

EPIDEMIOLOGICAL STUDY ON AMEBIASIS IN THE DISTRICT OF HAWIJA IN KIRKUK

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Abstract:

Entamoeba histolytica is a common parasite worldwide, particularly in Iraq. This statistical study adds new findings confirming human infection with this parasite and its role in causing gastrointestinal disorders. The study was conducted in Kirkuk, Hawija District, at the Hawija General Hospital laboratory, on 120 samples randomly collected between February 2024 and February 2026. Direct examination of stool samples revealed that the cause of diarrhea was *Entamoeba histolytica* infection. Microscopic examination using wet swabs and flotation/sedimentation methods was performed to detect larval and cystic stages. The study also revealed the infection rates between males and females, with males showing a higher rate of 65%. The highest infection rates were recorded in November (40%) and June (14%). The most affected age group was children aged 1 to 10 years (45%), followed by adolescents aged 11 years and older (20.8%).

Keywords: Epidemiological, amebiasis, *Entamoeba histolytica*, Amebiasis-dysentery.

Introduction

Amebiasis is an intestinal infection caused by the parasite *Entamoeba histolytica*, and is usually transmitted through water or food contaminated with human feces. While many cases are asymptomatic, symptoms can include diarrhea (often bloody), abdominal pain, and cramps. (Alam *et al* ,2013). It is a widespread parasite, found in various regions of the world, including developing countries in the Indian subcontinent, Central and South America, and tropical Africa (Bhattacharya , A. and Bhattacharya, P. J.(2002)). The parasite causing the infection was discovered by Loch in 1875 in the stool of a patient suffering from severe diarrhea. In 1903, Schauden distinguished it from *Entamoeba histolytica*. In 1913, Walker and Sellers demonstrated its pathogenic effects. (David 2007) The global infection rate is estimated to be between 0.2% and 50%, and it is more prevalent among the poor, possibly due to malnutrition or poor sanitation. It is a widespread parasite, infecting humans, monkeys, dogs, birds, cats, and

