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Frequency and Molecular Detection of *Giardia intestinalis* in Children Attending Pediatrics of Punjab, Pakistan

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Abstract

Background: *Giardia intestinalis* is the most common protozoan infecting the small intestine of human beings and a major cause of enteric infection, especially in children throughout the world. It is a highly diverse protozoan, which comprises a complex of eight genetic assemblages that are further differentiated into sub-assemblages.
Objectives: A cross-sectional study was conducted to detect the frequency, molecular detection and assemblage identification of *G. intestinalis* in children of Punjab, Pakistan.
Methods: A total of 800 stool samples were collected from children ranging 0 - 10 years of age with gastrointestinal disturbances and subjected to direct microscopy, enzyme-linked immunosorbent assay and polymerase chain reaction targeting small-subunit ribosomal RNA (SSU rRNA) and triosephosphate isomerase (*tpi*) genes. A predesigned questionnaire was filled prior to sampling from the guardian of each child to collect information.
Results: The results indicated that the prevalence of 9.5% (76/800) was achieved by microscopy, ELISA and PCR targeting SSU-rRNA gene. The genetic DNA from 69 out of 76 (90.80%) was successfully amplified by *tpi* gene. Among these *tpi* gene-positive samples, 38 were successfully typed in assemblage B (55.07%) followed by 20 (28.98%) in assemblage A and 11 (15.94%) in mixed type assemblages (A & B). Residency and socioeconomic status were statistically associated with giardiasis. Among the clinical presentations, abdominal pain is prominent in assemblage B (57.89%) and vomiting in assemblage A (40%) type infections.
Conclusions: Advanced molecular tools for giardiasis are well-adapted to get true prevalence, better discrimination of assemblages and their correlation with clinical signs.

Keywords: Molecular Detection, *Giardia intestinalis*, Assemblages, Children, Pakistan

1. Background

Giardia intestinalis is one of the commonest causes of gastrointestinal infections in children. It is the most vital drinking water contaminant and re-emerging as a cause of food-related illness (1). *Giardia intestinalis*, which is also famous as *G. duodenalis* or *G. lamblia* is a flagellated protozoan parasite that not only invades small intestine of humans but also resides in domestic and wild animals (2). *Giardia* is a parasite, which reproduces only in vertebrate host and it exhibits two clinically important forms, the cyst and trophozoite. Cysts are resistant to the environment and remain viable for several months even after treating with different disinfectants (3), while the trophozoites shows clinical signs and symptoms in the hosts (4). There are eight different assemblages of *G. intestinalis* (A-H) and among these types, assemblage A and B have potential to infect human and other mammals, while other genetic groups (C-H) showed host specificity for domesticated animals, livestock and wild animals. The type A and B have sub-groups Al, All, Blll, and BlV which are mostly related to human isolates (5-7).

The prevalence of *G. intestinalis* is variable: in developed areas of the world, it ranges from 2% to 5% (8), while in developing countries the prevalence level is quite high form 15% - 55% (9). In Pakistan, the reported prevalence of *G. intestinalis* is between 9% - 10% (10, 11). A major part of this prevalence consists of children less than 10 years of age, particularly those who are malnourished (12). Rou-

tinely the laboratory diagnosis of *G. intestinalis* is done by direct or concentrated microscopic method to detect *Giardia* cysts/trophozoites but this method has many limitations, including low sensitivity. On the other hand, antigen detection immunoassays are also a choice used in public health laboratories having better sensitivity and less time-consuming ability but they are unable to discriminate between the genetic assemblages of *G. intestinalis*. In order to improve the sensitivity, speed and specificity in comparison to microscopy and immunoassays, molecular diagnostic techniques based on polymerase chain reaction (PCR) are the better option to characterize *Giardia* in stool samples and to differentiate between its genetic assemblages (13, 14)

2. Objectives

The aim of this study was to detect the frequency of *G. intestinalis* in pediatric children and to determine the prevalence of genetic assemblages in Pakistan.

