

Article

# Molecular and Statistical Study to Detect Infection with *Entamoeba histolytica* Isolated from Children's Feces in The City of Nasiriyah

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**Abstract:** *Entamoeba histolytica* is a significant gastrointestinal protozoan parasite worldwide, particularly in developing nations, where it leads to amoebiasis and severe health complications. It is primarily transmitted through the fecal-oral route by ingesting contaminated food or water. This study was conducted at Bint Alhuda Hospital in Nasiriyah City between February 2023 and November 2024 to determine the prevalence of *E. histolytica* infection among children with diarrheal symptoms. Stool samples were analyzed using microscopic examination and polymerase chain reaction (PCR) techniques. Limited data exist on the molecular and statistical prevalence of *E. histolytica* infections in children in Nasiriyah City, particularly regarding age- and gender-specific infection rates. The study aimed to assess the incidence of *E. histolytica* infection in 2112 children using microscopic and molecular methods and to analyze infection rates by gender and age groups. Infection rates were 13.5% in 2023 and 16.1% in 2024, with statistically significant differences ( $P < 0.01$ ). Males had a higher infection rate than females ( $P < 0.05$ ). The highest prevalence was in children under one year, while the lowest was in those over six years. PCR confirmed that 75% of microscopically positive samples contained *E. histolytica*. This study provides new epidemiological insights into *E. histolytica* infection trends in Nasiriyah City using molecular and statistical approaches. The findings highlight the need for improved public health interventions, including better sanitation and targeted health education, to reduce infections, particularly among vulnerable pediatric populations.

**Citation:** Lhwak, N. S. Molecular and Statistical Study to Detect Infection with *Entamoeba histolytica* Isolated from Children's Feces in The City of Nasiriyah. Central Asian Journal of Medical and Natural Science 2025, 6(2), 516-522.

Received: 15<sup>th</sup> Jan 2025  
Revised: 22<sup>nd</sup> Jan 2025  
Accepted: 30<sup>th</sup> Jan 2025  
Published: 23<sup>th</sup> Feb 2025



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**Keywords:** Diarrhea, Amoebiasis, PCR, Microscopic Examination

## 1. Introduction

Over three billion people worldwide are affected via the prevalence the gastrointestinal protozoa parasites, which is one of the most prevalent major health issues [1–7]. Most the human illnesses affect children and newborns, according to the World Health Organization (WHO). [8] reports that over 450 million neonates and children worldwide are infected with these parasites [9]. *Entamoeba histolytica* is the intestinal parasite that the majority common. These illnesses are common and widespread in developing countries. They are in charge of fatal, severe diseases that ultimately lead for public health problems [10]. *Entamoeba histolytica* is causative agent of amoebiasis, also known as amoebic dysentery, an infection that primarily causes diarrhea or dysentery in newborns in underdeveloped nations. The amoebiasis claims the lives of about 100,000 children each year [11], [12]. The fecal-verbal route, which involves ingesting feed or water

polluted with a protozoan cyst, is the usual way that *Entamoeba histolytica* is spread. Additionally, living in regions where the disease is endemic, swimming in contaminated water, coming into direct contact with people, fresh vegetables, and dirt can all spread it. [13], [14]. Some hazards including poverty, poor diet, and lack of access to healthcare, are commonly associated with the high detection of *Entamoeba histolytica* infection. The bloody diarrhea, severe dysentery, weight loss, muscular depletion, fatigue, and the abdominal pain are the majority common symptoms of *Entamoeba histolytica* infections [15]. Negligence will result in severe amoebic dysentery and its related consequences, making asymptomatic *Entamoeba histolytica* infections more deadly than symptomatic ones. Patients who are asymptomatic are usually affected with trophozoites, a non-invasive infection that is found in gut lumen. In certain cases, trophozoites exhibiting clinical symptoms penetrate the intestinal mucosa (intestinal sickness) or reach extra-intestinal organs such as the brain, liver, and lungs via the bloodstream (extraintestinal tract illness) [16]. The purpose of this study for ascertain the incidence of *Entamoeba histolytica* in 2112 children's visited the Bint Alhuda hospitals in Nasiriyah City, Iraq, in 2023 and 2024, regardless of age or sex and detected with microscopic and PCR.

