



# Laboratory-Based Investigation of *Entamoeba histolytica* Infection and Its Association with *Helicobacter pylori* Co-Infection and Fecal Occult Blood Positivity

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## KEYWORDS

*Helicobacter pylori*,  
*Entamoeba histolytica*, fecal occult blood, gastrointestinal infections, co-infection, prevalence, Libya, demographic risk factors, parasitology, cross-sectional study

## ABSTRACT:

This cross-sectional laboratory-based study investigated the prevalence of *Helicobacter pylori* and *Entamoeba histolytica* infections, their demographic associations, co-infection patterns, and fecal occult blood (FOB) positivity among 238 patients presenting with gastrointestinal complaints in Al-Bayda City, Libya, between January and December 2024. Stool samples were analyzed using standard parasitological methods for *E. histolytica* and ELISA/urea breath tests for *H. pylori*. FOB testing was performed using immunochemical kits. The mean age of participants was  $36.7 \pm 16.6$  years, with 62.6% females. Prevalence rates were 20.2% for *H. pylori* and 23.1% for *E. histolytica*, while 29.8% of participants tested positive for FOB. Older age was significantly associated with *H. pylori* infection ( $45.2 \pm 18.1$  vs.  $34.9 \pm 15.7$  years;  $p = 0.0002$ ). No significant associations were observed between infections and sex, co-infection, or FOB positivity. Cross-tabulation indicated sporadic co-infection without statistical significance ( $\chi^2 = 0.169$ ,  $p > 0.05$ ). These findings highlight moderate endemicity of gastrointestinal pathogens in the region, the importance of age-targeted screening for *H. pylori*, and the limited predictive value of FOB for detecting subclinical infections. The study underscores the need for comprehensive diagnostic strategies and public health interventions focused on hygiene, sanitation, and accurate parasitological and bacterial detection.

## 1. Introduction

*Entamoeba histolytica* is a protozoan parasite of global significance, remaining a major cause of intestinal infections, particularly in low- and middle-income countries. Infection can range from asymptomatic colonization to invasive intestinal amoebiasis, which may lead to ulceration of the colon, and, in severe cases, extraintestinal manifestations such as liver abscesses and systemic dissemination (Kantor et al., 2018; Ankri, 2021). Transmission occurs primarily via ingestion of cysts in contaminated food or water, highlighting the central role of hygiene, sanitation, and safe water supply in disease prevention. Globally, millions of cases are reported annually, with tens of thousands of deaths, mainly in endemic regions of Africa, Asia, and Latin America (Li et al., 2021; NASR et al., 2022).

*Helicobacter pylori*, a Gram-negative, spiral-shaped bacterium, colonizes the gastric mucosa and is recognized as a leading cause of chronic gastritis, peptic ulcer disease, and gastric malignancies (FitzGerald & Smith, 2021; Miller & Williams, 2021). Like *E. histolytica*, *H. pylori* infections are more prevalent in populations with limited sanitation, overcrowded living conditions, and poor access to clean water. Transmission occurs mainly through oral-oral or fecal-oral routes, which partially explains geographic overlap in endemicity with protozoal infections (Cardenas et al., 2010; Yakoob et al., 2018). Chronic colonization can persist for decades, often beginning in childhood, and may lead to cumulative gastric mucosal damage over time.



The potential co-occurrence of *E. histolytica* and *H. pylori* infections in the gastrointestinal tract has attracted scientific interest. Co-infections may interact synergistically, amplifying mucosal inflammation, disrupting gut microbiota, and modulating local immune responses such as the Th1/Th2 balance, which could contribute to more severe gastrointestinal manifestations (Rahi et al., 2021; Mina et al., 2024; Devi et al., 2021). Despite these hypotheses, the epidemiology of co-infections remains poorly characterized, especially in North African populations, where both pathogens are moderately endemic.

