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Prevalence of *Giardia duodenalis* assemblages and sub-assemblages in symptomatic patients from Damascus city and its Suburbs

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Abstract

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Giardia duodenalis is one of the most important human enteric parasites worldwide and is endemic throughout the world with a vast range of mammalian hosts. However, there is limited information on the prevalent genetic variability of *G. duodenalis* in Syria. This study aimed to evaluate the predominance of *G. duodenalis* assemblages /sub-assemblages causing humans infection in the city of Damascus and its suburbs. 40 symptomatic giardiasis patients were recruited in this study. Fecal samples were genotyped using PCR/RFLP assay targeting the *β-giardin* and glutamate dehydrogenase (*gdh*) genes. *HaeIII, BspL1* and *RsaI* restriction enzymes were used to differentiate between *G. duodenalis* assemblages /sub- assemblages. Our data showed that 65% of isolates were of assemblage A; 45% belonged to sub-assemblage AII and 20% to sub-assemblage AI. Assemblage B was detected in 27.5% of isolates; 12.5% fit in sub-assemblage BIV, 5% fit in sub-assemblage BIII and 10.5% fit in Discordant genotype BIII/BIV. Mixed genotypes (AII+BIII and AI+BIV) was identified in 3 isolates (7.5%). Significant correlation was found between *Giardia* AII sub-assemblage and weight loss symptom (P-value = 0.05) as well as between contact with domestic animals (cats, P-value = 0.027). Moreover, a significant correlation was found between sub-assemblage AI and livestock breeding (P-value = 0.000). In conclusion genotyping of human *Giardia duodenalis* isolates suggests anthroponotic transmission for the route of infection in Damascus and its suburbs. Further studies are need to screen a wide geographic areas in Syria and to estimate the prevalence of *G. duodenalis* infection in our population.

Keywords: *Giardia*, Prevalence, *β-giardin*, glutamate dehydrogenase, Damascus, Assemblage

1. Introduction

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Giardia duodenalis (syn: *G. lamblia*, *G. intestinalis*) is a flagellate protozoan parasite which infects a wide range of mammalian hosts, including humans through ingestion of infective cysts (Carmena, 2010; Roointan et al., 2013). Approximately 200 million people have symptomatic giardiasis in Asia, Africa and Latin America, and about 500,000 new cases are reported each year mostly from children (Kosek et al., 2003). Therefore, *G. duodenalis* has been included in the Neglected Diseases Initiative of the World Health Organization since 2004 (Savioli et al., 2006).

G. duodenalis exhibit a high degree of genetic diversity, and have been grouped into genetic assemblages A to H (Lasek-Nesselquist et al., 2010; Saksirisampant et al. 2012). Molecular studies revealed the existence of further sub genetic structure within these major assemblages (Sprong et al., 2009; Torres-Romero et al., 2014). Assemblage A commonly subdivided into AI, which is mainly of zoonotic transmission. AII is commonly known as an anthroponotic transmission, and sub-assemblage AIII which is restricted to animals only (Al-Mohammed, 2011, Atherton et al., 2013). Assemblage B, was classified into sub-assemblages BIII and BIV which were found with a very similar frequencies in human isolates, as well as in animals (dogs, cattle, horses) (Xiao and Fayer, 2008). Thus, sub-assemblages AI, AII, BIII and BIV are potentially considered of zoonotic transmission (Caccio and Ryan, 2008; Bowman and Lucio-Forster, 2010). Most studies relied on genotyping *G. duodenalis* assemblages using more than one genetic markers such as: small-subunit *ssrRNA, β-giardin*, glutamate dehydrogenase "*gdh*" or triosephosphate isomerase "*tpi*" genes (Wielinga and Thompson, 2007; Minetti et al., 2015).

Giardia epidemiology, infection sources and the way of pathogen transmission in Syria are unknown yet. Although we had previously reported a preliminary data on giardiasis, our aim in this study was to focus on the prevalence and diversity of *Giardia duodenalis* assemblages, and sub- assemblages in symptomatic patients with giardiasis from the city of Damascus and its suburbs.

2. Materials and methods

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2.1. Clinical samples

Samples were collected between June and September 2014 from three main hospitals at the city of Damascus, Syria, which receive all medical conditions from the capital and its surrounding areas (rural and urban). Forty patients with symptomatic giardiasis participated in the study, fecal specimens were collected in clean sterile flasks. *Giardia* cysts and /or trophozoites, were detected by wet smear stained with Lugol's iodine using light microscope at 100X magnification.

Information about the patients' gender, age, symptoms, contact with household pets and history of travel was collected via a questionnaire. Patients and their families were informed about the study and they signed a written consent. This study has been approved by the ethical committee of Damascus University and Atomic Energy Commission – Syria

2.2.DNA extraction

Whole fecal DNA was extracted directly from the specimens using a commercial kit (QIAamp® DNA Stool Mini Kit, QIAGEN) following the manufacturer's instructions with only minor modifications as described by Skhal et al., (2016). The DNA was eluted in 200 µl of elution buffer and stored at −20 °C.

