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Exploring the co-occurrence of Entamoeba histolytica and Escherichia coli infection in diarrheal patients from Al-Rifai and Al-Nasiriyah in Thi-Qar province

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Abstract

Enteric infections, whether accompanied by apparent diarrhea or not, significantly impact gastrointestinal absorption, nutrition, childhood development, and global mortality rates. This study attempts to answer the question (Is there co infection between *E. histolytica* and *E. coli* in diarrheal samples?). This study, conducted from October 2024 to January 2025 in Al-Rifai and Al-Nasiriyah in Thi-Qar, province the possible co-infection of *E. histolytica* and *E. coli* in 130 diarrheal patients. The samples were examined by direct examination. The findings reveal a correlation between the parasite and the bacterium in Al-Rifai and Al-Nasiriyah districts.The infection prevalence of *E. histolytica* was 66 out of 130 (50.76%). Conversely, 54 out of 130 (41.53%) *E. coli* showed positive culture growth confirmed by morphological characteristics. However, 28/130 (21.53%) were found to be positive for both pathogens. It is clear that the co-existence of these two pathogens follows the same trend (both were highly prevalent in the age group (> 20 years old), the co-existence of the two pathogenesis was higher in Al-Refai than in Al-Nasiriyah (28.94% vs.71.06%). Conclusions, there is co infection between *E. coli* and *E. histolytica* in the two districts.

Key words: *Escherichia coli, Entamoeba histolytica,* coinfection, diarrheal patients, Al-Rifai, Al-Nassiryah, Thi-Qar,

الملخص:

تؤثر العدوى المعوية، سواء كانت مصحوبة بإسهال واضح أم لا، بشكل كبير على الامتصاص المعوي والتغذية ونمو الأطفال ومعدلات الوفيات العالمية. تحاول هذه الدراسة الاجابة عن هذا التساؤل (هل هناك عدوى مشتركة بين *E. histolytica* و*E. coli* في إصابات الاسهال؟). أجريت هذه الدراسة من تشرين الاول 2024 الى كانون الثاني 2025 في الرفاعي والناصرية في محافظة ذي قار من 130 مريضا مصابا بالإسهال. فحصت العينات بالفحص المجهري المباشر. تظهر النتائج وجود علاقة عدوى بين *هذا الطفيلي وهذه البكتيريا* في قضائي الناصرية والرفاعي .بلغ معدل انتشار الإصابة بطفيلي *E.histolytica* 66 حالة من أصل 130 حالة (50.76%)، وعلى العكس من ذلك ،أظهرت 54 عينة من أصل 130 عينة (41.53%) من *E.coli* نموا أيجابياً على الوسط الزرعي أكدته الخصائص الشكلية ومع ذلك ،وجد أن 28 عينة من اصل 130 عينة (21.53%)ايجابيا لكلا الممرضين .ومن الواضح ان أنتشار هذين الممرضين يتبع نفس الاتجاه (كلاهما كان منتشرا للغاية في الفئة العمرية اكبر من 20 عاما) ،وكان انتشارهما أعلى في قضاء الرفاعي مما هو علية في قضاء الناصرية (28.94% مقابل 71.06%). الاستنتاج هو وجود ظهور مشترك لكلا الممرضين *E.*coli و*E.histolytica* في المنطقتين .

الكلمات المفتاحية : *E. coli*، ***E. histolytica*، العدوى المشتركة، مرضى الإسهال، قضاء الرفاعي، قضاء الناصرية، ذي قار**

Introduction

Enteric infections, whether accompanied by apparent diarrhea or not, significantly impact gastrointestinal absorption, nutrition, childhood development, and global mortality rates(1). Any form of diarrhea is a clinical sign that the intestines are secreting more water and electrolytes than they are absorbing (2). The most common causes of gastrointestinal infections in children, including bacteria, viruses, parasites, and protozoans (3). Gastrointestinal protozoa and helminthic parasites are significantly contributing to medical and public health

