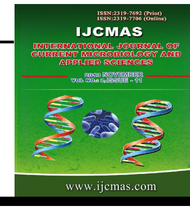
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 11 (2013) pp. 539-550**
http://www.ijcmas.com



Original Research Article

Intestinal parasitic infestations among people living with HIV/AIDS in Nsukka, Southeast Nigeria

Dibua, Uju Marie-Esther[1], Ejere, Vincent[2], Lijoka, Ilemobayo[3] and Uma Akunnaya[4]

[1, 3, 4]Department of Microbiology, University of Nigeria, Nsukka, Nigeria
[2]Department of Zoology, University of Nigeria, Nsukka
*****Corresponding author

A B S T R A C T

Keywords

Gastrointestinal parasitoses; Prevalence; Human immuno-deficiency virus; morbidity and mortality rates.

To investigate the prevalence of and co-infectivity paradigm of parasitic opportunistic infections and their demographics among HIV positive individuals. Case study of 100 individuals screened for HIV 1 and 2 antibodies using the Enzyme Linked Immunosobent Assay (ELISA) at two major HIV Referral Centres in Nsukka was undertaken. Fifty of the HIV seropositive persons were screened for intestinal parasites using the Formol-ether concentration technique. Data was collected on pre-designed proforms and analysed using SPSS 17.0 (Chicago, USA). Gender profile of HIV infection indicated preponderance among the females (66%). Co-infection of HIV and parasitic infections was established: prevalence was significant among different occupational groups; highest mong drivers and traders (100%), with no age difference or bias ($P<0.001$). Implicated risk factors included ignorance/illiteracy, poor sources of potable-water and unhygienic lifestyle including indiscriminate disposal of sewage and domestic wastes ($P<0.05$). The most predominant debilitating parasitic infections among the dually infected were *Ascaris lumbricoides (*28%) and hookworm (20%). No significant difference was observed in the rate of infection by *Trichuris trichuria* (4%) and *Giardia limbia* (4%). Prevalence of, and public health importance of parasitic infections as serious AIDS-defining condition which need urgent attention in the rural communities of Nigeria is here underscored. The study further advocates the incorporation of stringent screening for intestinal parasites in the management of people living with HIV/AIDS in resource poor communities of Nigeria and the entire sub-Saharan Africa where poverty and poor living standards predispose to severe coinfection of HIV and parasitic opportunistic pathogens.

Introduction

HIV-immunesuppression is very closely associated with severe gastrointestinal parasitic infestations; highly debilitating opportunistic infections particularly common in the developing countries of the sub- Saharan Africa, with the highest concentration of HIV and associated morbidities. Co-infection of HIV with parasitic infections dramatically enhance progressive decline of the immune system, causing a more rapid progression to AIDS, as a result of more rapid decline of the $CD4^+$ T-lymphocyte counts which characteristically falls below 200 cells/ml (Morris *et al*., 2004; Ramakrishnan *et al*., 2007) Such co-infections, generally, are the proximate cause of death of AIDS patients WHO, 2002; Chan *et al*., 1994).

Parasitic infections are the hallmark of HIV disease epecially in the rural and resource poor communities of Nigeria, and these pose serious public health threat as previously reported by several authors who presented varying prevalence rates depending on their geographical locations and nature of their surveys (Dibua *et al*., 2007; Okodua *et al*., 2003; Adesiji *et al*., 2007).

One of the major debilitating conditions associated with severe immune suppression is diarrhoea, caused by several intestinal parasites. It is defined as loose, watery stools bowel movements, sometimes with unusual colours occurring more than three times in one day is a common problem associated with parasitic infestation.[8] Implicated gastrointestinal parasites in diarrheal condition include: *Cryptosporidium parvum, Isospora belli, Microsporidia* species, *Giardia intestinalis, Entamoeba histolytica*, Cyclospora species, Others include Nematodes: *Strongyloides stercoralis.*[9] Presenting clinical conditions associated with diarrhoea include weight loss, iron deficiency anaemia. These conditions usually assume more extraordinarily debilitating proportions following immune suppression and the resultant decline in the $CD4^+$ T-lymphocytes.

