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Research

*Corresponding author
Kingsley Badu, BSc, MPhil, PhD
Department of Theoretical and Applied Biology
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
E-mail: kingsbadu@gmail.com

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Intestinal Parasitemia and HIV/AIDS Co-infections at Varying CD4+ T-cell Levels

Samuel CK Tay, BSc, DVM, MSc, MPH[1]; Eric Nii Okai Aryee, BSc, MSc[2]; Kingsley Badu, BSc, MPhil, PhD[3*]

[1]*Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*Madina Polyclinic- Kekele Ghana Health Service, Madina, Accra, Ghana*

[3]*Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

ABSTRACT

Background: Intestinal parasites, especially coccidian parasites, cause gastrointestinal symptoms such as severe diarrhoea which increases morbidity and mortality rates in people living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS), particularly in Sub-Saharan Africa. We examined the prevalence of intestinal parasites in people living with AIDS at different CD4+ T-cell levels.
Method: Case-control studies were conducted over a four month period including a total of 672 participants, between the ages of 8 and 72 years. HIV screening and confirmatory tests were done. We examined stool samples by wet mount, followed by formol-ether concentration and staining with Modified Field's and Ziehl Neelsen techniques. We also carried out fluorescence-activated cell sorting (FACS) analyses to obtain their CD4+ T-cell levels.
Results: The prevalence of intestinal parasites were significantly higher (25.2%) among HIV seropositives than HIV seronegative individuals (13.3%), ($p<0.001$). Coccidian parasites: *Cystoisospora belli* (formerly *Isospora belli*), Cryptosporidium and the round worm *Strongyloides stercoralis* infections were found exclusively in HIV seropositives. Cryptosporidium infections were more frequently observed in the rural cohort ($p=0.039$). *C. belli, Cryptosporidium*, *Giardia lamblia* and *Strongyloides stercoralis* infections were significantly higher in diarrhoeic stools. Microsporidia and *Cystoisospora belli* were found mostly in individuals with CD4+ T-cell levels of ≤200 cells/μL. Participants with CD4+ T-cell count of ≤50 cells/μL were associated with diarrhoea.
Conclusion: The prevalence of opportunistic coccidian parasites remains high in HIV-infected individuals with low CD4+ T-cell counts. Routine diagnosis is recommended to ensure comprehensive care for HIV patients.

KEY WORDS: HIV/AIDS; Intestinal parasites; *Diarrhoea,* CD4+ T-cell count.

ABBREVIATIONS: HIV/AIDS: Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; NSAID: Non-Steroidal Anti-Inflammatory Drugs; ART: Antiretroviral Therapy; CI: Confidence Interval.

INTRODUCTION

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) and intestinal parasites co-infections are linked in a vicious cycle which, results in a major public health burden for developing countries.[1] Currently, about 23.5 million people, an estimated 69% of all people living with HIV and AIDS, live in sub-Saharan Africa.[2,3] Moreover, 90-92% of all pregnant women and children living with HIV are reportedly living on the continent.[3,4]

In Ghana, the HIV prevalence is relatively low, but the rising trend in the last three years is a matter of concern.[5] According to HIV sentinel survey report for 2016, the national

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prevalence is estimated to be 2.4% representing "a second consecutive upsurge from the 2014 prevalence of 1.6 % and 1.8 % in 2015".[5] The survey also found that HIV prevalence is higher in urban areas (2.5%) than rural (1.9%). New infections remained unchanged at 1.1%. The prevalence of the disease was highest among the 45-49 age groups at 5.6%, followed by 35-39 year group at 3.5% whilst 15-19 being the lowest at 0.6%.[5] The survey also found that the proportion of HIV subtype 1 is 98.5% compared to 1.5% for dual HIV type 1 and 2 infections but no subtype 2 sole infections.[5]

Although, antiretro viral therapy is available in Ghana, the coverage hardly reaches all the patients who need them. According to the Centers for Disease Control and. Prevention (CDC), 66,366 adults were receiving Antiretroviral Therapy (ART) at the coverage of 62% by 2012 (http://www.cdc.gov/globalaids/global-hiv-aids-at-cdc/countries/ghana/default.html). In the 2015 UN Sustainable Development Goals, the world committed itself to stopping HIV/AIDS by 2030, it is therefore imperative that infections that aggravate disease progression, such intestinal parasites are closely investigated.

