International Journal of Infectious Diseases 122 (2022) 130–135

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

The implementation of the Kinyoun staining technique in a resource-limited setting is feasible and reveals a high prevalence of intestinal cryptosporidiosis in patients with HIV

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ARTICLE INFO

Article history:
Received 11 February 2022
Revised 4 May 2022
Accepted 5 May 2022

Keywords:
Intestinal parasites
Cryptosporidia
Gregaria
Stool concentration
Africa
Ethiopia

ABSTRACT

Objectives: In resource-limited settings, intestinal Cryptosporidial or coccidian infections are common causes of chronic diarrhea but usually remain undiagnosed by routine stool investigation. Here, the addition of the Kinyoun staining technique after stool concentration was evaluated as an easy and inexpensive method for diagnosis of intestinal parasitic infection in patients with HIV.

Methods: This cross-sectional study investigated patients with HIV with diarrhea and randomly selected patients with HIV without diarrhea as controls. Stool samples were examined by wet mount microscopy and Kinyoun staining after stool concentration. Clinical, sociodemographic, and behavioral data were collected. Statistical analysis was performed using chi-squared test and multivariate regression analysis.

Results: In total, 163 participants were included (62.0% female, mean age 38.2 [SD ± 10.7] years). Diarrhea was present in 52.1% (85/163). The prevalence of intestinal parasites was 18.4% (30/163). Cryptosporidial infections were more frequent among patients with diarrhea (12.9% [11/85] vs 1.3% [1/78], $P = 0.005$) and in patients with CD4$^+$ cell count <200 cells/µl (25.9% [7/27] vs 3.7% [5/136], $P = 0.001$). Risk factors for intestinal parasitic infections were diarrhea and the habit of regularly eating uncooked food. Kinyoun staining was necessary for the detection of cryptosporidiosis.

Conclusion: In our cohort, the prevalence of intestinal parasitic infection was high, especially after additional use of Kinyoun staining for detection of Cryptosporidia or intestinal coccidia. Considering its clinical relevance, particularly in individuals at risk, the implementation of this technique should be considered in resource-limited settings.

Introduction

Intestinal parasitic infections are common in many tropical regions and therefore are a major public health challenge in affected countries (Brooker, 2010; Karagiannis-Voules et al., 2015). Different climatic conditions and effects of climate change on regional climate contribute to the varying but overall increasing incidence of intestinal parasitosis (Cissé, 2019; Karagiannis-Voules et al., 2015). Frequently, the pathogens causing parasitic enteritis are not identified in resource-limited health care systems with restricted diagnostic capacities. In particular, intestinal gregarines of the subclass Cryptogregaria (*Cryptosporidia* species) or coccidia (*Cystoisospora* or *Cyclospora* species), hereafter summarized with the clinical term "coccidian infections," are common causes of opportunistic infections. However, these pathogens cannot be reliably detected by the widely practiced wet mount stool microscopy techniques (ten Hove et al., 2007). In general, patients with an impaired immune response, which is frequently caused by an HIV infection in af-

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https://doi.org/10.1016/j.ijid.2022.05.009

fected countries, are at a greater risk for developing severe and chronic opportunistic intestinal parasitic infections (Agholi et al., 2013; Laksemi et al., 2019). As a consequence, these opportunistic infections are a common cause of diarrhea in HIV-infected individuals (Low et al., 2016), causing up to half of the cases of diarrheal disease within this group of patients (Nissapatorn and Sawangjaroen, 2011). Once symptomatic, they often lead to significant impairments in quality of life or even death (Costa et al., 2018; Siddiqui et al., 2007). In addition, chronic courses of cryptosporidiosis and cystoisosporidiosis are considered AIDS-defining illnesses.

