Tikrit Journal of Pure Science Vol. 30 (2) 2025

DOI: https://doi.org/10.25130/tjps.v30i2.1667

IRAQI Academic Scientific Journals

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: https://tjpsj.org/

Tikrit Journal of Pure Science

Evaluation of *Entamoeba histolytica* In Stool Samples of Children in Baghdad City Using of Polymerase Chain Reaction (PCR)

Haneen Mohanad Maher

Department of Biology, College of Science, Tikrit University, Tikrit, Iraq

Received: 2 Jul. 2024 **Received in revised form:** 18 Sep. 2024 **Accepted:** 24 Sep. 2024

Final Proofreading: 13 Oct. 2024 **Available online:** 25 Apr. 2025

ABSTRACT

Amoebic dysentery is a frequent infectious disease that is acquired by contaminated food and water carrying the infective stage of the parasite. *Entamoeba histolytica* is a parasite that has spread internationally as a generating growing illness and death in underdeveloped nations. The disease is considered more frequent in conditions were insufficient cleanliness and congested population is present. Although the first diagnostic methods of the parasite in infected patient is microscopy, it is not feasible to depend on this approach since it is not able to discriminate between amoebic forms that imitate this parasite. Thus, the requirement for a more advanced approach to offer accurate diagnosis of the parasite is important to represent the genuine frequency of the parasite. The present research includes the assessment of (220) fecal samples from children under (15 years) over the period of 1st December 2023 to 1st of February 2024. It involves microscopic inspection of fecal samples confirmation of diagnosis with two distinct Enzyme Linked Immunosorbent Assay test (ELISA) that catch *E. histolytica*/disbar and *E. histolytica* alone. Also, microscopic positive samples were submitted to nucleic acid identification of *E. histolytic* by Real Time-Polymerase Chain Reaction (RT-PCR) The findings indicated that the proportion of microscopic positive samples were 93(42.27 %), with males representing (68.82 %) and females by (32.18 %). The most afflicted age group was between (1-5 years) with an infectivity rate (47.31 %). Most of the patients with amoebic dysentery (66.67 %) were dwelling in urban areas, while (33.33 %) were from rural areas. Regarding *E. Histolytica* /dispar stool antigen ELISA, this test was positive in (63.44 %) of a total of (93) microscopy positive specimens with sensitivity and specificity of (73.17 %) and (96.42 %) correspondingly. On the other hand, *E. Histolytica* specific ELISA test was positive in (25.81 %) out of (93) microscopy positive fecal samples with a sensitivity and specificity of (69.28 %) and (97.91 %), respectively. As far as RT-PCR is involved, *E. histolytica* nucleic acid was positive in (20.44 %) out of (93) microscopy positive fecal samples. In conclusion, microscopy positive Entamoeba complex is a crude mean of detection of Entamoeba complex and diagnosis and should always be validated using better means like ELISA or PCR.

Keywords: *Entamoeba histolytica*, Stool samples, ELISA, PCR

Name: **Hasan Mohammed Qasim** **E-mail:** **hanin.m.maher@tu.edu.iq**

تقييم الكشف عن المتحولة الحالة للنسيج في عينات براز الأطفال في مدينة بغداد باستخدام تفاعل البلمرة المتسلسل

