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Original Article

Molecular Detection and Genotyping of *Dientamoeba fragilis* from Human Stool Specimens in Nineveh Governorate

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Abstract

Background: We investigated *Dientamoeba fragilis* molecular distribution and genetic types among Nineveh Governorate patients with symptoms through SSU rRNA gene PCR amplification and sequencing.

Methods: Fifty stool samples were collected from symptomatic patients aged 3–68 years attending the Medical Research Hospital in Nineveh Governorate, northern Iraq in 2024. Genomic DNA was extracted using the Presto™ Stool DNA Extraction Kit. The PCR reaction used species-specific primers DF400/DF1250 to produce ~850 bp amplicons. Positive products were sequenced and examined using BLAST, MEGA 11, DnaSP, and PopART. The Maximum Likelihood method used the Tamura 3-parameter model to perform phylogenetic analysis. PCR detection indicated *D. fragilis* in 12/50 stool samples, corresponding to a prevalence of 24%.

Results: The BLAST analysis showed that the sequence had 97%-99.7% similarity to global reference strains (AY730405). The study identified three haplotypes which contained three mutations while showing a haplotype diversity (Hd) of 0.318 that indicated minimal genetic diversity. The phylogenetic analysis showed that all isolated strains belonged to genotype 1 but U37461 formed a separate lineage as genotype 2. The Iraqi isolates showed sequence similarity to genotype 1 reference strains reported globally.

***Conclusion*:** This study provides additional molecular evidence of *D. fragilis* in northern Iraq and the second report nationally. Genotype 1 was identified among all analyzed isolates with minimal genetic variation between different groups. The molecular detection methods delivered vital diagnostic data but scientists need to expand their surveillance activities to study animal disease transmission to humans and animal disease progression.

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Introduction

For over a century since its discovery, *Dientamoeba fragilis* has remained a taxonomically intriguing and clinically overlooked intestinal protozoan parasite. The parasite is globally distributed; however, its biological and epidemiological characteristics remain insufficiently understood due to its fragile trophozoite stage and the historical reliance on microscopy-based diagnostic approaches (1-3). The organism exists in the human large intestine where it reaches sizes between 5–15 µm, and scientists now place it among the trichomonads based on both physical characteristics and genetic data (4).

The predominant form of *D. fragilis* is the binucleated trophozoite, whereas cyst formation is rarely observed, thereby complicating assumptions regarding its transmission dynamics (2,3). The trophozoite stage shows environmental instability, which makes it difficult to detect through traditional microscopy according to Barratt et al. (5). Human populations have probably underreported this condition for many years. Molecular studies have become vital for understanding how the parasite spreads, which hosts it infects, and the extent of its disease-causing ability.

The clinical presentation of *D. fragilis* infection is variable, ranging from asymptomatic carriage to gastrointestinal manifestations such as diarrhea, abdominal pain, nausea, and anorexia (3). The clinical improvement of patients after receiving targeted antiparasitic treatment supports the idea that parasites cause gastrointestinal disorders (6,7). The human host mechanisms that cause infection and maintain persistence remain unknown. Current hypotheses suggest that *Enterobius vermicularis* ova may serve as a potential transmission vehicle because *D. fragilis* trophozoites have a short survival period outside the human body (2).

The SSU rRNA gene serves as the main genetic marker for *D. fragilis* taxonomic research and evolutionary studies (4). Research has identified two main genotypes of the parasite, with genotype 1 being the most common form that affects humans and animals, while genotype 2 exists as a rare, distinct genetic variant (1,3,8). The global molecular epidemiology of this protozoan has shown progress highlighting the limited availability of molecular epidemiological data from Iraq and the broader Middle East.

Molecular studies in this situation serve two purposes—confirming diagnosis and revealing how the parasite has evolved through time. PCR-based assays have enhanced detection sensitivity through their ability to identify weak infections and multiple genotypes (9). Scientists conduct molecular genotyping tests with phylogenetic analysis to track geographic lineages and assess zoonotic risks in areas where humans interact with animals, such a domestic and farm environment (10).

