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Detection of *Entamoeba* species among Amoeba diagnosed infection in Nablus District

Amjad Hussein[1,], Sireen Hamad[1], Motasem Al-Masri[2] & Abdel Fattah Hasan[3]*

[1]Medical Laboratory Sciences Department, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. [2]Microbiology, Immunology and Pathology Department, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. [3]Environmental Research Lab, Civil and Architectural Engineering Department, An-Najah National University, Nablus, Palestine.

*Corresponding author: amjadhs@najah.edu

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ABSTRACT

Amoebiasis is still of serious concern for public health in developing countries. The main issue in diagnosing amoebiasis infection through stool samples is the inability to differentiate the morphologically indistinguishable *Entamoeba histolytica* from the nonpathogenic Amoeba. This study aimed at detecting *Entamoeba* species (*E. histolytica / dispar*) among the microscopically diagnosed amoebiasis infections in the Nablus district using nonconventional immunoassay methods. The study utilized 101 Amoeba-positive stool samples based on the microscope examination. The stool specimens were collected from patients who sought medical investigation and diagnosis regardless of age or gender. The consented patients answered questionnaires. *The immunoassay method detected E. histolytica / E. dispar in 36.2% of stool specimens out of the tested samples*. There was a variation in the prevalence of amoebiasis among the different age groups. Noteworthy, infants positive, 9.9 % and 20.6 % microscopically and using the immunoassay, respectively. In conclusion, this study stressed the urgent need to revise the conventional technique used to diagnose amoebiasis for its serious impact on health. The immunoassay kits could be a good and fast screening technique.

Keywords: Amoebiasis, *E. histolytica*, *E. dispar*, Immunoassay, Palestine.

INTRODUCTION

Amoeba of medical concern is *Entamoeba histolytica*, the main pathogenic protozoan parasite affecting 50 million individuals globally (1). The *Entamoeba* genus includes other species with disputed pathogenicity, including *E. dispar*, *E. moshkovskii*, *E. hartmanni*, *E. coli*, and *E. polecki*. *E. dispar* species is nonpathogenic morphologically indistinguishable from *E. histolytica*, making the conventional microscopic diagnosis challenging in identifying the real causative agent of the disease. Until now, microscopy is still the most globally used method for routine diagnosis, especially in poor sanitation and hygiene infrastructure environments (2, 3, 4, 5)

Entamoeba histolytica infection is endemic in many parts of the world with poor sanitation and hygiene infrastructure. Still, this infection is likely restricted in certain areas, such as risk groups residents in mentally handicapped institutions (6). Although the effective therapy availability, mortality, and morbidity associated with amebic infection continue, almost all interventions designed to eliminate or limit the disease are ineffective (7).

Amoebiasis is one of the important global diseases. Amoebiasis is a gut infection characterized by intestinal mucosa invasion that sometimes spreads to other organs, mostly the liver, causing a liver abscess (2, 3, 5). Despite the parasite's worldwide distribution and high prevalence rates, more than 10% of the population has been reported from different developing countries (7).

Amoeba diagnosis is tricky due to the indistinguishable nonpathogenic species, degenerated polymorphonuclear cells, and artifacts. *E. dispar* appears to be almost 10 times more common than *E. histolytica*. Accordingly, amoebiasis needs to be justified for the nonpathogenic *E. dispar* and the pathogenic *E. histolytica* infection (2, 8, 9, 10, 11, 12). Most of the available data were gained using incapable methods for distinguishing between the morphologically identical species that need more capable techniques like PCR. Even the most

usable screening immunoassay techniques cannot differentiate between them (RIDA QUICK ENTAMOEBA (N1703) KIT, Germany). *Entamoeba histolytica* identification vs. Amebiasis detection uses variable techniques from the basic microscopic serological to the more advanced and developed molecular techniques. Moreover, the most important point to take care of is the sample, as sample preparation for detecting the specific antigen and antibodies in stool and the other blood antibodies (7).

There are many limitations of conventional diagnostic ways, such as the limitation of microscopic detection is the insensitive differentiation of pathogenic strains of Amoeba from other nonpathogenic. Diagnosis by culture is more specific and sensitive but is time-consuming and hard; it needs several weeks if successful. Amoebic culture may be negative for many reasons, like the delay in processing, antiamoebic therapy before stool collection, and the parasite nature (13).