In Ethiopia, an example of a country with relevant risk for parasitic infections and with limited resources in the health care system, only wet mount light microscopy of stool samples (without previous stool concentration) is routinely performed in health care facilities to identify causative pathogens in patients with diarrhea. The sensitivity of this technique to diagnose intestinal coccidian infections is very limited (Ahmed and Chowdhary, 2013; Tuli et al., 2010). Thus, reliable epidemiological data about the prevalence of opportunistic intestinal coccidian infections among patients with HIV are scarce, and the prevalence is likely to be underestimated. Nevertheless, accurate information is needed to provide guidance for empirical treatment and the development of strategies to improve HIV/AIDS care. Increasingly, more advanced laboratory techniques such as polymerase chain reaction–based testing are being proposed for the diagnosis of intestinal coccidian infections (Abd-Ella, 2014; ten Hove et al., 2007; Masur et al., 2014), but the widespread use of such techniques is limited to high-income countries without the diagnostic limitations often present in low- and middle-income countries. However, easily applicable acid-fast staining techniques of stool samples, such as Kinyoun staining, have been evaluated for the diagnosis of intestinal coccidian infections and might be of use in settings with the unavailability of polymerase chain reaction–based diagnostic capacities (Chalmers et al., 2011; Checkley et al., 2015). In the absence of strong evidence, as far as can be judged, their performance seems satisfactory (Ahmed and Chowdhary, 2013). In the local setting, choices for treatment of wet mount microscopy-negative diarrheal disease in affected patients are limited and mostly rely on the empirical use of cotrimoxazole. This could result in the unnecessary prescription of antimicrobials with potential side effects (e.g., allergies, exanthema, or intestinal disorders) and the development of antimicrobial drug resistance.

Therefore, this study aimed to assess the prevalence of intestinal parasitic infection in patients with HIV after implementation of Kinyoun (modified acid-fast) staining after stool concentration in comparison with standard wet mount stool microscopy for the identification of intestinal parasites from stool samples of HIV-infected individuals. The objective was also to demonstrate that the introduction of enhanced stool diagnostics leads to an increase in diagnostic yield and is possible in the low-resource setting.

Methods

Study design, population, and sampling techniques

This single-center cross-sectional study was conducted at the HIV outpatient clinic of the Asella Teaching and Referral Hospital (ATRH). The ATRH serves as a referral hospital for a catchment population of around 3.5 million people in the Arsi Zone, central Ethiopia. During a period of 10 months, HIV-positive patients presenting for routine follow-up were interviewed for the presence of diarrheal disease, defined as ≥3 loose bowel movements per day on ≥3 consecutive days. Patients with diarrhea, according to self-report, were offered inclusion in the study. The first patient with HIV who presented to the HIV clinic for a routine check-up and who did not have diarrhea after the offer of study participation to a patient with diarrhea was also offered participation in the study. No other selection criteria such as age, gender, or disease stage were taken into account. Thus, a control group of patients without diarrhea was formed with the intention to include an equal number of patients with and without diarrhea. Using a standardized questionnaire, clinical, sociodemographic, and behavioral data were collected from all participants.

Laboratory tests

Stool and blood samples were collected from all study participants. Stool examination for parasitic infections was done by wet mount light microscopy and light microscopy after modified acid-fast (Kinyoun) staining at a maximum magnification of 1000-fold (oil immersion) before and after processing the stool samples with the Telemann concentration technique. For the Telemann concentration, 1-2 g of stool sample was dissolved in 6 ml 10% HCl. Then, 6 ml of ether was added, and the mixture was sieved through double-layer gauze to remove large stool particles. After the centrifugation at 1000 *g* for 3 minutes, the remaining sediment was used for wet mount examination and staining. For the Kinyoun staining, a thin fecal smear prepared from the homogenized sediment was fixed with methanol for 5 minutes. Afterward, air-dried slides were stained with a phenol-fuchsine solution for 20 minutes. HCl-ethanol was used as a decolorizer, and the preparation was counter-stained with methylene blue for 5 minutes. Wet mount microscopic examination was also performed from native stool samples to detect trophozoite and larval stages of parasites. Samples with putative detection of oocysts in wet mount microscopy were stained according to the Kinyoun method for confirmation. Microscopy was performed by trained and experienced investigators, who were blinded to the patient's clinical data. *Entamoeba histolytica* was differentiated from other *Entamoeba species* by detecting hematophagic trophozoites, confirming *E. histolytica* infection. *Pentatrichomonas hominis* infection was identified according to typical motility.