HIV/AIDS infection is associated with high prevalence of gastrointestinal infections including parasite-associated diarrhoea due to apparent dysfunctional immunity.[6-8] The progressive decline of the mucosal immunologic defense mechanisms in HIV/AIDS patients predisposes them to early, intermediary, or late gastrointestinal infections.[9] Progressive HIV infection have been characterized by an increase in Th2-like responses, which may either be a consequence or a cause of the immune deterioration.[10-15] This has been shown to be responsible for increasing host susceptibility to a myriad of intestinal opportunistic agents, such as *Cryptosporidium parvum*, *Cystoisospora belli* and *Microsporidia* species.[16] Diarrhoea affects up to 90% of HIV patients, increasing in frequency and magnitude as the disease progresses.[17,18] Helminths are known to cause T-cell dysfunction, thereby worsening the already compromised immune system.[19] Diarrhoea, as a result, has been recognized as one of the most frequent causes of morbidity and death in people living with HIV.[20] At the national level, data on intestinal parasites in people living with HIV/AIDS is virtually non-existent since there are no specific guidelines that require standard investigation and diagnosis of intestinal parasitic infections in HIV patients. The objective of the study was to determine the patterns of intestinal parasitic infections in people living with HIV/AIDS, and its association to diarrhoea at different $CD4^+$ T cell levels.

METHODS

Study Area

This study was based on two cross-sectional surveys conducted at two hospitals in the Ashanti region of Ghana which is known to have HIV prevalence of 2.6%.[5] The surveys were conducted at HIV/AIDS Voluntary Counseling and Testing centers (VCT) at the two hospitals. The Nyinahin Government Hospital was located in a rural area and St. Patrick's Hospital at Offinso was located in a peri-urban area. The surveys were carried out between April and July, 2011.

The study was conducted in two districts of the Ashanti region (forest zone) in the middle belt of Ghana; the Atwima Mponua District and the Offinso Municipality. The Atwima Mponua District is located in the western part of Ashanti Region, and lies between longitude 2°00'W and 2°32'W and latitude 6°32'N and 6°75'N. The district is within the wet-semi equatorial zone and has two peaks of rainfall in the year. About 92% of the people reside in the rural countryside, with only about 8% living within an'urban'settlement. The Offinso Municipality, on the other hand, is located in the north-western stretch of Ashanti. It is about 40 km away from the regional capital of Kumasi, and lies between longitude 1°65'W and 1°45'E and latitudes 6°45'N and 7°25'S. The district covers an area of 1255 km². The high population growth rate in these localities can be attributed to high immigration and the spillover population from the Kumasi metropolis giving rise to about 40% of the populace in urban dwellings. The district represents a peri-urban settlement. Nyinahini and Offinso are district capitals of the Atwima Mponua District and Offinso Municipality, Ashanti Region, Ghana, respectively.

Sample Size

This was determined based on assumptions of the binomial distribution to estimate the confidence interval (CI) of HIV prevalence at 95% with an estimated population size of less than 3000, described earlier.[21,22] $n=z^2(pq)/d^2$, where z is the critical value of standard normal distribution, p is the baseline parasite prevalence and d is the level of precision.[21,22] For 95% CI and precision level of 5%, the minimum number of participants required to achieve adequate statistical power was 323.

Inclusion and Exclusion Criteria

All patients attending the VCT clinic, who were at least one year old, regardless of their HIV status (seropositive or negative) and gender, and not on non-steroidal anti-inflammatory drugs (NSAID) were eligible to be included in the study. Informed consent was sought from all eligible patients, and participants were only recruited when consent was given.