An immunochromatographic kit is used for in vitro diagnostic use and for the qualitative determination of *Entamoeba histolytica* or *E. dispar* in stool samples like RIDA QUICK *Entamoeba* Kit. This rapid assay is a single step. Sensitive immunological test with specific antibodies against antigens of Amoeba has many advantages. This diagnostic method is not dependent on subjective evaluation and is more sensitive as the formation of antibodies caused only by the invasive form of *Entamoeba.* Since antibody titers can be detected with the onset of the clinical symptoms, a specific antibody determination can be used to identify *E. histolytica*. Also, the ability to differentiate between the size of the titers of intestinal and extraintestinal amoebiasis is important for deciding on the choice of therapy (RIDA QUICK *Entamoeba* (1703) Kit, Germany)

This study aims to correlate the microscopic dependent techniques for diagnosis with the clinical presentation. Elucidate the risk factors associated with *Entamoeba* spp. Infections. Use immunochromatographic assay techniques to confirm and estimate the frequency of Amoebia infection.

MATERIALS and METHODS

Consent and questionnaire form

The institutional review board (IRB) permission from An-Najah National University was acquired on October 31, 2018, and is identified by the archived number 57 October w. Prior to completing the questionnaire, verbal agreement was obtained from each participant. The survey instrument was designed to include the relevant domain of inquiry, encompassing the participants' socioeconomic circumstances. The questionnaire included general questions concerning personal information such as age, gender, and marital status. This study examines the living conditions of residents, specifically focusing on the location (town, village, or camp), the source of water, the type of flooring, the water and drainage systems, the number and type of functional baths within the home, and the health status of the individuals. The health conditions considered include hypertension, diabetes mellitus, the use of cortisol medication, organ transplant history, Irritable Bowel Syndrome (IBS), and thyroid gland issues.

Samples collection

From March to June 2019, 101 stool samples were collected from patients who sought medical consultation at Nablus hospitals and medical labs for abdominal pain, diarrhea, and medical checkups. Patients were diagnosed as amoeba-positive during the visit. Regardless of their gender, age, and living or residence, samples were collected, preserved, and handled according to the detailed protocols below (3, 14).

Microscopic examination

The stool specimens were examined immediately after collection. The microscopic examination was in 0.9% saline and Lugol's wet mount examination. Microscopic examination of stool specimens in saline wet mount were examined immediately after collection time within half an hour, seeking the motile trophozoites and cysts (14).

Concentration was done using centrifugal techniques for comparison using the flotation (Faust technique) and/or the sedimentation (modified Ritchie technique) methods. In the

Faust method, 10 mL of fecal sample suspension in normal saline was filtered by gauze and then spun for settlements. The settled pellet was suspended by vigorous shaking in the gradient zinc sulfate solution (1,180 g/mL) and immediately centrifuged to separate the components to investigate the supernatant for the cyst by the light microscope. In the Ritchie technique, fecal suspensions were filtered by gauze and then spun for settlements. The settled pellet was suspended in 5 mL water, and then 5 mL ethyl-acetate was added to remove the light and mucoid ingredients. The sedimentation technique is less harsh for the cyst than the flotation technique, so the sediment was investigated using the light field microscope (3). Several characteristics were reported for each diagnosed case during the microscopic examination, such as leukocytes, red blood cells, and cysts.

Immunochromatographic assay

The immunochromatographic assay kit (RIDA QUICK *Entamoeba* (N 1703) Kit, Germany) was used to determine *E. dispar / E. histolytica* in stool samples qualitatively. A volume of 1 mL of the extraction buffer (diluent) was pipet into a test tube, and then 100 µL of stool sample was added; the sample was homogenized on a vortex mixer, stool sample was settled for 3 minutes, then four drops of the clear supernatant were added into the round opening of the cassette. Finally, the results were read after 5 minutes. If a red test band (T) appears along with the blue control band (T), the result is positive, but if only the blue control band appears, the result is considered negative and not valid; if no blue control band appears, in this case, the assay must be repeated with a new cassette (RIDA QUICK *Entamoeba* (N 1703) Kit, Germany).

RESULTS

Microscopic examination

This study included only stool specimens found through microscopic examination to contain *Entamoeba* spp. The specimens were collected from medical labs in the Nablus district. However, none of these samples gave positive microscopic results after reexamining at An-Najah as the used concentration techniques, which are specific and applicable for cyst detection. After examining stool samples, the samples were preserved in three types of media: Iodine Formalin (IF), Potassium dichromate (K2CrO3), and Brig for further and later usage.