## 2. Materials and Methods

### 2.1 The study design and sample

Between February 2023 and November 2024, stool samples from 2112 children with clinically diagnosed primary enteritis and diarrheal symptoms were taken from the Bint Alhuda Hospital in Nasiriyah City. Sterile plastic bottles bearing precise personal information, such as name, age, gender, and collection date, were used to collect these specimens. The feces were noted for their colors (brown, yellow, semi-brown, and verdant) and textures (serous, mucus, viscous, and the bloody). A glass slide drop of physiological saline or Lugol's iodine solution was placed next to a small sample of freshly void feces, and the samples were examined under a light microscope using a direct saline technique made with a sodium chloride solution consisting of 0.9 percent. The direct smear method was used to carefully inspect this slide under a microscope in order to ascertain whether cysts or trophozoite of *Entamoeba histolytica* were present. The different colors (brown, yellow, semi-brown, and greenish) and textures (mucus, serous, greasy, and the bloody) of the school were observed [17]. The fecal samples were divided into two components: one for microscopy inspection of the parasite and another for two hundred mg and straight-line PCR at -20°C for molecular analysis [18], [19].

### 2.2 The study design and sample

With a p-value less than 0.05, the Chi-square test in SPSS version 25 (IBM Franklin D. Roosevelt, New York, NY) was used to identify the significant relationship.

### 2.3 PCR Steps and Components

Five microliters of DNA template; a series of oligonucleotide primers unique to the 18S rRNA gene in *E. histolytica*, PCR water, 7 µL; master mix, 5 µL; forward primer (GGGGAGTATGGTCACAAGGC), 1.5 µL; and reverse primer (TGTGTACAAAGGGCAGGGAC), 1.5 µL. Taq DNA polymerase, dNTPs, TrisHCl pH: 9.0, KCl, MgCl<sub>2</sub>, stabilizer, and tracking dye are among the other ingredients required for the PCR process that are included in the AccuPower® PCR PreMix Kit. All of the PCR tubes were then placed in an Eppendorf centrifuge and spun for three minutes at 3000 rpm. After that, it was put in a PCR thermocycler (BioRad T100 Thermal Cycler, USA). PCR procedures, annealing, 58°C, 30sec, 30cycle; extension, 72°C, 1min, 30cycle; final extension, 72°C, 5min, 1cycle; cold, 4°C, forever; initial denaturation, 95°C, 5min, 1cy.

## 3. Results

Stool exams were conducted on 2112 patients who visited Bint Alhuda Hospital in Nasiriyah City through February 2023 and November 2024. Table (1) shows the total

number of the *E. histolytica* parasite infections over this time period. 137 (13.5%) of the 1010 samples examined in 2021 had *E. histolytica* infections that were statistically significant ( $P < 0.01$ ) at the probability level. 178 (16.1%) of the 1102 samples that were examined in 2022 had positive *E. histolytica* infection tests and there was a statistically significant difference ( $P < 0.01$ ).

**Table 1.** Number of *E. histolytica* infections from 2023 and 2024.

Parasite species	<i>E. histolytica</i> Found/total	P-value
2023	137/1010	
2024	178/1102	$P < 0.01$
Total	315/2112	

**Table 2.** *E. histolytica* infectious with respect to age group between 2023 and 2024.

Age group (years)	2023	2024
	No. of <i>E. histolytica</i> % Found/total	No. of <i>E. histolytica</i> % Found/total
Less than 1 year	45/137 (32.8)	68/178 (38.2)
1-2	42/137 (30.6)	60/178 (33.7)
2-4	18/137 (13.1)	25/178 (14)
4-6	16/137 (11.6)	12/178 (6.7)

