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Original Article

Dientamoeba fragilis diagnosis by fecal screening: relative effectiveness of traditional techniques and molecular methods

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Abstract

Introduction: *Dientamoeba fragilis,* an intestinal trichomonad, occurs in humans with and without gastrointestinal symptoms. Its presence was investigated in individuals referred to Milad Hospital, Tehran.

Methodology: In a cross-sectional study, three time-separated fecal samples were collected from 200 participants from March through June 2011. Specimens were examined using traditional techniques for detecting *D. fragilis* and other gastrointestinal parasites: direct smear, culture, formalin-ether concentration, and iron-hematoxylin staining. The presence of *D. fragilis* was determined using PCR assays targeting 5.8S rRNA or small subunit ribosomal RNA.

Results: *Dientamoeba fragilis*, *Blastocystis* sp.*, Giardia lamblia, Entamoeba coli*, and *Iodamoeba butschlii* were detected by one or more traditional and molecular methods, with an overall prevalence of 56.5%. *Dientamoeba* was not detected by direct smear or formalin-ether concentration but was identified in 1% and 5% of cases by culture and iron-hematoxylin staining, respectively. PCR amplification of SSU rRNA and 5.8S rRNA genes diagnosed *D. fragilis* in 6% and 13.5%, respectively. Prevalence of *D. fragilis* was unrelated to participant gender, age, or gastrointestinal symptoms.

Conclusions: This is the first report of molecular assays to screen for *D. fragilis* in Iran. The frequent finding of *D. fragilis* via fecal analysis indicated the need to include this parasite in routine stool examination in diagnostic laboratories. As the length of amplification target correlates to the sensitivity of PCR, this assay targeting the *D. fragilis* 5.8S rRNA gene seems optimal for parasite detection and is recommended in combination with conventional microscopy for diagnosing intestinal parasites.

Key words: *Dientamoeba fragilis*; intestinal parasites; PCR; Iran.

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Introduction

Dientamoeba fragilis Jepps and Dobell 1918 (*Monocercomonadidae*, Sarcomastigophora) is a trichomonad parasite infecting the gastrointestinal tract of humans and other vertebrates, including sheep, pigs, and birds [1,2]. This microorganism shows extensive genetic diversity, comprising variants morphologically related but distinct in their pathogenicity [3-5]. Although described about century ago, *D. fragilis* biology, virulence, pathogenicity, epidemiology, and mode of transmission are not well understood, and findings can be conflicting [4,6,7].

Dientamoeba fragilis infections range from asymptomatic to causing acute or chronic disease in children and adults. The most common symptom of dientamoebiasis is diarrhea, followed by abdominal pain, fatigue, anorexia, and flatulence [8-10]. Dientamoebiasis may occur at any age and has a cosmopolitan distribution. Prevalence of *D. fragilis* infection varies considerably and is influenced by factors including geographic location, population density, living conditions, and level of hygiene and sanitation [1]. Data on the international prevalence of *D. fragilis* are limited. Worldwide, the prevalence has been reported to range from 0.4% to 71% [2,8,11,12], making it a more frequent cause of gastrointestinal infection than *Giardia lamblia* [13-15]. The sensitivity of diagnostic techniques and the expertise of testing laboratories affect the reported prevalence rate of *D. fragilis* [14,16]. Common methods such as direct smear and culture are challenging and require experience to distinguish *D. fragilis* from other gastrointestinal parasites [17]. Accurate identification depends on detection of the trophozoites in permanently stained stool smears, since the nuclear structure cannot be demonstrated in unstained stool samples [18]. The

staining technique is generally laborious, time consuming, and relatively insensitive. The development of PCR has provided a highly sensitive and specific method for diagnosis of pathogenic protozoa. PCR-based assays using species-specific primers offer a convenient and reliable technique for the detection of *D. fragilis* [17,19].

