United International Journal for Research & Technology

Volume 07, Issue 10, 2026 | Open Access | ISSN: 2582-6832

Prevalence of Entamoeba histolytica and Entamoeba dispar in Tripoli – Libya using ELISA

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***Abstract*—** Entamoeba histolytica is one of the most common intestinal parasites, it's an intestinal pathogenic parasite considered the causative agent of amoebiasis, E. histolytica is morphologically indistinguishable from Entamoeba dispar, a closely related organism that is not able to invade tissues, and therefore, the differential diagnosis under conventional microscopy is impossible. The main purpose of this study was to estimate the proportions between E. histolytica and E. dispar in patients with amoebic infection, using the Enzyme-Linked Immunosorbent Assay (ELISA). 500 Human fecal samples were collected randomly from different laboratories in Tripoli City-Libya, samples were divided into 2 parts, a portion of each sample was quickly conserved in the freezer at (-20oC) until used for ELISA analysis, and the other part was microscopically examined immediately or fixed in 10% formalin-saline to be further examined by direct smear and Formalin-ether sedimentation concentration technique. The result of this study revealed the prevalence rate of E. histolytica / E. dispar infection in Tripoli city was 70 (14%) using microscopy. While ELISA results that from 70 patients with an initial diagnosis of E. histolytica/E. dispar by microscopy only 26 patients (5.2%) were E. histolytica positive, while the remaining 44 (62.6%) samples out of 70 samples were E. dispar.

***Keywords*—** differential diagnosis, E. histolytica, E. dispar, ELISA, Tripoli-Libya.

INTRODUCTION

The genus Entamoeba has a range of species that reside in the human intestinal tract, each species having distinct implications for human health. Noteworthy species found in the human intestinal lumen include Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli, and Entamoeba hartmanni. Among these species, Entamoeba histolytica is the sole one that has been definitively associated with harmful effects on human health, leading to considerable concerns. The remaining species are commonly considered nonpathogenic, indicating that they are not usually linked to disease [1, 2]. However, the identification of E. dispar in individuals experiencing gastrointestinal problems has prompted inquiries regarding their potential impact on human health [3, 4, 5, 6, 7]. Nevertheless, despite these discoveries, the existing evidence does not conclusively establish a clear cause-and-effect connection between these species and the gastrointestinal symptoms found in patients.

Amebiasis, a severe parasite illness, is predominantly caused by Entamoeba histolytica. This syndrome has been acknowledged as a substantial factor in human illness and death worldwide [8, 9, 10]. The disease presents itself in different manifestations, ranging from the presence of the parasite in the intestines without any symptoms to more severe forms, such as amoebic dysentery and invasive extraintestinal amebiasis. Amebiasis can lead to the formation of liver abscesses, which are a serious complication that can be life-threatening. Each year, invasive amebiasis affects an estimated 50 million individuals, resulting in nearly 100,000 fatalities globally [9, 10]. E. histolytica is found worldwide, but certain underdeveloped nations have very high incidence rates, often surpassing 10% of the population [11]. Recent research has additionally indicated that infections produced by E. histolytica can have a negative impact on the growth and development of children, specifically through the occurrence of diarrheal illnesses [12]. Although there are effective therapies available, the continued presence of high rates of illness and death indicates that the present efforts to control the condition may not be adequate. Given that humans are the sole identified host for E. histolytica, the possibility exists for a well planned and

executed control program to entirely eliminate amebiasis.

Recently, there have been notable advancements in the techniques employed for the detection of E. histolytica in medical environments. The accuracy of identifying this parasite has been enhanced by recent developments in molecular diagnostics, namely in the identification of species-specific antigens and DNA in stool and other clinical samples.

Methods such as conventional and real-time PCR have been devised to accurately distinguish E. histolytica from E. dispar in clinical samples. The utilization of these genetic methods has stimulated a reassessment of the epidemiology of amebiasis, particularly in areas where the disease is more prevalent, yielding more precise information on the frequency and consequences of the disease [13]. The primary objective of this study was to differentiate between Entamoeba histolytica and Entamoeba dispar in stool samples using ELISA, and to compare the results with those obtained through the sedimentation method. Additionally, the study aimed to assess the prevalence of amoebiasis in Tripoli, Libya.

