

Causes for diarrhoea and infection with intestinal parasites among HIV positive patients at a referral hospital in Central Ethiopia

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Research article

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Abstract

Background: Intestinal parasitic infections are a major public health challenge in many tropical countries. Opportunistic intestinal coccidia such as *Cryptosporidia*, *Cytoisospora* or *Cyclospora* species are common pathogens which are regularly missed using widely practiced wet mount stool microscopy techniques. Therefore, treatment choices are limited and mostly rely on empirical use of cotrimoxazole. The aim of this study was to determine the prevalence of intestinal parasitosis among HIV-infected individuals with and without diarrhoea at the Asella Teaching and Referral Hospital in Ethiopia. Methods: This institution-based cross sectional study was conducted among 163 ambulatory HIV-infected patients with and without diarrhoea. Stool samples were processed for both wet mount and Kinyoun stain. EDTA blood was collected for analysis of CD4 cell count using BD FACSCount™ Flow Cytometer. Sociodemographic and behavioural data was collected using a standardized questionnaire. Chi-squared test was used for statistical analysis. Results: The majority of study participants (62.0%, n=101) were female and the mean age was 38.2 (SD +10.7) years. 52.1% (n=85) of the participants suffered from diarrhoea. The overall prevalence of intestinal parasitic infection in the study population was 18.4% (n=30). Protozoa (*Cryptosporidium* spp., *E. histolytica*, *G. lamblia* and *Pentatrichomonas hominis*) and helminths (*Taenia* spp., *A. lumbricoides*, *S. stercoralis*, *T. trichuria* and *H. nana*) were detected in 12.9% (n=21) and 5.5% (n=9) of patients, respectively. The likelihood for having a parasitic infection was more than eight times higher in participants having diarrhoea. No oocysts of coccidian parasites were detected in the routinely performed wet mount stool microscopy, as expected. Conclusions: There was a high prevalence of opportunistic intestinal parasitic infection in the studied population. Considering the clinical relevance of opportunistic infections particularly in individuals with low CD4 cell count and diarrhoea, the implementation of both stool concentration and modified acid fast staining techniques should be considered to enhance the quality of health care service for HIV-infected patients in resource-limited settings as Ethiopia.

Background

Intestinal parasitic infections are a major public health challenge in many tropical countries. Frequently, the pathogen causing parasitic enteritis is not identified in resource-limited health care systems with restricted diagnostic capacities. Opportunistic intestinal coccidia such as *Cryptosporidia*, *Cystoisospora* or *Cyclospora* species are common causes of those infections which are regularly missed using widely available wet mount stool microscopy techniques. In general, patients with an impaired immune response, primarily due to a Human Immunodeficiency Virus (HIV) infection in this study setting, are at greater risk for developing severe and chronic infections (1). Opportunistic parasite-related intestinal infections are the most common reasons for the development of diarrhoea in HIV-infected individuals; causing about half of the cases of diarrhoeal disease within this group of patients (2). Once

symptomatic, they often lead to significant impairments in quality of life or even death (3,4). In addition, cryptosporidiosis and cystoisosporidiosis are considered AIDS-defining illnesses.

In Ethiopia, the prevalence of opportunistic parasitic infections among HIV-infected patients is likely to be under-estimated, as only wet mount microscopy of stool samples is routinely performed in health care facilities. Therefore, choices for treatment of wet mount microscopy negative diarrhoeal disease in HIV-infected patients are limited and mostly rely on empirical use of cotrimoxazole.

Regional differences in the frequency of causing pathogens are to be expected. Epidemiological evaluation of disease prevalence in specific populations or regions is needed for development of guidelines for empirical treatment and necessary data for planning and evaluation of HIV/AIDS care. To our best knowledge, no reliable data on the epidemiology of intestinal parasitosis of HIV-infected individuals exists from the studied area. The aim of this study was to determine the prevalence of parasitic infection among HIV-infected individuals at the Asella Teaching and Referral Hospital (ATRH), the referral hospital for a catchment population of around 3.5 million people at Arsi Zone in Central Ethiopia.