Intestinal parasitic infections are a critical public health problem in Iran; however, research on *D. fragilis* has been limited. Its reported prevalence, as determined by the direct smear method, varies from 0.5 to 2.4% depending on area of the country [20-22]. Using the iron-hematoxylin staining method, Jamali and Khademvatan [23] reported prevalence of 13.2%. As *D. fragilis* is a significant human pathogen, further research on its occurrence and effects is warranted [2,24]. We therefore aimed to investigate *D. fragilis* infection in individuals referred to Milad Hospital in Tehran, comparing traditional and molecular methods of detection.

Methodology

Sample collection

In a cross-sectional study, three fresh fecal specimens, separated by at least one day, were collected from each of the 200 participants referred to the clinical laboratory of Milad Hospital in Tehran, from March through July 2011. Participants provided informed consent and the study was approved by Ethics Committee under number IR.IUMS.FMD.REC 1390.1065. Fecal specimens were immediately submitted to the research laboratory of the Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences. All specimens were investigated for parasites by direct wet-mount microscopy, formalin-ether concentration, culture, modified iron-hematoxylin staining, and two PCR assays for *D. fragilis*.

Microscopic examination

Direct wet-mount microscopy and formalin-ether concentration methods

Stool specimens were investigated microscopically for trophozoites forms of intestinal protozoan parasites using direct wet-mount in saline and iodine-solution (Lugol's iodine) [10]. Formalin-ether concentration was conducted to identify ova and cysts or oocysts [25,26].

Sample preparation and culture

To a 10-20 g fecal sample, 50 mL of phosphate buffered saline (PBS) pH 7.4 was added and thoroughly mixed. The suspension was filtered through two layers of gauze and centrifuged at 800 × g for 5 min. Sediments were re-suspended in ~2 mL of PBS before combining with culture medium and fixing in either sodium acetate-acetic acid-formalin (SAF) or 80% ethanol [27-29].

For isolation of intestinal protozoa to be cultivated in an axenic medium, feces were cultured in a diphasic medium as described by Clark and Diamond [30]: slope of heat-inactivated horse serum (kindly provided by the Faculty of Veterinary Medicine, University of Tehran, Iran) overlaid with 5 mL of Ringer's solution and supplemented with ~1 mg rice starch (HSr+S). Penicillin-streptomycin (Sigma–Aldrich, Steinheim, Germany) was added to control the growth of human bacterial flora. A 300 µL sample of washed and unpreserved stool were added to culture tubes containing medium and rice starch and incubated in a vertical position at 35.5 °C. A drop of sediment from the tube was examined on a microscope slide three times at 48 hours intervals at 100× and 400× magnification.

Staining

The stool samples fixed in SAF were stained with modified iron-hematoxylin stain according to methods for identification of protozoa [9,27,31]. Precise microscopic diagnosis of *D. fragilis* was based on morphological characters from permanent stained smears at 400× and 1000× magnification. All slides were examined by two independent examiners.

Molecular examination

DNA extraction

One mL of stool preserved in 80% ethanol was centrifuged at 1000 × g for 5 minutes, and the sediment was re-suspended in PBS and washed twice in sterile PBS to remove ethanol. After washing, the sediment was re-suspended in 200 µL 2% polyvinylpolypyrrolidone (PVPP) (Sigma–Aldrich, Steinheim, Germany) in PBS, combined thoroughly, and stored at -20 °C for 24 hours [28]. The samples were heated for 10 minutes at 100 °C before submitting to DNA extraction using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, modified according to Verweij *et al.* [28].

Polymerase chain reactions

Conventional PCR was performed on samples to amplify a *D. fragilis* 98 bp 5.8S rDNA product [28] and an 887 bp SSU rDNA fragment as previously described [27]. To target 5.8S rRNA, the primers DF-124 (5'-

CAACGGATGTCTTGGCTCTTTA-3') and DF-221 (5'-TGCATTCAAAGATCGAACTTATCAC-3') [28] were used in 15 µL of an amplification reaction mixture with 7.5 µL Taq DNA polymerase 2X-preMix (GeneOn, Germany), 2 µL of genomic DNA, and 0.4 µM of each primer. Reaction conditions were 3 minutes at 95 ºC followed by 50 cycles of 95 ºC for 15 s and 60 ºC for 60 s, with a final extension of 72 ºC for 2 minutes. Amplification products were separated on 2.5% (W/V) agarose gel by electrophoresis.

