

# Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria

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This study was carried out to determine the presence of intestinal parasites and their correlation with CD4<sup>+</sup> T-cell counts and demographics among human immunodeficiency virus (HIV)-positive patients in Benin City, Nigeria. Stool specimens from 2,000 HIV-positive patients and 500 controls (HIV-negative individuals) were examined for ova, cysts, or parasites, using standard procedures. In addition, patient's blood samples were analyzed for CD4 counts by flow cytometry. An overall prevalence rate of 15.3% was observed among HIV-positive patients while 6.2% was noted among non-HIV subjects. HIV status was a significant ( $P < 0.0001$ ) risk factor for acquiring intestinal parasitic infections. Male gender, CD4 count  $< 200$  cell/ $\mu$ l, and diarrhea were significantly associated with an increased prevalence of intestinal parasitic infections among HIV-positive patients. The level of education, occupation, and source of water among HIV patients significantly ( $P < 0.0001$ ) affected the prevalence of intestinal parasitic infections. *Ascaris lumbricoides* was the most predominant parasite in both HIV-positive patients and controls. A CD4 count  $< 200$  cells/ $\mu$ l was significantly associated with only *Isospora belli* and *Cryptosporidium* infections. The presence of pathogenic intestinal parasites such as *A. lumbricoides*, hookworm, *Giardia intestinalis*, *Entamoeba histolytica*, *Trichuris trichiura*, and *Taenia* species among HIV-infected persons should not be neglected. *Cryptosporidium* species and *I. belli* were the opportunistic parasites observed in this study. Routine screening for intestinal parasites in HIV-positive patients is advocated.

Keywords: *intestinal parasites; HIV; CD4 count; Demographics; Benin City*

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Parasitic infections are among the most widespread of all chronic human infections worldwide. The rate of infection is remarkably high in sub-Saharan Africa, where the majority of human immunodeficiency virus (HIV) and AIDS cases are concentrated (1, 2). Individuals with HIV/AIDS are threatened by a great number of diseases including those caused by different kinds of biological agents. The progressive decline and ultimate collapse of immune system functions, which are characteristic for AIDS, usually result in morbidity and ultimately death due to opportunistic bacterial, viral, and parasitic infections (3). Helminths and HIV infection have major effects on the host immune response and co-infection is widespread (4). With the progressive development of AIDS, especially once CD4<sup>+</sup> T-lymphocyte counts have fallen below 200 cells/ $\mu$ l, patients often become co-infected by bacteria, parasites, or viruses (5).

Such co-infections, generally, are the proximate cause of death of AIDS patients (6–8). Several parasites have been implicated as major contributors to morbidity in HIV-infected persons living in developing countries, and the parasites frequently encountered include: *Cryptosporidium* spp., *Isospora belli*, Microsporidia spp., *Entamoeba histolytica*, *Giardia intestinalis*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, and hookworm, which are associated with diarrhea and iron deficiency anemia (9). Parasitic infections in HIV-infected patients are common in many regions and populations across Nigeria and represent a lasting public health challenge. It has long been recognized that interactions between HIV and other infective agents, including parasites, influence the health status of people living with HIV/AIDS. There are a number of studies on parasites among HIV/AIDS patients in Nigeria, but with

conflicting prevalence. Some authors reported a low prevalence of 28.4% in Abeokuta, Nigeria (10), while others observed a high prevalence of 79.3% in Osun state, Nigeria (11). There is a paucity of information on the prevalence of parasites among HIV-positive patients in Benin City. Against this background, this study focuses on these intestinal parasitic infections in relation to clinical manifestations, demographics, and immune status.