2.3. PCR amplification and RFLP analysis

A nested PCR targeting *ß-giardin* gene was performed using two pairs of primers G7/G759 to amplify a 753 bp fragment (Caccio et al., 2002), and GiarF/GiarR to amplify a second 514 bp PCR fragment (Lalle et al., 2005). PCR products were digested using *HaeIII* restriction enzyme (Promega, USA) .PCR conditions and RFLP digestion method was described previously by Skhal et al., (2016).

The amplification of *gdh* gene was performed by a semi-nested PCR protocol. A first PCR reaction was generated using the following primer pairs GDHeF (5´-TCAACGTYAAYCGYGGYTTCCGT-3´) and GDHiR (5´GTTRTCCTTGCACATCTCC-3´), while the second PCR reaction amplified a fragment of 432base pairs using a third primer GDHiF (5´-CAGTA CACCTCYGCTCTCGG-3´) as forward along with GDHiR as reverse (Read et al., 2004).

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In general, PCR reactions mixture (25 µl final volume) consisted of 12.5 µl Go Taq Green Master Mix 2X (Promega, USA), and 1 µl of each primer pairs. A total of 7.5 µl of extracted DNA template was used for the first PCR reaction, While 5 µl of PCR product from the first reaction was added for the second reaction.

The amplification condition for the first PCR reaction was as follows: initial denaturation at 95°C for 15 min, followed by 35 cycles (95°C for 3 min, 56°C for 1 min and 72°C for 2 min), The final extension was at 72°C for 7 min. The second PCR reaction was performed under the same previous conditions except for cycling time (95°C for 1 min, 56°C for 20s and 72°C for 45s). All PCR reactions were carried out using Eppendorf Master Cycler.

Nuclease-free water was used as a negative control for contamination detection. PCR products were electrophoresed on 1.5% agarose gels containing ethidium bromide, visualized and photographed using a UV transilluminator.

Diagnostic genotyping was performed by RFLP digestion method to distinguish between *Giardia* assemblages and sub-assemblages. PCR products of *gdh* gene was digested by *BspLI* (10 U/µl) (Thermo Fisher Scientific, USA) restriction enzyme, to differentiate between groups I and II of assemblages A and assemblage B, and by *RsaI* restriction enzyme (10 U/µl) (Thermo Fisher Scientific, USA), to distinguish between sub-assemblages BIII and BIV. Reaction conditions were performed according to manufacturer's instructions. Restriction fragments were separated by 15% polyacrylamide gel electrophoresis using 50 bp DNA ladder (Sigma, USA) as size standard, visualized and photographed using a UV transilluminator.

2.4. Statistical analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 22. Percentage rates were used to describe the prevalence of *G. duodenalis* assemblages and sub-assemblages. Pearson's chi-squared and Fisher's Exact Test were used to assess the correlation between assemblages / sub-assemblages and different variables. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

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All 40 human fecal samples were successfully amplified in nested and semi-nested PCR for *β-giardin* and *gdh* genes respectively. *HaeIII* restriction enzyme digestion of *β-giardin* fragments identified assemblages A (67.5%), assemblages B (10%) and a mixed of assemblages A+B (22.5%).

A further classification of *Giardia duodenalis* isolates into A and B sub-assemblages using *BspL1* and *Rsa1* restriction enzymes showed that 18/40 (45%) belonged to sub-assemblage AII, 8/40 (20%) belonged to sub-assemblage AI, 2/40 (5%), 5/40 (12.5%) and 4/40 (10%) belonged to BIII, BIV and BIII/BIV groups respectively. Finally, only 2/40 (5%) and 1/40 (2.5%) isolates were identified as AII+BIII and AI+BIV mixed sub-assemblages respectively (Table 1; Fig. 1).

Table1: Diagnostic fragment sizes of *Giardia duodenalis* sub-assemblages after digesting with restriction enzymes

Enzyme	Assemblages	Sub-assemblages	diagnostic fragment sizes (bp)
BspL1	**A**	**AI** **AII**	39, 87, 123, 145 bp 39,68,77,87,123 bp
	B	 **Mixed (AII+B)** **Mixed (AI+B)**	123,287 bp 39,68,77,87,123,287 bp 39,87,123,145,287 bp
RsaI	**B**	**BIII** **BIV** **BIII / BIV**	130,297 bp 427 bp 130,297,427 bp

Patient's ages ranged from 4 months to 75 years; 19 were females and 21 were males. The distribution of genotypes in relation to gender among the 40 patients, was as follows: 10 females had assemblage A (AI 7.5% ; AII 17.5 %), 7 assemblage B (BIV 10%; BIII+BIV 7.5%) and two females had a mixed sub-assemblages (AII+BIII 5 %). 16 males had assemblage A (AI 12.5%; AII 27.5 %), 4 assemblage B (BIII 5%; BIV 2.5%; BIII+BIV 2.5 %) and only one male had a mixed sub-assemblages (AI+BIV 2.5 %).

