Microbes and Infectious Diseases 2021; 2 (2): 378-385

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Molecular genotyping of *Giardia lamblia* assemblages by conventional polymerase chain reaction in rural and urban areas in Egypt

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ARTICLE INFO

Article history:
Received 10 December 2020
Received in revised form 12 January 2021
Accepted 14 January 2021

Keywords:
Giardiasis
Gastroenteritis
Genotyping
Microscopy
PCR

ABSTRACT

Background: Giardiasis is one of the most important parasitic gastro-intestinal infections that affect humans worldwide. Children are the most affected age group either in developing or developed countries. Genotyping of *Giardia lamblia* by molecular techniques classified it into eight assemblages; of which, assemblages A and B are potentially zoonotic pathogens. This study was done to investigate the prevalence of different *Giardia lamblia* genotypes by conventional polymerse chain reaction (PCR), and to explore the environmental and patients' sociodemographic factors that may affect the disease prevalence. **Methods**: Two hundreds fecal samples were collected at Damanhour General Hospital from patients with gastrointestinal diseases. All samples were examined microscopically, and the positive ones were investigated by conventional PCR. **Results:** Giardiasis was detected in 92 (46%) samples. Eighty-eight samples gave positive results by PCR, 18% of which were assemblage A, 70.5% were assemblage B, and 11.5% were mixed infection of both assemblages. The infection was more prevalent in males, rural patients and the highly educated ones. **Conclusion:** This study presents critical and demonstrative data regarding public health in Egypt. The results reveal that the prevalence of giardiasis is high among both rural and urban patients, particularly in children. It also prevails in patients of all education levels, and patients dealing and not dealing with animals. Moreover, we recommend PCR amplifying the triose phosphate isomerase (*tpi*) gene in fresh fecal samples as a very effective method for the diagnosis and genotyping.

Introduction

The lack of sanitation and hygiene measures is a major cause for the dissemination of protozoal intestinal diseases; therefore, they are most investigated in developing countries [1]. Giardiasis is the most common human parasitic intestinal infection worldwide. It's caused by *Giardia lamblia* (*G. lamblia*); a flagellated, bi-nucleated protozoa, being a major health concern worldwide. Children are the most affected persons either in developing or developed countries with prevalence rates 30% and 2-5%, respectively [2,3]. In the United States, it was reported that the average number of *G. lamblia* infected cases during 1995–2016 was 19781 cases per year [4]. Giardiasis clinical manifestations may be asymptomatic or symptomatic with acute or chronic diarrhea associated with abdominal cramps, vomiting, abdominal pain, fatigue, nausea, weight loss, damage of the small intestinal mucosal surface, and malabsorption [5,6]. Giardiasis is usually self-limited in immune-competent individuals; however, it can cause severe and fatal diarrhea in immune-compromised individuals and children, depending on

DOI: 10.21608/MID.2021.55720.1103
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host immune response and the virulence of *Giardia* genotype [7].

Life cycle of *G. lamblia* has a simple two stages: cysts and trophozoites. Cysts are the infective stage of this parasite. Giardiasis occurs through consumption of food or water harboring *Giardia* cysts or by the fecal-oral route. Trophozoites are the vegetative active stages that live and multiply in the upper small intestine where they adhere and cause giardiasis signs and symptoms [5].

Giardia contains at least six species that infect animals and/or humans [8]. *Giardia lamblia* is the main *Giardia* spp. documented in humans and most domestic animals such as: cattle, sheep, dogs, cats and horses [9]. The current taxonomy depends mainly on the morphology of *Giardia* spp. without any consideration for the genetic heterogeneity [10]. Polymerase chain reaction (PCR) and other molecular techniques revealed that there are eight morphologically indistinguishable *G. lamblia* assemblages (A - H) that differ in the sequence of the glutamate dehydrogenase, small subunit rRNA, and triose phosphate isomerase (*tpi*) genes. So far, assemblages C-H have not been detected in humans; however, assemblages A and B or "Polish" and "Belgian" are potentially zoonotic human pathogens [5,11]. To date, the distribution of assemblages of *G. lamblia* in different countries have been presented [7].

