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Research Article

Prevalence and Associated Risk Factors of Opportunistic Intestinal Parasites among HIV Positive and Negative Individuals in South Ethiopia: A Case Control Study

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Abstract

Introduction: *Cryptosporidium*, *Isospora* and *Cyclospora* are the most known opportunistic intestinal infections that frequently causes diarrhea in patients with AIDS.

Methods: A case-control study was conducted among of 273 subjects (139 HIV positive and 134 HIV negative) from March to June, 2011. Questionnaire and clinical document reviews were utilized to collect data. Stool samples were examined by direct saline, and formol-ether sedimentation and modified Ziehl-Neelsen staining techniques. The level of significance was set at P value of 0.05.

Result: Accordingly, intestinal parasites were observed in 42.5% and 42.5% of HIV positive and negative subjects, respectively. Nine types of intestinal parasites were identified in HIV positive individuals. Two of these, *Cryptosporidium* spp. (9.4%) and *Isospora belli* (5%) were opportunistic parasites while the remaining were non-opportunistic. The overall prevalence of opportunistic intestinal parasites was significantly ($P < 0.05$) higher among HIV positive than negative subjects. This study signified a significant ($P < 0.001$) correlation between the immune status of HIV positive subjects and enteric parasitic infection. Among the risk factors, only contact with animals was found to be significantly ($P < 0.05$) associated with *Cryptosporidium spp* infection in HIV positive patients.

Conclusion: Opportunistic intestinal parasites were more common in HIV positive than HIV negative subjects and that they were associated with diarrhea and low immune status. In addition, contact with animals was an important risk factor for opportunistic intestinal parasites. Therefore routine screening of HIV patients for these parasites and avoiding contact with animals is essential for effective prevention of opportunistic parasites infection.

Keywords: Opportunistic parasites; *Cryptosporidium spp*; *Isospora belli*; Risk factors.

Introduction

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 acquired immunodeficiency syndrome (AIDS) cases are concentrated [1].

HIV/AIDS compromises the immunity of the body. This predisposes to various opportunistic infections of viral, bacterial, fungal and parasitic origins. *Cryptosporidium parvum*, *Isospora belli* and *Cyclospora cayetanensis* are the most frequent causes of diarrhea and they are well documented in patients with AIDS [2].

Environmental contamination with protozoan occurs through water and food-borne transmissions are the major risk factor of the community as well as the immunocompromised individuals [2]. HIV-positive patient who owned dogs had two fold higher risk of cryptosporidiosis and HIV positive patients who owned cats and consumption fecal contaminated water and food also at risk for isosporiasis. On the other hand, children with diarrhea, contact with farm animals especially calves, swimming in public swimming pool are also risk factors of cryptosporidiosis [3,4].

In Ethiopia, even though the prevalence of opportunistic parasites is expected to be high due to poor living standard and spread of HIV/AIDS, there are only few published data on the subject matter. The available information suggests that infection with opportunistic intestinal parasites such as *Cryptosporidium*, *Isospora* and *Cyclospora* are common in HIV positive and negative individuals from different parts of the country [5-9]. Most of the studies carried out so far concentrated only on certain parts of the country and there is paucity of information especially in Southern, Nations, Nationalities and Peoples' Regional State. The present study was, therefore, designed to determine the prevalence of opportunistic intestinal parasites in people with and without HIV infection and also their association with diarrhea, immune status and suspected risk factors in Yirgalem Hospital.

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Methods

Study area

The study was conducted in Yirgalem Hospital which is located at a distance of 347km south of Addis Ababa, the capital of Ethiopia.

Study design and population

This was a case-control study which carried out from March to June 2011. The study population consisted of selected patients with confirmed HIV sero activity who had not received any anti protozoal or anti retroviral treatment within 3 weeks prior to sampling and an equal number of HIV negative controls who didn't taken any medication prior to sampling were selected from patients came to Yirgalem Hospital in search of to know their HIV status and to enable health care providers offer specific medical services.