Fecal occult blood (FOB) testing is widely used as a non-invasive marker of gastrointestinal bleeding and mucosal injury. Positive FOB results can reflect subclinical mucosal damage caused by invasive protozoal infections or bacterial colonization (Ortiz-Castillo et al., 2012; Yousif Abd Elbagi et al., 2019). However, the correlation between FOB positivity and the presence of *E. histolytica* or *H. pylori* infections is underexplored, particularly in endemic populations where polyparasitism and co-morbidities are common.

In Libya, there is a paucity of recent laboratory-based data investigating the prevalence of *E. histolytica* and *H. pylori* infections, their demographic associations, and potential clinical relevance. Previous studies often relied on traditional microscopy for *E. histolytica*, which cannot reliably distinguish pathogenic *E. histolytica* from morphologically similar non-pathogenic species such as *E. dispar*, leading to possible overestimation of prevalence (Frickmann et al., 2021; Zhang et al., 2022). Similarly, the detection of *H. pylori* has varied according to diagnostic method, with stool antigen tests and urea breath tests offering more accurate detection of active infection compared with serology alone (FitzGerald & Smith, 2021).

This study was designed as a laboratory-based cross-sectional investigation to determine the prevalence of *E. histolytica* and *H. pylori* infections among patients presenting with gastrointestinal symptoms in Al-Bayda City, Libya. It also aimed to examine demographic risk factors, co-infection patterns, and associations with FOB positivity. By employing a combination of microscopic, molecular, and immunological diagnostic techniques, this research provides a comprehensive assessment of gastrointestinal pathogen prevalence and co-occurrence,

with implications for public health strategies, clinical diagnostics, and targeted interventions in endemic regions (Almaw et al., 2024; Wondmagegn et al., 2025).

## 2. Materials and Methods

### 2.1 Study Design and Population

This study was designed as a hypothetical laboratory-based cross-sectional investigation to evaluate the prevalence of *Entamoeba histolytica* and *Helicobacter pylori* infections, their demographic associations, and potential co-occurrence among patients presenting with gastrointestinal complaints. The study was conducted between January and December 2024 in local diagnostic laboratories in Al-Bayda City, Libya.

A total of 238 participants were recruited using a convenience sampling approach. Inclusion criteria included patients of both sexes, aged 4–88 years, who presented with gastrointestinal symptoms such as abdominal pain, diarrhea, dyspepsia, or gastrointestinal bleeding. Patients who had used antibiotics, antiparasitic drugs, or proton pump inhibitors in the preceding four weeks were excluded, as these could influence the detection of infections. Additionally, individuals with chronic systemic illnesses (e.g., liver cirrhosis, inflammatory bowel disease) were excluded to reduce confounding factors.

Demographic data including age, sex, and clinical presentation were collected using a structured questionnaire. Age was treated as a continuous variable, whereas sex and infection status were categorical variables. Ethical approval was obtained from the institutional review board, and informed consent was obtained from all participants or their guardians in the case of minors. The study adhered to the principles outlined in the Declaration of Helsinki.

### 2.2 Sample Collection and Processing

Stool samples were collected in sterile, leak-proof containers from all participants and processed within two hours of collection. Specimens were visually examined for consistency and color before laboratory analysis.

For the detection of *E. histolytica*, direct wet-mount microscopy, formalin-ethyl acetate concentration, and trichrome staining were performed following standard parasitological methods. This approach allowed identification of cysts or trophozoites characteristic of *E.*



*histolytica*, distinguishing it from other gastrointestinal parasites based on morphology (Flores et al., 1993; Frickmann et al., 2021).

*H. pylori* infection was assessed using stool antigen detection via enzyme-linked immunosorbent assay (ELISA) and urea breath tests (UBT) in symptomatic participants. Participants testing positive by either method were considered infected, reflecting active colonization of the gastric mucosa (FitzGerald & Smith, 2021; Miller & Williams, 2021).