There is a dearth of information on the prevalence of gastrointestinal parasitosis among HIV-positive patients in Nsukka. In spite of the daily increasing incidence of gastrointestinal disorders in the Nsukka metropolis, little or no studies have been carried out to correlate the emerging incidence of life threatening infestations (with parasitic pathogens) with the regularly reported cases of diarrhoea and the associated anaemia, malnutrition, weight loss, intestinal obstruction and other HIV/AIDS associated gastrointestinal disorders. It is against this background, that this study, which investigated the prevalence of gastrointestinal intestinal parasitic infections in relation to clinical manifestations, demographics, and immune status. The study further aims at determining the level or prevalence of these parasites and the relationship between clinical findings and the laboratory diagnosis of these parasites in Nsukka where the socioeconomic, socio-cultural and agricultural practices of the indigenes, the ecosystem degradation resulting from erosion as well as the constraint of inadequate water and the associated poor sanitary and hygienic conditions predispose the inhabitants to intestinal parasitic infections. The findings would serve as reference data to health personnel in the community and environs constrained by trained personnel and good laboratory facilities in the course of management (Dibua *et al*., 2007).

Materials and Methods

Study Population and Design

This was a cross sectional study of 100 patients at two HIV Referral Centres in Nsukka:

the District Hospital in Enugu-Ezike, Igbo-Etiti Local Government Area (LGA), and Shanahan Hospital in Nsukka metropolis. The hospitals are centres for HIV/AIDS management, with a referral status and also serve as medical centres for the local communities of Nsukka and Igbo-Etiti LG and the neighbouring towns in Kogi State. The inhabitants of these communities are mostly farmers and traders with very minimal income per capita. Patients between 8 to 71 years attending the clinics for HIV voluntary testing and counselling (VTC) as well as others on routine medical check or treatment were recruited into the study between January 2011 and June 2012.

Ethical Consent

Verbal informed consent was elicited from the volunteer participants or their guardians (for those below 18 years of age) to whom the nature and significance of the study was explained before inclusion in the study. Reasons given by participants for preference of verbal consent were for fear of societal rejection or stigmatization; no participant or guardian wanted documented evidence of seropositive HIV status. However, discretion was used in data collection and handling including careful recording in which each specimen was carefully coded.

The study was carried out according to the Declaration of Helsinki (World Medical Association and Council for International Organizations of Medical Sciences (CIOMS), and the International Guidelines for Human Experimentation in Clinical Research, as well as due permission from the Research Ethics Committee of the University of Nigeria, and the Ethical Board of the participating hospitals.

Sampling Procedure

Stool sample were collected from the general population, including people living with HIV/AIDS and non-infected persons (the control group). Participation was voluntary, and bio-data such as age, gender, educational level, occupation, marital status, was collected from their hospital records following history taking, oral discussion and questionnaire sessions.

Socio-demographic Data

A pre-designed structured questionnaire was employed in collecting the socio-demographic characteristics of the subjects.

Sample Analysis

HIV Screening: Detection of HIV 1 and 2 antibodies

One hundred (100) participants were screened for HIV. Antibodies to HIV 1 and 2 were determined by abridged Enzyme Linked Immunosorbent Assay (ELISA) using commercially available abridged ELISA Kits: (ACON HIV ½, ACON Diagnostics' USA REF HH - 401, Bio System, USA N0 098 KE) and confirmed by a second stage confirmatory tests of two - three rapid test kits with different principles (Capillus HIV ½ Assay, Trinity Biotech Ireland and Determine kit list No 7D 23-43, Abbot Japan Co. Ltd) of antibodies and antigen testing methods as recommended by WHO for resource low countries including Nigeria at 99.7% Confidence Intervals. [10]

Stool Collection and Analysis

Fresh faecal samples were collected from participants in sterile open-mouthed universal containers and analyzed within

24h of collection. Saline and iodine wet preparations, modified Ziehl Neelseen staining technique and microscopy were carried out using standard procedures as described (Akinbo *et al*., 2010). Concentration of stool was carried out using modified formol - ether concentration method (Dibua *et al*., 2007). Details of the tests are indicated below.

Macroscopic examination

Preliminary macroscopic examination of samples was carried out to determine the colour, consistency and/or texture as well as presence of blood, mucus, pus and worms in stool, samples.

