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Original Article

Prevalence of amoebiasis and associated risk factors among population in Duhok city, Kurdistan Region, Iraq

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Abstract

Introduction: *Entamoeba histolytica*, a protozoan parasite, is the third major contributor to human mortality and morbidity outside of malaria and schistosomiasis. The purpose of this cross-sectional study was to estimate the prevalence of *Entamoeba* spp. among outpatients of two teaching hospitals in Duhok city who agreed to participate in the study from April 2021 to March 2022 to assess the impact of associated risk variables on the infection rate.

Methodology: Stool specimens were collected from outpatients suffering from diarrhea and other gastrointestinal symptoms in two teaching hospitals: Azadi and Heevi Pediatric in Duhok city, Kurdistan Region- Iraq. The collected stool specimens were examined macroscopically, followed by microscopic examination using the direct wet mount and zinc sulfate flotation methods, respectively.

Result: Infection with *Entamoeba* species was recorded in 21.68% (562/2592) of the analyzed specimens. Males had a significantly higher infection rate than females (67.43% vs. 32.56%). This difference was statistically significant ($p < 0.000$). The highest rate was seen in the age group 1–10 years ($p < 0.001$). Lower levels of education, low incomes, eating unwashed fruits and vegetables, drinking well water, eating frequently outside of homes, not using antidiarrheal medications and living in overcrowded families were risk factors that showed high levels of infection ($p < 0.0001$).

Conclusions: The present study concluded that improving living conditions, providing clean water, and promoting health education programs are essential to reduce the rate of this disease among the population.

Key words: prevalence; parasitic infection; *Entamoeba* spp.; risk factor.

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Introduction

Entamoeba histolytica is a protozoan parasite causing the infectious disease amoebiasis [1]. An estimated 50 million individuals are affected annually, 100,000 of them lose their lives [2]. *Entamoeba histolytica* causes intestinal and extraintestinal invasion of the host tissues [3]. This parasite primarily lives in the intestine, and the vast majority of infected persons show no symptoms whatsoever, but it may damage the tissues of host's intestinal wall and result in intestinal discomfort, gastrointestinal pain, intermittent diarrhea or constipation, chills, fever, and irregular bowel movement characterized by blood or mucoid stool [4]. The average duration of these symptoms is 1 to 3 weeks, however, in a small number of cases, severe and even deadly complications can arise from extraintestinal infections, such as liver abscesses, lung abscesses, brain abscesses, etc. [5]. Nine species of the genus *Entamoeba* live in the human intestinal lumen, namely *E. histolytica*, *E. coli*, *E. dispar*, *E. moshkovskii*, *E. bangladeshi*, *E. polecki*, *E. hartmanni*, *E. struthionis* and *E. chattoni*. Among them, the pathogenic species are *E. histolytica*, *E. moshkovskii* and *E. Bangladeshi;* while *E. dispar* is nonpathogenic. These four species have similar morphology and are known as the *Entamoeba* complex. Furthermore, these species cannot be differentiated by microscopic examination [6,7].

Entamoeba histolytica can attach to and subsequently destroy epithelial tissue. Amoebiasis is transmitted through acquiring mature cysts in contaminated food or water by fecal materials of infected people [8]. *Entamoeba histolytica* has two distinct phases. The cyst is able to thrive outside the host under cold and harsh conditions for several months [6]. After being ingested, the cyst walls rupture and the released trophozoites become active in the intestine and multiply producing many generations of trophozoites. Trophozoites form and colonize the colonic region in

the small intestine before adhering to the mucosal layer of the large intestine. Intestinal and extraintestinal infections are brought on by *E. histolytica* trophozoite [9]. Additionally, some trophozoites may be passed outside the human host in diarrheic stool, but they are unable to survive. Uncertainty exists regarding the signaling pathways that result in excystation or encystation [10]. In extraintestinal infections, *Entamoeba* can cause hepatic abscesses that can be lethal if left untreated. In addition, the parasite can disseminate from the liver via the circulatory system or directly due to the rupture of these abscesses to other organs like the lungs, brain or other parts [11]. Although amoebiasis has a worldwide distribution, it is mostly prevalent in the developing countries, tropical and subtropical regions of the world, due to poor sanitation and hygiene, and overcrowded living conditions which facilitate the transmission in impoverished nations, while travelers from endemic nations are the main source of transmission in industrialized nations [12,13].