Sociodemographic characteristics of Entamoeba-infected patients

Microscopically diagnosed infected individuals were 57 males and 44 females (Table 1). The age range of 30-60 years showed a notable percentage of infection, 30.6%, regardless of gender. In contrast, patients under two years represent 9.9%, and a low percentage of 2.9% was detected in patients over 60 years old.

The questionnaire, as shown in Table 1, inquired about the community, income levels, supply of drinking water, and sanitary facilities. Concerning the drinking water source, the highest infection rate percentage was noticed in people who use municipal water at 78.2%. In contrast, mineral water represents 0.9%, while Israeli (from Mekorot Co.) primary water source was 13.8%. None of the studied population used harvested rainwater only as a source of drinking water, and people who used rainwater also used municipal water, representing 6.9%. The drinking water source and sanitation facilities are the most important factors in Amoeba infection. Patients' use of latrines was investigated and found to use two types: Arabic and Frankish. Only 0.9% use the traditional Arabic toilet, 83.1% use the Frankish, and 15.8% use both. So, the type of bath and the way of sanitation determine the frequency of Amoeba infection; 32.6% use collection pits and 67.3% use the municipality network. In contrast, there is no open defecation (exposed waste water channels), which is good to decrease the chance of infection since this way of sanitation prevents Amoeba cyst diffusion.

According to residency data (Table 1), the highest Amoeba infection (62.4%) was in patients who live in Nablus city. In contrast, those living in villages were 35.6% of the cases, and 2.0% of patients live in refugee camps. The higher rate among the city residents could be only due to demographic differences, and the water source is mainly municipal water, and few use other sources such as mineral water. However, villagers use rainwater stored in a cistern or Mekorot water that

may be less contaminated than the municipal supply. The people who live in the camp also use municipal water, but the infection rate of 2.0% is not reflected in the patients involved in this study.

The relationship between the income strata and Amoeba infection indicated that people who are classified as not poor (income more than 5000 NIS) have the lowest percent of infection others, 9.9% rate, that means if the standards of living are good, there may be a decrease in the chance of Amoeba infection, due to availability of healthy life such as the use of clean water as a source of drinking water either mineral or rainwater collected in wells, and availability of a big house with more than one bathroom that may decrease the infection with Amoeba. High income helps people take medical tests periodically, which helps for early diagnosis of Amoeba. Contraindicatable, the mid-income showed the highest infection rate of 61.4% compared to poor people at 28.7%, as reflected in education and the ability to seek diagnosis and treatment.

Concerning drug treatment for Amoeba infection, all patients who used Flagyl medicine got better on the treatment. There is no relationship between Amoeba infection and any other disease, such as diabetes or heart disease; only 12 patients have another health problem, such as Irritable Bowel Syndrome (IBS), and were diagnosed as Amoeba positive. However, all 12 IBS patients were negative for the immunodiagnosis that questioned the microscopic result as they came to the hospital to complete their treatment for their health condition, and they made a routine test to find they were infected with Amoeba. IBS and Amoeba infection share some symptoms, such as abdominal pain. Still, in general, many patients who came to hospitals for diagnosis did not know they had Amoeba. Others made the clinical test routine, repeating it every 6 months to ensure no diseases or health problems.

Table (1): Sociodemographic characteristics of the studied population, Nablus, 2019.

Characteristics	Total number of 101 n (%)	Immunoassay test positive 34/ 94 * n (%)
Gender		
Female	44 (43.5)	20 (58.8)
Male	57 (56.4)	14 (41.2)
Age group (years)		
<2	10 (9.9)	7 (20.6)
2-6	14 (13.8)	4 (11.8)
6-12	9 (8.9)	4 (11.8)
12-18	11 (10.8)	4 (11.8)
18-22	7 (6.9)	2 (5.9)
22-30	16 (15.8)	3 (8.8)
30-60	31 (30.6)	10 (29.4)
>60	3 (2.9)	0 (0.0)
Patients residence		
City	63 (62.3)	17 (50)
Village	36 (35.6)	17 (50)
Camp	2 (1.9)	0 (0.0)
Income strata		
Poverty (<2500 NIS)	29 (28.7)	7 (20.6)
Middle income (2500-5000 NIS)	62 (61.3)	22 (64.7)
High income (>5000 NIS)	10 (9.9)	5 (14.7)

