Food and Waterborne Parasitology 36 (2024) e00241

Contents lists available at ScienceDirect

Food and Waterborne Parasitology

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

Prevalence of *Entamoeba histolytica/dispar* and *Giardia lamblia* infections and their associated factors among schoolchildren in the Amhara region, Northwest Ethiopia

Tadesse Hailu *, Getaneh Alemu

Department of Medical Laboratory Science, College of Medicine and Health Sciences, Bahir Dar University, P. O. Box: 79, Ethiopia

A R T I C L E I N F O

Keywords:
Prevalence
Schoolchildren
Protozoa
Entamoeba histolytica
Giardia lamblia
Amhara region
Ethiopia

A B S T R A C T

Globally, intestinal protozoa *E. histolytica/dispar* and *Giardia lamblia* are the cause of amoebiasis and giardiasis, respectively. Despite their important medical importance and common occurrence in Ethiopia, they are minimally addressed in terms of their prevalence, sensitive diagnostic methods, and associated risk factors. Infections with *E. histolytica/dispar* and *G. lamblia* are often misdiagnosed and underreported in impoverished countries. Thus, the purpose of this study was to ascertain the prevalence of *Giardia lamblia* and *E. histolytica/dispar* infections as well as related variables among schoolchildren in the Amhara region. A cross-sectional study was conducted among 844 schoolchildren in the Amhara region from April to December 2019. A stool sample was collected from each study participant and processed via the formol ether concentration technique (FECT) and spontaneous tube sedimentation techniques (STST). Data were entered in EpiData and analysed by SPSS statistical software. The prevalence of *E. histolytica* and *G. lamblia* infections using each diagnostic method and composite reference was determined by descriptive statistics. The association of risk factors with *E. histolytica/dispar* and *G. lamblia* infections was analysed by logistic regression and variables with $p < 0.05$ were considered to have statistical significance. From the total, 243 (28.8%) schoolchildren were found to be infected by at least one of *E. histolytica/dispar* or *G. lamblia*. The prevalence of *E. histolytica/dispar* and *G. lamblia* infections was 201 (23.8%) and 62 (7.3%), respectively. The co-infection prevalence with both *E. histolytica/dispar* and *G. lamblia* was 22 (2.6%). The sensitivity (78.6%) and negative predictive value of STST (19.6%) were higher than FECT sensitivity (65.4%) and negative predictive value (13.1%). Children in 10–14 years of age (AOR = 1.66;95%CI: 1.16–2.38), lived in the rural (AOR = 1.97;95%CI: 1.12–3.49), used latrine improperly (AOR = 1.49;95%CI: 1.04–2.13), did not wash hands before meal (AOR = 2.10; 95%CI:1.08–4.10), and after latrine (AOR = 1.51;95%CI: 1.05–2.19), ate unwashed raw vegetables (AOR = 1.85;95%CI:1.26–2.70), and played with soil (AOR = 1.48;95%CI:1.06–2.06) were associated with *E. histolytica/dispar* and *G. lamblia* infection. These findings revealed high prevalence of *E. histolytica/dispar* and *G. lamblia* infections was high in the Amhara region. Therefore, proper implementation of water, sanitation and hygiene should be advocated at the community and school levels to mitigate the disease burden.

* Corresponding author.
E-mail address: tadessehailu89@gmail.com (T. Hailu).

https://doi.org/10.1016/j.fawpar.2024.e00241
Received 24 June 2024; Received in revised form 9 August 2024; Accepted 12 August 2024
Available online 13 August 2024

1. Introduction

Intestinal protozoa infections caused by *Entamoeba histolytica* and *Giardia lamblia* are common worldwide, especially in developing countries. Ninety percent of those affected are carriers of amoebiasis without any symptoms, according to Stanley (2003). The silent infection of *E. histolytica* seems to be more dangerous than the symptomatic illness because neglect might lead to severe amoebic dysentery. Trophozoites in the intestinal lumen (a non-invasive infection) usually only affect carriers who do not exhibit any symptoms (Zaki, 2022). The California Department of Public Health estimated that 411 cases of amoebiasis had been documented in this state alone in 2007 and that the prevalence of *E. histolytica* infection nationwide was roughly 4% (California MSR, 2007). Protozoa of the genus *G. lamblia* are comparable to this and are capable of infecting a wide range of animals. The prevalence of *G. lamblia* is at least 30% in poorer nations, compared to the 2%–5% range in industrialized nations (Tektook et al., 2019).

