EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
E
MEDICAL ENTOMOLOGY & PARASITOLOGY

ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 17 No. 2 (2025)

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.17(2) pp **109-115**(2025)*
DOI: 10.21608/EAJBSE.2025.456773

Egypt. Acad. J. Biology. Sci., 17 (2):109– 115 (2025)

Egyptian Academic Journal of Biological Sciences
E. Medical Entomology & Parasitology

ISSN: 2090–0783
www.eajbse.journals.ekb.eg

Comparative Evaluation of Diagnostic Methods for *Entamoeba species* Detection in Zakho, Kurdistan Region of Iraq

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ARTICLE INFO
Article History
Received:26/8/2025
Accepted:3/10/2025
Available:7/10/2025

Keywords:
Entamoeba histolytica, wet mount, zinc sulfate flotation, ELISA.

ABSTRACT

Objective: In this study, we compared the effectiveness of three detection methods: direct wet mount microscopy, zinc sulfate floatation, and enzyme-linked immunosorbent assay (ELISA) to detect *Entamoeba*. *histolytica* among inhabitants of Zakho city, Kurdistan Region of Iraq. **Methods:**The collection of faeces was carried out between September 2024 and May 2025 (615 samples, 515 symptomatic patients, and 100 asymptomatic patients). Direct wet mount microscopy, zinc sulfate flotation, and ELISA target *E. histolytica*-specific antigen were used to examine each specimen. **Results:**Wet mount revealed 105 positive results (17.07%), whereas zinc sulfate flotation was able to detect significant number of positive cases 149 of them (24.22%, $p < 0.001$). Only 35 cases (5.69%) were positive using *E. histolytica*-specific antigen detected by ELISA, which is more specific and can rule out deliberately nonpathogenic species that otherwise resemble pathogens morphologically, such as *Entamoeba dispar* and *E. moshkovskii*. The prevalence of infection was the highest in children between the ages of 1 to 10 years with the absence of any significant differentiation between the genders. Socioeconomic factors, in general, and low level of education and low income were found to be significantly associated with the infection rates. **Conclusion:** Wet mount microscopy is not very sensitive, and zinc sulfate flotation lacks specificity, although it improves sensitivity. ELISA gives valid validation of pathogenic *E. histolytica*. A combination of flotation and ELISA should be adopted to enhance better accuracy of diagnosis, prevent overtreatment and provide better and reliable epidemiological data in endemic settings.

INTRODUCTION

Intestinal parasitic infections are a major worldwide complication, especially in developing countries where conditions such as poor sanitation, overcrowding and inadequate access to clean water are contributing factors to the high transmission rates. Among the most important of them is amoebiasis which is caused by *Entamoeba histolytica*. It is estimated that the extent of the infections resulting in the death of nearly 100 000 per year is around 50 million people, making amoebiasis the third death leading parasitic disease besides malaria and schistosome infections to which deaths occur on the scale of 100 000 per year (Jannati *et al.,* 2024 & Kantor *et al*., 2018). The means of transmission mainly lies in ingestion of cysts of *E. histolytica* in contaminated food, water, or even in the hands, and this is directly connected with the economic and environmental background conditions (Abbas *et al*., 2023, Rawat *et al*., 2020).

The clinical manifestation of amoebiasis varies in severity between asymptomatic intestinal carriage and invasive intestinal disease (dysentery, colitis) and extraintestinal complications (amoebic liver abscesses). The presentation also varies, which makes early detection complicated, and reliable diagnostic methods are needed. Conventionally direct wet mount stool microscopy has remained the most important diagnostic tool in endemic areas. Although cheap and fast, wet mount microscopy has its weaknesses. Its sensitivity is low, and detection relies on the operator, and most importantly cannot differentiate between pathogenic *E. histolytica* and morphologically identical but nonpathogenic *E. dispar and E. moshkovskii* (Servián *et al*., 2024 & Calle-Pacheco *et al*., 2022). This leads to a high rate of false positive diagnosis of pathogenic infection and unnecessary application of anti-amoebic treatment.