We investigated *D. fragilis* molecular epidemiology in Nineveh Governorate, northern Iraq, through SSU rRNA-based amplification and sequencing of symptomatic patients to study genetic diversity and phylogenetic patterns. We employed PCR to produce SSU rRNA gene amplifications, analyzed through sequencing and phylogenetic methods. The research presents the second molecular evidence of *D. fragilis* in Iraq since Al-Qassab (11) and adds new information that helps scientists understand the parasite's molecular spread and genetic variation across the region.

Materials and Methods

Sample Collection

Fifty stool samples were collected from patients attending the Medical Research Hospital in Nineveh Governorate, northern Iraq in 2024. The study participants spanned from 3 to 68 yr old while showing signs of intestinal parasitic infection through their abdominal pain and diarrhea symptoms. The research team placed

each sample into sterile containers before storing them at –20 °C for preservation until they performed additional analysis (9). The researchers documented demographic information about each patient through age and sex and symptom patterns to study how infections spread throughout the population.

DNA Extraction

Genomic DNA was extracted directly from stool samples using the Presto™ Stool DNA Extraction Kit (Geneaid, Korea) according to the manufacturer's protocol. The extraction process was optimized to eliminate PCR inhibitors known to be present in fecal samples. The NanoDrop spectrophotometer from Thermo Fisher Scientific (USA) measured DNA purity and concentration through optical density (OD) ratio assessment at 260/280 which should fall between 1.7 and 2.0 for high-quality nucleic acids. The DNA samples received storage at –20 °C temperatures until the amplification process started.

Polymerase Chain Reaction (PCR) Amplification

Detection and molecular identification of *D. fragilis* were achieved through the amplification of a partial region of the small subunit ribosomal RNA (SSU rRNA) gene (10). The specific primer pairs were:

Forward primer (DF400): 5'–TATCGGAGGTGGTAATGACC–3' Reverse primer (DF1250): 5'–CATCTTCCTCCTGCTTAGACG–3'

These primers amplify an ~850 base pair fragment corresponding to the SSU rRNA gene region commonly used for molecular genotyping of the parasite (1,3,8).

The total reaction volume was 20 µL, consisting of 4 µL of DNA template, 1 µL of each primer (10 pmol/µL), 10 µL of 2× Master Mix (Promega, USA), and 4 µL of nuclease-free water (Promega, USA). The thermal cycling conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1.5 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min.

Each PCR run included a positive control containing previously confirmed *D. fragilis* DNA and a negative control containing nuclease-free water instead of template DNA to monitor contamination and validate amplification specificity.

All PCR reactions were performed using a Thermo Scientific Thermocycler, and amplified products were visualized by 2% agarose gel electrophoresis stained with ethidium bromide. The presence of a distinct 850 bp band confirmed the positive amplification of *D. fragilis* DNA.

Gel Electrophoresis and Visualization

Amplified products were separated on 2% agarose gels prepared in 1× Tris–acetate–EDTA (TAE) buffer and run at 100 V for 45 min. The gels were subsequently examined using a Gel Documentation System (Bio-Rad, USA) to verify the expected amplicon size. The team recorded positive samples which they processed for sequencing analysis.

DNA Sequencing and Genotypic Characterization

The researchers conducted DNA sequencing on twelve PCR-positive samples. The company Psomagen Inc. (USA) received amplified fragments together with their respective primers for bidirectional Sanger sequencing. The researchers inspected the resulting chromatograms to verify both sequence quality and proper alignment of the data. Clean sequences were then compared with previously deposited *D. fragilis* sequences in the National Center for Biotechnology Information (NCBI) database using BLASTn. The accession numbers for the sequences obtained in this study were PX069430–PX069441. The research used reference sequences from human and animal isolates including ON242172 and OP375693 and

MN560150 and AY730405 and U37461 for phylogenetic analysis (8,1).