As a diagnostic method, PCR has a high sensitivity and specificity compared to direct microscopy; moreover, it can also detect giardiasis in patients with low parasitic cysts and differentiate the different *G. lamblia* genotypes [12]. Genotyping of *G. lamblia* by PCR in fecal samples depends greatly on concentration of DNA and presence of inhibitors such as: phenolic compounds, bilirubin, cellulose, hemoglobin, and polysaccharides, which may inhibit the *taq* polymerases. Fecal samples for parasitologic analysis are frequently collected and maintained by adding preservatives such as: 10% formalin or formalin derivatives. However, it's advisable to use fresh fecal samples for PCR procedures as it was reported that formalin may interact with DNA, or inhibit polymerases and amplification giving false negative results [10].

This study aimed to investigate the prevalence of giardiasis in rural and urban areas in El Behira governorate, Egypt, and molecularly genotype *G. lamblia* by conventional PCR. Moreover, it aimed to assess the possible environmental and patients' sociodemographic factors that may affect the disease prevalence.

Materials and Methods

Sample collection and microscopic examination

Two hundred fecal samples were collected from 145 male and 55 female patients with gastroenteritis at Damanhour General Hospital, El-Behira, Egypt, between June and September 2018. The patients belonged to various age groups starting from 4 to 62 years. The samples were examined, both unstained and iodine stained, by light microscopy to detect *G. lamblia* oval tetranuclear cysts and trophozoites at Damanhour General Hospital, El-Behira, Egypt. In addition, the patients' sociodemographic characteristics were collected. Patients were considered suffering from diarrhea if they experienced loose or watery gastrointestinal movements for a few days, at least, three times per day. High education was considered for post-secondary education in this study, while low education was considered for any other lower education level. The DNA extraction and PCR processes were performed at the Microbiology Laboratory at Faculty of Pharmacy, Damanhour University.

DNA extraction and genotyping of *G. lamblia* using conventional two-step PCR

DNA extraction from fresh stool samples was performed according to the manufacturer's instructions using a FavorPrep Stool DNA Isolation Mini Kit (Favorgen Biotech Corp., Vienna, Austria.). The DNA extracts were stored at -20 °C till PCR performance. A conventional two-step PCR was performed to detect *tpi* gene, and genotype *G. lamblia* as reported by **Amar et al.** [13]. The *tpi* gene was amplified in thermal cycler (Thermo Fisher Scientific, US) using a PCR master mix (ThermoScientific DreamTaq Green PCR Master Mix (2X), California, USA), and specific primers (Thermo Fisher Scientific, US) **(Table 1)**. One µl of PCR product from the first step is used as a template for the second step. The PCR cycling conditions were: 94 °C for 3 min for initial denaturation, 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. All PCR products were loaded onto 1.5% agarose gel, stained with ethidium bromide, and visualized by ultraviolet illumination.

Table 1. The primers used for *G. lamblia* detection and genotyping by conventional PCR [12].

	The gene	The primers	The amplicon size (bp)
The first step (Duplex PCR)	*tpi* assemblage A	*tpiA*-FI: 5'-CGAGACAAGTGTTGAGAT G-3'	576 bp
		tpiA-R: 5'-GGTCAAGAGCTTACAACA CG-3'	
	tpi assemblage B	*tpiB*-FI: 5'-GTTGCTCCCTCCTTTGTGC-3'	208 bp
		tpiB-R: 5'-CTCTGCTCATTGGTCTCGC-3'	
The second step (Uniplex PCR)	*tpi* assemblage A	*tpiA*-FII: 5'-CCAAGAAGGCTAAGC GTGC-3'	476 bp
		tpiA-R: 5'-GGTCAAGAGCTTACAACA CG-3'	
	tpi assemblage B	*tpiB*-FII: 5'-GCACAGAACGTGTATCTG G-3'	140 bp
		TPIB-R: 5'-CTCTGCTCATTGGTCTCG C-3	

Statistical analysis

The correlation between giardiasis and different sociodemographic characteristics of patients was determined using the χ 2 test and Monte-Carlo method. All statistical analyses were performed using IPM SPSS version 20.0 (IBM Corp., Armonk, NY). $P < 0.05$ was considered statistically significant.