Consuming food or water contaminated with the parasites' is the commonly route to transmit infections with *E. histolytica* and *G. lamblia*. They can also be directly transferred from person to person by polluted water, fresh produce, soil, and residing in endemic areas (Mama and Alemu, 2016; Callixte et al., 2019). Poor nutrition, a lack of health services, and poverty are key risk factors for the high occurrence of *E. histolytica* and *G. lamblia* infections, according to studies by Bahrami et al. (2018) and Callixte et al. (2019).

Most amoebiasis and giardiasis diagnoses are obtained from stool samples by molecular, serological, or parasitological techniques (Saidin et al., 2019; Hooshyar et al., 2019). The formol ether concentration (FECT) and spontaneous tube sedimentation technique (STST) are two alternative parasitological diagnostic methods for intestinal parasitic infections (Allam et al., 2021; Tello et al., 2012). While STST is inexpensive, easy to use in remote areas, and has a better sensitivity, it is not widely used as a routine diagnostic method for intestinal parasitic infections.

Countries like Ethiopia deal with a variety of public health challenges, such as the prevalence of intestinal parasitic infections and the dearth of reliable diagnostic instruments. Increasing the detection rates of intestinal parasites in people living in severe poverty is an urgent need. Nevertheless, in order for a diagnostic technique to be successful in a low-income society, it must be highly sensitive and reasonably priced. In clinical settings, intestinal parasite infection is diagnosed via direct saline microscopy. As a result, infections with *G. lamblia* and *E. histolytica/dispar* are underdiagnosed and underreported nationwide. Therefore, it's essential to search for new user-friendly, cost-effective, and sensitive diagnostic methods to identify giardiasis and amoebiasis. Additionally, the advantages of establishing water, sanitation, and hygiene (WASH) in areas where *G. lamblia* and *E. histolytica/dispar* are endemic are poorly understood. This study attempted to examine the field performance of FECT and STST in identifying both protozoan species, as well as the prevalence and variables related to infection with *G. lamblia* and *E. histolytica/dispar* among schoolchildren in the Amhara region.

2. Materials and methods

2.1. Study design, period and area

From April to December 2019, schoolchildren in the Amhara region participated in a school-based cross-sectional study to find out how common illnesses with *G. lamblia* and *E. amoeba/dispar* were. The region's yearly mean temperature ranges from 15 °C to 21 °C. There is 1145 mm of rain on average every year. In the Amhara region, June to August and October to May are the wettest and driest months, respectively.

2.2. Sample size and sampling technique

To calculate the sample size, a 95% confidence interval (CI), a 5% margin of error, $p = 50\%$, the design effect 2 and 10% of the non-response were used since there was no previous prevalence research on amoebiasis or giardiasis in the Amhara region. The total sample size was 844.

The districts, primary schools, and schoolchildren in the Amhara region were selected by using a multistage sampling technique. Seven districts (Toledere, Gosamen, Dangila Zuria, Fogera, Bahir Dar, Metema, and Jawi) were randomly selected. Within the seven districts, thirteen primary schools (Bededo, Korke, Wonka, Gultabeshikan, Dokemit, Nora, Zege, Robit, Aftit, Azobahir, and Workmeda) were also selected by a simple random sampling technique. The study comprised 6–14-year-old students whose parents or guardians volunteered and gave their consent. Students who had taken anti-protozoa medications within the three months preceding the data collection period were not allowed to participate in the research. Each primary school's study courses were distributed proportionately to the total number of students enrolled in the institution. Lastly, students who were willing to participate in the study were chosen by a rigorous random sampling process.

2.3. Data collection

Parents provided demographic and environmental data via questionnaires that were gathered by trained health workers. Stool cups were used to collect fresh stool samples from research participants. The samples were then brought to the medical facility and processed using modified FECT and STST to identify *G. lamblia* and *E. histolytica/dispar.*

Modified FECT: Approximately 0.5 g of stool, 1 ml of ethyl acetate and 1.5 ml of 10% formalin were added to the collection tube and well mixed. The mixture was thoroughly combined, and was spun at 123 g, and 3 min. One drop of the sediment was placed on a microscope slide, covered with a cover slip, and examined under a microscope to look for *G. lamblia* and *E. histolytica/dispar* (Young et al., 1979; Jember et al., 2022).

Table 1
Socio-demographic characteristics of schoolchildren with their *E. histolytica/dispar* and *G. lamblia* infections in Amhara region, 2019.