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issues in poor nations (4). The impact of intestinal parasites on morbidity and mortality (5). Entamoeba histolytica (*E. histolytica*) is a unicellular protozoan recognized as a prevalent cause of dysentery in humans (6). *E. histolytica* infects approximately 45-50 million people each year, causing 40,000 to 100,000 deaths (7). Amebiasis, a worldwide intestinal parasite affliction, is caused by E. histolytica. This parasite, which consumes bacteria in the large intestine of its human host, can elicit a robust inflammatory response upon penetrating the colonic mucosa (8). The clinical manifestations of *E. histolytica* exposure might significantly differ, presenting as diarrhea, dysentery, or amebic liver abscess. Host and parasite (9). *Escherichia coli* (*E, coli*) are facultative or aerobic anaerobes, rod-shaped, and Gram-negative. The majority of the participants are lactose fermenters that are non-spore forming, flagellated (10). The majority *E. coli* strains exist normally in the intestines and hardly induce disease in healthy persons (11). Nonetheless, certain strains may be harmful, leading to both intestinal and extraintestinal infections in people (12). *E. coli* is the primary cause of septicaemia, enteritis, urinary tract infections, and other clinical infections, including neonatal meningitis (13). This situation is caused by the household and recreational utilization of contaminated surface waters, deficiencies in hygiene, limited access to healthcare and potable water, and inadequate environmental and health monitoring systems(14).Upon entering the intestines, which are abundant in bacteria, *E. histolytica* initiates its invasion by eliminating the protective mucus layer, subsequently attaching to and detaching enterocytes, resulting in the rupture and deterioration of the epithelial barrier (15). *E. histolytica* consumes germs in the gastrointestinal tract. Rising evidence supports the importance of gut bacteria in disease development (16). Epithelial monolayers subjected to enteropathogenic bacteria exhibit increased vulnerability to E. histolytica damage. Simultaneously, the phagocytosis of harmful microorganisms by amoebae exacerbated epithelial cell injury (17). A presence of intestinal pathogenic bacteria influences the motility and mechanical force development of the parasite, both of which are crucial to its pathogenicity (18)

Materials and methods

Research region:

Thi-Qar Province in southern Iraq encompasses the districts of Nasiriyah and Al-Rifai. Nasiriyah serves as the

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administrative hub of the province, distinguished by a high population density and efficient administrative operations, with an estimated population of around 850,000 inhabitants. The Al-Rifai District is located in the northern region of the province, housing around 250,000 inhabitants. Thi-Qar province encompasses around 12,900 km² and possesses a semi-arid climate that influences the environmental and public health issues in the region.

Study Participants and Design

The research was conducted from October 2024 to January 2025. Samples were collected from Al-Rifai General Hospital and Al-Nasiriyah Teaching Hospital in Thi-Qar province. One hundred thirty stool samples were obtained from patients with severe to moderate diarrhea. The samples were analyzed by direct microscopy. Data was gathered regarding the patient, including age, sex, and location.

The isolation and designation of *E. histolytica*

The stool is dissolved in normal saline to facilitate the examination of the parasite's morphology and movement (19). A compound light microscope capable of 1000x magnification was used to diagnose the parasite. On glass slides, a drop of 0.9% normal saline solution was combined with a swab taken from the stool of patients experiencing acute diarrhea. The slides were inspected under a microscope with a 40x magnification lens after being covered with coverslips (20).

The isolation and designation of *E. coli*

Samples were inoculated onto MacConkey agar and Eosin Methylene Blue agar (EMB) and incubated at 37 °C for 24 hours. After culturing and purifying the bacterial isolates on various culture media, including MacConkey agar, eosin methylene blue, and MacConkey sorbitol, the morphological features of the isolated colonies were examined. The study took into account the isolated bacterial colonies' dimensions, heights, edges, texture, color, and shape (21). Lactose-fermenting colonies were selected and re-cultured on fresh MacConkey agar plates to achieve pure, well-isolated colonies. The isolated colonies were streaked on Eosin Methylene Blue to examine the presence of green metallic sheen colonies **(22).**

Microscopic designation:

After spreading a small portion of the developed bacterial colonies on microscopic slides with a drop of distilled water, the smear was stained with Gram stain and examined under a light microscope using an oil-based lens with a magnification of 100x to determine the color of the bacteria (23). The staining process distinguishes species within the domain Bacteria based on their cell wall construction, Gram-positive cells possess a robust peptidoglycan layer and exhibit a blue to purple coloration upon staining, Gram-

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negative cells possess a slender peptidoglycan layer and exhibit red to pink staining (24).

