Cryptosporidium and other enteric parasitic infections in HIV-seropositive individuals with and without diarrhoea in Osogbo, Nigeria.

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ABSTRACT

The primary objective of this cross-sectional study is to correlate the presence of *Cryptosporidium* and other gastrointestinal parasites with the presence of diarrhoea in human immunodeficiency virus (HIV)-infected patients. Stool samples from 96 HIV-seropositive cases were examined for non-opportunistic parasites using the direct and formol-ether concentration methods, while the modified Ziehl-Neelsen technique was used to detect *Cryptosporidium* spp. The overall prevalence of *Cryptosporidium* spp. was 54.2%. Other intestinal parasites detected included *Ascaris lumbricoides* (9.4%), hookworm (5.2%), *Entamoeba histolytica* (3.1%), *Strongyloides stercoralis* (1%) and *Taenia* spp. (1%). Infection in males was more common (68.2%) than in females (55.4%) but the difference was not statistically significant. There was a significant association between *Cryptosporidium* infection and CD4+ count (P=0.0001), with the highest parasite prevalence (90%) observed among patients who had the lowest CD4+ count (<200 cells/mm^3). Forty-five (86.5%) patients with *Cryptosporidium* infection presented with diarrhoea and the difference between those with and without diarrhoea was statistically significant (P=0.0001). There was a statistically significant difference (P=0.0001) among the age groups, with the 41–50 group showing the highest prevalence (84.6%) of infection. Co-infection was observed in 13.5% of the patients. As no drug is currently available for the treatment of cryptosporidiosis, emphasis should be placed on educating HIV-infected individuals about prevention.

KEY WORDS: Antigens, CD4.
Cryptosporidium.
Diarrhea.
HIV.
Parasites.

Introduction

Infections in human immunodeficiency virus (HIV)-infected individuals can reduce their quality of life and life span, especially in those who are severely immunosuppressed and have a low CD4+ T-lymphocyte count (<200 cells/mm^3).[1] Immunodeficiency due to HIV can predispose to severe and prolonged diarrhoea from intestinal infections, including parasites. Diarrhoea is a significant cause of morbidity in the majority of studies and is most strongly associated with low CD4+ count.[2,3]

Intestinal parasitic infections that are asymptomatic or cause self-limiting diarrhoea in immunocompetent individuals can cause profuse diarrhoea in immunocompromised individuals, generally accompanied by weight loss, anorexia, malabsorption and, in some cases, fever and abdominal pain.

Gastrointestinal involvement in HIV/AIDS is almost universal, and significant disease occurs in 50–90% of patients, while diarrhoea can sometimes be a presenting manifestation or a life-threatening complication in HIV patients during the course of the disease.[4]

Several species of protozoa have been associated with acute and chronic diarrhoea in association with HIV-related disease; for example, *Cryptosporidium* spp., *Isospora belli*, *Microsporidia* spp., *Giardia duodenalis, Entamoeba histolytica, Cyclospora cayetanensis, Blastocystis hominis* and *Dientamoeba fragilis*.[5] Nematodes such as *Strongyloides stercoralis* can also cause diarrhoea and overwhelming infestation in patients with a variety of immunosuppressive disorders including HIV/AIDS.[6,7] Helminths, including hookworm, *Ascaris lumbricoides, Taenia saginata* and *Trichuris trichiura*, are considered non-opportunistic except on rare occasions.[8,9]

Cryptosporidium spp. is one of the enteric microorganisms most commonly associated with persistent diarrhoea and wasting in immunologically compromised individuals.[10,11] This parasite causes morbidity and mortality in AIDS patients worldwide, and these outcomes would be expected to be appreciably higher in developing countries due to a higher prevalence of infection in the general population. With the increasing number of HIV/AIDS-positive individuals, investigating the influence of HIV-induced immunodeficiency on the epidemiology of intestinal parasites infection is essential.