Characteristics		Total n (%)	*E. histolytica/dispar* and *G. lamblia*		χ^2/ *p*-value
			Positive n (%)	Negative n (%)	
Age (year)	**6–9**	253 (30.0)	54 (21.3)	199 (78.7)	10.06/0.001
	10–14	591 (70.0)	189 (32.0)	402 (68.0)	
Sex	**M**	436 (51.7)	147 (33.7)	289 (66.3)	9.86/0.001
	F	408 (48.3)	96 (23.5)	312 (76.5)	
Residence	**Rural**	745 (88.3)	227 (30.5)	518 (69.5)	7.52/0.003
	Urban	99 (11.7)	16 (16.2)	83 (83.8)	
Family size	**1–4**	161 (19.1)	47 (29.2)	114 (70.8)	0.01/0.50
	5–9	683 (80.9)	196 (28.7)	487 (71.3)	
Family educational status	**Illiterate**	109 (12.9)	35 (32.1)	74 (67.9)	062/0.24
	Educated	735 (87.1)	208 (28.3)	527 (71.7)	
Family income in birr	**<1000**	271 (32.1)	76 (28.0)	195 (72.0)	0.15, 0.38
	≥1000	573 (67.9)	167 (29.1)	406 (70.9)	
Total		**844 (100)**	**243 (28.8)**	**600 (71.1)**	

STST: About 10 ml of saline solution and 3 g of stool samples were mixed and filtered in a 50 ml Falcon tube. The Falcon tube was tightly sealed, loaded with more saline solution, vigorously shaken and left to stand for 45 min. One drop of sediment from the bottom was put on the microscope slide, coved with a cover slip, and inspected under a microscope to check for *G. lamblia* and *E. histolytica/dispar* cysts and trophozoites (Tello et al., 2012; Jember et al., 2022).

2.4. Performance evaluation of diagnostic methods

The performance of FECT and STST was evaluated against the composite reference using the following metrics: sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV). This is because there was no available "Gold" standard diagnostic method for the detection of *E. histolytica/dispar* and *G. lamblia*. The diagnostic agreement of the techniques was evaluated using the Kappa value (McHugh, 2012).

2.5. Data quality assurance and data analysis

Laboratory staff and health officers received training on how to collect data prior to data collection. During sample collection, the quantity of stool samples was verified and stool cups were appropriately labelled. In $<$1 h after collection, the sample was delivered to the laboratory of the adjacent medical facility. For every laboratory test, a standard operating protocol was followed to. Microscopic examination of stool slides was performed by two laboratory technologists independently in order to remove observer bias. The findings of their observations were noted on different sheets for future comparison. The inconsistent outcomes were re-examined. Generally, data quality was checked during the pre-, analytical, and post-analytical phases.

The Statistical Package for Social Sciences (SPSS) version 23 was used to analyze the data once they were entered into the EpiData program. A combination of descriptive statistics and Chi-square was used to determine the overall prevalence of giardiasis and amoebiasis. Both univariate and multivariate logistic regression models were used to calculate the degree of correlation between the dependent and independent variables. In the multivariate analysis, variables with $p < 0.05$ were deemed statistically significant.

2.6. Ethical considerations

The Ethical Review Committee at Science College, Bahir Dar University granted ethical approval. The Amhara Public Health Institute, the Amhara National Regional Education Bureau, and the zonal education offices provided supportive correspondence. Letters of authorization were obtained from every elementary school and district education office that were chosen. Written informed consent was acquired from schoolchildren's parents or guardians. Individuals in the study who tested positive for any intestinal parasite were referred to medical professionals for treatment.

3. Results

3.1. Socio-demographic characteristics of the study participants

In all, 844 students took part in the research. Participants in the study ranged in age from 6 to 14 years old, with a mean age of 10.3. Of the 591 study participants, the majority (70.0%) were in the age range of 10 to 14 years. There were more men 436 (51.7%) than women among the participants. The majority of research participants, 745 (88.3%), 683 (80.9%), 735 (87.1%), and 573 (67.9%), were educated to a formal level, had five to nine family members, resided in rural areas, and earned $>$1000 Birr per month for their families (Table 1).

Table 2
The detection of *E. histolytica/dispar* and *G. lamblia* by formol-ethyl acetate test, spontaneous tube sedimentation test and combined diagnostic methods among primary schoolchildren in Amhara region, 2019.