The primers DF400 (5'-TATCGGAGGTGGTAATGACC-3') and DF1250 (5'-CATCTTCCTCCTGCTTAGACG-3') targeting SSU (18S) rRNA [27] in 20 µL final PCR reaction [10 µL of Taq DNA polymerase 2X-preMix (GeneOn, Ludwigshafen, Germany), 2 µL genomic DNA, and 0.4 µM of each PCR primer] with the reaction conditions of 3 minutes at 94 ºC followed by 30 cycles of 94 ºC for 1 minutes, 57 ºC for 1.5 minutes, and 72 ºC for 2 minutes and a final step of 7 minutes at 72 ºC. The PCR products were detected on ethidium bromide stained 1.5% agarose gels. All PCR reactions included a negative control containing sterile distilled water instead of DNA template and a positive control containing genomic DNA extracted from a stool specimen microscopically confirmed to be infected with *D. fragilis*. Some *D. fragilis* PCR-positive samples were confirmed by sequencing an 887 bp amplified SSU rRNA gene fragment in both directions (MWG-Biotech Company, Ebersberg, Germany). The sequence results were read by CHROMAS (Technelysium Pty Ltd., Queensland, Australia) and aligned using DNASIS MAX v. 3.0 (Hitachi, Yokohama, Japan). The final SSU rDNA sequencing results were compared with the Genbank database using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analysis was performed in MEGA7 (www.megasoftware.net) using the neighbor-joining method, and the evolutionary distances were computed using the Kimura 2-parameter method and a bootstrap value of 1000.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, USA). A descriptive analysis was conducted to determine the prevalence of parasites by gender, age group, clinical symptoms, and reason for referral. Associations between qualitative variables were evaluated using the chi-square (χ^2) test to reveal statistically significant values (p-value < 0.05).

Results

Participant enrollment

Two-hundred participants were enrolled in the study, 50.5% female and 49.5% male. The mean age was 27.6 ± 19.1 years, ranging from one to 79 years. Most participants lived in Tehran Province (86%), with

Table 1. Characteristics and clinical features of participants positive and negative for *D. fragilis*

	Participants			
	Positive n = 27	**Negative n =173**	**Total n =200**	***p*-value**
Age years (Mean ± SD)	31.7 ± 19.8	26.9 ± 19.0	27.6 ± 19.1	0.23
Gender				
Male (%)	13 (6.5)	86 (43.0)	99 (49.5)	0.88
Female (%)	14 (7.0)	87 (43.5)	101 (50.5)	
Clinical symptoms				
Diarrhea (%)	5 (18.5)	26 (15.0)	31 (15.5)	0.21
Anorexia (%)	2 (7.4)	12 (6.9)	14 (7.0)	0.93
Abdominal pain (%)	8 (29.6)	54 (31.2)	62 (31.0)	0.87
Flatulence (%)	4 (14.8)	27 (15.6)	31 (15.5)	0.92
Cramping (%)	8 (29.6)	38 (22.0)	46 (23.0)	0.92
Nausea (%)	4 (14.8)	11 (6.4)	15 (7.5)	0.42
Vomiting (%)	1 (3.7)	5 (2.9)	6 (3.0)	0.82
Urticaria (%)	2 (7.4)	6 (3.5)	8 (4.0)	0.33
Constipation (%)	0 (0.0)	4 (2.3)	4 (2.0)	0.43
Reason for referral				
Routine exam (%)	14 (12.2)	101 (87.8)	115 (57.5)	0.43
Gastrointestinal disorder (%)	12 (16.0)	63 (84)	75 (37.5)	
Non-gastrointestinal disorder (%)	1 (10.0)	9 (90)	10 (5.0)	
Participant home				
Tehran province (%)	23 (13.4)	149 (86.6)	172 (86.0)	0.54
Other province (%)	4 (14.3)	24 (85.7)	28 (14.0)	

Clinical symptom percentages do not total 100% as some participants had multiple symptoms.