The study participants were selected by simple random sampling technique from those individuals visiting Yirgalem hospital for antiretroviral therapy, HIV test and other medication. Initially 300 patients were enrolled (150 HIV positive and 150 HIV negative); however, those participated in the study were 273 (139 HIV positive and 134 HIV negative). This is because 11 of the selected HIV positive patients were withdrawn from the study as they had taken anti-retroviral treatment during sampling and 16 of the HIV negative patients were not willing to give stool sample though they filled up the questionnaire.

Sample collection and processing

A single fresh stool sample was collected with leak proof and tightly cupped sterile stool cup. A portion of the sample was used for direct microscopy to detect the presence of motile parasites and trophozoites while the rest of the sample was preserved in 10% formalin. The sample was concentrated by formalin-ether sedimentation for the detection of ova. The sample was also subjected to modified ziehl-Neelsen technique for intestinal coccidian identification [10].

Socio-demographic and clinical information were obtained from the study participants by questionnaires. Data about $CD4^+$ T-cell counts for HIV positive participants were collected from their medical records at the hospital. $CD4^+$ T-cell counts were considered only when it is done at the time of or very close to stool sample collection. The HIV seropositives study subjects were further divided in to three immune status categories based on their $CD4^+$ T- cell counts according to CDC, HIV Surveillance Supplemental Report [11].

Data analysis

The data were entered in to Microsoft excel spreadsheet and analyzed using STATA version 11 and EpiCalc 2000 Version 1.02 statistical software's. The association between the prevalence of intestinal parasites and the presumed risk factors was analyzed using univariable logistic regression. Factors found significant in the univariable analysis were entered to multivariable regression model. Furthermore, Chi–square (χ^2) and Fisher's exact tests were also used to evaluate the differences between proportions. Student's t-test was used for the comparison of mean $CD4^+$ T-cell counts between groups. All p-values < 0.05 at 95% confidence interval were considered for significance.

Ethical considerations

The Ethical Review Committee of College of Health and Medicine, Hawassa University was approved the study proposal. Prior to the enrolment into the study, an informed written consent was obtained from all people involved in the study.

Results

Socio-demographic characteristics

The socio-demographic characteristics of the study participants has been described in Table 1. From the total of 273 study participants, 57.1% were males and 42.9% were females; 52% lived in rural areas while 48% in urban areas and 44.3% were aged less than 30 and 55.7% above 30. The mean age of the study participants was 35.5 year (ranged: 16 to 70 years). Based on their educational status, a higher proportion (45.8%) of them were illiterate, about 40% had attended primary or secondary education and the remaining 13.9% were diploma and degree graduates. Occupationally, farmers occupied the greatest proportion (44.3%) while students comprised the smallest (8.8%).

Prevalence of intestinal parasites

Parasitological stool examination showed that infections with various protozoan parasites and intestinal helminthes were common in both HIV positive and HIV negative study participants. Of the 139 HIV positive study participants, 59 (42. 5%) were infected with one or more intestinal parasites. Similarly, 57 (42.5%) individuals from the 134 HIV negative study participants had one or more intestinal parasites. The overall prevalence of parasitic infection showed no significant ($P > 0.05$) difference between HIV positive and HIV negative individuals included in this study. Nine different types of intestinal parasites were detected from the HIV seropositives. Of which, five were helminthes *(Ascaris lumbricoids, Hook worms*, *Strongyloides stercolaris*, *Trichuris trichuria* and *Taenia* spp) whereas the remaining four were protozoan parasites (*Cryptosporidium spp*, *Isospora belli*, *Giardia lamblia,* and *Entamoeba histolytica/E. dispar*). Also, the same spectrum of intestinal parasites with the exception of *Isospora belli* and *Strongyloides stercolaris* were recovered from the HIV seronegatives. Protozoan parasitic infection were significantly ($P < 0.05$) higher in HIV seropositives than HIV seronegatives and this difference was mainly attributed to *Cryptosporidium spp* and *I. belli*. In contrast, no significant ($P > 0.05$) difference was observed in the prevalence of helminthes infection between HIV positive and HIV negative individuals (Table 2).