Characteristics		Total (n)	FECT [n (%)]		STST [n (%)]		Combined total n [(%)]	
			E. h/d	*G. l*	*E. h/d*	*G. l*	*E. h/d*	*G. l*
Age (year)	**6–9**	253	35 (13.8)	7 (2.8)	32 (12.6)	14 (5.5)	45 (17.9)	16 (6.3)
	10–14	591	106 (17.9)	27 (4.6)	133 (22.5)	33 (5.6)	156 (26.4)	46 (7.8)
Sex	**M**	436	86 (19.7)	24 (5.5)	88 (20.2)	32 (7.3)	118 (27.1)	40 (9.2)
	F	408	55 (13.5)	10 (2.5)	77 (18.9)	15 (3.7)	83 (20.3)	22 (5.4)
Residence	**Rural**	745	131 (17.6)	31 (4.2)	152 (20.4)	44 (5.9)	187 (25.1)	57 (7.7)
	Urban	99	10 (10.1)	3 (3.0)	13 (13.1)	3 (3.0)	14 (14.1)	5 (5.1)
Total		844	141 (16.7)	34 (4.0)	165 (19.5)	47 (5.6)	201 (23.8)	62 (7.3)

FECT = formol-ethyl acetate, *STST* = spontaneous tube sedimentation test, *E. h/d* = *E. histolytica/dispar, G. l* = *G. lamblia.*

Table 3
The performance of formol-ethyl acetate test, spontaneous tube sedimentation test in *E. histolytica/dispar* and *G. lamblia* detection compared to the composite reference, 2019.

Methods	Composite reference to amoebiasis or giardiasis						
	Pos (N)	Neg (N)	SN (95%CI)	SP (95%CI)	PPV (95%CI)	NPV (95%CI)	K value
FECT	159	7	65.4 (59.1–71.4)	98.8 (97.6–99.5)	99.91 (99.80–99.96)	13.1 (11.2–15.2)	0.710
	84	596					
STST	191	7	78.6 (72.9–83.6)	98.8 (97.6–99.5)	99.92 (99.84–99.96)	19.6 (16.0–23.6)	0.820
	52	594					
	Composite reference to amoebiasis						
FECT	140	1	69.7 (62.8–75.9)	99.8 (99.1–100.0)	99.99 (99.92–100.0)	14.8 (12.3–17.6)	0.774
	61	642					
STST	161	4	80.1 (73.9–95.4)	99.4 (98.4–99.8)	99.96 (99.89–99.98	20.8 (16.6–25.8)	0.847
	40	639					
	Composite reference to giardiasis						
FECT	32	2	51.6 (38.6–64.5)	99.7 (99.1–100.0)	99.87 (99.89–99.99)	9.8 (7.7–12.3)	0.648
	30	780					
STST	46	1	74.2 (61.5–84.4)	99.9 (99.3–100.0)	99.99 (99.94–100.0)	16.9 (11.8–23.7)	0.834
	16	781					

Pos (N) = positive (number), Neg (N) = negative (number), SN = sensitivity, CI = confidence interval, SP = specificity, PPV = positive predictive value, NPV = negative predictive value.

3.2. Prevalence of E. histolytica/dispar and G. lamblia

From the total, 243 (28.8%) schoolchildren were infected at least by one of the *E. histolytica/dispar* or *G. lamblia* parasites. High prevalence of *E. histolytica/dispar* and *G. lamblia* (189 (32.0%), 147 (33.7%), and 227 (30.5%) were obtained in the respective 10–14 age groups, male participants, and rural dwellers. The prevalence rate of *E. histolytica/dispar* and *G. lamblia* co-infections was 22 (2.6%) (Table 1).

Using a combination of FECT and STST, the prevalence of *G. lamblia* and *E. histolytica/dispar* was 62 (7.3%) and 201 (23.8%), respectively. Using FECT and STST, the corresponding prevalences of *E. histolytica/dispar* were 141 (16.7%) and 165 (19.5%). The FECT and STST detection rates for *G. lamblia* infection were 34 (4.0%) and 47 (5.6%), respectively. When it came to the identification of *G. lamblia* and *E. histolytica/dispar,* there was a significant difference ($p < 0.01$) between FECT and STST (Table 2).

3.3. Performance of E. histolytica/dispar and G. lamblia diagnostic methods

High sensitivity (78.6%) and negative predictive value (19.6%) in at least one of amoebiasis or giardiasis detection were obtained by STST by utilizing the combination of FECT and STST procedures as composite references. STST yielded higher amoebiasis detection sensitivity (80.1%) and negative predictive value (20.8%). In the detection of *G. lamblia,* STST yielded improved sensitivity (74.2%), specificity (99.9%), and negative predictive value (16.9%). The agreements of STSTS were almost perfect in both amoebiasis and giardiasis detections. FECT also had a substantial agreement with amoebiasis and giardiasis detections (Table 3).