3. Methods

3.1. Study Design

The present cross-sectional study was conducted on 800 children admitted to the Pediatric wards of different hospitals of Punjab, Pakistan between 2016 and 2017. The children included in this study were ranging between 0 - 10 years of age showing gastrointestinal disturbances. Written informed consent was obtained before sample collection from the parents of each child after explaining the objectives and purpose of the study to them. Epidemiological and clinical data from each child was recorded in a predesigned questionnaire. The data includes some demographic variables like age, gender, residential area and socioeconomic status as well as disease variables like vomiting, nausea, and abdominal pain.

3.2. Collection and Processing of Samples

A fresh stool sample of 5 - 10 grams was collected in sterile 50 mL screw-cap containers from each child by a trained hospital staff member using non-probability convenience sampling technique. The sample size was estimated according to a formula devised by (WHO) in manual of epidemiology for district health management (15). Each sample was divided into three equal parts, one for microscopic examination of *Giardia* cysts/trophozoites using Lugol's iodine and concentration methods (16), second for Ridascreen *Giardia* enzyme immunoassay (R-Biopharm AG, Germany) for antigen detection of *G. intestinalis*, and the third part was used for molecular detection (17). All the Giardia cyst/trophozoite positive samples by microscopy and ELISA were preserved in 10% formalin solution and stored at -20°C for molecular analysis. The *Giardia* cysts were purified by sucrose gradient method. The walls of the cysts were disrupted by freeze/thaw method, five to seven cycles were done to achieve proper disruption of cell wall (18).

3.3. Detection of Giardia intestinalis by Conventional Methods

All the collected stool specimens were rapidly screened for the presence of *G. intestinalis* cyst/trophozoite followed by concentration techniques for detection of giardiasis. The second portion of the samples was subjected to Ridascreen *Giardia* enzyme immunoassay to detect *G. intestinalis* antigens, according to the manufacturer's instructions (11).

3.4. Detection of Giardia intestinalis by Molecular Methods

DNA Extraction: The genomic DNA of *G. intestinalis* was extracted by Favor Prep stool DNA isolation Kit (Favorgen Biotech Corporation, Taiwan) according to the manufacturer's instructions.

Amplification of (SSU-rRNA) and (*tpi*) Genes for Detection of *G. intestinalis*: The extracted DNA was subjected to SSU-rRNA polymerase chain reaction using specific primers RH11 (5'-CATCCGGTCGATCCTGCC-3') and RH4 (5'-GTCGAACCCTGATTCTCCGCCAGG-3') to amplify a 292 bp product as described previously with slight modifications (19). The thermal cycler profiling was adjusted to 95°C for 05 min/1 cycle, 95°C for 30 sec/35 cycles, 58°C for 30 sec/35 cycles, 72°C for 45 sec/35 cycles and final extension at 72°C for 07 min/01 cycle using (Optimus 96G, Gradient Thermal Cycler, UK). The detection of assemblages was done by amplification of the triosephosphate isomerase (*tpi*) gene using primers TPIA-F (5'-GGAGACCGACGAGCAAAGC-3') and TPIA-R (5'-CTTGCCAAGCGCCTCAA-3') for assemblage A while TPIB-F (5'-AATAGCAGCACARAACGTGTATCTG-3') and TPIB-R (5'-CCATGTCCAGCAGCATCT-3') for assemblage B as previously described (20). The thermal cycler condition varies and they were adjusted at 95°C for 15 min followed by 50 cycles 94°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec and final extension was performed at 72°C for 7 min. A 148-bp fragment of the assemblage A gene and 81-bp fragment of assemblage B gene were obtained with these primers. The PCR products were run on a 2% agarose gel with 5 μL of the reaction solution and visualized by staining the gel with ethidium bromide (21).