حنين مهند ماهر

قسم علوم الحياة، كلية العلوم جامعة تكريت، تكريت، العراق

الملخص

الزحار الأميبي هو مرض معد شائع تتم الإصابة به من خلال تناول الأغذية والمياه الملوثة التي تؤوي الطور المعدي للطفيلي. المتحولة الحالة للنسيج (*Entamoeba histolytica*) هو طفيلي منتشر على مستوى العالم ويسبب زيادة معدلات الإصابة بالأمراض والوفيات في البلدان النامية، كما ان عوامل سوء النظافة والكثافة السكانية العالية تزيد من انتشار المرض وصعوبة السيطرة عليه. على الرغم من أن طريقة التشخيص الأولية للطفيل لدى المريض المصاب هي الفحص المجهري، إلا أنه لا يمكن الاعتماد على هذه الطريقة حيث صعوبة التمييز بين الشكل الأميبي الذي يحاكي هذا الطفيل وبالتالي، فإن الحاجة إلى طريقة أكثر تقدمًا لتوفير تشخيص دقيق للطفيلي أمر ضروري لتعكس الانتشار الحقيقي للطفيلي. تضمنت الدراسة الحالية فحص (220) عينة براز من أطفال تقل أعمارهم عن (15 عامًا) خلال الفترة من 1 ديسمبر 2023 حتى 1 فبراير 2024. تضمن الفحص المجهري اختبارين باستخدام تقنية ELISA لعينات البراز تأكيد تشخيص أصابتهم بـ *E. histolytic/disbar* و *E. histolytic alone*. تم تقديم عينات مجهرية إيجابية للكشف عن الحمض النووي لطفيلي *E. histolytic* عبر Real Time PCR. أظهرت البيانات أن نسبة العينات الإيجابية المجهرية بلغت (42.27 %)93 بنسبة ذكور (68.82 %) والإناث (32.18 %). وكانت الفئة العمرية الأكثر تأثراً هي الأطفال ما بين 1–5 سنوات، وبلغت نسبة الإصابة بالمرض (47.31 %) معظم المرضى الذين يعانون من الزحار الأميبي (66.67 %) يقيمون في المناطق الحضرية (33.33 %) كانوا من المناطق النائية. فيما يتعلق بـ *E. Histolytica*/مستضد البراز المتباين ELISA كان هذا الاختبار إيجابيا في (63.44 %) من إجمالي (93) عينة مجهرية إيجابية مع حساسية ونوعية (73.17 %) و (96.42 %) على التوالي. من ناحية أخرى، كانت مقايسة ELISA النوعية لـ *E. Histolytica* إيجابية في (25.81 %) من أصل (93) عينة برازية إيجابية مع حساسية ونوعية (69.28 %) و(97.91 %) على التوالي. بقدر ما يتعلق الأمر بـ RT–PCR، كان الحمض النووي لطفيلي *E. Histolytica* إيجابيًا في (20.44 %) من أصل (93) عينة برازية إيجابية. في الختام، فإن الفحص المجهري الإيجابي لمركب المتحولة هو وسيلة بدائية للكشف عن مركب المتحولة ويجب تأكيد التشخيص دائمًا باستخدام طريقة متفوقة مثل ELISA أو PCR.

INTRODUCTION

Amoebiasis is the term for Entamoeba histolytica infection, both with or without scientific signs and symptoms [(1)]. Amoebiasis is one of the maximum unusual parasite international sicknesses [(2, 3)]. Even though the parasite has unfolded over the sector, large occurrence quotes of more than (10 %) of the population were reported in some growing international locations [(4-6)]. *E. Histolytica* is typically observed in humans and is greater popular in tropical and subtropical populations. When ingested, cysts from fecal infection may additionally contaminate meals and water [(7, 8)].

The disease is considered as the second well-known motive of mortality from parasitic diseases[(9)]. Although amoebiasis affects more than 500 million human beings yearly, signs and symptoms most

effective appear in (10 %) of instances [10]. Human intestinal lumen can exist in six species of Entamoeba (*E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. coli*, and *E. hartmanni*). The other species are believed to be nonpathogenic, however simple *E. histolytica*, it is absolutely related to medical effects in humans [11, 12]. There are a multitude of medical signs and symptoms, which includes amoebic dysentery, asymptomatic colonization, and invasive extraintestinal amoebiasis—which are frequently manifested as liver abscesses—which can be introduced by way of *E. histolytica*. Invasive ailments declare the lives of 100,000 human beings yearly, affecting around 50 million people [13,14]. Genetic distinctions have made it viable to split *E. dispar* and *E. histolytica* from one another. Despite sharing the same morphology, the genetic composition and mechanisms of action of the two protozoa are different. *E. dispar* colonizes the frame asymptomatically and does no longer need scientific remedy, but *E. histolytica* is invasive and can motivate situations including colitis and liver abscesses [15,16]. The analysis of amoebic colitis calls for the presence of *E. histolytica* inside the stool or colonic mucosa of the patient. The use of microscopic parasite detection in stool for amoebiasis prognosis is insensitive and is not differentiated among the invasive parasite *E. histolytica* and the commensal parasite *E. dispar*[17, 18]. Since microscopic inspection is not able to distinguish between these species, it is no longer needed to be used to diagnose amoebiasis. Sensitive and targeted molecular strategies that distinguish *E. dispar* from *E. histolytica* have evolved lately. These methods consist of the usage of stool sample lifestyles followed by way of isoenzyme evaluation, PCR to amplify amoebic DNA, and an enzyme-linked immunosorbent check (ELISA) to pick out an *E. histolytica* antigen [19-21].

To some distance, a huge range of molecular diagnostic assays, inclusive of serological techniques, have been used for amoeba immunodiagnosis. Complement fixation (CF), the amoebic gel diffusion, the indirect fluorescence assay (IFA), the enzyme linked-immunosorbent assay (ELISA), the oblique hemagglutination (IHA), and counter immuno-electrophoresis (CIE) are a number of these techniques. Real-time PCR has been shown to be the maximum touchy test for figuring out *E. histolytica* in feces when compared to the sensitivities of traditional nested PCR[22]. Furthermore, *E. dispar* and *E. histolytica* in scientific samples may be diagnosed and differentiated using ELISA, which has been designed for this reason. These genetic methods have led to a reevaluation of the amoeba epidemiology in phrases of occurrence and morbidity, in particular in the ones located within excessive endemic rates [23-25].