Table 1: Socio-demographic characteristics of the study participants (N=273) Yirgalem, 2011

Socio demographic variables		HIV sero status Sero positive, (%) (n=139)	Sero negative, (%) (n=134)	Total	Percentage (%)
Sex	Female	66(47.58)	51(38.1)	117	42.9
	Male	73(52.5)	83(61.9)	156	57.1
Age	16- 30 yrs	65(46.86)	56(41.8)	121	44.3
	> 30 yrs	74(53.2)	78(58.2)	152	55. 7
Residence	Rural	53(38.1)	89(66.4)	142	52.
	Urban	86(61.9)	45(33.6)	131	48
Education	Uneducated	41(29.5)	84(62.7)	125	45.8
	Primary	39(28.1)	18(13.4)	57	20.9
	Secondary	39(28.1)	14(10.4)	53	19.4
	Diploma & degree	20(14.4)	18(13.4)	38	13.9
Occupation	Un employee	30(21.6)	8(6)	38	13.9
	Farmer	32(23.)	89(66.4)	121	44.3
	Student	6(4.3)	18(13.4)	24	8.8
	Merchant	18(12.9)	8(6)	26	9.5
	Gov. employee	24(17.3)	7(5.2)	31	11.4
	Private employee	29(20.9)	4(3)	33	12.1
Marital status	Married	105(75.5)	89(66.4)	194	71.1
	Un married	25(18)	31(23.1)	56	20.1
	Divorced	9(6.5)	14(10.4)	23	8.4

Table 2: Prevalence of opportunistic and non-opportunistic intestinal parasites in HIV positive and HIV negative individuals, Yirgalem, 2011

Type of parasite	HIV positives (N =139)		HIV negatives (N =134)		Test for differences in prevalence	
	№ Pos	Prevalence (%)	№ Pos	Prevalence (%)	χ^2	P-value
Hook worms	5	3.6	10	7.5	1.97	0.160
S.stercoralisis	5	3.6	0	0	4.91	0.027
Ascaris lumbricoids	21	15.1	29	21.6	1.94	0.164
T. trichuria	4	2. 9	4	3	0.24	0.626
Taenia spp.	2	1.4	2	1.5	0.00	0.967
Cryptosporidium spp	13	9.4	3	2.2	6.25	0.012
Isospora belli	7	5.	0	0	6.93	0.008
Giardia lamblia	18	13	16	11.9	0.06	0.800
Entamoeba histolytica/dispar	3	2.2	2	1.5	0.17	0.680
All helminthes	34	24.5	37	27.6	0.34	0.559
All protozoa	36	25.9	21	15.7	4.29	0.038
Overall	59	42.5	57	42.5	0.00	0.989

Association of intestinal parasitic infection with immune status in HIV seropositives

The immune status of the 139 HIV seropositives study subjects was assessed by measuring the CD4+ T-cell count. The overall mean CD4+ T-cell count was 318 ± 165.8 cells/μL ranging from 39 to 799 cells/μL. The t-test analysis showed that the mean CD4+ count was significantly ($P < 0.05$) lower among individuals infected with one or more intestinal parasites (mean, 245 ± 127.7 cells/μL) than among those not infected with any of the parasites (mean, 371 ± 171cells/μL) (Table 3).