A complication to diagnosis is the fact that the nonpathogenic *E. dispar and E. moshkovskii* are morphologically similar to *E. histolytica*. These species cannot be distinguished using traditional microscopy, which remains the most easily accessible method of diagnosis and therefore results in overreporting of pathogenic infections (Hasan *et al*., 2023 & Paul *et al*., 2023). Direct wet mount stool microscopy is the diagnostic gold standard in endemic countries that has been in use in decades. Sensitivity is low with it and it is operator-dependent (Barwari and Ismael, 2011). Zinc sulfate flotation, a concentration technique, enhances detection of cysts and trophozoites by lowering fecal debris and higher parasite recovery hence giving more sensitivity compared to direct wet alone (Jannati *et al.,* 2024).

Though microscopy will continue to be crucial even in resource-poor areas, it suffers limitations that require more specific diagnostic techniques to be introduced. Immunological tests, mostly enzyme linked immunosorbent assays (ELISAs) have been devised to identify some rather specific parasite antigens in stool samples. These assays provide greater specificity to *E. histolytica* and can differentiate it with other morphologically similar *Entamoeba* species (Ohanu *et al*., 2022 & Bayoumy *et al*., 2019). This has been confirmed by various studies that have shown that the detection methods of the antigens are better than microscopy in symptomatic and asymptomatic people since it lowers the false positives caused by the non-pathogenic *Entamoeba* spp. (Abbas *et al*., 2023). Moreover, ELISA-based stool antigen tests would be useful in large-scale epidemiological surveillance, which makes it highly useful in monitoring and controlling the health of the population.

Amoebiasis is a common intestinal infection in Iraq, and its prevalence is inconsistent throughout the country and demographic groups. In Duhok, Erbil, Kirkuk, and other governorates in Iraq, the studies reported prevalence rates between 12 and 43% (depending upon the type of diagnostic techniques utilized and population examined (Chalabi, 2024 & Servián *et al*., 2024 & Al Saqur *et al*., 2017 & Barwari and Ismael, 2011). *Entamoeba histolytica* has been reported as a frequent pathogen in both children and adults with diarrhea in previous studies in Kurdistan (Obaid, 2022 & Bayoumy *et al*., 2019). Few included immunological testing, like ELISAs, meaning there was a gap in the epidemiological gap of actual pathogenic infection.

MATERIALS AND METHODS

Study Design and Population:

This was a cross-sectional study in Zakho city, Kurdistan Region, Iraq that was carried out throughout September 2024 to May 2025. A total of 615 stool samples were received: 515 were symptomatic patients with diarrhea, abdominal pain, or gastrointestinal complaints in Zakho General Teaching Hospital or in private laboratories, and 100 volunteers who were asymptomatic. The age of the participants was 1-60 years and was of both sexes.

Sample Collection:

Fresh stool samples were collected in sterile screw-capped containers and divided into three portions. One gram was used immediately for direct wet mount microscopy, another gram was processed by the zinc sulfate flotation technique, and the remaining gram was stored at –20 °C until testing by ELISA.

Direct Wet Mount Microscopy:

Each sample was prepared as Saline, Lugol,s iodine and Giemsa stain preparations. The cyst, trophozoites, red blood cells, and mucus were observed using: ×10 and ×40 objectives in slides. Giemsa stain enhanced the observation of nuclear and cytoplasmic features.

Zinc Sulfate Flotation:

Approximately 1 g of stool was homogenized with 10 mL of saline, filtered, and centrifuged at 3,000 rpm for 2 minutes. Sediment was suspended in zinc sulfate solution (specific gravity 1.18) and centrifuged again. The surface layer was taken and treated microscopically at x4o to search for cysts and trophozoites.

ELISA Antigen Detection:

Stool supernatants were prepared (1:10 diluted in PBS). The samples were then tested by an ELISA commercial kit (SunLong Biotech), which is specific to *E. histolytica* lectin antigen. Plates were incubated with HRP conjugate reagent then washed and then developed with chromogen A/B and then stopped solution. Absorbances were measured on 450 nm. Samples whose OD value exceeded the cut off were assumed to be positive.

Statistical Analysis:

Analysis of data was done using SPSS v25 the prevalence rates were cross checked amongst the diagnostic methods using the Chi square test. A p-value of <0.05 level was regarded as statistically significant.