MATERIALS AND METHODS

Sample collection was collected randomly from different hospitals in Tripoli-Libya, 500 sample were divided into 2 parts, one portion of each sample was quickly conserved in the freezer at (-20oC) until used for ELISA and the other portion was examined immediately by Formalin-ether sedimentation technique [14]. Formalin-ether sedimentation concentration technique is considered the best technique used in diagnostic parasitology laboratories for the detection of cysts, ova, and larvae.

Generally, 10% formalin is used to kill and preserve diagnostics. Diethyl ether collects most of the debris in a separate layer, all diagnostic stages that apply to this technique were concentrated at the bottom of the analysis centrifuge tube [15] Antigen detection (ELISA):

All conserved fecal samples were tested with ELISA for E. histolytica lectin-specific antigen. The commercially available kit E. histolytica II (Techlab, Blacksburg, USA) was used according to the manufacturer's instructions, Statistical analysis: All data were subjected to statistical analysis using SPSS.

RESULTS

The total infectivity with E. histolytica/E. dispar using the microscopic examination (Formalin-ether sedimentation technique) method was (14%) which represented 70 positive cases out of 500 patients, leaving the non-infected (negative cases) 430 (86%) Fig. 1. While using ELISA only 26 (5.2%) positive samples for E. histolytica antigen out of 500 cases. Fig. 2.

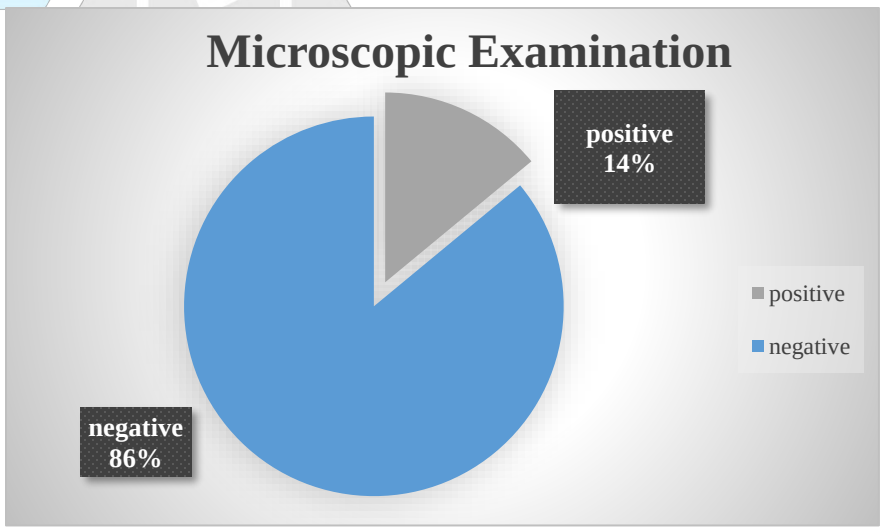

Fig. 1. *The infection rate of E. histolytica/E. dispar in patients by microscopy*

