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Molecular genotyping of *Giardia duodenalis* in municipal waste workers in Ahvaz, southwestern Iran

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Abstract. *Giardia duodenalis* is one of the most common intestinal parasites in a wide range of vertebrates, including humans and animals. It is estimated that there are approximately 200 million symptomatic giardiasis per year globally. The aim of the present study was to evaluate the prevalence and molecular diversity of *G. duodenalis* in municipal waste workers in Ahvaz County, southwestern Iran. This cross-sectional study was conducted among municipal waste workers aged 21 to 59 years in Ahvaz County, southwestern Iran in 2015. Stool samples collected from 400 workers were examined initially by microscopy and sucrose flotation methods, and then *G. duodenalis* isolates were confirmed by *SSU* rRNA and subsequently the genotypes were identified by triose phosphate isomerase (*tpi*) gene of the parasite. In total, a prevalence of 4.0% was found for *G. duodenalis* by microscopy and sucrose flotation methods. All microscopic-positive samples were successfully amplified at the *SSU* rRNA gene while *tpi* gene was amplified in 13 (81.25%) samples. Out of the 13 amplified isolates at *tpi* gene, 10 (76.9%) were typeable while the other three (23.1%) were untypeable. Assemblage A, sub-assemblage AII, was found in nine (69.2%) typeable isolates, and one (7.7%) was assemblage B, sub-assemblage BIII. The current study represents the first molecular epidemiological data on the occurrence and genotypic diversity of *G. duodenalis* in municipal waste workers in southwestern Iran. Assemblage A, sub-assemblage AII was the predominant assemblage of *G. duodenalis*. Since all infected subjects were asymptomatic, periodic examinations are recommended to prevent and control giardiasis.

INTRODUCTION

Giardiasis, one of the most common protozoan diseases with a worldwide distribution, is considered a neglected tropical disease (Savioli *et al.*, 2006). It is caused by *Giardia duodenalis*, an enteric protozoan of humans, and domestic and wild animals (Thompson, 2000). This cosmopolitan protozoan is one of the most common agents of diarrhea in developing countries (Ramirez *et al.*, 2015). In developed countries, *G. duodenalis* has been also reported from 2% to 7% in different populations (Fletcher *et al.*, 2012). Children, especially those living in developing countries and rural areas, are more at the risk of *Giardia* infection (Ramirez *et al.*, 2015). Approximately 200 million people in Asia, Africa, and Latin America experience symptomatic giardiasis, and according to the World Health Organization (WHO), about 500,000 new cases have been reported annually (Savioli *et al.*, 2006). *Giardia duodenalis* is mainly transmitted indirectly through the fecal-oral route by ingestion of contaminated food, water, and also by direct contact with domestic animals and infected individuals (Robertson *et al.*, 2010, Sprong *et al.*, 2009).

Currently, *G. duodenalis* has been classified into eight assemblages (A to H),

which differ in host range and specificity. Assemblages A and B are considered zoonotic. Other assemblages have strong animal host specificity; however, assemblages C–F are sporadically isolated from human infections. Based on isoenzyme and phylogenetic analyses, *G. duodenalis* is classified into at least five sub-assemblages (AI-III, BIII-IV) (Feng & Xiao, 2011).

In Iran, several studies have been carried out on giardiasis in recent years (Hazrati Tappeh *et al.*, 2014; Hooshyar *et al.*, 2017, Rayani *et al.*, 2014). Based on socioeconomic status and geographical location, the prevalence rate of giardiasis was reported from 1.4% to 59.6% (Hatam-Nahavandi *et al.*, 2017). However, data on the prevalence of *G. duodenalis* in human communities in Khuzestan Province are insufficient and available data on the molecular diversity of *G. duodenalis* is limited to a single study (Roointan *et al.*, 2013).

Therefore, the current study aimed to evaluate the prevalence and genotyping of *G. duodenalis* among Ahvaz municipal waste workers, southwest Iran. Municipal solid waste (MSW), which contains household and commercial waste, could be contaminated with infectious agents such as bacteria, fungi, viruses, and parasites (Alvarado-Esquivel *et al.*, 2008; Athanasiou *et al.*, 2010). In Iran, collecting solid waste from households, hospitals, factories, or cleaning streets and green spaces is performed by municipal waste workers. Thus, workers who are involved in collecting solid waste are at risk of infection.

MATERIALS AND METHODS

Ethics statement

The protocol of this study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Approval No IR.AJUMS.REC. 2015-244).

