

# Correlation between *Entamoeba histolytica* and *Escherichia coli* infection in patients of Wasit Province

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**Keywords:**

*E. histolytica*, *E.coli*, Diarrhea, Feces, Human

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**ABSTRACT**

This study was carried out at Wasit Province / Iraq in cooperation with Fairouz Hospital/ unit of microbiology. Samples were collected from many places at Wasit province. It included 177 stool samples from Iraqi patients with diarrhea, and 30 healthy subjects as a control. The study was conducted during the period from October to December, 2021 to investigate the prevalence of intestinal parasites and bacteria. Only 54 stool sample were positive for *E.histolytica* and *E.coli*. The results showed that the percentage of parasitic infection among patients and control were 54 (30%) and 0 (0%) respectively, with significant differences. For the parasitic infection, the higher rate of infection was recorded for the group (1-10) years which reached 27.8%, while the lower was recorded for the group (60-70) years it reached 3.7% without significant differences. Regarding gender males rate was 72.3% and females rate was 27.8% in this study without significant differences. According to the residence, urban areas were reported 59.3% and rural areas were 40.7% without significant differences. Correlation between *E.histolytica* parasite infection and *E.coli* bacterial infection in patients group were that 100% and revealed significant differences.

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## 1. INTRODUCTION

In countries of endemicity, the human protozoan parasite *Entamoeba histolytica* has become a common resident of the large intestine, where it survives by feeding on dead cells and bacteria without causing harm to the host [1], [2]. However, for reasons not quite understood, *E. histolytica* transition to an invasive form and following invasion gives rise to amebic colitis with symptoms ranging from diarrhea, ameboma, and life-threatening extraintestinal invasion to the liver [3], [4]. This suggests that changes in the gut environment may contribute to the pathogenesis of *E. histolytica*, leading to invasive amebiasis [5], [6].

Coinfection with pathogenic organisms, especially enteropathogenic bacteria, may be an important factor that contributes to alteration of normal enteric microbiota and immune regulation, enhancing the virulence of *E. histolytica* in disease pathogenesis [7], [8]. Epidemiological studies have reported frequent presence of enteropathogenic bacteria in coinfection with symptomatic intestinal amebic infection [9]. Under *in vitro* culture conditions, *E. histolytica* interaction with enteropathogenic bacteria for as little as 1 h enhanced parasite adherence and cysteine protease activity, with increased cytopathic activity [10]. Another study showed that short-term coculture (12 h) of a pathogenic *Escherichia coli* serotype with an *E. histolytica* strain

that had lost its capacity to produce amebic liver abscess (ALA) in hamsters restored parasite virulence by producing ALA [11]. Likewise, *Entamoeba dispar* strain ADO cultured under axenic conditions did not produce ALA, but when it was maintained in culture with microbiota from patients, it produced liver damage in hamsters similar to that produced by axenic *E. histolytica* [12].

The influence of bacteria on intestinal amebiasis has been observed in gnotobiotic athymic mice [13], in which the percentage of cecum colonization with *E. histolytica* strains HK-9 and NIH:200 increased when they interacted with *E. coli* or *Clostridium perfringens*. Similarly, in C3H/HeJ mice, cecum damage produced by *E. histolytica* increased from 17% to 39% when parasites were cocultured with bacterial organisms [14]. Furthermore, innate host immune responses also play a key role in susceptibility to *E. histolytica* infection. In HT-29 cells, exposure to *E. histolytica* in the presence of *E. coli* DH5 $\alpha$  resulted in a synergistic increase in the expression of interleukin 8 (IL-8), IL-1 $\alpha$ , and granulocyte-macrophage colony-stimulating factor (GM-CSF) [15].

## 2. Materials and Methods

### 2.1 Samples collection

This study was carried out in Fairouz Hospital in the city of Wasit province. This study included the collection of 177 feces samples, which were obtained from various areas in Wasit province during the period from the beginning of October, 2021 to the end of December, 2021. The stool samples were collected from diarrheal patients who attended Fairouz Hospital and some private laboratories in clean, airtight plastic bottles and examined directly. The following information was recorded on each sample, such as: name, age, gender, as well as the residence of patients.

