Original Article

Epidemiological Study of Intestinal Protozoan Infections: A Cross-sectional Study in Zakho, Kurdistan, Iraq, during 2018-2022

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ABSTRACT

Intestinal protozoan parasitic infections are considered one of the most frequent types of infection caused by these parasites and remain a major health problem for communities. This study aims to detect the frequency of intestinal protozoan infections infection among Zakho general population from October 2018 to June 2022. This cross-sectional study was performed on 2,118 patients referring to private medical diagnostic laboratories in Zakho, Kurdistan Region, Iraq. Samples of fecal matter were collected and subjected to analysis using two different techniques: direct observation under the microscope (wet mount) and formalin-ether concentration methods. Morphological characteristics of trophozoites and cysts were used to identify *E. histolytica* using microscopical examination. Out of 2,118 recruited samples, 1,155 (54.53%) were male, and 963 (45.47%) were female. The mean age of participants was 20.41 (±19.12), with ages ranging from 1 to 63 years. The overall prevalence of protozoan infections was 395/2118 (18.65%). Out of these, *Entamoeba histolytica* was the predominant pathogenic protozoa infection 271 (68.61%), followed by *Giardia lamblia* 100 (25.31%). A significant association was seen between *Entamoeba histolytica* and age groups (P=0.003) and gender (P=0.004). The highest infection rate was reported among the age group <15 years (55.72%). We also found a higher rate of *Giardia* infection among age group < 15 years (46%) with significant differences (P=0.002) and a higher rate of *Giardia* seen in females (55%) with significant association (P=0.014). The frequency of *Giardia lamblia* and *E. histolytica* infections in our study was higher than other studies reported in Iraq and other countries, and these infections continue to pose a difficult public health issue and necessitate the implementation of stronger and more effective preventative measures.

Keywords: *E. histolytica* and *Giardia lamblia*, *General population*, Intestinal protozoa, *Zakho*

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1. Introduction

Protozoan infections affecting the intestines persist as the most widespread infectious agents globally and continue to be a significant public health concern in tropical and subtropical regions, primarily in rural areas (1). Particularly in children, these intestinal parasitic infections can cause malnutrition, anemia, mental growth retardation, and physical impairments among infected people (2). It is known that low economic status, cultural conditions, illiteracy, inadequate or poor sanitation conditions, insufficient water supply, low educational performance of infected children, and lack of health awareness are attributed to the high-frequency rates of protozoal infection, especially in developing countries (3). The identification of intestinal protozoan infections is crucial in executing efficient prevention and control programs aimed at preventing these overlooked protozoal infections in our country. Additionally, variances in demographic characteristics among people in various societies emphasize the need for population-based investigations of protozoal infections from various geographical areas. The prevalence and transmission of intestinal protozoan infections are influenced by various factors such as environmental conditions, socioeconomic status, demographic factors, and hygiene-related behaviors (4). Intestinal protozoal infections occur with varying frequency and epidemiological features across different countries worldwide. In many developing nations, including Iraq, the prevalence of these infections is particularly high (5). The most prominent intestinal protozoa infections are *Giardia intestinalis*, *Entamoeba histolytica*/*Entamoeba dispar*, and *Blastocystis* sp. (6). Globally, for example, it has been suggested t*hat E. histolytica* infects around 480 million people, with its prevalence ranging from 5-81% (4). Although the significance of *Entamoeba* infections in public health is widely recognized, their geographic distribution and regional impact have yet to be fully understood. In this regard, a recent study has revealed a high prevalence of *E. histolytica* in patients with liver abscesses (7). More than one million people are affected by *G. lamblia* annually, making it the most prevalent intestinal parasite in the United States (8). *Giardia intestinalis* is a widespread cause of parasitic diarrhea in both humans and animals, with an estimated 280 million people affected by the parasite worldwide (7). The global frequency rate of these intestinal protozoal infections in humans is estimated at 1 to 8% in developed countries and 8 to 30% in developing countries (8). In the Kurdistan region, Iraq, similar to other developing countries, parasitic and other infectious diseases are a significant public health concern among the population (9, 10, 11, 12). Most studies examining the prevalence of intestinal parasitic infections in Iraq have focused on the urban population and school-age group. Due to the absence of population-based surveys, there is inadequate information on the frequency and prevalence rates of intestinal protozoan infections in various rural and urban regions across the country. Since there is a shortage of studies in our region, the current research aimed to assess the prevalence rate of intestinal protozoan infections, including *Entamoeba histolytica* and *Giardia lamblia*, among various age groups and genders in the Zakho districts of Kurdistan Region, Iraq, from 2018-2022.

