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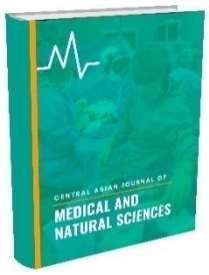

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Epidemiological Study of *Entamoeba Histolytica* among Children in Nasiriyah City, Thi-Qar, Iraq

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Abstract: Diarrhea is characterized by increased stool frequency or the passage of watery stool more than three times in 24 hours. *Entamoeba histolytica* is a global gastrointestinal parasite that causes amebiasis, a common cause of diarrhea in humans. This study aimed to determine the prevalence of *E. histolytica* in stool samples from children with diarrhea in Nasiriyah City, Thi-qar Province, Iraq. From August 2022 to May 2023, a total of 2,639 stool samples were collected from children under seven years old. Microscopic examination and PCR techniques were used to analyze the samples. The results showed that 16.33% of the samples tested positive for *E. histolytica* using microscopic examination, while 83.67% tested negative. Among the positive samples, the highest percentage of infected patients (18.81%) was found in females, and the lowest percentage (14.66%) was found in males. The rural area had the highest percentage of infected patients (16.40%), while the urban area had the lowest percentage (16.22%). In terms of age groups, the highest percentage of infected patients (16.92%) was in the 6-7 years age group, and the lowest percentage (15.40%) was in the 0-1 years age group. PCR analysis of 90 samples showed that 74.44% tested positive for *E. histolytica*, while 25.56% tested negative. These findings provide insights into the prevalence of *E. histolytica* among diarrheic patients in the study area during the specified time period.

Key words: Diarrhea, *Entamoeba histolytica*, amebiasis.

1. Introduction

Entamoeba histolytica, a protozoan parasite, is responsible for causing amoebiasis, a disease that remains a significant global health concern primarily affecting tropical and subtropical regions (1) where this particular amoeba is endemic (2). The transmission of amoebiasis in developing countries is often attributed to inadequate sanitation, poor hygiene practices, and crowded living conditions, while

in developed countries, it is primarily transmitted by individuals who have traveled from endemic regions (3,4). Among young children, intestinal protozoan infections are a leading cause of mortality (5,6), and those who survive may experience long-term adverse effects (7,8–10).

It is estimated that approximately 500 million people worldwide are affected by amoebiasis (11). Previous studies have suggested an annual incidence of 50 million cases of amoebiasis and an annual death toll of 100,000 individuals due to this disease (12,13). As a result, amoebiasis ranks as the third highest cause of death among human parasitic infections (14,15).

Nasrallah, J., Akhoundi, M., Haouchine, D., Marteau, A., Mantelet, S., Wind, P., ... & Izri, A. (2022). Updates on the worldwide burden of amoebiasis: A case series and literature review. *Journal of Infection and Public Health*.

E. histolytica is a protozoan parasite primarily found in the intestinal tract that has the ability to attach to and destroy epithelial tissue. The disease caused by this parasite often presents with various symptoms such as bloody diarrhea, fever, abdominal pain, colitis, malaise, fatigue, flatulence, and weight loss (16). *E. histolytica* has two forms, namely trophozoites and cysts, in its life cycle. Infection occurs when cysts are ingested through contaminated water or food. Once in the small intestine, trophozoites emerge from the cysts, develop, and colonize the colon before adhering to the mucosal layer of the large intestine. *E. histolytica* is responsible for causing both intestinal and extra-intestinal infections (17). While some trophozoites may be excreted in stool without the ability to survive, the signaling pathways leading to excystation or encystation are not yet fully understood (18). In cases of extra-intestinal infections, *E. histolytica* parasites can breach the intestinal wall and travel through the portal circulation to reach the liver, where they form hepatic abscesses. If left untreated, these abscesses can be fatal. Additionally, the abscesses may spread to other organs, including the lungs and brain (15).

This study aimed to assess the prevalence of amoebiasis over an 10-month period in the study area of Nassiriah City, located in Thi-Qar Province, Iraq. Additionally, the study sought to compare the infection rates among different factors such as gender, age groups, and other relevant variables. By examining these factors, the research aimed to gain insights into potential variations in amoebiasis infection based on demographic characteristics.