Methods

Study design, population and sampling techniques

This institutional-based cross sectional study was conducted at the HIV outpatient clinic of the ATRH. Between July 2015 and April 2017, HIV-positive patients presenting for routine follow-up were interviewed for clinical signs of diarrhoeal disease, defined as ≥ 3 loose bowel movements per day on ≥ 3 consecutive days. If diarrhoea was present the patient was offered inclusion in the study. Randomly, patients without diarrhoea visiting the HIV clinic for routine follow-up were also offered inclusion as asymptomatic controls. It was intended to include equal numbers of patients with and without diarrhoea. Stool samples and complete data from 85 HIV positive patients with and 78 without diarrhoea were available and eligible for analysis.

Laboratory test

Stool and blood samples were collected from all study participants. Stool examination for parasitic infections was done by wet mount light microscopy at a maximum magnification of 400-fold and modified acid-fast (Kinyoun) staining after processing the stool samples with Telemann concentration technique. Wet mount microscopic examination was also performed from native stool samples to detect trophozoite and larval stages of parasites. CD4 cell count was determined from EDTA blood samples using BD FACSCount™ Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Data collection and statistical analysis

Sociodemographic and behavioural data was collected using a standardized questionnaire to identify possible predictors of and risk factors for parasitic infection. Collected data was analysed using IBM SPSS Statistics for Windows, version 21.0 (IBM corp., Armonk, NY, USA). Prevalence of parasitic infection among different age groups and sex was analysed by simple frequency distribution. Chi-squared test and multivariate regression analysis were used to identify significant predictors and risk factors. A *p*-value of <0.05 was considered statistically significant.

Ethical considerations

Ethical clearance to conduct this study was obtained from the appropriate institutional ethical review board at Arsi University, College of Health Science and collected data was used only for the purpose of this study. All study participants signed written informed consent before data and specimen collection commenced. Positive results were communicated with treating physicians for further treatment.

Results

Demographic characteristics and clinical status among the study participants

A total of 163 HIV-infected patients were included into the study. The majority of study participants (62.0%, n=101) were female and the mean age was 38.2 years (SD \pm 10.7). 68.7% (n=112) were living in urban areas. 90.2% (n=147) of the participants were treated with combined antiretroviral therapy (cART). The mean CD4 cell count was 482 cells/ μ l (SD \pm 286.1) with minimum and maximum of CD4+ cell count of 21 cells/ μ l and 1,742 cells/ μ l, respectively. 52.1% (n=85) of the participants suffered from diarrhoea. Of those, 11.0% (n=18) reported

ongoing diarrhoea for more than two weeks. 11.0% (n=11) of participants reported that they did not have a latrine facility and 17.8% (n=29) to defecate in open field. 25.2% (n=41) had repeated contact with animal excreta and 32.5% (n=53) had the habit of regularly eating uncooked food (for more details, see Table 1).

Prevalence and risk factors of intestinal parasitosis

The overall prevalence of intestinal parasitic infection in the study population was 18.4% (n=30). Protozoa (*Cryptosporidium* spp., *E. histolytica*, *G. lamblia* and *Pentatrichomonas hominis*) and helminths (*Taenia* spp., *A. lumbricoides*, *S. stercoralis*, *T. trichuria* and *H. nana*) were detected in 12.9% (n=21) and 5.5% (n=9) of patients, respectively. The most commonly detected parasite was *Cryptosporidium* spp. (7.4% in study population, 40.0% of isolated pathogens, n=12), followed by *Giardia lamblia* (4.3% in study population, 23.3% of isolated pathogens, n=7) (see Figure 1). As expected, all cases of Cryptosporidiosis were only diagnosed by detection of oocysts in Kinyoun-stained preparations of stools samples but not by wet mount microscopy. All patients with opportunistic infection and low CD4 count were symptomatic with diarrhoea. The utilization of Kinyoun staining led to an increased detection rate of intestinal parasites from 11.0% to 18.4%. No infection with multiple intestinal parasites was found.

Socioeconomic and clinical factors significantly associated with intestinal parasitosis were having diarrhoea (p<0.001), not receiving cART (p=0.038), CD4+ cell count < 200 cells/ μ l (p=0.01), repeated contact with cattle excreta (p=0.03) and having the habit of regularly eating uncooked food (p=0.001) (see table 2). Using multivariate logistic regression, risk factors significantly associated with intestinal parasitic infections were having diarrhoea (Adjusted odds ratio [AOR] 9.28; 95% CI 2.44-35.20) and having the habit of regularly eating uncooked food (AOR=4.90; 95% CI 1.83, 13.11) (see Table 3). The positive predictive value for

opportunistic infection in patients with diarrhoea and compromised immune status was low (29.2%).