3.5. Statistical Analysis

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for all descriptive statistics. Chi-square test was used to ac-

cess the association between variables and outcome. The P value < 0.05 was considered statistically significant.

4. Results

The results of the present study showed that *G. intestinalis* prevalence was 9.5% (76/800) among pediatric children of Pakistan by microscopy, ELISA, and PCR targeting SSU-rRNA loci. On the basis of assemblage-specific *tpi* gene PCR, 90.80% (n = 69) samples were amplified successfully. The details of collected samples and positive samples by different methods are given in (Table 1). The assemblage specific *tpi* gene PCR categorized the highest (n = 38) samples into assemblage B (55.07%) followed by 20 (28.98%) as assemblage A, whereas 11 samples (15.94%) showed a mixed-type infection with both assemblages A and B. Statistically the difference between the prevalence of these assemblages was statistically significant (P < 0.05) as shown in Table 2. The highest number of assemblage B was detected from Khanewal, while assemblage A was more prevalent in Multan. Rawalpindi showed the elevated prevalence of mixed (A + B) infections.

The association between *G. intestinalis* assemblages and sociodemographic characters were also evaluated in this study as shown in Table 2. No statistical association was found between assemblages of Giardia and demographic variables (gender, residence and socioeconomic status). Overall the giardiasis was statistically associated with residence and socioeconomic status of the host (P < 0.05). The clinical variables associated with *G. duodenalis* assemblages were investigated and listed in Table 3. The abdominal pain was more prevalent in assemblage B (57.89%), while vomiting is more frequent in assemblage A (40%) infections. Bloody diarrhea is almost equally found in both types of infections.

5. Discussion

In the present study, we applied conventional and molecular methods for *G. intestinalis* diagnosis from children attending pediatric wards of Punjab, Pakistan. In the routine laboratory diagnosis, microscopic methods are used mostly but these tests have less sensitivity, require experienced laboratory technicians, are and unable to differentiate between the assemblages of *G. intestinalis* (22). The results indicated the prevalence of *G. intestinalis* in children as 9.5% (76/800), which was similar to the findings showed 9% in population of Kabul, Afghanistan (10). These findings also resemble the results of 11.8% in Pakistan (23), 8% in Cuba (17), and 6.8% in Portugal (24). The difference in these results is due to variety of factors such as drinking water, sewerage system and hygienic conditions of that area.

The clinical signs of giardiasis are due to nutritional status, immune status, nature of GIT microbiota of host, and virulent potential of the parasite, which affect the disease outcome (25). When PCR was applied to the positive samples targeting SSU-rRNA gene, all the samples were detected positive in terms of *Giardia* genome, showing 100% efficacy. These results are in accordance with the results of some recent findings that recorded more than 80% efficiency of SSU-rRNA PCR (17, 26). The reason is SSU-rRNA locus proved a greater sensitivity and traditional gene sequence for identification of *G. intestinalis* from stool samples due to its multicopy nature (27). On the other hand, this sequence is more conserved as compared to others (22).

It was observed in this study that genotype-specific *tpi* PCR detected 69 out of 76 samples (90.80%) positive for *G. intestinalis* genome. Very familiar results were achieved with 96% (20), 91% (28), and 92.6% (17) in France, Iran, and Cuba, respectively. Many scientists and coworkers also suggested that the *tpi* gene proved itself a better candidate for detecting *G. intestinalis* cysts from human stool samples in comparison to glutamate dehydrogenase (gdh) and 18S rRNA genes (28, 29). There is a variation in the performance of these different genes but the cause is yet unclear; the difference might be due to the presence of certain PCR inhibitors in stool samples as well as difference in thermal cycling profiles adopted by different researchers. The *tpi* gene is a polymorphic gene and has been established as a profitable marker for genotyping of *G. intestinalis* (30).