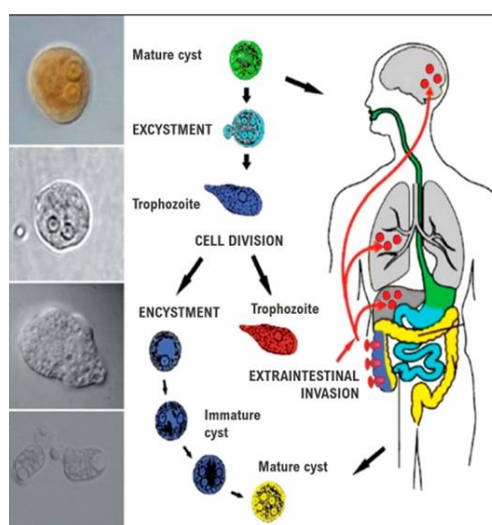


rodents. It lives in the intestinal wall and the lumen of the large intestine, particularly the cecum, causing dysentery.(Pinilla *et al.*, 2008) . According to the World Health Organization, amoebiasis is one of the most widespread diseases in the world, affecting approximately 45 to 55 percent of the world's population annually. (W H O, 1995) . The World Health Organization considers amoebiasis the third leading cause of death after malaria and schistosomiasis, and its spread is linked to climate and contaminated food and water. (WHO , 1985) Approximately 100,000 people die annually from this disease. (Lampel *et al.*, 2012) . The infection rate in Turkey was recorded at 2.2 percent according to Özer *et al.* ,(2013), which is lower than the rate recorded in Iran, where it reached 4.28 percent according to Fallah *et al.* (2014). In Saudi Arabia, 120 cases of infection were detected among children and infants out of 1,038 cases examined, according to a study by Hegazi *et al.* (2013). These are countries neighboring Iraq. In Arab countries such as Egypt, the infection rate reached 21%, according to a study by Abd-Alla *et al.* (2006). In a ten-year Palestinian study, the infection rate ranged from 8.2% to 18.2%, according to a study by Badir and Adwan (2010). In Iraq, the prevalence rate in Basra Governorate was 29.2%, according to a study by Al-Shaheen *et al.* (2007). A five-year study in Thi-Qar Governorate showed the highest prevalence rate in 2015 at 26.1% and the lowest at 8.1%, according to a study by Falah *et al.* (2021). In Qadisiyah Governorate, the prevalence rate was 5.6%, according to a study by Abdullah (2013). A study conducted on 800 children with diarrhea at Kirkuk Children's Hospital and Kirkuk General Hospital showed an infection rate of 23.57 percent, according to the study by (Hameed *et al.*,2021). In a study conducted in Baghdad on 1712 stool samples taken from children suffering from diarrhea who visited the Central Children's Hospital in the capital, the infection rate was 18.72 percent, according to (Aljanabi and Altikrity , 2012). In Tikrit Governorate, a study by (Qader *et al.*, 2018) of 1100 children who visited health care centers suffering from diarrhea and flatulence in Tikrit City between 2010 and 2012 found an incidence rate of 9.3 percent. According to a study conducted by Al-Rawi and Al-Omar (2026) in Mosul on patients who visited some hospitals in the city center during 2022 and 2023, through examining 371 samples, the percentages were (78%). According to a study conducted by (Al-Daoudy *et al.*,2024), a study was conducted on 23,600 samples in the central laboratory in Erbil Governorate, and it was found that 3.6 percent of them were infected with the Entamoeba histolytica parasite during 2023. In a study conducted during 2021 and 2022 in the holy city of Karbala on 3,748 children aged between 1 and 15 years, the overall rate of parasite infection was 13 percent, while the rate of Entamoeba histolyticus infection was 10.54 percent.(Al-Sultany *et al.* ,2023) Another study conducted at Heet General Hospital and its affiliated laboratories in Anbar Governorate during 2021 on 398 stool samples from children suffering from diarrhea showed that the total number of parasitic infections was 64.3 percent, while the percentage of Entamoeba histolytica parasites was 56.7 percent.(Ali. *et al.* ,2023) This parasite goes through four different stages during its life cycle: the trophozoite stage, the pre-cystic stage, the cystic stage, and finally the post-cystic metaxy stage.(Marianne, 2010) . The pathogenic stage is 20 to 60 micrometers in size and consists of an irregularly shaped protoplasmic mass resulting from variable extensions called pseudopodia, which extend



in all directions. In *Entamoeba histolytica*, its movement is forward-directed, unlike other amoebas where movement is non-directional. (Guillen , 2021) The precystic stage is round or oval, ranging in size from 10 to 20 micrometers, and represents a transitional stage between the pathogenic and cystic stages, where the pseudopodia and food vacuoles disappear. The cystic stage is oval, ranging in size from 6 to 20 micrometers, and contains one nucleus until it reaches four nuclei in the mature cystic stage. This stage is the most dangerous because it is the source of infection. (Ankri, 2021) . Healthy humans ingest cystic stages through contaminated water, vegetables, food contaminated by flies, or contaminated hands. (Al-Khaysee and Sultan 2008) .The cyst withstands the harsh conditions of the digestive tract because it is unaffected by gastric juices . (Ximénez *et al* .,2011) Figure_1.

It moves through the digestive tract to the lower part of the small intestine, where its outer wall dissolves under the influence of alkaline and neutral juices, and the cyst begins to emerge. The post-cystic stage is when the parasite emerges from the cyst into the lumen of the small intestine. Here, it divides from its tetranuclear stage into four mononuclear, feeding amoebae, which in turn divide directly to produce eight small, active amoebae.(Tanyuksel and Petri, 2003) These amoebae then move down the large intestine with the intestinal current, attacking the mucous membrane. The pathological effects of tissue amebiasis depend on the severity of the infection and the body's resistance. The colon, particularly the coiled portion, is the primary site of infection due to its slow motility, which allows the parasite to attack the intestinal mucosa. The trophozoite stage can attack tissues with its lytic enzymes, and the infection begins with small necroses in the superficial layer of the mucosa, causing a flask-shaped ulcer the size of a pinhead . (Nagaraja and Ankri,2019)(Vaisusuk, *et al* .,2021) . If the condition worsens, it may cause enteritis, which can progress to appendicitis. Alternatively, the infection may spread from the intestine via the bloodstream to the liver, causing amebic hepatitis, or it may spread to the lungs via the inferior vena cava, causing pulmonary amebiasis. In rare cases, the infection may reach the brain, causing a brain abscess. Although this parasite is widespread, only 10% of it is able to invade tissues and cause amoebiasis, specifically amoebiasis, while 90% remain asymptomatic and are associated with *E.dispar* and *E. moshkovskii* (Solaymani *et al*.,2006)