Results

Out of 200 clinical stool samples, 92 (46%) were identified as *Giardia* spp. by light microscopy depending on the morphological characteristics of *Giardia* cysts and trophozoites **(Figure 1)**. All patients suffered from diarrhea, nausea, vomiting, abdominal cramps, and weight loss. *Giardia* assemblages A and B couldn't be differentiated microscopically.

DNA extraction was performed for 92 fresh stool samples, and 88 (95.7%) of them tested positive by PCR. Regarding genotyping, 16/88 (18%) samples were positive for *G. lamblia* assemblage A, 62/88 (70.5%) were positive for *G. lamblia* assemblage B, and 10/88 (11.5%) were mixed infection of assemblages A and B. Polymerase chain reaction products were loaded onto 1.5% agarose gels, stained with ethidium bromide, and visualized by ultraviolet illumination **(Figures 2 and 3)**.

In the present study, giardiasis was more prevalent in patients from rural areas; however, *G. lamblia* assemblage B isolates were more prevalent in urban patients. The prevalence of giardiasis was higher in males than females and was higher among children patients aged from 4-12 years. Surprisingly, the prevalence of giardiasis was higher in patients having sanitation facilities for sewage disposal; however, all mixed infections were prevalent in patients lacking sanitation facilities. In addition, the prevalence of giardiasis was higher in patients not dealing with animals; however, all mixed infections were prevalent in patients dealing with animals. Moreover, the prevalence of giardiasis was higher in patients with high education levels; however, all mixed infections were prevalent in patients with low education levels. Statistically, we found a statistically strong positive correlation between giardiasis and age, sex, and education levels ($P<0.05$); however, no significant correlation was observed between giardiasis and community, dealing with animals, and the presence of sanitation facilities ($P > 0.05$) **(Table 2)**.

Figure 1. Cyst and trophozoite of *G. lamblia* by light microscopy.

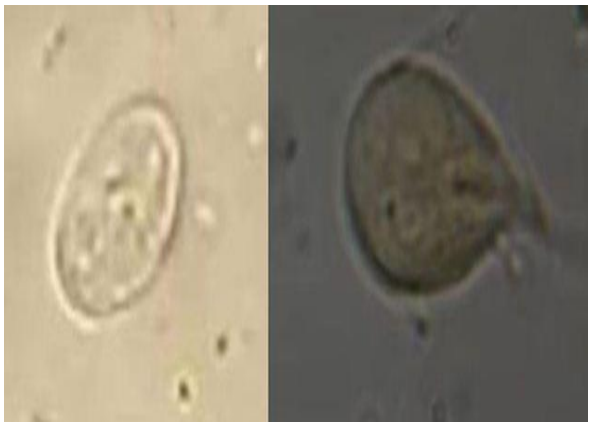

Figure 2. Detection of *G. lamblia* assemblage A by conventional PCR. PCR products were visualized in 1.5% agarose gel with ethidium bromide staining. Lanes 1 and 3: two *G. lamblia* assemblage A positive fecal samples at 476 bp; Lanes 2, 4-7: negative fecal samples; Lane 8: a 100-bp DNA ladder.

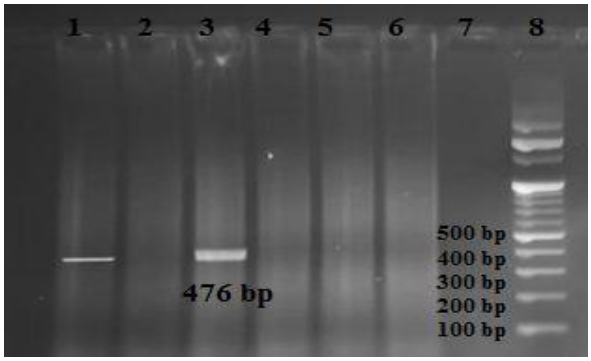

Figure 3. Detection of *G. lamblia* assemblage B by conventional PCR. PCR products were visualized in 1.5% agarose gel with ethidium bromide staining. Lanes 1 - 5: five *G. lamblia* assemblage B positive fecal samples at 140 bp; Lane 6: negative fecal sample; Lane 7: a 100-bp DNA ladder.