The *tpi* gene PCR found 38 samples (55.07%) positive as assemblage B of *G. intestinalis* followed by assemblage A (28.98%), while the mix infection type (A + B) was detected in 11 samples making (15.94%). Similar trend of results was observed previously showing 64% assemblage B and 36% assemblage A in 2005 (20), 16% assemblage B and 10% assemblage A in 2013 (31), 67.9% assemblage B and 32.1% as assemblage A in 2016 (19) and 50.8% as type B followed by 27% as type A and 22.2% as mix types in 2017 (17). In contrast, a higher prevalence of assemblage A (54.8%) and (80%) was detected in 2011 and 2014, respectively (26, 28). The difference in the prevalence of *G. intestinalis* assemblages was credited to geographical locations of the study area. Secondly, it also depends upon virulence of parasite such as assemblage A is mostly present in cases with intermittent diarrhea, whereas the assemblage B is profound in persistent diarrheal subjects (32). Thirdly, it was also reported that children infected with assemblage B shed more cysts in their feces as compared to assemblage A (33). Other factors might be the immune system and diet of the host (25).

Table 1. Geographical and Assemblage-Based Frequency of *Giardia intestinalis* in Children of Punjab, Pakistan[a]

Geographical Area/ District	Total Giardia Positive Samples	Positive by *tpi* Gene PCR	Different Assemblages in Positive Samples		
			Assemblage "B"	Assemblage "A"	Assemblage "A + B"
Faisalabad (n = 200)	13	12 (92.30)	6	4	2
Multan (n = 200)	21	19 (90.47)	10	7	2
Khanewal (n = 200)	25	23 (92.00)	15	5	3
Rawalpindi (n = 200)	17	15 (88.23)	7	4	4
Total (n = 800)	76	69 (90.80)	38 (55.07)	20 (28.98)	11 (15.94)
P value	non-significant (> 0.05)		significant (< 0.05)		

[a]Values are expressed as No. (%).

Table 2. Frequency of *Giardia intestinalis* on the Basis of Demographic Characteristics in Children of Punjab, Pakistan[a]

Demographic Variables	Assemblage A (N = 20)	Assemblage B (N = 38)	Assemblages A + B (N = 11)	Total (N = 69)
Gender				
Male	15 (75)	21 (55.26)	6 (54.54)	42 (60.87)
Female	5 (25)	17 (44.74)	5 (45.46)	27 (39.13)
Residence				
Rural	18 (90)	30 (78.95)	8 (72.72)	56 (81.16)[b]
Urban	2 (10)	8 (21.05)	3 (27.28)	13 (18.84)
Socioeconomic status				
Poor	17 (85)	32 (84.21)	9 (81.81)	58 (84.06)[b]
Good	3 (15)	6 (15.79)	2 (18.19)	11 (15.94)

[a]Values are expressed as No. (%).
[b]Statistically significant at (P < 0.05).

Table 3. Frequency of *Giardia intestinalis* on the Basis of Clinical Signs in Children of Punjab, Pakistan[a]

Clinical Variables	Assemblage A (N = 20)	Assemblage B (N = 38)	Assemblages A + B (N = 11)	Total (N = 69)
Abdominal pain	11 (55)	22 (57.89)	4 (36.36)	37 (53.62)
Vomiting	8 (40)	5 (13.15)	4 (36.36)	17 (24.63)
Bloody Diarrhea	2 (10)	4 (10.52)	1 (9.09)	7 (10.14)

[a]Values are expressed as No. (%).

The prevalence of *G. intestinalis* was statistically associated with residence and socioeconomic status of host. Similarly, abdominal pain was more prevalently found in assemblage B (57.89%), while vomiting is more frequent in assemblage A (40%) infections. This result also favors by the findings of some researchers that abdominal pain in *Giardia* infected children is more prominent in assemblage B infections (34).