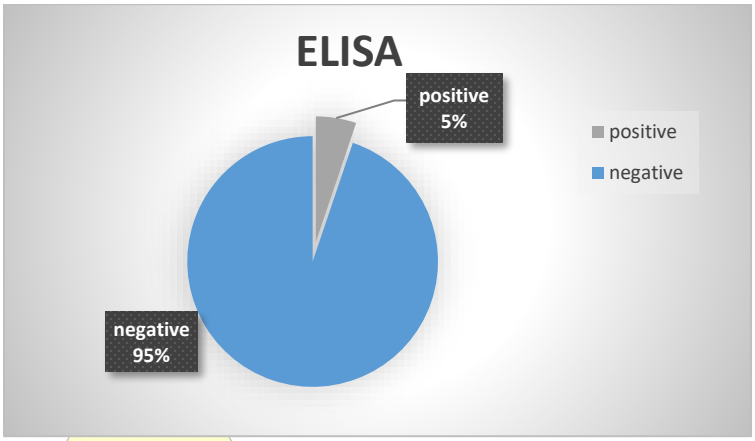

Fig. 2. *The infection rate of E. histolytica in patients by ELISA E.*

The infection rate of E. histolytica/E. dispar infection according to gender (using microscopic examination) was 18.1% in males and 9.3% in females (P-value = 0.032). While the prevalence of amoebiasis according to the sex was 5.3% in males and 5.09% in females, P-value = 0.584 by ELISA (table 1). The infection rates of E. histolytica/E. dispar according to age groups were (5.6%, 21.6%, 11.5%, 10.7%, 20%) in age groups (5-14, 15-24, 25-34, 35-44, ≥45 years) respectively by using concentration method , P-value = 0.163 and this means that there were no significant differences recorded between age groups, based on One-Way ANOVA test. The rate of infection by Entamoeba histolytica regarding age group were (5.6%, 5.4%, 1.6%, 1.8%, 11.7%) in age groups (5-14, 15-24, 25-34, 35-44, ≥45 years) respectively, P-value = 0.092 and this means that there were no significant differences recorded between age groups (Table 2).

Table 1. *Distribution of E. histolytica/E. dispar among patients regarding gender*

Method	Sex	Negative	Positive	Total	P. Value
sedimentation	Male	81.80% (216)	18.20% (48)	100% (264)	0.032
	Female	90.70% (214)	9.30% (22)	236	
	Total	86% (340)	14% (70)	500	
ELISA	Male	94.70% (250)	5.30% (14)	264	0.584
	Female	93.91% (224)	5.09% (12)	236	
	Total	94.80% (474)	5.20% (26)	500	

Table 2. *Distribution of E. histolytica/E. dispar complex among patients regarding age group*

Method	Age group	Results		Total	P-value
		Negative	Positive		
Sedimentation	5-14	68 (94.4%)	4 (5.6%)	72 (14.4%)	0.163
	15-24	58(78.4%)	16(21.6%)	74(14.8%)	
	25-34	108 (88.5%)	14 (11.5%)	122 (24.4%)	
	35-44	100 (89.3%)	12 (10.7%)	112 (22.4%)	
	≥45	96 (80%)	24(20%)	120 (24.0%)	
	Total	430 (86%)	70(14%)	500	
ELISA	5-14	68 (94.4%)	4 (5.6%)	72 (14.4%)	0.092
	15-24	70 (94.6%)	4 (5.4%)	74 (14.8%)	
	25-34	120 (98.4%)	2 (1.6%)	122 (24.4%)	
	35-44	110 (98.2%)	2 (1.8%)	112 (22.4%)	

	≥45	106 (88.3%)	14 (11.7%)	120 (24.0%)	
	Total	237 (94.8%)	13 (5.2%)	500	

The distribution of E. histolytica/E. dispar according to the consistency of the samples were (9.2%, 15.9%, 26.7%, and 100.0 %) in states (Formed, Semi-formed, Diarrhea, Dysentery) respectively(by sedimentation method) where P-value was 0.0047. while the infective rate of E. histolytica were (1.5%, 4.5%, 20.0%, and 100.0%) in states (formed, Semi-formed, Diarrhea, and Dysentery) respectively, with P-value 0.0001 (table 3).

Table 3. *Distribution of E. histolytica/E. dispar among patients regarding consistency of the samples*

Method	**Consistency of Sample**	**Results**		**Total**	**P-value**
		Negative	Positive		
Sedimentation	formed	238 (90.8%)	24 (9.2%)	262 (52.4%)	0.0047
	Semi-formed	148 (84.1%)	28 (15.9%)	176 (35.2%)	
	Diarrhea	44 (73.3%)	16 (26.7%)	60 (12.0%)	
	Dysentery	0 (0.0%)	2 (100.0%)	2 (0.04%)	
	Total	430 (86%)	70 (14%)	500	
ELISA	formed	258 (98.5%)	4 (1.5%)	262 (52.4%)	< 0.0001
	Semi-formed	168 (95.5%)	8 (4.5%)	176 (35.2%)	
	Diarrhea	48 (80.0%)	12 (20.0%)	60 (12.0%)	
	Dysentery	0 (0.0%)	2 (100.0%)	2 (0.04%)	
	Total	474 (94.8%)	26 (5.2%)	500	

Out of 500 samples examined in this study (Table 4), 20 samples were revealed as true positive (TP) and 50 as false positive (FP). However, 424 were true negative (TN) and 6 samples were false negative. The sensitivity was (76.9%), specificity was (89.4%), the positive predictive value PPV was (28.6%), and the negative predictive value NPV was (98.6%).

