization recommendations.[14] The prevalence of intestinal infections in Adamaoua was the same for HIV-positive and HIV-negative patients. Parasites like *Cryptosporium parvum* known as opportunistic parasites were identified in the overall population, but not specific to PLHIV which reflected the overall poor hygiene conditions of the entire population. Even the parasite density was the same in the two groups. This finding was contradictory to what has been found in other studies, where the prevalence of parasitic diseases was higher in PLHIV.[2,5–7]

The 32.3% prevalence of parasitic infections found in the Adamaoua general population was greater than 14.6% in West Region of Cameroon,[7] but less than 57.5% in the Center Region.[15] Note that the techniques used for biological analyzes of the study conducted in the West Region

TABLE 1
Overall population demographic, clinical and laboratory data

Variable	Overall population *N* (%)	HIV-infected patients *N* (%)	Non-HIV-infected patients *N* (%)	*P* value between HIV-infected and noninfected patients	Infected with protozoans/ helminths *N* (%)	HIV patients infected by parasites *N* (%)	Non-HIV patients infected by parasites *N* (%)	*P* value between HIV and non-HIV infected by parasites
N (%)	235	68 (28.94)	167 (71.06)	×	76 (32.34)	22 (32.35)	54 (32.34)	0.99
Sex								
Male	77 (32.77)	21 (30.88)	56 (33.53)	0.69	26 (34.21)	6 (27.27)	20 (37.04)	0.41
Female	158 (67.23)	47 (69.12)	111 (66.47)	0.69	50 (65.79)	16 (72.73)	34 (62.96)	0.41
Average age ± SD (year)	31.61 ± 11.96	32.62 ± 8.90	31.2 ± 13	0.017	32.66 ± 12.21	31.86 ± 7.56	32.98 ± 13.71	0.41
Marital status								
Single	74 (31.49)	14 (20.59)	60 (35.93)	0.02	19 (25.00)	4 (18.18)	15 (27.78)	0.38
Married	133 (56.6)	39 (57.35)	94 (56.29)	0.88	46 (60.53)	13 (59.09)	33 (61.11)	0.87
Cohabitation	4 (1.70)	1 (1.47)	3 (1.80)	1.00	1 (1.32)	0 (0.00)	1 (1.85)	1.00
Widowed	11 (4.68)	6 (8.82)	5 (2.99)	1.00	3 (3.95)	0 (0.00)	3 (5.56)	1.00
Divorcee	13 (5.53)	8 (11.76)	5 (2.99)	1.00	7 (9.21)	5 (22.73)	2 (3.70)	1.00
Education level								
Illiterate	50 (21.28)	24 (35.29)	26 (15.57)	0.00	19 (25.00)	8 (36.36)	11 (20.37)	0.14
Primary	57 (24.26)	24 (35.29)	33 (19.76)	0.01	17 (22.37)	6 (27.27)	11 (20.37)	1.0
Secondary	86 (36.6)	18 (26.47)	68 (40.72)	0.04	25 (32.89)	7 (31.82)	18 (33.33)	0.90
Postsecondary	42 (17.87)	2 (2.94)	40 (23.95)	0.00	15 (19.74)	1 (4.55)	14 (25.93)	1.00
Occupation								
Student	44 (18.72)	3 (4.41)	41 (24.55)	0.00	12 (15.79)	1 (4.55)	11 (20.37)	1.00
Farming	9 (3.83)	7 (10.29)	2 (1.20)	0.00	1 (1.32)	0 (0.00)	1 (1.85)	1.00
Housewife	83 (35.32)	35 (51.47)	48 (28.74)	0.00	28 (36.84)	11 (50.00)	17 (31.48)	0.13
Official	21 (8.94)	1 (1.47)	20 (11.98)	0.01	2 (2.63)	0 (0.00)	2 (3.70)	1.00
Others	78 (33.19)	22 (32.35)	56 (33.53)	0.86	33 (43.42)	10 (45.45)	23 (42.59)	0.82
Personal hygiene wash of hands								
Always	167 (71.06)	50 (73.53)	117 (70.06)	0.59	43 (56.58)	12 (54.55)	31 (57.41)	0.82
Sometimes	67 (28.51)	18 (26.47)	49 (29.34)	0.65	33 (43.42)	10 (45.45)	23 (42.59)	0.82
Not at all	1 (0.43)	0 (0.00)	1 (0.60)	1.00	0 (0.00)	0 (0.00)	0 (0.00)	
Nail cleaning								
Always	118 (50.21)	37 (54.41)	81 (48.50)	0.41	33 (43.42)	10 (45.45)	23 (42.59)	0.82
Sometimes	106 (45.11)	28 (41.18)	78 (46.71)	0.44	41 (53.95)	11 (50.00)	30 (55.56)	0.66
Not at all	11 (4.68)	3 (4.41)	8 (4.79)	1.00	2 (2.63)	1 (4.55)	1 (1.85)	
Cleaning after defecation								
Wash with water	78 (33.19)	25 (36.76)	53 (31.74)	0.46	39 (51.32)	12 (54.55)	27 (50.00)	0.72
Use toilet paper	95 (40.43)	13 (19.12)	82 (49.10)	2.164e-05	22 (28.95)	4 (18.18)	18 (33.33)	0.18
Others methods	62 (26.38)	30 (44.12)	32 (19.16)	8.27e-05	15 (19.74)	6 (27.27)	9 (16.67)	1.00
Source of water consumed								
Borehole	61 (25.96)	14 (20.59)	47 (28.14)	0.23	32 (42.11)	7 (31.82)	25 (46.30)	0.25
Tap	174 (74.04)	54 (79.41)	120 (71.86)	0.23	44 (57.89)	15 (68.18)	29 (53.70)	0.25
ART								
Yes	38 (16.17)	38 (55.88)	0 (0.00)	< 2.2e-16	11 (14.47)	11 (50)	0 (0.00)	1.00
No	197 (83.83)	30 (44.12)	167 (100)	< 2.2e-16	65 (85.53)	11 (50)	54 (100)	1.00