5.1. Conclusions

In conclusion, the current study reveals a high prevalence of *G. intestinalis* in pediatric children of Punjab, Pakistan. It is also demonstrated that PCR is a rapid and sensitive method for detection of *Giardia* cysts from human stools and plays an important role in understanding the epidemiology, discrimination of assemblages and transmission dynamics of *G. intestinalis* infections.

Footnotes

Authors' Contribution: Study concept and design: Zeeshan Nawaz and Sultan Ali. Acquisition of data: Aneeqa Naz and Muhammad Uzair Mukhtar. Critical revision of the manuscript for important intellectual content: Muhammad Hidayat Rasool. Drafting of the manuscript: Abu Baker Siddique and Muhammad Asif Zahoor. Analysis and interpretation of data: Zeeshan Nawaz and Muhammad Asif Zahoor.

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Approval: This study was approved by Institutional Ethics Review Committee of Government College University Faisalabad, Pakistan under code GCUF/ERC/4155, and the samples were collected in accordance with international safety rules and ethical standards.

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Informed Consent: Written informed consent was obtained from the parents of each child before sample collection and after explaining the objectives and purpose of the study to them.

References

1. Thompson RC, Hopkins RM, Homan WL. Nomenclature and genetic groupings of Giardia infecting mammals. *Parasitol Today*. 2000;**16**(5):210–3. doi: 10.1016/s0169-4758(99)01624-5. [PubMed: 10782081].
2. Bouzid M, Halai K, Jeffreys D, Hunter PR. The prevalence of Giardia infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. *Vet Parasitol*. 2015;**207**(3-4):181–202. doi: 10.1016/j.vetpar.2014.12.011. [PubMed: 25583357].
3. Vernile A, Nabi AQ, Bonadonna L, Briancesco R, Massa S. Occurrence of Giardia and Cryptosporidium in Italian water supplies. *Environ Monit Assess*. 2009;**152**(1-4):203–7. doi: 10.1007/s10661-008-0308-4. [PubMed: 18528772].
4. Cook C. Giardiasis: A review. *Int Child Rights Monitor*. 2002;**15**(1):26–9. doi: 10.1023/a:1014271625511.
5. Lebbad M, Petersson I, Karlsson L, Botero-Kleiven S, Andersson JO, Svenungsson B, et al. Multilocus genotyping of human Giardia isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children. *PLoS Negl Trop Dis*. 2011;**5**(8). e1262. doi: 10.1371/journal.pntd.0001262. [PubMed: 21829745]. [PubMed Central: PMC3149019].
6. Li N, Xiao L, Wang L, Zhao S, Zhao X, Duan L, et al. Molecular surveillance of Cryptosporidium spp., Giardia duodenalis, and Enterocytozoon bieneusi by genotyping and subtyping parasites in wastewater. *PLoS Negl Trop Dis*. 2012;**6**(9). e1809. doi: 10.1371/journal.pntd.0001809. [PubMed: 22970334]. [PubMed Central: PMC3435239].
7. Monis PT, Andrews RH, Mayrhofer G, Ey PL. Genetic diversity within the morphological species Giardia intestinalis and its relationship to host origin. *Infect Genet Evol*. 2003;**3**(1):29–38. doi: 10.1016/s1567-1348(02)00149-1. [PubMed: 12797970].
8. Oberhuber G, Kastner N, Stolte M. Giardiasis: A histologic analysis of 567 cases. *Scand J Gastroenterol*. 1997;**32**(1):48–51. doi: 10.3109/00365529709025062. [PubMed: 9018766].
9. Alum A, Sbai B, Asaad H, Rubino JR, Khalid Ijaz M. ECC-RT-PCR: A new method to determine the viability and infectivity of Giardia cysts. *Int J Infect Dis*. 2012;**16**(5):e350–3. doi: 10.1016/j.ijid.2012.01.004. [PubMed: 22390842].
10. Tariq MT. Prevalence of Giardiasis in Afghan population. *Pak Pediatr J*. 2013;**37**(3):180–4.
11. Naz A, Nawaz Z, Rasool MH, Zahoor MA. Cross-sectional epidemiological investigations of Giardia lamblia in children in Pakistan. *Sao Paulo Med J*. 2018;**136**(5):449–53. doi: 10.1590/1516-3180.2018.0350060918. [PubMed: 30570096].
12. Al-Mekhlafi MS, Azlin M, Nor Aini U, Shaik A, Sa'iah A, Fatmah MS, et al. Giardiasis as a predictor of childhood malnutrition in Orang Asli children in Malaysia. *Trans R Soc Trop Med Hyg*. 2005;**99**(9):686–91. doi: 10.1016/j.trstmh.2005.02.006. [PubMed: 15992838].