Wet Mount

Aliquots of stool samples were examined for presence of trophozoites, cysts, oocysts, larvae and ova of intestinal parasites using normal saline and Lugol's iodine smear.

Formol – ether Concentration Technique

The rapid formol ether concentration techniques which involves the removal of large debris as well as the concentration of wide range of parasite with minimum damage to their morphology was used to detect cysts, oocysts and ova. Further concentration and extraction of parasites was carried out using modified Zeihl - Neelsen technique.

Data Analysis

Result of oral discussions and questionnaire responses were analyzed using the software SPSS (version 17.0; Chicago, USA) and reported as percentile ratios. Relationship between different variables in the questionnaire responses was analyzed by the Chi-square test at P = 0.05 using SPSS, while available results of the laboratory screening were presented as frequencies and percentages; a p value of < 0.05 was also considered statistically significant.

Result and Discussion

Results of HIV Screening

Out of the one hundred patients sampled, 50 were seropositive for HIV/AIDS antibodies: females (66%), males (34%); with preponderance on the females. Seronegative individuals included: females 28%, and males, 36% (Table 1).

Coinfection of Intestinal Parasites among HIV infected Persons

Percentage distribution of intestinal parasitosis among HIV infected persons in the surveyed area indicated the preponderance of *Ascaris lumbricoides* (26%), Hookworm (18%) and *Entamoeba coli* (10%) ($p<0.05$). However there was no significant difference in the observed rates of infection by *Trichuris trichuria* (4%) and *Giardia limbia* (4%). Similarly, the difference between the established rate of infection with *Schistosomia mansoni* (6%) and *Cryptosporidium parvum* (8%) were not statistically significant ($p>0.05$). *Strongyloides stercoralis* (2%) had the least prevalence rate (Figure 1).

Investigation of gender distribution of HIV and urban parasitaemia indicated a high prevalence of *Ascaris lumbricoides* among the males, (20%). However, no significant statistical difference was established in the rates of infection of the parasites between both sexes (13.33%) ($p<0.05$) (Table 2).

Table.1 Profile of hiv infected individuals in surveyed population

Number tested	HIV Seropositive			HIV Seronegative		
100	**Females**	**Males**	**Total**	**Females**	**Males**	**Total**
	33 (66%)	**17 (34%)**	**50**	**14 (28%)**	**36 (72%)**	**50**

Figure.1 Profile of HIV and intestinal parasites co-infection in surveyed area
(No positive for HIV=50)