However, the distribution of genotypes in relation to age groups revealed that AII sub-assemblage was mostly frequent amongst patients aged between 1-10 years (12, 30%), whereas it was less detected amongst patients 11-15 and older (3, 7.5%) for each age group. AI sub-

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assemblage was less detected amongst patients older than 16 years of age (1, 2.5%), while found amongst patients between 1-10 and 11-15 years (4, 10%; 3, 7.5%) respectively. Furthermore, BIV sub-assemblage was detected in 3 patients aged between 1-10 years (7.5%), while existed in only 2 patients aged between 11-15 years (5%). BIII sub-assemblage was found equally amongst patients between 1-10 and 11-15 years old (1, 2.5% for each age group). In addition, a mixture of sub-assemblages BIII+BIV has been found in 4 patients belonging to aged group 1-10 (10%), and a mixture of sub-assemblages AII+BIII was identified equally amongst patients between 1-10 and 11-15 years(1, 2.5%). Whereas a mix of sub-assemblages AI+BIV was only found in one patient belonging to 11-15 age group (1, 2.5%). Finally, no assemblage B nor its sub-assemblages were detected in patients over the age of 16 years.

Our results showed that AII sub-assemblage was associated mostly with domestic animals contact, while AI sub-assemblage was correlated with livestock breeding. However, BIII and BIV sub-assemblages were generally linked with direct contact with domestic animals.

Statistical results showed no significant correlation between *G. duodenalis* sub-assemblages and the different variables studied, except between AII sub-assemblage and weight loss symptom and between AII and domestic animals contents. Furthermore, a significant correlation was found between sub-assemblage AI and livestock breeding (Table 2).

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Table 2: **Statistical results between sub-assemblages and some different variables**

		Age		Symptom			Animals	
Sub-assemblage	<1-10	11-15	16≤	Diarrhea	Weight loss	Cats	Dogs	Livestock
AI								
N (%)	4 (10%)	3 (7.5%)	1 (2.5%)	8 (20%)	6 (15%)	2 (5%)	5 (12.5%)	8 (20%)
95% CI	0.03 - 0.25	0.02-0.22	.001 - 0.14	0.08-0.39	0.06 - 0.33	0.01-0.18	0.04 - 0.29	0.08-0.39
P value		0.713		0368	0.0686	0.428	0.229	0.000*
AII								
N (%)	12 (30%)	3 (7.5%)	1 (2.5%)	16 (40%)	7 (17.5%)	17 (42.5%)	11 (27.5%)	-
95% CI	0.15 - 0.52	0.02-0.22	.001 - 0.14	0.23-0.65	0.07-0.36	0.25-0.68	0.14-0.49	
P value		0.230		0.579	0.05*	0.027*	0.267	
BIII								
N (%)	1 (2.5%)	1 (2.5%)	-	2 (5%)	1 (2.5%)	2 (5%)	2 (5%)	1 (2.5%)
95% CI	0.00 - 0.14	0.001 - 0.14		0.01-0.18	0.001- 0.14	0.01-0.18	0.01-0.18	0.001 - 0.14
P value	0.722			1.000	1.000	1.000	1.000	1.000
BIV								
N (%)	3 (7.5%)	2 (5%)	-	5 (12.5%)	5 (12.5%)	4 (10%)	5 (12.5%)	2 (5%)
95% CI	0.02-0.22	0.01-0.18		0.04 - 0.29	0.04 - 0.29	0.03-0.26	0.04-0.29	0.01-0.18
P value	0.637			1.000	0.137	1.000	1.000	1.000
BIII/BIV								
N (%)	4 (10%)	-	-	3 (7.5%)	3 (7.5%)	3 (7.5%)	4 (10%)	-
95% CI	0.03 - 0.25			0.02-0.22	0.02-0.22	0.02-0.22	0.03-0.26	
P value	0.264			0.277	1.000	1.000	0.297	
AI+BIV								
N (%)	-	1 (2.5%)	-	1 (2.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)
95% CI		0.001 - 0.14		0.001 - 0.14	0.001 - 0.14	0.001 - 0.14	0.001 - 0.14	0.001 - 0.14
P value		0.259		1.000	1.000	1.000	1.000	0.325
AII+BIII								
N (%)	1 (2.5%)	1 (2.5%)	-	2 (5%)	2 (5%)	2 (5%)	1 (2.5%)	1 (2.5%)
95% CI	0.001 - 0.14	0.001 - 0.14		0.01-0.18	0.01-0.18	0.01-0.18	0.001 - 0.14	0.001 - 0.14
P *value*	0.722			1.000	0.519	1.000	0.515	1.000

N: number of isolates; **∗**: Statistically significant difference.

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4. Discussion

Giardia duodenalis is the etiologic agent of giardiasis in humans and animals (Solieman et al., 2011; Tappeh et al., 2014). This disease causes a major public and veterinary health concern worldwide due to the high prevalence and disease burden of the infection and its tendency in causing major propagation and emergency responses (Feng and Xiao, 2011). Transmission is either direct (fecal-oral route) or indirect (ingestion of contaminated water or food) (Fallah et al., 2008).