## Materials and methods

### Study population

The study was carried out between August 2007 and August 2009 at the University of Benin Teaching Hospital, Benin City – a teaching hospital with a referral status and center for HIV/AIDS management under the President's Emergency Plan for AIDS Relief (PEPFAR). A total of 2,500 subjects consisting of 2,000 (668 males and 1,332 females) HIV-positive patients attending HIV clinics and 500 (209 males and 291 females) apparently healthy HIV-negative individuals, who were contacted through HIV outreach programs in their homes and offices and served as controls, were included in this study. The age of the study subjects ranged from 21 to 70 years (mean  $\pm$  standard deviation = 37.06  $\pm$  9.19 years). Verbal informed consent was obtained from all subjects prior to specimen collection. The study was approved by the Ethics Committee of the University of Benin Teaching Hospital, Benin City, Nigeria.

### Socio-demographic data

A pre-designed structural questionnaire was utilized to collect the socio-demographic characteristics of the subjects.

### Sample selection and collection

Blood and stool specimens were collected from each subject. The blood specimens were placed in ethylene diamine tetraacetic acid containers and the stool specimens were collected in clean wide-mouthed containers.

Stool specimens were examined microscopically for ova, cysts, or parasites, using saline and iodine mounts on grease-free slides. Following this, each fresh stool sample was preserved in 10% formol saline. The fecal sample preserved in 10% formol saline was concentrated and used to detect oocysts of *Cryptosporidium* species, *I. belli*, and *Cyclospora cayetanensis* using the modified Ziehl-Neelsen staining technique described by Akinbo et al. (12). Giemsa solution stain was used in detecting the spores of *Microsporidium* species using an earlier described method (13).

Blood samples were analyzed for CD4<sup>+</sup> T lymphocyte cell estimation using flow cytometry (Partec, GmbH, Germany). Briefly, 20  $\mu$ l of CD4 PE antibody was placed

into a Partec test tube and 20  $\mu$ l of well-mixed whole EDTA blood was added, mixed gently and incubated in the dark for 15 min at room temperature. The mixture was agitated during incubation every 5 mins. Eight hundred microliters of CD4 buffer was added to the mixture of antibody and sample and mixed gently. This was then plugged to the counter for counting.

### Statistical analysis of data

The data were analyzed using the Chi square ( $\chi^2$ ) test and odds ratio (OR) analysis using the statistical software INSTAT<sup>®</sup>.

## Results

The prevalence of intestinal parasitic infection among HIV-infected patients and non-HIV subjects was 15.3 and 6.2%, respectively. HIV status was a significant ( $P < 0.0001$ ) risk factor for acquiring an intestinal parasitic infection (HIV-positive vs HIV-negative: 15.3 vs 6.2% OR = 2.722, 95% CI = 1.855, 3.995). In a similar vein, the prevalence of a parasitic infection among HIV-infected patients in relation to gender was 18.3 and 13.7% (male and female, respectively) ( $P = 0.0096$ ), while 4.8 and 7.2% was observed among non-HIV subjects (male and female, respectively) ( $P = 0.3476$ ). The prevalence of an intestinal parasitic infection among HIV-infected patients with a CD4 count  $< 200$  cell/ $\mu$ l and  $\geq 200$  cell/ $\mu$ l was 24.7 and 13.9%, respectively ( $P < 0.0001$ ). Diarrhea was significantly associated with an increased prevalence of intestinal parasitic infections among HIV-positive patients (Table 1).

The effect of demographics on the prevalence of intestinal parasitic infections among HIV-positive patients is shown in Table 2. The level of education among HIV-positive patients significantly ( $P < 0.0001$ ) affected the prevalence of intestinal parasitic infections, and patients with no formal education had the highest prevalence of the infection. Artisans, farmers, and security officers were significantly affected (41, 40, and 37.5%, respectively) while traders (2.9%) were ( $P < 0.0001$ ) least affected with intestinal parasitic infections among HIV-positive patients. HIV-infected patients whose source of water was from a stream/river (54.6%) and the municipal water supply (50%) were significantly affected with these infections ( $P < 0.0001$ ). Defecating in nearby bushes resulted in a significantly increased prevalence of intestinal parasitic infections among HIV-positive patients. The rate of infection among those who had contact with animals ranged from 15.4% (dog) to 25% (cats and goats) while for those who had no contact with animals, the rate was 14.7%.