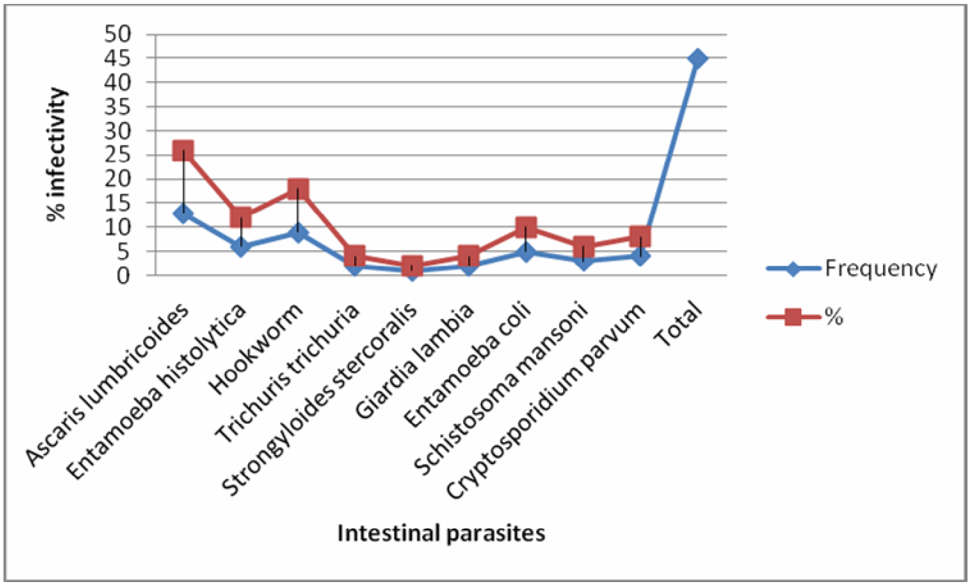


Table.2 Gender distribution of urban parasitaemia

Parasite	No co-infected	M (%)	F (%)
Ascaris lumbricoides	5	3 (20)	2 (13.33)
Entamoeba histolytica	3	1 (6.67)	2 (13.33)
Hookworm	2	2 (13.33)	-
Trichuris trichuria	-	-	-
Strongyloides stercoralis	1	1 (6.67)	-
Giardia lambia	-	-	-
Entamoeba coli	2	1 (6.67)	1 (6.67)
Schistosoma mansoni	-	-	-
Cryptosporidium parvum	2	2 (13.3)	0
Total	15	10	5

While infestation by *Ascaris lumbricoides* was prevalent among the co-infected males in the rural areas of Nsukka (20%), there was an observed prevalence of Hookworm (6.67%) and *Ascaris lumbricoides* (6.67%) in co-infected females. Least prevalence rates occurred in the infestation by *Giardia lambia* and *Trichuris trichuria* in males (3.33%). Similarly, in females, there was no significant difference in the rate of infestation with *Entamoeba histolytica* (3.33%), *Entamoeba coli* (3.33%) and *Schistosoma mansoni* (3.33%) ($P<0.05$). *Strongyloides starcoralis* was not observed for both males and females in the rural communities ($p>0.05$). *Cryptosporidium parvum* was not also observed among the female population within the rural environment (Table 3).

In relation to age bracket, HIV and parasitic infestation was a common occurrence in the population studied. Highest prevalence of HIV and parasitosis was however observed among the age groups; 21-30, 51-60 and >61 (100%), and in those aged 31-40 years (75%). Nonetheless, the difference in the observed prevalence rates was not statistically significant (Figure 2).

Preponderance of co-infection in the different occupational groups was observed among Drivers (100%) and Traders (100%). No significant statistical difference was recorded in the prevalence rates among students (92%) and civil servant (91%). Prevalence was however high among house- wives (80%) ($p>0.05$) Nevertheless, low prevalence rate was recorded among farmers (75%) ($p>0.05$) (Figure 3).

The observation that intestinal parasitic infection constitutes a serious AIDS-defining clinical condition with gastro-intestinal involvement, with resultant diarrhoea abdominal pains, dysentery, weight loss etc. is elucidated below. *Ascaris lumbricoides*, causative agent of diarrhoea, abdominal pain, constipation, vomiting ,weight loss and intestinal disorder was observed in individuals in the different age groups with 0-10 (30%)>11-20(23%)>41-50(15%). *Entaomeba histolytica* , agent of dysentery and epi-gastric pain was observed in all but these age groups (21-30), (41-50), (51-60). Moreover, Hookworm causative agent of severe anaemia, abdominal pain, mental inertia, debility, weight loss and retarded growth was shown to affect all age brackets except (11-20), (21-30). Nonetheless, the least observed parasites include *Trichuris trichuria, Strongyloides stercoralis, Giardia lambia.*

Clinical symptoms associated with identified parasitic infestation among the dually infected persons, and the distribution of parasites among different age groups are shown in Table 4. *Ascaris lumbricoides* had the higest prevalence, infecting 13 of the 45 individuals (28%); 9 others had hookworm (20%), 6 had *Entamoeba histolytica (13%)*, 5 had *Entamoeba coli (11%). Stongyloides starcoralis* was the least (1 person (2%)). *Ascaris lumbricoides* was more prevalent among those aged 0-10 years old with a prevalence of 30% and 11-20 years old had a prevalence of 23% respectively. *Entamoeba histolytica* in age group >61 had a prevalence of 50% among those co-infected. In age bracket 41-50 years, hookworm had a percentage prevalence of 33%. However, no significant statistical difference was found in the prevalence of *Ascaris lumbricoides* among the 0-10 and 11-20 age bracket (8.89% and 6.67% respectively) ($p>0.05$). Diarrhoea,