Risk factors for Cryptosporidiosis

The prevalence of *Cryptosporidium* spp. was significantly higher among patients with diarrhoea (12.9% [11/85] vs. 1.3% [1/78], $p=0.005$) and CD4+ cell count lower than 200 cells/ μ l (25.9% [7/27] vs. 3.7% [5/136], $p=0.001$) compared to their counterparts. Patients receiving cART were infected with *Cryptosporidium* spp. in 6.1% (9/147) compared to 18.8% (3/16) of patients being infected who were not receiving cART. However, there was no statistically significant association ($p=0.066$), probably because numbers were low. Using multivariate logistic regression, the only risk factor significantly associated with *Cryptosporidium* spp. infection was a CD4+ cell count < 200 cells/ μ l, leading to a more than fivefold increased risk (AOR=5.41; 95% CI 1.48-19.73). There was no evidence for infection with *Cryptosporidium* spp. in immune-compromised patients (CD4 cell count < 200 / μ l) without diarrhoea.

Discussion

Parasitic infections, especially in immunocompromised individuals, are considered to be common in Ethiopia. Due to inadequate laboratory testing, exact numbers are largely unknown and a high number of missed diagnoses is to be expected. Adding Kinyoun staining increased the detection rate of intestinal parasites in HIV patients in this study cohort. The findings of this investigation show infection with intestinal parasites in more than 18% of the HIV-infected participants. This prevalence was higher than in similar studies e.g. from Nigeria (5) or southern India (6), but lower than the prevalence described in previous studies from Ethiopia (i.e. from Desie (7), Butajira (8) and Bahir Dar (9)). The highest prevalence of intestinal parasitosis among HIV-positive individuals (80.3%) was described from an investigation in Bahir Dar in northwest Ethiopia (9). These distinctive regional differences could be triggered by different climatic conditions. The study site is situated in the town of Asella, roughly 2,400 m above sea level in the Eastern Ethiopian Highlands. Despite low numbers, this study showed

evidence for four different protozoan parasites and five helminths circulating in the community.

In study participants with diarrhoea, the likelihood of parasitic infection was more than eight times higher in comparison to participants without diarrhoea. Similar results were reported from different studies [9-11]. Eating dishes prepared from uncooked beef or vegetables is common in Ethiopian cuisine. Besides low CD4+ cell count, regular consumption of uncooked food was identified as one of the main risk factors for parasitic infection, leading to a 5-fold increase of opportunistic parasitosis. Presumably, constricted hygienic conditions and limited supply of clean water might be significant contributors leading to contaminated food and a higher incidence of parasitic infections. Also, the common practice of open field defecation should be considered as possible contributor for transmission of intestinal parasites in the communities.

Despite regular empiric treatment or prophylaxis of HIV-infected individuals with antiparasitic substances such as cotrimoxazole, the prevalence of opportunistic parasitic infections among the investigated patients is considerably high. As it has to be expected, the prevalence of *Cryptosporidium* species was higher among patients with CD4+ cell count < 200 cells/ μ l. This finding is in line with findings from similar studies (1,10).

Both wet mount stool microscopy and Kinyoun stain were used for detection of parasites in the patients' stool samples but oocysts of *Cryptosporidium* spp. were only detectable after staining. Without application of the Kinyoun staining technique all patients with diarrhoea and cryptosporidiosis would have been left without exact diagnosis. Despite regular availability and cost-effectiveness of required consumables and the simple and reliable applicability of Kinyoun stain, only wet mount stool microscopy is routinely carried out in the study centre's hospital laboratory or many other comparable facilities in the country to date. This finding is underlining the need of enhanced stool investigations for accurate diagnosis and improvement of HIV patient care.

Conclusion

The prevalence of opportunistic intestinal parasites was high in the studied population. In HIV-infected patients with diarrhoea and low CD4+ cell count the risk for parasitic or specifically opportunistic parasitic infection was highest, indicating the need for circumstantial stool diagnostics. Cultural practices as the habit of eating uncooked food in the studied population are contributors for infections caused by faeco-orally transmitted parasites. All opportunistic parasite infections were missed in the routinely performed wet mount stool microscopy. Considering the high prevalence and clinical relevance of opportunistic intestinal infections particularly in individuals with low CD4 cell count and diarrhoea, the implementation of stool concentration and modified acid fast staining techniques in HIV programs should be considered to enhance the quality of service for HIV patients in highly endemic settings as Ethiopia. Adaption of diagnostic techniques should be easily achievable; as acid-fast staining is regularly applied in most of the countries' laboratories for TB diagnostics. The easy accessibility and low costs of required reagents makes the technique practical in Ethiopia or similar settings.