General giardiasis surveillance data are critical in assessing the disease prevalence and epidemiologic characteristics of giardiasis. In light of the crisis our country faces, there is a big demographic changes due to the migration of people from their permanent residency areas and staying in evacuation shelters, making it difficult to restrict sources of infection, as well as the short-infection emergence of the parasite. On the other hand, in Syria, few studies was done using mostly conventional methods in the routine diagnosis of giardiasis (Almerie et al., 2008). However, these methods are unable to distinguish between genetically distinct *G. duodenalis* isolates (Amar et al., 2002; Skhal et al., 2016). In this current study, we evaluated the different prevalence of assemblages and sub- assemblages of *G. duodenalis*, using PCR-RFLP analysis at the *β-giardin* and *gdh* gene loci. These genes have been mostly used as markers for their genetic variability which allow to discriminate between assemblages and sub-assemblages of *G. duodenalis* (Caccio et al., 2002; Babaei et al., 2008). The *gdh* locus has been shown to recognize and verify assemblages A and B isolates into four subgroups, AI, AII, BIII and BIV (Amar et al., 2002); whereas other markers like the *ssurRNA*, and elongation factor 1α gene can only be used to discriminate major assemblages of *Giardia* (Babaei et al., 2008). Furthermore, this method of detection proved to be reliable, easy and cost-effective method to identify *G. duodenalis* isolates directly from stool (Fallah et al., 2008; Pestehchian et al,. 2012).

Our data showed that *G. duodenalis* assemblage A was predominant (65%), while assemblage B and a mixture of genotypes A+B were less detected (27.5%, 7.5%) respectively. The predominance of assemblage A in our study is similar to the previous results of many neighboring regions such as: Palestine, Turkey and Iraq, however it was in contrast to some

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other studies showing the predominance of assemblage B (Canada, England), (Table 3). This may be due to transmission dynamics of the parasite.

Table 3. Prevalence of assemblages A and B in human cases of giardiasis from many different countries

Assemblage A		Assemblage B	
Italy (n=30; 80%)	Caccio et al., 2002	Australia (n=23 ;70%)	Read et al., 2002
Iran (n=38 ; 87%)	Babaei et al., 2008	Canada (n=15; 60%)	Guy et al., 2004
Egypt (n=41; 75.5%)	Helmy et al., 2009	Bangladesh (n=35; 91%)	Ng et al., 2005
Palestine (n=8; 62.5%)	Hussein et al., 2009	France (n=25 ; 64%)	Bertrand et al., 2005
Saudi-Arabia (n=40; 57.5%)	Al-Mohammed, 2011	Norway (n=63; 93.6%)	Robertson et al., 2007
Turkey (n=22 ; 50%)	Tamer et al., 2015	Brazil (n=58; 74%)	Kohli et al., 2008
Iraq (n=73 ; 30.2%)	Turki et al., 2015	Nepal (n=35; 74%)	Singh et al., 2009
Syria (n= 40 ; 65%)	Current study	England (n=158; 64%)	Minetti et al., 2015

(n: number of isolates).

The molecular genotyping of human isolates of *Giardia* from different regions of the world, demonstrated that in almost all cases, only *G. duodenalis* assemblage A and B are related to human infections (Caccio and Ryan, 2008; Mbae et al., 2016).

Furthermore, most of assemblage A isolates were assigned to the sub-assemblage AII, which is consistent with an anthroponotic origin of infection (45%), followed by sub-assemblages AI (20%), BIV and BIII (12.5%; 5%) respectively. This data correspond with the findings of other reports like, Caccio et al., (2002) from Italy; Babaei et al., (2008) and Etamadi et al., (2011) from Iran. As well as with Brazilian study (Souza et al., 2007), which showed that *Giardia* parasite detected in humans was mostly belonged to sub-assemblage AII.

It is important to emphasize that this current study is compatible with our previous report (Skhal et al. 2016), with a slight difference. One isolate was identified as assemblage A by *β-giardin* gene loci, whereas the *gdh* gene loci indicated that it is belonging to sub-assemblage BIV/ BIII. This difference may be due to a mixed infection showing preferential amplification of single genotype over others at a specific locus (Read et al., 2004; De Lucio et al., 2016). This explains the different results we obtained using only one single locus, and indicates the need to use more than one locus when genotyping *Giardia* isolates

Interestingly, a mixture of sub-assemblages AII+BIII (5%) and AI+BIV (2.5%) has been recorded in 3 isolates. Our data is in agreement with previous studies performed in Australia, United Kingdom, India, Italy, and Peru, where the percentages of mixed infections range from

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2.0% to 21.0% (Caccio et al., 2002; Traub et al., 2004; Lalle et al., 2005; Helmy et al., 2009; Molina et al., 2011). These mixed assemblages may reflect ingestion of sources contaminated by heterogeneous mixtures of *Giardia* and a complex life cycle of the parasite (Babaei et al., 2008; Pestehchian et al., 2012).

Our data also showed a discordant genotype results BIII/BIV in 4 isolates (10%). A current study by De Lucio et al., (2015) assumes a recombination occurrence between these subgroups which most likely explain this phenomenon.