Characteristics	Total number of 101 n (%)	Immunoassay test positive 34/ 94 * n (%)
Source of drinking water		
1. Mekorot	14(13.8)	4 (11.8)
2. Municipal water	79(78.2)	30 (88.2)
3. Others (Mineral water)	1(0.9)	0 (0.0)
4. Municipal and mineral water	7(6.9)	0 (0.0)
Type of latrine		
Arabic latrine	1(0.9)	-
Frankish latrine	84(83.1)	23 (67.6)
Both	16(15.8)	11 (32.4)
Sanitation facilities		
Collection pits	33(32.6)	14 (41.2)
Pipes for municipality	68(67.3)	20 (58.8)

Note: NIS refers to money currency used in Palestine (Shekel)

* 94 samples out of 101 stool samples were used in the immunoassay test due to the kit capacity of 100 assays. Six were used as a test to decide the best preservative material; the remaining 94 cassette was used for stool sample analysis.

General symptoms of infection with *E. histolytica* colitis include abdominal pain and/or watery, bloody, or mucous diarrhea. All patients have at least one of these symptoms, which could explain the presence of Amoeba in Palestine, but a suitable condition is not available to study it molecularly and the presence of other species.

This study involved 101 patients; all had positive microscopic examination results. There are 10 patients less than 2 years from those patients, which was reflected in the positive immunoassay screening of 20.5%, as shown in Table 1. Ordinarily, kids do not drink mineral or artificial milk, except for one kid who uses only mineral water. Also, all the kids have extreme diarrhea, which is inconsistent with the medical opinion that it is difficult and almost impossible for a baby to be infected with Amoeba if he breastfed.

Most people use municipal water as a drinking water source and depend on it more than other sources, reflected by the 62.4% of the patients living in Nablus city who use it more than others who live in a village, for example. When people are infected with Amoeba, the primary source is related to water since Amoeba is the only human source that is transferred by the presence of the infective cyst stage in water. As a result, for the way of Amoeba cyst transfer, the expected type of sanitation is one of the most important ways for Amoeba infection. According to the questionnaire results, there are no open defecation, exposed wastewater channels, and low users for Arabic latrines. Still, there is an Amoeba infection, and there are cases diagnosed with Amoeba regardless of the infection sources and factors.

No relationship was detected between Amoeba infection and a patient's health status, such as those who suffered from chronic diseases such as diabetes and IBS. Most patients sought medical consultation for real clinical needs and apparent symptoms. The most prominent symptoms ranged from abdominal pain watery or bloody diarrhea, and the microscopic examination of stool samples showed an Amoeba infection incidentally. Few patients followed their health status and treatment, which resulted in efficiently curing the symptoms.

All 12 IBS patients were negative for the immunodiagnosis that questioned the microscopic result as they came to the hospital to complete their treatment for their health condition, and they made a routine test to find they were infected with Amoeba. IBS and Amoeba infection share some symptoms, such as abdominal pain. Still, in general, many patients come to hospitals for diagnosis or to

continue their health status, and they do not know they have Amoeba. Others made the clinical test routine, repeating it every six months to ensure no diseases or health problems.

It has almost become a population notion and routine practice to seek Amoeba treatment without medical consultation. People expect to have Amoeba for previous history and symptoms even though people who ask for medical consultation do not complete the treatment course, especially if the symptoms disappear and the patient feels well.

Immunochromatographic assay

An immunoassay was used to screen the sample for the actual Amoeba infection. From 94 stool samples preserved in IF (Iodine Formalin), only 34 samples (Table 2) were found positive for *Entamoeba histolytica* or *Entamoeba dispar*, which means 34 samples taken from patients have the morphologically indistinguishable Amoeba. Ninety-four stool samples were used for immunoassay instead of 101 samples. Initially, we used 6 samples as a test to decide the most suitable preserved material, so we lost six cassettes for this step and continued with 94 stool samples preserved in IF. IF preserved three positive samples were found negative in PDC (Potassium dichromate) and Brig preserved samples. Therefore, the recommendation is to use IF but not the PDC and Brig to preserve the used kit immunodiagnosis.

Table (2): Immunoassay positive result samples symptoms other than the main abdominal pain and diarrhea symptoms.

Symptoms Sample No.	Vomiting	Fever	Dizziness	Other symptoms
3		*		
4		*		
6		*		
8		*		
9		*		
13		*		
17			*	*
18			*	
20			*	
22		*		
23		*		
24	*			*
25				*
27		*		
28		*		
29	*			*
31			*	

All 34 immunoassay-positive samples correlated to the typical Amoeba symptoms in patients with abdominal pain and diarrhea. In addition, some patients (Table 2) of the 34 immunoassay-positive samples had vomiting, fever, dizziness, and other symptoms such as headache since the main symptoms of Amoeba are abdominal pain and bloody or watery diarrhea, and other symptoms are uncommon.