The objective of this study is to determine the rate of occurrence of *Cryptosporidium* and a range of other intestinal parasites commonly associated with diarrhoea in individuals with HIV/AIDS in Osogbo, Nigeria.

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Materials and methods

This cross-sectional study was conducted at the HIV clinic of Ladoke Akintola University of Technology (LAUTECH), Osogbo. This clinic routinely delivers HIV counselling, testing for HIV status and treatment of those already infected. Osogbo is the capital city of Osun State, Nigeria. It is located in the rain forest zone and has a population of approximately 845,000. Stool samples were collected from confirmed, consenting HIV-positive patients attending the HIV clinic for routine check-up, drug collection or other clinical complaint who volunteered to have a stool investigation. A clean sterile universal bottle was given after educating the patients on the mode of sample collection. Ethical approval was obtained from the LAUTECH ethics committee.

A modified Ziehl-Neelsen technique was used to detect *Cryptosporidium* spp.[12] Air-dried stool smears were fixed in methanol for 2 min, stained in carbol fuchsin for 15 min, decolourised with 3% acid alcohol for 10 sec and counterstained with 0.5% methylene blue for 30 sec. Stained smears were air-dried and examined microscopically for oocysts of *Cryptosporidium* using a x100 objectives.

To detect other intestinal parasites, samples were processed using the direct saline and the formol-ether concentration techniques. In the direct saline method, fresh stool samples were emulsified with physiological saline and examined microscopically for ova or larvae using a x10 objective. For the formol-ether concentration technique, approximately 1 g faeces was emulsified in 10% formol-water and then strained to remove large faecal particles. The sieved suspension was mixed with diethyl ether and centrifuged at 800 *xg* for 5 min. The sediment was examined microscopically for the presence of larvae and ova using a x10 objective.

Data obtained were analysed using GraphPad (GraphPad Software, San Diego USA). Statistical significance was estimated using the χ^2 test. $P<0.05$ were taken as significant.

Table 1. General characteristics and distribution of intestinal parasite among HIV patients in Osogbo, Nigeria.

Patient's characteristics	Number (%)
Number of subject studied	96
Male:Female	22:74
Mean age (years)	24.7
Mean CD4+ count (cells/mm^3)	352.6
CD4+ count <200/mm^3	30 (31.3)
CD4+ count ≥200/mm^3	66 (68.8)
Subjects positive for *Cryptosporidium*	52 (54.2)
Subjects positive for other intestinal parasites	19 (19.8)
With diarrhoea	60 (62.5)
Cryptosporidium spp.	52 (54.2)
Ascaris lumbricoides	9 (9.4)
Hookworm	5 (5.2)
E. histolytica	3 (3.1)
S. stercoralis	1 (1.0)
Taenia spp.	1 (1.0)
Co-infection (*Cryptosporidium* + other parasites)	13 (13.5)
Cryptosporidium + *A. lumbricoides*	4 (4.2)
Cryptosporidium + Hookworm	5 (5.2)
Cryptosporidium + *E. histolytica*	3 (3.1)
Cryptosporidium + *S. stercoralis*	1 (1.0)

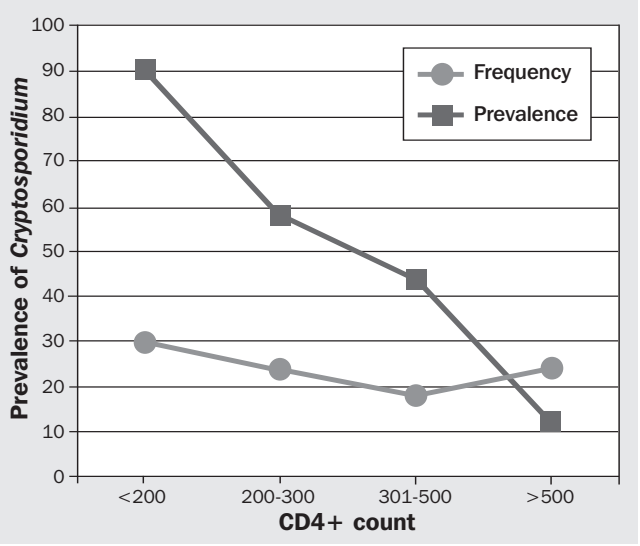

Fig. 1. Frequency and prevalence of *Cryptosporidium* spp. in relation to CD4+ count.