2. Materials and methods

2.1. sample collection

During the study period, a total of 2639 stool samples were collected from patients with diarrhea who sought medical attention at Bint Al-Huda, Mohammed Al-Mosawy Hospitals and private clinics in Nassiriah City. Detailed medical information related to amoebiasis was recorded for a duration of 10 months, covering the period from August 2022 to May 2023. The information collected included the patient's sex, date of presentation, age, and residential area. The age range of the patients included in the study was from 1 day to 7 years, with 1576 males and 1063 females. The fecal samples were collected using sterile containers and promptly transported to the laboratory of the respective hospitals for further analysis. The fecal samples were divided into two portion, the first portion was for the microscopic examination of parasites while the other portion of 200 mg and stored directly at -20°C for molecular analysis by conventional PCR (19).

2.2. Direct wet

For the direct smear, a small drop of normal saline (0.9%) or an Iodine stain was placed on the glass slide. A small portion of feces was mixed thoroughly with the saline or Iodine using a wooden stick. A cover slide was then placed over the mixture, and the prepared slide was examined under a microscope

using a magnification power of 40X. This allowed for the observation and analysis of the parasite's shape and motility (20).

2.3. PCR Components and steps

DNA template, 5µL; a set of oligonucleotide primers specific for 18SrRNA gene *E. histolytica*, Forward primer (GGGGAGTATGGTCACAAGGC), 1.5 µL; Reverse primer (TGTGTACAAAGGGCAGGGAC), 1.5 µL,; Master mix 5 µL; PCR Water, 7 µL. The **AccuPower® PCR PreMix Kit** that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, TrisHCl pH: 9.0, KCl, MgCl2,stabilizer, and tracking dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000 rpm for 3 minutes. Then placed in PCR Thermocycler (T100 Thermal cycler. Biorad. USA). PCR steps, Initial denaturation, 95C, 5min, 1cycle; denaturation, 95C, 30sec. 30cycle; annealing, 58C, 30sec, 30cycle; extension, 72C, 1min, 30cycle; final extension, 72C, 5min, 1cycle; cold, 4C, forever.

3. Results

Microscopic examination of stool samples is one of the earliest and most commonly used methods for the diagnosis of amebiasis, caused by the protozoan parasite *E. histolytica*. During microscopic examination, we examine the stool samples under a microscope to identify the presence of trophozoites or cyst. The trophozoites are the active feeding stage of the parasite and can be observed in the stool samples. (21,22).

3.1. Microscopic Examination.

3.1.1. Percentage of infected and non-infected children with *E. histolytica*

Based on the our information, the study examined 2,639 stool samples from patients with diarrhea using a direct wet preparation method and Lugol's iodine for *E. histolytica* detection under a light microscope. The results revealed that out of the 2639 samples, 431 of them (16.33%) tested positive for *E. histolytica* infection. The remaining 2,208 samples (83.67%) were found to have other causes of diarrhea. This information is represented in Figure (3-1).

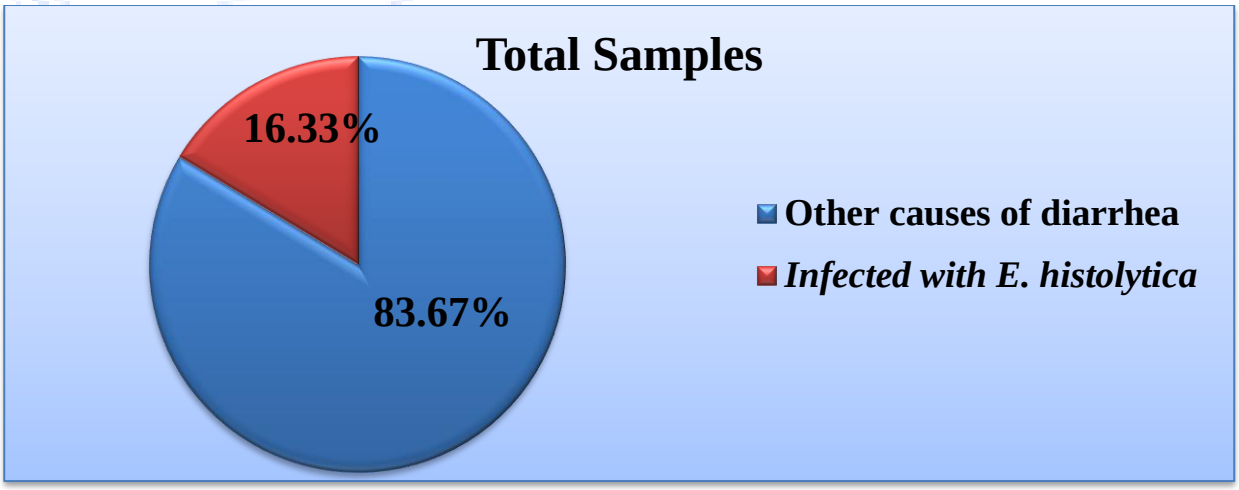

Figure (3-1): *E. histolytica* percentage among children.