Phylogenetic and Haplotype Analysis

Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method implemented in MEGA version 11 software. The ClustalW algorithm was used to align 23 *D. fragilis* nucleotide sequences which included study isolates and reference strains. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method based on the Tamura three-parameter model. The phylogeny stability was evaluated through a bootstrap analysis which ran 1000 times.

The researchers analyzed isolate clustering through evolutionary methods to identify genotypes while studying geographic relationships between them. The analysis used DnaSP ver. 6 (Arnoux et al., (13) to calculate haplotype diversity (Hd) and number of mutations and nucleotide polymorphism indices. The PopART software produced a median-joining haplotype network which showed both genetic connections and mutation distances between local and reference isolates.

Ethical Considerations: Ethical approval for the present study was obtained from the Ethical Committee of the College of Medicine, University of Mosul (Approval No.: 53,2024), prior to sample collection, in accordance with the principles of the Helsinki Declaration.

The researchers obtained consent from all participants and their legal guardians before starting the sample collection process. The research protected patient confidentiality absolutely while keeping all data completely anonymous.

Stool samples were stored at –20 °C prior to DNA extraction due to laboratory infrastructure limitations; although –80 °C storage is generally recommended for long-term preservation of nucleic acids in fecal specimens, amplification quality was verified by NanoDrop spectrophotometry and agarose gel electrophoresis prior to downstream molecular analysis.

Results

Molecular Detection of Dientamoeba fragilis

The SSU rRNA fragment PCR analysis detected *D. fragilis* DNA in 12 out of 50 stool samples, corresponding to a molecular detection rate of 24%. The molecular test results confirm that the parasite exists at levels which standard microscopic tests fail to detect during Nineveh Governorate surveillance.

The parasite infection occurred in 32 female participants (64%) and 18 male participants (36%) without any statistical differences based on age or sex. The amplified products produced a single 850 bp band which matched the predicted fragment length (Fig. 1). The DNA banding pattern showed distinct uniform bands confirming successful amplification of the target gene.

The sharp and uniform banding pattern confirmed the reliability of the DNA extraction and amplification protocol.

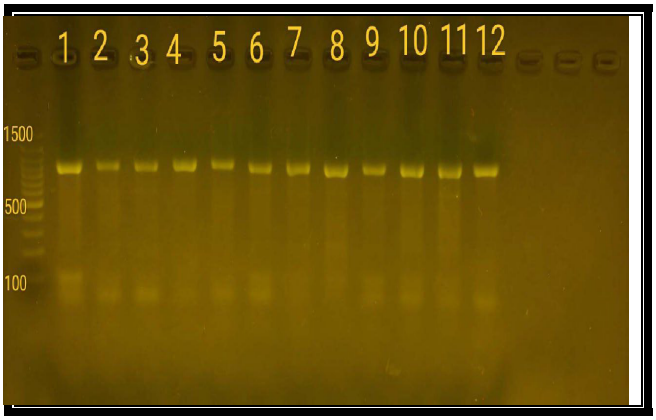


Fig. 1: Agarose gel electrophoresis showing PCR amplification of the *Dientamoeba fragilis* SSU rRNA gene (850 bp). Lanes 1–12 represent positive samples from human stool specimens, while the leftmost lane shows the DNA molecular weight marker (100–1500 bp). The presence of a clear 850 bp band confirms the successful amplification of the target gene

Sequencing and BLAST Analysis

All twelve PCR-positive amplicons were subjected to Sanger sequencing. High-quality

bidirectional reads were obtained and compared against reference sequences in the NCBI GenBank database using BLASTn. All sequences shared 97.0% to 99.7% identity with the reference strain AY730405. The analysis results for coverage and alignment length and E-values matched the homologous SSU rRNA fragments found in human isolates deposited in worldwide databases (Table 1). The percentage of similarity for all the sequences included in this study was equal to or greater than 97%, therefore the studied isolates are of high sequence similarity to each other and all of them belong to the predominate genotype 1.