The $CD4^+$ cell count was determined from EDTA blood samples using BD FACSCount™ Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) during a routine investigation. No information on current HIV viral load was available from study participants, as this investigation was not part of routine investigation at the study center.

Data collection and statistical analysis

Clinical, sociodemographic, and behavioral data were collected using a standardized questionnaire to identify possible risk factors for parasitic infection. Data were analyzed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Chi-squared test and multivariate regression analysis were used to identify risk factors. A *P*-value of <0.05 was considered statistically significant.

Results

Demographic characteristics and clinical status among the study participants

A total of 163 patients with HIV were recruited and included in the study. Most study participants (62.0%, n = 101) were female, and the mean age was 38.2 years (SD ± 10.7). Overall, 68.7% (n = 112) lived in urban areas (Table 1). According to clinical data analysis, 90.2% (n = 147) of participants were treated with combination antiretroviral therapy (cART) (Table 2). The mean $CD4^+$ cell count was 482 cells/µl (SD ± 286.1, range 21-1742 cells/µl)

Table 1
Demographic and socioeconomic characteristics of study participants

Variables	% (n)	Mean (SD)
Sex		
Male	38.0 (62)	
Female	62.0 (101)	
Age group		
<21	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		
No	11.0 (18)	
Regular practice of open field defecation		
Yes	17.8 (29)	
Educational status		
Illiterate	28.2 (46)	
Primary school	65.6 (107)	
High school and above	6.1 (10)	
Occupation		
Farmer	22.1 (36)	
Government employee	14.7 (24)	
Student	3.1 (5)	
Day laborer	31.3 (51)	
Other	9.2 (15)	
No regular employment	19.6 (32)	
CD4⁺ cell count (cells/μl)		482 (±286.1)

(Table 1). Diarrhea was present in 52.1% (n = 85) of the participants, and 47.9% (n = 78) reported not experiencing diarrhea (Table 2). Within the group of symptomatic patients, 11.0% (n = 18) described ongoing diarrhea for more than two weeks (Table 2). In terms of living conditions, 11.0% (n = 18) of the participants reported that their household did not have a latrine facility of its own, 25.2% (n = 41) had repeated contact with animal excreta, and 32.5% (n = 53) had the habit of regularly eating uncooked food (Table 2).

Prevalence and risk factors of intestinal parasitic infection

The overall prevalence of intestinal parasitic infection in the study population was 18.4% (n = 30). Protozoa (*Cryptosporidium* species, *E. histolytica, G. lamblia,* and *Pentatrichomonas hominis*) were detected in 12.9% (n = 21), and helminths (*Taenia* species, *A. lumbricoides, S. stercoralis, T. trichuria,* and *H. nana*) were detected in 5.5% (n = 9) of patients, respectively. *Cryptosporidium* species was the most commonly detected parasite (7.4% in the study population, 40.0% of isolated pathogens, n = 12), followed by *Giardia lamblia* (4.3% in the study population, 23.3% of isolated pathogens, n = 7) (Figure 1). As expected, all cases of cryptosporidiosis were only diagnosed by detection of oocysts in Kinyoun-stained preparations of stool samples but not by wet mount microscopy. In comparison, the CD4⁺ cell count of patients with cryptosporidiosis was significantly lower than in patients without evidence of opportunistic intestinal infection (mean CD4⁺ cell count 236.2 cells/μl [SD ± 229.1] vs 501.6 cells/μl [SD ± 281.7]; $P = 0.002$). All patients with this opportunistic infection and low CD4⁺ count (i.e., <200 cells/μl) were symptomatic with diarrhea. In 25.0% (3/12) of the patients with cryptosporidiosis, diarrhea lasted for >2 weeks. Overall, the use of Kinyoun staining led to an increased detection rate of intestinal parasites (11.0% vs 18.4%, P <0.001). No infections with multiple intestinal parasites in a single participant were detected.

