



# The prevalence and diagnostic utility of molecular methods for identifying *Entamoeba* and *Giardia lamblia* in Iraq

Ali Isam Najm<sup>1\*</sup>, Sura S. Alwan<sup>1</sup>

<sup>1\*</sup>Department of General Sciences / College of Basic Education/ Missan University

## Summary

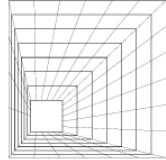
Intestinal parasite infections are the most common cause of infectious disease worldwide, and they have been identified as a public health concern in developing countries. Globally, intestinal *Entamoeba histolytica* and *Giardia lamblia* are responsible for amoebiasis and giardiasis, respectively. Although their significant health impact and widespread incidence in Iraq, they receive little attention in terms of prevalence, sensitive diagnostic tools, and associated risk factors. Different techniques for diagnosis are available for detecting intestinal parasites, with microscopic stool testing being the most prevalent and appropriate method. Nonetheless, one limitation is that it lacks the precision of molecular approaches. Therefore, this study aimed to highlight the prevalence of *Entamoeba histolytica* and *Giardia lamblia* infections, as well as the molecular detection methods used.

**Keywords:** Intestinal Parasites, *Entamoeba histolytica*, *Giardia lamblia*, Molecular Methods

## Introduction

A human body's digestive system consists of various types of microbes, which include bacteria, protozoa, viruses, and fungi (Wilson et al., 2019). An intestinal parasite disease is one of the most neglected tropical diseases that result in severe consequences and affect nearly 450 million people worldwide (Muñoz et al., 2023). Parasite disease is one of the most common issues today due to poor sanitation. Such a concern exists everywhere around the globe, depending on various economic and geographic influences. Such determinants include poor sanitation, wearing shoes, lack of good toilet facilities, and other similar factors (Duguma et al., 2023).

In developing countries, these infections pose a significant public health challenge, resulting in considerable morbidity and mortality among preschool and school-aged children (Mathy et al., 2022). School-aged children exhibit a tendency to play on contaminated soil, maintain inadequate personal cleanliness, and possess an underdeveloped immune system. Consequently, they possess an elevated likelihood of contracting gastrointestinal parasitic infections (Debash, Alemu, and Bisetegn, 2023). The primary etiological agent of amoebiasis in humans is the parasite protozoan *Entamoeba histolytica*. *Giardia lamblia* is a widespread parasite protozoan flagellate responsible for watery diarrheal illness in humans (Hajari et al., 2022). In Iraq, they are regarded as the most prevalent intestinal parasites, particularly in youngsters (Wielinga et al., 2023). Amoebiasis is prevalent, resulting in around 67,900 fatalities annually. It significantly influences the morbidity and mortality rates among children (Suleiman and Ralston, 2022). In cases where the disease is severe, there is usually a spread from the digestive system to another organ, such as the brain, lung, or liver. *Entamoeba histolytica* and *Giardia lamblia* are tropical parasites responsible for causing serious illnesses among humans. They are considered more dangerous than any other parasites since they tend to be more lethal (Carrero et al., 2020). *Giardia lamblia* is an intestinal parasitic disease caused by a flagellate protozoon, which infects the small intestines of many humans, leading to health issues like diarrhea and stomachache (El-Kady et al., 2021). Diarrhea is considered the major symptom,



and, in extreme situations, patients can also have bloody stools. For young or immuno-suppressed patients, the consequences of the condition can be fatal in some cases (Singh et al., 2023).

### The Prevalence of Intestinal Parasites in Iraq

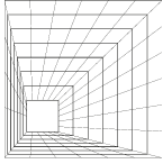
Parasitic infections of the intestines mainly occur as a result of poor hygiene conditions such as consumption of foods and fluids that have been infected, consumption of eggs and cysts orally from hands and nail-biting behavior, and penetration of larvae in poorly hygienic areas. (Debash, Alemu & Bisetegn, 2023). Researchers investigated the prevalence of intestinal parasitic infections among Iraqis over a twenty-year period, studied the distribution of intestinal parasites in different communities, and noticed that parasitic infections are the most common type of infection in Iraq (Table1). This situation signifies a public health issue, particularly for children. Consequently, it requires more focus for the implementation of control measures. (Mahdi, 2022).

