Molecular Diagnosis and Phylogenetic Investigation of *Entamoeba histolytica* and *Giardia intestinalis* among Diarrheal Patients in Iraq

Ola Nasrat A. Al-Rawi* ⓘD, Najah Subhi N. Al-Omer ⓘD

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

ABSTRACT

Background and Aim: *Entamoeba histolytica* and *Giardia intestinalis* are two common parasites associated with gastrointestinal infections and diarrheal diseases worldwide. This study investigated the prevalence, molecular characterization, and phylogenetic relationships of *E. histolytica* and *G. intestinalis* among patients with diarrhea and intestinal disorders in hospitals across Iraq.

Materials and Methods: Stool samples were collected from individuals presenting with gastrointestinal symptoms, and a microscopic examination was performed to screen for the presence of *E. histolytica* and *G. intestinalis*. DNA was extracted from positive samples, and PCR amplification targeting the 16S rRNA gene for *E. histolytica* and the triose phosphate isomerase (TPI) gene for *G. intestinalis* was carried out. The resulting PCR products were subjected to agarose gel electrophoresis, purified, and sequenced. Phylogenetic trees were constructed using the Maximum Likelihood method based on the Tamura-Nei model, and bootstrap analysis was performed.

Results: Among the studied samples, 27.8% (n=85) were positive for *E. histolytica*, while 19.6% (n=60) were positive for *G. intestinalis*. Sequence analysis confirmed the presence of *E. histolytica* and *G. intestinalis* by matching the obtained sequences with those available in the NCBI gene bank. The phylogenetic analysis revealed the genetic diversity and relatedness of the *E. histolytica* and *G. intestinalis* isolates. Clustering patterns specific to the Iraqi population were observed, suggesting potential regional transmission dynamics.

Conclusion: This study provides valuable insights into the prevalence, molecular characterization, and phylogenetic relationships of *E. histolytica* and *G. intestinalis* in Iraqi patients with diarrhea and intestinal disorders.

Keywords: *Entamoeba histolytica*, *Giardia intestinalis*, Diarrhea, Intestinal Disorders, Molecular Characterization, Phylogenetic Analysis

Ola Nasrat A A , Najah Subhi N A. Molecular Diagnosis and Phylogenetic Investigation of *Entamoeba histolytica* and *Giardia intestinalis* among Diarrheal Patients in Iraq. Iran J Med Microbiol. 2023; 17(3):361-8.

Use a device to scan and read the article online

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks

Send citation to: Mendeley Z Zotero RefWorks

1. Introduction

Intestinal parasitic infections are a global public health concern, particularly in developing countries **(1)**. The World Health Organization (WHO) reports that approximately 3.5 billion people worldwide are infected with intestinal parasites responsible for significant morbidity and mortality **(2)**. Intestinal parasitic infections pose significant health challenges worldwide, particularly in developing countries with inadequate sanitation and limited access to clean water **(3, 4)**. Among the numerous parasitic pathogens affecting the gastrointestinal tract, *E. histolytica* and

Giardia lamblia are two prominent causes of diarrhea and intestinal disorders **(5, 6)**. These parasites are responsible for substantial morbidity and mortality, particularly in regions where socio-economic conditions hinder effective disease control and healthcare delivery **(7, 8)**.

Iraq, a country with a history of conflict and limited resources, is confronted with a high burden of intestinal parasitic infections **(9, 10)**. The prevalence of *G. lamblia* infections remains a pressing concern,

especially among individuals presenting with diarrhea and intestinal disorders in hospitals across Iraq **(11)**. Only a little data is available regarding the prevalence of *E. histolytica.* According to the study conducted in 2011 by Hussein et al. **(12)**, the prevalence of *E. histolytica* among 730 fecal samples was 24.3%. It became over a decade; no studies have been performed on the prevalence and molecular detection of *E. histolytica* at the level of Iraq.