3.1.2. Distribution of the infection with *E. histolytica* according to gender

According to the statistical analysis results of the current study, there were significant differences observed in the infection rates based on sex (gender) at "P≥0.05". Specifically, among the patients, the highest infection rate was recorded in females, amounting to 18.81%. On the other hand, the lowest infection rate was observed among males, with a rate of 14.66%. These findings are summarized in

Table (3-1), which presents the infection rates for males and females separately, along with relevant statistical measures.

Table(3-1): Percentage of the *E.histolytica* infection according to the gender.

Gender	Number of examination	Number of infected	Percentages (%)	Number of not infected	Percentages (%)
Male	1576	231	14.66	1345	85.34
Female	1063	200	18.81	863	81.19
CalX^2=1.67 TabX^2= 1.64 Df=1 P. value 0.196					

3.1.3 Distribution of the infected patients with *E.histolytica*, according to age group

According to the results of the current study, there were no significant differences observed in the infection rates based on age groups at "P≥0.05". Among the different age groups examined in the study, the highest infection rate was recorded in the age group (6-7), with a rate of 16.92%. On the other hand, the lowest infection rate was observed among children in the age group (1day-1), with a rate of 15.40%. Table (3-2), summarized these findings for different age groups along with relevant statistical measures.

Table(3-2): Percentage of the *E.histolytica* infection according to age groups

Age groups	Number of examination	Infected		Non-infected	
		No.	(%)	No.	(%)
1 day -1	734	113	15.40	621	84.60
2-3	679	114	16.79	565	83.21
4-5	564	92	16.31	472	83.69
6-7	662	112	16.92	550	83.08
Cal X^2= 0.74, TabX^2= 4.11, Df=3 , P. value 0.863					

3.1.4 Distribution of the infected patients with *E. histolytica* according to months of study

Among the months included in the study, the highest infection rate was recorded in September, with a rate of 37.91% . This suggests that September had the highest number of individuals testing positive for the infection (58 patients). On the other hand, the lowest infection rate was observed in January, with a rate of 6.28%. This indicates that January had the lowest number of individuals testing positive for the infection (12 patients). Based on the results of the current study, no significant differences were observed in the infection rates based on the months of study, at (P≤0.05). These findings are summarized in Table (3-3), which presents the infection rates for each month along with the corresponding number of infected patients.

Table(3-3): Percentage of the *E.histolytica* infection according to months.

Months of study	Number of examination	Number of infected	Percentages (%)	Number of not infected	Percentages (%)
August	149	52	34.90	97	65.10
September	153	58	37.91	95	62.09
October	461	67	14.53	394	85.47
November	385	64	16.62	321	83.38
December	307	27	8.79	280	91.21
January	191	12	6.28	179	93.72
February	152	20	13.16	132	86.84

March	221	19	8.60	202	91.40
April	189	26	13.76	163	86.24
May	431	86	19.95	345	80.05
Cal X^2= 133.56 , TabX^2= 29.67, Df=9 , P. value 0.0001					

3.1.5. Distribution of the infected patients with *E. histolytica* according to residence

The results show the number of patients with diarrheal that infected by *E. histolytica* according to habitation at "P≥0.05" where the highest rate of infection 16.40% (273 patients) in rural compared with 16.22% (158 patients) in urban. This results show non-statistical significant differences between rural and urban, as a Table (3-4).

Table(3-4): Percentage of the *E.histolytica* infection according to residence.

Residence	Number of examination	Number of infected	Percentages (%)	Number of not infected	Percentages (%)
Urban	974	158	16.22	816	83.78
Rural	1665	273	16.40	1392	83.60
Cal X^2=0.01, TabX^2= 1.32 , Df=1 , P. value 0. 907					

3.2. Molecular study

3.2.1. Polymerase Chain Reaction (PCR).

The current study includes an examination of 90 stool samples that are positive microscopically by PCR technique. Figure (3-2) shows positive *E. histolytica* in agarose gel electrophoresis with m.w. 501bp.