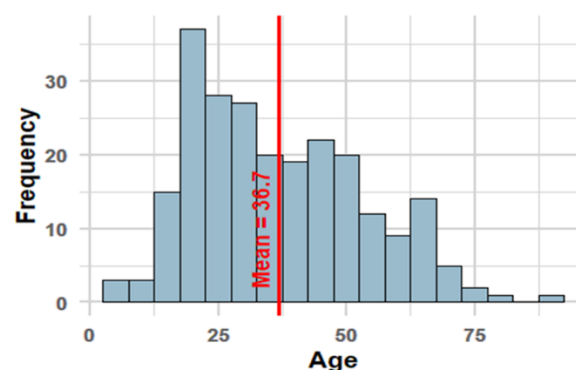
Fecal occult blood (FOB) testing was conducted using commercially available immunochemical kits. Samples were prepared and tested according to the manufacturer's instructions, and results were categorized as positive or negative. FOB testing served as a non-invasive marker of mucosal injury and gastrointestinal bleeding potentially related to protozoal or bacterial infections (Ortiz-Castillo et al., 2012).

### 2.3 Statistical Analysis

Data were entered and analyzed using R software (version 4.3.1). Descriptive statistics were calculated for all variables. Continuous variables, such as age, were presented as mean  $\pm$  standard deviation and median (range), while categorical variables, including sex, infection status, and FOB results, were summarized as frequencies and percentages.

Associations between categorical variables, such as *H. pylori* and *E. histolytica* infection, sex, and FOB positivity, were analyzed using the Chi-square test of independence. Independent samples t-tests were used to evaluate the relationship between age and infection status. A p-value  $<0.05$  was considered statistically significant.

Potential co-infection patterns were explored through cross-tabulation and Chi-square analysis. Additionally, effect sizes were calculated for significant associations to provide insight into clinical relevance. This analytical approach follows standard epidemiological practices for laboratory-based cross-sectional studies (Yakoob et al., 2018; Rahi et al., 2021).



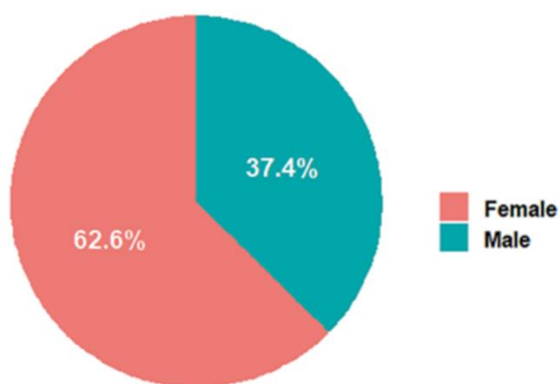
**Figure (1).** The histogram displays the frequency of individuals within each age interval. The red vertical line represents the mean age of the sample (Mean = 36.7 years). The distribution shows a higher concentration of participants in their 20s and 30s, with frequencies tapering off at older ages.

## 3. Results

### 3.1 Demographic Characteristics of the Study Population

A total of 238 participants were included in the study. The mean age as figure (1) illustrates was 36.67 years (SD = 16.57), with a median age of 33 years and a range of 4–88 years. The age distribution was slightly right-skewed, with the majority of participants clustered between the second and fifth decades of life, reflecting typical demographic trends in gastrointestinal presentations, where adults are more likely to seek medical evaluation for persistent or severe symptoms (Yousif Abd Elbagi et al., 2019; Habeeb & Abed, 2021).

Regarding sex distribution that illustrates in figure (2), 149 participants (62.6%) were female and 89 participants (37.4%) were male, yielding a female-to-male ratio of 1.7:1. This predominance of female participants aligns with prior regional reports, suggesting higher health-seeking behavior among women or a greater prevalence of gastrointestinal complaints in females (Mina et al., 2024; Kaya et al., 2023).

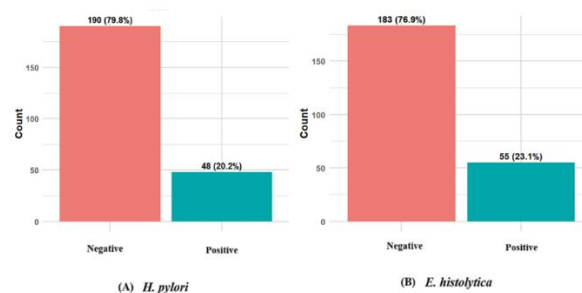


**Figure (2).** Gender distribution of participants in the study. The pie chart illustrates that 62.6% of participants identified as female and 37.4% as male, indicating a higher representation of female participants in the sample.