Conventional microscopy has long been the primary method for diagnosing intestinal parasitic infections **(13)**. However, its limitations in terms of sensitivity and specificity have led to the development and adoption of molecular techniques for improved diagnostic accuracy **(6)**. Polymerase chain reaction (PCR) and molecular-based assays offer enhanced sensitivity and specificity, enabling the detection of low-level infections and the differentiation of closely related species **(14)**. Such molecular techniques have proven invaluable in parasitology, allowing for rapid and reliable detection of *E. histolytica* and *G. lamblia* **(15)**.

Microscopy enables the direct visualization of parasites in clinical samples, allowing for immediate diagnosis **(16)**, while PCR amplifies specific DNA sequences of the parasites, improving diagnostic sensitivity and providing species-specific information **(17)**. Furthermore, gel electrophoresis facilitates the separation and visualization of PCR products, enabling the identification of subtypes within *E. histolytica* and *G. lamblia* populations **(18)**.

The current study aims to investigate the prevalence and diagnosis of *E. histolytica* and *G. lamblia* infections among individuals suffering from diarrhea and intestinal disorders in hospitals throughout Iraq. The study employs a comprehensive approach utilizing conventional microscopy and molecular examination

techniques, including PCR, to enhance diagnostic accuracy.

2. Materials and Methods

Study Design and Sample Collection

This study was a cross-sectional study conducted in hospitals in the city of Mosul, Iraq, between February 2022 and July 2023. Patients with diarrhea or other intestinal disorders who met the inclusion criteria (The inclusion criteria are people suffering from digestive disorders and diarrhea of varying severity, from the age of one year to the age of 70 years) were invited to participate in the study. Written informed consent was obtained from each participant or their parents/guardians if the participant was a child. A total of 371 stool samples were collected from consenting patients and transported to the laboratory in sterile containers.

Microscopic Examination

All stool samples were subjected to direct wet mount preparation, and saline and iodine mounts were used to detect the presence of *E. histolytica* and *G. lamblia* cysts and trophozoites. The samples were examined under a microscope by experienced technicians who were blinded to the participants' clinical information.

Molecular Examination

DNA was extracted from stool samples using a commercial kit (Qiagen, Germany) following the manufacturer's instructions. A set of primers was designed to amplify various fragments of gene specific for *E. histolytica* and *Giardia lamblia* (Table 1).

Table 1. The sequence of primers used for the detection of *E. histolytica* and *Giardia lamblia*

Name of parasite	The primer	Sequence (5'-3')	No. base pair	References
E. histolytica	UEH-F	TAAGATGCACGAGAGCGAAA	20	(19)
	UEH-R	GTACAAAGGGCAGGGACGTA	20	
	Ehis-F	ATGCACGAGAGCGAAAGCAT	20	(20)
	Ehis-R	GATCTAGAAACAATGCTTCTCT	20	
G. lamblia	AL3543	AAATIATGCCTGCTCGTCG	19	(21)
	AL3546	CAAACCTTITCCGCAAACC	19	
	AL3544	CCCTTCATCGGIGGTAACTT	20	
	AL3545	GTGGCCACCACICCCGTGCC	20	

DNA amplification

PCR amplification was performed in a 20 μL reaction volume containing [10 μL 2X master mix, 1 μL Forward primer, 1 μL Reverse primer, 6 μL PRC grade water, 2 μL DNA sample]. The PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 1 min., annealing at [55°C for each of AL3543, AL3544, AL3545 and AL3546, while 58°C was used for UEH and 50°C for Ehis] for 45 s, and extension at 72°C for 1 min., with a final extension at 72°C for 10 min. The PCR products were visualized on a 1.5% agarose gel electrophoresis using a safe stain. The gel band for PCR product of *E. histolytica* samples was 166 bp while the band size for *G. intestinalis* was 530 bp.