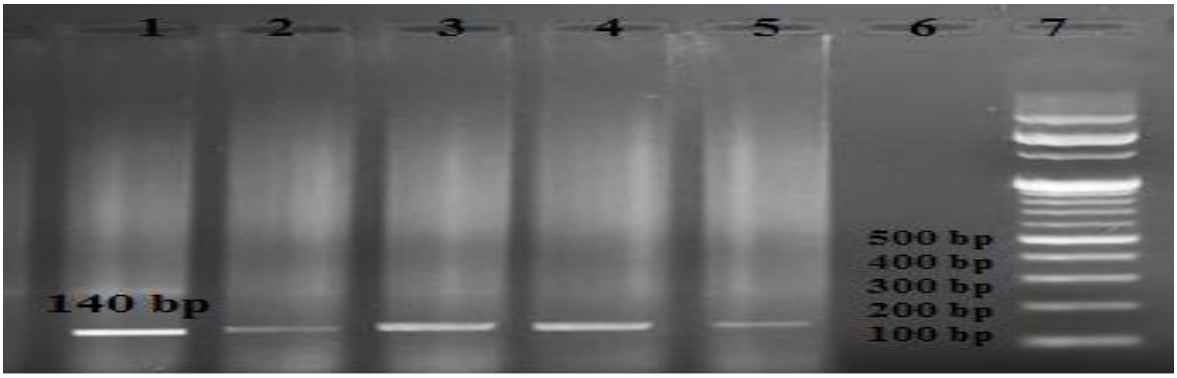

Table 2: The sociodemographic characteristics of the 88 patients genotyped into *G. lamblia* assemblages A and B by conventional PCR.

	***G. lambilia* assemblage A**	***G. lambilia* assemblage B**	**Mixed infections (*G. lamblia* assemblages A and B)**	**Total**
Sex				
• Male	12 (18%)	44 (67%)	10 (15%)	66 (100%)
• Female	4 (18%)	18 (82%)	0 (0%)	22 (100%)
Age groups				
• 4-12 yrs.	6 (10%)	46 (74%)	10 (16%)	62 (100%)
• 13-30 yrs.	6 (37%)	10 (63%)	0 (0%)	16 (100%)
• 31-65 yrs.	4 (40%)	6 (60%)	0 (0%)	10 (100%)
Community				
• Rural area	12 (25%)	26 (54%)	10 (21%)	48 (100%)
• City	4 (10%)	36 (90%)	0 (0%)	40 (100%)
Education level				
• Low	0 (0%)	8 (44%)	10 (56%)	18 (100%)
• High	16 (23%)	54 (77%)	0 (0%)	70 (100%)
Sanitation facilities				
• Latrine	16 (21%)	62 (79%)	0 (0%)	78 (100%)
• Open defecation	0 (0%)	0 (0%)	10 (100%)	10 (100%)
Dealing with domestic animals	0 (0%)	0 (0%)	10(100%)	10 (100%)
• Dealing	16 (21%)	62 (79%)	0 (0%)	78 (100%)
• Not dealing				

Discussion

Giardiasis is the most frequent human parasitic intestinal infection worldwide primarily affecting children in either developing or developed countries [10]. Diagnosis of giardiasis is commonly performed in Egypt and other countries by examining patients' fecal samples using light microscopy to detect *G. lamblia* tetranuclear cysts and trophozoites. However, microscopy cannot determine or differentiate *G. lamblia* genotypes. It was reported that fecal samples that will be processed by PCR are frequently collected without preservatives as they may degrade DNA in samples and reduce PCR sensitivity. Hence, the use of fresh fecal samples would inhibit the false negativity in results [10]. In this study, we used the two-step conventional PCR reported by **Amar et al.** [13] to detect and genotype *G. lamblia* in fresh fecal samples. Of 92 microscopically positive fresh fecal

samples, 88 (95.7%) displayed positive results by PCR. Similarly, **Molina et al.** [10], **Abbas et al.** [5], and **Chakarova et al.** [7] reported that 82%, 93%, and 94% of their fecal samples revealed positive results by PCR in Argentina, Iraq, and Bulgaria, respectively. The failure of DNA amplification and false negative results for some samples may be due to the low concentration of DNA, handling error and presence of some PCR inhibitors [5].