### 2.2 Isolation and identification of *E. histolytica*

All stool specimens were examined under microscope after taking small amount on glass slide with normal saline and covered with cover slip to examined under 40x for trophozoite and cyst forms [16].

### 2.3 Isolation and identification of *E. coli*

#### 2.3.1 Culture

The stool samples were cultured on MacConkey agar, eosin methylene blue and blood agar [17].

### 2.4 Triple Sugar Iron (TSI) medium

The TSI medium contains phenol-red reagent, ferrous sulfate, sodium thiosulfate (to detect the production of hydrogen sulfide gas) and three sugars glucose, lactose and sucrose, the concentration of glucose in the medium is 0.1 concentration of the other two sugars [18].

## 3. Results and Discussion

The total number of samples were 177 feces samples collected from people with diarrhea who attended Fairouz Hospital in Wasit province, from those patients only 54 (30%) samples were positive for *E. histolytica* under microscope examination who showed trophozoite form, figure (1).



**Figure 1.** Trophozoite of *E. histolytica*

All *E. histolytica* samples were positive for *E. coli* (100%) bacteria test, in addition to 30 healthy people for the period from the beginning of October 2021 until the end of December 2021. *E. coli* was diagnosed based on morphological, culture and biochemical tests, where it appeared in the form of short gram-negative bacilli and fermented with lactose sugar on the MacConkey agar, colonies are circular, moist, smooth and of entire margin, colonies appear flat and pink, they are lactose fermenting colonies [17], while on the center of the eosin methylene blue it appeared green in color and Green Metallic sheen colonies are formed, which is a distinctive feature of *Escherichia coli* from the rest of the genera of the intestinal family, figure (2).



**Figure 2.** Growth of *E. coli* bacteria on MacConkey and EMB culture media.

While on blood agar colonies are big, circular, grey and moist, Beta ( $\beta$ ) hemolytic colonies are formed [17] on TSI culture medium *E. coli* was able to ferment glucose, lactose and sucrose, with CO<sub>2</sub> production, bubbles and cracks and even displacement of the environment are observed, the whole tube becomes acidic and the culture medium turns yellow [18] figure (3).



**Figure 3.** A: Growth of *E. coli* bacteria on Blood agar; B: *E. coli* bacteria results on TSI medium

The study showed that the rate of infection with intestinal parasites was 30% among diarrheal cases for *E. histolytica*. and 0.0% among control subjects. The results of the statistical analysis showed that there is a

significant difference at the level ( $P \leq 0.05$ ) between the two groups as shown in Table (1).

**Table 1.** Percentage of infection with intestinal parasites under study.

Groups	Number of Examined Samples	Positive Samples No.(%)
Diarrheal Group	177	54 (30%)
Control Group	30	0 (0%)
Total	200	54 (30%)

Significant \*\*

The study agreed with [19] who showed a difference in the infection rate, as the percentage of tissue-type amoeba infection was recorded at 24.85% in the city of Kirkuk. but it disagreed with [20], because they showed that the total infection rate with intestinal parasites was 13.64%. The incidence of infection in the current study is high compared to results of previous studies. The reason for the difference may be attributed to many reasons, the most important of which are the service and environmental situation, as well as public hygiene, attention to personal culture, and the difference in the standard of living and social.

The stool examined samples were collected from different age groups, starting from one year to 70 years, and the results showed no significant difference at the probability level of 0.05 between infection rates and *E.histolytica* parasite, as the percentage was 27.8% for the age group (1-10) years old, which recorded the highest rate of infection, and the lowest percentage of infection in the age group (60-70) years, as it was 3.7% as shown in table (2).

**Table 2.** Distribution of *E.histolytica* infection according to age groups.

Age groups / Years	Number of examined samples	No. of <i>E.histolytica</i> infection %	P.value
1-10	45	15 (27.8%)	P $\geq$ 0.05
11-20	20	9 (16.7%)	
21-30	33	10 (18.5%)	
31-40	20	5 (9.3%)	
41-50	20	8 (14.8%)	
51-60	15	5 (9.2%)	
61-70	24	2 (3.7%)	
Total	177	54 (30%)	

Non-significant

Table (2) shows the total number of people examined and the infection rates for parasite according to the age groups under study. The total infection rate with *E.histolytica* was 30 %. It was found that the highest rate of infection with *E.histolytica* was within the age group (1-10) years, it reached 27.8 %, and the lowest infection rate was within the age group (61-70) years with a rate of 3.7 %.