Sequencing

A subset of PCR products (13 samples for each parasite PCR product) was selected for sequencing to confirm the accuracy of the PCR results. The PCR products were purified using a PCR purification kit (Qiagen, Germany), and sequencing was performed using an automated DNA sequencer (ABI 3730, Applied Biosystems, USA). The obtained sequences were compared with sequences available in public databases using BLAST software.

Phylogenetic analysis

The phylogenetic analysis assessed the genetic diversity and relatedness of *E. histolytica* and *G. intestinalis* isolates. The obtained DNA sequences, including the 16S rRNA gene for *E. histolytica* and the triose phosphate isomerase (TPI) gene for *G. intestinalis*, were aligned with reference sequences using nucleotide Blast in NCBI. Phylogenetic trees were constructed using the Maximum Likelihood method with the Tamura-Nei model in software such as MEGA11. Bootstrap analysis with 1000 replicates was performed to evaluate the robustness of the inferred phylogenetic relationships. The resulting trees were visualized using tree visualization software, and interpretation was based on the clustering and genetic distances between sequences.

Data Analysis

Data were analyzed using SPSS version 22 (IBM, USA). Descriptive statistics were used to calculate the prevalence of *E. histolytica* and *G. lamblia* infections. The chi-square test was used to compare the prevalence of infections between different groups. P-values less than 0.05 were considered statistically significant.

3. Results

The results of laboratory tests for 371 collected samples showed that the percentage of infection with parasites was 212 samples, with a rate of (78%) for the amoeba parasite and (22%) for the giardia parasite. Among the infected samples, 166 samples were infected with *E. histolytica*, 46 samples were infected with *G. lamblia*, and 82 samples were co-infected with both parasites.

The prevalence of *E. histolytica* infection was significantly higher than that of *G. lamblia* infection (p < 0.001). The age and gender of the participants did not significantly affect the prevalence of infection with either parasite.

Sequencing results

Sequencing results for the TPI gene of *G. intestinalis* and 16S rRNA of E. histolytica isolates revealed the presence of the species in the samples. The obtained TPI gene sequences were submitted to the NCBI gene bank, and accession numbers were obtained for future reference (Figures 1 and 2).

To elucidate the genetic diversity and relationships of *E. histolytica* isolates from Iraq, sequencing of the 16S rRNA gene was performed. The resulting sequences were used to construct a phylogenetic tree using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software. Bootstrap analysis with 1000 re-samplings was conducted to assess the robustness of the tree. Partial DNA sequences of concatenated partial 16S rRNA gene were utilized as input data for the phylogenetic analysis (Figure 3).

The phylogenetic tree of *E. histolytica* demonstrated the evolutionary relationships among the isolates from Iraq. The tree provided insights into the genetic diversity and relatedness of the identified *E. histolytica* isolates. The bootstrap values indicated the confidence level of the branching patterns within the tree.

(Figure 3) displays the phylogenetic tree of *G. intestinalis* based on the TPI gene sequences. The tree depicts the grouping of the identified *G. intestinalis* isolates from the study samples and their placement within the broader evolutionary context of *G. intestinalis* isolates worldwide.

Giardia intestinalis isolate 3 triose-phosphate isomerase (TPI) gene, partial cds
Sequence ID: KY613606.1 Length: 530 Number of Matches: 1