Indole A red ring that appears on the medium's surface indicates a positive test (25).

Methyl Red: The color changing to red signifies a successful outcome (26). Citrate utilization: The color changing from green to blue signifies a successful outcome indicates negative test. This experiment was conducted to evaluate the bacteria's capacity to use ammonium salts as a source of nitrogen and citrate as its sole source of carbon (27).

Voges -Proskauer's Test: tubes containing Methyl Red-Voges-Proskauer's (MR-VP) media, the tubes were incubated for 24 hours at 37 °C. Subsequently, five drops of VP2 (KOH) were added after ten drops of VP1 (alpha-naphthol), and a shake the tubes vigorously. After half an hour, the tubes were checked for color changes. A favorable outcome was indicated by the color changing from yellow to red. This test examined the bacteria's capacity to ferment sugars and produce acetoin (27).

which is indicative of *E. coli*

Statistical Analysis:

Descriptive statistics were utilized, with categorical variables (cities, age groups, and identification approaches) represented as percentages (%). Fisher's exact test was employed to compare categorical variables. The odds ratio was computed using the Baptista-Pike method, while the relative risk was determined by Koopman's asymptotic score. The significance threshold was set at 0.05, and a two-sided significance value was selected for all statistical tests. All statistical analyses and graphical representations were conducted using GraphPad Prism version 9

Results and Discussion

The total number of samples collected was 130 fecal samples from individuals with diarrhea who attended two districts: Al-Nasiriyah and Al-Rifai. Of these patients, only 66 (50.76%) samples tested positive for *E. histolytica* upon microscopic examination. Conversely, 54 out of 130 (41.53%) of *E. coli* demonstrated positive growth on agar media (MacConkey agar, Eosin Methylene Blue agar and biochemical testing). while 76 (58.47%) showed no growth. Statistical analysis indicated no significant differences ($P \leq 0.05$) in the detection rates of the two pathogens based on morphological identification.

This study's findings align with those of a study carried out in Basra city by(28), who found that 47% of diarrhea patients had an *E. histolytica* infection. But it disagreed with study(4) in Al-Fhood district of Thi - Qar because they showed that the total infection rate with *E. histolytica* was 17.5%.(29)

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reported an infection rate of 26.66% in Tikrit. This study(30) showed that the incidence of *E. histolytica* was 35.3% in Baghdad. Highlights the effectiveness of public health initiatives in curbing the spread of these pathogens. Additionally, economic and societal factors, along with varying environmental conditions, levels of health awareness, and diagnostic techniques, may all contribute to the discrepancies observed in the results. Also, the rate of *E. coli* isolation in this study was consistent with what was found by a study conducted by(31) where they recorded *E.coli* infection rates of 57 (44.2)% in in Al Musayyib District.(32) reported that the incidence of coliform bacteria 21/150 (14%). But it disagreed with study(33) in Wasit because they showed that the total infection rate with *E. coli* 36.25%. The difference in results might be caused by several things, such as different environmental conditions, varying health awareness, the diagnostic methods used, and economic and social factors that impact how these organisms spread.

Coexistence of *E. histolytica* and *E. coli* Among Age Groups

The findings presented in Table (1) reveal that out of 130 patients, 54 (41.53%) were infected with *E. coli*, while 66 (50.76%) tested positive for *E. histolytica*. Additionally, 28 out of 130 patients (21.53%) were found to be positive for both pathogens. It is evident that the co-existence of these two pathogens follows a similar trend, as both were highly prevalent in the age group over 20 years old.

These results are consistent with (34) study showed that the infection rate is higher in adults than in children. However, a study conducted in Basra City by(35) found *E. histolytica* a higher incidence among adults. This finding aligns more closely with the results of the current study, suggesting that employment and lifestyle factors may contribute to increased exposure in adults.A study (36)in Wasit, Iraq, found that the incidence of *E. histolytica* infection was 30%, and that the incidence of coinfection with E. coli was significant. It was noted that infection was high in young age groups, but not limited to them. The study confirms the existence of a statistically significant relationship between the presence of bacterial and parasitic infections in patients. Study(28) found that children under the age of one are more susceptible to infections. In a regional review,(37) highlighted that contamination in food products disproportionately impacts children under five, owing to their developing immune systems and dietary habits. However, clinical data from Arab countries frequently indicate higher infection rates among adults, particularly women of reproductive age. The current study corroborates regional trends that reveal

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elevated infection rates in both young children and adults. Conversely, this pattern contradicts various epidemiological studies that generally indicate higher infection rates among younger children. These children are particularly vulnerable due to their immature immune systems and their frequent exposure to contaminated environments.