Study Participants and Recruitment

The study took place in two hospitals, (The Nyinahin Government hospital and St. Patrick's Hospital) specifically at HIV VCT clinic.

Researchers visited the clinics twice per week until desired sample size was realized. Researchers approached all persons attending the clinic including family members for consent and participation. The study was duly explained to them and participation was purely voluntary. Informed consent mainly adult

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consent forms and parental/guardian/assent consent forms were administered.

A total of 341 HIV patients (seropositives) consented to the study and these constituted the case group. Another 331 HIV seronegative gave their consent to participate in the study; this group constituted the negative control group. Demographic data and antiretroviral drug usage information were obtained for each individual who consented to the study. Each participant was provided with sterile screw-capped containers to provide stool samples (collected in the morning) on 2 consecutive days during their scheduled visits. The stool samples were examined for ova, larvae and cyst of parasites regardless of the presence of diarrhoea. In addition, 3 mL of blood taken from participants were put in ethylenediaminetetraacetic acid (EDTA) anti-coagulated tube for $CD4^+$ T-cell count.

HIV Testing

Screening: All consenting study participants regardless of the knowledge of their status were screened for the presence or otherwise of HIV-1 and HIV-2 or both, using the First Response HIV Card Test (PMC Ltd, Shree Indl Estate, India). Briefly, (according to manufacturer's instructions) 10 µl of serum was dropped into the sample well and 35 µl of assay diluent was added. The results were read and interpreted within 5-15 minutes. The presence of only one band in the control line in the result window indicates a negative result. However, two-color bands, one control and the other for HIV-1 indicated reactivity for antibodies to HIV-1. Two-color bands, one control and the other for HIV-2 indicated reactivity for antibodies to HIV-2. All three color bands indicated reactivity for antibodies to HIV-1 and HIV-2.

Confirmation: All seropositive individuals were re-tested with the Qualitative Immunoassay test for confirmation of their HIV status. The HIV-1/2 Oral Quick Rapid Test (OraSure Technologies, Inc., Bethlehem, PA 18015, USA) was used according to manufacturer's instructions. Briefly, the rapid test device was removed from its pouch and the padded-end was used to swab the upper and lower gums of participants. The padded-end of the rapid test device was inserted into the buffer, and the test result was read and interpreted after 20 minutes.

Stool Examination

A total of 1,344 stool samples were obtained from 672 participants. These were subjected to routine stool examination, which included saline and iodine mounts to screen for helminth ova and larvae, protozoan cyst, and trophozoites.

Direct wet mount of stool sample in normal saline (0.85% NaCl) was prepared immediately upon arrival in the laboratory and examined under light microscopy (x10 and x40 objectives) for the presence of vegetative forms, larvae, and ova of helminthes. Field's stain and Lugol's iodine staining was used to detect *Giardia lamblia* flagellates and cysts of protozoa, respectively. The formol-ether concentration technique was employed to concentrate stool samples for further confirmatory microscopic examination. Examination of fecal smears after special staining (Modified Zhiel Neelsen and Modified Field's staining techniques for the detection of *Cryptosporidium*, *Isospora belli*, *Cyclospora cayetanensis* and Microsporidia spores, respectively) was done according to Chessbrough.[23]

$CD4^+$ T-cell Count

The fluorescence-activated cell sorting (FACS) count (Becton Dickinson Immunocytometry system, Singapore) was used for the immunophenotyping of lymphocytes. Briefly, CD4 reagent tubes were vortexed and opened with the coring station for 50 µl of whole blood to be added. These were vortexed and incubated for one hour in the dark at room temperature. The tubes were uncapped and 50 µl of fixative added. The tubes were recapped and vortexed for 5 seconds upright before subjecting it to the FACS Count instrument for the immunophenotyping of lymphocytes.

Data Analysis

Statistical analysis was carried out using SPSS (2007) version 17.0. Data was summarized using frequency tables. The proportions of parasites were compared between the $CD4^+$ T-cell counts with chi-square test. The relationship between the $CD4^+$ T-cell count and the presence of diarrhoea were assessed using the chi-square analysis with significance level set at 0.05.