14% referred from other provinces. Participants were categorized according the reason for referral: routine medical examination (115), gastrointestinal symptoms (75), or non-gastrointestinal disorders (10). Gastrointestinal symptoms were diarrhea (41.3%, 31/75), anorexia (18.7%, 14/75), abdominal pain (82.7%, 62/75), flatulence (41.3%, 31/75), cramping (61.3%, 46/75), nausea (20%, 15/75), vomiting (8%, 6/75), and constipation (5.3%, 4/75) (Table 1).

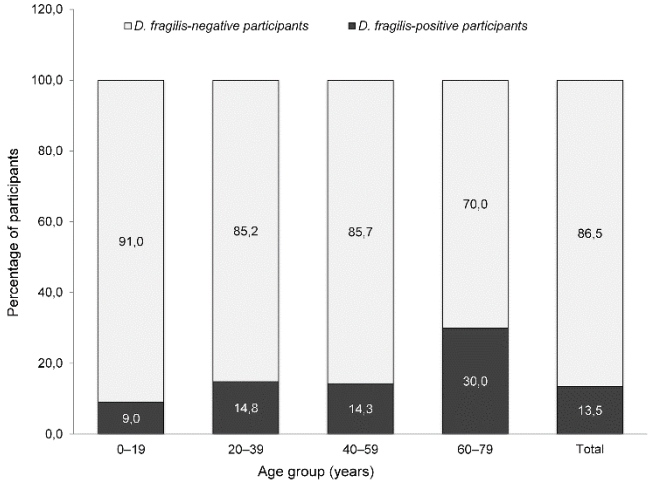
Microscopic analysis

Examination by direct microscopy, formalin-ether concentration, and culture and staining techniques revealed 69 (34.5%; 95% CI: 28.3%–41.3%) participants infected with at least one of the species of intestinal protozoon detected: *Dientamoeba fragilis*, *Blastocystis* sp., *Giardia lamblia, Entamoeba coli*, and *Iodamoeba butschlii* (Table 2). Helminth infection was not detected. *Blastocystis* sp. was the most commonly detected protozoon (31.5%; 95% CI: 25.5–38.2%). *Dientamoeba fragilis* was detected in ten (5%; 95% CI: 2.7%–9.0%) and two (1%; 95% CI: 0.3%–3.0%) subjects by modified iron-hematoxylin staining and culture methods, respectively; while no *D. fragilis* infection was found by direct microscopy or formalin-ether concentration methods (Table 2).

Molecular analysis

The PCR assays detected *D. fragilis* in 12 (6%; 95% CI: 3.5%–10.2%) and 27 (13.5%; 95% CI: 9.4%–18.9%) subjects with SSU rRNA and 5.8S rRNA gene amplification, respectively (Table 2). *Dientamoeba fragilis* was diagnosed in 14 (13.9%; 95% CI: 8.4%–21.9%) females and 13 (13.1%; 95% CI: 7.8%–21.2%) males. The mean age of *D. fragilis*-positive patients was 31.7 years (SD = 19.8), ranging from one to 66 years (Table 1). The highest prevalence of *D. fragilis* infection (30%; 95% CI: 10.8%–60.3%) was found in participants 60-79 years of age (Figure 1). *D. fragilis*-positive patients had been referred for clinical symptoms associated with *D. fragilis* (44.4%; 95% CI: 27.6%–62.7%), for routine medical examination (51.9%; 95% CI: 34.0%–69.3%), and for non-gastrointestinal disorders (3.7%; 95% CI: 0.7%–18.3%) (Table 1). Gastrointestinal complaints were diarrhea (18.5%, 5/27), anorexia (7.4%, 2/27), abdominal pain (29.6%, 8/27), flatulence (14.8%, 4/27), cramping (29.6%, 8/27), nausea (14.8%, 4/27), and vomiting (3.7%, 1/27) (Table 1). Chi-square analysis have revealed no relationship among *D. fragilis* infection and gender, age, reason for referral, or clinical symptoms The age distribution of *D. fragilis*-positive individuals compared to participating individuals is shown in Figure 1.