Figure_1.



Materials and methods

Sample Collection

Between February 2024 and February 2026, 120 stool samples were collected from patients of various ages at Hawija General Hospital. All patients complained of gastrointestinal problems, bloating, and abdominal pain. With the consent of the patients and their guardians, both children and the elderly, stool samples were collected for medical diagnosis and for use in the study. Patient data, including name, age, sex, and date of sample collection, was recorded. Laboratory tests were performed directly in the hospital laboratory, including visual examination and recording of stool color. Normal stool color is yellowish-brown due to bilirubin metabolism. Other colors are considered abnormal, such as bright red, yellow, green, black, blue, and clay-colored. Stool consistency was also recorded, as it varies from person to person depending on diet, age, and overall health. Loose stools suggest the presence of active trophozoite stages, while semi-solid stools may contain both bacteria and active trophozoite stages. Solid stools are the least likely to contain vegetative trophozoite stages. Laboratory tests were performed using the wet swab method and flotation/sedimentation methods.

Laboratory Examinations

Stool samples were visually inspected prior to microscopic examination, where stool consistency and color were assessed.

Liquid stools are likely to contain active stages, while solid stools are likely to contain cyst stages. Observation revealed that stools containing *Entamoeba histolytica* were characterized by a foul odor and the presence of mucus and blood.

After preparing the fresh sample, it was placed in 85% saline solution. Three slides were taken from each sample using the wet swab method with the addition of a drop of iodine solution. The cytoplasm would stain light brown, while the nucleus would appear dark brown. The slides were examined under a microscope to detect the vegetative and cyst phases. The slides were examined at 10x magnification to look for the vegetative and cyst phases, and then at 40x magnification to confirm their presence.

Using the zinc sulfate flotation method (Harry, 1938), the presence of cysts was detected. The specific gravity was set to 1.20, which is higher than that of most parasitic cysts, causing them to float to the surface of the liquid. Maintaining a room temperature of approximately 20°C was also necessary, as specific gravity is affected by heat. Three grams of fecal matter were collected with the zinc sulfate solution and centrifuged for two minutes. A portion of the supernatant was then immediately examined under a microscope after the addition of iodine stain.

Results and Discussion

Because the region is largely rural, with widespread animal husbandry, and because dysentery is a zoonotic disease, the negative effects of transmission between humans and animals are extensive. The spread of these diseases leads to increased mortality rates over time in both



humans and animals, and there are economic repercussions for animals due to decreased productivity. (Samad, 2011)

Patients described their condition as acute diarrhea, ranging in consistency from mucous to watery, and in color from brown to reddish-brown, and in some cases, bloody diarrhea. Other symptoms included intestinal disturbances, weight loss, and abdominal pain ranging from severe to crampy, accompanied by a slight fever.

After collecting 120 random stool samples from patients visiting Hawija General Hospital and recording the data, the number of males was 78 (65%) and the number of females was 42 (35%), The results showed that the number of infected males was higher than the number of females (65% and 35% respectively), and we attributed this to males going to work or eating contaminated food outside the home, such as fast food prepared in unsanitary conditions no statistically significant difference was observed between the sexes, where χ^2 (10.8) showed a statistically significant difference ($p < 0.01$) in the percentage of amoebiasis infection. This is consistent with the findings of Hassan and Al-Salami (2023), whose study showed that the ratios of males and females are 65.4% and 34.5% respectively. according to the following Table 1.