MATERIALS AND METHODS

Feces samples had been accrued from 220 children below the age of (15) who were introduced to the Pediatric Hospital in Baghdad City due to signs and symptoms of diarrhea and/or stomach disenchanted during the present-day study, which lasted from January 12, 2023, to January 2, 2024. To further discover microscopy-superb samples utilizing DRG ELISA based antigen detection of *E. histolytica* / *E. dispar*, nice specimens were investigated regarding the use of TechLab *E. histolytica* II monoclonal ELISA primarily based antigen detection. Positive amoebic check samples had been submitted for PCR identity of the *E. histolytica* gene and DNA extraction .

Stool samples were analyzed using a wet mount preparation to check for trophozoites and/or cysts of *E. dispar* and/or *E. histolytica*. There were two prepared slides for each sample. After adding a little amount of normal saline to one of the slides, the sample was well mixed with a wooden stick. Lugol's iodine (1 %), ordinary saline (0.9 %), and direct wet mount (90) made it possible to identify the parasite with precision.

The isolated DNA from *E. histolytica* positive antigen samples were amplified by PCR using an improved protocol. The amplification process was performed in an Italian-made Sacace Biotechnologies Sacycler qPCR thermal cycler. The Carboxyfluorescein FAM channel was chosen for gene detection in order to show the target DNA's propagation, and the apparatus was built and calibrated to carry out the amplification process under ideal circumstances.

Statistical analysis: The statistical analysis was carried out using the analytical program Graph Pad Prism, and comparisons were done using tTest and Chi-square as needed. If the P value was less than (0.05), the data difference was deemed significant, and if it was more than (0.05), the data were deemed non-significant.

RESULTS AND DISCUSSION

Results

To decide the prevalence of *E. histolytica* / *E. dispar* in children and babies with diarrhea, 220 stool specimens have been analyzed. The research protected each widespread microscope inspection and complex techniques to detect amoebic dysentery, which includes ELISA, which could perceive both *E. histolytica* and *E. dispar* alone. Additionally, high-quality cases diagnosed by using ELISA were established using RT PCR, which amplifies genomic DNA particular to *E. histolytica*.

According to our results, 93 (42.27%) of the (220) stool samples that underwent microscopy and iodin coaching analysis (Table 1) examined high quality for E. Histolytica/E. Dispar cysts and trophozoites, while the remaining 127 (57.73 %) examined poor for amoebia as in Table (1). The trophozoite stage of the numerous amoebic form levels become observed to have the most important percentage (65.59 %), followed through the cystic and trophozoite ranges (25.88 %), while the cystic shape represented (8.60 %), as (Table 2) illustrates.

Table 1: The proportion of positive to negative specimens for *E. dispar*/*E. histolytica*.

E. histolytica / *dispar*	No.	%
Positive	93	42.27
Negative	127	57.73
Total	220	100.00

Table 2: Microscopic Phases of *E. dispar* / *E. histolytica* in positive specimens.

Amoebic stages	No. positive	Positive %
Trophozoite	61	65.59
Trophozoite and cyst	24	25.88
Cyst	8	8.60
Total	93	100.00

Table (3) suggests that 64 children (82 %) had a higher incidence of *E. histolytica* / *E. dispar* contamination than (29) girls (31.18 %). The statistical analysis, which used chi-square (X^2), found out a huge distinction between the genders with (P=0.013).

Table (3): Distribution of gender in *E. histolytica* and *E. dispar*.

Gender distribution	distribution No.	No. positive	Positive %	P value
Male	126	64	68.82	0.013
Female	94	29	31.18	

According to Table (4), the bulk of amoebiasis patients (66.67 %) were city dwellers, with the remaining individuals (33.33 %) being from rural areas. When (P=0.047) was applied with Chisquare (X^2), the yield was dramatically altered.

Table 4: Distribution of *E. histolytica* and *E. dispar* according to residents.

infection residency	No. examined	No. Positive	Positive %	P value
Urban	141	62	43.97	
Rural	79	31	39.24	**0.047**

DOI: https://doi.org/10.25130/tjps.v30i2.1667

Table (5) exhibited the percentage of positive specimens that were tested with *E. histolytica* / *E. dispar* ELISA, as these samples were microscopy positive and further confirmed with DRG ELISA. Out of (93) stool specimens, 59(63.44 %) were positive, while the remaining specimens 34(36.56%) were negative despite tested positive by microscopy. The DRG stool ELISA revealed sensitivity and specificity (73.17 % and 96.42 %) respectively and predictive value of (98.97 %)

Table 5: *E. histolytica* and *E. dispar* antigen found in stool samples using DRG ELISA.