Table 3: CD4+ T cell counts in HIV positive individuals with and without intestinal parasites as analyzed by t-test, Yirgalem, 2011

Parasitic infection	No	Mean CD4+± SD (cells/μL)	CD4+ Range (cells/μL)	95% CI mean (cells/μL)	t-test	P-value
Non infected	80	371±171.0	39 – 799	333 — 409		
Infected	59	245±127.7	54 – 522	212 — 278	4.77	0.0000
Total	139	318±165.8	39 – 799	290 — 346		

The prevalence of intestinal parasitic infection among HIV positive subjects with different immune status is given in Table 4. From the 139 HIV seropositives, 22 (15.8%), 85 (61.2%) and 32 (23%) had CD4 T cell counts > 500/μL, 200-499/μL and < 200/μL, respectively. A correlation between CD4+ T cell counts and parasitic infection was evident. That is, the overall prevalence of intestinal parasitic infection was significantly ($P < 0.001$) higher among HIV seropositives with low immune status (CD4+ < 200/μL) than those with better immune status.

Analysis of enteric parasites infection with hypothesized risk factors

In univariable logistic regression various hypothesized risk factors (sex, age, residence, water source presence of animals at home, educational status, toilet facility, eating raw vegetables and swimming in river) were analyzed, of these, three factors such as water source, presence of animal at home and swimming in river were found to be significantly associated with intestinal parasitic infection in HIV positive study subjects ($P < 0.05$ for each factor). However, when these factors entered into the multivariable regression, only the presence of animals at home was found to be significantly associated ($P < 0.001$). The odds of intestinal parasitic infection in HIV seropositives having contact with animals was 5.3 times higher than that of individuals who did not have contact with domestic animals (Table 5).

When opportunistic parasites alone were analyzed with different risk factors, *Cryptosporidium* and *I. belli* were significantly ($P < 0.05$ in each case) associated with only one factor, presence of animals at home and swimming in river, respectively (Table 6).

Discussion

This study demonstrated a high prevalence of intestinal parasites among HIV seropositive (42.4%) and HIV seronegative (42.5%) study subjects without a statistically significant difference ($P > 0.05$) between the two study groups. However, previous studies conducted in Ethiopia and elsewhere documented higher prevalence in HIV positives as compared with the HIV negatives [6-8,12]. The prevalence observed in HIV positive study subjects in the present study is comparable to a previous report of 44.8% from southwest Ethiopia [8]. However, it is lower than other studies in the country viz. 48% from three areas in eastern Ethiopia [6], 52.6 - 69.2% from Jimma University Hospital in southwest Ethiopia [13,14] and 59.8% from Hawassa Referral Hospital in south Ethiopia [7].

Nine types of intestinal parasites were observed in HIV positive study subjects, five of which were helminthes and the remaining four were protozoan parasites. The spectrum of parasites observed in this study is in agreement with previous studies in different parts of the country [6,13-15]. In agreement with most of the aforementioned studies in the country, *Ascaris lumbricoids* was the most frequent parasite observed in both HIV positive and HIV negative individuals in the current study. *G. lamblia* was the second most abundant parasite. *Taenia spp* was the least prevalent in the present study.

Table 5: Multivariable logistic regression analysis of enteric parasites infection with different risk factors in HIV seropositives, Yirgalem, 2011

Risk factors	Category	Odds ratio	95% CI	P- value
Water source	Tap water	1		
	Spring	2.1	0.5–9.2	0.322
	Shallow well	0.9	0.3–3.1	0.895
	River	0.3	0.1–1.6	0.163
Presence of animals at home	No	1		
	Yes	5.3	2.2–12.4	0.000
Swimming in river	No	1		
	Yes	1.1	0.5–2.8	0.890

Table 6: Analysis of the prevalence of *Cryptosporidium spp* and *Isospora belli* infection with different risk factors in HIV positive individuals, Yirgalem, 2011

