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ORIGINAL ARTICLE

Molecular and epidemiological characterization of *Giardia Intestinalis* assemblages detected in Djelfa, Algeria

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Abstract *Giardia intestinalis* is a flagellated protozoan that lives and proliferates in the small intestine of the host causing giardiasis. The route of transmission is the fecal–oral route, either directly or indirectly. Limited genetic information on *G. intestinalis* is known in Algeria. This study aimed to estimate the prevalence of *G. intestinalis* assemblages in the city of Djelfa. A total of 355 fecal samples were collected from symptomatic and asymptomatic school children aged ranged between 6 and 11 years old. Genotyping was done to the *Giardia* positive samples (n = 30) targeting the *beta-giardin* gene by applying PCR/RFLP assay. Our data showed that most of the cases were asymptomatic (56.7%). Co-infection with other intestinal parasites was found in 16.6% of cases. We obtained 28/30 positive PCR products while two samples only showed false-negative results, and only 20 samples have shown strong PCR products suitable for RFLP analysis. Assemblage A (70%) was more prevalent than assemblage B (30%) and was more expressed by signs than assemblage B. Moreover, only assemblage A was associated with close contacts with domestic animals and birds. In conclusion, this study gave the first molecular data on *G. intestinalis* isolates in the city of Djelfa. Further expanded studies using more genes and covering other cities in Algeria are mostly needed.

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Introduction

Giardiasis is a disease caused by a microscopic parasite; a protozoan that affects many animal species, including domestic carnivores and humans. The disease is often asymptomatic and can be the cause of chronic enteritis with a syndrome of poor digestion and absorption, which may be accompanied by stunted growth (Wielinga et al. 2015; Squire and Ryan 2017). *Giardia intestinalis* is the only species within the genus *Giardia* that infects humans, causing intestinal infections. Children are at most risk of *Giardia intestinalis* infection, mostly those in developing countries and living in poor community settings (Sirize et al. 2008; Ramírez et al. 2015). Molecular characterization of *Giardia intestinalis* has classified it into seven assemblages depending on particular genes (Saksirisampant et al. 2012; Skhal et al. 2016). Assemblages A and B infect a broad range of hosts including humans, livestock, cats, dogs, and wild mammals (de Lucio et al. 2015).

In Algeria, intestinal parasites are of public health concern and still a reason for consultation in medical practice (Hamaidi et al. 2010). Most of the epidemiological studies are based on microscopic examination (Hamaidi et al. 2010; Benouis et al. 2013). So far only one study was performed using molecular tools (Lalle et al. 2009). Therefore, this present study aimed to determine the different genotypes of *G. intestinalis* isolates from a sample of school children in the city of Djelfa, in an effort to attain a better understanding of the genetic diversity and transmission of this disease.

Materials and methods

Population and sampling

The present study was carried out at the Djelfa province of Algeria. It is considered as the seventh-most-populous city in Algeria. Djelfa is the capital of Djelfa Province, approximately 300 km from Algiers, it has a population of about three hundred thousand people in the 2008 census. Djelfa has a chilly semi-dry climate (Fig. 1). Official approval was obtained from the Directorate of Education in the province state of Djelfa for obtaining samples from school children. An information sheet for each patient has been filled out by an adult family member, containing information about age, gender, socioeconomic background, personal hygiene, specifically regarding hand washing and food consumption, types of water supply, sewage disposal system, and if there is close contact with household pets.

Stool collection and microscopic examination

A total of 355 fecal samples were collected from three different schools at the city of Djelfa with parental consent during the first semester of the school year 2018–2019, in a labeled and sterile container. Stool smears were stained by Lugol's iodine and examined under a light microscope at × 40 and × 100 magnifications. The intensity of infection was estimated by average cyst count per high power field (HPF) of a light microscope. Samples' score was divided into three categories: 1–5 per field (+), 6–10 (++) and more than 10 cysts per field (+++). All positive samples for *Giardia* were stored at 4 °C in 2.5% potassium dichromate solution (1:3) for further analysis.