Figure (3-2): Agarose gel electrophoresis image that shows PCR product analysis for 18S ribosomal RNA gene in *E. histolytica* isolates.

M (Marker ladder 2000-100bp). Lane (1-10) some positive *E. histolytica* stool samples at 501bp product size.

3.2.1.1. Percentage of *E. histolytica* infection by PCR

The result shows percentage of infected children were 67 (74.44%) positive samples and 23 (25.56%) other causes of diarrhea from the 90 of microscopically positive samples that examined by PCR. as Figure (3-3).

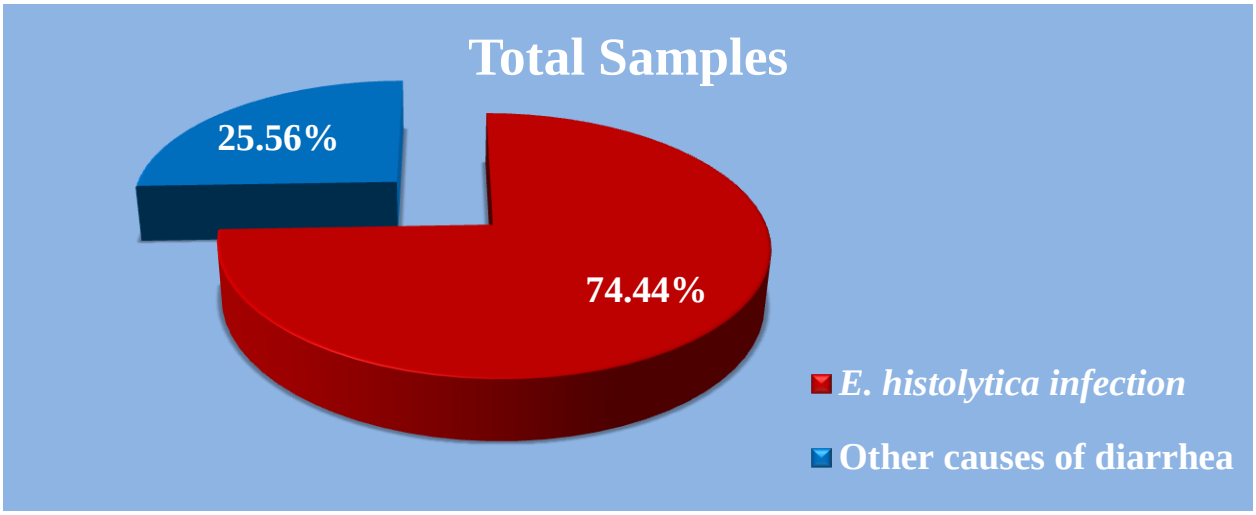

Figure (3-3): Percentage of samples with *E. histolytica* by using PCR.

3.3. Comparison between PCR and microscopic examination according to infectious samples

The results show there are statistical differences between PCR technique and microscopic examination. The total of 2639 samples of stools are examined by the microscope; and a total of 90 stool samples that are microscopically positive, are examined by PCR technique. The results show the percentage of positive cases for *E. histolytica* is 431(16.33%) that recorded by microscope while the percentage of positive cases 67 (74.44%) by PCR.

Table (4-9): Percentage of *E. histolytica* infection by microscope and PCR

Examination	Number of examination	Positive		Negative	
		Number of infected	Percentages (%)	Number of not infected	Percentages (%)
Microscopy	2639	431	16.33	2208	83.67
Molecular	90	67	74.44	23	25.56
Cal X^2=197.01, TabX^2= 12.12 , Df=1 , P. value 0.0001					

4. Discussion

Amoebiasis remains a significant health problem in developing countries, especially among children.