Table 2
Univariate analysis of risk factors for intestinal parasitic infections (dependent variable) among study participants

Variable	All participants% (n)	Parasitic infection		*P*-value
		Yes% (n)	No% (n)	
Having diarrhea				
Yes	52.1 (85)	31.8 (27)	68.2 (58)	**<0.001**[a]
No	47.9 (78)	3.8 (3)	96.2 (75)	
Duration of diarrhea				
More than 2 weeks	21.7 (18)	33.3 (6)	66.7 (12)	0.934
Up to 2 weeks	78.3 (65)	32.3 (21)	67.7 (44)	
Receiving cART				
Yes	90.2 (147)	16.3 (24)	83.7 (123)	**0.038**[a]
No	9.8 (16)	37.5 (6)	62.5 (10)	
Sex				
Male	38.0 (62)	22.6 (14)	77.4 (48)	0.30
Female	62.0 (101)	15.8 (16)	84.2 (85)	
Area of residence				
Urban	68.7 (112)	17 (19)	83 (93)	0.51
Rural	31.3 (51)	21.6 (11)	78.4 (40)	
CD4⁺ cell count (cells/μl)				
<200	16.6 (27)	37 (10)	63 (17)	**0.01**[a]
≥200	83.4 (136)	14.7 (20)	85.3 (116)	
Repeated contact with cattle excreta (self-assessment)				
Yes	25.2 (41)	29.3 (12)	70.7 (29)	**0.03**[a]
No	74.8 (122)	14.8 (18)	85.2 (104)	
Having latrine facility				
Yes	89.0 (145)	16.6 (24)	83.4 (121)	0.10
No	11.0 (18)	33.3 (6)	66.7 (12)	
Having the habit of eating uncooked food (self-assessment)				
Yes	32.5 (53)	37.7 (20)	62.3 (33)	**0.001**[a]
No	67.5 (110)	9.1 (10)	90.9 (100)	

[a] statistically significant association.cART = combination antiretroviral therapy.