Table 1: Representative BLAST alignment results for *D. fragilis* isolates from the current study

Isolate ID	Query Cover (%)	E-value	Identity (%)	Accession Length (bp)
PX069435.1	51	0	99.65	864
PX069439.1	51	0	99.41	863
PX069432.1	50	0	99.76	834
PX069441.1	49	0	99.75	810
PX069437.1	49	0	99.75	816
PX069434.1	42	0	99.71	691
PX069431.1	42	0	99.71	691
PX069430.1	42	0	99.71	695
PX069438.1	41	0	99.71	679
PX069440.1	39	0	99.54	646
PX069436.1	26	0	97.00	443
PX069433.1	23	0	98.94	377

The similarity values between all sequences reached at least 97% indicating high sequence similarity among the analyzed isolates. The analyzed isolates exhibited predominance of genotype 1 with high sequence similarity.

Haplotype and Genetic Diversity Analysis

Alignment of the 12 nucleotide sequences revealed three distinct haplotypes with three nucleotide substitutions across the analyzed SSU rRNA fragment. The final aligned sequence length used for haplotype diversity estimation was 812 base pairs.

The haplotype diversity calculation (Hd = 0.318) showed that the studied isolates displayed minimal genetic diversity (Fig. 2).

Each haplotype was represented by multiple isolates differing at only one or two mutational sites. The median-joining network revealed close clustering among all Iraqi isolates, with short mutational branches connecting them to the *AY730405* reference strain, further confirming the predominance of genotype 1 in the studied population.

The computed haplotype diversity (Hd=0.318) indicated a low genetic variability within the isolates. This restricted diversity likely reflects either a recent introduction of the parasite into the local population or a strong selective pressure maintaining genetic uniformity (Fig. 2). The haplotype network was constructed using PopART ver. 1.7.

Median-joining haplotype network constructed from 12 Iraqi isolates of D. fragilis based on partial SSU rRNA sequences based on partial SSU rRNA sequences (812 bp). Three haplotypes (H1–H3) were identified with three mutational steps separating them. Circle size corresponds to the number of identical sequences, and hash marks indicate point mutations. Constructed using PopART ver. 1.7.

The computed haplotype diversity (Hd=0.318) indicated a low genetic variability within the isolates (Fig. 2).

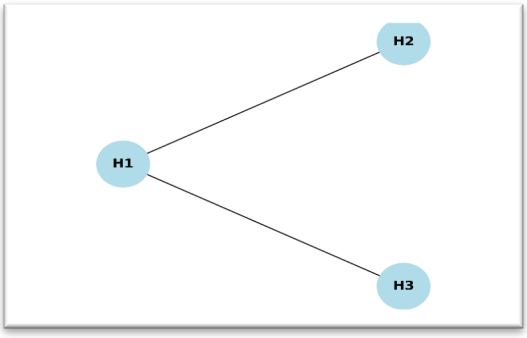


Fig. 2: Median-joining haplotype network of *Dientamoeba fragilis* isolates constructed based on partial SSU rRNA gene sequences using PopART version 1.7

Each haplotype was represented by multiple isolates differing at only one or two mutational sites. The median-joining network revealed close clustering among all Iraqi isolates, with short mutational branches connecting them to the *AY730405* reference strain, further confirming the predominance of genotype 1 in the studied population (Figs. 3,4).