Co-existence of Two Pathogens Regarding Residency

The results in Figure (8) indicate that the co-existence of the two pathogens was higher in Al-Refai City compared to Al-Nasiriyah City (28.94% vs. 71.06%). This difference was statistically significant ($P < 0.05$).

A study conducted in Wasit Province by(38) reported a 30% co-infection rate among diarrheal patients, revealing a strong correlation between the presence of *E. coli* and *E. histolytica*. The researchers suggested that the interaction between bacteria and parasites might render *E. histolytica* more harmful, particularly for individuals with weakened immune systems or those residing in poor environmental conditions. Both studies indicate a significant co-infection rate and underscore the impact of environmental exposure. The Wasit study specifically focused on *E. coli O157* and employed PCR diagnostics, whereas it utilized broader clinical detection methods. Differences in diagnostic specificity and the sanitation conditions of the region may explain the variations in prevalence observed. Similarly, a study in Egypt by(39) found that co-infection rates were highest among adults in urban slums. Regional studies indicate that areas with inadequate sanitation frequently experience co-infection, with adults being disproportionately affected. However, some studies suggest that children, particularly those under five years of age, may exhibit higher co-infection rates. The variation in prevalence related to age could be attributed to differences in immunity, exposure patterns, and health-seeking behaviours. Around the world, having *E. histolytica* along with harmful bacteria like *E. coli* is linked to worse disease symptoms. (40) suggest that bacterial co infection enhances the virulence of *E. histolytica* by modifying gut microbiota and encouraging more invasive.

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Figure (1): The geographical characteristics, where study was conducted

(https://www.google.come)