Range 1: 33 to 528 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
895 bits(992)	0.0	496/496(100%)	0/496(0%)	Plus/Plus

```
Query  1    ATCGCTCGACTTCATTAAGAGCCACGTAGCGTCCATCGCCTCCCATAAGATCCCCGAGTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  33   ATCGCTCGACTTCATTAAGAGCCACGTAGCGTCCATCGCCTCCCATAAGATCCCCGAGTC  92

Query  61   CGTGGACGTTGTTGTTGCTCCCTCCTTTGTGCACCTTTCTACAGCTATTGCGGCGAACAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  93   CGTGGACGTTGTTGTTGCTCCCTCCTTTGTGCACCTTTCTACAGCTATTGCGGCGAACAC  152

Query  121  CTCGAAGTGTCTGAAAATAGCAGCACAGAACGTGTATCTGGAGGGGAACGGTGCATGGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  153  CTCGAAGTGTCTGAAAATAGCAGCACAGAACGTGTATCTGGAGGGGAACGGTGCATGGAC  212

Query  181  CGGCGAGACAAGCGTCGAGATGCTGCTGGACATGGGGCTGAGCCATGTAATAATAGGACA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  213  CGGCGAGACAAGCGTCGAGATGCTGCTGGACATGGGGCTGAGCCATGTAATAATAGGACA  272

Query  241  CTCTGAAAGACGTAGAATCATGGGCGAGACCAATGAGCAGAGTGCTAAGAAGGCGAAGCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  273  CTCTGAAAGACGTAGAATCATGGGCGAGACCAATGAGCAGAGTGCTAAGAAGGCGAAGCG  332

Query  301  TGCTCTGGACAAAGGTATGACTGTTATCTTCTGCACCGGAGAGACCCTGGATGAACGCAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  333  TGCTCTGGACAAAGGTATGACTGTTATCTTCTGCACCGGAGAGACCCTGGATGAACGCAA  392

Query  361  GGCCAATAACACTATGGAGGTGAATATTGCTCAGCTCGAGGCTCTTAAGAAGGAGATTGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  393  GGCCAATAACACTATGGAGGTGAATATTGCTCAGCTCGAGGCTCTTAAGAAGGAGATTGG  452

Query  421  AGAATCAAAGAAGTTATGGGAGAACGTTGTAATTGCCTATGAGCCGGTGTGGTCTATCGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  453  AGAATCAAAGAAGTTATGGGAGAACGTTGTAATTGCCTATGAGCCGGTGTGGTCTATCGG  512

Query  481  CACGGGCGTGGTGGCC  496
            ||||||||||||||||
Sbjct  513  CACGGGCGTGGTGGCC  528
```

Figure 1. Sequencing results for the triose phosphate isomerase gene (TPI) of *G. intestinalis*

Range 1: 922 to 1071 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
249 bits(275)	9e-66	145/150(97%)	0/150(0%)	Plus/Plus

```
Query  1     GCATTTCACTCAACTCTGTCCATTAATCAAGAACGAAAGTTAGCGGATCGAAGACGATCA  60
             |||||||||||||||| ||||||||||||||||||||||||||| |||||||||||||||
Sbjct  922   GCATTTCACTCAACTGTGTCCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA  981

Query  61    GATACCGTCCTAGTCCTAACTATAGACGATGTCAACCAAGCATTGGATGAAATTCAGATG  120
             |||||||| ||||||||||||||| ||||||||||||||| ||||| |||||||||||||
Sbjct  982   GATACCGTCGTAGTCCTAACTATAAACGATGTCAACCAAGGATTGGATGAAATTCAGATG  1041

Query  121   TACAAAGATAGAGAAGCATTGTTTCTAGAT  150
             ||||||||||||||||||||||||||||||
Sbjct  1042  TACAAAGATAGAGAAGCATTGTTTCTAGAT  1071
```

Figure 2. Sequencing results for the 16S rRNA for *E. histolytica*.
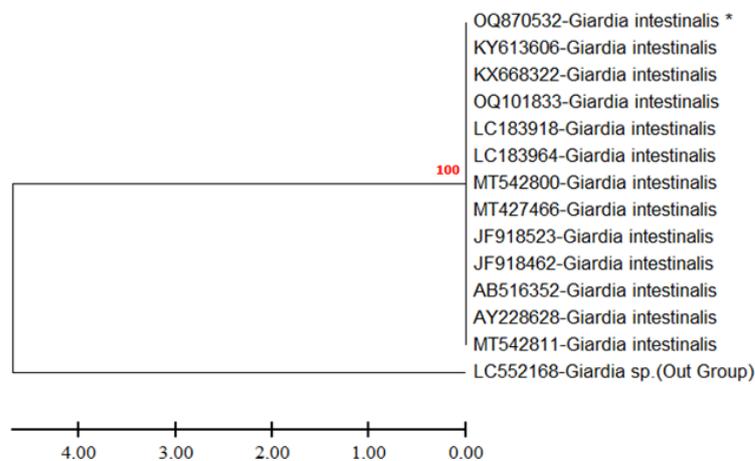