Study area

This cross-sectional study was conducted among municipal waste workers in Ahvaz County, Khuzestan Province, southwestern Iran in 2015. Ahvaz, the biggest city of Khuzestan Province, has an estimated population of 1,112,021 million people and extends over an area of 528 km^2. The average annual rainfall in Ahvaz is around 250 mm. It is located at 31°192 N 40°092 E and has an elevation of 20 meters above sea level. The climate is warm and dry, and occasionally humid in summer (Zarasvandi *et al.*, 2011). Ahvaz is divided into seven municipal districts, and approximately 2419 workers are responsible for collecting solid waste in the county.

Study population, fecal sample collection and processing

Of the 2419 municipal waste workers aged 21 to 59 years in Ahvaz County, southwestern Iran, 400 workers were selected randomly. All participants were male (in Iran, all municipal waste workers must be male). Initially, information about the aims of the study was provided to the participants, and then they were asked to complete a questionnaire. The associated risk factors with *Giardia* transmission, such as age, educational status, and clinical symptoms were included in the questionnaire. A plastic stool container labelled with individual identification was given to each participant. They were asked to return the stool sample the following day. The collected fecal samples were transported to the Department of Parasitology, Ahvaz Jundishapur University of Medical Sciences. Samples were processed on the same day of collection.

Microscopic examination and sucrose flotation method

For the detection of intestinal parasites, all stool samples were examined microscopically using normal saline and lugol's iodine stain at 100X and 400X magnification. Additionally, the isolation of *Giardia* cyst was performed on all samples by sucrose flotation (1 M) method. Approximately 5 grams of each fecal sample was suspended in 50 mL of tap water and mixed thoroughly. The fecal suspension was passed through a four-layer surgical gauze (10×10 cm) and centrifuged at 1000× g for 5 min. After discarding the supernatant, 30 mL distilled water was added to the pellet and completely homogenized

with vortex. The suspension was gently added to 15 mL of sucrose solution and centrifuged for 10 min at 800× g. Thereafter, the superior layers (upper and middle layers) were transferred to a 50 mL clean tube, and the final volume was reached to 50 mL with distilled water and centrifuged for 5 min at 1000× g. The supernatant was discarded, and the sediment was washed three times with 50 mL distilled water and then centrifuged at 1000× g for 5 min. The obtained pellet was examined microscopically using normal saline and lugol's iodine stain at 100X and 400X magnification. Approximately 200 µL of the sediment containing *G. duodenalis* cysts were transferred to a 2 ml microtube and stored at -20°C until further analyses.

DNA extraction

Genomic DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Before extraction, seven cycles of freeze/thaw (7 min in liquid nitrogen and 7 min in boiling bath each time) were performed to disrupt the cyst wall. DNA extracts were eluted in 200 µL elution buffer and stored at -20°C until further analyses.

Molecular detection

Nested PCR was conducted for amplification of the small subunit of ribosomal RNA (*SSU* rRNA) and *tpi* genes to identify *G. duodenalis* according to previous descriptions (Gillhuber *et al.*, 2013; Sulaiman *et al.*, 2003).

A fragment of 130-bp of the *SSU* rRNA gene was amplified using the primer pairs RH11 (52-CATCCGGTCGATCCTGCC-32) and RH4 (52-AGTCGAACCCTGATTCTCCG CCAGG-32) in the primary reaction, and GiarF (52-GACGCTCTCCCCAAGGAC-32) and GiarR (52-CTGCGTCACGCTGCTCG-32) in the secondary reaction (Gillhuber *et al.*, 2013).

The PCR reaction was conducted in a 25 µL reaction using 12.5 µL of the Taq DNA Polymerase (Ampliqon-Biomol, Hamburg, Germany), 3 µL of template DNA for the primary and 1 µL for the secondary reactions, 1 µL of each forward and reverse primers (10 µM), and 7.5 and 9.5 µL of distilled water for the primary and secondary reactions, respectively (Gillhuber *et al.*, 2013). A nested PCR for the amplification of a 530-bp fragment of *tpi* gene was performed using the primer pairs AL3543 (52-AAAT IATGCCTGCTCGTCG-32) and AL3546 (52-CAAACCTTITCCGCAAACC-32) in the primary reaction, and AL3544 (52-CCCTT CATCGGIGGTAACTT-32) and AL3545 (52-GTGGCCACCACICCCGTGCC-32) in the secondary reaction (Sulaiman *et al.*, 2003). PCRs were carried out in 25 µL volume containing 12.5 µL Taq DNA Polymerase (Ampliqon-Biomol, Hamburg, Germany), 3 and 2.5 µL of template DNA for the primary and secondary reactions, 1 µL of forward and reverse primers (10 µM), and 7.5 and 8 µL of distilled water for the primary and secondary reactions, respectively (Sulaiman *et al.*, 2003). The PCR conditions were identical to those described previously (Gillhuber *et al.*, 2013; Sulaiman *et al.*, 2003). PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide, and visualized using a UV transilluminator imaging system (Syngene, Cambridge, UK).