Molecular analysis

Potassium dichromate was washed off by distilled water, and samples were centrifuged at 3800 rpm for 10 min. This procedure was repeated between 8 and 10 times approximately, to ensure the removal of all dichromate. Genomic DNA was extracted from almost 250 mg of each fecal sample using QIAamp DNA stool mini kit (QIAamp® DNA Stool Mini Kit, QIAGEN) as described by Skhal et al. (2016). We applied PCR–RFLP method using *beta-giardin* gene to differentiate *Giardia* assemblages. Briefly, a nested PCR method was used to amplify a 514 bp fragment of the gene using the following primer pairs: G7/G759 and GiarF/GiarR respectively (Caccio et al. 2002; Lalle et al. 2005). The PCR reaction (25 µl final volume) contained 12.5 µl One PCR™ master mix 2X (GeneDirex

Fig. 1 Map of Algeria where the location of the study is marked by a star

Inc, Taiwan ROC), 1 µl of each primer pairs, 10.5 µl nuclease- free water, and 3 µl of the extracted gDNA. PCR conditions were previously described by Skhal et al. (2016). The amplified products were digested by *HaeIII* restriction enzyme (Thermo Fisher Scientific, USA) for 1 h at 37 °C followed by 20 min inactivation at 80 °C. Restriction fragments for all samples were electrophoresed in 2% agarose gel stained with ethidium bromide (Sigma-Aldrich, USA) along with a 100 bp DNA ladder (Gene-Direx Inc, Taiwan ROC) as a size standard. The final results were visualized under a UV transilluminator and photographed for documentation.

Results

Our 30 positive samples consisted of 70% males and 30% females. The presence of weight loss (23.3%) was the most significant symptom in all symptomatic cases. However, most of our studied cases were asymptomatic (Table 1).

Cysts and/or trophozoites of *Giardia*, were observed in all fecal samples after staining by Lugol's iodine (Fig. 2). Among the 30 children infected with giardiasis, co-infection with other parasites was found in 5 cases (one case *Entamoeba coli* plus *Entamoeba spp*; one case: *Blastocystis hominis* plus *Taenia spp*; two cases: *Blastocystis hominis* alone and one case *Chilomastix*).

PCR amplification of the 514 bp fragment of the *β-giardin* gene was successfully obtained from 28 out of the 30 studied cases (Fig. 3a), while only two samples showed no amplification. Furthermore, only 20 samples have shown strong PCR products suitable for RFLP analysis. Our genotyping results showed different restriction patterns (Fig. 3b); 70% of the samples were belonging to assemblage A (14/20) and 30% belonging to assemblage B (6/20). Our total results are summarized in Table 2.

Table 1 Summary of the studied samples from Djelfa city, by age, sex and clinical symptoms

	No. of samples	%
Variable age		
< 6–7	17	56.7
8–9	9	30%
10–11 >	4	13.3%
Gender		
Female	9	30%
Male	21	70%
Presence of symptoms		
Yes	13	43.3%
No	17	56.7%
Abdominal cramps	5	16.7%
Diarrhea	5	16.7%
Weight loss	7	23.3%
Anorexia	5	16.7%
Fever	4	13.3%

Our data revealed that the most age group associated with Giardiasis was 6–7 years old (56.7%) followed by 8–9 then 10–11 (30% and 13.3% respectively) (Table 1).

No assemblage B was noted in females of any age groups. We noted the dominance of male sex regarding assemblages distributions (Table 3).

Regarding the relation between assemblages and symptoms, our results revealed the presence of assemblages A at 7 symptomatic and 7 asymptomatic cases also. The symptomatic cases were characterized by the frequent presence of weight loss (57.1%), diarrhea (42.9%), anorexia and fever (28.6%) and abdominal cramps (14.3%). On the other hand, assemblages B were detected at 4 asymptomatic and 2 symptomatic cases. The latter shows weight loss, anorexia, abdominal cramps, and fever.

The comparison between assemblages A and B according to close contact with animals showed that there was a difference between the two assemblages. Six cases of assemblage A were associated with domestic animals (cats), poultry and birds; however, there were no cases for assemblage B.

Discussion

This study provides for the first time, information about the distribution of *G. intestinalis* genotypes in the city of Djelfa. *G. intestinalis* is the most common intestinal parasite of humans in several countries (Caccio and Ryan 2008; Lane and Lloyd 2002; Abdullah et al. 2016). Recently, this protozoa is included in the 'Neglected Diseases Initiative' by the WHO (Savioli et al. 2006).