Sex	No.	%
Male	78	65%
Female	42	35%
Sum.	100	100%

According to data collected over two years, from February 2024 to February 2026, an increase in incidence rates was observed during months that witnessed a climatic transition between summer and winter. The highest incidence rate was recorded in November, reaching 40%, which is attributed to the onset of winter and the drop in temperatures in the region. The percentage of males was 38% and females 43% (see Table 2). In June, the incidence rate was 14%, a period that also witnesses climatic changes, as it indicates the beginning of summer and the rise in temperatures in the region. The percentage of males was 15% and females 10%. No statistically significant difference was observed between months, as the chi-squared value (18.96) showed a statistically significant difference with a level of freedom ($p < 0.05$) in the percentage of tissue amoebiasis infection. This is consistent with (Al-Doody *etal.*,2024)



Table-2

Month	male		female		total	
	No.	%	No.	%	No.	%
Feb.	2	3%	0	0%	2	2%
Mar.	2	3%	2	5%	4	3.3%
Apr.	0	0%	0	0%	0	0%
May.	10	13%	0	0%	10	8.3%
Jun.	12	15%	5	10%	17	14.1%
Jul.	6	8%	8	19%	14	12%
Aug.	1	1.2%	0	0%	1	0.8%
Sep.	0	0%	2	5%	2	2%
Oct.	0	0%	0	0%	0	0%
Nov.	30	38%	18	43%	48	40%
Dec..	10	13%	7	17%	17	14.1%
Jan.	5	6.4%	0	0%	5	4.1%
Sum.	78	65%	42	35%	120	100%

Regarding age group, the highest infection rate was recorded in the 1-10 year age group (45%), with males accounting for 46.1% and females 42.8%. Children in this age group are often unaware of the importance of personal hygiene. Due to the rural nature of the area, children may play with or be in close proximity to animals, contributing to the higher infection rate among younger age groups. The calculated chi-squared value was 7.013, and the p-value ($p < 0.05$) was statistically significant. This is consistent with the study by Al-Kubaisi et al. (2014), which found that children are more susceptible to infection, recording an infection rate of 23.44% among children in three hospitals in central Baghdad. This was also confirmed by Dalabieh et al. (2020), Nath et al. (2015), and Saeeda (2016).

Table-3

age group	male		Female		total	
	No.	%	No.	%	No.	%
1-10	36	46.1%	18	42.8%	54	45%
11-20	15	19.2%	10	23.8%	25	20.8%
21-30	9	11.5%	4	9.5%	13	10.8%
31-40	1	1.2%	3	7.1%	4	3.3%
41-50	5	6.4%	3	7.1%	8	6.6%
51-60	7	8.9%	3	7.1%	10	8.3%
61-70	3	3.8%	0	0	3	2.5%
71-80	2	2.5%	1	2.3%	3	2.5%
Sum.	78	65%	42	35%	120	100%