Table (4): *Comparison between sedimentation & ELISA for detection of E. histolytica*

Method	**result**	**ELISA**		**Sensitivity**	**Specificity**	**PPV**	**NPV**
		Positive	Negative				
sedimentation	Positive	20	50	76.9%	89.4%	28.6%	98.6%
	Negative	6	424				

DISCUSSION

To begin with, it is important to note that there have been very few studies reporting the prevalence of Entamoeba infections in Libya. In these studies, microscopic examination was the primary method used to identify the organisms, typically reported as Entamoeba histolytica/Entamoeba dispar. However, relying solely on microscopic examination has significant limitations, as it cannot distinguish between the two species, which could potentially lead to false-positive results.

One of the most significant advantages of using the Enzyme-Linked Immunosorbent Assay (ELISA) as a diagnostic tool is its ability to differentiate between E. histolytica and E. dispar in regions where other Entamoeba species are also prevalent. Additionally, ELISA is known for its accuracy and reliability, making it particularly valuable when there is a need to understand the epidemiology of Entamoeba histolytica and Entamoeba dispar infections in a specific area.

Furthermore, the findings of the current study suggest that infections caused by E. histolytica, and E. dispar are associated with gastrointestinal symptoms such as diarrhea. However, it is crucial to recognize that not all E. histolytica infections lead to clinical disease, as has been demonstrated in previous studies [16].

There have been relatively few studies that have explored the prevalence of amoebiasis in Libya. In the studies that have been conducted, microscopic examination was commonly used, with the organisms typically identified as E. histolytica or E. dispar. However, relying exclusively on microscopic examination has significant limitations, as it cannot differentiate between these two species, leading to a high risk of false-positive results. One of the key advantages of using the Enzyme-Linked Immunosorbent Assay (ELISA) as a diagnostic tool is its ability to accurately distinguish between E. histolytica and E. dispar in regions where other E. species are also present. Additionally, ELISA is known for its precision and reliability, making it particularly valuable in understanding the epidemiology of E. histolytica and E. dispar infections.

The observations from the current study suggest that symptoms such as diarrhea and other gastrointestinal disorders are associated with infections caused by E. histolytica and E. dispar. However, it is important to remember that not all infections caused by E. histolytica result in clinical illness, as demonstrated in previous research [16].

According to the World Health Organization (WHO), it is crucial to differentiate between E. histolytica and E. dispar whenever possible. This distinction is particularly important because patients should not be treated based solely on microscopy findings. To reduce the risk of the disease progressing into an invasive form, treatment should be administered to any cases that are presumptively diagnosed or confirmed to be caused by E. histolytica, regardless of the presence or absence of symptoms. Conversely, cases confirmed to involve only E. dispar do not require treatment, as this species has not been associated with invasive disease [17].

To facilitate accurate diagnosis, several commercial ELISA kits have been developed, such as the TechLab Entamoeba test, which detects both E. histolytica and E. dispar. These kits, along with other diagnostic methods like ELISA and PCR, have significantly contributed to our understanding of the epidemiology of these parasites and have proven valuable in investigating disease outbreaks. This advancement in diagnostic techniques has led to a reevaluation of previously investigated cases. Upon further examination, it was found that many individuals initially believed to have asymptomatic infections with E. histolytica were actually carrying E. dispar, a species not known to cause invasive disease in humans [18].

The results of this study in Tripoli / Libya showed a significant difference in E. histolytica/E. dispar regarding gender using sedimentation, meanwhile there was no significant difference in the overall of E. histolytica amongst the gender using ELISA or the age group using sedimentation and ELISA. The detected infection rate was 18.2% (48/264) in males meanwhile 9.3% (22/236) in females with P-value 0.032 using sedimentation method, while the detected infection rate was 5.3% in males and 5.09% in females using the ELISA, with P-value 0.584, these results agree with the other studies [19, 20], while in another study in Nepal no significant relationship was found between gender and age group with the infection of E. histolytica, this may imply that both males and females have a similar predisposition to E. histolytica infection due to their sharing the same community and generally engage with the similar activities [21].