The Kurdistan Region and other parts of Iraq are considered as endemic regions for many parasitic diseases, mainly caused by protozoa, including amoebiasis, and most of the recent studies dealt with the distribution of these parasites among various communities, such as hospital outpatients, day-care centers, primary school children, general population, food handlers, immunocompromised and malnourished patients residing in urban and rural areas [14]. Moreover, the prevalent intestinal parasites in Iraq vary in their density and species from one place to another depending on several factors [14]. Therefore, this study evaluated the prevalence of amoebiasis, and its correlation with gender, age and some risk factors in Duhok province, Kurdistan Region - Iraq in order to get a more precise insight about the situation in this area.

Methodology

Samples and data collection

Between April 2021 to March 2022, 2592 stool samples were collected from outpatients who visited two teaching hospitals, the Azadi and the Heevi Pediatric in Duhok city and used in a descriptive cross-sectional analysis. After obtaining verbal consent from each participant and the parents of infants and small children, fresh stool specimens were collected in clean, clearly labeled screw-topped sample tubs and maintained cold on a cold pack fully labeled with the patients' information.

The use of data and samples in this study was approved by the ethical committee of the Duhok General Directorate of Health (0652021-2-4).

A structured questionnaire was designed to collect data on participants' demographics such as gender, age, level of education, application of hygiene, type of drinking water, source of food eaten, use of antidiarrheal medications and family size. The samples were taken within one hour daily for macroscopic and microscopic analysis to the Postgraduate Microbiology Laboratory, in the Biology Department, Faculty of Science, Zakho University.

Sample examination: macroscopic

The samples were analyzed macroscopically for characteristics like color, consistency, texture, presence of blood, mucus, and the adults or segments of helminths. Macroscopical inspection of feces revealed all of these features, as well as a wide range of colors, including yellow, brown, semi-brown, and greenish.

Sample examination: microscopic

In order to look for parasite stages such as cysts and trophozoites, pus cells, red blood cells (RBCs), and epithelial cells, a small fleck was taken from the specimen and placed in a drop of normal saline (0.85%) in the center of a clean slide. A drop of diluted Lugol's iodine solution (stock solution) was then added as it stains the cytoplasm yellow or light brown. The nucleus becomes dark brown, and the arrangement of peripheral chromatin and karyosome can be seen and the objects remain clear. The trophozoite can also be detected by this method in fresh stool specimens. The two drops were thoroughly mixed with a wooden stick before being covered with a coverslip and examined under a light microscope, with 10x, 40x and then carefully with 100x if required. Three slides were prepared from different parts of each specimen, and every *Entamoeba* stage observed was reported. The disadvantage of this method is that light infection cannot be detected.

Sample examination: concentration technique (Zinc sulfate floatation)

About 2 grams from each stool specimen were mixed with 10-12 mL of normal saline and mixed with a glass rod to detect protozoan cysts. This approach can also be used to detect helminths ova, and larvae [15]. Although protozoan trophozoites cannot be detected in this method as they will disintegrate, the mixture was passed through two layers of damp surgical gauze to remove large particles. The supernatant was centrifuged for two minutes at 1500-2000 rpm. This procedure was performed three times; each time the supernatant liquid was decanted and the sediment was resuspended in normal saline and centrifuged. The zinc sulfate solution

Table 1. Distribution of amoebiasis according to the age and gender.

Age groups (years)	Total no.	Infected no.	%	Males		Females	
				No. positive / No. examined	%	No. positive / No. examined	%
1-10	1627	304	54.09	219/304	72.03	85/304	27.96
11-20	287	152	27.04	93/152	61.18	59/152	38.81
21-30	337	57	10.14	35/57	61.40	22/57	38.59
31-40	135	22	3.91	14/22	63.63	8/22	36.36
41-50	71	8	1.4	5/8	62.5	3/8	37.5
51-60	67	11	1.9	7/11	63.63	4/11	36.36
Up to 60	68	8	1.4	6/8	75	2/8	25
Total	562/2592		21.68	379/562	67.43	183/562	32.56

χ^2: 190.357; p = 0.000.

was prepared by dissolving 331 g of $ZnSO_4.7H_2O$ in 1000 mL of deionized distilled water and mixed until dissolved. The centrifuge tube was filled with zinc sulfate solution up to the rim, covered with a cover slide, and then centrifuged at 2500 rpm once more for one minute. The cover slide was inverted onto a glass slide with a drop of Lugol's iodine, viewed at 10x and 40x magnification, and the organism(s) detected were reported as mentioned above. The advantage of this method is that it concentrates the parasite stages present in the stool specimens, while its disadvantage is that the trophozoites cannot be detected as they rupture.