Ethical Considerations

The study was conducted with the approval of the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

RESULTS

Demographic Characteristics

A total of 672 patients gave their consent to participate in the study; these comprised of 341 HIV seropositives and 331 seronegatives. Overall, there were 81.5% females (n=548) 18.5% males (n=124). The percentage of male and female participants under each study group (seropositive or seronegative) was similar, and followed the same trend as that mentioned for the overall percentages (Figure 1).

When the study population was stratified into age groups, it was realized that majority of the HIV seropositives 65% (n=225) belonged to the most productive age bracket 25-45 years. Only 3.2% (n=11) belonged to the paediatric population, that is less or equal to 14 years (Figure 2).

Parasite Prevalence among HIV Seropositive and Seronegative

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Figure 1: Total Number of Participants Stratified into Gender and HIV Status.

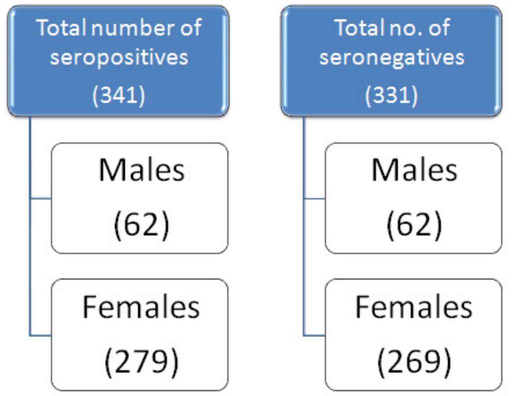

Figure 2: Age Distribution among HIV Seropositive (in Red) and HIV Seronegative in Black.

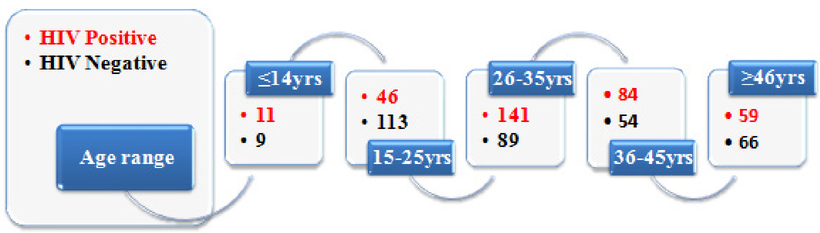

Table 1: Parasite Prevalence among HIV Seropositive and Seronegative Participant.

Parasite	HIV seropositive N=341 (%)	HIV seronegative N=331 (%)	Total (N=672)	*p*-value
Giardia lambia	39 (11.4)	39 (11.8%)	78 (11.6)	0.905
Entamoeba histolytica	4 (1.2)	0 (0)	4 (0.6)	0.124
Cystoisospora belli (formerly *Isospora belli*),	9 (2.6)	0 (0)	9 (1.3)	0.004
Cryptosporidium spp.	7 (2.1)	0 (0)	7 (1.0)	0.032
Microsporidia	1 (0.3)	0 (0)	1 (0.1)	1
Cyclospora cayetanensis	1 (0.3)	0 (0)	1 (0.1)	1
Strongyloides stercoralis	13 (3.8)	0 (0)	13 (1.9)	<0.001
Enteriobius vermicularis	1 (0.3)	1 (0.3)	2 (0.3)	1
Ascaris lumbricoides	0 (0)	1 (0.3)	1 (0.1)	0.493
Hookworm	3 (0.9)	2 (0.6)	5 (0.7)	1
G. lambia and *E. coli*	1 (0.3)	0 (0)	1 (0.1)	1
G. lambia and *E. histolytica*	4 (1.2)	1 (0.3)	5 (0.7)	0.373
C. belli and *S. stercoralis*	1 (0.3)	0 (0)	1 (0.1)	1
Microsporidia and *C. belli*	2 (0.6)	0 (0)	2 (0.3)	0.499

HIV^+=HIV Positive; HIV^-=HIV Negative; N=Number of Participants, *Coccidian parasites: *p*-value of <0.001 with HIV positive participants.