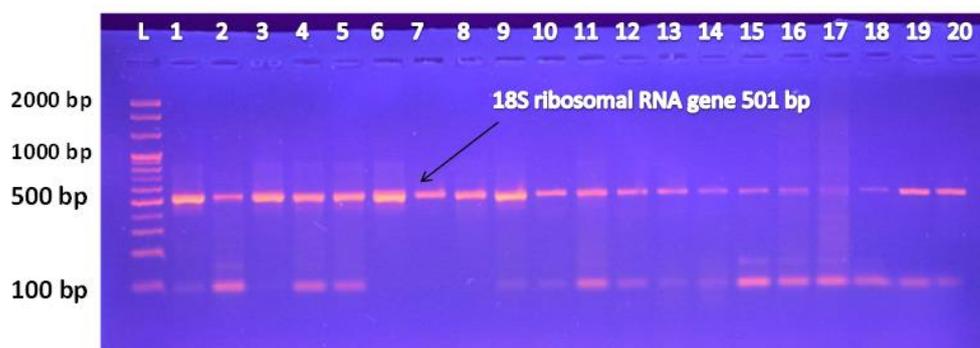
**Table 3.** *E. histolytica* infectious with respect to gender through 2023 and 2024.

Years	No. % of <i>E. histolytica</i> Found/Total	No. % of Males Found/Total	No. % of Females Found/Total	P-value
2023	137/1010 (13.5)	75/137 (54.7)	62/137 (45.2)	
2024	178/1102 (16.1)	113/178 (63.4)	65/178 (36.5)	$P < 0.05$
Total	315/2112 (14.9)	188/315 (59.6)	127/315 (40.3)	

Table 2 shows the analysis of the relationship between the ages of *E. histolytica* infections in 2021 and 2022. The age categories with the highest and lowest prevalence rates of *E. histolytica* infection in 2021 were those aged 0–1 year 45 (32.8%) and older than 6 years 16 (11.6%). There was statistical significance in the infection rate ( $P < 0.01$ ) through the *E. histolytica* infection and different age groups. Additionally, infection prevalence rates in 2024 were comparable to those in 2023 with just slight changes. Those younger than one year old had the highest prevalence of *E. histolytica* infection (38.2%), whereas those older than six years had the lowest incidence (6.7%). Additionally, a statistically significant correlation ( $P < 0.01$ ) was seen through the rate of infection and different age groups for *E. histolytica*.

The relationship between *E. histolytica* infections by gender in 2023 and 2024 is shown in Table 3. According to the data, males were found to have the virus at a higher rate than females. In 2021, the detected of *E. histolytica* infection was higher in males (75, 54.7%) than in females (62, 45.2%). Furthermore, at a rate that is statistically significant ( $P < 0.05$ ). According to the examination of data from 2024, men had a larger prevalence of *E. histolytica* (113, or 63.4%) than women (65, or 36.5%). One hundred stool samples that test positive under a microscope using the PCR technique are examined as part of the current investigation. Using m.w. 501bp, Figure 1 illustrates positive *E. histolytica* in agarose gel electrophoresis. Out of the 100 microscopically positive samples that were analyzed by

PCR, the results indicate that 75 (75%) of the children who were sick had positive samples, and 25 (25.0%) had other causes of diarrhea.



**Figure 1.** Analysis of the 18S ribosomal RNA gene using PCR isolates of *E. histolytica* is displayed in the agarose gel electrophoresis image. L (2000-100 bp ladder). Some positive stool samples of *E. histolytica* at 501 bp product size were found in Lanes 1–20.