2. Materials and Methods

2.1 The area of study

A cross-sectional study was conducted across several districts within Zakho, located in the Kurdistan Region of Iraq, from October 2018 to June 2022. Zakho City is situated on the border with Turkey, specifically at the crossing point named Ibrahim Khalil. The distance between the center of Zakho and the Turkish border is roughly 10 km, while the Syrian border (Peshkhabor) is approximately 28 km away (Figure 1). Zakho serves as a transit point for refugees who are on their way to Western countries via the Turkish border, resulting in the city hosting a significant number of internally displaced persons and refugees.

2.2 Sample Collection and Processing

A total of 2,118 stool samples were collected from patients who were referred to a Private Health Center in Zakho, Iraq. The participants were invited to participate voluntarily in the study and were provided with a clear explanation of the study's objectives. The confidentiality of the participants' details was ensured. Stool samples were collected in screw-capped containers and labelled with individual numbers to maintain anonymity. A container was given to each participant for the collection of stools and sent to the laboratory. Protozoan cysts or trophozoites were initially detected by direct smear (wet mount) of fresh samples. Microscopic examination was used to identify *E. histolytica* based on the morphological characteristics of its trophozoites and cysts. The morphological characteristics of *E. histolytica* include the presence of one to four spherical vesicular nuclei, each containing a central karyosome, a nuclear membrane lined with a thin layer of chromatin, the presence of chromatoidal bars in the cytoplasm, and measurement ranging from 10-20 micrometers (13). The formalin-ether concentration method and Lugol's iodine staining were utilized to examine the stool samples, and direct smears (wet mount) were used to identify protozoan trophozoites in diarrheal samples under a microscope. The direct smear method involves mixing a small amount of stool sample with a drop of distilled water on a slide, covering it with a

Figure 1. Map viewing of the location of Zakho City included in the study

coverslip, and then examining it under a microscope. In the formalin-ether concentration method used in the study, a small amount of the fresh stool sample weighing 1 gram was dissolved in a tube containing 10 ml of 10% formalin solution. The mixture was then thoroughly mixed with 7 mL of the filtered suspension using a vortex mixer. After centrifugation at 3,000 rpm for 5 min, the supernatant was discarded and 3 ml of ether was added to the remaining sediment. The mixture was centrifuged again at 3,000 rpm for 5 min, and the supernatant was removed. Then, a drop of Lugol's iodine was added to the remaining sediment and examined under a microscope.

2.3 Statistical analysis

The collected data was analyzed using the statistical software GraphPad Prism version 8.0 (San Diego; California, USA). The Chi-square test was conducted to find the associations between infections and age and gender. A *P*-value less than 0.05 was considered statistically significant.

3. Results

The participants had an average age of 20.41 years with a standard deviation of 19.12 years, and their ages ranged from 1-63 years. More than half of the participants (55.45%) belonged to the age group of < 15 years. Out of 2,118 participants, 1,155 (54.53%) were male, and 963 (45.47%) were female. Out of the 2,118 stool samples collected, 395 were positive for intestinal protozoan infections, resulting in an overall prevalence of 18.65%. The majority of these infections were caused by *Entamoeba histolytica*, with 271 (68.61%) participants testing positive for this pathogenic protozoon, followed by *Giardia lamblia* 100 (25.31%) and both protozoan 24 (6.08%) (Table 1). We also found a significant association between *Entamoeba histolytica* and different age groups (P=0.003; Table 2). The highest infection rate was reported among the age group < 15 years 151/271 (55.72%), followed by the age group 62/271 (22.89%). We also found a higher rate of *Giardia* infection among the age group < 15 years 46/100 (46%) with statistically significant differences among them (P=0.002; Table 2). However, the results indicated no statistically significant difference between the prevalence of *Giardia lamblia* and *Entamoeba histolytica* infections across different age groups (P=0.72) (more prevalent in < 15-year-old participants) (Table 2). Regarding gender, the highest rate of *Entamoeba histolytica* infection was reported among males 165 (60.89%), and statistically significant differences among them (P=0.004; Table 3). However, the higher infection rate of *Giardia* was found in females 55 (55%) with significant differences (P=0.014). Similarly, 13 (54.17%) was the highest percentage of both infections found in females; however, it was statistically not significant (P=0.39; Table 3).