Table.3 Gender distribution of rural parasitaemia

Parasite	No infected	M (%)	F (%)
Ascaris lumbricoides	8	6 (20)	2 (6.67)
Entamoeba histolytica	3	2 (6.67)	1 (3.33)
Hookworm	7	5 (16.67)	2 (6.67)
Trichuris trichuria	2	1 (3.33)	1 (3.33)
Strongyloides stercoralis	-	-	-
Giardia lambia	2	1 (3.33)	1 (3.33)
Entamoeba coli	3	2 (6.67)	1 (3.33)
Schistosoma mansoni	3	2 (6.67)	1 (3.33)
Cryptosporidium parvum	2	2 (6.67)	-
Total	30	21	9

Figure.2 Age paradigm of HIV/Intestinal parasite co-infection

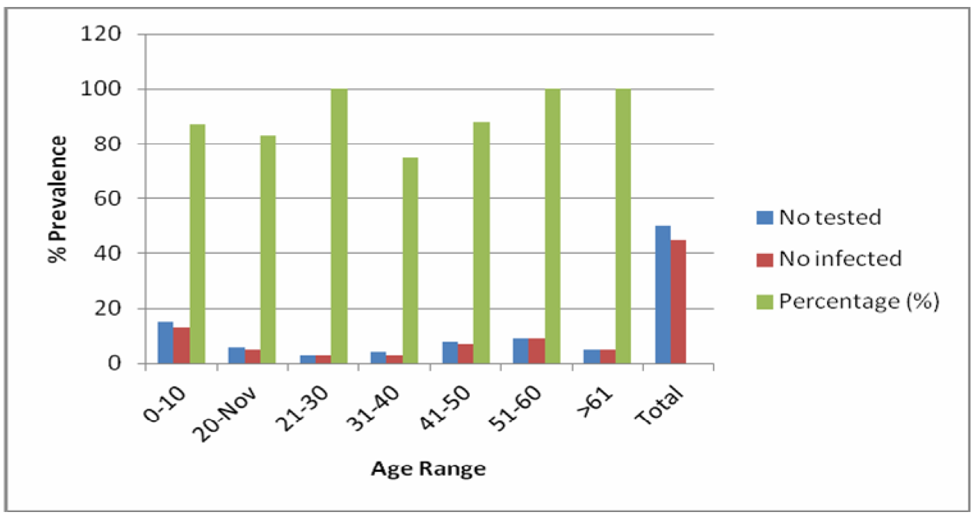


Figure.3 Profile of occupational distribution of coinfection with HIV and parasitic infection

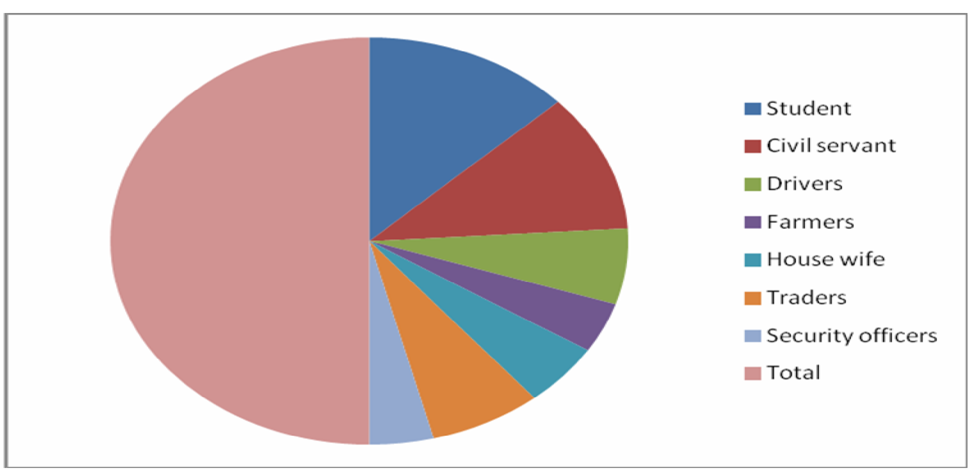


Table.4 Clinical presenting symptoms of intestinal parasitoses among the study group and the implicated Parasites