Figer (2): *E. histolytica* under microscopic

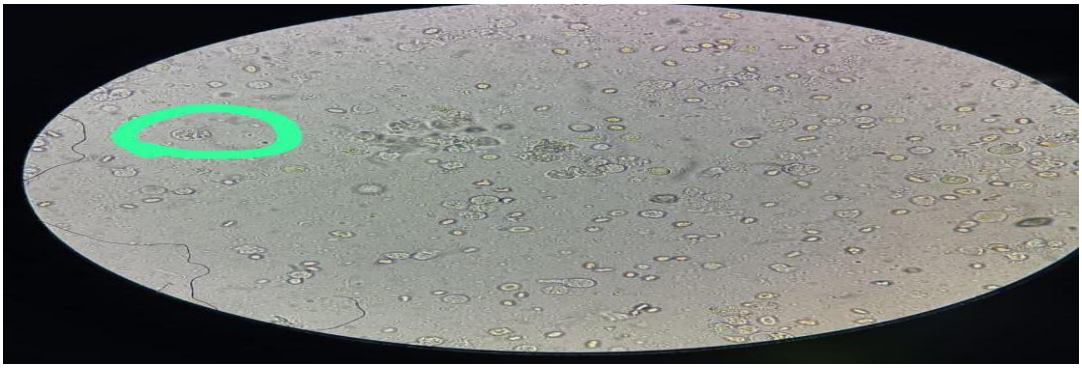

Figure (3): Demonstrates the growth of *E. coli* on MacConkey

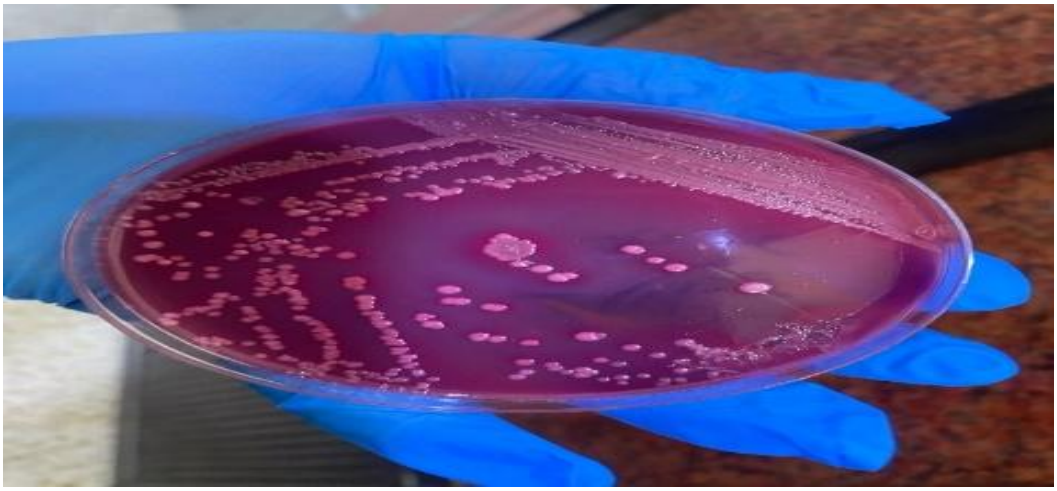

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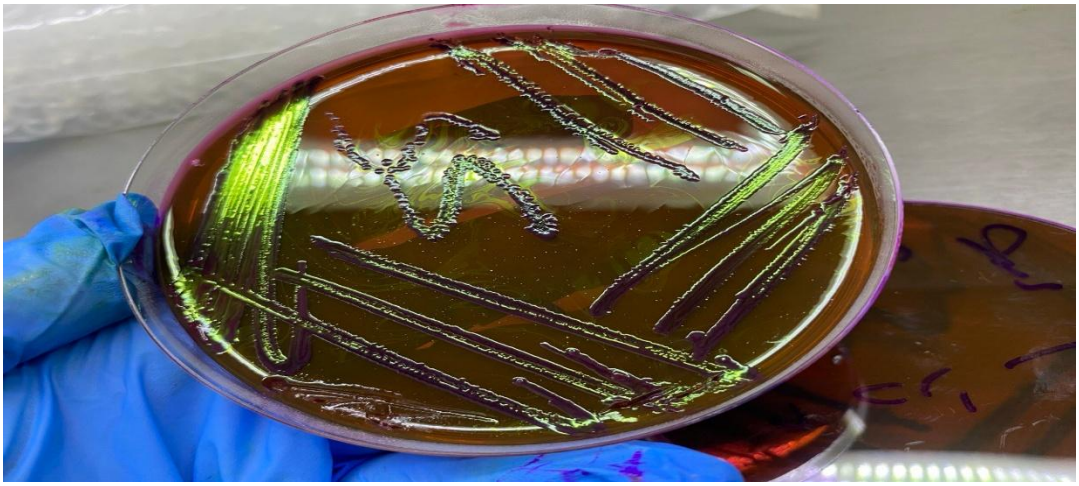
Figure (4): Demonstrates the growth of *E.coli* on EMB