Table 1: Frequency of *E. histolytica* and *Giardia lamblia* in Zakho City, Iraq (n=2118)

Protozoa Species	Frequency	Percent (%)
Entamoeba histolytica	271	68.61
Giardia lamblia	100	25.31
Mixed *E. histolytica and G. Lamblia*	24	6.08
Total	**395**	**100**

Table 2: Frequency of *E. histolytica* and *Giardia lamblia* protozoan based on age groups

		Age group (year) No. (%)				
	Total No. (%)	**<15**	**15-24**	**25-44**	**>45**	***P value**
Entamoeba histolytica	271 (68.61)	151 (55.72)	20 (7.38))	62 (22.89)	38 (14.02)	0.003
Giardia lamblia	100 (25.32)	46 (46.0)	18 (18.0)	14 (14.0)	22 (22.0)	0.002
Mixed *E. histolytica and G. lamblia*	24 (6.07)	11 (45.83)	2 (8.33)	5 (20.83)	6 (25)	0.72
Total No. (%)	**395 (100)**	**208 (52.65)**	**40 (10.13)**	**81 (20.51)**	**66 (16.71)**	

*p-value is determined using the Chi-square test

Table 3: Frequency of *E. histolytica* and *Giardia lamblia* protozoan based on gender

		Gender No. (%)		
	Total No. (%)	**Male**	**Female**	***P value**
Entamoeba histolytica	271 (68.61)	165 (60.89)	106 (39.11)	0.004
Giardia lamblia	100 (25.32)	45 (45)	55 (55)	0.014
Mixed *E. histolytica and G. Lamblia*	24 (6.07)	11 (45.83)	13 (54.17)	0.39
Total No. (%)	**395 (100)**	**221 (55.95)**	**174 (44.05)**	

*p-value is determined using the Chi-square test

4. Discussion

Intestinal protozoan infections caused by parasites continue to be a significant global public health issue; thereby, the infection possibly contributes to socioeconomic disaster and hampers community health, especially in rural areas (1). The present study demonstrated a relatively high prevalence rate of intestinal protozoan parasites among the general population in Zakho, Iraq. The overall frequency rate of protozoal infections was 18.65%, and such a prevalence rate has been consistently reported by several studies performed in other countries (5). In the present study, the most prominent intestinal protozoa infections were *E. histolytica* (68.61%), followed by *G. lamblia* (25.31%) and mixed infections (6.08%). It is worth noting that while *E. histolytica* and *G. lamblia* are recognized as public health issues, there is still limited understanding of their geographic distribution and regional impact (8). Earlier studies in Iraq have revealed that the most prevalent intestinal protozoan infections among patients who visited Azadi Teaching Hospital in Duhok City in Kurdistan Region were *E. histolytica* and *G. lamblia* (5). The prevalence of intestinal protozoan infections and the reported species vary in different studies conducted in Iraq. However, most of these studies have reported that the highest prevalence of infection was caused by *E. histolytica* (5). The current study is consistent with a previous study conducted in Erbil, Iraq, which reported similar species of intestinal parasites with slightly lower prevalence rates than those found in the present study (13). This study reported that 61.24% and