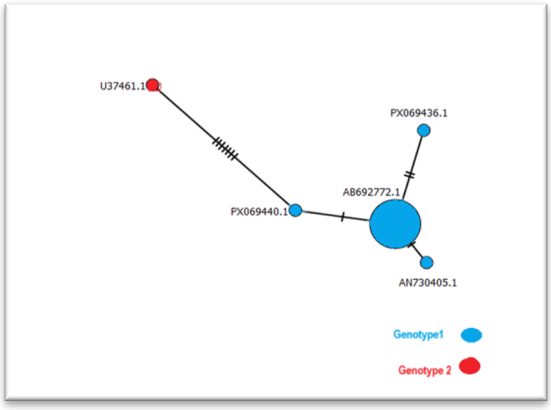
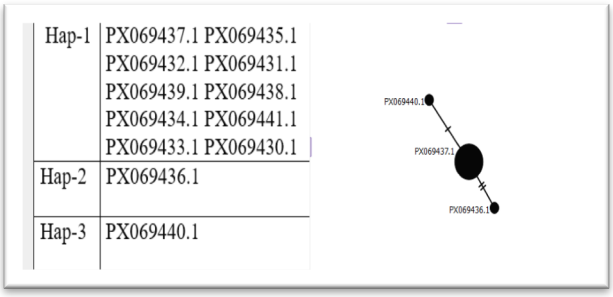


Fig. 3: Median-joining haplotype network of *Dientamoeba fragilis* isolates illustrating genotype 1 (blue) and genotype 2 (red) clusters constructed using PopART ver. 1.7



Hap-1	PX069437.1 PX069435.1 PX069432.1 PX069431.1 PX069439.1 PX069438.1 PX069434.1 PX069441.1 PX069433.1 PX069430.1
Hap-2	PX069436.1
Hap-3	PX069440.1

Fig. 4: Haplotype network of *Dientamoeba fragilis* isolates identified in the present study based on SSU rRNA gene sequence variation (Hd = 0.318).4

Phylogenetic Analysis

The phylogenetic relationships inferred by the Maximum Likelihood (ML) method based on the Tamura 3-parameter model demonstrated that all isolates from the present study grouped within a well-supported cluster (bootstrap=88%) corresponding to genotype 1 of *D. fragilis* (Fig. 5).

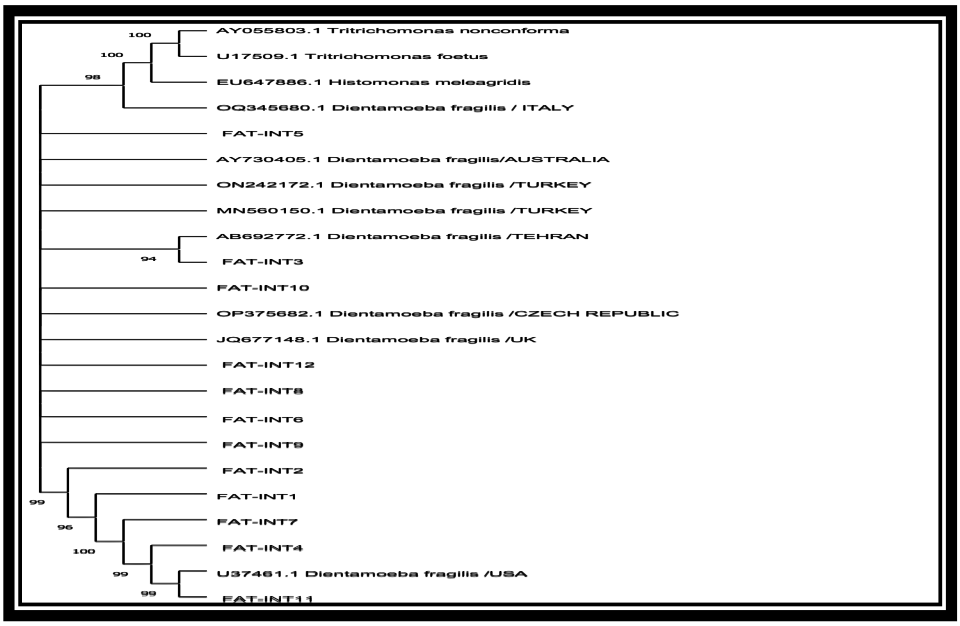


Fig. 5: The evolutionary history was inferred using the Maximum Likelihood (ML) method based on partial SSU rRNA gene sequences of *Dientamoeba fragilis*. The bootstrap consensus tree inferred from 1000 replicates is shown, and only branches supported by ≥70% bootstrap values are displayed. Evolutionary distances were computed using the Tamura 3-parameter model and expressed in units of the number of base substitutions per site. The analysis involved 23 nucleotide sequences with ambiguous positions removed using the pairwise deletion option. All evolutionary analyses were conducted using MEGA ver. 11