Participants

The overall prevalence of intestinal parasites among the study subjects was 19.3%. A total of 130 participants had at least one parasite. *Giardia lamblia* was the most common parasite encountered, and had similar prevalence in HIV seropositives (11.4%) and seronegatives (11.8%) (Table 1). Parasite prevalence was significantly higher in HIV seropositive participants (25.2%), than the control group (13.3%) [(χ^2=15.3, *p*=0.000) (Table 1)]. This confirms the susceptibility of HIV patients to opportunistic parasitic infections.

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Among the HIV seropositive cohort, 21 (6.2%) were infected with the coccidian parasites (*Cystoisospora belli*, *Cryptosporidium, Cyclospora cayetanensis*) including the fungi-like unicellular intracellular parasite Microsporidia. There were single infections as well as co infections with more than one parasite. Parasites such as *Cystoisospora belli* (2.6%), *Cryptosporidium sp* (2.1%), Microsporidia (0.3%) and *C. cayetanensis* (0.3%) as well as *S. stercoralis* were single infections exclusively found with HIV seropositives. The predominant helminths was *S. stercoralis* (3.8%) followed by Hookworm (0.9%) and *E. vermicularis* (0.3%) among seropositive participants. Again, there were co-infections with *Giardia lamblia* and *E. coli*, *Giardia lamblia* and *Entemoeba histolytica*, *C. belli* and *S. stercoralis* as well as Microsporidia and *C. belli* albeit in low prevalence. *Ascaris lumbricoides* occurred in only 1 HIV negative participant (0.3%) Table 1.

CD4⁺ T-cell Levels among Parasite and HIV Co-infected Participants

Using $CD4^+$ T-cell estimate as a marker of relative risk of developing HIV related opportunistic infections,[24] we observed 29.3% of HIV seropositives were in the acute (asymptomatic) infection stage, 44.3% were in the intermediate (symptomatic) stage, and 19.7% were in the late (symptomatic) disease stage. Still 6.7% were in the most advanced HIV disease stage (Table 2). *Giardia lamblia* infections were found at all $CD4^+$ T-cell levels with lower prevalence (4.3%) among the participants with the least $CD4^+$ T-cell count (<50 cells/µl), but this was not statistically significant (p=0.852) (Table 2). *C. belli*, Microsporidia, and *C. cayetanensis* were also found in patients with the least $CD4^+$ T-cell count. Although, *S. stercoralis* occurred exclusively among HIV seropositives (p<0.001) (Table 1), its occurrence was not significantly associated with $CD4^+$ T-cell level.

CD4⁺ T-Lymphocyte Levels and Diarrhoea

Out of 341 participants belonging to the HIV seropositive group, 110 presented diarrhoeic stools; representing 32.3%. Intestinal parasites were observed in 32.7% of the diarrhoeal stools. The highest incidence of diarrhoeic stools (78.3%) was observed among participants with $CD4^+$ T-cell count <50 cells/µL (Table 3). Contrary, only 2% of participants with $CD4^+$ T-cell count of ≥500 cells/µL presented diarrhoeal stools.

Coccidian parasites were detected more commonly in HIV seropositives with diarrhoea than in participants with hel-

Table 2: CD4⁺ T-cell Levels among Parasite and HIV Co-Infected Participants.