The routine diagnostic method for giardiasis is the microscopic detection of *Giardia* cysts and/or trophozoites in stools. Our data revealed that most of microscopic positive samples were identified by nested PCR, only two samples (6.7%) with low numbers of cysts were negative after nested PCR. False-negative results using PCR were mentioned in many previous studies (Amar et al. 2002; Bertrand et al. 2005; Hatam Nahavand et al. 2011). Although there is no clear explanation for such false-negative results, it could be due to low concentration level of DNA, the presence of PCR inhibitors in some of the fecal samples and the existence of a robust wall that inhibits release of DNA from the cysts or degradation of parasite material during storage (Babaei et al. 2008; Roointan et al. 2013).

PCR–RFLP is usually a sensitive and powerful analytical tool, that is capable of discriminating between and within assemblages by targeting some loci such as *gdh, tpi*,

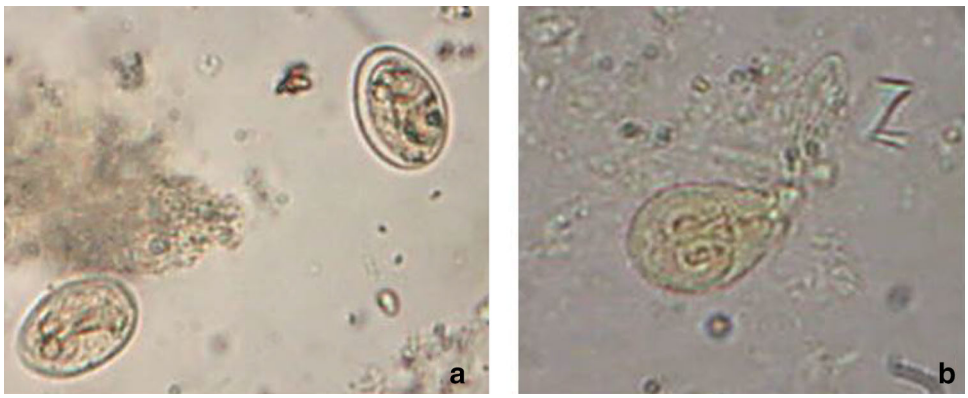

Fig. 2 *Giardia intestinalis* in stool sample. **a** Cysts; **b** trophozoites (× 100)

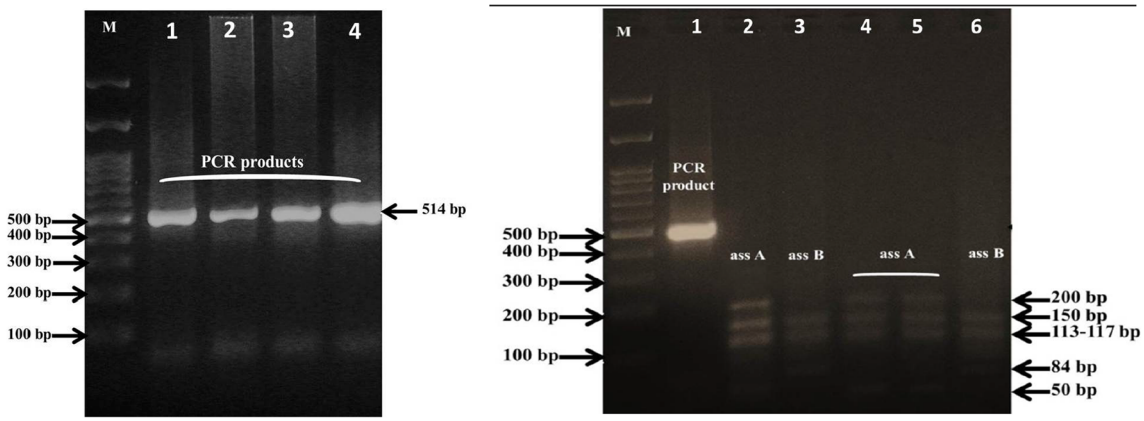

Fig. 3 Ethidium Bromide-Stained Agarose Gel 2%. **a** PCR Products. Lane M, molecular weight marker (100 bp); lanes 1–4, PCR products from different samples. **b** Enzyme digestion of *β-giardin*- PCR products. Lane M, molecular weight marker (100 bp); lane 1, uncut PCR product; lanes 2.4.5 represent Assemblage A; lanes 3,6, represent Assemblage B