13. Amar CF, Dear PH, Pedraza-Diaz S, Looker N, Linnane E, McLauchlin J. Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of Giardia duodenalis in human feces. *J Clin Microbiol*. 2002;**40**(2):446–52. doi: 10.1128/jcm.40.2.446-452.2002. [PubMed: 11825955]. [PubMed Central: PMC153413].
14. Read CM, Monis PT, Thompson RC. Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. *Infect Genet Evol*. 2004;**4**(2):125–30. doi: 10.1016/j.meegid.2004.02.001. [PubMed: 15157630].
15. Vaughan JP, Morrow RH; World Health Organization. *Manual of epidemiology for district health management*. Geneva: World Health Organization; 1989.
16. Abrar Ul Haq K, Gul NA, Hammad HM, Bibi Y, Bibi A, Mohsan J. Prevalence of Giardia intestinalis and Hymenolepis nana in Afghan refugee population of Mianwali District, Pakistan. *Afr Health Sci*. 2015;**15**(2):394–400. doi: 10.4314/ahs.v15i2.12. [PubMed: 26124784]. [PubMed Central: PMC4480483].
17. Jerez Puebla LE, Nunez FA, Santos LP, Rivero LR, Silva IM, Valdes LA, et al. Molecular analysis of Giardia duodenalis isolates from symptomatic and asymptomatic children from La Habana, Cuba. *Parasite Epidemiol Control*. 2017;**2**(3):105–13. doi: 10.1016/j.parepi.2017.05.003. [PubMed: 29774288]. [PubMed Central: PMC5952668].
18. Babaei Z, Oormazdi H, Rezaie S, Rezaeian M, Razmjou E. Giardia intestinalis: DNA extraction approaches to improve PCR results. *Exp Parasitol*. 2011;**128**(2):159–62. doi: 10.1016/j.exppara.2011.02.001. [PubMed: 21315715].
19. Faria CP, Zanini GM, Dias GS, da Silva S, Sousa Mdo C. Molecular characterization of Giardia lamblia: First report of assemblage B in human isolates from Rio de Janeiro (Brazil). *PLoS One*. 2016;**11**(8). e0160762. doi: 10.1371/journal.pone.0160762. [PubMed: 27517469]. [PubMed Central: PMC4982690].
20. Bertrand I, Albertini L, Schwartzbrod J. Comparison of two target genes for detection and genotyping of Giardia lamblia in human feces by PCR and PCR-restriction fragment length polymorphism. *J Clin Microbiol*. 2005;**43**(12):5940–4. doi: 10.1128/JCM.43.12.5940-5944.2005. [PubMed: 16333079]. [PubMed Central: PMC1317193].
21. Asgarian F, Tavalla M, Teimoori A, Zebardast N, Cheraghian B. Evaluation of three protocols of DNA extraction for detection of Giardia duodenalis in human fecal specimens. *Jundishapur J Microbiol*. 2018;**11**(4). e63096. doi: 10.5812/jjm.63096.
22. Nantavisai K, Mungthin M, Tan-ariya P, Rangsin R, Naaglor T, Leelayoova S. Evaluation of the sensitivities of DNA extraction and PCR methods for detection of Giardia duodenalis in stool specimens. *J Clin Microbiol*. 2007;**45**(2):581–3. doi: 10.1128/JCM.01823-06. [PubMed: 17122010]. [PubMed Central: PMC1829074].
23. Chaudhry ZH, Afzal M, Malik MA. Epidemiological factors affecting prevalence of intestinal parasites in children of Muzaffarabad District. *Pakistan J. Zool*. 2004;**36**(4):267–71.
24. Julio C, Vilares A, Oleastro M, Ferreira I, Gomes S, Monteiro L, et al. Prevalence and risk factors for Giardia duodenalis infection among children: A case study in Portugal. *Parasit Vectors*. 2012;**5**:22. doi: 10.1186/1756-3305-5-22. [PubMed: 22284337]. [PubMed Central: PMC3275531].
25. Cotton JA, Beatty JK, Buret AG. Host parasite interactions and pathophysiology in Giardia infections. *Int J Parasitol*. 2011;**41**(9):925–33. doi: 10.1016/j.ijpara.2011.05.002. [PubMed: 21683702].
26. Rayani M, Zasmy Unyah N, Hatam G. Molecular identification of Giardia duodenalis isolates from Fars Province, Iran. *Iran J Parasitol*. 2014;**9**(1):70–8. [PubMed: 25642262]. [PubMed Central: PMC4289883].
27. Ryan U, Caccio SM. Zoonotic potential of Giardia. *Int J Parasitol*. 2013;**43**(12-13):943–56. doi: 10.1016/j.ijpara.2013.06.001. [PubMed: 23856595].
28. Nahavandi KH, Fallah E, Asgharzadeh M, Mirsamadi N, Mahdavipour B. Glutamate dehydrogenase and triose-phosphate-isomerase coding genes for detection and genetic characterization of Giardia lamblia in human feces by PCR and PCR-RFLP. *Turk J Med Sci*. 2011;**41**(2):283–9.