	CD4⁺ T- cell count (cells/ul)				
Parasite	**>500 (N=100) (29.3%)**	**200-500 (N=151) (44.3%)**	**50-200 (N=67) (19.6%)**	**<50 (23) (6.7%)**	***p*-value**
Giardia lambia	50 (11.9%)	16 (9.9%)	11 (16.4%)	1 (4.3%)	0.852
Entamoeba histolytica	0 (0%)	3 (1.9%)	1 (1.5%)	0 (0%)	0.079
Cystoisospora *belli*	0 (0%)	0 (0%)	3 (4.5%)	6 (26.1%)	<0.001
***Cryptosporidium* spp.**	2 (0.5%)	1 (0.6%)	3 (4.5%)	1 (4.3%)	0.442
Microsporidia	0 (0%)	0 (0%)	0 (0%)	1 (4.3%)	0.002
Cyclospora cayetanensis	0 (0%)	0 (0%)	0 (0%)	1 (4.3%)	0.002
Hookworm	4 (1.0%)	1 (0.6%)	0 (0%)	0 (0%)	0.343
Strongyloides stercoralis	2 (0.5%)	5 (3.1%)	5 (7.5%)	1 (4.3%)	<0.001
Enteriobius vermicularis	1 (0.2%)	0 (0%)	1 (1.5%)	0 (0%)	0.423
Ascaris lumbricoides	0 (0%)	1 (0.6%)	0 (0%)	0 (0%)	0.571
G. lambia* and *E. coli	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	0.503
Cystoisospora *belli* and *S. stercoralis*	0 (0%)	0 (0%)	1 (1.5%)	0 (0%)	<0.001
Microsporidia and Cystoisospora *belli*	0 (0%)	0 (0%)	0 (0%)	2 (8.7%)	<0.001
G. lambia and E. histolytica	1 (0.2%)	2 (1.2%)	2 (3.0%)	0 (0%)	0.068

HIV^+=HIV Positive; HIV^-=HIV Negative; N=Number of Participants; ND=Not Determined.

Table 3: Correlation between CD4⁺ T-Lymphocyte Levels and Diarrhoea.

	CD4 T⁺-cell count(cells/L)					
	>500	**200-500**	**50-200**	**<50**	**Total**	***p*-value**
Diarrhoea Stools	2 (2%)	6 (4.0%)	23 (34.3%)	18 (78.3%)	49 (14.4%)	<0.001
Non Diarrhoea Stools	98 (98%)	145 (96.0%)	44 (65.7%)	5 (21.7%)	292 (85.6%)	
Total	100 (100%)	151 (100%)	67 (100%)	23 (100%)	341 (100%)	

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Table 4: Presence of Parasites in Diarrhoeic Stools of HIV Positive and Negative Participants.

	HIV$^+$ (N=341)			HIV$^-$ (N=331)		
Parasite	**D**	***ND***	***p*-value**	**D**	**ND**	***p*-value**
Giardia lambia	10 (40.0%)	29 (9.2%)	<0.001	18 (62.1%)	21 (7.0%)	<0.001
Entamoeba histolytica	0 (0%)	4 (1.3%)	0.736	0 (0%)	0 (0%)	nd
Cystoisospora *belli*	8 (16.3%)	1 (0.3%)	<0.001	0 (0%)	0%	nd
***Cryptosporidium* spp.**	3 (6.1%)	3 (1.0%)	0.04	0 (0%)	0 (0%)	nd
Microsporidia	1 (2.0%)	0 (0%)	0.14	0 (0%)	0 (0%)	nd
Cyclospora cayetanensis	1 (2.0%)	0 (0%)	0.14	0 (0%)	0 (0%)	nd
Hookworm	0 (0.0%)	3 (1.0%)	1	0 (0%)	2 (0.6%)	1
Strongyloides stercoralis	5 (10.2%)	8 (2.7%)	0.026	0 (0%)	0 (0%)	nd
Enteriobius vermicularis	1 (2.0%)	0 (0%)	0.14	0 (0%)	1 (0.3%)	1
Ascaris lumbricoides	0 (0%)	0 (0%)	nd	0 (0%)	1 (0.3%)	1
G. lambia* and *E. coli	2 (4.1%)	2 (0.7%)	1	0 (0%)	1 (0.3%)	1
Cystoisospora *belli* and *S.stercoralis*	1 (2.0%)	0 (0%)	0.14	0 (0%)	0 (0%)	nd
Microsporidia and Cystoisospora *belli*	2 (4.1%)	0 (0%)	0.02	0 (0%)	0 (0%)	nd
G. lambia* and *E. histolytica	2 (4.1%)	2 (0.7%)	0.1	0 (0%)	1 (0.3%)	1
Total diarrhoea (143) (21.3%)	36/110 (32.7%)	52/283 (18.3%)		18/33 (54.5%)	27/325 (8.3%)	