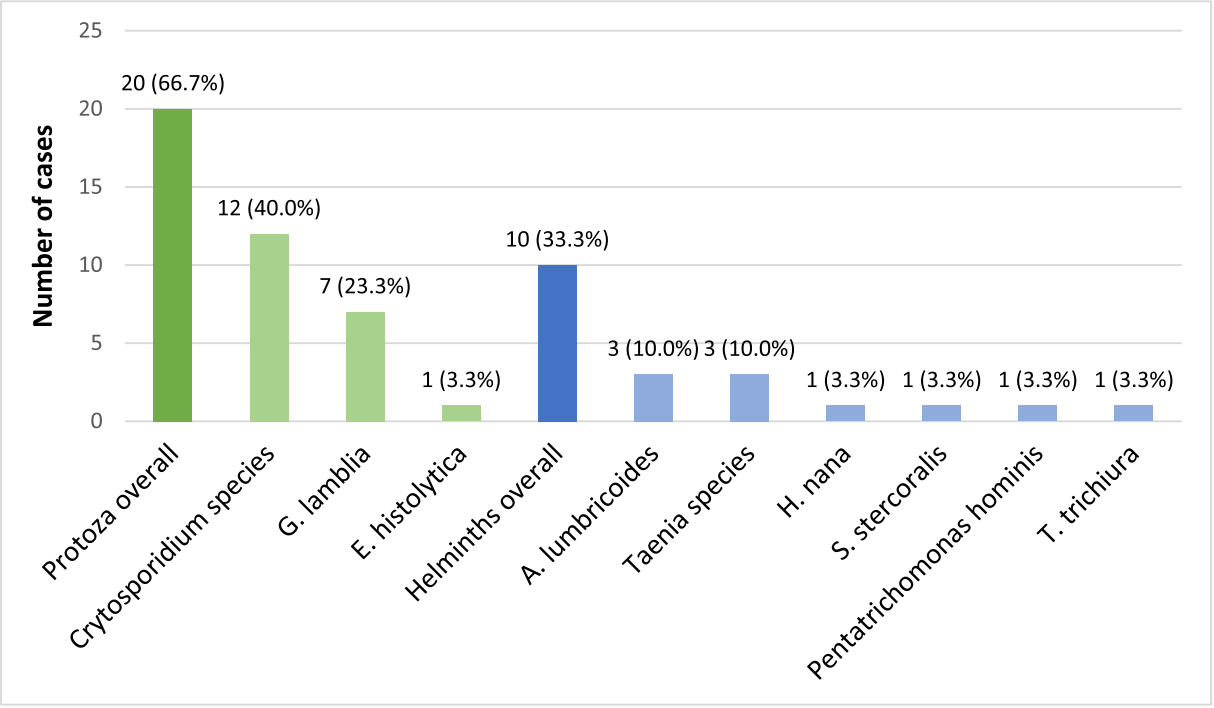

Figure 1. Distribution of parasites among study participants.

Table 3
Regression analysis of predictors for intestinal parasitic infection among the study population

Variable	Parasitic infection		Odds ratio		*P*-value
	Yes% (n)	No% (n)	COR (95% CI)	AOR (95% CI)	
Having diarrhea					**0.001**[a]
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	1	
No	37.5 (6)	62.5 (10)	3.07 (1.02–9.26)	2.23 (0.60–8.21)	
CD4⁺ cell count (cells/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 (self-assessment)					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 (self-assessment)					**0.002**[a]
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	

[a] statistically significant association.AOR = adjusted odds ratio; cART = combined antiretroviral therapy; CI = confidence interval; COR = crude odds ratio.

Socioeconomic and clinical factors significantly associated with intestinal parasitic infection were reporting diarrhea (P <0.001), not receiving cART (P = 0.038), CD4⁺ cell count <200 cells/μl (P = 0.01), repeated contact with cattle excreta (P = 0.03), and having the habit of regularly eating uncooked food (P = 0.001) (Table 2).

Using multivariate logistic regression, risk factors significantly associated with intestinal parasitic infections were having diarrhea (adjusted odds ratio [AOR] 9.28; 95% confidence interval [CI] 2.44-35.20) and having the habit of regularly eating uncooked food (AOR = 4.90; 95% CI 1.83-13.11) (Table 3). The positive predictive value for opportunistic infection in patients with diarrhea and compromised immune status (i.e., CD4⁺ cell count <200 cells/μl) was 29.2%.

The prevalence of intestinal coccidian infections (i.e., cryptosporidiosis in our cohort) was significantly higher among patients with diarrhea (12.9% [11/85] vs 1.3% [1/78], P = 0.005) and CD4⁺ cell count <200 cells/μl (25.9% [7/27] vs 3.7% [5/136], P = 0.001). Patients receiving cART seem to be infected less frequently with *Cryptosporidium* species in comparison with those not receiving cART (6.1% [9/147] vs 18.8% [3/16]). However, this difference was not significant (P = 0.066). Using multivariate logistic regression, the only risk factor significantly associated with *Cryptosporidium* species infection was a CD4⁺ cell count <200 cells/μl, which led to a more than fivefold increased risk (AOR = 5.41; 95% CI 1.48-19.73) (Table 4).

Discussion

Parasitic infections, especially in immunocompromised individuals, are considered to be common in Ethiopia. Because of 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

Table 4
Regression analysis of predictors for intestinal cryptosporidiosis among the study population

Variable	Intestinal cryptosporidiosis		Odds ratio			
	Yes% (n)	No% (n)	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Having diarrhea						
Yes	12.9 (11)	87.1 (74)	11.44 (1.44–90.87)	0.005[a]	6.56 (0.76–56.24)	0.086
No	1.3 (1)	98.7 (77)	1		1	
CD4⁺ cell count (cells/μl)						
<200	25.9 (7)	74.1 (20)	9.17 (2.65–31.69)	0.001[a]	5.42 (1.48–19.73)	0.010[a]
≥200	3.7 (5)	96.3 (131)	1		1	
Receiving cART						
Yes	6.1 (9)	93.9 (138)	1	0.099		
No	18.8 (3)	7.4 (12)	3.53 (0.85–14.71)			