3.4. Factors associated with E. histolytica/dispar and G. lamblia infections

The probability of acquiring *G. lamblia* and *E. histolytica/dispar* infections was 1.66 times (AOR = 1.66;95%CI: 1.16–2.38) higher in children between the ages of 10 and 14. Compared to children in urban regions, those in rural areas had 1.97 times (AOR = 1.97;95% CI: 1.12–3.49) higher risk of acquiring *G. lamblia* and *E. histolytica/dispar* infections. Similarly, compared to their peers, children who used the latrine inappropriately had a 1.49-fold (AOR = 1.49;95%CI: 1.04–2.13) higher risk of contracting *G. lamblia* and *E. histolytica*

Table 4
Univariate and multivariate analyses of factors associated with *E. histolytica/dispar* and *G. lamblia* infections among schoolchildren in the Amhara region, 2019.

Variables		Number (n)		COR (95%CI)	*P-value*	AOR (95%CI)	*P-value*
		Pos	Neg				
Age	**6–9**	54	199	1			
	10–14	189	402	1.73 (1.23–2.45)	0.002	1.66 (1.16–2.38)	0.006
Sex	**M**	147	290	1.64(1.21–2.22)	0.001	1.28 (0.92–1.78)	0.139
	F	96	311	1			
Residence	**Rural**	227	518	2.27 (1.30–3.97)	0.004	1.97 (1.12–3.49)	0.019
	Urban	16	83	1			
Proper utilization of latrine	**No**	75	124	1.72 (1.23–2.40)	0.002	1.49 (1.04–2.13)	0.028
	Yes	168	477	1			
Handwashing before meal	**No**	21	25	2.18 (1.20–3.97)	0.010	2.10 (1.08–4.10)	0.029
	Yes	222	576	1			
Handwashing after latrine	**No**	82	150	1.53 (1.11–2.12)	0.01	1.51 (1.05–2.19)	0.028
	Yes	161	451	1			
Eating raw vegetables	**Yes**	191	416	1.63 (1.15–2.32)	0.006	1.85 (1.26–2.70)	0.002
	No	52	185	1			
Play with soil	**Yes**	155	303	1.73 (1.28–2.35)	<0.001	1.48 (1.06–2.06)	0.021
	No	88	298	1			

COR = crude odds ratio, AOR = adjusted odds ratio.

infections. In a similar way, schoolchildren who were unable to wash their hands prior to meals had a 2.10 (95% CI: 1.08–4.10) increased risk of infection with *G. lamblia* and *E. histolytica/dispar* compared to those who could. Similarly, children who could not wash their hands after using the restroom were 1.51 times (AOR = 1.51;95%: 1.05–2.19) more likely to be infected with *G. lamblia* and *E. histolytica/dispar* than those who could. Children who consumed raw, unwashed veggies were 1.85 times (AOR = 1.85;95% CI:1.26–2.70) more likely to become sick than children who did not. Children who played in soil had 1.48 times higher infection rates than those who did not (AOR = 1.48;95%CI:1.06–2.06) (Table 4).

4. Discussion

In the present study, 28.8% of schoolchildren were infected at least by one of the *E. histolytica/dispar* or *G. lamblia* parasites. The current results are in agreement with a previous 25.8% prevalence finding in Africa (Hajissa et al., 2022a, 2022b), but higher than previous findings of 20% in northwest Ethiopia (Hailu and Ayele, 2021) and 13.64% in Iraq (Jaeffer, 2011). In the current study, the prevalence of *E. histolytica/dispar* was 23.8%, which is higher than prior findings of 18.1% in northwest Ethiopia (Hailu and Ayele, 2021), 10.9% in northern Ethiopia (Yeshitila et al., 2020), 13.17% in southern Ethiopia (Abate et al., 2023), 16.7% in Ecuador (Tapia-Veloz et al., 2023), and 7.7% in Nepal (Gupta et al., 2020). However, *the* 7.3% prevalence of *G. lamblia* in this study was lower than the 15.4% found in Nepal (Gupta et al., 2020) and the 27.1% in southern Ethiopia (Hajare et al., 2022). The difference in geography, personal hygiene circumstances of the study subjects, diagnostic techniques utilized, and the application of water, sanitation, and hygiene (WASH) all contribute to the variances in the findings from the previous studies.