Figure 3. Phylogenetic tree of *G. intestinalis* based on TPI gene sequences

([Figure 4](#)) shows the phylogenetic tree of *E. histolytica* from Iraq, based on the 16S rRNA gene sequences. The tree illustrates the clustering of the isolates from Iraq and their relationship with other known *E. histolytica* isolates from different geographic regions.
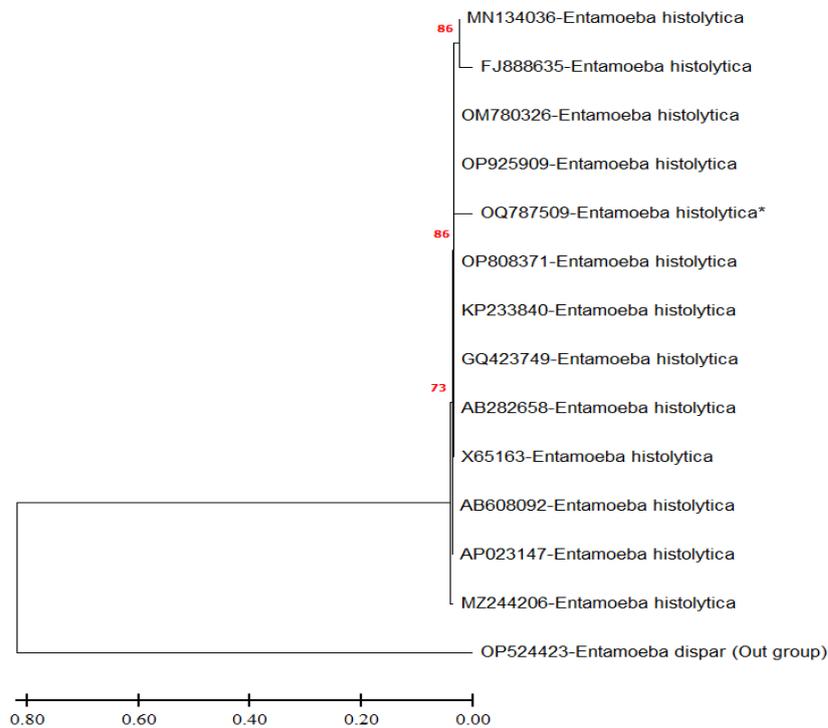

Figure 4. Phylogenetic tree of *E. histolytica* from Iraq based on 16S rRNA gene sequences.

4. Discussion

The current study aimed to investigate the prevalence of *E. histolytica* and *G. lamblia* infections in patients with diarrhea and intestinal disorders in hospitals in Mosul, Iraq, using conventional microscopy and molecular techniques. The results showed that the prevalence of parasitic infections in the studied population was high, with *E. histolytica* being the most prevalent parasite. In this study, we employed molecular techniques, including sequencing and phylogenetic analysis, to gain insights into the prevalence, genetic diversity, and relatedness of these parasites among individuals presenting with diarrhea and intestinal disorders in hospitals across Iraq.

The prevalence of *E. histolytica* in this study was consistent with previous studies conducted in Iraq and other developing countries [22]. About 48% of diarrheal patients were positive in Wasit governate-Iraq [23]. Infection with Entamoeba species was recorded in Kurdistan region of Iraq in 21.68% (562/2592) of the analyzed studied specimens [24]. Poor sanitation and hygiene practices are the main contributing factors to the high prevalence of *E.*

histolytica infection in these areas. However, this study's lower prevalence of G. lamblia infection could be attributed to differences in the transmission routes and environmental factors affecting cyst survival [25].