References

1. Abd-Alla, M .D. ; Stauffer, W . ; Ravdin, J. I. (2006). Prevalence and Incidence of Entamoeba histolytica Infection in South Africa and Egypt. Am. Trop. Med.;37(2): 265–268.
2. Alam, S.; Khanum, H.; Zaman, R. F.; Haque, R.(2013). prevalence of different protozoan parasites inpatients visiting at icddr'b hospital, Dhaka. J. Asian. Soc. Bangladesh, Sci.; 39(1): 117-123.
3. Alaa Nusrat Abdulrahim Al-Rawi and Najah Subhi Nayef Al-Omar (2026) Epidemiological study of the parasites Entamoeba histolytica and Giardia lamblia in the city of Mosul, Rafidain Journal of Science, Vol. 35, No.1, pp.1-8, 2026 (March)
4. Ayoub Ibrahim Ali, Abdalwahab Bdewi Hussain, Saleem Obaid Gatia Almawla, Nabaa Adnan Muhammed, Rawaa Adnan Muhammed(2023). Prevalence of Entamoeba histolytica and Giardia lamblia infection among patients attending Heet Hospital in Al-Anbar, Iraq. Samarra J. Pure Appl. Sci., 2023; 5 (4): 140-146.
5. Ankri, S. Entamoeba histolytica—Gut Microbiota Interaction: More Than Meets the Eye. Microorganisms 2021, 9, 581. [CrossRef]
6. Al-Khaysee, G.H. and Sultan, A.A. (2008). The factors that effect the epidemiology of Entamoeba histolytica and Giardia lamblia among population of Khalis and Baledrose. Diala. Jour., 27: 92-99.
7. Al-Sultany, Aseel Kariem; Al-Morshidy, Kassim Abdulla ,(2023) An Epidemiological Study of Intestinal Parasites in Children Attending the Pediatric Teaching Hospital in the Holy City Of Karbala, Iraq., *Medical Journal of Babylon* 20(1):p 95-100, Jan–Mar 2023. | DOI: 10.4103/MJBL.MJBL_276_22.
8. Badir , S. and Adwan, G.(2010). Prevalence of intestinal parasitic infections in Jenin governorate ,Palestine. As. Pac. J.Trop. Med .; 3(9): 745-747 .
9. Bhattacharya , A. and Bhattacharya, P. J.(2002). Close sequence identity between Ribosomal DNA episomes of the nonpathogenic Entamoeba dispar and pathogenic Entamoeba histolytica. J. Bio.sci.; 27 (3): 619–627.
10. Mohammed A. Kadir , Abdul-Karim M. Ismail (2016) Detection and clinical characteristics of Entamoeba histolytica infection among children in Kirkuk-Iraq Sch. J. App. Med. Sci., 2016; 4(2B):451-459
11. Fallah , E.; Shahbazi, A.; Yazdanjooi, M.; Rahimi-Esboei , B.(2014). Differential detection of Entamoeba histolytica from Entamoeba dispar by parasitological and nested multiplex polymerase chain reaction methods. J. Anal. Res. Clin. Med.; 2(1): 25-9.
12. Mohammed Hassan Flaih1, Ruaa Majid Khazaal1, Manar Karem Kadhim2, Khwam Reissan Hussein1, Falah Abd Bashir Alhamadani. (2021). The epidemiology of amoebiasis in Thi-Qar Province, Iraq (2015-2020): differentiation of Entamoeba histolytica and Entamoeba dispar using nested and real-time polymerase chain reaction J. Epidemiol Health ,Volume: 43, Article ID: e2021034, 7 pages