In the present study, STST outperformed FECT in terms of detection rates and sensitivity for both *G. lamblia* and *E. histolytica/dispar*. Previous investigations (Tello et al., 2012; Gonçalves et al., 2014) made similar conclusion. The rationale might be that STST used a larger stool sample (three gram) than FECT, which only used half a gram of stool. It seems that the likelihood of finding parasites improves when a larger sample size is used. Although it enables detection of motile trophozites, the direct saline wet mount microscopy is poorly sensitive in detecting intestinal protozoa, particularly *E. histolytica/dispar* and *G. lamblia* (Obaid, 2022) Therefore, it is crucial in developing nations to use the best available diagnostic techniques, like STST. As an alternate diagnostic approach for intestinal parasitic infections, STST can be employed in this case since it is less expensive, easy to use, and more sensitive to intestinal helminths and protozoa (trophozoite and cysts) than direct saline microscopy and FECT.

In the current study, male and female pupils aged 10 to 14 were, respectively, 1.66 and 1.39 times more likely to be infected with *E. histolytica/dispar* and *G. lamblia* than were lower-aged and female participants. A possible explanation could be that pupils become more independent in using the toilet as they get older and participate in more outdoor activities, both of which may raise the risk of *E. histolytica/dispar* and *G. lamblia* infections. Additionally, compared to females, males participate in more outdoor activities, including irrigating, caring for pets, and playing games, which could raises their risk of contracting intestinal parasites like *E. histolytica/dispar* and *G. lamblia.*

Intestinal parasitic infection is common among people who practice open defecation and in rural areas (Muluneh et al., 2020). In the present study, schoolchildren who lived in rural areas and improperly utilized latrine facilities were 1.97 and 1.49 times more likely to be exposed to *E. histolytica/dispar* and *G. lamblia* infections, respectively. These results are in line with those of earlier research findings, where the prevalence was higher among rural participants than urban participants (40.0% vs. 30.0%, $p = 0.005$) and a higher prevalence of 42.9% was reported among participants who had latrines compared to 29.8% among participants who defecated in the open field ($p = 0.04$) (Gupta et al., 2020). Similarly, participants who did not have latrine were 2.33 times more likely to be infected than their counterparts, according to a previous study in Ethiopia (Sitotaw et al., 2019). This can be explained by the fact that rural areas have limited knowledge on how to construct and use latrine and that most people there practice open defecation when engaging in outdoor activities.

The spread of intestinal parasitic diseases in children is influenced by poor sanitation and hygiene (Gebru et al., 2023). In the present investigation, *E. histolytica/dispar* and *G. lamblia* infections were 2.10 times more likely to occur in schoolchildren who did not wash their hands before meals. This result is in line with research conducted in Jawi town (AOR = 5; 95%CI: 1.34–14.94, $p < 0.001$) (Sitotaw et al., 2019) and Rama town, Ethiopia (AOR = 2.3; 95%CI: 1.32–4.0, $p = 0.001$) (Tektook et al., 2019). Similarly, *E. histolytica/dispar* and *G. lamblia* infections were 1.48 times more likely to develop in children who played in the soil. These results are consistent with those from an earlier research (Sitotaw et al., 2019). This is understandable given that *E. histolytica/dispar* and *G. lamblia* cysts spread through stool and remain contagious up to a few months in soil and water. Playing in the dirt, not washing hands before eating, not washing hands after using the latrine, and drinking contaminated water are all typical problems that increase the risk of contracting *E. histolytica/dispar* and *G. lamblia* infections. Consumption of raw vegetables and fruits acts as a potential source for the spread of various parasitic diseases (Alemu et al., 2020). In the current study, children who ate unwashed raw vegetables and fruits were 1.85 times more likely to develop *E. histolytica/dispar* and *G. lamblia* infections. This is consistent with previous results (Shahrul Anuar et al., 2012; Sitotaw et al., 2019). The rationale could be that fruits and vegetables are more likely to be contaminated with protozoa cyst stages when they are sold in open markets (on the street) in impoverished nations. Fruits and vegetables typically become contaminated from infected person either directly by stool or indirectly via soil or water. Furthermore, since access to clean water is a major challenge in developing nations, wash water could be a source of contamination for fruits and vegetables. When asked about the source of water for drinking, most participants responded that they used both tap water and surface water (streams, wells, rivers). So, we were unable to analyze the association between intestinal protozoan infection and drinking water sources.

In conclusion, infections with *E. histolytica/dispar* and *G. lamblia* were very common in the Amhara region. However, we were unable to distinguish *E. histolytica* from non-pathogentic species of amoeba, particularly *E. dispar* and *E. moshkovskii.* Similarly, we were unable to identify *G. lamblia* assemblages circulating in humans in the region. We recommend that future molecular studies estimate the actual prevalence of *E. histolytica* and types of *G. lamblia* in the study area. Compared to FECT, STST demonstrated a greater detection rate and sensitivity for *E. histolytica* and *G. lamblia* infections. Risk factors for *E. histolytica/dispar* and *G. lamblia* infections include children between the ages of 10 and 14, living in rural areas, improper latrine use, lack of hand washing before meals and after using the latrine, eating raw vegetables and fruits, and playing in soil. Thus, promoting potable water, proper implementation of WASH, personal hygiene, good latrine use and a one health approach at the community and school levels may aid in reducing *E. histolytica/dispar* and *G. lamblia* infections.