Abbreviations

AOR	Adjusted odds ratio
ATRH	Asella Teaching and Referral Hospital
cART	Combined antiretroviral therapy
COR	Crude odds ratio
HIV	Human Immunodeficiency Virus

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the appropriate institutional ethical review board of Arsi University in Asella, Ethiopia prior to conducting the described research. The study is listed under the reference number A/U/H/S/C/120/351. Data collection and per protocol stool examination were only performed if a participant signed informed consent.

Consent for publication

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Million Getachew Mesfun:

Conception and design, acquisition, analysis and interpretation of data, drafting of the manuscript

Andre Fuchs:

Conception and design, acquisition and interpretation of data, drafting and revision of the manuscript

Martha Charlotte Holtfreter:

Supervision and quality control of data acquisition and interpretation, revision of the manuscript

Torsten Feldt:

Accountability for the published work, conception and design, interpretation of data, critical revision of the manuscript

Dieter Häussinger:

Interpretation of data, critical revision of manuscript and approval of final version to be published

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Tables

Table 1: Demographic and socioeconomic characteristics of study participants

Variables	% (n)
Sex	
Male	38.0 (62)
Sex	62.0 (101)
Age group	
<=20	3.1 (5)
21-40	63.8 (104)
>40	33.1 (54)
Marital status	
Single	19.0 (31)
Married	46.6 (76)
Divorced	19.0 (31)
Widowed	15.3 (25)
Area of residence	
Urban	68.7 (112)
Rural	31.3 (51)
Source of drinking water	
Tap water	87.7 (143)
Protected well	5.5 (9)
Unprotected well	3.1 (5)
Having latrine facility	
Yes	89.0 (145)
No	11.0 (18)
Regular practice of open field defecation	
Yes	17.8 (29)
No	82.2 (134)
Educational status	
Illiterate	28.2 (46)
Primary school	65.6 (107)
Highschool and above	6.1 (10)
Occupation	
Farmer	22.1 (36)
Government employee	14.7 (24)
Student	3.1 (5)
Unemployed(House wife)	19.6 (32)
Day laborer	31.3 (51)
Other	9.2 (15)

Table 2: Possible contributors for parasitic infections among study participants (with *: statistically significant association; cART: combined antiretroviral therapy)

Variable	Parasitic infection		p value
	Yes	No	
	% (n)	% (n)	
Having diarrhoea			<0.001*
Yes	38.8 (27)	68.2 (58)	
No	3.8 (3)	96.2 (75)	
Duration of diarrhoea			0.934
More than 2 weeks	33.3 (6)	66.7 (12)	
Up to 2 weeks	32.3 (21)	67.7 (44)	
Receiving cART			0.038*
Yes	16.3 (24)	83.7 (123)	
No	37.5 (6)	62.5 (10)	
Sex			0.30
Male	22.6 (14)	77.4 (48)	
Female	15.8 (16)	84.2 (85)	
Area of residence			0.51
Urban	17 (19)	83 (93)	
Rural	21.6 (11)	78.4 (40)	
CD4+ cell count (/µl)			0.01*
<200	37 (10)	63 (17)	
≥200	14.7 (20)	85.3 (116)	
Repeated contact with cattle excreta			0.03*
Yes	29.3 (12)	70.7 (29)	
No	14.8 (18)	85.2 (104)	
Having latrine facility			0.10
Yes	16.6 (24)	83.4 (121)	
No	33.3 (6)	66.7 (12)	

Having the habit of eating uncooked food		0.001*	
Yes	37.7 (20)	62.3 (33)	
No	9.1 (10)	90.9 (100)	

Table 3: Regression analysis of predictors for intestinal parasitic infection among HIV-infected patients in the study population (with *: statistically significant association; COR: crude odds ratio; AOR: adjusted odds ratio; cART: combined antiretroviral therapy)