and *β-giardin* (Amar et al. 2002; Cedillo-Rivera et al. 2003; Read et al. 2004; Itagaki et al. 2005; Skhal et al. 2016). Despite the small number of samples studied, our data indicated that assemblage A (14/20; 70%) was more prevalent than assemblage B (6/20; 30%). This result is in agreement with many previous studies conducted in different countries as in Iran (Roointan et al. 2013; Hooshyar et al. 2017; Kashinahanji et al. 2019); Syria (Skhal et al. 2016); Saudi Arabia (Al-Mohammed 2011); Eygpt (Helmy et al. 2009) and South Asia, including India (Bertrand et al. 2005; Geurden et al. 2009). Also, the predominance of assemblage A in wastewater has been reported in Italy (Yong et al. 2000; Cedillo-Rivera et al. 2003). On the other hand, the results of a study conducted in the south west of Iran showed that the majority of cases belonging to assemblage B (Rafiei et al. 2013). Similar results were obtained in Egypt (Soliman et al. 2011) and in Baghdad (Qader and Bakir 2011), as well as in Algeria, results of the study conducted on 120 stool samples from Sahrawi children indicated the dominance of assemblage B (56.2%) comparing to assemblage A (37.5%) (Lalle et al. 2009). It has been reported that these differences in the prevalence of assemblages may be attributed to the geographical location, or the contamination of drinking water, in addition, the lifestyle of the studied population who may be in close contact with animal wastes, especially in rural areas. (Lalle et al. 2005; Feng and Xiao 2011; Gasparinho et al. 2017; Al-Shehri et al. 2018).

Our data indicated the presence of assemblages A and B in both asymptomatic and symptomatic patients, so it is difficult to link the clinical outcome to a specific assemblage. However, no consensus is currently available on the correlation between the occurrence of symptoms and the genetic variations of *G. intestinalis* involved in the infection (Kasaei et al. 2018). Nonetheless, the prevalence of assemblage A in symptomatic cases was more than symptomatic cases at assemblage B in our study. This result is in agreement with some previous studies which have correlated assemblage A with the occurrence of symptoms (Sahagun et al. 2008; Robertson et al. 2010; Cardona et al. 2011; Laishram et al. 2012; Anuar et al. 2015; Skhal et al. 2017; Kasaei et al. 2018). Despite the fact that in some other studies, assemblage A has been associated with asymptomatic patients (Sarkari et al. 2012;

Table 2 Microscopic and molecular results

Sample number	Gender/Age (year)	Microscope examination categories	Notes	PCR amplification	Ass. A	Ass. B	
1	M/7	++	Cyst	+	A		
2	M/7	++	Cyst	+	A		
3	M/7	++	Cyst	+			Poor PCR product
4	M/8	++	Cyst	+	A		
5	M/9	+	Cyst	–			
6	M/9	++	Cyst	+	A		
7	M/7	++	Trophozoite	+		B	
8	F/8	++	Cyst	+			Poor PCR product
9	M/7	+++	Cyst	+	A		
10	M/7	+	Cyst	+			Poor PCR product
11	M/9	++	Cyst	+	A		
12	F/6	+	Cyst	–			
13	M/6	+	Cyst	+			Poor PCR product
14	F/7	+++	Cyst	+	A		
15	M/6	++	Cyst	+	A		
16	M/10	+++	Cyst	+		B	
17	M/11	++	Cyst	+		B	
18	M/7	++	Cyst	+			Poor PCR product
19	F/7	+++	Cyst	+	A		
20	M/8	+++	Cyst	+		B	
21	M/6	+++	Cyst	+		B	
22	M/6	++	Cyst	+			Poor PCR product
23	M/11	++	Cyst	+		B	
24	F/9	+	Cyst	+			Poor PCR product
25	F/7	++	Cyst	+	A		
26	F/6	++	Cyst	+	A		
27	M/6	++	Trophozoite	+	A		
28	M/8	++	Cyst	+	A		
29	F/11	++	Cyst	+	A		
30	F/9	+	Cyst	+			Poor PCR product
Total		30		28	14	6	8