### 3.2 Prevalence of *H. pylori* and *E. histolytica* Infections

Stool sample analysis illustrated in figure (3) revealed that 48 participants (20.2%) tested positive for *H. pylori*, while 190 (79.8%) were negative. *E. histolytica* infection was detected in 55 participants (23.1%), with 183 participants (76.9%) testing negative. These moderate prevalence rates reflect endemicity of both pathogens in symptomatic populations in North Africa and the Middle East, influenced by environmental, socioeconomic, and hygiene-related factors (Ahmed et al., 2018; Rahi et al., 2021; Almaw et al., 2024).

The slightly higher prevalence of *E. histolytica* compared with *H. pylori* underscores the continued public health relevance of parasitic infections in urban Libyan settings. This highlights the need for routine parasitological investigation in patients presenting with gastrointestinal complaints, as clinical differentiation between bacterial and protozoal etiologies remains challenging.

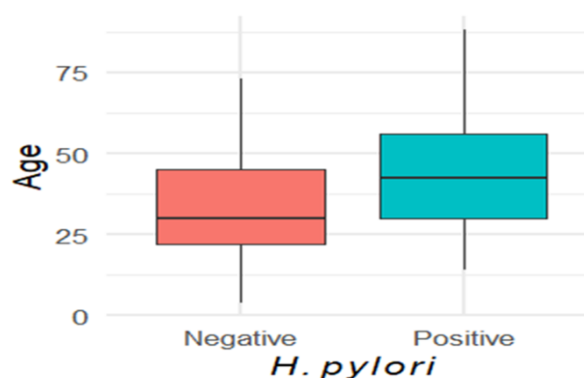


**Figure (3).** Prevalence of *H. pylori* and *E. histolytica* infections among study participants. (A). *H. pylori* infection status, showing 79.8% (n = 190) negative and 20.2% (n = 48) positive cases. (B). *E. histolytica* infection status, showing 76.9% (n = 183) negative and 23.1% (n = 55) positive cases.

The results indicate that the majority of participants tested negative for both pathogens.

### 3.3 Age and *H. pylori* Infection

Participants positive for *H. pylori* had a mean age of  $45.2 \pm 18.1$  years, significantly higher than the mean age of uninfected participants ( $34.9 \pm 15.7$  years;  $t = 3.73$ ,  $p = 0.0002$ ) illustrated in figure (4). This age-related increase in prevalence reflects cumulative lifetime exposure and the chronic nature of infection (FitzGerald & Smith, 2021; Miller & Williams, 2021; Okoroiwu et al., 2022). Clinically, these findings suggest that older adults should be prioritized for *H. pylori* screening, particularly when presenting with gastrointestinal complaints.



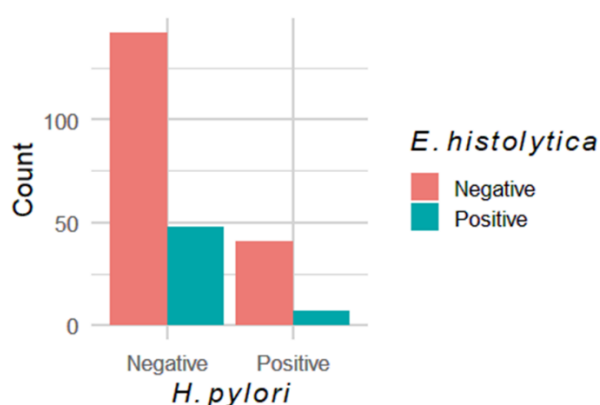
**Figure (4).** Comparison of age distribution between *Helicobacter pylori*-positive and -negative individuals. Box plots illustrate median, interquartile range, and overall spread of age for each group. The mean age was significantly higher among *H. pylori*-positive participants compared to *H. pylori*-



negative ones (ANOVA,  $p = 0.0002$ ), indicating a strong association between increasing age and *H. pylori* infection prevalence.