A research conducted by Naqid (2024) in Zako City, Kurdistan, Iraq, examined the prevalence of intestinal protozoan infections among the general population of Zakho from October 2018 to June 2022. Among them, *Entamoeba histolytica* was the most prevalent harmful protozoan infection 68.61%, followed by *Giardia lamblia* 25.31%. Chalabi's (2024) investigation, performed over 11 years in northern Iraq, found only two protozoan parasites at medical centers in Erbil province: *E. histolytica* and *G. lamblia*. The prevalence of the protozoans *Entamoeba histolytica* and *Giardia lamblia* was 60.92% and 29.54%, respectively. A direct microscopic investigation conducted by Hasan et al., (2024) on stool samples from children's and general hospitals in western Iraq revealed an infection rate of 9.5% for *G. lamblia*.

A study was conducted by Latif, Al-Talib, and Al-Akelyin, (2020) in Baghdad province to estimate the prevalence of intestinal protozoa among human stools. The highest level of infection was observed for *Entamoeba histolytica*, with an infection rate of 48.48% and followed by *Giardia intestinalis* at 33.33%. Parasite diseases' incidence in a span of seven years was assessed in the Babylon Governorate of Iraq. Subjects of the samples had an age range of less than one year old to over 71 years old. According to the study, the prevalence of infections for pathogenic parasites such as *Entamoeba histolytica* and *Giardia lamblia* was found to be 88.00% and 10.80%, respectively (Al-Taei, 2019). In a study carried out by Abd AL-Khalek and Hraija (2025), *Entamoeba histolytica* and *Giardia lamblia*, which are the main etiological factors responsible for causing bowel diseases in humans, were found to have prevalence levels of 66.67% and 11.46%, respectively, in central Iraq. Results from PCR tests carried out using the 18S rRNA genes of the intestinal parasites show that there was 62.0% infection of *E. histolytica* and 12.0% for *G. lamblia* (Al-Saady and Lefta, 2025). The results obtained from the study conducted by Harb et al. (2029) in the Dhi Qar Governorate of southern Iraq among the population of children below five years revealed that *Entamoeba* spp. was 13.5% and *Giardia* spp. was 7.1%. Higher prevalence of infections caused by *E. histolytica* and *G. lamblia* has been recorded, thus highlighting the necessity of stronger preventive methods against these infections (Naqid, 2024). Present-day health issues include parasitic diseases, development problems in poor countries, living standards, poverty rates, availability of healthcare, hygiene, sanitation, and availability of safe water, all of which have a link to intestinal parasites being widespread (Norhayati et al., 2003). The first thing one should remember is that despite *Entamoeba histolytica* and *Giardia lamblia* being known as public health problems, knowledge of how widespread these organisms are globally or even in particular regions is rather insufficient (Hooshyar et al., 2019).

Table 1: Shows the prevalence of intestinal parasites in some Iraqi governorates

NO.	Region	Samples	% Infected		Reference
			<i>Entamoeba histolytica</i>	<i>Giardia lamblia</i>	
1.	Erbil	Samples of various ages during 2011 to 2021	60.92	29.54	Chalabi, (2024)



2.	Zakho	Patients from 19 year to the age of 63 years from 2018 to 2022.	68.61	25.31	Naqid, (2024).
3.	Mosul	Patients from one year to the age of 70 years from 2022 to 2023.	78	22	Al-rawi and Al-Omar (2023)
4.	Al-Anbar	Samples of various ages	56.7	7.5	Ali et al., (2023).
5.	Baghdad	Samples of various ages	48.48	33.33	Latif, Al-Talib, and Al-Akelyin, (2020)
6.	Babylon	Ages range from less than one year to more than 71 years old, from 2011 to 2017.	88	10.8	Al-Taei, (2019)
7.	Wasit	Children aged 1 day to 16 years.	66.67	11.46	Abd AL-Khalek and Hraija, (2025)
8.	Al-Diwaniyah	Samples of various ages	62	12	Al-Saady and Lefta, (2025)
9.	Thi Qar	Patients under 12 years of age from 2015-2020			Flaih et al., (2021)