Figer (5): Under microscopic using Gram stain

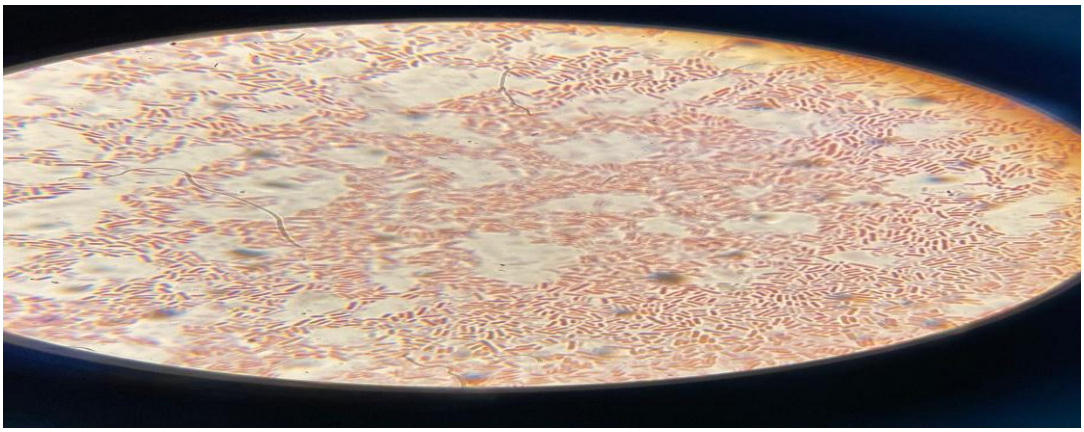


Figure (6): Demonstrates the growth of *E. coli* with IMVIC

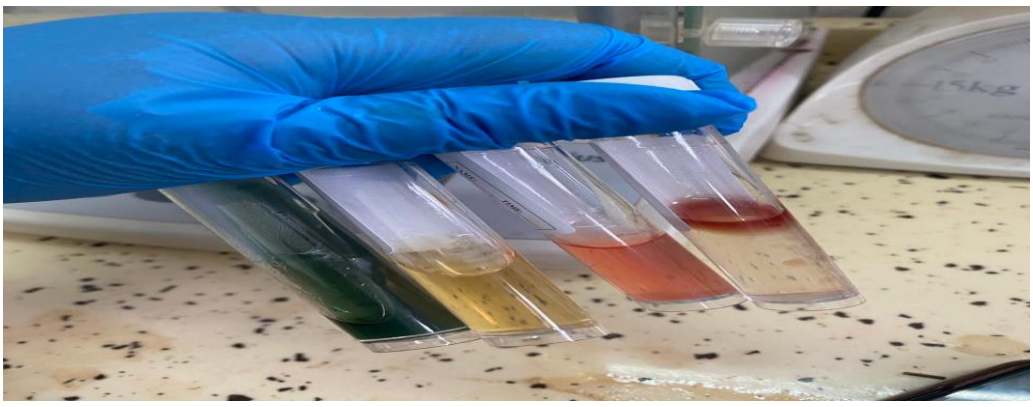


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Figure (7): Detection rates of *E. histolytica* and *E. coli* from stool samples based on morphological identification

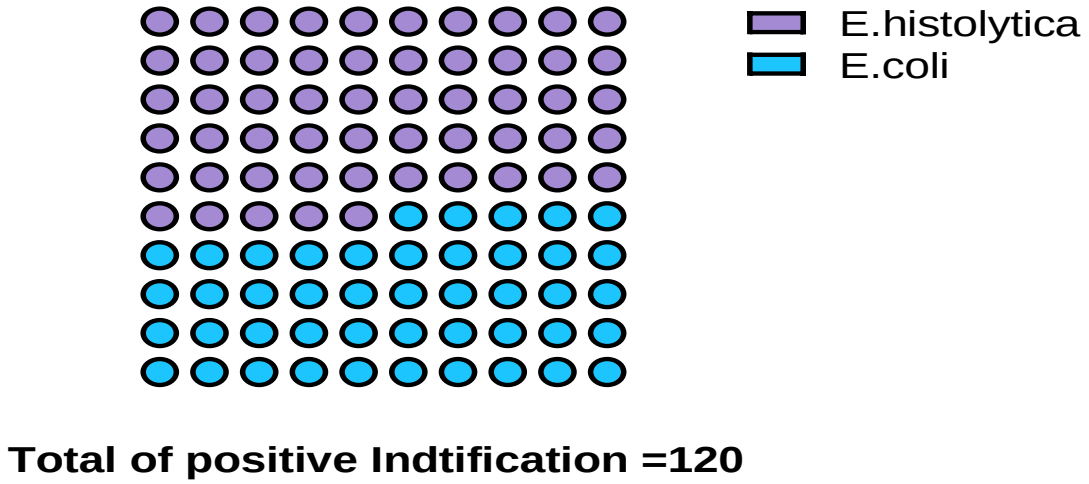

Table (1): Co-existence of *E. histolytica* and *E. coli* among the age groups

Age groups	*E. coli* %	*E. histolytica* %	Co-existence of both pathogens %
Less than 1 year old	5 (9.25)	9(13.63)	4 (14.28)
1-5 years	9 (16.7)	19(28.7)	7(25)
6-9 years	7 (12.96)	3(4.5)	2 (7.14)
10-15 years	3(5.5)	2(3)	2 (7.14)
16-20 years	3(5.5)	5(7.5)	3(10.71)
>20 years	27 (50)	28(42.42)	10 (35.71)
Total	54 (41.53%)	66 (50.76%)	28 (21.53%)

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