The reference sequence U37461 which represents genotype 2 showed independent evolutionary development through its distinct molecular characteristics.

Summary of Key Findings

The study showed that D. fragilis infected 24% of patients who displayed symptoms in Nineveh Governorate indicating its presence in the area.

The detected *D. fragilis* isolates exhibited high sequence identity ranging from 97 to 99.7% and low haplotype diversity (Hd=0.318), indicating limited genetic variability with predominance of genotype 1.

The analysis produced three haplotypes which show three-point mutations yet maintain a close phylogenetic relationship.

The phylogenetic tree and median-joining network demonstrate that Iraqi isolates cluster into one monophyletic group which belongs to the worldwide D. fragilis genotype 1 lineage.

Discussion

The present molecular study provides novel insights into the prevalence and genetic diversity of *D. fragilis* among symptomatic patients in Nineveh Governorate, Iraq. The study detected the parasite in 24% of tested samples which shows how little researchers understood about local parasite spread because they used to depend on microscopy for detection. The study confirms that *D. fragilis* maintains its local spread which supports previous research indicating *D. fragilis* infections remain undiagnosed worldwide because of restricted diagnostic

methods (3,4). The detection rate aligns with European research from Italy and the Czech Republic which demonstrated 21.4% and 24% infection rates through molecular assays that provided better results than conventional microscopy (5,14).

The molecular detection rate of 24% observed in the present study is comparable to those reported in Italy (21.4%) and the Czech Republic (24%) using PCR-based assays (5,14).

The high homology values of the analyzed isolates revealed a genetically stable population which is in line with earlier genotyping studies. The high sequence identity (97–99.7%) of the Iraqi isolates with each other as well as with isolates from world-wide locations that have been genotyped before underlines the fact that genotype 1 is a highly stable genotype in human populations all over the world (1,10,15).

The infection rates did not vary between different age groups or between male and female participants which suggests that exposure happens everywhere in the population through either direct contact with feces or through contaminated surfaces that affect people of all ages and sexes (2,5).

The study depends on PCR amplification of the SSU rRNA gene to achieve precise detection results. The fragile nature of *D. fragilis* trophozoites makes them difficult to detect through conventional microscopic examination because they deteriorate quickly when removed from their host environment (2). The parasite's actual spread in the population remains underreported because traditional detection methods fail to identify it properly. The study employed PCR to detect *D. fragilis* because this method enables sensitive and specific detection of the parasite through its targeting of a stable ribosomal sequence (9). Molecular parasitology experts recommend using these methods for diagnosing intestinal protozoa that were previously overlooked (1,6). The PCR detection method received additional validation through sequencing and BLAST analysis which showed that the detected sequences matched global *D. fragilis* isolates at 97%–99.7% genetic level. The research results confirm the detection method while showing minimal genetic variation between different *D. fragilis* specimens which supports the organism's single genetic strain (1).

The Iraqi *D. fragilis* population shows low genetic diversity because three haplotypes exist with a diversity index of 0.318. The population shows restricted genetic diversity because it could have expanded quickly or because host-to-host disease transmission remains restricted rather than because of evolutionary separation.

The observed pattern suggests that a single dominant genotype has spread throughout the population through clonal reproduction or the population went through a genetic bottleneck because of limited transmission patterns.