DRG ELISA *E. histolytica/ dispar*	No.	%
Positive	59	63.44
Negative	34	36.56
Total	93	100.00
Sensitivity	Sensitivity	
Specificity	96.42%	
Predictive value	98.97%	

The cutting-edge end result established that every specimen had superb microscopy consequences and have been submitted for amoebic DNA genetic extraction and in addition to the amplification of the *E. histolytica* particular gene utilizing Tech-Lab ELISAs as well as DRG. Table 6 and Figure 1 show that of the 93 specimens that exceeded microscopy best, 19 (20.44 %) tested effective for the *E. histolytica* genome by way of RT PCR, while the ultimate 74(79.50 6 %) had no detectable *E. histolytica* DNA. The RT PCR device's FAM channel became used for the amplification method with the intention to pick out amoebic DNA.

Table 6: RT PCR amplification for the identification of the *E. histolytica* gene in fecal specimens.

RT PCR for E. Histolytica	No.	%
Positive	19	20.43
Negative	74	79.57
Total	24	100.00
Sensitivity	74.22%	
Specificity	96.74%	
Predictive value	96.91%	

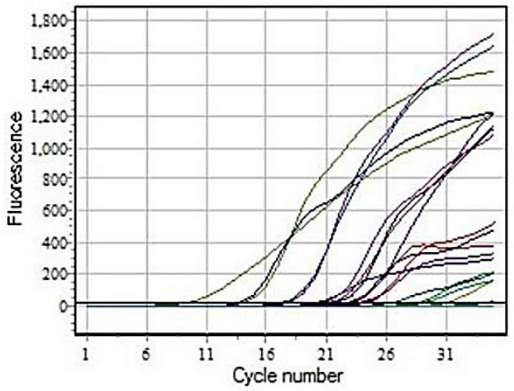

Fig. 1: RT- PCR amplification of *E. histolytica* gene on FAM channel.

Our results revealed that different methods of diagnosis of patient's infected with amoebiasis could have a variable result as shown in Table (7). Microscopy positive E. histolytica/dispar were (42.27 %), DRG ELISA for *E. histolytica*/ *E. dispar* positive result were (63.44 %), while TechLab ELISA has produced (25.81 %) positive *E. histolytica*. On the other hand, RT PCR results were only (20.44 %). Chi-square analysis was applied and yielded significant difference the method of diagnosis with (P=<0.0001).

Table 7: Comparison of different techniques for *E. histolytica* diagnostic.

Examination method	Total No.	+ve	%	−ve	%	P value
Microscopy wet mount	220	93	42.27	127	57.73	
DRG E. Histolytica / E. dispar stool antigen	93	59	63.44	34	36.56	<0.0001
TechLab E. Histolytica II stool antigen	93	24	25.81	69	74.19	
RT PCR for E. histolytica	93	19	20.44	74	79.56	

Discussion

The current studies used microscopy to detect the presence of *E. histolytica*/ *dispar* in stool samples from children underneath the age of (15). Following an ELISA test that identified both *E. histolytica* and *E. dispar*, the specimens were transferred to an

ELISA that most effective diagnosed *E. histolytica* in stool samples if the check ended in an advantageous result. It also established that the DNA specific to *E. histolytica* became a gift within the superb pattern from those microscopies.

It is clear that (42.27 %) of the (220) stool specimens have been microscopically positive. This end result is in line with Uslu et al., who tested that stool specimens assessed by using trichrome staining had a (31.1 %) presence price of *E. histolytica*/ *dispar*. Our outcomes, however, struggle with a study by means of Das *et al*. This observed that stool samples from handiest three. (17 %) of tested patients were microscopy-positive and that stool samples from numerous indicated the presence of *E. histolytica*/ *dispar*[26]. It is also different from what isconducted in Malaysia with the aid of Ngui *et al*. 2012, who discovered that (17.6 %) of the samples exhibited amoebic shape which changed into microscopy-wonderful. Furthermore, previous studies have proven a high-quality prevalence of Entamoeba contamination in rural areas, with rates varying among (9.4 %) and (21.0 %) [11, 27].

Because amebiasis microscopy detection by and large depends on man or woman expertise, there can be discrepancies within the facts displayed above. As an end result, there can be fake advantageous or even fake poor reviews of the amoebic shape, and no precise facts can be produced through microscopic exam. Furthermore, when you consider that only a tiny portion of the stool material may be utilized, most preparation tactics for microscopic detection do not cover the complete specimen, even supposing large volumes of s may want to cowl more types of stools of the parasite. Nevertheless, although being on hand in many healthcare settings, microscopy nonetheless lacks the required sensitivity and from time-to-time specificity [28-30]. As it cannot distinguish between great amoebic species based totally on morphological tendencies, most medical facilities offer microscopy to resource within the identity of the amoebic shape; but it does require a positive level of knowledge, and similar affirmation of an acceptable result is essential to permit correct occurrence of the parasite and represent the authentic photograph of ailment occurrence [31].