Figure 1. Age distribution of *D. fragilis*-infected patients.



Microscopic examination and PCR showed 79 (39.0%; 95% CI: 32.5–45.9%) patients with intestinal protozoa. Single parasite infections were observed in 60 (30%; 95% CI: 24.1–36.7%) cases. Nineteen patients (9.5%; 95% CI: 6.2–14.4%) had mixed parasite infections, with 15 (7.5%; 95% CI: 4.6–12.0%) infected with two, and four (2%; 95% CI: 0.8–5.0%) infected with three, parasites (Table 3). Among the 27 *D. fragilis*-infected patients, 14 (51.9%; 95% CI: 34.0–69.3%) were co-infected with other intestinal protozoa. Ten showed double infection with *Blastocystis*. In four,

Table 2. Number positive and prevalence (%) of intestinal parasites in participants referred to the clinical laboratory of Milad Hospital, Tehran, from March to July 2011.

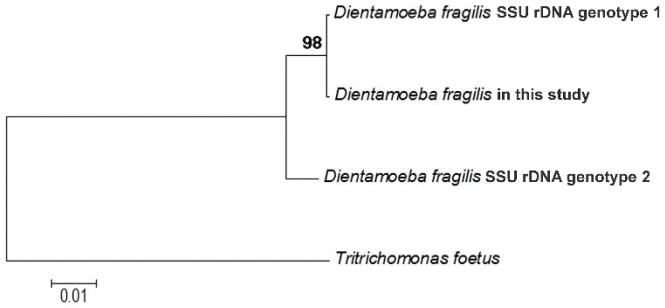
Parasite species	Direct microscopy	Formalin-ether concentration	Culture	Iron-hematoxylin	*Dientamoeba* PCR		Total infection	95% CI[a] of total
					5.8S rRNA	SSU rRNA		
Dientamoeba fragilis	0 (0)	0 (0)	2 (1)	10 (5)	27 (13.5)	12 (6)	27 (13.5)	9.4–18.9
Blastocystis sp.	21 (10.5)	22 (11)	58 (29)	ND	ND	ND	63 (31.5)	25.5–38.2
Giardia lamblia	2 (1)	2 (1)	0 (0)	ND	ND	ND	2 (1)	0.3–3.6
Entamoeba coli	4 (2)	6 (3)	4 (2)	ND	ND	ND	7 (3.5)	1.7–7.0
Iodamoeba butschlii	3 (1.5)	1 (0.5)	1 (0.5)	ND	ND	ND	3 (1.5)	0.5–4.3
Total	**26 (13)**	**29 (14.5)**	**58 (29)**	**10 (5)**	**27 (13.5)**	**12 (6)**	**78 (39)**	**32.5–45.9**

[a] CI, Confidence Intervals; ND, not done; Infection percentages do not total 100% as some participants had multiple infections.

triple infection was observed: *Blastocystis* and *G. lamblia* in one, *Blastocystis* plus *E. coli* in two, and *Blastocystis* and *I. butschlii* in one (Table 3).

Sequence analysis of SSU rRNA gene amplicons confirmed the *D. fragilis* infections. The sequences were aligned with the published sequences in GenBank using DNASIS MAX v.3.0; (Hitachi, Yokohama, Japan). The three sequences showed 100% homology without variation and were submitted to DDBJ/EMBL/GenBank databases under accession nos. AB692771–AB692773. The sequences showed a 98% identity with that of the *D. fragilis* genotype 1 accession no. AY730405.1 (Figure 2), with a single substitution of a cytosine with a thymine at position 305 and an extra guanine in nucleotide position 239 of the first nucleotide of AY7304050.1.

Figure 2. Phylogenetic tree of *D. fragilis* genotypes constructed by neighbor-joining analysis, based on small subunit ribosomal DNA (SSU rDNA) sequences retrieved from this study (AB692771–3) compared with *D. fragilis* genotype 1 (AY730405.1), *D. fragilis* genotype 2 (U37461.1), and *Tritrichomonas foetus* (M81842.1) from Genbank. Bootstrap values obtained from 1000 replicates are indicated on branches in percentage. The length of the scale bar is equivalent to a sequence difference of 1%. The evolutionary distances were computed using the Kimura 2-parameter method and are expressed as the number of base substitutions per site. There were 805 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.



