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ORIGINAL ARTICLE

Prevalence and risk factors of *Entamoeba histolytica* and *Giardia lamblia* infection among patients with gastrointestinal complaints

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ABSTRACT

BACKGROUND
Intestinal parasitic infections remain a significant public health problem in developing regions, primarily driven by inadequate hygiene and sanitation standards. The most prevalent protozoan parasites causing gastrointestinal illness are *Entamoeba histolytica* and *Giardia lamblia*. This study aimed to investigate the prevalence of these infections and evaluate their association with socio-demographic factors and clinical stool consistency.

METHODS
A total of 122 subjects aged 1-60 years were involved in this cross-sectional study. Data was collected via direct interviews and parasitological diagnosis using standard laboratory protocols to identify the presence of cysts or trophozoites. Data were stratified by age group, gender, and stool consistency, and a Chi-square test was used for data analysis.

RESULTS
The findings revealed a high overall prevalence, with *E. histolytica* being the main parasite at 84.42% (103/122) compared to *G. lamblia* at 15.57% (19/122). Age-specific data showed that the 31-40 year age group had the highest infection rate for *E. histolytica* (92.31%), while the 21-30 year age group showed the highest occurrence of *G. lamblia* (25.81%). Gender-wise, males exhibited a significantly higher infection rate for *E. histolytica* (91.93%) compared to females (76.66%) ($p=0.038$). Regarding stool consistency, a highly significant association was observed ($p<0.001$).

CONCLUSION
The study highlights a substantial burden of protozoan infections, particularly *E. histolytica*. The significant association between stool consistency and parasite type emphasizes the importance of clinical presentation in diagnostic suspicion. Enhanced community-level health education and improved sanitation infrastructure are urgently needed to mitigate the transmission of these intestinal parasites.

Keywords: *E. histolytica, G. lamblia,* gastrointestinal, patients

INTRODUCTION

Intestinal protozoan parasites, including *Entamoeba histolytica* and *Giardia lamblia,* are among the most common causes of gastrointestinal disease worldwide. *Entamoeba histolytica* is the causative agent of amebiasis, which can manifest as diarrhea, dysentery and in severe cases, extraintestinal complications, such as liver abscesses.[1,2] Similarly, *G. lamblia* is responsible for giardiasis, a condition characterized by persistent diarrhea, abdominal cramps, and malabsorption.[3] The transmission of these parasites is often associated with fecal oral contamination, inadequate sanitation, and poor personal hygiene. Developing countries, particularly those with limited access to clean water and health education, bear the highest burden of infection.[4,5] The prevalence of these infections varies significantly across regions. Studies in Iraq have reported high rates of intestinal protozoa, particularly in children and young adults.[6-8,10] Globally, both species of protozoa remain significant contributors to gastrointestinal morbidity.[4,9.11-13] Several risk factors, including age, gender, and stool consistency, have been associated with infection risk and clinical presentation.[14]

Previous studies have indicated that factors such as age, gender, and stool consistency are important indicators of infection risk and clinical outcomes.[15-17] Unlike previous research studies that may have focused on a single parasite, this study examines how stool consistency directly correlates with dual infections in a private sector context. Furthermore, age, gender, and stool consistency were prioritized as the primary risk factors in this research study because they represent the most immediate clinical determinants for rapid diagnosis in Zakho clinics, establishing a baseline before considering secondary environmental variables.[18]

A cross-sectional study conducted among 844 schoolchildren found that the 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.[19] A study involving children presenting with symptoms of diarrhea, that was conducted at Heevi Paediatric Hospital in Duhok City, Kurdistan Region, Iraq, found that the prevalence rates of *E. histolytica* and *G. lamblia* infection were the highest and the lowest in the age groups of less than 1 year and over 12 years, respectively.[20] Another study found that *G. lamblia* has the highest incidence at ages 5-14 years, but that *E. histolytica/dispar* is more common at older ages than at the *Giardia* infection ages and extends to include ages 15-45 years.[21] By contrast, the results of another study found results that are far removed from all of above findings by indicating the ages 40-60 years to be the most vulnerable to *Entamoeba histolytica* and *Giardia lamblia* infections.[22]

These inconsistent results indicate that further study is necessary to determine the cause of the variability. The aim of the present study was to evaluate the prevalences of *E. histolytica* and *G. lamblia* among patients attending private health centers using microscopic examination and to determine the risk factors of infections such as age, gender, stool consistency, and clinical characteristics, and to associate them with gastrointestinal complaints.