The research conducted in Turkey and China produced similar findings, as most D. fragilis isolates belonged to genotype 1 and showed low nucleotide diversity (10,16). This restricted diversity may reflect either a recent introduction of the parasite into the local population or strong selective pressure maintaining genetic uniformity. The restricted polymorphism may also be related to the low mutation rate and slow evolutionary change of the SSU rRNA gene (7).

The Iraqi isolates showed close similarity to genotype 1 sequence reported globally; however, broader conclusions regarding evolutionary stability require analysis of additional genetic loci and larger sample sizes (1,3). The median-joining network showed that all isolates shared three-point mutations with the reference strain AY730405 which placed them in a single tight genetic cluster. The observed clustering pattern shows that the transmission happened recently because the genetic variation stays small due to the small population size which is common for protozoa that reproduce directly (17).

The Maximum Likelihood model-based phylogenetic reconstruction showed that all study isolates belonged to genotype 1 which matches most global reports (1,8).

A high bootstrap value of 88% separated genotype 2 *(U37461)* from genotype 1. This genotype formed an independent evolutionary line of the organism. Genotype 1 forms a consistent evolutionary line which is supported by previous studies conducted in the USA in 1996 (7) and Turkey in 2021 (10). The Iraqi isolates form evolutionary relationships with some of the SSU rRNA gene sequences from Turkish and UK isolates of the organism. However, this relatedness is due to the conserved nature of the SSU rRNA gene sequences and is not indicative of disease transmission between individuals in different parts of the world. Previous molecular studies have also shown similar type of relatedness (8,18).

D. fragilis exists in nonhuman hosts including cattle and pigs (8), but it is unclear how much these hosts affect human infections. Previous research shows no distinct phylogenetic separation between human and animal isolates which indicates possible zoonotic transmission but needs additional experimental proof to confirm this route of transmission.

D. fragilis exists as a commensal organism but scientific evidence shows it can cause disease. Research studies show that patients experience symptom improvement following antiparasitic treatment which supports the idea that *D. fragilis* infection causes gastrointestinal diseases (6,5). Abdominal pain and diarrhea were the most common symptoms in positive patients which confirmed the disease-causing nature of the pathogen. The different symptoms that infected people show indicate that host factors including immune status, microbiome composition, and *Blastocystis* spp. co-infection might affect pathogenicity (14). The observed low prevalence of this infection might be caused by the short survival time of trophozoites in the environment because they break down quickly after being excreted, which makes transmission through the fecal–oral route difficult unless proper sanitation exists (2).

The current research study provides the second molecular proof of *D. fragilis* infection in Iraq since Al-Qassab (11), while expanding the small collection of intestinal protozoa data in the country. The molecular epidemiological profile of Iraq matches neighboring and European regions because genotype 1 dominates the population and the genetic diversity within the population remains low. Research should investigate human populations who do not show symptoms and animal species to determine how *D. fragilis* spreads between species and how it evolves. MLST or whole-genome sequencing methods would provide detailed information about microevolutionary processes and species transmission according to (3,19). The presence of genotype 1 in human populations can be confirmed through environmental water source and domestic animal sampling, which would also reveal if the genotype exists in peridomestic areas.

The findings of this study should be interpreted in light of certain limitations, including the relatively small sample size, analysis based on a single genetic locus (SSU rRNA), absence of asymptomatic controls, and storage of stool samples at –20 °C prior to DNA extraction. These factors may have influenced haplotype resolution and should be considered in future large-scale molecular epidemiological investigations.

Conclusion

The present study provides molecular evidence of the presence of *D. fragilis* and its genetic variation among symptomatic patients in Nineveh Governorate. The predominance of genotype 1 with low haplotypic diversity suggests limited genetic variability within the analyzed isolates and PCR-based molecular detection proved useful for identifying D. fragilis infections in the present study. The SSU rRNA gene detection using PCR revealed predominance of genotype 1 among the analyzed isolates with 97%–99.7% sequence similarity and low haplotype diversity at 0.318. Sequence

similarity with previously reported regional and international isolates was observed within the analyzed SSU rRNA fragment. Further studies involving larger sample sizes and multilocus molecular approaches are required to better characterize the epidemiology and genetic diversity of *D. fragilis* in the region.