Parasites	**Age range**								
Infecting parasites	**0-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**>61**	**Total infected**	**Associated clinical symptoms**
Ascaris lumbricoides	4	3	1	1	2	1	1	13	Diarrhoea, abdominal pain, constipation, vomiting, weight loss and intestinal disorder
Entamoeba histolytica	1	1	-	1	-	-	3	6	Dysentery and epi-gastric pain
Hookworm	2	-	-	1	3	2	1	9	Diarrhoea, severe anaemia, abdominal pain, mental inertia, debility, weight loss and retarded growth
Trichuris trichuria	1	-	-	-	-	-	1	2	Blood tingled diarrhoea, anaemia, weakness and abdominal pain.
Strongyloides stercoralis	-	-	-	-	-	1	-	1	Itching rashes, bloody diarrhoea, nausea, anaemia, weakness and abdominal pain.
Giardia lambia	2	-	-	-	-	-	-	2	Diarrhoea, with pale fatty stools, flatulence and nausea.
Entamoeba coli	1	1	-	-	1	2	-	5	
Schistosoma mansoni	1	-	1	-	-	1	-	3	
Cryptosporidium parvum	1	-	1	-	1	1	-	4	Large volume, non bloody, watery diarrhoea, severe abdominal cramp and anaemia.
Total infected	13	5	3	3	7	9	5	45	

abdominal pains, and cramps, weight loss and anaemia were the major presenting symptoms of intestinal parasitoses observed among the study group (Table 4)

Coinfection with gastrointestinal opportunistic parasitic infestations with HIV has become a public health concern with increasing morbidity and mortality rates in the local communities of Nigeria. Parasitic infestations have thus remained important cause of gastrointestinal tract problems with associated diarrhoea and other AIDS-related abnormalities among HIV- infected persons in developing countries (WHO, 1981). The prevalence of intestinal parasites among HIV/AIDS patients in Nsukka was therefore investigated. This study demonstrated the vulnerability and/or susceptibility of HIV patients to a mirage of intestinal parasites due particularly to their reduced immune response which makes them more susceptible to these infections, and further suggests that the increasing incidence of intestinal parasitemia in Nsukka rural and urban communities are largely due to the lack of safe, portable drinking water, poor hygienic and sanitary conditions as previously reported (Dibua *et al*., 2007). In addition, we observed that HIV infection was a significant risk factor for acquiring an intestinal parasitic infection; a relationship was observed to exist between the nature of infecting parasites, the parasitic load and severity of presenting clinical signs and symptoms. This is because HIV infection leads to loss of $CD4^{+}$T cells, which leaves affected individuals mortally susceptible to opportunistic infections, especially gastrointestinal problems which often present as diarrhoea and weight loss syndrome, which significantly enhance progression of HIV disease to AIDS. This view is in consonance with previous findings (Oguntibeju, 2006; Kuppamattus *et al*., 2007).

It is important to note that the prevalence (90%; 45 out of 50) observed among HIV-infected patients in this study is not in agreement with the 15.3% and11.4% reported by some authors in Benin, and Ethopia (Akinbo *et al*., 2010; Mohammad *et al*., 2004). However, other investigators reported 42.9% in Abeokuta, Nigeria and 84.3% in South Africa (Udeh *et al*., 2008; Zelalem *et al*., 2008). The difference could be due to sample size as our study presented small sample size than that reported by other authors. Gender significantly affected the prevalence of intestinal parasitic infections among HIV-infected patients. This finding is inconsistent with previous reports (Mohammad *et al*., 2004). The reason for this association between gender and intestinal parasites may be adduced to more males being exposed than females based on occupational grounds.

An interactive synergy was thus established between gastrointestinal parasites and HIV: while parasitic infestation can cause drastic suppression of the immune system, probably as part of the mechanism by which they protect themselves against host immune responses, damaged intestinal walls, thus enhance viral entry and multiplication. In addition, parasites and associated intestinal damage can cause malabsorption and resultant malnutrition, which further weaken the immune system. Cellular immunity is the major defence against intestinal parasitic infections (Omalu *et al*., 2005). Therefore, the reduction in CD4 count by the HIV virus predisposes HIV-infected patients to opportunistic intestinal parasitic infections (Wiwanitkit, 2001). It is generally accepted that a CD4 count below 200cells/ml predisposes HIV-infected persons to opportunistic infections (Lee et *al*., 2005). In HIV infection, diarrhoea is a major sign of progression to AIDS, which results from opportunistic infections, and this may explain the findings in this study, which demonstrated the significant association of diarrhoea with intestinal parasitic infections among the observed HIV-positive patients. Others reported similar findings (Endeshaw *et al*., 2004). One of the observed major risk factors of parasitic infestations in HIV patients was participants' occupation; this significantly affected the prevalence of intestinal parasitic infections, with Traders and Drivers having the highest prevalence (100% each). Traders and Drivers 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 explain the observed high prevalence in this group.

