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Prevalence of *Entamoeba histolytica* Infection and Its Association with Gender and Occult Blood in Stool among Patients in Al-Bayda City, Libya

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Abstract
Amoebiasis, caused by *Entamoeba histolytica*, remains a major parasitic disease in developing countries, with significant health impacts across Africa and the Middle East. Despite improvements in sanitation, intestinal protozoal infections continue to be widespread in Libya. This study aimed to determine the prevalence of *E. histolytica* infection among patients in Al-Bayda City, Libya, and to evaluate its association with gender, age, and the presence of fecal occult blood (FOB) in stool samples. A cross-sectional study was conducted on 238 stool samples collected during 2024. Samples were examined for *E. histolytica* cysts and trophozoites and tested for occult blood using a rapid immunochromatographic method. Statistical analyses (chi-square and t-tests) were performed using R software, with $p < 0.05$ considered statistically significant. The prevalence of *E. histolytica* was 23.1% (55/238). There was no significant association between infection and gender (χ^2=0.00, p=0.983) or age (t=1.05, p=0.296). However, a strong association was found between *E. histolytica* infection and FOB positivity (χ^2=21.9, p=0.0000193). The relationships between gender and FOB (χ^2=0.00, p=0.99) and between age and FOB =0.42, p=0.673) were not significant. The high prevalence of *E. histolytica* and its strong association with occult blood indicate ongoing transmission and mucosal invasion among infected individuals in Al-Bayda City. Improved sanitation, public health education, and molecular confirmation of *E. histolytica* are essential to reduce infection rates and prevent severe outcomes.
Keywords: *Entamoeba histolytica*, Prevalence, Al-Bayda, Libya, Gender, Fecal Occult Blood, Intestinal Parasites.

Introduction

Amoebiasis, caused by *Entamoeba histolytica*, is a major parasitic disease affecting millions of people worldwide, particularly in tropical and subtropical regions. Globally, it is estimated that up to 50 million people are infected annually, with over 100,000 deaths attributed to invasive amoebiasis [1]. The infection is transmitted via the fecal–oral route, primarily through ingestion of cysts in contaminated food or water. Despite progress in water and sanitation, *E. histolytica* continues to pose a serious public health challenge, especially in developing countries where diagnostic limitations and environmental conditions facilitate its persistence [2]. The disease manifests in diverse clinical forms, ranging from asymptomatic colonization to severe dysentery and extra intestinal abscesses [3]. Previous studies have reported variable infection rates depending on diagnostic methods and study populations. For instance, in Egypt, infection rates range from 12% to 25% depending on the diagnostic tool [4]. In sub-Saharan Africa, *E. histolytica* remains among the most prevalent protozoa detected in stool examinations [5]. In India, similar findings were reported with infection rates around 20% in both genders [6].

In Libya, limited epidemiological data exist. A recent study reported that *E. histolytica* and *Giardia lamblia* are among the most frequently detected intestinal protozoa in both Libya and Egypt [7]. Al-Bayda City, located in eastern Libya, is characterized by mixed urban and rural populations with varied sanitation practices, which may contribute to continued parasite transmission. This study investigates the prevalence of *E. histolytica* infection among patients in Al-Bayda City and explores its relationship with gender, age, and the presence of occult blood in stool samples. The association with FOB serves as a clinical indicator of mucosal invasion, providing insight into the pathogenic potential of local *E. histolytica* strains.

Methods

Study Design and Population

This cross-sectional study was conducted in Al-Bayda City, Libya, between January and December 2024. A total of 238 stool samples were collected from patients presenting to local diagnostic laboratories for routine parasitological examination. The inclusion criteria were patients of both genders, aged 4–88 years, who provided stool samples for

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investigation. All samples were collected under sterile conditions and processed within two hours of collection to ensure the reliability of results.

Laboratory Procedures

Each stool specimen was examined macroscopically to assess color, consistency, and the presence of mucus or visible blood. For microscopic analysis, direct wet mounts were prepared using normal saline and Lugol's iodine solution to detect *Entamoeba histolytica* cysts and trophozoites under light microscopy at 10× and 40× magnifications. In addition to microscopic examination, fecal occult blood (FOB) was detected using a rapid immunochromatographic test cassette (standard guaiac-based kit). The procedure was performed according to the manufacturer's instructions as follows:

Step 1 – Preparation of Materials

Before starting, all materials were assembled, including the FOB rapid test cassette, stool collection tube containing buffer solution, stool sample, timer, and personal protective equipment (PPE) such as gloves and laboratory coats.

Step 2 – Collection of Stool Sample

A small amount of stool was collected using the stick or applicator attached to the cap of the collection tube. The applicator was dipped into different parts of the stool sample to ensure proper representation, then reinserted into the tube containing buffer solution. The cap was screwed tightly, and the tube was shaken to mix the sample thoroughly.

Step 3 – Application to the Test Cassette

The test device was removed from its foil pouch and placed on a clean, flat surface. The tip of the collection tube was broken off, and 3–5 drops of the stool-buffer mixture were added to the sample well (S) of the test cassette.