Earlier reports indicated that AI sub-assemblage and assemblage B (regardless BIII and BIV) have a broad host range including pets and livestock, while AII sub-assemblage is more limited to humans (Sprong et al., 2009; Pestehchian et al., 2012). This is consistent with our findings which revealed that most AII sub-assemblage was associated mostly with domestic animals, and AI sub-assemblage was largely correlated with livestock breeding.

No significant differences between distribution of assemblages /sub-assemblages and gender was found in our study. This result is in agreement with other molecular epidemiological studies carried out in Philippines (Yason and Rivera, 2007), Ethiopia (Gelanew et al., 2007) and Iraq (Turkiet al., 2015). However, Bertrand et al., (2004) demonstrated that most males were infected with assemblage B. while, a study in Malaysia showed that females were infected more than males by assemblage B (Mahdy et al., 2009), which may be explained by women's role as care takers of children and of direct contact with infected children (Espelage et al., 2010).

On the other hand, our results recorded a high prevalence of assemblage A and AII sub-assemblage amongst patients aged between 1-10 years. Although, this finding disagrees with the results of Mbae et al., (2016) from Kenya and Boontanom et al., (2011) from Thailand where assemblage B, sub-assemblage BIV was found to be the most common in preschool children, our finding agrees with few previous studies showing an association between assemblage occurrence and the age of patients. For example, in Turkey and in Egypt assemblage A was significantly more frequent in children aged between 1-13 years and 2-8 years respectively (Tamer et al., 2015; El Basha et al., 2016). The high infection rate with *Giardia* parasite in younger age groups may be due to a weakened immune system, bad health habits (such as putting fingers in the mouth; eating unwashed fruits and vegetables; not

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washing hands before eating and after defecation) and the low socio-economic status (Ojonoma,2008).

No clear correlation between symptoms and sub-assemblages found in our study, except between AII sub-assemblage and Weight loss. This data is in agreement with other reports showing no association between clinical symptoms and particular assemblages in people with giardiasis (Ajjampur et al., 2009; Mateo et al., 2014). Although some studies have suggested a strong correlation between severe diarrhea, abdominal pain, nausea and vomiting with A assemblages in comparison with assemblages B (Sarkari et al., 2012; Anuar et al., 2015), as well as, abdominal pain was statistically associated with infection with sub-assemblage BIII (Jerez-Puebla et al., 2015) and an association between assemblage B infection and diarrhea with flatulence (Lebbad et al., 2011).

In conclusion, our results offer primary data on the distribution of *G. duodenalis* in Syria. However, sequencing is needed to confirm the assemblages/sub-assemblages of the strains. In addition, further studies with a larger series of fecal samples (human and animals) can lead to better knowledge of the distribution of these assemblages in humans as well as the role of domestic animals and livestock as a potential source of infection for humans.

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6. References

Ajjampur, S.S., Sankaran, P., Kannan, A., Sathyakumar, K., Sarkar, R., Gladstone, B.P., Kang, G., 2009. *Giardia duodenalis* assemblages associated with diarrhea in children in South India identified by PCR-RFLP. Am. J. Trop. Med. Hyg. 80, 16-19.

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Almerie, M.Q., Azzouz, M.S., Abdessamad, M.A., Mouchli, M.A., Sakbani, M.W., Alsibai, M.S., Alkafri, A., Ismail, M.T., 2008. Prevalence and risk factors for giardiasis among primary school children in Damascus, Syria. Saudi. Med. J. 29, 234–240.

Al-Mohammed, H.I., 2011. Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. Parasitol. Res. 108: 1375–1381.

Amar, C.F., Dear, P.H., 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. Microbial. 40: 446–52.

Anuar, T.S., Moktar, N., Salleh, F.M., Al-Mekhlafi, H.M., 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):835-43.

Atherton, R., Bhavnani, D., Calvopiña, M., Vicuña, Y., William Cevallos, W., Joseph Eisenberg, J., 2013. Molecular identification of *Giardia duodenalis* in Ecuador by polymerase chain reaction-restriction fragment length polymorphism. Mem. Inst. Oswaldo Cruz. Rio de Janeiro, 108(4): 512-515.

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

Bertrand, I., Gantzer, C., Chesnot, T., Schwartzbrod, J., 2004. Improved specificity for *Giardia lamblia* cyst quantification in waste water by development of a real-time PCR method. J. Microbiol. Methods. 57:41–53.

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. 43:5940–5944.

Boontanom, P., Mungthin, M., Tan-Ariya, P., Naaglor, T., Leelayoova, S., 2011. Epidemiology of giardiasis and genotypic characterization of *Giardia duodenalis* in preschool children of a rural community, central Thailand. Trop. Biomed. 28: 32–9

ACCEPTED MANUSCRIPT

Bowman, D.D., Lucio-Forster, A., 2010. Cryptosporidiosis and giardiasis in dogs and cats: veterinary and public health importance. Exp. Parasitol. 124: 121–127.

Caccio, S.M., De Giacomo, M., Pozio, E., 2002. Sequence analysis of the *β-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: 1023–1030

Caccio, S.M., Ryan, U., 2008. Molecular epidemiology of giardiasis. Mol. Bioch. Parasitol. 160: 75–80.