[a] statistically significant association.AOR = adjusted odds ratio; cART = combined antiretroviral therapy; CI = confidence interval; COR = crude odds ratio.

patients with HIV in this study cohort. In particular, none of the frequently symptomatic cases of cryptosporidiosis were detected by the wet mount microscopy, which is usually the only available standard diagnostic for detecting intestinal parasites. The findings of this study show a high rate of infections with intestinal parasites in more than 18% of the HIV-infected participants, especially among those with self-reported diarrhea. This prevalence was higher than in studies from Nigeria (11.4%) (Jegede et al., 2014) or southern India (9%) (Kaniyarakkal et al., 2016) but lower than the prevalence described in previous studies from Ethiopia, that is, from Desie (28.3%) (Missaye et al., 2013), and Butajira (35.9%) (Gedle et al., 2017). The highest prevalence of intestinal parasitic infections among HIV-positive individuals (80.3%) was described in an investigation in Bahir Dar in northwest Ethiopia (Alemu et al., 2011). These distinctive regional differences could possibly be triggered by different climatic, environmental, and hygienic conditions, as this can positively or negatively influence the extraintestinal life cycle of the parasites (Karagiannis-Voules et al., 2015). Our study site was situated in the town of Asella, roughly 2400 m above sea level in the eastern Ethiopian Highlands. In addition, seasonal changes in climate could influence the incidence of intestinal coccidian infections (de Oliveira-Silva et al., 2007) and therefore explain differences between those distinct observations within Ethiopia. Long-term monitoring would be required to elucidate such possible effects clearly. As expected in this study from a country with a known high incidence of intestinal parasitic infections in the general population, this study showed evidence for a wide variety of four different protozoan parasites and five helminths circulating in the community.

Here, in study participants with diarrhea, the likelihood of parasitic infection was more than eight times higher than participants without diarrhea. This was higher than previously reported in another study from France, with the risk being four times increased (Costa et al., 2018). However, individual habits such as washing hands before eating influence the incidence of intestinal parasitic infections (Eyamo et al., 2019). Eating dishes prepared from uncooked beef or vegetables, a known risk factor for intestinal parasitic infections (Dorny et al., 2009), is common in Ethiopian cuisine. In addition, in our cohort, regular consumption of uncooked food was identified as an important risk factor for parasitic infection and low CD4⁺ cell count, which led to a fivefold increase of opportunistic infections, which were all caused by Cryptosporidia in our cohort. High infestation rates with intestinal parasites in livestock, constricted hygienic conditions, and limited supply of clean water should be considered as possible reasons for a high contamination rate with intestinal parasites in uncooked food. In addition, the common practice of open field defecation should be considered a possible contributor to the 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. However, cotrimoxazole is known to have limited activity against Cryptosporidia, which were the only opportunistic parasites detected in our cohort. As expected, the prevalence of *Cryptosporidium* species was higher among patients with CD4⁺ cell count <200 cells/μl, and all patients with intestinal cryptosporidiosis and low CD4⁺ cell count had diarrhea. This finding is in line with findings from similar studies (Agholi et al., 2013; Nsagha et al., 2016).

Both wet mount stool microscopy and Kinyoun stain were used to detect parasites in the patients' stool samples, but oocysts of *Cryptosporidium* species were only detectable after Kinyoun staining. Using only the wet mount technique, none of the patients with diarrhea and cryptosporidiosis would have been diagnosed. Acid-fast staining techniques are regularly used for diagnosis of tuberculosis in most of the laboratories in affected countries as Ethiopia. Despite regular availability and cost-effectiveness of required consumables and the simple and reliable applicability of the Kinyoun stain, the method is not widely implemented in most resource-limited settings. Given the high prevalence of chronic diarrhea in HIV-infected persons and its importance to disease progression, particularly in Africa (DuPont and Marshall, 1995), this finding underlines the need for enhanced stool investigation methods for the accurate diagnosis of coccidian infections, especially in high HIV-prevalence settings.