Table 3 Distribution of *Giardia* assemblage A and B according to gender and age

	G. intestinalis assemblages						
	Assemblage A			Assemblage B			Total
Sex	♀	♂	%; N = 14	♀	♂	%; N = 6	N = 20
Age							
< 6–7	4	5	9 (64.3%)	0	2	2 (33.3%)	11
8–9	0	4	4 (28.6%)	0	1	1 (16.7%)	5
10–11 >	1	0	1 (7.1%)	0	3	3 (50%)	4
Total	5 (35.7%)	9 (64.3%)	14 (100%)	0 (0%)	6 (100%)	6 (100%)	20

Nasiri Goorabi et al. 2017). It is worth to mention that, other studies showed a clear association between assemblage B and symptoms like diarrhea, abdominal pain, and flatulence (Lebbad et al. 2011; Jerez-Puebla et al. 2015).

Our data have shown that the most age group associated with Giardiasis was 6–7 years old (56.7%), this could be due to the young age of this group of patients and the lack of awareness of personal hygiene.

Co-infection with other gastrointestinal parasites is commonly noticed. In this study, 16.6% of our studied cases had one or two parasites along with *G. intestinalis*. The reason for such co-infections is due to drinking or eating contaminated food or water, this case is of great significance for child health especially in countries with limited resources (Acosta et al., 2014). Previous studies revealed that in such countries, infection with Giardia appeared to protect children against diarrhea via unknown mechanisms (Veenemans et al. 2011; Muhsen et al. 2014). This can explain the limited number of diarrhea cases observed in our study sample.

It is well known that *G. intestinalis assemblages* have a broad spectrum of hosts including domestic animals, and livestock (Sprong et al. 2009; Pestehchian et al. 2012; Skhal et al. 2017). Our data revealed that only assemblage A was associated with close contacts with domestic animals and birds.

In conclusion, determining the genetic base of *G. intestinalis* is a useful way to understand the infection route, to prevent infection effectively, and to reveal the critical issues in the molecular epidemiology of this parasite in Algeria. Further studies are recommended on a larger number of individuals as well as animals. In addition, more studies are required in other regions of Algeria in order to determine the distribution pattern of this parasite in the country.

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Compliance with ethical standards

Conflict of interest We declare that no conflicts of interest concerning the work reported in this paper.

References

Abdullah I, Tak H, Ahmad F, Gul N, Nabi S, Sofi TA (2016) Predominance of gastrointestinal protozoan parasites in children: a brief review. J Health Educ Res Dev 4:4. https://doi.org/10.4172/2380-5439.1000194

Acosta AM, Chavez CB, Flores JT, Olotegui MP, Pinedo SR, Trigoso DR et al (2014) The MAL-ED Study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-Poor environments. Clin Infect Dis 59:193–206. https://doi.org/10.1093/cid/ciu653

Al-Mohammed HI (2011) Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. Parasitol Res 108:1375–1381. https://doi.org/10.4297/najms.2011.3362

Al-Shehri H, LaCourse EJ, Klimach O, Kabatereine NB, Stothard JR (2018) Molecular characterization and taxon assemblage typing of giardiasis in primary school children living close to the shoreline of Lake Albert, Uganda. Parasite Epidemiol Control 3:e00074. https://doi.org/10.1016/j.parepi.2018.e00074

Amar CF, Dear PH, Pedraza-Diaz S, Looker N, Linnane E, McLauchlin J (2002) Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. J Clin Microbiol 40(2):446–452. https://doi.org/10.1128/JCM.40.2.446-452.2002

Anuar TS, Moktar N, Salleh FM, Al-Mekhlafi HM (2015) Human Giardiasis in Malaysia: correlation between the presence of clinical manifestation and *Giardia intestinalis* assemblage. Southeast Asian J Trop Med Public Health 46(5):835–843