Carmena, D., 2010. Waterborne transmission of *Cryptosporidium* and *Giardia*: detection, surveillance and implications for public health. In: Mendez-Vilas A. Curr. Res. Technol. Educ. Topics Appl. Microbiol. Microb. Biotechnol. 1(2):3-14.

De Lucio, A., Martínez-Ruiz, R., Merino, F.J., 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(12): e0143981. doi:10.1371/journal.pone.0143981

De Lucio, A., Amor-Aramendía, A., Bailo, B., Saugar, J.M., Anegagrie, M., Arroyo, A., López-Quintana, B., Zewdie, D., Ayehubizu, Z., Yizengaw, E., Abera, B., Yimer, M., Mulu, W., Hailu, T., Herrador, Z., Fuentes, I., Carmena, D., 2016. Prevalence and genetic diversity of *Giardia duodenalis* and *Cryptosporidium spp*. among school children in a rural area of the Amhara Region, North-West Ethiopia. PLoS ONE 11(7): e0159992. doi:10.1371/journal. pone.0159992

El Basha, N.R., Zaki, M.M., Hassanin, O.M., Rehan, M.K., Omran, D., 2016. *Giardia* Assemblages A and B in diarrheic patients: A Comparative study in Egyptian children and adults. J. Parasitol. 102 (1): 69-74.

Espelage, W., Heiden, M.A.D., Stark, K., Alpers, K., 2010. Characteristics and risk factors for symptomatic *Giardia lamblia* infections in Germany. BMC Public Health. 10: 41–50.

Etamadi, S., Zia-Ali, N., Babaei, Z., Harandi M.F., Zia–Ali, A., Salari, Z., 2011. The correlation between clinical signs and genotypes of *Giardia duodenalis* isolated from patients with giardiasis in Kerman city. J. Kerman Univ. Med. Sci.18: 330-8.

ACCEPTED MANUSCRIPT

Fallah, E., Nahavandi, K.H., Jamali, R., Mahdavi, B., Asgharzadeh, M., 2008. Genetic characterization of *Giardia intestinalis* strains from patients having sporadic giardiasis by PCR assay. J. Med. Sci. 8: 310-5.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24: 110-40.

Gelanew, T., Lalle, M., Hailu, A., Pozio, E., Caccio, S.M., 2007. Molecular characterization of human isolates of *Giardia duodenalis* from Ethiopia. Acta Trop. 102: 92-99.

Guy, R.A., Xiao, C., Horgen, P.A., 2004. Real-time PCR assay for detection and genotype differentiation of *Giardia lamblia* in stool specimens. J. Clin. Microbiol. 42: 3317-3320.

Helmy, M.M., Abdel-Fattah, H.S., 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.

Hussein, A.I., Yamaguchi, T., Nakamoto, K., Iseki, M., Tokoro, M., 2009. Multiple sub-genotype infections of *Giardia intestinalis* detected in Palestinian clinical cases using a sub-cloning approach. Parasitol. Int. 58: 258–262.

Jerez-Puebla, L.E., Núñez, F.A., 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. Volume 2015: Article ID 378643, 8 pages.

Kohli, A., Bushen, O.Y., Pinkerton, R.C., Houpt, E., Newman, R.D., Sears, C.L., Lima, A.A., Guerrant, R.L., 2008. *Giardia duodenalis* assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. Trans. R. Soc. Trop. Med. Hyg. 102: 718-725.

Kosek, M., Bern, C., Guerrant, R.L., 2003. The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. Bull. World Health Organ.

Lalle, M., Cardosa, E.J., Caccio, S.M., Pozio, E., 2005. Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a *β-giardin* nested polymerase chain reaction assay. J. Parasitol. 9 (1), 201–203.

ACCEPTED MANUSCRIPT

Lasek-Nesselquist, E., Welch, D.M., Sogin, M.L., 2010. The identification of a new *Giardia duodenalis* assemblage in marine vertebrates and a preliminary analysis of *G. duodenalis* population biology in marine systems. Int. J. Parasitol. 40: 1063–1074.

Lebbad, M., Petersson, I., Karlsson, L., Botero-Kleiven, S., Andersson, J.O., Svenungsson, B., Svard, S.G., 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.

Mateo, M., Mateo, M., Montoya, A., Bailo, B., Saugar, J.M., Aguilera, M., Fuentes, I., Carmena, D., 2014. Detection and molecular characterization of *Giardia duodenalis* in children attending day care centers in Majadahonda, Madrid, Central Spain. Medicine (Baltimore). 93 (15): e75.

Mbae, C., Mulinge, E., Guleid, F., Wainaina, J., Waruru, A., Njiru, Z.K., Kariuk, S., 2016. Molecular characterization of *Giardia duodenalis* in Children in Kenya. BMC. Infect. Dis. 16: 135.

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

Mahdy, A.K.M., Surin, J.,. Wan, K.L., Mohd-Adnan, A., Al-Mekhlafi, M.S., Lim, Y.A., 2009. *Giardia intestinalis* genotypes: risk factors and correlation with clinical symptoms. Acta Trop. 112: 67–70.