26.60% of people were infected with *E. histolytica* and *G. lamblia*, respectively (13). Another study conducted on 2,296 participants in Mosul, Iraq, showed that the rate of *E. histolytica* and *G. lmblia* in children associated with diarrhea was 14.5% and 0.96%, respectively (14). Another study performed in Duhok, Northern Iraq, revealed that the frequency rate of *E. histolytica* and *G. lmblia* among patients attending Azadi Teaching Hospital was 28.3% and 1.1%, respectively (5). Another study conducted in the Mazandaran Province of Iran reported that the prevalence of *G. lamblia* infection among the general population was 4.6% (15). Furthermore, another study conducted in Duhok province among children found that the rate of *E. histolytic* was 15% using ELISA technique (16). This rate was lower than the rate recorded by the present study. This variation in the rate of parasitic infections could be due to differences in the number of samples, design of the study, different laboratory tests, sanitary conditions, type of consumed water, economic standards, climate and environmental factors, hygiene levels, behavior style, and type of drinking water. In the present study, we found a positive relationship between *E. histolytica or G. lamblia* and different age groups (P=0.001). The highest parasitic infection rate was reported among young age < 15 years (55.72%). However, no positive association was seen between mixed parasitic infections and different age groups (P=0.72). The present results are consistent with a study conducted in Zakho among primary school children, in which they found the highest infection rate occurred in 9-year-old children (17). The higher prevalence of infection among young people in the present study may be attributed to less attention paid by their mothers towards personal hygiene, as children in this age group are more active and tend to play outdoors, exposing them to more dirt and potential sources of infection. On the other hand, older individuals tend to be more conscious of personal hygiene and take better care of themselves. Similar findings were reported in another study, which showed a significant relationship between age and *E. histolytica* infection. The study indicated that the parasite had the highest prevalence (30.82%) in the age group of < 15 years, while the lowest prevalence (17.34%) was observed in the age group of 31-45 years (P<0.05) (18). Our results are also in accordance with a study conducted in Iran found the most prevalence rate of *G. lamblia* was found among people aged between 5-9 years (9.3%) (15), and this is also in agreement with several other studies performed in Iran and Malaysia (19, 20). The higher prevalence of infection among young people in the present study may be due to their increased exposure to various sources of infection and their potentially lower standards of personal hygiene compared to adults. The prevalence rate of *E. histolytica* was found to be significantly higher among males (60.89%) than females in this study (P=0.004). However, a higher rate of *G. lamblia* was reported in females in comparison with males (55%) (P=0.014). These results were consistent with other studies conducted in Iraq; the study found that the prevalence of *E. histolytica* and *G. lamblia* was higher in males than females, and this difference was statistically significant (P<0.05) (14). Several studies have reported a higher prevalence of giardiasis among males compared to females, which is in contrast to the findings of our study. For example, one study reported that the prevalence of giardiasis in males was 4.7% and in females 4.5% (14). Additionally, a study conducted in Palestine found a higher prevalence of giardiasis among males (24%) compared to females (21). Another study conducted in Duhok, northern Iraq, found a higher rate of giardiasis among males (41.6%) than in females (35.6%) (22). The results of the present study were in line with other studies, which found that the prevalence of *E. histolytica* was higher in males (48.8%) than females (34.44%) (23). Other studies also reported that the rate of *E. histolytic* (61.7%) in males was higher than the rate of infection in females (24). Our findings are in agreement with previous studies that have shown a significant difference *in E. histolytica* infection between genders, with a higher proportion of men being affected by invasive amebiasis. This may be due to the greater susceptibility of men to invasive diseases, as demonstrated in one study (23). Additionally, another study found that *E. histolytica* infection was more prevalent in male hosts (22.36%) compared to female hosts (20.9%), but the difference was not statistically significant (P>0.05). The present finding is consistent with the results of a previous study (23), which reported a higher prevalence of *E. histolytica* in males than in females. The higher prevalence of *E. histolytica* in males could be attributed to their increased susceptibility to infection compared to females. The differences observed between genders concerning susceptibility to infections could be due to variations in endocrine immune interactions, specifically in the levels of sex steroid hormones, such as androgens and estrogens. These hormones have been shown to modulate different aspects of the host immune response by regulating the expression of Toll-like receptors and the production of humoral immunity (25). The lower prevalence rate of parasitic infections among female participants may be due to their higher level of concern for hygiene care. Females tend to pay more attention to personal hygiene, which includes washing hands before meals, using clean water for washing and cooking, and keeping the environment clean. Additionally, females are generally more cautious about what they eat and tend to avoid consuming contaminated food and water. These behaviors can reduce the risk of contracting parasitic infections, especially those transmitted through the fecal-oral route. The obtained results are also supported by the results of another study, which found a higher rate of infection among males due to sociocultural lifestyles (23). Furthermore, it is explained by the fact that males tend to be more active and have more outdoor activities, which may increase their exposure to sources of parasitic infections or unhygienic conditions. In contrast, females may spend more time indoors and pay more attention to personal hygiene, which could reduce their risk of infection (3). the present study reported a higher prevalence of *E. histolytica* and *Giardia lamblia* infections among the general population than previous studies conducted in Iraq and other countries. The highest infection rate was reported among younger age groups and females. The high prevalence of *E. histolytica* and *Giardia lamblia* infections in the present study highlights the continued challenge these infections pose to public health in our community. More effective preventive and control measures are urgently needed to reduce the burden of these infections on the population. Public health education and awareness campaigns on personal hygiene practices, such as handwashing with soap and clean water, safe food and water handling, and proper sanitation can effectively reduce the transmission of intestinal parasitic infections. In addition, routine screening and treatment of infected individuals and environmental interventions, such as proper waste management, can also contribute to the control and prevention of these parasitic infections in the community.

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Authors' Contribution
Ibrahim Naqid developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and is guarantor.

Ethics
The protocol and design of study was approved by the Ethics Committee of the College of Medicine, University of Zakho, Kurdistan Region, Iraq. Informed consent was obtained from all participants before sampling.

Conflict of Interest
The author declares that there is no conflict of interest.

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Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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