Risk factors	Category	No examined	Cryptosporidium spp		Isospora belli	
			Prevalence, N (%)	P -value	Prevalence, N (%)	P- value
Sex	Female	66	7(10.61)		1(1.52)	
	Male	73	6(8.22)	0.629	6(8.22)	0.119
Age	16-30	65	9(13.85)		3(4.62)	
	>30	74	4(5.41)	0.142	4(5.41)	1.000
Residence	Rural	53	4(7.55)		5(9.43)	
	Urban	86	9(10.47)	0.766	2(2.33)	0.105
Water source	River	14	3(21.43)		1(7.14)	
	Spring	19	1(5.26)		2(10.53)	
	Shallow well	9	0		1(11.11)	
	Tape water	97	9(9.28)	0.294	3(3.09)	0.187
Presence of animals at home	No	86	3(3.49)		3(3.49)	
	Yes	53	10(18.87)	0.005	4(7.55)	0.427
Eating raw vegetables	No	44	2(4.55)		1(2.27)	
	Yes	95	11(11.58)	0.227	6(6.32)	0.432
Swimming in river	No	77	5(6.49)		1(1.30)	
	Yes	62	8(12.9)	0.219	6(9.69)	0.045

Cryptosporidium spp and *Isospora belli* were the only opportunistic intestinal parasites identified in the present study. Other opportunistic parasites reported in previous studies like *Blastocytis spp* [6,13,14,16] and *Cyclospora spp* [8]6] were not observed in the present study. This might be due to low sensitivity of the tests used or due to absence of the parasites in the study area. However this needs further confirmation.

The prevalence of *Cryptosporidium spp* was significantly ($P < 0.05$) higher in HIV seropositives (9.4%) than seronegatives (2.2%). *Cryptosporidium spp* was the predominant parasite observed in diarrheic subjects. In both HIV seropositives and seronegatives, all *Cryptosporidium spp* infections were observed only in subjects with diarrhea. No asymptomatic carriage was seen even in HIV seronegatives. Furthermore, the majority of *Cryptosporidium spp* infection (92.3%) in HIV seropositives in the current study was seen in those with low immune status ($CD4^+$ counts < 200 cells/μL). These findings are consistent with what has already been established about the parasites in several researches. It has been recognized that cryptosporidiosis is an opportunistic parasite characterized by self-limiting diarrhea in immunocompetent patients but persistent and life-threatening diarrhea in immunocompromised subjects such as HIV/AIDS patients [17]. The observed prevalence in HIV patients is lower than that of previous similar studies in Ethiopia, which have reported a higher prevalence of 11% to 26.9% [7,8,14,18]. On the other hand, the current prevalence in HIV seropositives lies within the range (3.8 % to 73.6%) reported from HIV-infected individuals in 12 countries in Africa [17].

Isospora belli was observed only in HIV seropositives. This finding is consistent with previous studies in the country [7,14] and other studies in Nigeria [19] and Thailand [12]. Most of the cases (62.5%) were recorded in subjects with CD4+ T-cell counts < 200/μL. This is in agreement with previous reports [7,9]. It has been reported that *I . belli* is one of the most common causes of diarrhoea in HIV-infected individuals [20] and endemic in tropical and subtropical regions [21]. A French study conducted to determine the incidence of *I. belli* infection in HIV-infected patients and to study risk factors, revealed that the risk of infection was significantly higher among patients from sub-Saharan Africa [22]. The present prevalence (5%) was similar to the 5% prevalence reported by one research from Ethiopia [6]. But some researches' from Ethiopia were also reported different *Isospora belli* prevalence from present studies, which some of them [7,8] have reported a higher prevalence of 7.4% and 12.2%, respectively while others [14] found a lower prevalence (3.9%) than the present. Most studies outside Ethiopia have reported a lower prevalence of *I. belli* ranging from 1.3% to 3.1% [12,19,23,24].

Like *I. belli*, *S. stercoralis* was also recorded only in HIV seropositives. This finding is consistent with the prevalence reported by one research from Ethiopia [14]; however, the present prevalence (3.6%) is lower than previous findings of 5.1% [13] and 11.5% [14] in different parts of the country. *S. stercolaris* is a well known cause of diarrheal infections but it is not clear whether the parasite is an opportunistic or not [12].