Results

A total of 96 confirmed HIV-positive individuals were enrolled in this study. Mean age was 24.7 years (range: 3–58 years; 74 [77%] females, 22 [23%] males). Mean CD4+ cell count was 352.6 cells/mm^3 and 31.3% were severely immunosuppressed (CD4+ count <200 cells/mm^3). Seventy-one (73.9%) harboured at least one parasite. The recovered parasites included *Cryptosporidium* spp. (54.2%), *A. lumbricoides* (9.4%), hookworm (5.2%), *E. histolytica* (3.1%), *S. stercoralis* (1%) and *Taenia* spp. (1%). Co-infection with *Cryptosporidium* was observed in 13 (13.5%) patients. Table 1 shows the general characteristics of the HIV-positive subjects and the distribution of intestinal parasites detected in stool samples. The prevalence of the parasites with respect to age and gender is shown in Table 2.

Table 3 shows the relationship between intestinal parasitic infection and diarrhoea in the study population. Figure 1 shows the frequency and distribution of *Cryptosporidium* infection in relation to CD4+ cell count. The pattern shows a gradual decrease in the prevalence of *Cryptosporidium* infection as CD4+ cell count increases. The association between *Cryptosporidium* spp. infection and CD4+ count was significant ($P=0.0001$).

Discussion

Human immunodeficiency virus infection is now a major threat to people in sub-Saharan Africa and it has also been associated with chronic diarrhoea, usually as a result of opportunistic parasitic infection.[13,14] Intestinal parasitic infections are among the leading opportunistic parasitic causes of morbidity and mortality in patients infected with

Table 2. Intestinal parasitic infection among HIV-positive patients according to age and gender.

Age	Male		Female		*P* value
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	
<21	3	2 (67)	5	3 (60)	
21–30	4	2 (50)	35	22 (63)	
31–40	5	4 (80)	26	9 (53)	0.0001
41–50	6	5 (83)	7	6 (86)	
>50	4	2 (50)	1	1 (100)	
Total	22	15 (68.2)	74	41 (55.4)	
P value		0.3404			

HIV. *Cryptosporidium* is a well-established cause of diarrhoea among HIV-infected patients worldwide, with prevalence of infection ranging from 3% in developed countries to over 60% in developing countries.[15–17]

In the present study, the prevalence of *Cryptosporidium* was 54.2%, which is comparable to the infection rate previously reported among HIV-infected individuals in this area[17] and in other African countries.[18] However, a lower rate (3.1%) was previously reported from Jimma (south-west Ethiopia).[5] Presently, the increasing population of immunocompromised individuals and the various outbreaks of cryptosporidiosis through infection by waterborne *Cryptosporidium* oocysts (often in drinking water) have placed an even greater emphasis on this pathogen. Little is known about the pathogenesis of the parasite, and no safe and effective treatment has been developed to combat cryptosporidiosis.[19]

As in previous studies, it is confirmed that the rate of opportunistic *Cryptosporidium* infection is inversely proportional to CD4+ count.[20] Patients with CD4+ count <200cells/mm^3 had the highest infection rate. This also shows that *Cryptosporidium* infection can be a marker of a severe immune deficiency in HIV,[21] and that intracellular protozoan infection is associated with a reduction in CD4+ T lymphocytes. Only HIV infection is specifically associated with CD4+ T-lymphocyte deficiency, while other conditions (e.g., malignancy and malnutrition) can adversely affect various elements of the immune system, including non-specific and specific mechanisms, and the humoral and cellular response.[22,23]

This study also showed that 86.5% of *Cryptosporidium*-infected patients had diarrhoea, and confirmed that the protozoan is an important opportunistic parasitic disease causing diarrhoea among HIV-infected patients in Nigeria. A strong statistical association was observed between *Cryptosporidium* and diarrhoea.