Variable	Parasitic infection		Odds ratio		p value
	Yes	Yes	COR (95% CI)	AOR (95% CI)	
	% (n)	% (n)			
Having diarrhoea					0.001*
Yes	38.8 (27)	68.2 (58)	11.63 (3.36-40.25)	9.28 (2.44-35.20)	
No	3.8 (3)	96.2 (75)	1	1	
Receiving cART					0.226
Yes	16.3 (24)	83.7 (123)	1 3.07 (1.02-9.26)	1	
No	37.5 (6)	62.5 (10)		2.23 (0.60-8.21)	
CD4+ cell count (/µl)					0.883
<200	37 (10)	63 (17)	3.41 (1.36-8.50)	1.08 (0.34-3.39)	
≥200	14.7 (20)	85.3 (116)	1	1	
Repeated contact with cattle's excreta					0.128
Yes	29.3 (12)	70.7 (29)	2.39 (1.03-5.52)	2.05 (0.77-5.42)	
No	14.8 (18)	85.2 (104)	1	1	
Habit of eating uncooked food					0.002*
Yes	37.7 (20)	62.3 (33)	6.06 (2.57-14.25)	4.90 (1.83-13.11)	
No	9.1 (10)	90.9 (100)	1	1	

Figures

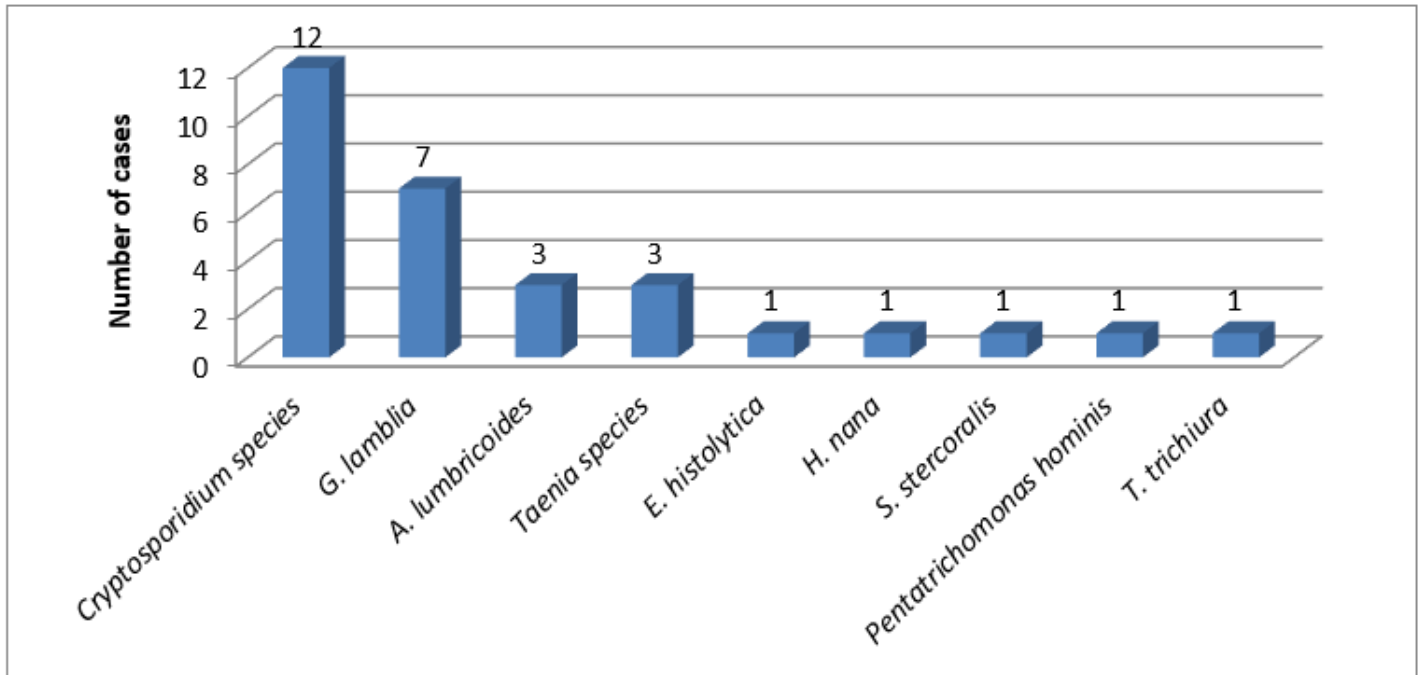


Figure 1

Distribution of parasites detected among study participants