RESULTS

Overall Prevalence by Diagnostic Method:

A total of 615 stool specimens were tested with the three diagnostic methods of examination (wet mount microscopy, zinc sulfate flotation, and ELISA). Among these, 149 (24.22%), 105 (17.07%), and 35 (5.69%), respectively, were positive by zinc sulfate flotation, wet mount microscopy, and ELISA (Table 1). The sensitivity of zinc sulfate flotation provides significantly higher levels of detection when compared with wet mount microscopy ($p < 0.001$). On the other hand, ELISA showed a lesser rate, which translates to its high level of specificity regarding *E. histolytica* antigen.

Table 1. Comparison of diagnostic methods.

Method	Symptomatic (n=515)	Asymptomatic (n=100)	Total (n=615)	Positive %
Wet mount microscopy	92 (17.86%)	13 (13.0%)	105	17.07%
Zinc sulfate flotation	127 (24.66%)	22 (22.0%)	149	24.22%
ELISA antigen test	26 (5.04%)	9 (9.0%)	35	5.69%

Symptomatic vs. Asymptomatic Individuals:

Of the symptomatic patients (n=515), zinc sulfate flotation methods identified 24.66% of the positive cases, whereas the percentage was 17.86% and 5.04% for wet mount, and ELISA respectively. Among asymptomatic individuals (n=100), sensitivity was 22 % by flotation, 13 % by wet mount, and 9 % by ELISA. These findings explain that asymptomatic carriers could not be devoid of *Entamoeba* spp. but considerably fewer of them would be true *E. histolytica* infection based on ELISA.

Age and Gender Distribution:

Of the 127 symptomatic positive individuals, there was the peak infection in children aged 1-10 years old; almost 3 out of 10 women and men were infected (Table

2). The prevalence declined with age with the lowest positivity among the patients aged above 50 years. Chi-square tests showed that age was highly associated with infection ($p < 0.001$) but the gender was not associated with infection ($p = 0.935$).

Table 2. Distribution of amoebiasis by age and gender (symptomatic group)

Age (years)	Males positive (%)	Females positive (%)	Total positive (%)
1–10	28.35	29.92	29.1
11–20	13.38	14.17	13.8
21–30	4.72	3.15	3.9
31–40	0.79	1.57	1.2
41–50	0	0.79	0.4
51–60	0.79	0.79	0.8
>60	1.57	0	0.9

Seasonal and Monthly Variation:

Each month of analyzing symptomatic patients showed a distinct seasonal variation. The most favorable prevalence was higher in April 2025 (26.8%) because of warmer weather conditions, and the least favorable were in February 2025 (3.1%). There were also gender specific differences on incidence levels of infections monthly where by females were more infected in October 2024 than on the November of the same year when only male infections were reported. The analysis of variance proved that there was a significant relationship between infection rate and the month ($p = 0.028$).

Socioeconomic and Educational Factors:

Prevalence was greatly linked to lack of education and lack of income. In symptomatic patients, 61.4% of the infections were found in patients with pre- or primary school level of education, whereas 19.7% had high school level and 18.9% had college level ($p < 0.001$). Likewise, 66.9% of infections were experienced by the low-income segment, against 30.7% by the moderate-income and 2.3% by high-income people ($p = 0.033$). Family size and residence in urban or rural areas were not statistically significant associations.

Clinical Symptoms:

Watery diarrhea (88.2%) was the most frequent positive symptomatic presentation followed by fever (74.8%), vomiting (68.5%), abdominal pain (22.8%) and dysentery (11.8%). There was no statistically significant symptom distribution in males compared to those of females ($p = 0.595$).

DISCUSSION

The current study has served as a comparative analysis of three approaches to the diagnosis of amoebiasis and the analysis should show crucial implications to the epidemiology of *Entamoeba* infection in Zakho. The results are clear in that outcomes of diagnoses using different methods differ significantly, and this makes a lot of difference both in the clinical world and in the surveillance system of society.

Direct wet mount microscopy showed a poor sensitivity of 17%, which is associated with lack of sensitivity and dependence on the person examining. Wet mount is not adequate to make a correct diagnosis despite its common use in endemic regions owing to its simplicity and low cost. Flotation with zinc sulfate resulted with better results of 24%, similar with that of Duhok (Hasan *et al*., 2023), India (Paul *et al*., 2023), and Kirkuk (Obaid, 2022) where concentration techniques were used to achieve high cyst and trophozoites detection. The results of this experiment are an indication of the better use of flotation

method in regular stool scan especially in epidemiological studies.