Generally, amoebiasis with high positive results using the microscopy gets lower confirmed positive samples, possibly due to misdiagnosis and nonpathogenic Amoeba. However, the used immunoassay kit detects both *E. histolytica* and *E. dispar*. PCR is a valuable diagnostic tool for species differentiation, but at the same time, it cannot replace routine microscopy. The immunoassay and the clinical pattern that needs more deep analysis can partially resolve the need for a rapid, simple, and sensitive method for diagnosing amoebiasis.

However, this study shed light on the gap between the microscopically and immunoassay positive results.

Immunoassay assay gave positive results for 34 samples out of 94 samples (33.6%); the explanation of these results emphasizes high misdiagnosis for Amoeba; all of these samples were preserved in IF, Brig, and PDC, but IF only gave positive results. The recommendation is that IF is the best Amoeba preservative substance. In contrast, Brig and PDC can cause a degenerative effect on Amoeba.

DISCUSSION

E. histolytica, an etiological agent of amoebiasis, is one of the main parasitic infections causing morbidity and mortality, especially in developing, low-hygienic countries. In the 1980s, the infection with *E. histolytica* was estimated to infect around 10% of the world population, of which around 90 % are asymptomatic carriers and causing up to one hundred thousand deaths per year (15).

Microscopic results were very tricky as a routine diagnosis for similar species needs professionals and training to add extra measures with staining and size estimation to differentiate them from *E. histolytica* and *E. dispar*. However, without advanced techniques, it is almost impossible to distinguish *E. histolytica* and *E. dispar*. Therefore, the microscopic examination should not be used as the sole technique for amoebiasis detection; it is insensitive, incapable of differentiating pathogenic *E. histolytica* from nonpathogenic *E. dispar*, and with a tendency to get false-positive results (10). It is critical to use specific methods like antigen detection tests and PCR to get more accurate results.

In this study, the confirmed true diagnosis of the infected infants < 2 years old raises the need to increase the awareness for hygiene control as Amoebiasis infection sources are only human contamination (15, 16, 17, 18). In Bangladesh, a study involved a group of infants observed for the first year of life in an urban slum; roughly 10.9% of children had at least one diarrheal episode positive for *E. histolytica*, children were born malnourished and more likely to be infected with *E. histolytica* (19).

The healthier the life, the less infection with Amoeba or other diseases caused by other parasites or microbes is expected. In this study, the high-income people were 9.9 %, microscopically Amoeba positive, followed by low income 28.7% and moderate income 61.3 %. In contrast, a study by Haque (2006) reported no differences in family size, income, and nutritional status between children with or without *E. histolytica* infection (20).

This study shows that only 34 samples were confirmed positive using the immunoassay kit among 94 positive microscopic samples. Microscopic examination for stool samples is very tricky. In order to reduce the obstacles due to the undistinguishable nonpathogenic Amoeba, the recommendation is to use routine diagnosis and screening techniques, including immunoassay and molecular techniques. In a similar study in 1998, 98 stool specimens from patients with diarrhea were examined by the microscope, where 68 stool specimens were positive for Amoeba. Still, from those 68 microscopically positive specimens, only 46 samples could be confirmed positive (10). This gave strong evidence for low accuracy for microscopic examination and the subjective nature of this test concerning the more advanced specific and sensitive techniques.

The setup of the validated protocols is essential, as indicated in the low detection using screening techniques for positive microscopic samples, including careful sample collection, preparation, and preservation. If an immediate analysis is not possible for the screening immunoassay, the best and most successful preservative chemical substance was Iodine Formalin (IF) but not Brig or Potassium Dichromate. It seems IF preserves well and does not affect Amoeba cyst or trophozoite. Routinely, *E. histolytica* diagnosis depends on the microscopic examination searching for the morphological features of the protozoan parasite, which is considered very convenient for diagnosing amoebiasis because the basic technical requirements are simple and cheap (21). Still, this technique is as insensitive as the new redescription of the two species. It cannot distinguish between the nonpathogenic *E. dispar* and the pathogenic *E. histolytica* (22). This has triggered the researchers to develop new

and efficient techniques based on either molecular biology methods or antigenic determinants for successful differentiation between the two species in the human clinical samples.