*A. lumbricoides* was the most predominant parasite, followed by *Cryptosporidium* species and *Gardia intestinalis* was the least prevalent. *G. intestinalis* was recovered only from females (Table 3). Among

**Table 1.** Prevalence and risk factors of intestinal parasitic infections among HIV patients

Characteristics	No. tested	No. with infection	OR	95% CI	P-value
<b>HIV status</b>					
HIV patients	2000	305 (15.3)	2.722	1.855, 3.995	
Non-HIV subjects	500	31 (6.2)	0.367	0.250, 0.539	<0.0001
<b>Gender (HIV patients)</b>					
Male	668	122 (18.3)	1.403	1.092, 1.803	
Female	1332	183 (13.7)	0.713	0.555, 0.916	0.0096
<b>Non-HIV</b>					
Male	209	10 (4.8)	0.646	0.298, 1.403	
Female	291	21 (7.2)	1.548	0.713, 3.360	0.3476
<b>CD4 count (cells/<math>\mu</math>l) of HIV patients</b>					
<200	255	63 (24.71)	2.038	1.487, 2.794	
$\geq$ 200	1745	242 (13.87)	0.491	0.358, 0.673	<0.0001
<b>Clinical manifestation of HIV patients</b>					
Diarrhea	374	82 (21.93)	1.767	1.332, 2.344	<0.0001

HIV-negative subjects, *A. lumbricoides* and hookworm were the only intestinal parasites detected and the ratio was 3:1. Males had more *A. lumbricoides* while females had more hookworm infections (Table 3).

A CD4 count <200 cells/ $\mu$ l was significantly associated with only *I. belli* and *Cryptosporidium* infections ( $P<0.0001$ ) (Table 4).

## Discussion

Parasitic infections remain an important cause of morbidity and mortality in developing countries especially among HIV-infected persons (14). In the present study, HIV infection was a significant risk factor for acquiring an intestinal parasitic infection. This is in agreement with previous findings (15–17). It is important to note that the prevalence (15.3%) observed among HIV-infected patients in this study is within the range of 11.4 and 18.4% reported by some authors in Iran and Ethiopia (18, 19). However, other investigators reported 27.9% in Apulia (Italy), 28.1 and 42.9% in (Abuja and Abeokuta, respectively) Nigeria, 50% in Thailand, 63.9% in Rio de Janeiro (Brazil), 69.2% in Ethiopia, and 84.3% in South Africa (20–25). The difference could be due to sample size as our study presented a larger sample size than that reported by other authors. Gender significantly affected the prevalence of intestinal parasitic infections among HIV-infected patients while it was not a risk factor for acquiring these infections among non-HIV subjects. This finding is inconsistent with Mohammad et al. (18). The reason for this association between gender and intestinal parasites may be adduced to more males being exposed than females based on occupational grounds. The CD4 count of HIV-infected patients with intestinal parasitic

infections was significantly lower than those of HIV-seronegative patients ( $383.57 \pm 136.15$ ,  $1029.84 \pm 117.42$ , respectively). Cellular immunity is the major defense against intestinal parasitic infections (13). Therefore, the reduction in CD4 count by the HIV virus predisposes HIV-infected patients to opportunistic intestinal parasitic infections (22, 26). It is generally accepted that a CD4 count <200 cells/ $\mu$ l predisposes HIV-infected persons to opportunistic infections (27). In this study, a CD4 count <200 cells/ $\mu$ l resulted in a significantly higher prevalence of intestinal parasitic infections ( $P<0.0001$ ).

In HIV infection, diarrhea is a major sign of progression to AIDS, which results from opportunistic infections, and this may explain the findings in this study. We found diarrhea significantly associated with intestinal parasitic infections among HIV-positive patients. Others reported similar findings (28–33).