Although the rate of occurrence of non-opportunistic helminthes (*A. lumbricoides,* hookworms, *T. trichuria* and *Taenia spp*) tended to be higher in HIV seronegatives than seropositives, the differences were not significant ($P > 0.05$ for each parasite). It was further noticed that the prevalence of hookworms and *T. trichuria* was significantly higher among HIV seronegatives. Similarly, among HIV seropositives, *T. trichuria* was found only in diarrheic subjects especially in those with $CD4^+$ T-cell counts < 200cells/μL suggesting that this parasite is an important cause of diarrhea in both immunocompetent and immunocompromised individuals.

There was a significant correlation between $CD4^+$ T-cell counts and the prevalence of intestinal parasitic infections. That is, the mean $CD4^+$ count was significantly ($P < 0.0001$) lower in parasite infected (mean, 245 ± 127.7) than non-infected subjects (mean, 371 ± 171). It was demonstrated that the prevalence of intestinal parasites, except *G. lamblia, S. stercoralis, E. histolytica/dispar* and *Taenia spp*, was significantly ($P < 0.05$) higher among HIV seropositives with low immune status ($CD4^+$ < 200 cells/ μL) than in those with higher $CD4^+$ counts. Fifty seven (96.6%) of the HIV seropositives infected with the intestinal parasite had a CD4+ T-cells count below 500/μL (of which 38.6% with a count below 200). Other studies have also reported a significantly ($P<0.05$) higher prevalence of intestinal parasites in subjects with low CD4+ T-cell counts [23-25].

Regarding the risk factors for acquiring intestinal parasites, about nine hypothesized factors were assessed in HIV seropositives, out of which three variables: drinking water source, swimming in river and contact with animals were observed to be significant in the univariable logistic regression analysis. However, only contact with domestic animals was found to be significant in the final multivariable logistic regression analysis. When each opportunistic parasite was analyzed separately this was true only for *Cryptosporidium spp.* infection. In other similar study, residence in slum, public toilet and homosexual were reported as risk factors for enteric parasites in addition to contact with domestic animals [23]. Hunter [4] also reported that children with diarrhea, contact with farm animals especially calves, swimming in public swimming pool are also risk factors of cryptosporidiosis. On the other hand, swimming in river was the determined risk factor for acquiring isosporiasis in this study. According to Goodgame [3], poor sanitation and fecal (cat feces) contaminated food and water are the most likely sources of infection for *Isospora belli*, but waterborne transmission has not been confirmed.

Conclusion

The prevalence of opportunistic parasites, *Cryptosporidium spp* and *Isospora belli* was significantly higher among HIV positive than HIV negative individuals. The entire *Cryptosporidium spp.* infection was recorded in patients with lower $CD4^+$ T-cell counts. Besides those opportunistic parasites, the non opportunistic parasite *Strongyloides stercoraris* was also more common in HIV/AIDS groups.

Assessment of the immune status of HIV/AIDS patients showed a significant correlation between $CD4^+$ T-cells count and intestinal parasitic infection. The prevalence of enteric parasites except few non opportunistic ones was significantly higher in patients with low immune status. About 96.6% of HIV infected subjects with intestinal parasites had $CD4^+$ counts bellow 500 cells/μL.

Among the hypothesized risk factors, exposure to domestic animals was the single factor found to be associated with increased risk of *Cryptosporidium spp.* infection among HIV positive patients. Swimming in river was also the associated risk factor for *Isospora belli* infection.

In light of the above findings, it is recommended that proper diagnosis and treatment of those opportunistic and non opportunistic parasites are important to reduce the associated morbidity and mortality especially in HIV positive individuals. As there is no effective drug and vaccine for cryptosporidiosis, people with HIV should be advised how to avoid infection, including the potential benefits of drinking boiled water and avoiding contact with animals. A further longitudinal study with larger sample size is needed to generate a complete data set about the type and distribution of opportunistic and non opportunistic intestinal parasites in the study area.

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