These data were in agreement with (37) in Turkey, who recorded that the age groups most exposed to infection with parasite are (1-14) years. And the study by [18] as they mentioned the same outcomes. Also [21] who concluded that amebiasis was a substantial burden on the overall health of the cohort children. But this study differed with what It was recorded [22] there findings alluded to the high prevalence of *E. histolytica* and other unidentified enteropathogens as major potential causes of pediatric AGE in hospitalized Lebanese children. This should drive us to widen our diagnostic panel by adopting new diagnostic techniques other than the routinely used ones (particularly specific for the pathogenic amoeba *E. histolytica* and for the unidentified enteropathogens), and to improve health services in this unfortunate area of the world where insanitary water supplies and lack of personal hygiene represent a major problem. The results of present study revealed that the males were 39 (72.2 %) and 15(27.8%) of the females with no significant differences as shown in table (3).

**Table 3.** Distribution of *E.histolytica* infection according to the gender

<b>Gender</b>	<b>Number of examined samples</b>	<b><i>E.histolytica</i> infection %</b>
Male	99 (55.93%)	39 (72.2%)
Female	78 (44.07%)	15 (27.8%)
Total	177 (100%)	54 (100 %)

Non-significant

The current study was compatible with [23] showed that the infection rate was higher in males as it is in females. And with [24] in the city of Kirkuk, where the rate of infection with *Entamoeba histolytica* among males was 61.26%, and in females it was 37.74%. But these did not agreed with the study of [25], [26], as each of them recorded a higher incidence of intestinal parasites in females than males. Also [27] showed that the rate of infection with stomach germs was higher in females compared to males,. Also, the study [28] showed that the infection rate of parasite was higher in females compared to the infection rate in males.

Also according to the residence study was implicated in number of parasitic infection rate, patients were divided into two groups according to residence. Urban relation to infection was 59.3% out of the number of samples examined, while Rural relation to infection was 40.7%. The results of the statistical analysis showed that there were no significant differences (Table 4).

**Table 4.** percentages of *E.histolytica* according to the residence.

<b>Residence</b>	<b>Number of examined samples</b>	<b><i>E.histolytica</i> infection %</b>
Urban	95 (53.67%)	32 (59.3 %)

Rural	82 (46.33%)	22 (40.7%)
Total	177 (100%)	54 (100%)

Non-significant

Our information was compatible to [29], It was recorded a high rate of amoebic dysentery infection in some villages of Baghdad. And [30] who mentioned the same outcomes. In [31] study one hundred and eighteen (41.8%) of the 282 rural subjects had anti - *E. histolytica* IgG antibodies. While [32] concluded that the progression of infection to invasive disease is contingent on the unique interplay between host and pathogen factors, such as the strength of host-immunity to overcome infection and inherent pathogenicity of the *Entamoeba* species.

As a preventable illness, *E. histolytica* complications such as ALA impose a significant burden on the healthcare system. The last one result was to clarify the correlation between *E.histolytica* parasite infection and *E.coli* bacterial infection in patients group, outcomes were that 100% of parasite infection have the bacterial infection in this study, outcomes of statistical analysis revealed significant differences (Table 5).

**Table 5.** Correlation between *E.histolytica* and *E.coli* infections

Infection types	No. (%)	Correlation %
Parasitic	54 (100%)	1
Bacterial	54 (100%)	1
Total	54 (100%)	1

Very significant\*\*\*

These information were compatible to [33] as they revealed that a number of differentially expressed genes encoding metabolic enzymes, ribosomal proteins, virulence factors and proteins related with cytoskeletal and vesicle trafficking were found. These results suggest that *E. coli* 055 has a nutritional role that strongly supports the amoebic growth, and is also able to modulate some biological activities related with amoebic virulence. But the percentage of samples positive for bacterial 14.83% of the all number of examined samples and the number of different infections with parasitic species was 12.44%, among the patients included in the study [34]. The study [35] showed that the incidence of Coliform bacteria, reaching 62.6%.

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