CRediT authorship contribution statement

Tadesse Hailu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Getaneh Alemu:** Writing – review & editing, Software, Methodology, Investigation, Data curation.

Declaration of competing interest

Dear editor, We declare that there have no competing interests.

References

Abate, M., Eriso, F., Kalyankar, V.B., Padwal, N.D., Singh, S.C., Upadhye, V., Humbe, A.S., Jadhav, S.D., Hajare, S.T., 2023. Prevalence of Entamoeba histolytica among schoolchildren in Dilla town, Gedeo zone, Ethiopia. Infect. Dis. Now. 53 (1), 104621.

Alemu, G., Nega, M., Alemu, M., 2020. Parasitic contamination of fruits and vegetables collected from local markets of Bahir Dar City, Northwest Ethiopia. Res. Rep. Trop. Med. 11, 17–25.

Allam, A.F., Farag, H.F., Lotfy, W., Fawzy, H.H., Elhadad, H., Shehab, A.Y., 2021. Comparison among FLOTAC, Kato-Katz and formalin ether concentration techniques for diagnosis of intestinal parasitic infections in school children in an Egyptian rural setting. Parasitology 148 (3), 289–294.

Bahrami, F., Haghighi, A., Zamini, G., Khadem-Erfan, M., Azargashb, E., 2018. Prevalence and associated risk factors of intestinal parasitic infections in Kurdistan province, Northwest Iran. Cogent Med. 5, 1503777.

California MSR, 2007. Selected Reportable Diseases: Report Weeks 49–52 (Dec 2–Dec 29, 2007) Sacramento. Division of Communicable Disease Control Surveillance and Statistics Section. California Department of Public Health, CA.

Callixte, C., Ayubu, A., Lestari, P., Daniel, N., Budhy, T.I., 2019. Epidemiological prevalence of Entamoeba histolytica infections among the patients attending Nyanza District hospital, Rwanda in 2018. Int. J. Epidemiol Res. 6 (4), 149–153.

Gebru, H., Deyissia, N., Medhin, G., Kloos, H., 2023. The association of sanitation and hygiene practices with intestinal parasitic infections among under-14 children in rural Dire Dawa, eastern Ethiopia: a community based cross-sectional study. Environ. Health Insights 17, 11786302231180801.

Gonçalves, A.Q., Abellana, R., Pereira-da-Silva, H.D., Santos, I., Serra, P.T., Julião, G.R., Orlandi, P.P., Ascaso, C., 2014. Comparison of the performance of two spontaneous sedimentation techniques for the diagnosis of human intestinal parasites in the absence of a gold standard. Acta Trop. 131, 63–70.

Gupta, R., Rayamajhee, B., Sherchan, S.P., Rai, G., Mukhiya, R.K., Khanal, B., Rai, S.K., 2020. Prevalence of intestinal parasitosis and associated risk factors among school children of Saptari district, Nepal: a cross-sectional study. Trop. Med. Health 48, 73.

Hailu, G.G., Ayele, E.T., 2021. Assessment of the prevalence of intestinal parasitic infections and associated habit and culture-related risk factors among primary schoolchildren in Debre Berhan town, Northeast Ethiopia. BMC Public Health 21, 112.

Hajare, S.T., Chekol, Y., Chauhan, N.M., 2022. Assessment of prevalence of *Giardia lamblia* infection and its associated factors among government elementary school children from Sidama zone, SNNPR, Ethiopia. PLoS One 17 (3), e0264812.

Hajissa, K., Islam, M.A., Sanyang, A.M., Mohamed, Z., 2022a. Prevalence of intestinal protozoan parasites among school children in africa: A systematic review and meta-analysis. PLoS Negl. Trop. Dis. 16 (2), e0009971.

Hajissa, K., Muhajir, A.E.M.A., Abdall, T., Zakeia, M.S., Hamza, A.E., Elnzer, E., Nahied, E., Jafer, M., Abdulwali Sabo, A., Maki, A., 2022b. A. Prevalence of Entamoeba histolytica and Giardia lamblia among schoolchildren in Um-Asher Area, Sudan. Intern. Med. J. 29 (5), 286–290.