Conclusion

The prevalence of intestinal parasitic infections in general, and opportunistic intestinal coccidian infections in particular, in this cohort of patients with HIV, was high, indicating the need for improved stool diagnostics. Diarrhea was the most important risk factor for intestinal parasitic infection. Other risk factors included consumption of uncooked food, but behavioral factors, for example, the habit of eating uncooked food, were also contributing to the high prevalence. Cryptosporidiosis was common, especially in patients with severe immunodeficiency and an important etiology for diarrhea, but exclusively detected using the Kinyoun staining method. The implementation of stool concentration and Kinyoun staining techniques is feasible with low training effort and despite limited resources. The use of these techniques significantly increases the detection rate of intestinal parasites compared with standard examination. Thus, the implementation should be considered to enhance the quality of service for HIV patients in highly endemic settings such as Ethiopia.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

Ethical clearance for the study was obtained from the appropriate institutional ethical review board at Arsi University, College of Health Science (reference number A/U/H/S/C/120/351), and collected data were used only for the purposes of this study. All study participants gave written informed consent before enrollment into the study.

Acknowledgments

The authors would like to thank the patients of the HIV clinic for their participation in the study, all the staff at the Hirsch Institute of Tropical Medicine, and the nursing staff of the HIV clinic at the Asella Teaching and Referral Hospital for their invaluable support.

Author contributions

Million Getachew Mesfun: Conception and design of the study; acquisition, analysis, and interpretation of data; drafting of the manuscript.

Andre Fuchs: Conception and design of the study; acquisition, analysis, and interpretation of data; drafting and revision of the manuscript.

Martha Charlotte Holtfreter: Analysis and interpretation of data, critical revision of the manuscript

Tafese Beyene Tufa: Data acquisition and critical revision of the manuscript

Hans Martin Orth: Interpretation of data and critical revision of the manuscript.

Tom Luedde: Critical revision of manuscript and approval of the final version to be submitted

Torsten Feldt: Conception and design of the study, interpretation of data, critical revision of the manuscript, and approval of the final version to be submitted

References

Abd-Ella OH. Diagnosis and treatment of cryptosporidiosis: an update review. J Egypt Soc Parasitol 2014;44:455–66.

Agholi M, Hatam GR, Motazedian MH. HIV/AIDS-associated opportunistic protozoal diarrhea. AIDS Res Hum Retroviruses 2013;29:35–41.

Ahmed NH, Chowdhary A. Comparison of different methods of detection of enteric pathogenic protozoa. Indian J Med Microbiol 2013;31:154–60.

Alemu A, Shiferaw Y, Getnet G, Yalew A, Addis Z. Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher clinic in Bahir Dar city. North West Ethiopia. Asian Pac J Trop Med 2011;4:661–5.

Brooker S. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers–a review. Int J Parasitol 2010;40:1137–44.

Chalmers RM, Campbell BM, Crouch N, Charlett A, Davies AP. Comparison of diagnostic sensitivity and specificity of seven Cryptosporidium assays used in the UK. J Med Microbiol 2011;60:1598–604.

Checkley W, White AC, Jaganath D, Arrowood MJ, Chalmers RM, Chen X-Met al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect Dis 2015;15:85–94.

Cissé G. Food-borne and water-borne diseases under climate change in low- and middle-income countries: further efforts needed for reducing environmental health exposure risks. Acta Trop 2019;194:181–8.

Costa D, Razakandrainibe R, Sautour M, Valot S, Basmaciyan L, Gargala Get al. Human cryptosporidiosis in immunodeficient patients in France (2015-2017). Exp Parasitol 2018;192:108–12.

de Oliveira-Silva MB, de Oliveira LR, Resende JCP, Peghini BC, Ramirez LE, Lages-Silva Eet al. Seasonal profile and level of CD4+ lymphocytes in the occurrence of cryptosporidiosis and cystoisosporidiosis in HIV/AIDS patients in the Triângulo Mineiro region. Brazil. Rev Soc Bras Med Trop 2007;40:512–15.