### **Molecular detection**

Microscopic diagnosis provides information on the presence of Entamoeba species, however it may fail to differentiate this pathogen from closely similar nonpathogenic species. In cases where discrimination is essential, PCR-based techniques could be a better option (Tanyuksel & Petri Jr, 2003). The identification of different Entamoeba species using PCR can be done through the amplification of the extrachromosomal rRNA gene, which is a highly repeated gene that has about 200 copies of itself within the cell (Faqe Mahmood & Mustafa, 2020). This method allows for the detection of infection much better than using single-copy gene areas in DNA. DNA sequencing gives important information on the genetics and evolution of major human parasites protozoa such as Giardia intestinalis and Entamoeba histolytica. The use of 18S ribosomal RNA (rRNA) was applied in studying the evolution and genetics of these parasites (Heyworth, 2016). The worldwide spread of Giardia may be comprehended exclusively through molecular identification and the dynamics of disease transmission. The PCR-RFLP and multi-locus sequence typing (MLST) are employed to define genotype types and monitor genetic variation (Mateusa et al., 2023). Compared to MLST, the PCR-RFLP method utilizing the beta-giardin gene exhibits a constrained discriminative ability, as it emphasizes the digestion patterns of restriction enzymes rather than the sequences of multiple loci (gdh, bg, and tpi genes), which reflect intra-assemblage diversity (Delling and Dausgies, 2022). Currently in Iraq, several molecular studies have been conducted to determine the infection and prevalence of intestinal parasites among symptomatic patients in Erbil by Mohammed et al. (2025), in Sulaimaniyah by Abdullah and Ali (2024), in Mosul Al-rawi and Al-Omar (2023), Kirkuk by Hasan, Rasheed and Ahmed (2024), in Al-Diwaniyah by Al-Saady and Lefta, (2025), in Wasit by Abd AL-Khalek and Hraija (2025), and in Thi-Qar by Harb et al. (2019).

### **Conclusion**

The prevalence rates of intestinal parasite diseases vary by region, influenced by personal and communal cleanliness, sanitation, and environmental conditions; nonetheless, they are rather frequent in Iraq as a whole. Intestinal infections are widespread throughout many populations and demographic samples in both urban and rural areas of Iraq. Individuals of all ages and genders are



infected. Consequently, prompt and effective preventative and control measures are needed (Mahdi, 2022). Despite the availability of effective treatments for amoebiasis and giardiasis, which are prevalent causes of diarrhea, morbidity remains elevated. The effects of invasive amoebiasis can be lethal, and giardiasis may lead to malabsorption in kids (John and Petri, 2006).

## References

1. Abd Abd AL-Khalek, E. J., & Hraija, B. A. (2025). Molecular Detection of Protozoal Infection in Diarrheic Children in Wasit Province. *Basrah Researches Sciences*, 51(1), 11-11.
2. Delling C, Dausgschies A (2022). Literature review: Coinfection in young ruminant livestock *Cryptosporidium* spp. and its companions. *Pathogens*, 11: 103.
3. Abdulraheem Al-rawi, O. N., & Subhi Naef Al-Omar, N. (2023). Molecular diagnosis and phylogenetic investigation of *Entamoeba histolytica* and *Giardia intestinalis* among diarrheal patients in Iraq. *Iranian Journal of Medical Microbiology*, 17(3), 361-368.
4. Abdullah, S. J., & Ali, S. A. (2024). Molecular detection of a pathogenic *Entamoeba* among symptomatic children in Eastern Kurdistan of Iraq. *Polish Journal of Microbiology*, 73(1), 99.
5. Mohammed, M. D., Karyagdi, T. K., Qneed, A. M., Jihad, I. A., Lahhob, Q. R., Mudhafar, M., ... & Ayada, A. A. (2025). Molecular characterization and zoonotic potential of giardia species in livestock with respect to their transmission dynamics and host adaptation. *J. Anim. Health Prod*, 13(s1), 411-421.
6. Harb, A., Abraham, S., Rusdi, B., Laird, T., O'dea, M., & Habib, I. (2019). Molecular detection and epidemiological features of selected bacterial, viral, and parasitic enteropathogens in stool specimens from children with acute diarrhea in Thi-Qar Governorate, Iraq. *International journal of environmental research and public health*, 16(9), 1573.
7. Mateusa M, Ozolina Z, Terentjeva M, Deksnė G (2023). *Giardia duodenalis* Styles, 1902 prevalence in cattle (*Bos taurus* Linnaeus, 1758) in Europe: A systematic review. *Microorganisms*, 11: 309.
8. Tanyuksel M, Petri WA Jr. (2003). Laboratory diagnosis of amebiasis. *Clin Microbiol Rev*;16(4):713–729.
9. Faqe Mahmood SA, Mustafa HB. (2020). Molecular identification and prevalence of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in Erbil City, Northern Iraq. *Pol J Microbiol*;69(3):263–272.
10. Hooshyar H, Rostamkhani P, Arbabi M, Delavari M. *Giardia lamblia* infection: review of current diagnostic strategies. *Gastroenterology and hepatology from bed to bench*. 2019;12(1):3–12
11. John DT, Petri WA (2006) *Markell and Voge Medical Parasitology*. Saunders Elsevier Inc.
12. Al-Saady, H. K. J., & Lefta, H. R. (2025). Molecular Detection and Phylogenetic Analysis of Gastrointestinal Protozoa from Diarrhea Patients in Al-Diwaniyah Hospital.
13. Debash, H., Alemu, M., & Bisetegn, H. (2023). The prevalence of intestinal parasites, undernutrition and their associated risk factors among school-age children in Sekota Town, Northeast Ethiopia: A community-based cross-sectional study. *Health Science Reports*, 6(3), e1137.
14. Mathy, N. W., Deng, S., Gong, A. Y., Li, M., Wang, Y., Burleigh, O., ... & Chen, X. M. (2022). The long non-coding RNA nostrill regulates transcription of *Irf7* through interaction with NF- $\kappa$ B p65 to enhance intestinal epithelial defense against *Cryptosporidium parvum*. *Frontiers in Immunology*, 13, 863957.
15. R. L. Suleiman and K. S. Ralston. (2022). "Growth and genetic manipulation of *Entamoeba histolytica*," *Current Protocols*, vol. 2, no. 1, pp.e327,
16. J. C. Carrero, M. Reyes-López, J. Serrano-Luna, M. Shibayama, J. Unzueta, N. León-Sicairos, and M. de la Garza.(2020). "Intestinal amoebiasis: 160 years of its first detection and