This is consistent with the results of Al-Damerchi *et al*. 2016, who tested that the sensitivity and specificity of microscopic examination had been (ninety-one, 44, and 61 %), respectively, and that the accuracy of wet mount turned into (60 %). Research on antigen detection and microscopic inspection have shown that ELISA and PCR-based totally antigen detection strategies are more sensitive and specific than microscopy within the identification of Entamoeba species [32, 33]. This is constant with the findings of these research. Therefore, reference labs must offer a higher technique than microscopy to detect parasites, and identity of the parasite must now not rely upon microscopic detection.

In terms of age distribution, the observer's conclusions confirm that youngsters less than 5 had the very best infection fee. These effects agree with the ones of Hamza *et al* 2021. [who pronounced a comparable infection occurrence in patients less than five years of age[15]. It does, however, go counter to a have a look at by using Flaih *et al* who discovered an extra contamination price inside the age variety of five to fourteen (5-14). Additionally, the examiner via Ngui et al. discovered that contamination charges differed by age category, with person prices (23.9 %) being more than the ones of children (15.3 %) [34, 35].

Aside from the core ELISA idea used within the assessment, which would possibly show versions in specificity and sensitivity[36], the fundamental purpose of stool antigen ELISA is to cap either *E. histolytica*/ *dispar* or *E. histolytica* by myself so that it is to stumble on the presence of any amoebic. There should be extremely good specificity in both situations. Disparate, moshkoviskii, and hitolytica[36,37] are the 3 Entamoeba species which can most be effectively identified by means of

microscopy, in assessment to the previous. The ELISA used to perceive *E. histolytica*/ The test for detecting may additionally have a decrease specificity fee considering it as handiest and able to be detected out of the (3 species). Furthermore, in order for the package to discover the presence of amoebic antigen inside the specimens underneath examination, it must have a selected load of the parasite; in any other case, incorrect or inadequate pattern reconstitution would possibly provide a fake bad end result [38, 39]. Furthermore, fake superb or fake negative consequences are viable because the ELISA approach necessitates that trained laboratory group of workers adhere to the ELSA protocol. Technicians lacking understanding may extensively contribute to the quantity of fake data accrued by using ELISA[40-42]. Furthermore, the antibody coat in each ELISA kit reacts to a particular goal; accordingly, fake negative findings could be generated if the antigen degree in the sample became below what can be detected. Because of this, an ELISA test has sure negative aspects despite its credible specificity and reliability, which includes the ability for infection from incorrect nicely washing strategies, errors within the addition of reagents, sample dilution, and contamination of wells with samples from other wells as a result of subpar technique [43-45].

There are a number of factors that may contribute to the high frequency of *E. histolytica*, including poor hygiene, tainted food and water, and direct transmission. Additionally, environmental, economic, and social reasons as well as malnutrition, dramatically increase children's vulnerability to *Entamoeba histolytica*. Lack of sanitary restrooms are a contributor to this situation[26, 46]. The results of the current study differ from those of previous studies, which may be attributable to differences in the effectiveness of the sewer system, personal hygiene, population density, geographic location, the number of tested samples, the length of the study, living habits, and ages [47].

Parasite from liquid stool is more readily extracted in the process of DNA extraction of parasite genome than semisolid stool and consequently less parasites will be obtained in the extraction process from those with semisolid ones. Another factor that may affect the PCR result is contamination of the reagents dedicated for PCR amplification or inadequate experience in the flow of the PCR steps can significantly alter the result interpretation and generate false data [48-50].

The PCR is considered a gold standard confirmatory method and it ranks superior to other techniques as it has the height specificity and sensitivity and the methods is labor intensive with limited time for generating the results with high accuracy and accendibility. Although this method is specific and sensitive still it has some sophisticated requirements like, PCR machine which is fairly expensive, extraction device and kits, specific primers to amplify the target genome, skilled laboratory staff to apply the master mix for the reaction and programming the thermocycler, in addition, data interpretation does require experts' personnel to approve the data [51, 52]. These factors are not abundant and not easily accessible so most of the reports are prone to personal judgement to decide whether the specimen positive for amoebic form or neglected as negative sample.

CONCLUSION

The common diagnostic tool in the health sector relies on microscopic examination and this method is not satisfactory as it cannot discriminate between pathogenic and nonpathogenic amoebas. Since PCR era is a gold standard method with high specificity and sensitivity, it could be used to assist in genetically differentiating between numerous species of amoebas. PCR era is commonly utilized in Iraqi hospitals.