Methods

Research design

A cross-sectional study was conducted at private clinics in Zakho city, Duhok governate, Iraq, between April 2025 and September 2025.

Study subjects

A total of 122 samples were enrolled in this cross-sectional study conducted at private clinics in Zakho city between April 2025 and September 2025. All participants filled out structured questionnaires, which captured sociodemographic characteristics and clinical symptoms related to the disease. To ensure data quality, all information was collected directly from patients through face-to-face interviews. The inclusion criteria were patients aged from 1 to 60 years who presented with clinical features of the diseases, including dysentery and bloody or soft watery stools, and who agreed to participate in our study. In the current study, the following exclusion criteria were applied: asymptomatic patients, and patients who did not agree to participate.

Data collection

A total 122 stool samples were collected from patients presenting with gastrointestinal complaints at private clinics in Zakho city, Duhok governate, Iraq. Patients of different age groups

and both genders were included in the study. Fresh stool samples were collected in separate sterile containers. Each sample was examined macroscopically for consistency (watery, liquid, soft, or solid) and the presence of blood. The entire smear area was initially examined at 100x magnification (using 10× objective and 10x ocular), then areas of interest were further magnified to 400x magnification (40× objective). The microscopic examination was performed using direct wet mounts and concentration techniques to detect cysts and trophozoites of *E. histolytica* and *G. lamblia*.

Statistical analysis

The prevalence of infection was calculated using SPSS version 12. Associations between infection and demographic and clinical variables were analyzed using Chi-square tests assessed associations between seropositivity and categorical variables. A p-value <0.05 was considered statistically significant.

Ethical approval

The protocol of this study was approved by the Ethics Committee of Shekhan Technical Collage of Health and Medical, Duhok Polytechnic University and private clinics in Zakho city (Zakho District 07508238913). All participants provided written informed consent prior to enrolment. To ensure privacy, all samples were identified by unique identification codes, and personal identifiers were removed from the final dataset. Participants were informed of their right to withdraw from the study at any time without consequence.

RESULTS

Out of 122 stool samples examined, *E. histolytica* was detected in 103 cases (84.42%), whereases *G. lamblia* was identified in 19 cases (15.57%). There were no samples that contained both parasites (co-infection). The study results indicate that each of the 122 subjects was infected with either *E. histolytica* or *G. lamblia*, but never both simultaneously. The highest prevalence of *E. histolytica* was recorded among individuals aged 31-40 years (92.31%) followed by children aged 1-10 years (89.66%) and young adult of 11-20 years (88.89%) as shown in Table 1. *G. lamblia* showed a peak in the 21–30 years group (8 cases), showing a non-significant different age-specific vulnerability compared to *E. histolytica* (p=0.339). Regarding gender, males had a significant higher prevalence of E. *histolytica* (91.93%) than females (76.66%). While female had significant lower rates of *E. histolytica*, they showed a higher incidence of *G. lamblia* (14 cases) compared to males (5 cases) (p=0.038). This suggests that gender-specific roles, daily activities, or biological factors may influence which parasite an individual is more likely to contract.

Table 1. Distribution of *E. histolytica* and *G. lamblia* infection according to age, gender and stool consistency

Risk factors		Total examined	*E. histolytica*		*G. lamblia*		p value
			Number of positives (n)	Percentage of positives (%)	Number of positives (n)	Percentage of positives (%)	
Age group (years)	1 -10	29	26	89.66	3	10.34	0.339
	11-20	36	32	88.89	4	11.11	
	21-30	31	23	74.19	8	25.81	
	31-40	13	12	92.31	1	7.69	
	41-50	7	6	85.71	1	14.29	
	51-60	6	4	66.67	2	33.33	
Gender	Male	62	57	91.93	5	8.06	
	Female	60	46	76.66	14	23.33	0.038
Consistency	Watery stool	31	24	77.41	7	22.58	
	Watery stool & blood	10	0	0	10	100	
	Liquid	42	40	95.23	2	4.76	<0.001
	Soft	18	18	100	0	0	
	Solid	21	21	100	0	0	

The association between stool consistency and parasite prevalence provides critical diagnostic insights within this study. Most notably, the 100% infection rate of *E. histolytica* in soft and solid stools highlights a significant trend of asymptomatic or chronic cyst shedding ($p<0.001$). This suggests that a large portion of the population may act as carriers, maintaining the transmission cycle even in the absence of active diarrheal symptoms. While *E. histolytica* remained the dominant finding in liquid stools (95.23%), its presence decreased as the severity of stool liquidity increased toward dysenteric presentations. However, *E. histolytica* prevalence was 0% in such samples, while *G. lamblia* was detected in 100% of the cases (10 out of 10).