Increased travel and migration from affected regions to developed countries have led to a rise in the occurrence of amoebiasis. Diagnosing and treating the disease can be challenging for clinicians due to the lack of symptoms in most patients, which can contribute to the ongoing transmission of the disease(23). The microscopic examination showed that 431 (16.33%) of total samples were positive samples and 2208 (83.67%) were negative samples. The present study corroborates Alyousefi's (2012) findings in Sana'a city, Yemen. When analyzing 503 positive diarrheic stool samples, the study revealed a prevalence of 17.1% for *E. histolytica* (24). The result of current study agrees with some previous studies, including the study of Ihsan *et al*. (2017) in Najaf, Wasit, Basra and Diwaniya provinces, showed the highest prevalence rates (18.6%, 10.4%, 10.2% and 10.3% respectively) (25). Khder (2018) in Saladin, Thi-qar, Wasit and Baghdad showed the prevalence rates of *E. histolytica* was (18.09%, 17.5%, 16.78% and 15.77% respectively) which agree with our result (26). Also our result agree with Al-Sultany and Al-Morshidy that showed the rates of *E. histolytica* was (10.54%) (27). Our study findings are consistent with the research conducted by Al-Waaly *et al*. (2020) in

Diwaniyah City. Their study reported a prevalence of 15.06% for *E. histolytica*, which aligns with our own results (28).

The results of the current study disagree with Shakir and Hussein (2014) in Baghdad province they registered the rate of *E. histolytica* (41.25%) (29). In addition to disagree with Akram (2018) in Thi-Qar he reported *E.*

histolytica rate (67.7%) (30). Also disagree with study by Ibrahim *et al*. (2019) in Egypt who mentioned *E. histolytica* (3.5%) (31).

The elevated occurrence of E.histolytica detected in microscopic examinations may be linked to the extended viability of cysts in the environment, as indicated by Nath *et al*. in their study conducted in 2015(9), and its direct mode of transmission, which does not necessitate an intermediate host. Additionally, factors such as inadequate emphasis on aseptic water treatment, scarcity of materials essential for water purification, and the use of pesticides contribute to the increased prevalence of *E. histolytica* (32). The mechanical carriage of cysts by flies can contribute to the escalation of the parasite's incidence rate (33). The finding of this study may be related to the fact that protozoa parasites are easier to transfer than other parasites that can grow and multiply in soil or an intermediate host before they infect people (34).

Furthermore, when employing the PCR technique on the 90 samples that tested positive in microscopic examination, it was found that 67 cases (74.44%) showed evidence of *E. histolytica* infection. This finding agree with the study conducted by Ngui *et al*. in 2012, where the rate of *E. histolytica* infection by PCR was reported to be 75.0% (35). According to Roy *et al*. in 2005, the PCR rate was found to be 72.0%, supporting the same conclusion (36). Furthermore, PCR confirmed of 74% positive samples diagnosed microscopically according to study of Shareef (2022) in the West Bank, that agree our finding (37). The results of the current study disagree with Ngosso *et al*. (2015) in Dar Es Salaam were detected 48 (33.3%) as *E. histolytica* from 144 stool samples by PCR (38). In addition to disagree with Salim (2018) in Thi-Qar he reported *E. histolytica* rate 5.0% (30). Also our result disagree with Alkhuzaey (2019) in Thi-Qar was detected *E. histolytica* rate as 38.1% (39). The present study revealed that the PCR results demonstrated a prevalence of (74.44%) for *E. histolytica*, which is significantly higher than the (16.33%) detected through microscopic examination. This disparity could be attributed to the enhanced accuracy and sensitivity of PCR in identifying of *E. histolytica*. It is plausible that during microscopic examination, the examiner encountered challenges in parasite diagnosis due to the presence of visual-obstructing substances.

The present study found no statistically significant difference based on gender, as the infection rate among females (18.81%) was slightly higher than that among males (14.66%). Additionally, there were no significant differences observed in the infection rates based on age groups, the highest rate was recorded in the age group (6-7) 16.92%, and the lowest rate was among children in the age group (0-1) 15.40%.Among the months included in the study there are no significant differences, the highest infection rate was recorded in September 37.91% and the lowest infection rate was in January 6.28%. Also, according to habitation the results show no statistical significant differences, where the highest rate 16.40% in rural compared with 16.22% in urban.

The research identifies multiple contributing factors to the observed outcome. Children in this age group are more likely to engage in activities near unsanitary conditions such as mud and stagnant water, where latrine waste is dumped without proper supervision. Their exploration of the environment exposes them to unclean surroundings. Furthermore, habits like putting fingers in their mouths and spending time outdoors increase the risk of soil-transmitted parasites. Factors such as inadequate healthcare, unhygienic living conditions, and lower education standards also contribute to their vulnerability. Interactions with pets like cats and birds, which can harbor parasites, further amplify the

risk. Additionally, the consumption of unwashed fruits and vegetables containing parasite cysts, particularly by children, significantly contributes to the spread of *E. histolytica* in this age group.

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