Sequencing

PCR products of *tpi* gene of *G. duodenalis* were directly sequenced at the Bioneer Co. (Daejeon, South Korea). Sequences were edited and aligned using ClustalW in MEGA software version 7.0. The phylogenetic tree was constructed using the neighbour-joining (NJ) method.

Statistical analysis

The statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

The mean age of the participants was 35.6 (standard deviation: 7.8). The overall prevalence of intestinal parasites was 15.0% (60/400), of which *Blastocystis hominis* (45/400; 11.2%) was the most common parasite, followed by *G. duodenalis* (16/400; 4.0%) and *Hymenolepis nana* (3/400; 0.7%) infections.

The maximum and minimum infection rate belonged to 20-40 (12/400; 3.0%) and 51-60 (1/400; 0.25%) age groups, respectively. *Giardia* infection was not significantly associated with age among the participants ($p>0.05$). Educational status was another variable that was taken into consideration in this study. Contrary to expectation, workers with high school educational level showed the highest (8.6%) and those attending middle school showed the lowest rate (1.5%) of *Giardia* infection. Although a statistical difference was not found in terms of infection and district ($p=0.53$), the workers belonging to District 6 were more infected (9.3%) than others (Table 1). Among the 400 participated workers, 388 (97.0%) were asymptomatic and 12 (3.0%) showed abdominal pain. All *Giardia* positive workers were asymptomatic.

The 16 positive isolates by microscopy were successfully amplified at the *SSU* rRNA gene while *tpi* gene was amplified in 13 (81.25%) samples. Of the 13 isolates successfully sequenced at *tpi* gene, 10 (76.9%) were typeable while the other three (23.1%) were untypable. Assemblage A, sub-assemblage AII, was found in nine (69.2%) isolates, and one (7.7%) isolate was assemblage B, sub-assemblage BIII (Fig. 1). Of the nine assemblage AII, six showed 100% identity with reference sequence U57897 (Baruch *et al.*, 1996) while the remaining three differed by two to four single-nucleotide polymorphisms (SNP) at positions 643 (A/T), 662 (A/G), 729 (A/G), and 770 (T/G). The only one isolate recognized as assemblage B, sub-assemblage BIII differed by a SNP at position of 57 (C/T) of reference sequence AF069561 (Monis *et al.*, 1999). Representative sequences were deposited in the GenBank under accession numbers LC329320 and LC329331.

Fig. 1 shows the phylogenetic tree resulting from the Neighbour-Joining analysis of *tpi* partial sequences obtained in the current study, and the representative reference sequences obtained from the NCBI

Table 1. Prevalence of *Giardia duodenalis* based on microscopy examination among municipal waste workers (n=400) Ahvaz county, southwestern Iran

Category	*G. duodenalis*			*p*- value
	No. examined (%)	No. Negative (%)	No. Positive (%)	
Age groups (years)				
20–30	121 (30.2)	115 (95.0)	6 (5.0)	0.866
31–40	175 (43.8)	169 (96.6)	6 (3.4)	
41–50	88 (22.0)	85 (96.6)	3 (3.4)	
51–60	16 (4.0)	15 (93.8)	1 (6.2)	
Educational status				
Primary school	39 (9.7)	37 (94.9)	2 (5.1)	0.029
Middle school	196 (49.0)	193 (98.5)	3 (1.5)	
High school	105 (26.3)	96 (91.4)	9 (8.6)	
Diploma	60 (15.0)	58 (96.7)	2 (3.3)	
Clinical symptoms				
Yes	12 (3.0)	12 (100.0)	0 (0.0)	0.473
No	388 (79.0)	372 (95.9)	16 (4.1)	
District				
1	84 (21.0)	82 (97.6)	2 (2.4)	0.533
2	44 (11.0)	42 (95.5)	2 (4.5)	
3	46 (11.5)	45 (97.8)	1 (2.2)	
4	65 (16.3)	63 (96.9)	2 (3.1)	
5	58 (14.5)	56 (96.6)	2 (3.4)	
6	54 (13.5)	49 (90.7)	5 (9.3)	
7	49 (12.3)	47 (95.9)	2 (4.1)	