Conflict of interests: The authors declared no conflicting interests.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

REFERENCES

1. Salih JM, Hassan AO, Al-Saeed ATMJJoDU. Intestinal parasites and associated risk factors among primary school children in Duhok city-Kurdistan region/Iraq. 2022;25(2):50-6. https://doi.org/10.26682/sjuod.2022.25.2.5

2. Solaymani-Mohammadi S, Lam MM, Zunt JR, Petri Jr WAJTotRSoTM, Hygiene. Entamoeba histolytica encephalitis diagnosed by PCR of cerebrospinal fluid. 2007;101(3):311-3. https://doi.org/10.1016/j.trstmh.2006.05.004

3. Zeyrek FY, Turgay N, Ünver A, ÜSTÜN S, Akarca U, Töz SJTPD. Differentiation of Entamoeba histolytica/Entamoeba dispar by the polymerase chain reaction in stool samples of patients with gastrointestinal symptoms in the Sanliurfa Province. 2013;37(3):174. https://doi.org/10.5152/tpd.2013.39

4. Ali MAJIJoFM, Toxicology. A Comparison of use of Microscopic Examination and PCR for Detection of Entamoeba Histolytica at Wasit Province. 2021;15(4). https://doi.org/10.37506/ijfmt.v15i4.16759

5. Bayoumy AMS, Elkeiy MTI, Zaalok TKI, Gad HM, Abd Elhamid WAM. Coproantigen Versus Classical Microscopy as a Diagnostic Tool for Entamoeba histolytica Infection in the Egyptian Patients %J The Egyptian Journal of Hospital Medicine. 2019;74(6):1423-7. https://dx.doi.org/10.21608/ejhm.2019.26982

6. AlAyyar RM, AlAqeel AA, AlAwadhi MSJJopr. Prevalence of Giardiasis and Entamoeba Species in Two of the Six Governorates of Kuwait. 2022;2022(1):5972769. https://doi.org/10.1155/2022/5972769

7. Bahrami F, Haghighi A, Zamini G, Khademerfan MJE, Infection. Differential detection of Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii in faecal samples using nested multiplex PCR in west of Iran. 2019;147:e96. https://doi.org/10.1017/s0950268819000141

8. Luo H, Jia T, Chen J, Zeng S, Qiu Z, Wu S, et al. The characterization of disease severity associated IgG subclasses response in COVID-19 patients. 2021;12:632814. https://doi.org/10.3389/fimmu.2021.632814

9. Dolabella SS, Serrano-Luna J, Navarro-García F, Cerritos R, Ximénez C, Galván-Moroyoqui JM, et al. Amoebic liver abscess production by Entamoeba dispar. 2012;11(1):107-17. DOI: 10.1016/S1665-2681(19)31494-2

10. Hooshyar H, Rostamkhani PJG, Bench HfBt. Accurate laboratory diagnosis of human intestinal and extra-intestinal amoebiasis. 2022;15(4):343. https://journals.sbmu.ac.ir/ghfbb/index.php/ghfbb/article/view/2496

11. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness JJCmr. Laboratory diagnostic techniques for Entamoeba species. 2007;20(3):511-32. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1932757/

12. Jackson TJIjfp. *Entamoeba histolytica* and *Entamoeba dispar* are distinct species; clinical, epidemiological and serological evidence. 1998;28(1):181-6. https://pubmed.ncbi.nlm.nih.gov/9504344/

13. Medina-Rosales MN, Muñoz-Ortega MH, García-Hernández MH, Talamás-Rohana P, Medina-Ramírez IE, Salas-Morón LG, et al. Acetylcholine upregulates Entamoeba histolytica virulence factors, enhancing parasite pathogenicity in experimental liver amebiasis. 2021;10:586354. doi: 10.3389/fcimb.2020.586354

14. Sardar SK, Ghosal A, Haldar T, Maruf M, Das K, Saito-Nakano Y, et al. Prevalence and molecular characterization of Entamoeba moshkovskii in diarrheal patients from Eastern India. 2023;17(5):e0011287.

https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0011287

15. Hamza DM, Ali Malaa SF, Alaaraji KKJM-LU. Real-Time-PCR Assay Based on Phosphoglycerate Kinase Gene for Detection of Entamoeba histolytica Trophozoites in Stool Samples in Holy Karbala, Iraq. 2021;21(1).
HTTPS://DOI.ORG/10.37506/MLU.V21I1.2558

16. Chou A, RL A. Entamoeba histolytica Infection.: StatPearls Publishing; National Library of Medicine.2024 Jan.
https://www.ncbi.nlm.nih.gov/books/NBK557718/.