#### 4. Discussion

Because of their higher rates of morbidity and mortality, intestinal parasite infections (IPIs) are known to be a global burden [20], [21], [22]. The WHO estimates that over 2 billion persons globally are now harboring IPI [23]. IPI propagation is more likely to occur in underprivileged groups having a low socioeconomic and academic level, inadequate poor hygiene practices, improper disposal of waste, limited access to clean water sources, and a lack of knowledge about appropriate personal hygiene [24], [25]. The results of the evaluation of the relationship between infection and age with *E. histolytica* during 2023 and 2024 are shown in Table 2. The age categories with the highest and lowest prevalence rates of *E. histolytica* infection in 2023 were those under one year old and those older than six. The frequency of infection rate in 2024 was comparable to that in 2019, albeit with considerable variation. The highest and lowest rates of *E. histolytica* infection were found in age groups under one year and older than six, respectively. These findings were consistent with research showing that the detected of *E. histolytica* was 11% in children in Delhi, India, and Pakistan, and 15.6% in preschool-aged children in Bangladesh [26]. 21.0% in seven Malaysian villages [27], [28] and [29]. Al-Karmah Teaching Hospital and Ibn Al-Ather Hospital in Iraq both reported similar outcomes by [30], [31], [18]. Compared to older adults, children in this age group may have lower resistance and poorer health, which could be the cause of the increased infection prevalence. Furthermore, it is more difficult for these kids to fend off illness because their vital defense systems are still developing. As a result, they are more vulnerable to parasites than older individuals. Furthermore, a number of variables contribute significantly to the occurrence, including a lack of social support, poor hygiene, toilet training, climate, large populations, and limited water supply of *E. histolytica* infection [13], [16], [31].

These results were in line with those of other studies that found that men experienced diarrhea at a higher rate than women [32]. Furthermore, our results showed that the prevalence rates of *E. histolytica* in males and females were 48.8% and 34.44%, respectively, in line with other research like Obadiah (2012) [33], [20]. Furthermore, other research indicates that men are more often than women to have this illness (27.7% versus 24.3%, respectively) [34]. The increased occurrence of *E. histolytica* in males may be due to the fact that males are more likely than females to suffer from parasitic infections. Males tend to exhibit a more severe infection than females, which is a result of their insufficient immunity [35]. Variations with hormonal roots in environmental and physiological facts these differences in immune responses to infection [36]. These disparities in infection between the sexes can also be explained by variations in the interaction between the immunological and endocrine systems. Similarly, androgens lower immunological

competence, and sex steroids, especially female estrogens and male testosterone, alter several facets of host immunity [37]. Steroid hormones affect genes and behaviors that are resistant to disease, making males more prone to illness [35]. The lower occurrence in females might partly result from their greater concern for medical care [16]. Additionally, 75 cases (75%) of the 100 samples that tested positive under a microscope had indications of an *E. histolytica* infection when the PCR technique was applied [38]. This result is consistent with a 2012 study by Ngui et al. that found a 75.0% PCR rate of *E. histolytica* infection [39]. The same conclusion was supported in 2005 by Roy et al., who reported a PCR rate of 72.0% [40]. According to a research by Shareef (2022) in the West Bank, PCR verified 74% of positive samples diagnosed microscopically, which is consistent with our findings [41]. The current study's findings are in conflict with those of Ngosso et al. (2015), who found that 48 (33.3%) of 144 stool samples contained *E. histolytica* using PCR [42]. Furthermore, he reported an *E. histolytica* rate of 5.0% in Thi-Qar, which contradicts Salim's (2018) findings [38]. Additionally, our findings conflict with those of Alkhuzaey (2019), who found that the rate of *E. histolytica* in Thi-Qar was 38.1% [43]. According to the current investigation, the PCR results showed a prevalence of *E. histolytica* that is noticeably greater than what was found by microscopic analysis. This discrepancy may be explained by PCR's improved sensitivity and accuracy in identifying *E. histolytica*. It's possible that the examiner had trouble diagnosing parasites during microscopic inspection because of the existence of visual-obstructing substances.

## 5. Conclusion

A study conducted an increased rate on the *Entamoeba histolytica* infection in children from 13.5% in the year 2023 to 16.1% in the year 2024 in Nasiriyah City, with a significant difference noted ( $P < 0.05$ ). The analyzed data had male appreciably infected compared to female, with 54.7% in 2023 and 63.4% in 2024. The majority of cases were found in children below one year of age, indicating increased susceptibility owing to probable immature immune systems and risk exposure. PCR proved to be a more sensitive technique than microscopy, as it detected *E. histolytica* in 75% of microscopically positive samples and this clearly indicates that advanced diagnostic methods are needed. These findings indicate the need for targeted public health interventions, especially in the areas of sanitation and hygiene education, in high-risk groups. The results of this study are valuable epidemiological data for *E. histolytica* and will certainly contribute to the development of a workable preventive measure in Nasiriyah City.

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