Babaei Z, Oormazdi H, Akhlaghi L, Rezaie S, Razmjou E, Soltani-Arabshahi SK, Meamar AR, Hadighi R (2008) Molecular characterization of the Iranian isolates of *Giardia lamblia:* application of the glutamate dehydrogenase gene. Iran J Publ Health 37(2):75–82

Benouis A, Bekkouche Z, Benmansour Z (2013) Epidemiological study of human intestinal parasitosis in the Hospital of Oran (Algeria). Int J Innov Appl Studies (IJIAS) 2(4):613–620

Bertrand I, Albertini L, Schwartzbrod J (2005) 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 43(12):5940–5944. https://doi.org/10.1128/JCM.43.12.5940-5944.2005

Caccio SM, Ryan U (2008) Molecular epidemiology of giardiasis. Mol Biochem Parasit 160:75–80

Caccio SM, De Giacomo M, Pozio E (2002) Sequence analysis of the *beta-giardin* gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human fecal samples. Int J Parasitol 32(8):1023–1030

Cardona GA, Carabin H, Goñi P, Arriola L, Robinson G, Fernández-Crespo JC, Clavel A, Chalmers RM, Carmena D (2011) Identification and molecular characterization of *Cryptosporidium* and *Giardia* in children and cattle populations from the province of Alava, North of Spain. Sci Total Environ 413:101–108. https://doi.org/10.1016/j.scitotenv.2011.09.076

Cedillo-Rivera R, Darby JM, Enciso-Moreno JA, Ortega-Pierres G, Ey PL (2003) Genetic homogeneity of axenic isolates of *Giardia intestinalis* derived from acute and chronically infected individuals in Mexico. Parasitol Res 90(2):119–123

de Lucio A, Martínez-Ruiz R, Merino FJ, Bailo B, Aguilera M, Fuentes I, Carmena D (2015) Molecular genotyping of *Giardia duodenalis* isolates from symptomatic individuals attending two major public hospitals in Madrid, Spain. PLoS One 10:e0143981. https://doi.org/10.1371/journal.pone.0143981

Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev 24(1):110–140. https://doi.org/10.1128/CMR.00033-10

Gasparinho C, Ferreira FS, Mayer AC, Mirante MC, Nery SV, Santos-Reis A, Portugal-Calisto D, Brito M (2017) Molecular characterization of Giardia lamblia in children less than 5 years of age with diarrhoea attending the Bengo General Hospital,

Angola. Trans R Soc Trop Med Hyg 111:497–503. https://doi.org/10.1093/trstmh/try004

Geurden T, Levecke B, Cacció SM, Visser A, De Groote G, Casaert S, Vercruysse J, Claerebout E (2009) Multilocus genotyping of *Cryptosporidium* and *Giardia* in non-outbreak related cases of diarrhea in human patients in Belgium. Parasitol 136(10):1161–1168. https://doi.org/10.1017/S0031182009990436

Hamaidi F, Chaouch A, Kais H, Zahraoui R, Benghrebia A, Hamaidi MS, Megatel S (2010) Etude des parasitoses digestives dans la région de Boufarik (Blida), Nord-Ouest d'Algérie. La science en liberté 4:13

Hatam Nahavand K, Fallah E, Asgharzadeh M, Mırsamad N, Mahdavıpour B (2011) Glutamate dehydrogenase and triose-phosphate-isomerase coding genes for detection and genetic characterization of *Giardia lamblia* in human feces by PCR and PCRRFLP. Turk J Med Sci 41(2):283–289

Helmy MM, Abdel-Fattah HS, Rashed L (2009) Real time PCR/RFLP assay to detect *Giardia intestinalis* genotypes in human isolates with diarrhea in Egypt. J Parasitol 95:1000–1004. https://doi.org/10.1645/GE-1670.1

Hooshyar H, Ghafarinasab S, Arbabi M, Delavari M, Rasti S (2017) Genetic variation of *Giardia lamblia* isolates from food-handlers in Kashan, Central Iran. Iran J Parasitol 12(1):83–89

Itagaki T, Kinoshita S, Aoki M, Itoh N, Saeki H, Sato N, Uetsuki J, Izumiyama S, Yagita K, Endo T (2005) Genotyping of *Giardia intestinalis* from domestic and wild animals in Japan using glutamate dehydrogenase gene sequencing. Vet Parasitol 133(4):283–287