Using molecular techniques such as PCR and sequencing has significantly enhanced the sensitivity and specificity of diagnosing parasitic infections. Moreover, combining microscopy and molecular techniques is essential for accurate diagnosis, particularly in cases where the parasite load is low [26].

The sequencing of the TPI gene for *G. intestinalis* confirmed the presence of this species in the studied samples. Our findings align with previous studies that have reported the high prevalence of *G. intestinalis* infections in Iraq [27]. Identifying *G. intestinalis* using molecular methods such as sequencing provides accurate species-level determination, enabling a better understanding of the epidemiology and potential transmission sources [28]. The availability of the obtained TPI gene sequences in the NCBI gene bank enhances the accessibility of this valuable data

for future research and comparison with other global isolates.

The phylogenetic analysis determined the genetic characteristics of the parasite in order to discover the source of contamination in human societies. Previous studies in Iraq and neighboring countries like Iran revealed that the source is mostly zoonotic **(29)**. Another study suggested that giardiasis disease is one of the endemic diseases in Iraq and the phylogenetic analysis showed that assemblage A was the most prominent type in Iraq **(30)**. In comparison, no data was available for the previous decade for the Phylogenetic analysis of Entamoeba histolytica in Iraq.

The findings of this study have important implications for managing and preventing parasitic infections in Iraq. Health education programs aimed at promoting hygiene practices, improving sanitation, and providing safe water sources could significantly reduce the burden of parasitic infections in the population. However, the study has some limitations, including the possibility of selection bias as the study was conducted in hospitals, which may not reflect the prevalence of infections in the general population. Furthermore, the study did not evaluate other potential risk factors for infection, such as socioeconomic status, hygiene practices, or water and food sources.

5. Conclusion

In conclusion, this study provides valuable information on the prevalence of *E. histolytica* and *G. lamblia* infections in patients with diarrhea and intestinal disorders in hospitals in Mosul, Iraq. The study concluded that more than a quarter of the studied patients were positive for *E. histolytica.* The sequencing results confirmed the presence of these parasites, and the phylogenetic analysis provided insights into their genetic characteristics. These findings contribute to understanding the epidemiology and genetic diversity of these parasites in Iraq.

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Mosul University. Written informed consent was obtained from all participants, and confidentiality was maintained throughout the study.

Acknowledgment

Not applicable.

Conflict of Interest

The authors declare any conflict of interest.

Author Contributions

All authors contributed to data collection, writing, and manuscript revision. All authors read and approved the final manuscript.

Funding

Not applicable.

Reference

1.   Wong LW, Ong KS, Khoo JR, Goh CB, Hor JW, Lee SM. Human intestinal parasitic infection: a narrative review on global prevalence and epidemiological insights on preventive, therapeutic and diagnostic strategies for future perspectives. Expert Rev Gastroenterol Hepatol. 2020;14(11):1093-105. [DOI:10.1080/17474124.2020.1806711] [PMID]

2.   World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021: accountability for the global health sector strategies 2016-2021: actions for impact. 2021.

3.   Fauziah N, Aviani JK, Agrianfanny YN, Fatimah SN. Intestinal parasitic infection and nutritional status in children under five years old: a systematic review. Trop Med Infect Dis. 2022;7(11):371. [DOI:10.3390/tropicalmed7110371] [PMID] [PMCID]

4.   Alasil SM, Abdullah KA. An Epidemiological Review on Emerging and Re-Emerging Parasitic Infectious Diseases in Malaysia. Open Microbiol J. 2019;13(1): 112-20. [DOI:10.2174/1874285801913010112]