ART = antiretroviral treatment; HIV = human immunodeficiency virus; SD = standard deviation.

were the same as those used in Ngaoundere. The only difference was that in the West, concentrated stools were not analyzed microscopically before the modified Ziehl–Neelsen staining. A prevalence of 12.3% was obtained in fresh wet mount microscopy, 23.5% after concentration, and 32.3% after concentration and staining. In the Center Region, in addition to KATO concentration technique, the same techniques of biological analysis as used in Adamawa were performed. Gold concentration formalin-ether takes into account the cysts and oocysts but not helminth eggs as the KATO technique.

TABLE 2
Parasites density with respect to HIV status

Density	PLHIV *N* (%)	HIV-negative patients *N* (%)
Nul	46 (67.65)	114 (68.26)
Light	2 (2.94)	11 (6.58)
Moderate	11 (16.18)	29 (17.37)
Heavy	9 (13.23)	13 (7.78)
Total	68	167

HIV = human immunodeficiency virus; PLHIV = people living with HIV. $P = 0.43$.

Contrary to the results found by Nkenfou and others,[7] no association ($\chi^2 = 0.00$) was found between parasitic infection and HIV status; 31.9% of parasitic infections in PLHIV compared with 32.5% in HIV-seronegative participants. However, a strong link was found between parasitic status and personal hygiene/hand washing ($\chi^2 = 17.57$; OR = 3.73 with 7.06; 1.97 at 95% confidence interval). Also, subjects with HIV have poor hygiene practice compared with HIV-negative subjects (19.1% of PLHIV against 49.1% of HIV-negative patients use toilet tissue after defecation; Table 1). Service providers at the treatment centers should educate PLHIV on hygienic living conditions. This is opposite to what was found in the West Region. Care providers should strive to educate PLHIV to adhere to ART treatment and to have a healthy lifestyle to minimize the risk of contracting opportunistic diseases.