Jerez-Puebla LE, Núñez FA, Martinez-Silva I, Rojas-Rivero L, Martinez-González M, Mendez-Sutil Y, Ayllon-Valdés L, Atencio-Millán I, Müller N (2015) Molecular characterization and risk factors of *Giardia duodenalis* among school children from La Habana, Cuba. J Parasitol Res 2015:378643. https://doi.org/10.1007/s12639-016-0816-z

Kasaei R, Carmena D, Jelowdar A, Beiromvand M (2018) Molecular genotyping of *Giardia duodenalis* in children from Behbahan, southwestern Iran. Parasitol Res 117(5):1425–1431. https://doi.org/10.1007/s00436-018-5826-6

Kashinahanji M, Haghighi A, Bahrami F, Fallah M, Saidijam M, Matini M, Maghsood AH (2019) *Giardia lamblia* assemblages A and B isolated from symptomatic and asymptomatic persons in Hamadan, West of Iran. J Parasit Dis 43(4):616–623. https://doi.org/10.1007/s12639-019-01139-x

Laishram S, Kang G, Ajjampur SS (2012) Giardiasis: a review on assemblage distribution and epidemiology in India. Indian J Gastroenterol 31(1):3–12. https://doi.org/10.1007/s12664-012-0161-9

Lalle M, Jimenez-Cardosa E, Cacciò SM, Pozio E (2005) Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a *β-giardin* nested polymerase chain reaction assay. J Parasitol 91(1):203–205

Lalle M, Bruschi F, Castagna B, Campa M, Pozio E, Cacciò SM (2009) High genetic polymorphism among *Giardia duodenalis* isolates from Sahrawi children. Trans R Soc Trop Med Hyg 103:834–838. https://doi.org/10.1016/j.trstmh.2009.04.017

Lane S, Lloyd D (2002) Current trends in research into the waterborne parasite *Giardia*. Crit Rev Microbiol 28(2):123–147

Lebbad M, Petersson I, Karlsson L, Botero-Kleiven S, Andersson JO, Svenungsson B, Svard SG (2011) Multilocus genotyping of human *Giardia* isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children. PLoS Negl Trop Dis 5(8):e1262. https://doi.org/10.1371/journal.pntd.0001262

Muhsen K, Cohen D, Levine MM (2014) Can *Giardia lamblia* infection lower the risk of acute diarrhea among preschool children? J Trop Pediatr 60(2):99–103. https://doi.org/10.1093/tropej/fmt085

Nasiri Goorabi L, Pirestani M, Sadraei J (2017) Genotyping of *Giardia duodenalis* by *β-giardin* gene in asymptomatic patients. J Mazandaran Univ Med Sci 27:27–34

Pestehchian N, Rasekh H, Babaei Z, Yousefi HA, Eskandarian AA, Kazemi M, Akbari M (2012) Identification of genotypes of *Giardia duodenalis* human isolates in Isfahan, Iran, using polymerase chain reaction—restriction fragment length polymorphism. Adv Biomed Res 1:84. https://doi.org/10.4103/2277-9175.105166

Qader AM, Bakir TY (2011) Molecular identification of *Giardia duodenalis* parasite isolates from human by polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP) in Baghdad province. Diyala J Pure Sci 7(4):54–66

Rafiei A, Roointan ES, Samarbafzadeh AR, Shayesteh AA, Shamsizadeh A, Pourmahdi Borujeni M (2013) Investigation of possible correlation between *Giardia duodenalis* genotypes and clinical symptoms in Southwest of Iran. Iran J Parasitol 8(3):389–395

Ramírez JD, Heredia RD, Hernández C, León CM, Moncada LI, Reyes P, Pinilla AE, Lopez MC (2015) Molecular diagnosis and genotype analysis of *Giardia duodenalis* in asymptomatic children from a rural area in Central Colombia. Infect Genet Evol 32:208–213. https://doi.org/10.1016/j.meegid.2015.03.015

Read CM, Monis PT, Thompson RC (2004) Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol 4:125–130