5.   Sengupta P, Chakraborty A. Infection of the gastrointestinal tract: Giardiasis and amoebiasis. InViral, Parasitic, Bacterial, and Fungal Infections. 2023, Academic Press: Elsevier. p. 365-373. [DOI:10.1016/B978-0-323-85730-7.00013-8]

6.   Khurana S, Gur R, Gupta N. Chronic diarrhea and parasitic infections: Diagnostic challenges. Indian J Med Microbiol. 2021;39(4):413-6. [DOI:10.1016/j.ijmmb.2021.10.001] [PMID]

7.   Othman N, Ujang JA, Ng YL, Kumarasamy G, Noordin R. Amebiasis. In Molecular Advancements

in Tropical Diseases Drug Discovery: Chapter 1 - Amebiasis. 2020, Academic Press: Elsevier. p. 1-19. [DOI:10.1016/B978-0-12-821202-8.00001-3]

8. Alia RA, Sadia N, Shammy NP, Tithy FA, Shelim R, Parvin R. Diarrhoeal Disease in Relation to Childhood Malnutrition and Its Impact on Socio-economic Condition in Emerging Countries Like Bangladesh. J Pediatr Perinatol Child Health. 2022; 6(3):370-9.

9. Atabati H, Kassiri H, Shamloo E, Akbari M, Atamaleki A, Sahlabadi F, et al. The association between the lack of safe drinking water and sanitation facilities with intestinal Entamoeba spp infection risk: A systematic review and meta-analysis. PloS One. 2020;15(11):e0237102. [PMID] [DOI:10.1371/journal.pone.0237102] [PMCID]

10. Al-Hasheme IH, Al-Tammime TA, Al-Morshidy KA. Study of some hematological and immunological parameters associated with the infection of intestinal parasites in the holy city of Kerbala, Iraq. Ann Trop Med Health. 2020;23:207-31. [DOI:10.36295/ASRO.2020.231207]

11. Falah AK, Almosawey H, Hameed R, Alhussain B, Ayit A, Al-Ibraheemi M, et al. Prevalence of Entamoeba histolytica and Giardia lamblia Associated with Infectious Diarrhea in Al-Shomally Population, Babil, Iraq. Biomed Biotechnol Res J. 2019;3(4):245. [DOI:10.4103/bbrj.bbrj_112_19]

12. Hussein, R. A., M. J. Shaker, and H. A. Majeed, Prevalence of intestinal parasitic infections among children in Baghdad City. Journal of College of Basic Education, 2011, 71, p. 130-147. Hussein RA, Shaker MJ, Majeed HA. Prevalence of intestinal parasitic infections among children in Baghdad City. J Coll Basic Educ. 2011;71:130-47.

13. Katagiri S, Oliveira-Sequeira TC. Comparison of three concentration methods for the recovery of canine intestinal parasites from stool samples. Exp Parasitol. 2010;126(2):214-6. [DOI:10.1016/j.exppara.2010.04.027] [PMID]

14. Köller T, Hahn A, Altangerel E, Verweij JJ, Landt O, Kann S, et al. Comparison of commercial and in-house real-time PCR platforms for 15 parasites and microsporidia in human stool samples without a gold standard. Acta Trop. 2020;207:105516. [DOI:10.1016/j.actatropica.2020.105516] [PMID]

15. Van den Bossche D, Cnops L, Verschueren J, Van Esbroeck M. Comparison of four rapid diagnostic tests, ELISA, microscopy and PCR for the detection of Giardia lamblia, Cryptosporidium spp. and Entamoeba histolytica in feces. J Microbiol Methods. 2015;110:78-84. [DOI:10.1016/j.mimet.2015.01.016] [PMID]

16. Momčilović S, Cantacessi C, Arsić-Arsenijević V, Otranto D, Tasić-Otašević S. Rapid diagnosis of parasitic diseases: current scenario and future needs. Clin Microbiol Infect. 2019;25(3):290-309. [DOI:10.1016/j.cmi.2018.04.028] [PMID]