Regarding genotyping, *G. lamblia* assemblage B was detected in 70.5% of fecal samples, the most abundant genotype in the current research. *G. lamblia* assemblage A and mixed infections of both assemblages A and B were detected in 18% and 11.5% of fecal samples, respectively. Similarly, **Wang et al.** [14] in Spain reported that 67%, 29%, and 4% of fecal samples were positive for assemblage B, assemblage A, and both assemblages, respectively. However, **Molina et al.** [10] and **Tak et al.** [15] reported that all their fecal samples were positive for assemblage B in Argentina and India, respectively; and no samples were positive for assemblage A or both assemblages. While, **Abbas et al.** [5] in Iraq, and **Chakarova et al.** [7] in Bulgaria detected assemblage B and both assemblages in their fecal samples; however, assemblage A wasn't detected at all. In contrast to our results, the incidence of assemblage A (76%) reported by **Helmy et al.** [16] in 2009 in Egypt was more prevalent than assemblage B (19.5%), and mixed infection of both assemblages was detected in 5% of fecal samples. Recently, it was reported that assemblage A or B were associated with the severity of infection in humans [7]. This was confirmed in our research as all samples were genotyped as assemblage A, B, or mixed genotypes, and they were all isolated from patients suffering acute severe diarrhea, abdominal cramps, nausea, and vomiting.

Of note, the prevalence of giardiasis was higher among children patients aged from 4-12 years in this study; however, the prevalence of assemblage A was the same in both 4-12 and 13-30 age groups. The high prevalence of infection among children may be due to the frequent contact with outdoor surroundings, and the underdeveloped immunological status and hygiene practices. Our findings were similar to those of **Wang et al.** [14] in Spain as the prevalence of assemblage B was higher among their children patients aged from 0-12; however, the prevalence of assemblage A was higher among their patients aged from 23-75. Similarly, **Samie et al.** [17] in South Africa and **Bahrami et al.** [11] in Iran reported that the prevalence of giardiasis was at its peak in children and young adults. In contrast, **Minetti et al.** [18] in England reported that their highest prevalence rate of giardiasis was observed in patients aged from 30-49 years.

The prevalence of all *G. lamblia* assemblages was obviously higher in males in this study, which may be due to their more exposure to the sources of infections than females, such as: outdoor food and water consumption and more contact with infected persons. Similarly, Wang *et al.* [14] in Spain, **Bahrami et al.** [11] in Iran, **Puebla et al.** [19] in Cuba reported that the prevalence of giardiasis was higher in males; however, the prevalence of giardiasis of mixed assemblages was the same in both sexes in Spain and Cuba. **Samie et al.** [17] in South Africa and **Colli et al.** [20] in Brazil countered our results as the prevalence of giardiasis of their fecal samples was higher in females.

Moreover, the overall prevalence of giardiasis was higher in patients from rural areas than urban patients in the current study. This may be due to the lack of hygiene practices and sanitation measures in this community which increases the transmission of infection; however, the prevalence of assemblage B was higher in urban patients. This may be due to the lack of hygiene practices and sanitation measures in this community which increases the transmission of infection. Similar results were reported by **Fatni et al.** [21] in Morocco and **Sorokman et al.** [22] in Ukraine. In contrast, **Puebla et al.** [19] in Cuba, **Bahrami et al.** [11] in Iran, and **Molina et al.** [10] in Argentina reported that the prevalence was higher among urban patients.