Hooshyar, H., Rostamkhani, P., Arbabi, M., Delavari, M., 2019. Giardia lamblia infection: review of current diagnostic strategies. Gastroenterol. Hepatol. Bed. Bench. 12 (1), 3–12.

Jaeffer, H.S., 2011. Prevalence of Gairdia lamblia and Entamoeba histolytic /Entamoeba dispare infections among Children in AL-Shulaa and. AL-khadimya –Baghdad-Iraq. J. Univ. Anbar Pure Sci. 5 (2), 06–12.

Jember, T.H., Amor, A., Nibret, E., Munshea, A., Flores-Chavez, M., Ta-Tang, T.-H., et al., 2022. Prevalence of *Strongyloides stercoralis* infection and associated clinical symptoms among schoolchildren living in different altitudes of Amhara National Regional State, Northwest Ethiopia. PLoS Negl. Trop. Dis. 16 (4), e0010299.

Mama, M., Alemu, G., 2016. Prevalence and factors associated with intestinal parasitic infections among food handlers of southern Ethiopia: cross sectional study. BMC Public Health 16 (1), 1–7.

McHugh, M.L., 2012. Interrater reliability: the kappa statistic. Biochem. Med. (Zagreb) 22 (3), 276–282.

Muluneh, C., Hailu, T., Alemu, G., 2020. Prevalence and associated factors of soil-transmitted helminth infections among children living with and without open defecation practices in Northwest Ethiopia: a comparative cross-sectional study. Am. J. Trop. Med. Hyg. 103 (1), 266–272.

Obaid, H.M., 2022. Assessment of direct wet mount and various concentration approaches for diagnosing of Entamoeba histolytica /dispar and Giardia lamblia cysts in human stool samples. Int. J. Biomed. Res. 7 (3), 17–21.

Saidin, S., Othman, N., Noordin, R., 2019. Update on laboratory diagnosis of amoebiasis. Eur. J. Clin. Microbiol. Infect. Dis. 38, 15–38.

Shahrul Anuar, T., M Al-Mekhlafi, H., Abdul Ghani, M.K., Osman, E., Mohd Yasin, A., Nordin, A., Nor Azreen, S., Md Salleh, F., Ghazali, N., Bernadus, M., Moktar, N., 2012. Prevalence and risk factors associated with Entamoeba histolytica/dispar/ moshkovskii infection among three Orang Asli ethnic groups in Malaysia. PLoS One 7 (10), e48165.

Sitotaw, B., Mekuriaw, H., Damtie, D., 2019. Prevalence of intestinal parasitic infections and associated risk factors among Jawi primary school children, Jawi town, north-West Ethiopia. BMC Infect. Dis. 19, 341.

Stanley, S.L., 2003. Amoebiasis. Lancet 361 (9362), 1025–1034.

Tapia-Veloz, E., Gozalbo, M., Guillén, M., Dashti, A., Bailo, B., Köster, P.C., Santín, M., Carmena, D., Trelis, M., 2023. Prevalence and associated risk factors of intestinal parasites among schoolchildren in Ecuador, with emphasis on the molecular diversity of *Giardia duodenalis*, *Blastocystis* sp. and *Enterocytozoon bieneusi*. PLoS Negl. Trop. Dis. 17 (5), e0011339.

Tektook, N.K., Al-abodi, H.R.J., Pirko, E.Y., 2019. Prevalence, risk factors and molecular investigation of Giardiasis amonginfants in Al-Shamia City, Al-Qadisiya Governorate. Iraq. Biomed. Res 30, 1–4.

Tello, R., Terashima, A., Marcos, L.A., Machicado, J., Canales, M., Gotuzz, E., 2012. Highly effective and inexpensive parasitological technique for diagnosis of intestinal parasites in developing countries: spontaneous sedimentation technique in tube. Int. J. Infect. Dis. 16 (6), e414–e416.

Yeshitila, Y.G., Zewde, H., Mekene, T., Manilal, A., Lakew, S., Teshome, A., 2020. Prevalence and associated risk factors of intestinal parasites among schoolchildren from two primary schools in Rama Town, Northern Ethiopia. Can. J. Infect. Dis. Med. Microbiol. 2020, 5750891.

Young, K.H., Bullock, S.L., Melvin, D.M., Spruill, C.L., 1979. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J. Clin. Microbiol. 10, 852–853.

Zaki, D.M., 2022. Prevalence of Entamoeba histolytica and Giardia lamblia associated with diarrhea in children referring to lbn AlAtheer Hospital in Mosul, Iraq. Arch. Razi. Inst. 77 (1), 73–79.