HIV$^+$=HIV Positive; HIV$^-$=Negative; N=Number of Participants; nd=Not Determined; D=Diarrhoea Stools; ND=Non Diarrhoeal Stools.

minths and other protozoa (Table 4). The incidence of diarrhoea among participants infected with *Giardia lamblia* only, was 40% and 62.1% in HIV seropositive and seronegatives respectively. The incidence of diarrhoea among HIV seropositives infected with *C. belli, Cryptosporidium* and *S. stercoralis* infections were 16.3%, 6.1% and 10.2%, respectively. Microsporidia, *C. cayetanensis* and *E. vermicularis* occurred with diarrhoea in at most 2% of participants.

DISCUSSION

Intestinal parasitic infections and HIV/AIDS have been the major public health problems and remain a vital cause of morbidity and mortality in developing countries. Both problems are linked in a vicious cycle.[1] The introduction of antiretroviral therapy has lessened the prevalence of gastrointestinal infections in HIV patients. This notwithstanding, several people with HIV infection still suffer from intestinal parasite infections.[1,6-8,25]

Intestinal parasitic infections found among HIV patients from low-income countries have been reported with prevalence 15% to over 80% in recent times.[1,6-8,25]

In the current study, the overall prevalence of intestinal parasite among the study population was 19.3%. However, the prevalence of intestinal parasites in the HIV seropositive group was significantly higher (25%) than that observed in the HIV seronegative group which was 13.3%. The observed prevalence in this study is similar to others carried out in Zambia which reported 25% prevalence among HIV-infected cohort.[26] Other reports from India, Ethiopia and Tanzania were comparably higher ranging from 30% and above.[27,28] However, much lower prevalence of 10.6% among HIV patients have also been reported elsewhere.[29]

It was also observed that opportunistic parasitic infections mainly the coccidian parasites occurred exclusively in HIV/AIDS patients with a corresponding depletion of CD4$^+$ T cell count. This has been attributed to the modulation of immune response in the advance stages of the disease.[10-15] The highest prevalence of parasitosis was observed among participants in the CD4$^+$ T-cell level ≤50 cells/μL. This category forms 56.5% of participants in the advanced stage of the disease. The most predominant parasites recovered among this group of participants belonged to the coccidian groups (47.8%), which are well known as opportunistic parasites in HIV disease. With the exception of one participant, all participants with mixed parasitic infections had CD4$^+$ T-lymphocyte level <200 cells/μl. This observation has been echoed by other studies.[6,30]

Typically, the dynamics of HIV-1 infection is known to follow a familiar pattern where there is the acute phase in which there is massive depletion of CD4$^+$ T cells of the gastrointestinal tract,[31] followed by the chronic phase, where there is a gradual reduction in CD4$^+$ T cells which results in high risk of opportunistic infections, and then AIDS sets in. Recently it has been found that there is significant preferential loss of Th17 cells within the gastrointestinal tract of HIV-infected individuals[32] as a result of microbial translocation after the initial structural and immunological disruption of the gut mucosa in the acute phase.[33] *Giardia lamblia* was the most common parasite among the participants. Its occurrence, among both HIV seropositive (11.4%) and seronegative (11.8%) was similar. Previous studies have demonstrated that although infection with *Giardia lamblia*

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and HIV correlated with enteritis or enterocolitis, its incidence does not differ amongst HIV-positive and negative patient populations.[34] This underscores the non-opportunistic nature of the *G. lamblia* reviewed by Cimerman et al.[35]

The helminths observed in this study were *A. lumbricoides*, *E. vermicularis*, hookworm and *S. stercoralis*. Helminths infections generally were low among the study groups when compared to findings of similar studies elsewhere reporting prevalence of 37.04%.[36] However, *S. stercoralis* was only associated with HIV seropositive individuals and hookworm infections were higher (5%). Modjarrad et al[36], reported relatively higher prevalence of intestinal helminths (24.9%) with *A. lumbricoides* and hookworm being prevalent among HIV-1 patients in an urban African setting.[37] Apart from *S. stercoralis*, other helminths had lower prevalence in our study when compared to others carried out in similar developing countries[20]; this may be due to the widespread administration of anti-helminths and co-tri-moxazole among the study participants (56% of participants were already on ART at the time of stool collection).