Step 4 – Waiting for Results

The test was allowed to run for 5–10 minutes at room temperature. Results were interpreted within the manufacturer's recommended time frame. The appearance of both control (C) and test (T) lines indicated a positive result, while the presence of only the control line indicated a negative result. The test was not interpreted after 15–20 minutes to avoid false results.

Data Collection and Variables

For each patient, demographic and laboratory data were recorded, including age, gender, *E. histolytica* infection status (positive/negative), and FOB test result (positive/negative). Age was treated as a continuous variable, while infection status, gender, and FOB were treated as categorical variables.

Statistical Analysis

Data were analyzed using R statistical software. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were calculated. The chi-square (χ^2) test was used to examine associations between categorical variables, while independent-sample t-tests were applied for comparisons involving continuous variables such as age. Statistical significance was set at $p < 0.05$.

Results

Among the 238 stool samples analyzed, 55 (23.1%) were positive for *E. histolytica,* while 183 (76.9%) were negative. The mean age of participants was 36.67 ± 16.57 years, with a range of 4 to 88 years. Females constituted 62.6% (149/238) of the sample, and males 37.4% (89/238). The prevalence of *E. histolytica* did not differ significantly between males and females (χ^2=0.002, p=0.983), as (Figure 1) illustrates. Likewise, (Figure 2) shows that there was no significant relationship between gender and the presence of occult blood (χ^2 =0.002, p=0.99).

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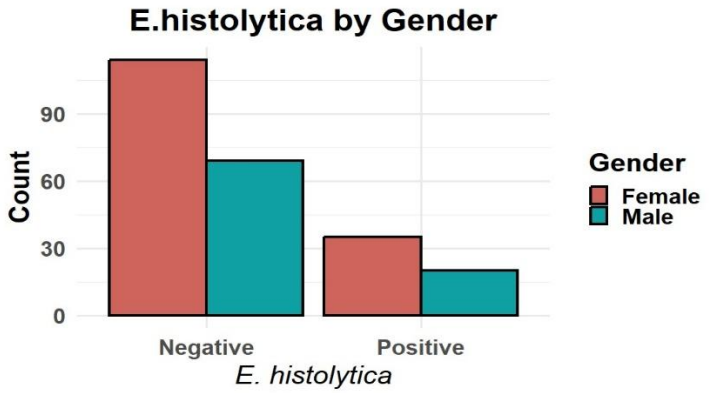

Figure 1. The grouped bar graph illustrates that E. histolytica did not differ significantly between males and females.

A significant association was found between *E. histolytica* infection and the presence of occult blood in stool (χ^2 =21.9, p=0.0000193), indicating that patients infected with *E. histolytica* were much more likely to test positive for FOB, as (Figure 3) shows.

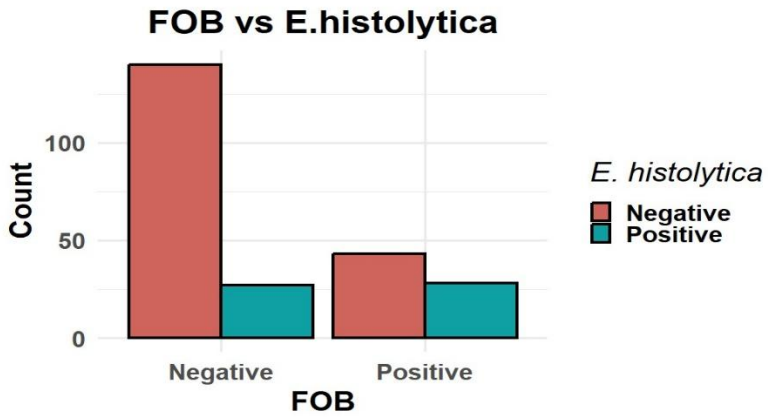

Figure 2. The grouped bar graph illustrates that there was no significant relationship between gender and the presence of occult blood

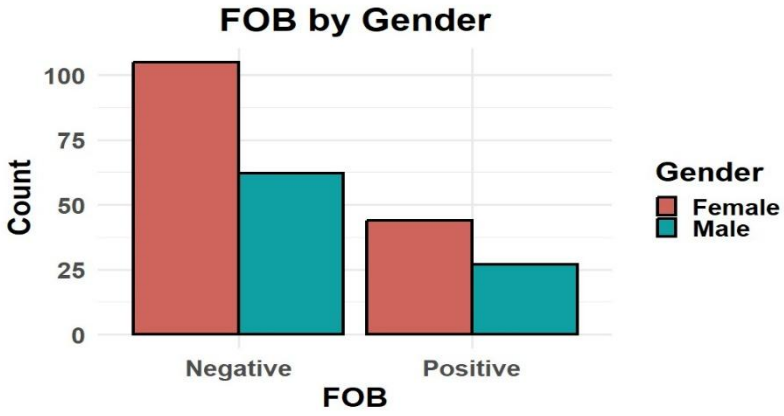

Figure 3. A grouped bar graph illustrates that there was A significant association found between E. histolytica infection and the presence of occult blood in stool

Age did not show a statistically significant difference between those infected and uninfected with *E. histolytica* (t =1.05, p=0.296) as figure (4) shows, nor between those with positive and negative FOB results (t =0.42, p=0.673). These

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findings indicate that while *E. histolytica* infection is relatively common in Al-Bayda; its distribution is not influenced by gender or age but is strongly associated with intestinal bleeding detected through FOB testing.