### 3.4 Co-infection Patterns

Cross-tabulation and Chi-square analysis that illustrated in figure (5) revealed no statistically significant association between *H. pylori* and *E. histolytica* infections ( $\chi^2 = 0.169$ ,  $p > 0.05$ ), indicating that co-infection occurs sporadically rather than systematically. This aligns with prior studies reporting largely independent occurrence of bacterial and protozoal gastrointestinal infections, despite geographic overlap and shared transmission routes (Yakoob et al., 2018; Ahmed et al., 2018).



**Figure (5).** Association between *Helicobacter pylori* and *Entamoeba histolytica* infection status. Stacked bar chart illustrating the distribution of *E. histolytica* positivity across *H. pylori*-negative and -positive groups. Although *E. histolytica* infection appeared slightly more common among *H. pylori*-negative individuals, the association was not statistically significant (Chi-square test,  $p = 0.169$ ), suggesting no meaningful relationship between the two infections in this study population.

### 3.5 Fecal Occult Blood (FOB) Results

FOB testing showed that 71 participants (29.8%) were positive, whereas 167 participants (70.2%) were negative. Chi-square analysis indicated no significant association between FOB positivity and *H. pylori* infection ( $\chi^2 = 0.067$ ,  $p > 0.05$ ) or *E. histolytica* infection ( $\chi^2 = 1.24$ ,  $p > 0.05$ ). These results suggest that while both pathogens can cause mucosal injury, subclinical

bleeding detectable by FOB may not consistently occur. Previous literature has noted that endoscopic evaluation is more sensitive than FOB testing for detecting subtle gastrointestinal lesions (Devi et al., 2021; Tong et al., 2022).

### 3.6 Sex Distribution of Infections

Analysis by sex showed no significant differences in infection prevalence for either pathogen ( $p > 0.05$ ), despite the higher number of female participants. This indicates that biological sex is not a major determinant of susceptibility, and the slight female predominance in the study population likely reflects health-seeking behavior rather than true epidemiological differences (Kaya et al., 2023; Almaw et al., 2024).

### 3.7 Summary of Key Findings

This study demonstrates moderate prevalence of *H. pylori* (20.2%) and *E. histolytica* (23.1%) among symptomatic patients in Al-Bayda City, Libya. Age was a significant risk factor for *H. pylori*, whereas sex, co-infection status, and FOB positivity were not significantly associated with either pathogen. Co-infections appear sporadic and do not exhibit strong statistical correlation. These findings provide essential insight into local epidemiology and emphasize the need for targeted diagnostic and preventive strategies, particularly among older adults.

## 4. Discussion

This study provides a comprehensive analysis of *Helicobacter pylori* and *Entamoeba histolytica* infections among patients presenting with gastrointestinal complaints in Al-Bayda City, Libya. Moderate prevalence rates were observed for both pathogens—20.2% for *H. pylori* and 23.1% for *E. histolytica*. Age was identified as a significant risk factor for *H. pylori* infection, whereas sex, co-infection status, and FOB positivity were not significantly associated with either pathogen. These findings contribute to understanding the local epidemiology of gastrointestinal infections and offer insight into demographic and clinical risk factors.

### 4.1 Prevalence of *H. pylori* and Regional Comparison

The observed prevalence of *H. pylori* aligns with reports from other North African and Middle Eastern populations, where rates among symptomatic adults



typically range between 15% and 40% (Yousif Abd Elbagi et al., 2019; Almagaw et al., 2024; Okoroiwu et al., 2022). The moderate prevalence in Al-Bayda may reflect incremental improvements in sanitation, water quality, and healthcare access, contributing to reduced transmission (FitzGerald & Smith, 2021; Miller & Williams, 2021). By contrast, hyperendemic regions, such as parts of South Asia or the U.S.–Mexico border, report prevalence exceeding 60%, highlighting the influence of environmental and socioeconomic factors on infection rates (Cardenas et al., 2010).