The mechanism by which *Cryptosporidium* causes diarrhoea is unclear. Inflammatory response to the infection is variable and may be modified by co-pathogens such as cytomegalovirus. However, histological evidence of gastrointestinal mucosal injury has been reported, with clinical manifestation influenced in part by anatomical distribution of the infection, with extensive infection involving both the small and large intestine producing the most severe illness.[24]

The prevalence recorded (73.9%) for intestinal parasitic infection among the HIV-positive subjects studied is similar to that found in other parts of Nigeria. However, a lower prevalence rate (28.4%) was observed in Abeokuta, western Nigeria.[25] The increased prevalence rate may be due in part to the fact that many opportunistic infections occur in HIV-infected patients, due to suppression of the immune system. Gastrointestinal parasitic infection is a universally recognised problem in these patients. Significant association

Table 3. Relationship between diarrhoea and intestinal parasites.

Parasites	Frequency *n*=96	Diarrhoea (%) *n*=60	*P* value
Cryptosporidium spp.	52	45 (86.5)	0.0001*
A. lumbircoides	9	6 (66.7)	0.0365*
Hookworm	5	5 (100)	1.000
E. histolytica	3	3 (100)	1.000
S. stercoralis	1	1 (100)	–
Taenia spp.	1	–	–
Co-infection			
Cryptosporidium + *A. lumbricoides*	4	4 (100)	1.000
Cryptosporidium + hookworm	5	5 (100)	1.000
Cryptosporidium + *E. histolytica*	3	3 (100)	1.000
Cryptosporidium + *S. stercoralis*	1	1 (100)	1.000

*Significant ($P<0.05$)

was also demonstrated between age group and intestinal parasitic infection, with those aged over 40 years showing more frequent episodes of infection. No significant association was shown between gender and intestinal parasitic infection. Gender and age-related disparities in prevalence of helminth infection in these areas could be attributed to various factors, including differential usefulness and unhygienic living conditions.

In conclusion, *Cryptosporidium* infection was responsible for diarrhoea in 86% of those infected with the parasite in this study population. *Cryptosporidium* infection was significantly more prevalent in those who had lower CD4+ T-cell counts. These results highlight the importance of evaluating HIV-infected individuals with diarrhoea for the presence of intestinal parasitic infection, which may help to provide better management for these patients. As no drug is currently available for the effective treatment of cryptosporidiosis, emphasis should be placed on educating HIV-infected individuals about prevention, including the potential benefits of drinking boiled water and avoiding contact with animals. □

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References

1 Smit C, Hallett TB, Lange J, Garnett G, de Wolf F. Late entry to HIV care limits the impact of anti-retroviral therapy in The Netherlands. *PLoS One* 2008; **3** (4): e1949.

2 Attili SV, Gulati AK, Singh VP, Varma DV, Rai M, Sundar S. Diarrhea, CD4 counts and enteric infections in a hospital-based cohort of HIV-infected patients around Varanasi, India. *BMC Infect Dis* 2006; **6**: 39.

3 Thom K, Forrest G. Gastrointestinal infections in immuno-compromised hosts. *Curr Opin Gastroenterol* 2006; **22** (1): 18–23.

4 Siddiqui U, Bini EJ, Chandarana K *et al*. Prevalence and impact of diarrhea on health-related quality of life in HIV-infected patients in the era of highly active antiretroviral therapy. *J Clin Gastroenterol* 2007; **41** (5): 484–90.