DISCUSSION

Our findings differ from many previous reports which often highlight children and young adults as the most vulnerable groups due to their immature immunity, poor hygienic practices and close contact in school.[3,4] The higher rate observed in the 31-40 years age group in the present study may be explained by occupational and behavioral exposure factors. Adults in this age range are often economically active, spending more time outside the home and potentially consuming contaminated food or water from street vendors, restaurants or communal workplaces. Such exposure increases the probability of encountering contaminated sources compared to other age groups. Additionally, adults in this age group may serve as asymptomatic carriers who continue daily activities while harboring the parasite, thus sustaining transmission cycles in the community.[1] This possibility is supported by McHardy et al.,[23] who reported that *E. histolytica* can persist in asymptomatic individuals, contributing to its spread in endemic areas. Comparatively, AL-Khikani et al.[13] in Iraq found the highest prevalence among young adults aged 21-30 years, while Tesfaw et al.[14] in Ethiopia reported the greatest burden among children under 15 years. These discrepancies between studies could reflect differences in local epidemiological factors such as water quality, sanitation, dietary habits, and healthcare seeking behavior. Moreover, variations in diagnostic methods may also contribute to differing prevalences across studies.

In contrast, *G. lamblia* infection was more frequent in young adults 21-30 years (25.81%) and in older adults 51-60 years (33.33%) as shown in Table 1. No cases were reported in infants less than one year old or elderly persons more than 61 years of age. Although statistical analysis revealed no significant association between age group and infection, previous findings in endemic regions found that 20-year-old adults are often exposed to infection through social, occupational, and dietary behaviors. Adults in their twenties are typically more mobile, frequently consuming food and beverages outside the home, particularly from street vendors or communal dining areas, which increases the probability of exposure to contaminated sources.[13,24] Furthermore, the above-mentioned age group may reflect lack of strict hygiene practices due to lifestyle factors which facilitate transmission. The second peak of infection observed in individuals aged 51-60 years could be attributed to age-related changes in immunity and chronic gastrointestinal susceptibility. Older adults may experience a decline in protective immune response, making them more prone to symptomatic giardiasis or recurrent infection.[25] In addition, comorbid conditions such as diabetes or gastrointestinal disorders, which are more common in this age group, may predispose them to higher infection rates. Another study reported a higher *Giardia* prevalence among adults particularly those engaged in outdoor occupations.[16,26] Other studies reported the highest prevalence rates among children due to immature immunity and poor hygiene practices.[14] These variations across studies underscore the complex interplay of behavioral, occupational, and immunological factors that shape giardiasis in different age groups. The dual peak pattern observed in young and middle-aged individuals suggests that giardiasis is not limited to childhood as often emphasized in epidemiological literature but also represents a risk in older age groups where immunity and comorbidities influence susceptibility. This emphasizes the need for targeted preventive measure across multiple age groups, particularly those with higher environmental exposure or compromised immunity.

Regarding gender, males showed a higher prevalence of *E. histolytica* (91.93%) compared to females (76.66%). Conversely, *G. lamblia* infection was more common in females (23.33%)

than in males (8.06%) with statistically significant differences as shown in Table 1. The higher prevalence of *E. histolytica* in males may be explained by greater environmental and occupational exposure, as men are often engaged in outdoor activities, agricultural work, or workplaces with poor sanitation where contact with contaminated water and soil is more likely.[13] Similar findings were reported in studies conducted in Iraq,[27] where males showed higher infection rates of amebiasis of around 50%. These results suggest that behavioral and occupational factors rather than biological susceptibility may account for the observed differences. In contrast, the higher prevalence of *G. lamblia* among females in this study is noteworthy. Some reports have indicated a similar trend, attributing the difference to house-related risk factors such as direct involvement in food preparation, care of children and water handling, which may increase exposure to contaminated food and water sources.[21,23]