The occupation of the HIV-infected persons significantly affected the prevalence of intestinal parasitic infections, with artisans having the highest prevalence (47.98%). Artisans are more likely to eat food and drink water from questionable sources as they carry out their work. They are also likely to have a poor educational background and to a large extent, poor hygiene standards. This may be the reason for the high prevalence in this group.

The source of water significantly affected the prevalence of intestinal parasitic infections with HIV-infected patients having streams/rivers and the municipal water supply as their sources of domestic water ( $P<0.0001$ ). Streams/rivers are not very hygienic sources of water for domestic use as a number of activities, such as bathing, defecating, and washing, are known to occur in these

**Table 2.** Effect of demographics on the prevalence of intestinal parasitic infections among HIV patients

Demographics	No. tested	No. infected (%)	P-value
<b>Level of education</b>			
No formal	57	27 (47.37)	
Primary	287	42 (14.63)	
Secondary	1281	174 (13.58)	
Tertiary	334	63 (18.86)	<0.0001
<b>Occupation</b>			
Civil servant	221	41 (18.6)	
Business man/woman	169	30 (17.8)	
Security officer	16	6 (37.5)	
Artisan	427	175 (41.0)	
Traders	1055	31 (2.9)	
Farmers	15	6 (40.0)	
Housewife	62	12 (19.4)	
Student	35	4 (11.4)	<0.0001
<b>Source of water</b>			
Municipal water	34	17 (50.0)	
Borehole	1811	252 (13.9)	
Well/rain	133	34 (25.6)	
Stream/river	22	12 (54.6)	<0.0001
<b>Type of toilet</b>			
Water cistern	1077	121 (11.24)	
Pit latrine	917	181 (19.24)	
Bush	6	3 (50.00)	<0.0001
<b>Animal contact</b>			
Cat	16	4 (25.0)	
Dog	26	4 (15.39)	
Birds	125	22 (17.6)	
Goat	44	11 (25.0)	
Cattle	2	2 (100.0)	
None	1787	262 (14.66)	0.0055

bodies of water. Similarly, the municipal water may be contaminated as it is usually an untreated source of water. Thus, these sources of water are likely sources of intestinal parasitic infections. This may explain, in part, the findings of the present study. However, contrary to our findings, Ikeh et al. (32) reported that the source of water did not affect the prevalence of intestinal parasitic infections among HIV-positive patients.

Type of toilet significantly affected the prevalence of intestinal parasitic infections with patient’s defecating in bushes having the highest prevalence of 50%.

Contact with animals was not a significant risk factor for acquiring intestinal parasitic infections in this study ( $P=0.0055$ ). This is discordant with previous reports (34–36). Among HIV-infected individuals, *Cryptosporidium*

species has been reported as the major zoonotic parasites (35, 36).

A total of 360 intestinal parasites were detected in HIV-infected individuals, with *A. lumbricoides* being the most prevalent (33.1%). Other workers (16, 22, 25) also reported *A. lumbricoides* as the most prevalent intestinal parasite in HIV-infected patients. The presence of pathogenic intestinal parasites such as *A. lumbricoides*, hookworm, *G. intestinalis*, *E. histolytica*, *T. trichiura*, and *Taenia* species among HIV-infected persons should not be neglected. *Cryptosporidium*, *Microsporidium*, *Cyclospora*, and *I. belli* are opportunistic infections that have been reported among HIV-infected persons (19). In the present investigation, *Cryptosporidium* species and *I. belli* were the opportunistic infections observed. We found *Cryptosporidium* species only among HIV-infected persons (22.2%). Similar findings have been reported by a number of authors (13, 16, 24, 25, 30, 31). The prevalence of *I. belli* among HIV-infected patients in this study was 7.8%. However, some authors reported similar prevalence (24, 31–36). The prevalence of hookworm (20.6%) observed in this study was higher than that previously reported (10, 25, 30). Zelalem et al. (24) observed a higher prevalence of *S. stercoralis* than was observed in our study. In a similar vein, the prevalence of *T. trichiura* was lower than that observed by other authors (16, 24). Furthermore, a higher prevalence of *E. histolytica* than that observed in this study (3.3%) has been reported (10, 24, 30). The prevalence of *Taenia* species (1.1%) and *Giardia intestinalis* (0.6%) observed in this study was lower than that previously reported (37–41).