Similarly, no association between intestinal parasitosis/ parasite density and ART treatment was found: $\chi^2 = 0.56$, $P = 0.51$; 35.5% of patients ART naive had a parasite infection and 28.9% of subjects on ART had an intestinal infection. The same results were obtained by Inyang-Etoh and

TABLE 3
Association between personal hygiene and parasitic infection

	Presence of parasites	Absence of parasites	*P* value
Hands washing			0.00
Always	43 (56.58)	124 (77.99)	
Sometimes	33 (43.42)	34 (21.38)	
Not at all	0 (0.00)	1 (0.63)	
Nail cleaning			0.14
Always	33 (43.42)	85 (53.46)	
Sometimes	41 (53.95)	65 (40.88)	
Not at all	2 (2.63)	9 (5.66)	
Cleaning after defecation			0.00
Washing with water	39 (51.32)	39 (24.53)	
Use of toilet paper	22 (28.95)	73 (45.91)	
Others methods	15 (19.74)	47 (29.56)	
Source of drinking water			9.471e-05
Borehole	32 (42.11)	29 (18.24)	
Tap	44 (57.89)	130 (81.76)	

HIV = human immunodeficiency virus.

others in Calabar, Nigeria.[10] Using the same biological techniques, namely direct microscopy, concentration formalin-ether and modified Ziehl–Neelsen coloring, they got 29% (116/400) of parasitic infections in HIV patients taking ART. But this result is opposite to those found by Nkenfou and others in the West Region of Cameroon, and Missaye and others in Ethiopia.[16] This could be explained by the overall poor hygienic conditions of the study population.

Dwellers of Adamaoua Region harbored mostly protozoa among which the most frequent one was *B. hominis* (18.3%). Infections by helminths were rarely detected (0.4% for *A. lumbricoides*). The proposed explanation for this is that inhabitants of Adamaoua were mainly animal breeders who do not eat raw meat and have less contact with soil compared with farmers. Monoinfection by protozoa was the rule compared with other studies where polyparasitism was more frequent.[17–21] The speculation for this observation may be linked to the main limitation of our study, the analysis of only one stool sample per patient with thus limited sensitivity for the detection of other parasites. The prevalence of monoinfection may also be due to constant competition in the microbiota, leading a particular organism to be dominant.

The clinical spectrum caused by intestinal parasites especially protozoa in HIV-positive patients ranged from asymptomatic infection to severe infection resulting in chronic diarrhea, dehydration, and malabsorption.[22] These could reduce the effectiveness of oral ART in case of intestinal parasites due to drug malabsorption. As factors associated to intestinal parasitic infections, three variables were identified: hand hygiene, cleaning after defecation, and water quality.

After Giemsa coloration, no microsporidia was found. In 2010, Anane and others in Tunisia have obtained an overall prevalence of 2.4% (14/572) microscoporidies. In PLHIV, the prevalence was 3.6% (10/279) and 1.4% (4/293) in seronegative patients.[23] The result obtained in our study could be explained by the fact that all participants had no evidence of disease worsening. Microsporidia are generally present in patients whose CD4 counts are low.[24]

A major limitation in our study was the fact that only one stool sample was analyzed per patient. This could affect the data as the sensitivity of the microscopy is limited in case of light infection. This can then underestimate the prevalence, the intensity of infection, as well as the degree of multiparasitism.

TABLE 4
Association between educational level and HIV status

	HIV-infected patients	HIV-noninfected patients	*P* value
Education level			
Illiterate	24 (35.29)	26 (15.57)	
Primary school	24 (35.29)	33 (19.76)	3.20158e-06
Secondary school	18 (26.47)	68 (40.72)	
Postsecondary school	2 (2.94)	40 (23.95)	
Total	68	167	

HIV = human immunodeficiency virus.

CONCLUSIONS

PLHIV have a depressed immune system and are susceptible to infections called opportunistic infections that can be bacterial, viral, fungal, or parasitic. In this work, we evaluated the prevalence of parasitic infections in people with or without HIV. The results showed that PLHIV and HIV-negative patients had similar prevalence rates for intestinal parasitic infections. The prevalence and parasite density were similar both in patients on ART and ART-naïve patients. As part of the fight against HIV, the inverse correlation between HIV status and level of education suggests the importance of developing a strategy for improving educational opportunities in the Adamaoua Region. As protective factors against intestinal parasitic infections, personal hygiene should be promoted and safe water made available to the populations.

Received November 14, 2016. Accepted for publication March 14, 2017.

Published online June 26, 2017.

Acknowledgments: We thank the study participants for their collaboration.

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