The present study has shown the importance of using a validated technique for Amoeba diagnosis with an urgent need for policies to include the rapid, sensitive, and appropriate techniques for diagnosing amebiasis that remain a major public health priority for the developing world. Obaid's study (2016) aimed to detect and distinguish between *E. dispar* and *E. histolytica* in human isolates using the ELISA technique to determine whether the patient requires treatment or not to avoid the side effects of unnecessary treatment. The study included 397 (212 males and 185 females) with mainly abdominal pain and diarrhea symptoms. Results illustrated that the overall prevalence of *E. histolytica* or *E. dispar* was 24.4%, with the infection rate being 21% in females and 27.4% in males. Among 97 microscopically positive samples, 87 (89.7%) were ELISA positive for *E. histolytica*,. The lowest infected male age group was among the 11-20 years with a 15% rate, while the highest range was the 41-50 years group, whereas female lowest infected age group was <1 year with an 11.9% rate, and the highest was> 50 years with the 34.6% rate (23). Another study for *E. histolytica* and *E. dispar* diagnosis involved 112 patients with gastrointestinal symptoms, mainly diarrhea and abdominal pain, using ELISA and microscopic examination. All patient samples of 112 samples were diagnosed by microscope as amoebiasis positive, while only three patients were positive for *E. histolytica* by ELISA; two patients out of the three ELISA positive showed invasive disease of dysentery and amebic liver abscess. Clinically, most of the 112 patients, 72 males, and 40 females, complained of mild symptoms, as 30.4% complained of diarrhea and 23.2% indicated abdominal pain (24).

In the present study, 20.6% confirmed amoebiasis of age <2 years despite the limited sample size. This indicates a real health situation for the household, who may not suffer but indicated by the immunocompetent patients who need further risk assessment for the sources for the high amoebiasis for the infants as humans are the only known definitive hosts of *Entamoeba histolytica* that stress the healthier life and hygiene measures to reduce the Amoeba infection (15, 16, 17, 18). The main events in the pathogenesis of *E. histolytica* infection are adhesion, attachment, and colonization, mainly at the cecum mucus layer that triggers cell-cell contact killing, as indicated in histolytic. However, Amoeba morbidity and mortality are more related to the ability for tissue invasion followed by dissemination to the extraintestinal soft organs, leading to liver abbesses. This denotes *E. histolytica* as one of the destructive protozoan parasites compared to the nonpathogenic Amoeba (18, 25).

A further limitation to sample size is the capability of the immunoassay kit that can only detect *E. dispar* / *E. histolytica*. Other nonpathogenic Amoeba other than E. dispar can be misdiagnosed with *E. histolytica*, which could partially explain the higher positive microscopic Amoebiasis results, considering the difficulties of Amoeba microscopic diagnosis. However, due to the immunoassay kit's high sensitivity and specificity, the sample size was enough to address this study's objectives.

CONCLUSION

The actual amoebiasis prevalence using the immunoassay technique is of higher detectability than the conventional microscopic techniques. Amoebiasis prevalence in different age groups, especially infants, stresses the urgent need for public health awareness and hygiene measures on amoebiasis infection, which is only known to be from human sources. This study highlights the need to consider revising the microscopic detection protocols for amoebiasis and the usage of an immunoassay as a complementary test for confirming the microscopically positive Amoeba. Further studies are needed to evaluate the real *Entamoeba* species situation using specific immunoassays or more advanced molecular techniques. Further studies are recommended to elucidate the critical issue due to pathogenic *E. histolytica* and other nonpathogenic Amoeba using other specific diagnostic techniques.

Ethics approval and consent to participate

Ethics approval was obtained from the Institutional Review Board of An-Najah National University. Each participant verbal

agreement and consent were obtained before completing the questionnaire.

Consent for publication

The authors have read and approved the paper for publication. The paper has not been published previously, nor does any other journal consider it.

Availability of data and materials

Data is available upon request.

Author's contribution

Amjad I.A. Hussein: conceptualization, writing first draft, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, and writing review and editing. **Sireen Hamad***: master student sampling, consent and questionnaire, writing first draft, methodology, funding acquisition. **Motasem Al-Masri**: formal analysis, inquiry, resources, software, supervision, validation, visualization, and writing review and editing. **A. Rasem Hasan**: conceptualization, methodology, resources, software, validation, visualization, writing review, and editing. *This research is based on student's work.

Competing interest

The authors declare that there is no conflict of interest.

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