With respect to stool consistency, there was a strong association between stool consistency and infection types. *E. histolytica* was more prevalent in liquid (95.23%), soft (100%), and solid (100%) stool samples. In contrast, *G. lamblia* was most common in watery stools with blood (100%) and watery stools without blood (22.58%), with a highly significant statistical difference as shown in Table 1. The high prevalence of *E. histolytica* in liquid and solid stool samples is consistent with parasite pathophysiology. *E. histolytica* trophozoites invade the intestinal mucosa, leading to dysentery-like diarrhea and sometimes more formed stools when chronic or asymptomatic infections are present.[28] The presence of the parasite in both liquid and solid stools has also been reported elsewhere,[13] thereby supporting the notion that *E. histolytica* infection may present in a range of stool consistencies, from asymptomatic cyst carriers to invasive disease.[29] On the other hand, the predominance of *G. lamblia* in watery and bloody diarrhea is in line with its non-invasive mechanisms of infection. *Giardia* trophozoites adhere to intestinal mucosa without penetrating the tissue, leading to malabsorption, osmotic diarrhea, and watery stools.[30] The high rates of watery stools particularly those with blood, may also reflect co-infections or irritation of the mucosa, as reported in previous studies.[24,26] Our findings highlight the diagnostic importance of stool consistency as an epidemiological marker. The significant statistical association suggests that stool examination should not only focus on parasite detection but also take into account the stool physical characteristics, which may provide valuable clues about the probable causative protozoa.[2,3]

However, the overall high prevalence of *E. histolytica* and *G. lamblia* in this study emphasizes the continued public health challenge posed by intestinal protozoa in resource-limited settings and underscores the need for improved sanitation, safe water supply, and health education to reduce transmission.

Despite the significant findings, this study has several limitations. First, the sample size of 122 participants is relatively small and was drawn exclusively from private clinical settings in Zakho City, which may not fully represent the wider population of the Kurdistan Region. Second, the diagnosis relied on microscopic examination (direct wet mount and concentration techniques). While these are standard laboratory protocols, they may have lower sensitivity compared to molecular methods such as multiplex polymerase chain reaction (PCR), potentially leading to an underestimation of low-intensity infections or the incapability to distinguish *E. histolytica* from the non-pathogenic *E. dispar*. Lastly, the cross-sectional design only provides a snapshot of the prevalence at a specific time period (April to September 2025) and cannot find a direct causal relationship between the identified risk factors and the onset of infection.

The findings of this study have direct implications for healthcare providers in the private sector. The 100% association between *G. lamblia* and watery stools with blood, and *E. histolytica* with soft/solid stools, provides a critical diagnostic indicator for rapid clinical suspicion before laboratory confirmation. Given the high prevalence of *E. histolytica* (84.42%) in this patient group, clinicians should order aggressive screening and pragmatic treatment strategies in adult males (31-40 years) who present with chronic gastrointestinal complaints. Furthermore, the high rates of infection in solid stools suggest a significant number of asymptomatic cyst passers in the community, highlighting a silent reservoir for transmission that requires targeted public health involvement.

Future research should aim to apply molecular diagnostic tools (such as multiplex PCR) to differentiate between pathogenic and non-pathogenic species and to detect potential co-infections that microscopy might miss.

Longitudinal studies are needed to track seasonal variations in parasite prevalence across both public and private sectors to better inform regional health policies. Additionally, further investigation into the environmental sources of infection such as testing local water supplies and fresh produce in Zakho City is essential to develop effective prevention and control strategies. Expanding the study to include a wider geographical area within the Duhok Governorate and Rural would also provide a more comprehensive epidemiological map of intestinal protozoa in the region.

CONCLUSION

This study demonstrated that *E. histolytica* is more prevalent than *G. lamblia* among patients with gastrointestinal complaints, particularly among children and young adults. Gender and stool consistency were significant risk factors, with males being more prone to *E. histolytica* and females to *G. lamblia*. The association of watery and dysenteric stools with protozoan infections highlights the clinical importance of stool examination in diagnosis.

Conflict of Interest

The authors declare that there are no financial or personal relationships with other people or organizations that could improperly influence or bias the findings of this study. There is no conflict of interest regarding the publication of this manuscript.

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Authors' Contribution

HAS: Conceptualization, Methodology, and Data collection. SOH: Formal analysis, Writing original draft, and Correspondence. GMF: Investigation, Justification, and Supervision. MIA: Visualization, Software, Writing review and Editing. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Shivan O. Haydar, upon reasonable request. The raw data are stored in a secure repository at the Medical Laboratory Technology Department, Shekhan Technical College of Health and Medical at Duhok Polytechnic University.

Declaration of AI Usage in Scientific Writing

The authors declare that no Artificial Intelligence (AI) technologies were used in the preparation of the scientific content, data analysis, or the formulation of the conclusions of this manuscript. The writing was performed uniquely by the human authors listed. (Note The authors used Gemini and ChatGPT for language editing and grammar refinement to improve the clarity of the manuscript. The scientific content and data analysis were performed solely by the authors)

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