Data management and analysis

All of the data were added into an Excel spreadsheet, and then imported to SPSS version 25 for data analysis. Frequencies of each variable were calculated. The prevalence of *Entamoeba* spp. was analyzed using a Chi-square (χ^2) test to see if there was a significant difference. Regarding the other parameters, the correlation between infection with *Entamoeba* spp. and potential risk factors was regarded as statistically significant at the $p < 0.05$ level.

Results

A total of 2592 stool specimens were examined both macroscopically and microscopically for the presence of *Entamoeba* spp. and the positive results were recorded. *Entamoeba* (cysts/trophozoites) were found in 21.68% (562/2592) of the examined specimens.

The prevalence of amoebiasis among both genders and different age groups is shown in Table 1. Overall, 54.09% of the specimen were positive for *Entamoeba* spp., and males were more likely to be infected than females (67.43% vs. 32.56%).

Regarding the relationship between gender and age, the rates in males over all ages were higher than that in

Table 2. Prevalence of *Entamoeba* spp. among the population in relation to some variables (N = 2592).

Variables	*Entamoeba* spp.			
	Total no.	Infected no.	% infected	Chi- square *p* value
Educational level				
Pre and Primary Schools	1151	296	52.66	χ^2:24.517
High school	914	185	32.91	$p = 0.000$
College and above	527	81	14.41	
Income status				
Low	1423	378	67.25	χ^2:48.961
Moderate	813	142	25.26	$p = 0.000$
High	356	42	7.47	
Washing fruits and vegetables				
No	1928	319	56.76	χ^2:116.933
Yes	664	243	43.23	$p = 0.000$
Source of Drinking water				
Well water	1618	387	68.86	χ^2:16.884
Chlorinated water	971	173	30.78	$p = 0.000$
Eating outside home				
Always	2425	484	86.12	χ^2: 114.873
Sometimes	167	78	13.87	$p = 0.000$
History of taking antidiarrheic drugs				
Did not take	828	319	56.76	χ^2:42.103
Did take	828	243	43.23	$p = 0.000$

females, with the highest in age group 1-10 years and those above 60 years of age (72.03% and 75%), respectively. On the other hand, females of the age groups 11-20 years and 21-30 years reported the highest rates of amoebiasis (38.81% and 38.59%), respectively. The differences in rate of infection between the genders and between age groups were statistically significant ($p < 0.000$).

Relationships between the prevalence of amoebiasis and some variables in the population

Table 2 shows the relationship between amoebiasis and some variables. Individuals with pre and primary school education reported the highest infection rate (52.66%), followed by those with a high school education or above (32.91% and 14.41%) respectively. The differences between levels of education were statistically significant ($p = 0.000$). There was a statistically significant ($p = 0.000$) difference between those with low incomes (67.25%) compared to those with moderate and high incomes (25.26% and 7.47), respectively. People eating unwashed fruit and vegetables experienced a higher rate of infection than those who ate them washed (56.76% vs 43.23%, $p = 0.000$) Well water users had a higher ($p = 0.000$) prevalence (68.86%) than those who drank chlorinated water (30.78%). Participants eating outside home had a higher rate (86.12%) of amoebiasis compared with those eating homemade food (13.87%; $p = 0.000$). Additionally, those who did not take antidiarrheal medications within the previous two weeks before the time of sample collection had a higher rate of infection (56.76%) compared to those who took such medications in the two weeks prior (43.23%).

The correlation between amoebiasis and family size is demonstrated in Table 3. Households with more than 8 members showed the highest rate of amoebiasis (40.39%), with a higher rate in males than females (93.19% vs 38.29%; $p < 0.000$).

Discussion

Numerous studies on the prevalence of intestinal parasites have been conducted throughout the world, and they have identified differences in infection rates based on locations, geographical areas, hygiene practices and living conditions of the population [16].