5 Awole M, Gebre-Selassie S, Kassa T, Kibru G. Prevalence of intestinal parasites in HIV-I-infected adult patients in south-western Ethiopia. *Ethiop J Health Dev* 2003; **17** (1): 71–8.

6 Pollok RC, Farthing MJ. Managing gastrointestinal parasite infections in AIDS. *Trop Doct* 1999; **29** (4): 238–41.

7 Ambroise-Thomas P. Parasitic diseases and immunodeficiencies. *Parasitology* 2001; **122** (Suppl l): S65–71.

8 Hung CC, Chen PJ, Hsieh SM *et al*. Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. *AIDS* 1999; **13** (17): 2421–8.

9 Gompels MM, Todd J, Peters BS, Main J, Pinching AJ. Disseminated strongyloidiasis in AIDS: uncommon but important. *AIDS* 1991; **5** (3): 329–32.

10 Dionisio D. Cryptosporidiosis in HIV-infected patients. *J Postgrad Med* 2002; **48** (3): 215–6.

11 Hunter PR, Nichols G. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clin Microbiol Rev* 2002; **15** (1): 145–54.

12 Cheesbrough M ed. *District laboratory practice in tropical countries* Part 1.2. Cambridge: Cambridge University Press, 2000.

13 Assefa S, Erko B, Medhin G, Assefa Z, Shimelis T. Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count. *BMC Infect Dis* 2009; **9**: 155.

14 Kulkarni S, Kairon R, Sane S *et al*. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immune suppression. *Indian J Med Res* 2009; **130**: 63–6.

15 Kurniawan A, Karyadi T, Dwintasari SW *et al*. Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia. *Trans R Soc Trop Med Hyg* 2009; **103** (9): 892–8.

16 Ng J, MacKenzie B, Ryan U. Longitudinal multi-locus molecular characterisation of sporadic Australian human clinical cases of cryptosporidiosis from 2005 to 2008. *Exp Parasitol* 2010; **125** (4): 348–56.

17 Adesiji Y, Lawal R, Taiwo S, Fayemiwo S, Adeyeba O. Cryptosporidiosis in HIV infected patients with diarrhoea in Osun State, Southwestern Nigeria. *Eur J Gen Med* 2007; **4** (3): 119–22.

18 Colebunders R, Francis H, Mann JM *et al*. Persistent diarrhea, strongly associated with HIV infection in Kinshasa, Zaire. *Am J Gastroenterol* 1987; **82** (9): 859–64.

19 Juranek DD. Cryptosporidiosis: sources of infection and guidelines for prevention. *Clin Infect Dis* 1995; **21** (Suppl 1): S57–61.

20 Taherkhani H, Fallah M, Jadidian K, Vaziri S. A study on the prevalence of *Cryptosporidium* in HIV-positive patients. *J Res Health Sci* 2007; **7** (2): 20–4.

21 El-Diffrawy M, Neanaa H, Eissa M, Sadaka H, Nomir A. Study of parasitic infections in immunocompromised patients in haematology department at main university hospital, Alexandria. *Exp Pathol Parasitol* 2002; **10**: 85–92.

22 Kavanaugh D, Carbone D. Immunologic dysfunction in cancer. *Hematol Oncol Clin North Am* 1996; **10**: 927–51.

23 Cunningham-Rundles S, Ananworanich J. Immune responses in malnutrition. In: Stiehm ER, Ochs HD, Winkelstein JA eds. *Immunologic disorders in infants and children*. Philadelphia: Elsevier Saunders, 2005.

24 Manabe YC, Clark DP, Moore RD *et al*. Cryptosporidiosis in patients with AIDS: correlates of disease and survival. *Clin Infect Dis* 1998; **27** (3): 536–42.

25 Okodua M, Adeyeba O, Tatfeng Y, Okpala H. Age and sex distribution of intestinal parasitic infection among HIV-infected subjects in Abeokuta, Nigeria. *Online J Health Allied Scs* 2003; **4**: 3.