Dorny P, Praet N, Deckers N, Gabriel S. Emerging food-borne parasites. Vet Parasitol 2009;163:196–206.

DuPont HL, Marshall GD. HIV-associated diarrhea and wasting. Lancet 1995;346:352–6.

Eyamo T, Girma M, Alemayehu T, Bedewi Z. Soil-transmitted helminths and other intestinal parasites among schoolchildren in Southern Ethiopia. Res Rep Trop Med 2019;10:137–43.

Gedle D, Kumera G, Eshete T, Ketema K, Adugna H, Feyera F. Intestinal parasitic infections and its association with undernutrition and CD4 T cell levels among HIV/AIDS patients on HAART in Butajira. Ethiopia. J Health Popul Nutr 2017;36:15.

Jegede EF, Oyeyi ETI, Bichi AH, Mbah HA, Torpey K. Prevalence of intestinal parasites among HIV/AIDS patients attending Infectious Disease Hospital Kano, Nigeria. Pan Afr Med J 2014;17:295.

Kaniyarakkal V, Mundangalam N, Moorkoth AP, Mathew S. Intestinal parasite profile in the stool of HIV positive patients in relation to immune status and comparison of various diagnostic techniques with special reference to Cryptosporidium at a tertiary care hospital in South India. Adv Med 2016;2016.

Karagiannis-Voules DA, Biedermann P, Ekpo UF, Garba A, Langer E, Mathieu Eet al. Spatial and temporal distribution of soil-transmitted helminth infection in sub-Saharan Africa: a systematic review and geostatistical meta-analysis. Lancet Infect Dis 2015;15:74–84.

Laksemi DA, Suwanti LT, Mufasirin M, Suastika K, Sudarmaja M. Opportunistic parasitic infections in patients with human immunodeficiency virus/acquired immunodeficiency syndrome: a review. Vet World 2019;13:716–25.

Low A, Gavriilidis G, Larke N, B-Lajoie MR, Drouin O, Stover Jet al. Incidence of opportunistic infections and the impact of antiretroviral therapy among HIV-infected adults in low- and middle-income countries: a systematic review and meta-analysis. Clin Infect Dis Off Publ Infect Dis Soc Am 2016;62:1595–603.

Masur H, Brooks JT, Benson CA, Holmes KK, Pau AK, Kaplan JEet al. Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: updated Guidelines from the Centers for Disease Control and Prevention, National Institutes of Health, and HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 2014;58:1308–11.

Missaye A, Dagnew M, Alemu A, Alemu A. Prevalence of intestinal parasites and associated risk factors among HIV/AIDS patients with pre-ART and on-ART attending Dessie Hospital ART clinic, Northeast Ethiopia. AIDS Res Ther 2013;10:7.

Nissapatorn V, Sawangjaroen N. Parasitic infections in HIV infected individuals: diagnostic & therapeutic challenges. Indian J Med Res 2011;134:878–97.

Nsagha DS, Njunda AL, Assob NJC, Ayima CW, Tanue EA, Kibu ODet al. Intestinal parasitic infections in relation to CD4(+) T cell counts and diarrhea in HIV/AIDS patients with or without antiretroviral therapy in Cameroon. BMC Infect Dis 2016;16:9.

Siddiqui U, Bini EJ, Chandarana K, Leong J, Ramsetty S, Schiliro Det al. Prevalence and impact of diarrhea on health-related quality of life in HIV-infected patients in the era of highly active antiretroviral therapy. J Clin Gastroenterol 2007;41:484–90.

ten Hove R, Schuurman T, Kooistra M, Möller L, van Lieshout L, Verweij JJ. Detection of diarrhea-causing protozoa in general practice patients in The Netherlands by multiplex real-time PCR. Clin Microbiol Infect 2007;13:1001–7.

Tuli L, Singh DK, Gulati AK, Sundar S, Mohapatra TM. A multiattribute utility evaluation of different methods for the detection of enteric protozoa causing diarrhea in AIDS patients. BMC Microbiol 2010;10:11.