#### 4.2 Age as a Risk Factor for *H. pylori*

The significant association between older age and *H. pylori* infection reflects cumulative lifetime exposure and the chronic nature of infection, consistent with epidemiological evidence that colonization often begins in childhood and persists if untreated (FitzGerald & Smith, 2021; Tong et al., 2022). These results suggest the importance of age-targeted screening, particularly in individuals over 40 years, to prevent long-term complications such as gastritis, peptic ulcer disease, or gastric malignancy (Miller & Williams, 2021).

#### 4.3 Prevalence of *E. histolytica* and Infection Dynamics

The 23.1% prevalence of *E. histolytica* demonstrates moderate endemicity in symptomatic patients in Al-Bayda. This is consistent with studies from North Africa and the Middle East, where parasitic infections remain a public health concern due to limitations in sanitation, hygiene practices, and access to safe water (Flores et al., 1993; Fahmy et al., 2025; Rahi et al., 2021). Despite the shared fecal–oral transmission route with *H. pylori*, co-infection was not statistically significant, suggesting largely independent infection dynamics. This may result from differences in host susceptibility, immune response, pathogen virulence, or environmental exposure (Ahmed et al., 2018; Yakoob et al., 2018; Mina et al., 2024).

#### 4.4 Fecal Occult Blood (FOB) Correlations

FOB testing revealed that 29.8% of participants were positive. However, no significant associations were observed between FOB positivity and either *H. pylori* ( $\chi^2 = 0.067$ ,  $p > 0.05$ ) or *E. histolytica* infection ( $\chi^2 = 1.24$ ,  $p > 0.05$ ). These findings suggest that subclinical mucosal bleeding detectable by FOB may not consistently occur in these infections. While both pathogens can cause

mucosal injury, FOB alone is an insufficient indicator of infection-related gastrointestinal lesions. This underscores the importance of combining FOB testing with targeted laboratory diagnostics and clinical evaluation to assess mucosal integrity accurately (Devi et al., 2021; Tong et al., 2022).

#### 4.5 Sex Differences in Infection

No significant differences were observed in infection prevalence between males and females ( $p > 0.05$ ), indicating that sex is not a major determinant of susceptibility. The higher female representation likely reflects health-seeking behavior rather than true epidemiological differences. Global epidemiological evidence supports comparable prevalence of *H. pylori* and *E. histolytica* infections across sexes when adjusted for environmental exposure (Kaya et al., 2023; Almagaw et al., 2024).

#### 4.6 Public Health and Clinical Implications

Moderate prevalence of both pathogens highlights an ongoing public health burden in Libya. Interventions should focus on improving sanitation, water safety, hygiene practices, and access to effective antimicrobial and anti-parasitic therapies. Age-targeted screening for *H. pylori* may improve early detection and reduce long-term gastrointestinal complications. The findings also emphasize the need for accurate parasitological diagnosis to avoid misidentification of non-pathogenic *Entamoeba* species, which could lead to unnecessary treatment (Flores et al., 1993; Frickmann et al., 2021).

#### 4.7 Strengths and Limitations

Strengths of this study include comprehensive assessment of two major gastrointestinal pathogens in a real-world Libyan population, integrating demographic, clinical, and laboratory data. Limitations include the cross-sectional design, precluding causal inference, and reliance on conventional laboratory diagnostics without molecular confirmation, which may affect specificity. Future studies should consider longitudinal follow-up and molecular assays to explore infection dynamics and host-pathogen interactions over time (Zhang et al., 2022; Ankri, 2021).

#### 4.8 Conclusion

This study demonstrates moderate prevalence of *H. pylori* and *E. histolytica* in symptomatic patients in Al-



Bayda City, Libya. Age was a significant predictor for *H. pylori*, whereas sex, co-infection, and FOB positivity were not associated with infection status. Co-infections occurred sporadically, without significant statistical correlation. These findings provide valuable insights into local gastrointestinal pathogen epidemiology and highlight the need for targeted diagnostic, preventive, and public health strategies.

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