Discussion

Gastrointestinal parasitic infections caused by helminths and protozoans are common worldwide and occur in most parts of Iran. Factors including method of sample fixation and examination may bias the diagnosis of *D. fragilis* and other protozoans in stool samples [32]. Identification and differentiation of these parasites by common techniques such as direct smear and formalin-ether concentration has been reported to lack accuracy and to be laborious and time consuming compared to molecular assays [17,27,28,33].

In this study we found *D. fragilis* infections in ~13% of individuals referred to Milad Hospital in Tehran, with no correlation to gender, age, clinical symptoms, or reason for referral. Little information with respect to *D. fragilis* in Iran is available, and this is the first report of molecular diagnosis in the area. The obtained prevalence agreed with the reported prevalence of 0.4% to 71% worldwide, in which observed variations are primarily dependent on diagnostic method, the studied population, and the geographic region [2,12]. The reported prevalence of *D. fragilis* in Iran varies from 0.5% [21,34] by direct microscopy to 2%-13.2% by iron-hematoxylin staining or trichrome staining in patients with intestinal symptoms [20,22,23]. Ghazanchaei *et al.* [20] and Sarafraz *et al.* [22] used nested-PCR to confirm *D. fragilis* identified by permanent staining (2% and 2.4%, respectively).

The impact of diagnostic methods on the reported prevalence of *D. fragilis* was clearly seen in our study. Although three stool samples from each individual were collected at different times to increase the probability of detecting *D. fragilis* and other protozoa [8,27], *D. fragilis* was not detected by direct smear or formalin-ether concentration methods, similar to previous studies [22,35,36]. Two *D. fragilis*-infected subjects (1%) were revealed by the culture method. It may be that the *D. fragilis* present were dead, or there may have been an over-growth of other protozoa in the stool samples that prevented *D. fragilis* replication [19]. The prevalence of *D. fragilis* was 5% with iron-hematoxylin staining.

Table 3. Number of single and multiple infections in participants referred to the clinical laboratory of Milad Hospital Tehran, from March to July 2011.

	Single and multiple infections			
Parasite species	**1**	**2**	**3**	**Total**
Dientamoeba fragilis (*Df*)	13	10 (*Df*+*B*)[a]	4 (*Df*+*B*+*Gl/Ec/Ib*)[b]	27
Blastocystis sp. (B)	44	15 (*B*+*Df/Ec/Ib*)[c]	4 (*B*+*Df*+*Gl/Ec/Ib*)	63
Giardia lamblia (*Gl*)	1	0	1 (*Gl*+*Df*+*B*)[d]	2
Entamoeba coli (*Ec*)	2	3 (*Ec*+*B*)	2 (*Ec*+*Df*+*B*)	7
Iodamoeba butschlii (*Ib*)	0	2 (*Ib*+*B*)	1 (*Ib*+*Df*+*B*)	3
Number of infected patients	60	15	4	79

[a] double infection of *Dientamoeba* with *Blastocystis;* [b] triple infection of *Dientamoeba* and *Blastocystis* with *Giardia* or *E. coli* or *Iodamoeba;* [c] double infection of *Blastocystis* with *Dientamoeba* or *E. coli* or *Iodamoeba;* [d] triple infection of *Giardia*, *Dientamoeba* and *Blastocystis*.

Grendon *et al.* [32] suggested that accurate and reliable detection of *D. fragilis* requires permanently stained preparations of fixed or fresh unpreserved stool specimens. However, the accuracy of this technique is low, as the trophozoites of *D. fragilis* can be easily overlooked due to pale staining of their nuclei, which may resemble those of *Entamoeba* spp. We used ethanol-preserved stool samples for PCR to prevent DNA fragmentation [17,28]. Prevalence varied from 6% when targeting the SSU (18S) rRNA gene to 13.5% with the 5.8S rRNA gene, likely reflecting the different size of amplicons of the 5.8S rRNA (98 bp) and the SSU rRNA (887 bp) genes. Verweij *et al.* [28] indicated that the amplification of large fragments can reduce the sensitivity of PCR for detecting *D. fragilis* directly from stool specimens.