17. El-Sheikh SM, El-Assouli SMJJoH, Population, Nutrition. Prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Jeddah, Saudi Arabia. 2001:25-30.
https://pubmed.ncbi.nlm.nih.gov/11394180/

18. Flores MS, Carrillo P, Tamez E, Rangel R, Rodríguez EG, Maldonado MG, et al. Diagnostic parameters of serological ELISA for invasive amoebiasis, using antigens preserved without enzymatic inhibitors. 2016;161:48-53.
https://pubmed.ncbi.nlm.nih.gov/26920240/

19. Ghosh S, Padalia J, Moonah SJCcmr. Tissue destruction caused by Entamoeba histolytica parasite: cell death, inflammation, invasion, and the gut microbiome. 2019;6:51-7.
https://pubmed.ncbi.nlm.nih.gov/31008019/

20. Hartmeyer G, Hoegh S, Skov M, Dessau R, Kemp MJJopr. Selecting PCR for the Diagnosis of Intestinal Parasitosis: Choice of Targets, Evaluation of In-House Assays, and Comparison with Commercial Kits. 2017;2017(1):6205257.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5695021/

21. Kadir MA, El-Yassin ST, Ali AJTJoPS. Detection of Entamoeba histolytica and Giardia lamblia in children with diarrhea in Tikrit city. 2018;23(6):57-64.
https://doi.org/10.25130/tjps.v23i6.672

22. Gomes TdS, Garcia MC, Souza Cunha Fd, Werneck de Macedo H, Peralta JM, Peralta RHSJTSWJ. Differential Diagnosis of Entamoeba spp. in Clinical Stool Samples Using SYBR Green Real-Time Polymerase Chain Reaction. 2014;2014(1):645084.
https://pubmed.ncbi.nlm.nih.gov/14557296/

23. saad Dahhaam S, Mohammed SAJTJoPS. Assessment of some immunological parameters for patients with diabetes mellitus infected with Entamoeba histolytica. 2022;27(4):1-6.
https://doi.org/10.25130/tjps.v27i4.26

24. Ahmed HM, Al-Nasiri FSJTJoPS. Serum level of Interleukin (IL)-8, Monocyte Chemoattractant Protein (MCP)-1 and Tumor Necrosis Factor (TNF)-α in children infected with Entamoeba histolytica. 2019;24(4):29-33.
https://doi.org/10.25130/tjps.v24i4.393

25. Uslu H, Aktas O, Uyanik MHJTEjom. Comparison of various methods in the diagnosis of Entamoeba histolytica in stool and serum specimens. 2016;48(2):124.
https://pubmed.ncbi.nlm.nih.gov/27551171/

26. Blessmann J, Buss H, Nu PAT, Dinh BT, Ngo QTV, Van AL, et al. Real-time PCR for detection and differentiation of Entamoeba histolytica and Entamoeba dispar in fecal samples. 2002;40(12):4413-7.
https://pubmed.ncbi.nlm.nih.gov/12454128/

27. Ngui R, Angal L, Fakhrurrazi SA, Lian YLA, Ling LY, Ibrahim J, et al. Differentiating Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii using nested polymerase chain reaction (PCR) in rural communities in Malaysia. 2012;5:1-7.
https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-5-187

28. Dans LF, Martínez EGJBce. Amoebic dysentery. 2007;2007.

29. Lin F-H, Chen B-C, Chou Y-C, Chien W-C, Chung C-H, Hsieh C-J, et al. The Epidemiology of Entamoeba histolytica Infection and Its Associated Risk Factors among Domestic and Imported Patients in Taiwan during the 2011–2020