Molina, N., Pezzani, B., Ciarmela, M., Orden, A., Rosa, D., Apezteguía, M., Basualdo, J., Minvielle, M., 2011. Intestinal parasites and genotypes of *Giardia intestinalis* in school children from Berisso, Argentina. J. Infect. Dev. Ctries. 5 (7): 527-534.

Ng, C.T., Gilchrist, C.A., Lane, A., Roy, S., Haque, R., Houpt, E.R., 2005. Multiplex real-time PCR assay using Scorpion probes and DNA capture for genotype-specific detection of *Giardia lamblia* on fecal samples. J. Clin. Microbiol. 43:1256–1260.

Ojonoma, O.L., 2008. A review of sexually transmitted diseases (STDs) of parasitic origin: The case of giardiasis. African J. Biotechnol. 7: 4979-4981.

Pestehchian, N., Rasekh, H., Babaei, Z., Yousefi, H.A., Eskandarian, A.A., Kazemi, M., Akbari, M., 2012. Identification of genotypes of *Giardia duodenalis* human isolates in Isfahan,

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Iran, using polymerase chain reaction – Restriction Fragment Length polymorphism. Adv. Biomed. Res. 1: 84.

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

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

Savioli, L., Smith, H., Thompson, A., 2006. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. Trends Parasitol. 22: 203–208.

Singh, A., Janaki, L., Petri, W.A. Jr., Houpt, E.R., 2009. *Giardia intestinalis* assemblages A and B infections in Nepal. Am. J. Trop. Med. Hyg. 81:538–539.

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.

Soliman, R.H., Fuentes, I., Rubio, J.M., 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.

Souza, S.L.P., Gennari, S.M., Richtzeriliain, L.J., Pem, H.F.J., Fumda, M.R., Cortez, A., Gregori, F., Soares, R.M., 2007. Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of Sao Paulo, Brazil by sequence analysis of fragments of glutamate dehydrogenase (*gdh*) coding gene. Vet. Parasitol. 149 (3–4): 258–264.

Sprong, H., Caccio, S.M., van-der-Giessen, J.W.B., on behalf of the ZOOPNET network and partners., 2009. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop. Dis. 3(12): e558.

Read, C., Walters, J., Robertson, I.D., Thompson, R.C.A., 2002. Correlation between genotype of *Giardia duodenalis* and diarrhea. Int. J. Parasitol. 32: 229–231.

ACCEPTED MANUSCRIPT

Read, C., Monis, P.T., Thompson, R.C.A., 2004. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect. Genet. Evol. 4: 125–130.

Robertson, L.J., Forberg, T., Hermansen, L., Hamnes, I.S., Gjerde, B., 2007. *Giardia duodenalis* cysts isolated from wild moose and reindeer in Norway: genetic characterization by PCR-RFLP and sequence analysis at two genes. J. Wildl. Dis. 43:576–585.

Roointan, E.S., Rafiei, A., Samarbaf-Zadeh, A.R., Shayesteh, A.A., Shamsizadeh, A., Pourmahdi Borujeni, M., 2013. Genotype analysis of *Giardia lamblia* isolated from children in Ahvaz, southwest of Iran. Jundishapur J. Microbiol. 6(3): 279-83.

Tamer, G.S., Kasap, M., Er, D.K., 2015. Genotyping and phylogenetic analysis of *Giardia duodenalis* isolates from Turkish children. Med. Sci. Monit. 21: 526-532.

Tappeh, K.H., Manafi, G., Asgharzadeh, M., Manafi, F., 2014. Incidence of *Giardia lamblia* subspecies by PCR-RFLP in stool specimens of hospitalized children at Urmia Mutahhari hospital, West Azerbaijan province, Iran. Iranian J. Parasitol. Vol. 9 (4): 541-547.

Torres-Romero, J.C., Euan-Canto, A.D.J., Benito-González, N., Padilla-Montaño, N., Huchin-Chan, C., Lara-Riegos, J., Cedillo-Rivera, R., 2014. Intestinal parasites and genotyping of *Giardia duodenalis* in children: first report of genotype B in isolates from human clinical samples in Mexico. Mem. Inst. Oswaldo Cruz. Rio de Janeiro. 109(3): 388-390.

Traub, R.J., Monis, P.T., Robertson, I., Irwin, P., Mencke, N., Thompson, R.C.A., 2004. Epidemiological and molecular evidence support the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitol. 128L 253–62

Turki, N.M., Mallah, M.O., Kremsh, Y.D., 2015. Iraqi genotyping of *Giardia lambila* (A,B,E,F) in human stool in AL-Muthanna province –Iraq. Int. J. Adv. Res. Vol 3 (10): 757 – 771.

WHO, 2004. Global Burden of Disease. http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_part2.pdf.

Wielinga, C.M., Thompson, R.C.A., 2007. Comparative evaluation of *Giardia duodenalis* sequence data. Parasitol. 134 (Pt12): 1795-821.

ACCEPTED MANUSCRIPT

Xiao, L., Fayer, R., 2008. Molecular characterization of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int. J. Parasitol. 38:1239–1255.