- still remains as a health problem in developing countries," *International Journal of Medical Microbiology*, vol. 310, no. 1, pp. 151358.
17. A.M. El-Kady, I. A. Abdel-Rahman, S. S. Fouad, K. S. Allemailem, T. Istivan, S. F. Ahmed, A. S. Hasan, H. A. Osman, and H. A. Elshabrawy. (2021). "Pomegranate peel extract is a potential alternative therapeutic for giardiasis," *Antibiotics*, vol. 10, no. 6, pp. 705.
  18. A. K. Singh, S. K. Ansari, A. Raghav, and V. Gaur. (2023). "Role and pathophysiology of protozoan parasites causing liver diseases," in *Pathobiology of Parasitic Protozoa: Dynamics and Dimensions*, Springer, Singapore, pp.45-60.
  19. Heyworth, M. F. (2016). *Giardia duodenalis* genetic assemblages and hosts. *Parasite*, 23, 13.
  20. Latif, B., Al-Talib, H., & Al-Akely, S. (2020). Prevalence of intestinal protozoa among humans, animals and vegetables in Baghdad, Iraq. *Int. Med. J.*, 27(2), 136-140.
  21. C. Muñoz-Antoli, M. J. Irisarri-Gutiérrez, L. Acosta, M. J. Bonet, J. G. Esteban, and R. Toledo. (2023). "Intestinal parasitic infections and their potential risk factors among prison inmates in Valencia, Spain," *BMC Infectious Diseases*, vol. 23, no. 1, pp. 616.
  22. T. Duguma, T. Worku, S. Sahile, and D. Asmelash. (2023). "Prevalence and associated risk factors of intestinal parasites among children under five years of age attended at Bachuma Primary Hospital, West Omo Zone, Southwest Ethiopia: A cross-sectional study," *Journal of Tropical Medicine*, vol. 2023, pp. 2268554,
  23. Chalabi, K. (2024). Prevalence of intestinal parasites in Erbil, Iraq. *Helminthologia*, 61(3), 214-223.
  24. Hasan, H., Rasheed, I., & Ahmed, N. (2024). Molecular Epidemiological Characterization of *Giardia Lamblia* in Kirkuk, Iraq. *Int. J. Multidiscip. Res. Anal.*, 7.
  25. Wilson IW, Weedall GD, Lorenzi H, Howcroft T, Hon CC, Deloger M, Guillén N, Paterson S, Clark CG, Hall N. (2019). Genetic diversity and gene family expansions in members of the genus *Entamoeba*. *Genome Biol Evol*;11(3):688–705.
  26. Hajari, S. T., Chekol, Y., & Chauhan, N. (2022). Assessment of prevalence of *Giardia lamblia* infection and its associated factors among government elementary school children from Sidama zone. *SNNPR, Ethiopia*, 17(3).
  27. Wielinga, C., Williams, A., Monis, P., & Thompson, R. (2023). Proposed taxonomic revision of *Giardia duodenalis*. *Infect Genet*, 111, 105430.
  28. Al-Taei, A. H. O. (2019, September). The prevalence of intestinal parasite among the attending peoples to Al-Hashimyah hospitals for seven years, Babylon province, Iraq. In *Journal of Physics: Conference Series* (Vol. 1294, No. 6, p. 062022). IOP Publishing.
  29. Norhayati, M.; Fatmah, M.S.; Yusof, S. and Edariah, A.B. (2003). Intestinal parasitic infections in man: a review. *Med J. Malaysia*. 58(2):296–305.