Result of the oral discussions and questionnaire responses demonstrated the significant relationship between parasitic infections and sources of domestic water supply; consisting of streams and rivers that are of common use among the local populace are places for bathing, defecating, and washing, in addition to the poorly treated municipal water supply in the area all of which constitute likely sources of intestinal parasitic infections. ($P < 05$). This may explain, in part, the findings of the present study and in agreement with other studies (Akinbo *et al*., 2010). However, contrary to our findings, other researcher] indicated that the source of water did not affect the prevalence of intestinal parasitic infections (Endeshaw *et al*., 2003). HIV-positive patients. A total of 9 intestinal parasites were detected in HIV infected individuals, with *A. lumbricoides* being the most prevalent (26%). Other workers also reported *A. lumbricoides* as the most prevalent intestinal parasite in HIV-infected patients (Oguntibeju, 2006; Wiwanitkit, 2001). The presence of pathogenic intestinal parasites such as *A. lumbricoides*, hookworm, *E. histolytica*, *T. trichiura*, and *S. Mansoni* and *E. coli* among HIV-infected persons are remarkably significant; *Cryptosporidium spp*, *Microsporidium spp*, *Cyclospora spp*, and *I. Belli* are opportunistic infections that have been severally reported among HIV-infected persons (Awole *et al*., 2003). In this present investigation, *Strongyloides starcoralis* was similarly reported as an important opportunistic intestinal parasitic infection observed, contrary to other reports (Oguntibeju, 2006; Zelalem *et al*., 2008; Omalu *et al*., 2005; Guptal *et al*., 2005). The prevalence of hookworm (18%) observed in this study was within the range that was previously reported (Okodua *et al*., 2003; Guptal *et al*., 2008; Oguntibeju *et al*., 2006). However, others (Zelalem *et al*., 2008) 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 (Oguntibeju, 2006; Oguntibeju *et al*., 2006). Furthermore, a high prevalence of *E. histolytica* was observed in this research (12%) as was previously reported in others studies (Oguntibeju, 2006; Zelalem *et al*., 2008; Guptal *et al*., 2008). The prevalence of *Giardia lambia* (4%) observed in this study was lower than that previously reported (Mohandas *et al*., 2002). Males had a higher prevalence (20%) of *A. lumbricoides* than their female counterparts (13.33%) among HIV-infected persons, though this was not statistically significant. This finding is contrary to previous work (Okodua *et al*., 2003), but in consonance with other reports (Mohandas *et al*., 2002). Preponderance of the parasite among males could be attributed to the higher number of males surveyed.

In relation to the urban and rural parasitic infestation difference, great significance was observed between prevalence in the rural (66.7%) and the urban (33.3%) areas. The higher difference in rural prevalence could be attributed to the highly unhygienic and/or less sanitary environment and poverty amongst other factors. There was a significant relationship between infection status with respondents sources of water ($P < 0.05$). While our study did not show any relationship between nutritional indicators and protozoan parasites such as *G. lamblia* and *E.histolytica*, similar studies showed a significant association between *G. lamblia* and nutritional status (Carvalho-costa *et al*., 2009). According to them, the social, economic and physical environment in which an individual lives are major determinants of the degree of association between intestinal parasites and nutritional status. These factors might be responsible for the differences observed in this study. There was however, a greater prevalence of infection by *Ascaris* (26%) than any other infectious intestinal parasite, the second most prevalent being Hookworm (18%). The persistence and prevalence of these intestinal parasites could be attributed to the general level of poor sanitary conditions especially from faecally polluted water bodies for domestic and agricultural purposes extensive use of pit toilet and surface latrines system in both the rural and urban communities. Other factors that could have contributed to their prevalence could be the problem of poor drainage system common to Nsukka. Nsukka has the poor habit of emptying refuse into the gutter as a result of poor sanitary inspection programs. 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.

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