It is important to investigate infections that threaten human health all over the world. For this reason, epidemiological studies on the prevalence of intestinal parasitic infections in different places have typically sought to identify at-risk communities and the diseases that cause great threat to human populations [17]. Parasitic infections are affected either directly or indirectly by a number of environmental, biological, behavioral, socioeconomic, and health-related factors. Infectiousness, disease transmission, and mortality rates are all affected by a number of factors, including source of drinking water, number of family members, income and level of education [18]. In endemic locations, a patient with fever, weight loss, right upper quadrant pain, and tenderness should always be suspected of having an amebic liver abscess [19].

Several studies have been conducted in Duhok city to show the extent of prevalence of intestinal parasites among the population in general and the majority of these studies witnessed high rates of *E. histolytica* as compared to other types of intestinal parasites [20-23]. Therefore, this comprehensive study was adopted to detect *Entamoeba* spp. in stool specimens and to investigate their relationship with some risk factors among outpatients who visited the two main teaching hospitals in Duhok city.

In this study, amoebiasis was recorded in 21.68% (562/ 2592) of the examined specimens, with a higher rate in males than females (67.43% vs 32.56%), indicating unbalanced gender distribution. The rate reported in this study is lower than the rates reported in previous studies conducted in this province, such as in Duhok and Zakho cities [20,21]. They reported rates of 25.67% and 48.67%, respectively among infants and children. The most probable reason for these high rates is that the studies were conducted on infants and children as these ages are more susceptible to infections. In addition, Murad *et al*. [22] reported much higher rate (36.7%) of amoebiasis by analyzing the outpatient hospital data from 2013-2017 in Duhok city. On the other hand, Al-berfkani [23] reported a lower rate (12.98%) of amoebiasis among the population in

Table 3. Relationship between amoebiasis and family size.

Number of family Members				Males		Females	
	Total no.	Infected no.	%	No. infected / no. examined	%	No. infected	%
1-4	919	147	26.15	103/188	54.78	60/227	26.43
5-8	930	188	33.45	139/227	61.23	51/147	34.69
More than 8	743	227	40.39	137/147	93.19	72/188	38.29
Total	562/2592		21.68	379/562	67.43	183/562	32.56

χ^2: 27.559; $p = 0.000$.

Zakho city. The difference might be due to the type of population involved in both studies and the number of samples tested. Additionally, the current study's results are higher than those of Muhsin *et al.* [24] in Karbala and Nayyef [25] in Baghdad who reported rates of 12.9% and 15.89%, respectively. In contrast to these findings, Hassan and Mero [26] found a significant prevalence of infection with *E. histolytica* and stated that it was the major protozoan among analyzed samples. Furthermore, higher rates of amoebiasis have been reported by Saida [17] in Erbil city, Nassar *et al.* [27] in Basrah city and Shallal [28] in Al-Anbar city, which were 61. 24%, 58.3% and 70%, respectively. While lower infection rates were reported in Iran and Iraq by Pestehchian *et al.* [29] and Farhan Al-Rwi *et al.* [30] which were 4.07% and 1.7%, respectively.

The variability in the prevalence of *Entamoeba* species in these investigations may be attributable to variations in hygienic, geographical and environmental conditions that permit the prolonged survival of the cysts in the stool. Moreover, the majority of refugee families in Duhok province reside in close proximity to domestic animals, which are regarded as significant risk factors since direct human contact with these animals facilitates the transmission of cysts, which may act as a mechanical vector for the disease [31]. Furthermore, the rate of infection and the recorded species of the parasite might vary depending on a number of factors, including the quality of the water, the family income, the standard of living, and the extent of hygienic application [32,33].

The rate of infection with *Entamoeba* species was significantly higher in males than females (67.43% vs. 32.56%). This is in accordance with other studies conducted in Duhok province and various parts of Iraq, including the cities of Duhok, Zakho, Karbala, and Baghdad [22,26,23-25]. They reported infection rates of 54.8% vs. 45.2%, 57.79% vs. 42.20%, 18.66% vs. 9.88% 13.56% vs 12.14%, and 51.89% vs 48.10%, respectively. In other countries also higher rates of amoebiasis were reported in males than females, such as in Iran, a rate of 22.36% vs 20.9% was reported by Mojarad *et al.* [34]. Esalem *et al.* reported that the prevalence of amoebiasis in males was 4% greater than that of females, with a higher rate of 6.66% in children between the ages of 8 and 11 years in Sebha, Libya [35]. In Uganda, Ekou *et al.* reported a considerably greater rate of *Entamoeba histolytica* infection in males (22.14%) than females (17.39%) [36]. Hegazi *et al.* [37], and Tasawar *et al.* [38] found that males were more likely than females to develop amoebiasis, because they are more socially active, and females may also, have a superior immune system than males due to genetic difference mediated by microRNAs found on the female X chromosome. Moreover, females are more attentive to cleaning and hygiene practices [39].