In addition to conflicting reports of *D. fragilis* worldwide prevalence, the influence of gender and age on vulnerability to infection is unclear. Our data showed no significant differences in *D. fragilis* infection associated with gender or age. Nevertheless, the highest rate of *D. fragilis* (30%) was detected in participants 60-79 years. These results may be related to the limited study population, particularly of older participants, or might reflect a correlation of age with *D. fragilis* infection. A more comprehensive study with a broad age distribution is needed to resolve this issue. These limitations aside, this finding is similar to studies showing trends of higher infection rates in adults [9,14,37] and in contrast to some reports suggesting that children are common *D. fragilis* carriers [9,38-40]. Other studies have shown no influence of gender or age on rates of *D. fragilis* infection [10,27]. As in most gastrointestinal infections, direct exposure to the parasite may play a crucial role. Therefore, it is probable that infection by *D. fragilis* is related to poor hygiene regardless of gender or age.

We found high overall prevalence of intestinal parasites (39%), including *D. fragilis*, *Blastocystis sp.*, *G. lamblia*, *E. coli*, and *I. butschlii* and their co-infections. The most frequently detected parasite was *Blastocystis* (31.5%) followed by *D. fragilis* (13.5%). The majority of *D. fragilis*-positive individuals showed co-infection with other parasites, most frequently *Blastocystis*. Co-infection of *D. fragilis* with other enteric protozoa, especially *Blastocystis*, has been widely reported [10,26,41] and could support the hypothesis of direct transmission of *D. fragilis* through the fecal-oral route [2,6,19,42]. Neither ova nor larvae of helminths were observed in the examined stool samples using the formalin-ether method, reflecting the decreasing incidence and prevalence of intestinal helminth infections in Iran during past two decades [43].

The presented data showed no significant relationship between infection with *D. fragilis* and clinical symptoms or reason for referral. Many studies have shown correlation of infection with *D. fragilis* and clinical symptoms [3,8,9,13,39], while others report no relationship between symptomatic infection and this parasite [44-46]. This disparity is not surprising, as manifestations ranging from subclinical to severe gastrointestinal symptoms is typically observed in parasitic enteropathogen infections. This phenomenon is suggested to be related to genetic diversity in *D. fragilis*, resulting in a heterogeneous species [2,4,8,47,48].

Currently, two genotypes are described for *D. fragilis*, with genotype 1 being the most common [48]. The investigation of genetic variation in *D. fragilis* SSU rRNA with respect to geographic area has shown that SSU rRNA gene variation is not sufficient to be used as an epidemiological marker [4,47,48]. The SSU rRNA gene sequencing analysis of three *D. fragilis* isolates were similar and revealed 98% identity between our isolates and two corresponding published reference sequences for *D. fragilis* (accession nos. AY730405.1 and FJ649228.1). These results indicated low level of polymorphism, in agreement with recent studies [47,49].

Conclusions

This study demonstrated high prevalence of *D. fragilis* in Tehran via laboratory fecal analysis. Hence, clinical diagnostic laboratories should include screening for this parasite in routine stool examination. The PCR assay targeting the 5.8S rRNA gene detected a significantly greater number of *D. fragilis*-infected patients than did other analyses and is recommended as an effective tool for the accurate diagnosis of *D. fragilis* that should be employed in combination with microscopic methods to obtain a complete assessment of intestinal parasite infection. The use of these methods will prevent a high number of undiagnosed infections. Therefore, further studies applying this method to obtain accurate data on the prevalence of infection in specific age groups, symptomatic and asymptomatic individuals, other animals, and possibly a population-wide study, are required to ascertain epidemiology, pathogenicity, and transmission routes, as well as to identify reservoirs of *D. fragilis*.

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