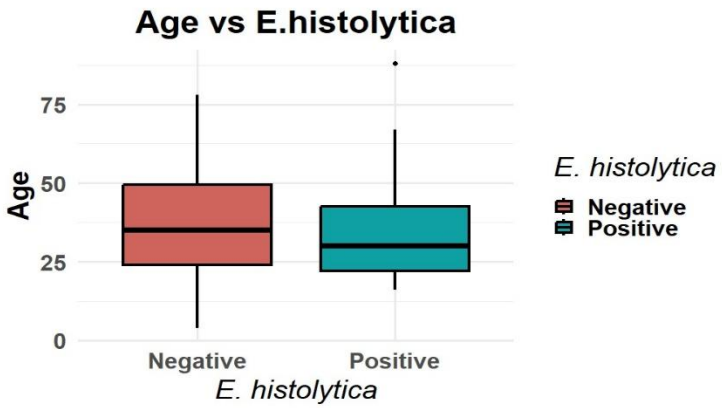

Figure 4. Grouped box plot illustrates that age did not show a statistically significant difference between those infected and uninfected with E. histolytica

Discussion

The 23.1% prevalence of *E. histolytica* observed in this study aligns with reports from other developing regions, where infection rates range between 15% and 30% [5,7]. This suggests that amoebiasis remains a public health concern in Libya, particularly in areas where sanitation infrastructure is limited. The absence of gender differences in infection rates (p=0.983) agrees with findings from India and Argentina, where no significant sex-related variation was reported by Singh [6] and Candela [8]. This consistency indicates that both men and women in endemic settings are equally exposed, likely due to shared environmental and behavioral risk factors such as contaminated water sources and food handling practices. In contrast, the strong correlation between *E. histolytica* and occult blood in stool (p<0.001) underscores the parasite's pathogenic potential. The presence of blood indicates mucosal invasion, consistent with *E. histolytic a's* ability to lyse host tissues through proteolytic enzymes and cytotoxic lectins [3]. This result is consistent with the observations of Kantor [1] and Ortiz [9], who reported that mucosal damage and inflammatory response are hallmarks of symptomatic amoebiasis.

Although molecular techniques can better distinguish between pathogenic *E. histolytica* and the non-pathogenic E. dispar, microscopy remains the most accessible diagnostic tool in developing countries [10,11]. The reliance on microscopy in this study, while practical, may slightly overestimate true infection prevalence since morphological similarities can lead to misidentification [12]. However, the significant association with FOB positivity strengthens the argument that most detected infections were indeed pathogenic. Our findings also highlight the importance of co-detection of occult blood as a clinical indicator. Patients with FOB-positive results should be evaluated for *E. histolytica*, as bleeding could reflect active invasion [13]. The pathophysiology involves trophozoite-mediated epithelial disruption, inflammation, and potential ulcer formation in the colon [14]. The lack of association between age and infection (p=0.296) suggests widespread exposure across all age groups, as reported in Ethiopia and Ghana [5,15]. Environmental contamination and communal water use likely play larger roles than age-related immunity or behavior.

Therapeutically, the persistence of infection highlights the need for preventive measures and novel anti-amoebic agents. Recent research has explored natural compounds such as p-coumaric acid for anti-amoebic activity [16], and the potential modulation of parasite virulence through gut microbiota interactions [3]. In the Libyan context, where municipal sanitation systems are often interrupted, surveillance and community-based education are essential. Integrating molecular diagnostic tools could also refine epidemiological data, as recommended by global studies [2].

Overall, the results emphasize that *E. histolytica* infection remains prevalent in Al-Bayda, with evidence of pathogenic activity reflected in FOB positivity. The findings underscore the urgent need for improved hygiene, diagnostic capacity, and targeted public health interventions to reduce transmission.

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Conclusion

This study demonstrated a 23.1% prevalence of *E. histolytica* among patients in Al-Bayda City, Libya. Infection was independent of gender and age but significantly associated with the presence of occult blood in stool, suggesting active intestinal invasion. Continued surveillance, improved sanitation, and molecular confirmation of *E. histolytica* are crucial to reduce misdiagnosis and prevent severe complications. Public health strategies in Libya should prioritize sanitation infrastructure, health education, and routine screening for intestinal parasites. These measures could substantially reduce the burden of amebiasis and other protozoan infections in the region.

Conflict of interest. Nil

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