Out of 500 stool samples examined from HIV-negative patients, *A. lumbricoides* (4.2%) was the commonest parasite followed by hookworm (1.4%). There were no opportunistic coccidian parasites encountered from the specimens of the non-HIV subjects.

Generally, females had a higher prevalence (59.72%) of *Cryptosporidium* than their male counterparts (40.28%) among HIV-infected persons, though this was not statistically significant. This finding is consistent with previous work of Okodua et al. (10), but disagreed with that of Mohandas et al. (37). The reason for this may have been that more females were tested in this study than previous works. Females have a higher prevalence of *I. belli* than males in this study (9.3 and 5.5%, respectively). This is not in agreement with Akinbo et al. (36), who found approximately the same prevalence between males and females. Females also have a higher prevalence of *A. lumbricoides*. Males have a higher prevalence of hookworm, *T. trichiura*, and *Taenia* species, while *G. intestinalis* was only detected in females. Prompt diagnosis of parasitic infections, especially intestinal parasitic infections, among HIV-infected persons is advocated in order to improve the management and quality of life of HIV-infected individuals.

**Table 3.** Prevalence of intestinal parasites among HIV-infected patients and HIV-negative persons in relation to sex

Parasite	HIV-infected			HIV-negative		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
<i>Entamoeba histolytica</i>	5 (3.5)	7 (3.3)	12 (3.3)	–	–	
<i>Giardia intestinalis</i>	0 (0.0)	2 (0.9)	2 (0.6)	–	–	
<i>Isoospora belli</i>	8 (5.5)	20 (9.3)	28 (7.8)	–	–	
<i>Cryptosporidium</i> spp.	31 (21.4)	49 (22.8)	80 (22.2)	–	–	
<i>Ascaris lumbricoides</i>	43 (29.7)	76 (35.4)	119 (33.1)	9 (90.0)	12 (66.7)	21 (75.0)
Hookworm	33 (22.8)	41 (19.07)	74 (20.6)	1 (10.0)	6 (33.3)	7 (25.0)
<i>Strongyloides stercoralis</i>	10 (6.9)	13 (6.05)	23 (6.4)	–	–	
<i>Trichuris trichiura</i>	12 (8.3)	6 (2.8)	18 (5.0)	–	–	
<i>Taenia</i> sp.	3 (2.07)	1 (0.5)	4 (1.1)	–	–	
Total	145 (40.28)	215 (59.72)	360			

**Table 4.** Effect of CD4 count on the prevalence of intestinal parasites among HIV-infected patients

Parasitic agents	CD4 count (cells/ $\mu$ l)		OR	95% CI	P-value
	<200	$\geq$ 200			
<i>Entamoeba histolytica</i>	0	12	0.283	0.017, 4.794	>0.05
<i>Giardia intestinalis</i>	0	2	1.422	0.068, 29.727	>0.05
<i>Isoospora belli</i>	11	16	5.085	2.331, 11.090	<0.0001
<i>Cryptosporidium</i> spp.	59	21	26.037	15.472, 43.817	<0.0001
<i>Ascaris lumbricoides</i>	16	103	1.115	0.647, 1.922	>0.05
Hookworm	9	65	0.987	0.485, 2.008	>0.05
<i>Strongyloides stercoralis</i>	3	20	1.070	0.316, 3.630	>0.05
<i>Trichuris trichiura</i>	4	15	1.916	0.631, 5.823	>0.05
<i>Taenia</i> sp.	1	3	2.382	0.247, 23.006	>0.05
Total	103	257			

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