Microscopy, whether direct or concentrated, is unable to differentiate pathogenic *E. histolytica* with morphologically identical nonpathogens *E. dispar* and *E. moshkovskii*. This constraint explains why ELISA, which is specific to *E. histolytica* antigen, produced far much lower prevalence 5.7%. Such inconsistencies are reported in Egypt (Bayoumy *et al*., 2019), Nigeria (EO & CU, 2021), and earlier studies in Kurdistan (Abdullah and Ali, 2024 & Barwari and Ismael, 2011). The use of ELISA in diagnosis as a confirmatory test can prevent over diagnosis and subsequent unnecessary use of anti-amoebic drugs.

Identification of *Entamoeba* in the asymptomatic population (22% by flotation, 9% by ELISA) highlights the existence of health carriers, who are a source of transmission. Nonetheless, the actual percentage of true *E. histolytica* among asymptomatic patients is less than that observed under microscopy (Abdullah and Ali, 2024 & EO & CU, 2021 & Bayoumy *et al*., 2019 & Barwari and Ismael, 2011),further demonstrating the prominence of antigen detection.The highest infection rates were among children of age 1-10 years which is comparable with other studies in Iraq (Mustafa *et al*., 2024 & Hasan *et al*., 2023 & Ali and Flaaeih, 2022). This susceptibility might arise because of immature immune system, lack of proper hygienic practices, and exposure to more contaminated surroundings. The trend of gender differences is not significant in this environment implying that both males and females are at the same level of exposure.

It was found that infection rates varied with seasons and were higher in warm seasons. These findings are also reported in southern Iraq (Chalabi, 2024) and Erbil (Mustafa *et al*., 2024). Warmer temperatures can increase exposure due to increased use of contaminated water or food and/or cyst survival in the environment. These results emphasize the significance of seasonal public health interventions especially during the spring and the summer.

Low income and a low level of education had a significant correlation with infection as in previous reports in Iraq (Hasan *et al*., 2023 & Hassan and Mero, 2020) and Egypt (Ibrahim *et al*., 2023). All of these are likely indicators of poor sanitation, access to clean water and poor understanding of hygiene. Interestingly, the size of families and those living on urban and rural areas did not have significant results, implying that socioeconomic status may be far stronger than household crowding in this region.

The most common was watery diarrhea (88%), fever and vomiting. These findings are similar to other research carried out in Iraq (Obaid, 2022 & Gharibi *et al*., 2017), implying that amoebiasis is not always accompanied by dysentery; rather, watery diarrhea is the most frequent manifestation in endemic regions. These unsexed distributions of symptoms also imply that there is no gender dependence to the intensity of the infection.

Conclusion

In general, the results indicate that microscopy-only is not sufficient in making accurate diagnosis. Zinc sulfate flotation may be used as a sensitive screening assay but critical confirmation using ELISA is required to determine true pathogenic *E. histolytica*. Both clinical and epidemiological applications should combine user preference with the use of combined approaches. Hygiene education, providing safe water, and enhancing socioeconomic status, especially among children and poor populations, should be the focus of public health interventions.

Declarations:

Ethical Approval: The study complied with ethical guidelines and was approved by ethics committees in both the College of Medicine, University of Zakho (SEP2024/E13) and the General Directorates of Health of Zakho District(No.7627 on 9 Sept 2024) to use

data and collected specimens in the current study.

Competing Interests: The authors declare that they have no conflict of interests that appear to be relevant to the content of this article.

Availability of Data and Materials: The data used in this study are available on request from the corresponding author.

Authors Contributions: I hereby certify that all the authors listed on the title page have significant contributions to the conception and design of the study, have read and deeply revised the manuscript, confirm the authenticity and precision of the data and its interpretation and agree to its submission.

Funding: No funding was received for conducting this study.

Acknowledgments: The author would like to extend his heart-warming appreciation to the Department of Biology, University of Zakho, due to their cost-effective academic guidance and support during this study. Appreciation to the staff of Zakho General Teaching Hospital is offered to them due to their help in collecting samples and laboratory work. We would also like to express our gratitude to all participants of the study because they cooperated with us and contributed to the research.

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