Robertson LJ, Hanevik K, Escobedo AA, Mørch K, Langeland N (2010) Giardiasis—why do the symptoms sometimes never stop? Trends Parasitol 26(2):75–82. https://doi.org/10.1016/j.pt.2009.11.010

Roointan ES, Rafiei A, Samarbafzadeh AR, Shayesteh AA, Shamsizadeh A, Pourmahdi Borujeni M (2013) Genotype analysis of *Giardia lamblia* isolated from children in Ahvaz, South west of Iran. Jundishapur J Microbiol 6(3):279–283

Sahagun J, Clavel A, Goni P, Seral C, Llorente MT, Castillo FJ, Capilla S, Arias A, Gomez-Lus R (2008) Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. Eur J Clin Microbiol Infect Dis 27:81–83. https://doi.org/10.1007/s10096-007-0404-3

Saksirisampant W, Boontanom P, Mungthin M, Tan-ariya P, Lamchuan D, Siripattanapipong S, Leelayoova S (2012) Prevalence of giardiasis and genotypic of *Giardia duodenalis* in hilltribe children, northern Thailand. Trop Biomed 29(3):331–338

Sarkari B, Ashrafmansori A, Hatam GR, Motazedian MH, Asgari Q, Mohammadpour I (2012) Genotyping of *Giardia lamblia* isolates from human in southern Iran. Trop Biomed 29(3):366–371

Savioli L, Smith H, Thompson A (2006) *Giardia* and *Cryptosporidium* join the neglected diseases initiative. Trends Parasitol 22(5):203–208. https://doi.org/10.1016/j.pt.2006.02.015

Sirize JY, Vitoux C, Holvort L, Carrie E (2008) Main pathologies of children returning from holiday in their country of origin. J Pediatr Pueric 21:67–77

Skhal D, Aboualchamat G, Al Nahhas S (2016) *Giardia duodenalis* in Damascus, Syria: identification of giardia genotypes in a sample of human fecal isolates using polymerase chain reaction and restriction fragment length polymorphism analyzing method. Acta Trop 154:1–5. https://doi.org/10.1016/j.actatropica.2015.10.008

Skhal D, Aboualchamat G, Al Mariri A, Al Nahhas S (2017) Prevalence of *Giardia duodenalis* assemblages and sub-assemblages in symptomatic patients from Damascus city and its suburbs. Infect Gen Evol 47:155–160. https://doi.org/10.1016/j.meegid.2016.11.030

Soliman RH, Fuentes I, Rubio JM (2011) Identification of a novel Assemblage B sub-genotype and a zoonotic Assemblage C in human isolates of *Giardia intestinalis* in Egypt. Parasitol Int 60:507–511. https://doi.org/10.1016/j.parint.2011.09.006

Sprong H, Caccio SM, van der Giessen JWB, On behalf of the ZOOPNET Network and Partners (2009) Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis 3(12):e558. https://doi.org/10.1371/journal.pntd.0000558

Squire SA, Ryan U (2017) *Cryptosporidium* and *Giardia* in Africa: current and future challenges. Parasit Vectors 10:195. https://doi.org/10.1186/s13071-017-2111-y

Veenemans J, Mank T, Ottenhof M, Baidjoe A, Mbugi EV, Demir AY, Wielders JP, Savelkoul HF, Verhoef H (2011) Protection against diarrhea associated with *Giardia intestinalis* Is lost with multi-nutrient supplementation: a study in Tanzanian children. PLoS Negl Trop Dis 5(6):e1158. https://doi.org/10.1371/journal.pntd.0001158

Wielinga C, Thompson RC, Monis P, Ryan U (2015) Identification of polymorphic genes for use in assemblage B genotyping assays through comparative genomics of multiple assemblage B *Giardia duodenalis* isolates. Mol Biochem Parasitol 201:1–4. https://doi.org/10.1016/j.molbiopara.2015.05.002

Yong TS, Park SJ, Hwang UW, Yang HW, Lee KW, Min DY, Min DY, Rim HJ, Wang Y, Zheng F (2000) Genotyping of *Giardia lamblia* isolates from humans in China and Korea using ribosomal DNA sequences. J Parasitol 86:887–891. https://doi.org/10.1645/0022-3395(2000)086%5b0887:goglif%5d2.0.co;2