Surprisingly, in the current research the prevalence of giardiasis was higher in patients having sanitation facilities for sewage disposal; however, all mixed infections were prevalent in patients lacking sanitation facilities. In contrast, **Molina et al.** [10] in Argentina and **Choy et al.** [23] in Malaysia reported that the prevalence of infection was higher in patients lacking latrine facilities for sewage disposal. In addition, the prevalence of giardiasis in our study was higher in patients with high education levels; however, all mixed infections were prevalent in patients with low education levels. **Choy et al.** [23] in Malaysia countered our results as the prevalence of infection was higher in patients with low education levels. It's supposed that low educated individuals have less knowledge concerning parasitic intestinal infections, their ways of transmission, and their prophylaxis-related hygienic practices compared with highly educated ones.

Assemblages A and B are potentially zoonotic human pathogens [16]. Dealing with domestic animals is an important risk factor, as they should be considered as a potential source of infection. Humans can acquire the infection easily as the surface of animals is contaminated by their fecal matter containing Giardia cysts. Unexpectedly, in the current research the prevalence of giardiasis was higher in patients not dealing with animals; however, all mixed infections were prevalent in patients dealing with animals. **Bahrami et al.** [11] in Iran and **Colli et al.** [20] in Brazil corroborated our results as the prevalence of infection was higher in their patients not dealing with domestic animals. However, **Choy et al.** [23] in Malaysia reported the prevalence of infection was higher among patients dealing with domestic animals.

In addition, giardiasis can be also transmitted by consumption of water contaminated by *G. lamblia* cysts. In this study, the tap water is the only water source for all patients. Therefore, the water supply pollution is a potential and important source of infection, as the water source of El Behira governorate relies on water derived from the river.

Conclusion

This study presents important and valuable data regarding the public health in Egypt. This is the first study to detect and distinguish *G. lamblia* assemblages in El Behira governorate. The results show that the prevalence of giardiasis is high among both rural and urban patients, particularly in children. It also prevails in patients of all education levels, and patients dealing and not dealing with animals. Several control measures are essential to struggle current infection levels, including education programs and awareness regarding the good personal hygiene measures and sanitation practices. Moreover, we recommend PCR amplifying the *tpi* gene in fresh fecal samples as very effective method for the detection and genotyping of giardiasis in routine diagnosis and epidemiological studies.

Ethics approval

The current research has followed the accepted principles of ethical conduct by the Research Ethics Committee of the Faculty of Pharmacy, Damanhour University, and it has been approved. Informed consent from the parents of young patients was obtained prior to undertaking testing and molecular investigation of their specimens.

Conflict of interest: The authors report no conflicts of interest.

Financial disclosures: This work received no specific grant from any funding agency.

References

1- **Calegar DA, Nunes BC, Monteiro KJ, Santos JP, Toma HK, Gomes TF, et al.** Frequency and molecular characterization of *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, and *Entamoeba hartmanni* in the context of water scarcity in northeastern Brazil. Mem Inst Oswaldo Cruz, Rio de Janeiro 2016; 111(2): 114-119.

2- **García EL, Galván SC, Cardoso JE.** Distancia filogenética de aislados de *Giardia intestinalis* de niños sintomáticos y asintomáticos. Rev Invest Clin 2002; 54: 113-118.

3- **García EL, Cortes-Campos A, Jiménez Cardozo E.** Genotype of *Giardia intestinalis* isolates from children and dogs and its relationship to host origin. Parasitol Res 2005; 97: 1-6.

4- **Coffey MC, Collier AS, Gleason EM, Yoder SJ, Kirk DM, Richardson MA.** Evolving Epidemiology of Reported Giardiasis Cases in the United States, 1995–2016. Clin Infect Dis 2020:1-6.

5- **Abbas MB, AL-Saqur MI, Majeed AH.** Detection and Genotyping of *Giardia lamblia* in Clinical and Environmental Samples in Some Regions of Baghdad city. Int J Curr Microbiol Appl Sci 2016; 5 (4): 459-468.

6- **Lalle M, Hanevik K.** Treatment-refractory giardiasis: challenges and solutions. Infect Drug Resist 2018;11: 1921-1933.

7- **Chakarova GB, Miteva DL, Stanilova AS.** Distribution of assemblages of *Giardia intestinalis* in Bulgaria. *Comptes rendus de* l'Académie bulgare des sciences: sciences mathématiques et naturelles 2011;64(2):293-298.