Studies have shown that, reconstitution of the immune system following ART administration alone resolves *Cryptosporidium* infections without specific treatment for the parasite.[38,39] This is because ART acts against the aspartyl-protease of the parasite depriving the parasite of an essential protein.[40,41] More than 56% of participants were already on ART at the time of stool collection. It is likely that as a patient's CD4+ T-cell level increases with the administration of ART, opportunistic infections are not established even if they are exposed to infection.

Diarrhoea is a life threatening complication often associated with HIV, causing severe weight loss; both conditions are independent predictors of mortality in HIV/AIDS.[42] The incidence of diarrhoea among HIV seropositives in this study was significantly high. Among the HIV cohort diarrhoea episodes increased with declining immunity, with the highest diarrhoea prevalence (78.3%) occurring at the least CD4+ T-cell count <50 cells/µL. (Table 4). *Giardia lamblia*, *Cystoisospora belli*, *Cryptosporidium*, and *S. stercoralis* were associated with diarrhoeal stools of HIV seropositive patients (Table 4). Among the opportunistic coccidian parasites in HIV seropositives *Cystoisospora belli* (3.5%) was predominant followed by *Cryptosporidium* (2.1%). Microsporidia and *C. cayetanensis* had a prevalence of 0.9% and 0.3%, respectively, these occurred exclusively among HIV seropositives. All participants with *Cystoisospora belli* infections presented with diarrhoea. This strong association with diarrhoea may be associated with patients who were ART naïve and presenting themselves very late to the hospitals. They often present with wasting, general weakness and diarrhoea. *Cyclospora cayetanensis*, an emerging parasite, was found in only one participant with diarrhoea.

On the other hand, the presence of diarrhoea without parasites in stool can be quite intriguing. About 32.7% of HIV seropositives had diarrhoea even in the absence of parasites (Table 4). This can arise from bacteria etiology, lactose intolerance or insufficient sensitivity of the diagnostic procedure.[39] It has been shown however, that no etiological agent is found in 15-50% of HIV patients with chronic diarrhoea.[41] Munnink et al observed that unexplained diarrhea in HIV- infected patients were not due to novel pathogens [immunodeficiency-associated stool virus (IAS virus)][42] or previously unknown pathogens, but may be due to HIV-1 itself having a "virotoxic" effect on the enterocytes that results in intestinal mucosal abnormalities leading to diarrhea.[40] Again some anti-retroviral agents especially the protease inhibitors have been reported to cause diarrhoea.[43] This appears to be changing the etiology of diarrhoea in some parts of the world where parasite related diarrhoea appear to be falling, while the number of unexplained and drug-induced diarrhea seems to be going up.[44] It is thus conceivable to state that the interpretation of diarrhoea associated with parasitic infections must be made cautiously.

In spite of the high prevalence (25%) of intestinal parasitosis in HIV patients, there are currently no clear guidelines that require its diagnosis. Moreover, the high burden of intestinal parasitosis results in diarrhea and weight loss which are independent predictors of mortality in HIV patients. In order for HIV patients to obtain comprehensive healthcare, it is recommended that efforts are made towards diagnosing intestinal parasites in HIV patients especially those with CD4+ T cell counts less than 50 cells/µL.[45]

COMPETING INTEREST

The authors declare no competing interest.

AUTHOR'S CONTRIBUTION

SCKT and KB conceived the study, participated in its design, supervised the field work, data analysis and drafted the manuscript; EOA conducted the field and laboratory data collection and performed the experiments. All authors read and approved the final manuscript.

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