In this study, we did not find any significant difference between age groups and this agrees with a study conducted in Kenya, it could be due to the fact that similar socio-economic activities of the people [22]. Also, a highly significant difference was found in E. histolytica regarding sample consistency using ELSA. The samples were formed with high prevalence in diarrhea and dysentery stool (20.0%, and 100.0%))

respectively with P-value 0.0001. These results disagree with a study conducted in India [23].

To determine whether the Enzyme-Linked Immunosorbent Assay (ELISA) is more effective than microscopic examination in identifying E. histolytica in clinical stool samples, the current investigation was conducted. ELISA is widely regarded as the gold standard due to its high sensitivity and its ability to accurately differentiate between different species of Entamoeba [24]. In a comparative study, the Indirect Hemagglutination Assay (IHA) demonstrated a sensitivity of 97.6% and a specificity of 97%, while the latex agglutination technique showed a sensitivity of 90.7% and a specificity of 95%. ELISA, on the other hand, exhibited a sensitivity of 93% and a specificity of 100% [25]. In this particular investigation, the microscopy technique displayed sensitivity, specificity, positive predictive value, and negative predictive value of 76.9%, 89.4%, 28.6%, and 89.6%, respectively, when compared to the reference assay. However, the results of the microscopic examination were found to have lower sensitivity, specificity, and predictive values compared to ELISA.

These findings suggest that positive results obtained through microscopy should not be relied upon as the sole criterion for identifying individuals in the community who are infected with E. histolytica. This conclusion aligns with a study conducted in Malaysia [26], which also reported low sensitivity and specificity values for microscopic examination. Similarly, Pillai et al. (1999) found that the specificity of microscopy for detecting E. histolytica was only 9.5% when compared to the antigen detection test performed using enzyme immunoassay (EIA) [27].

The results of this research indicate that the transmission of intestinal protozoa remains widespread within the population, particularly in the region studied, where E. dispar and E. histolytica were identified as the primary protozoa. The prevalence rate of E. histolytica/E. dispar infection in Tripoli was found to be 14%, with 70 cases detected using microscopic examination and the Formalin-ether sedimentation technique. However, when these cases were reexamined using the Enzyme-Linked Immunosorbent Assay (ELISA), only 26 individuals (5.2%) were confirmed to be positive for E. histolytica, marking a significant deviation from the initial findings. This suggests that the remaining 44 cases were likely due to E. dispar, highlighting the considerable rate of false-positive results that can occur with microscopic analysis.

These findings underscore the importance of using more reliable diagnostic methods, such as specific antigen assays in stool samples, for the accurate detection of E. histolytica. Consistent with previous studies [28, 29], our investigation also documented a high incidence of E. infections, with the majority attributed to E. dispar, a non-pathogenic amoeba. The relatively low incidence of disease may be partially explained by the higher prevalence of E. dispar rather than E. histolytica, despite the initially reported high rate of E. histolytica infection.

CONCLUSION

The findings of this research indicate that the prevalence rate of E. histolytica/E. dispar infection in Tripoli was 14%, with 70 cases identified through microscopy. However, upon re-examination of these 70 cases using the Enzyme-Linked Immunosorbent Assay (ELISA), only 26 individuals (5.2%) were confirmed to be positive for E. histolytica, highlighting a significant discrepancy from the initial microscopy findings. These results underscore the limitations of using microscopic examination as a screening method for detecting E. histolytica.

Given the potential for misdiagnosis, it is clear that the presence of E. histolytica/E. dispar cysts or trophozoites should be confirmed using more reliable techniques, such as antigen detection or PCR assays. Improving diagnostic methods for Entamoeba is crucial not only to reduce the morbidity and mortality associated with amebiasis but also to prevent unnecessary treatment of individuals infected with non-pathogenic Entamoeba species.

ACKNOWLEDGMENTS

The author would like to acknowledge the support provided by the Libyan Authority for Scientific Research, which was instrumental in facilitating this

study. This research was fully funded by the Libyan Authority for Scientific Research.

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