The current study demonstrated a strong correlation between amoebiasis and age in males and females. Males displayed higher rates than females across all age groups with the highest (75%) being among ages above 60 years. Furthermore, younger males and females (11-20 years and 21-30 years) showed high rates of infection (61.18%, 61.40%, and 38.81%, 38.59%) respectively. This indicates the presence of a disparity in infection rates between age groups, with the youngest ages and old ages (above 60 years) being more vulnerable to infection. Variable rates of infection have been reported by researchers based on age ranges. For example, Khalaf and Rashid reported the highest infection rates (4.1%) among the ages 11-20 years in Baghdad, which is partially consistent with the current study [40]. In addition, Mohamed reported the highest infection rate (51.7%) among the age group 10 -19 years in Kassala, Sudan [41], while in Erbil, Faqe Mahmood and Mustafa reported the highest rate of *E. histolytica* infection among the age group 1-4 years (13.8%) [42]. Flaih *et al.* reported the highest rate of the infection with *E. histolytica* among the ages of 5 to 14 years (11.1%) in Thi-Qar [43].

The high prevalence of amoebiasis among infants and children suggests the importance of implementing hygiene practices aimed at reducing the spread of this parasitic disease. Possible causes include suboptimal bottle sterilizing practices and the use of unfiltered tap water for the preparation of formula milk. Children are more vulnerable due to their immature immune system. In addition, they spend more time outside in playing and they take most of their meals away from home [26,44].

A strong correlation was also observed between *E. histolytica* infection and the participant's degree of education since participants with lower levels of education (pre and primary school) are at greater risks for acquiring infectious diseases than those with higher levels of education. This might be because people in these groups eat more street food, which is often not stored or prepared under adequate sanitary conditions. The current findings are consistent with those of Hassan and Mero who found that people with less than high school degree had the highest risk of infection (82.97%) [26]. Studies also showed that families with low-literacy were more likely to be infected than those with higher-literacy. This included both children and parents, and intestinal parasites are less prevalent in children of highly educated parents [45]. For this reason, it is important to implement programs that help

parents improve their health education so that they can contribute to the fight against parasitic infections and other infectious diseases.

Participants with poor sanitary practices, such as those who eat unwashed raw vegetables and fruits and drink non-potable water or well water, those who frequently eat either street food or in restaurants outside their homes and those who did not take any antidiarrheal medications had significantly higher infection rates. The causes of infection can be traced back to living conditions, unhygienic toilet facilities, eating unwashed fruits and vegetables, not washing hands before eating. All of these factors are associated with prevailing poverty, lack of exposure to health education program, low socioeconomic status, and low standards of sanitation and hygiene applications. Individuals in the Indian, Italian, and Yemeni populations have reported experiencing the same risk factors for the infections [46-48].

Amoebiasis was found to be more common in families with more than eight members (40.39%), with a higher prevalence in males (93.19%) than in females (38.29%). Despite the presence of significant ($p < 0.000$) difference between family sizes, the high prevalence of *Entamoeba* spp. among large families is in accordance with the findings of AL-Khlefawi [49] and Hassan and Mero [26]. When compared to smaller families, larger ones are more likely to spread parasites directly through sharing personal items, which is an important factor for parasites with a direct life cycle. In addition to personal hygiene, which has a significant effect on parasite infection, overcrowding circumstances at home can cause intra-family transmission due to a close contact between family members [50].

Conclusions

This study found that amoebiasis occurred in 21.68% of the Duhok population. Prevalence is significantly affected by factors such as age, gender, family size, education level, economic stratification, water source, eating unwashed raw fruits and vegetables, eating outside home, a history of not using antidiarrheal medications and living in large families. Therefore, reducing the infection rate can be greatly aided by improving family living conditions, environmental sanitation, and health education. The authors highlight the importance of disseminating health education programs among the community by the health authority in order to reduce the rate of infectious disease especially those with direct transmission.

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