Period.2022;58(6):820. https://doi.org/10.3390/medicina58060820
30. Shimokawa C, Kabir M, Taniuchi M, Mondal D, Kobayashi S, Ali IKM, et al. Entamoeba moshkovskii is associated with diarrhea in infants and causes diarrhea and colitis in mice. 2012;206(5):744
51. https://doi.org/10.1093/infdis/jis414
31. Ximénez C, González E, Nieves M, Magaña U, Morán P, Gudiño-Zayas M, et al. Correction: Differential expression of pathogenic genes of Entamoeba histolytica vs E. dispar in a model of infection using human liver tissue explants. 2019;14(1):e0210895.
https://doi.org/10.1371/journal.pone.0210895
32. Al-Damerchi ATN, Al-Ebrahimi HNJA-QMJ. Detection of major virulence factor of Entamoeba histolytica by using PCR technique. 2016;12(21):36-45.
https://doi.org/10.28922/qmj.2016.12.21.36-45
33. Pinilla AE, Lopez MC, Viasus DFJRMC. History of the Entamoeba histolytica protozoan. 2008;136(1):118-24.
http://dx.doi.org/10.4067/S0034-98872008000100015
34. Flaih MH, Khazaal RM, Kadhim MK, Hussein KR, Alhamadani FABJE, health. The epidemiology of amoebiasis in Thi-Qar Province, Iraq (2015-2020): differentiation of Entamoeba histolytica and Entamoeba dispar using nested and real-time polymerase chain reaction. 2021;43.
https://pubmed.ncbi.nlm.nih.gov/33971701/
35. Verma AK, Verma R, Ahuja V, Paul JJBm. Real-time analysis of gut flora in Entamoeba histolytica infected patients of Northern India. 2012;12:1-11.
https://doi.org/10.1186/1471-2180-12-183
36. Tanyuksel M, Petri Jr WAJCmr. Laboratory diagnosis of amebiasis. 2003;16(4):713-29.
https://pubmed.ncbi.nlm.nih.gov/14557296/
37. Mahdi MS, Seraj BL, 43(4):321-342. . Entamoeba histolytica Virulence Factors. Iraqi J. 2020;4(43):321-42.
https://doi.org/10.1016/s1369-5274(99)80076-9
38. Mendoza Cavazos C, Knoll LJJPp. Entamoeba histolytica: Five facts about modeling a complex human disease in rodents. 2020;16(11)
https://doi.org/10.1371/journal.ppat.1008950
39. Babuta M, Bhattacharya S, Bhattacharya AJPP. Entamoeba histolytica and pathogenesis: A calcium connection. 2020;16(5):e1008214.
https://dx.doi.org/10.1371/journal.ppat.1008214
40. Padilla-Vaca F, Anaya-Velázquez FJID-DT. Insights into Entamoeba histolytica virulence modulation. 2010;10(4):242-50.
https://dx.doi.org/10.2174/1871526107915916384
41. Servián A, Zonta ML, Navone GTJRadm. Differential diagnosis of human Entamoeba infections: Morphological and molecular characterization of new isolates in Argentina. 2024;56(1):16-24.
HTTPS://DOI.ORG/10.1016/J.RAM.2023.05.003
42. Ximénez C, González E, Nieves M, Magaña U, Morán P, Gudiño-Zayas M, et al. Differential expression of pathogenic genes of Entamoeba histolytica vs E. dispar in a model of infection using human liver tissue explants. 2017;12(8):e0181962.
http://dx.doi.org/10.1371/JOURNAL.PONE.0210895
43. Que X, Reed SLJCmr. Cysteine proteinases and the pathogenesis of amebiasis. 2000;13(2):196-206.
https://pubmed.ncbi.nlm.nih.gov/10458982/
44. Ralston KS, Solga MD, Mackey-Lawrence NM, Somlata, Bhattacharya A, Petri Jr WAJN. Trogocytosis by Entamoeba histolytica contributes to cell killing and tissue invasion. 2014;508(7497):526-30.
https://doi.org/10.1038/nature13242
45. Nichols GL. Entamoeba histolytica. In: Smithers GW, editor. Encyclopedia of Food Safety (Second Edition). Oxford: Academic Press; 2024. p. 480-8.
46. Petri Jr WA, Schaenman JM. WATERBORNE PARASITES| Entamoeba. 1999.
https://pubmed.ncbi.nlm.nih.gov/18952091/
47. Saidin S, Othman N, Noordin RJEJoCM, Diseases I. Update on laboratory diagnosis of amoebiasis. 2019;38:15-38.

https://pubmed.ncbi.nlm.nih.gov/30255429/

48. Al-Shaibani SW, editor Infection with Entamoeba histolytica and its effect on some blood parameters in Najaf City. Journal of Physics: Conference Series; 2020: IOP Publishing.: http://dx.doi.org/10.1088/1742-6596/1660/1/012008

49. Wilson I, Weedall G, Hall NJPI. Host–Parasite interactions in Entamoeba histolytica and Entamoeba dispar: what have we learned from their genomes? 2012;34(2-3):90-9. https://doi.org/10.1111/j.1365-3024.2011.01325.x

50. Shirley D-AT, Farr L, Watanabe K, Moonah S. A Review of the Global Burden, New Diagnostics, and Current Therapeutics for Amebiasis. Open Forum Infectious Diseases. 2018;5(7):ofy161. https://doi.org/10.1093/ofid/ofy161

51. Shirley D-AT, Farr L, Watanabe K, Moonah S, editors. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Open forum infectious diseases; 2018: Oxford University Press US. https://pubmed.ncbi.nlm.nih.gov/30046644/

52. Singh A, Banerjee T, Khan U, Shukla SKJPntd. Epidemiology of clinically relevant Entamoeba spp. (E. histolytica/ dispar/ moshkovskii/ bangladeshi): A cross sectional study from North India. 2021;15(9): HTTPS://DOI.ORG/10.1371/JOURNAL.PNTD.0009762