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Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Caccio SM. Molecular epidemiology of *Dientamoeba fragilis*. Acta Trop. 2017; 184:73–77.
2. Garcia LS. *Dientamoeba fragilis*, one of the neglected intestinal protozoa. J Clin Microbiol. 2016; 54:2243–2250.
3. Stark D, Barratt J, Chan D, Ellis JT. *Dientamoeba fragilis*, the neglected trichomonad of the human bowel. Clin Microbiol Rev. 2016;29:553–580.
4. Barratt JL, Harkness J, Marriott D, Ellis JT, Stark D. A review of *Dientamoeba* fragilis carriage in humans. Gut Microbes. 2011;2:3–12.
5. Calderaro A, Gorrini C, Montecchini S, et al. Prevalence of intestinal parasitoses revealed by molecular analysis. Microorganisms. 2022;10(5):1123.
6. Jones A, Peterson M, Walker T. Molecular detection of *Dientamoeba fragilis* cases in Utah, USA. Epidemiol Infect. 2025;153:e47.
7. Silberman JD, Sogin ML, Leipe DD, Clark CG. *Dientamoeba fragilis* shares a recent evolutionary history with the trichomonads. Mol Biochem Parasitol. 1996;76:311–314.
8. Yildiz I, Aynur ZE. First detection and molecular characterization of *Dientamoeba fragilis* in cattle. Zoonoses Public Health. 2022;69:897–903.
9. Rogers MJ, McManus DP, Muhi S, Gordon CA. Membrane technology for rapid point-of-care diagnostics for parasitic neglected tropical diseases. Clin Microbiol Rev. 2021;34(4):e00329–20.
10. Yildiz I, Aynur ZE, Korkmaz H. Investigation of *Dientamoeba fragilis* frequency in faecal samples by PCR. Turk Parazitol Derg. 2021;45:213–219.
11. Al-Qassab SE. Molecular detection of *Dientamoeba fragilis* among patients in Erbil City, Iraq. J Neonatal Surg. 2025;14(2):87–92.
12. Keklik G. Understanding evolutionary relationships and analysis methods through MEGA software. Int J New Horiz Sci. 2023;8:83–90.
13. Arnoux E, Eraud C, Navarro N, et al. Morphology and genetics reveal an intriguing pattern of differentiation at a very small geographic scale in a bird species, the forest thrush Turdus lherminieri. Heredity. 2014;113(6):514–525.
14. Jirku M, Ditrich O, Votypka J, Svobodova M. Prevalence of *Dientamoeba fragilis* in humans and animals using qPCR. Parasitol Res. 2022;121:2413–2422.
15. Dong HT, Chaijarasphong T, Barnes AC, et al. From the basics to emerging diagnostic technologies: what is on the horizon for tilapia disease diagnostics? Rev Aquacult. 2023;15:186–212.
16. Zhao Y, Zhang F, Liu W, Lu P, Zhang Y. Molecular detection of *Dientamoeba fragilis* in children in southern Xinjiang, China. Food Waterborne Parasitol. 2025;33:e00191.
17. Guadano-Procesi I, López A, García M, Pérez J, Gómez F. Detection and genotyping of *Dientamoeba fragilis* from symptomatic patients. Parasitol Int. 2023;98:102816.
18. Fernández-Miranda CM, Fernández JL, Arroyo M, Iglesias E. *Dientamoeba fragilis*: an emerging pathogen. Enferm Infecc Microbiol Clin. 2025;43(1):e12–e18.
19. Alabdaly YZ, Al-Hbiti TY, Kaleel HI. Fluconazole toxicity in a rat model: histopathological and neurobehavioral effects. Iran J Vet Med. 2025;19(3):459–470.