13. Nagaraja, S.; Ankri, S. Target Identification and Intervention Strategies against Amebiasis. *Drug Resist. Updates* 2019, 44, 1–14. [CrossRef]
14. Hegazia , M. A .; Patel ,T. A.; El-Deek, B. S. (2013). Prevalence and characters of Entamoeba histolytica infection in Saudi infants and children admitted with diarrhea at 2 main hospitals at south Jeddah: a re-emerging serious infection with unusual presentation. *Brazilian, J. Infect. Dis .*; 17(1):32–40 .
15. Nath J, Ghosh SK, Singha B, Paul J. Molecular epidemiology of amoebiasis: a cross-sectional study among North East Indian population. *PLoS Negl Trop Dis* 2015;9:e0004225.
16. Al-Dalabeeh EA, Irshaid FI, Roy S, Ali IK, Al-Shudifat AM. Identification of Entamoeba histolytica in patients with suspected amebiasis in Jordan using PCR-based assays. *Pak J Biol Sci* 2020; 23:166-172.
17. Ahmed Akil Al-Daoudy , Fattma A. Ali1 , Jihad A. Sulaiman , Bashdar Mahmud Hussien , Gashbin Ali Khalid , Avan Sharif Awlla , Rayan Esmail Aziz , Zhyan Hassan Asaad, (2024). Erbil City Food-Handler Entamoeba Histolytica Investigation . 5th International Conference on Biomedical and Health Sciences, (CIC-BIOHS'2024)
18. Marianne , L .(2010). Molecular Diagnosis and Characterization of Two Intestinal Protozoa: Entamoeba histolytica & Giardia intestinalis . *Karolinska Institutet , Stockholm*.pp:2-3.
19. Marwan A. H. Aljanabi and Ilham A. A. Altikrity (2012) The spread of Entamoeba histolytica infection among children attended the Central Child Hospital in Baghdad city. *Iraqi Journal of Science* 2014, Volume 55, Issue 3, Pages 979-985
20. Mohammed A. Kadir, Suhair T. El-Yassin, Ali AM (2018) Detection of Entamoeba histolytica and Giardia lamblia in children with diarrhea in Tikrit city, *Tikrit Journal of Pure Science* 23 (6) 2018
21. Hameed J. A., Omaima I. Mahmood , Adnan F. Al-azawy (2021) Epidemiological Study of Entamoeba Histolytica among Children in Kirkuk Province, Iraq *Annals of R.S.C.B.*, ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1915 – 1925 .
22. Lampel , K. A.; Al-Khalidi, S.; Cahill, S. M .(2012). Hand book of foodborne pathogenic Microorganisms and natural Toxins. 2nd Edition .Chapter : Entamoeba histolytica.pp:127.
23. Saida LA. Epidemiological study of intestinal parasites among population of Erbil city, Kurdistan, Iraq. *J Raparin Univ* 2016;3:75-86.
24. Solaymani , S.; Rezaian, M.; Babaei, Z .; Rajabpour, A.; Meamar, A. R.; Pourbabai, A. A.; Petri ,W. A.(2006). Comparison of a stool antigen detection Kit and PCR for diagnosis of Entamoeba histolytica and Entamoeba dispar Infections in asymptomatic cyst passers in Iranian. *J. of Cli. Microbial.*; 44(6): 2258–2261.
25. Samad , M. A.(2011). Public Health Threat Caused By Zoonotic Diseases In Bangladesh . *Bangl. J. Vet. Med.*; 9 (2): 95 – 120.



26. . Al-Taei AH. The prevalence of intestinal parasite among the at tending peoples to Al-Hashimyah hospitals for seven years, Bab ylon province, Iraq. *J Phys Conf Ser* 2019;1294:062022.
27. Al-Saqur IM, Al-Warid HS, Al-Qaisi AQ, Al-Bahadely HS. Prevalence of gastrointestinal parasites in Iraq during 2015. In *AIP Conf Proc* 2020;2290: 020005.
28. Vaisusuk, K.; Saijuntha, W. Intestinal Protozoa: Their Role as Human Pathogens and Zoonoses. In *Biodiversity of Southeast Asian Parasites and Vectors Causing Human Disease*; Petney, T.N., Saijuntha, W., Mehlhorn, H., Eds.; *Parasitology Research Monographs*; Springer Nature Switzerland AG: Cham, Switzerland, 2021; pp. 35–61. ISBN 978-3-030-71161-0.
29. W.H.O . (1995). Division of diarrheal and acute respiratory disease control, interim report, 1994. World Health Organization, Geneva. pp:5.
30. W.H.O. (1985). Amebiasis and its control. *Bull World Health Organ.*; 63(3):417–426.
31. Tanyuksel, M. and Petri, W.A. 2003. Laboratory Diagnosis of Amoebiasis. *Clin. Microbiol. Rev.*, 16, pp:713-729.
32. Guillen N. (2021). Signals and signal transduction pathways in *Entamoeba histolytica* during the life cycle and when interacting with bacteria or human cells. *Molecular microbiology*. 115(5):901-15.
33. Ximénez, C.; Morán, P.; Rojas, L.; Valadez, A.; Gómez, A.; Ramiro, M.; Cerritos, R.; González, E.; Hernández, E.; Oswaldo, P. Novelty on Amoebiasis: A Neglected Tropical Disease. *J. Glob. Infect. Dis.* 2011, 3, 166.