Yason, J.A., Rivera, W.L., 2007. Genotyping of *Giardia duodenalis* isolates among residents of slum area in Manila, Philippines. Parasitol. Res. 101: 681-687.

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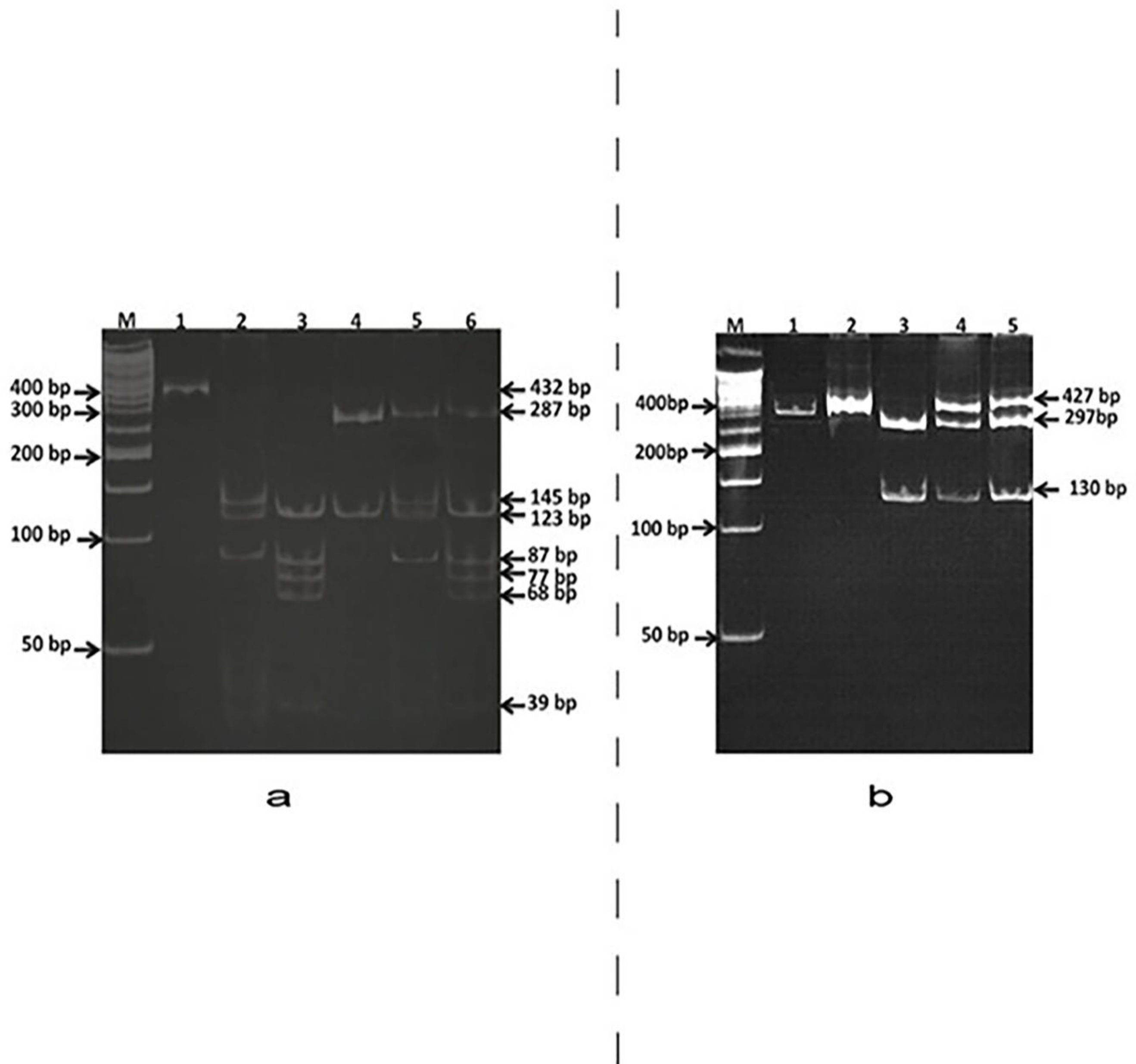
Figure 1 caption

Polyacrylamide gel electrophoresis of PCR products after enzymes digestion
a. *Bspl1* enzyme digestion. Lane 1: undigested gdh PCR products, Lane 2: subassemblage AI, Lane 3: sub-assemblage AII, Lane 4: assemblage B, Lanes 4,5: mixed assemblages/sub-assemblages. **b.** *Rsa1* enzyme digestion. Lane 1: undigested gdh PCR products, Lane 2: sub-assemblage BIV, Lane 3: sub-assemblage BIII, Lanes 4, 5: BIII / BIV. M: Molecular marker (50bp)



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Highlights

- *Giardia duodenalis* assemblage A/ sub-assemblage AII was prevalent in the city of Damascus and its suburbs.
- High prevalence of assemblage A / sub-assemblage AII amongst patients aged between 1-10 years.
- The route of transmission is mostly anthroponotic.
- Sequencing is needed to confirm assemblages/sub-assemblages of the strains.

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