8- **Ankarklev J.** Inter and intra-assemblage characterization of *Giardia intestinalis* from clinic to genome. Dissertation for the degree of

doctor of philosophy at the University of Uppsala. Uppsala: Acta Universitatis Upsaliensis.2012. p. 85

9-**Raza A, Iqbal Z, Muhammad G, Hanif K, Khan MA.** Giardiasis. Rev Vet Ani Sci 2013; 1(1): 15–20.

10-**Molina N, Polverino D, Minvielle M, Basualdo J.** PCR amplification of triosephosphate isomerase gene of *Giardia lamblia* in formalin-fixed feces. Rev Latinoam Microbiol 2007; 49 (1-2): 6-11.

11-**Bahrami F, Zamini GH, Haghighi A, Khademerfan MB.** Detection and molecular identification of human *Giardia* isolates in the West of Iran. Biomed Res 2017; 28 (13): 5687-5692.

12-**Sari Y, Suryawati B, Yudhani RD, Artama WT.** Comparison of microscopic and PCR for detection *Giardia* spp. in the human fecal sample at Bedog Watershed, Sleman, DIY. KnE Life Sciences 2019:103–108.

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):446–452.

14-**Wang Y, Gonzalez-Moreno O, Roellig DM, Oliver L, Huguet J, Guo Y, et al.** Epidemiological distribution of genotypes of *Giardia duodenalis* in humans in Spain. Parasites Vectors 2019;12: 432.

15-**Tak V, Mirdha BR, Yadav P, Vyas P, Makharia GK, Bhatnagar S.** Molecular characterisation of *Giardia intestinalis* assemblages from human isolates at a tertiary care centre of India. Indian J Med Microbiol 2014; 32: 19-25.

16-**Helmy MMF, Abdel-Fattah HS, Rashed L.** Real-Time PCR/RFLP Assay to detect *Giardia intestinalis* genotypes in human isolates with diarrhea in Egypt. J Parasitol 2009; 95(4): 1–5.

17-**Samie A, Tanih FN, Seisa I, Seheri M, Mphahlele J, ElBakri A, et al.** Prevalence and genetic characterization of *Giardia lamblia* in relation to diarrhea in Limpopo and Gauteng provinces, South Africa. Parasite Epidemiol Control 2020; 9: e00140.

18-**Minetti C, Lamden K, Durband C, Cheesbrough J, Fox A, Wastling JM.** Determination of *Giardia duodenalis* assemblages and multi-locus genotypes in patients with sporadic giardiasis from England. Parasites Vectors 2015; 8: 444.

19-**Puebla LE, Núñez FA, Santos LP, Rivero LR, Silva IM, Valdés LA, et al.** Molecular analysis of *Giardia duodenalis* isolates from symptomatic and asymptomatic children from La Habana, Cuba. Parasite Epidemiol Control 2017; 2(3):105-113.

20-**Colli CM, Bezagio RC, Nishi L, Bignotto TS, Ferreira ÉC, Falavigna-Guilherme AL, et al.** Identical assemblage of *Giardia duodenalis* in humans, animals and vegetables in an urban area in southern Brazil indicates a relationship among them. PLoS One 2015; 10(3): e0118065.

21-**El Fatni C, Olmo F, El Fatni H, Romero D, Rosales MJ.** First genotyping of *Giardia duodenalis* and prevalence of enteroparasites in children from Tetouan (Morocco). Parasite 2014; 21:48.

22-**Sorokman VT, Sokolnyk VS, Popelyuk VA, Bezrul TO, Bezruk VV, Popelyuk NO.** Giardiasis in children: molecular genotyping, growth and calprotectin levels. Arch Balk Med Union 2019; 54(3):522-531.

23-**Choy SH, Al-Mekhlafi HM, Mahdy MA, Nasr NN, Sulaiman M, Lim YA, et al.** Prevalence and Associated Risk Factors of Giardia Infection

among Indigenous Communities in Rural Malaysia. Sci Rep 2014; 4: 6909.

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