17. Mejia R, Vicuna Y, Broncano N, Sandoval C, Vaca M, Chico M, et al. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am J Trop Med Hyg. 2013;88(6):1041. [DOI:10.4269/ajtmh.12-0726] [PMID] [PMCID]

18. Shafi A, Farooq U, Akram K, Khan MZ, Hayat Z, Hayat K. Molecular epidemiology of foodborne diseases. Sequencing Technologies in Microbial Food Safety and Quality. 2021, CRC Press: Boca Raton, FL, USA. P. 109-54. [DOI:10.1201/9780429329869-4] [PMID] [PMCID]

19. Khairnar K, Parija SC. A novel nested multiplex polymerase chain reaction (PCR) assay for differential detection of Entamoeba histolytica, E. moshkovskii and E. dispar DNA in stool samples. BMC Microbiol. 2007;7(1):1-9. [DOI:10.1186/1471-2180-7-47] [PMID] [PMCID]

20. Hamzah Z, Petmitr S, Mungthin M, Leelayoova S, Chavalitshewinkoon-Petmitr P. Differential Detection of Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovskii by a Single-Round PCR Assay. J Clin Microbiol. 2006;44(9): 3196-200. [DOI:10.1128/JCM.00778-06] [PMID] [PMCID]

21. Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, et al. Triosephosphate Isomerase Gene Characterization and Potential Zoonotic Transmission of Giardia duodenalis. Emerging Infect Dis. 2003;9(11):1444-52. [DOI:10.3201/eid0911.030084] [PMID] [PMCID]

22. Dhubyan Mohammed Zaki Z. Prevalence of Entamoeba histolytica and Giardia Lamblia Associated with Diarrhea in Children referring to lbn Al-Atheer Hospital in Mosul, Iraq. Arch Razi Inst. 2022;77(1):73-9.

23. Kareem Kadhim D, Abdulsalam Hraija B, Aqeele G. Molecular-Genotyping Detection of Entamoeba histolytica in Diarrheic Patients. Arch Razi Inst. 2023;78(1):337-43.

24. Hasan HK, Mero WMS, Mohammed AB. Prevalence of amoebiasis and associated risk factors among population in Duhok city, Kurdistan Region, Iraq. J Infect Dev Ctries. 2023;17(4):542-9. [DOI:10.3855/jidc.17478] [PMID]

25. Chalabi KN. A meta-analysis of Entamoeba histolytica/dispar in Iraq. bioRxiv. 2018:435495. [DOI:10.1101/435495]

26. Wong SS, Fung KS, Chau S, Poon RW, Wong SC, Yuen KY. Molecular diagnosis in clinical parasitology: when and why?. Exp Biol Med. 2014; 239(11):1443-60. [DOI:10.1177/1535370214523880] [PMID]

27. Alhayali NS, Al-Amery AM, Hasan MH. DETECTION OF Giardia intestinals IN HUMAN, CALVES AND WATER SUPPLIES BY TRADITIONAL AND MOLECULAR METHODS AT BAGHDAD CITY, IRAQ. Iraqi J Agric Sci. 2020;51(5):1428-35. [DOI:10.36103/ijas.v51i5.1153]

28. Arshad S, Khatoon N, Warind JA, Khan A, Waheed S, Khan W. The prevalence of human intestinal protozoal and helminthic infection in Karachi. Int J Biol Biotechnol. 2019;16(2):319-23.

29. Eslamirad Z, Hajihossein R, Moslemi A. An Overview on the Main Assemblages and Sub-assemblages of Giardia intestinalis in the Western Half of Iran. Shiraz E-Med J. 2022;23(10):e116536. [DOI:10.5812/semj-116536]

30. Alasadiy YD, Gatea EA, Eabaid FA. Genotypes of from different Hosts and their Giardia lamblia Physiological Effects: A Review. Indian J Ecol. 2022; 49 Special Issue(18):188-191.