29. Gelanew T, Lalle M, Hailu A, Pozio E, Caccio SM. Molecular characterization of human isolates of Giardia duodenalis from Ethiopia. *Acta Trop*. 2007;**102**(2):92–9. doi: 10.1016/j.actatropica.2007.04.003. [PubMed: 17498637].
30. Sprong H, Caccio SM, van der Giessen JW, Zoopnet network; partners. Identification of zoonotic genotypes of Giardia duodenalis. *PLoS Negl Trop Dis*. 2009;**3**(12). e558. doi: 10.1371/journal.pntd.0000558. [PubMed: 19956662]. [PubMed Central: PMC2777335].
31. Roointan ES, Rafiei A, Samarbaf-Zadeh AR, Shayesteh AA, Shamsizadeh A, Pourmahdi Borujeni M. Genotype analysis of Giardia lamblia isolated from children in Ahvaz, Southwest of Iran. *Jundishapur J Microbiol*. 2013;**6**(3):279–83. doi: 10.5812/jjm.6443.
32. Homan WL, Mank TG. Human giardiasis: Genotype linked differences in clinical symptomatology. *Int J Parasitol*. 2001;**31**(8):822–6. doi: 10.1016/s0020-7519(01)00183-7. [PubMed: 11403774].
33. Kohli A, Bushen OY, Pinkerton RC, Houpt E, Newman RD, Sears CL, et al. Giardia duodenalis assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. *Trans R Soc Trop Med Hyg*. 2008;**102**(7):718–25. doi: 10.1016/j.trstmh.2008.03.002. [PubMed: 18485429]. [PubMed Central: PMC2963065].
34. Puebla LJ, Nunez FA, Fernandez YA, Fraga J, Rivero LR, Millan IA, et al. Correlation of Giardia duodenalis assemblages with clinical and epidemiological data in Cuban children. *Infect Genet Evol*. 2014;**23**:7–12. doi: 10.1016/j.meegid.2014.01.007. [PubMed: 24462623].