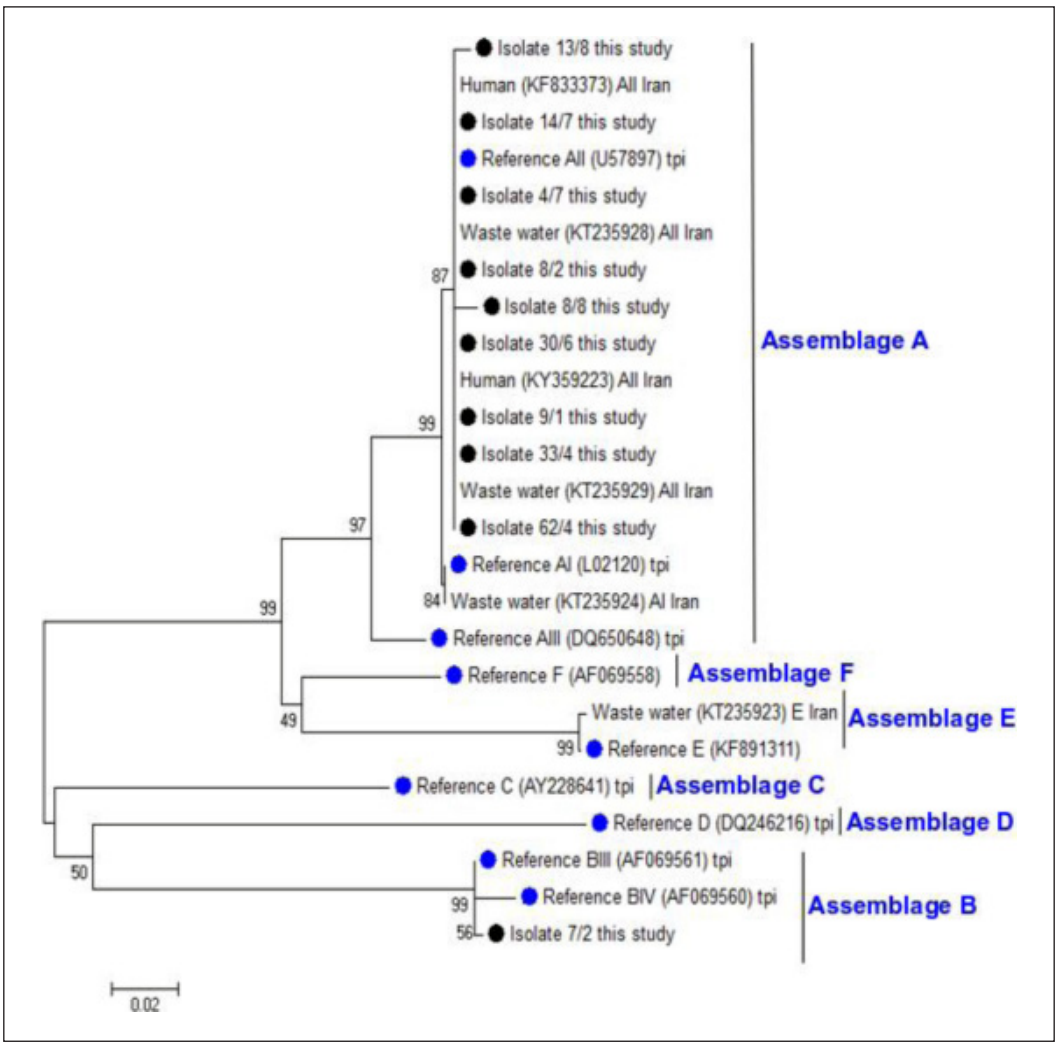

Figure 1. Phylogenetic tree for *Giardia duodenalis* based on *tpi* gene sequences. The evolutionary history was inferred using the Neighbor-Joining method. For each sequence used, GenBank accession numbers are provided. The bootstrap consensus tree was inferred from 1,000 replicates. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site.

database. A cluster was obtained from our AII isolates with Iranian human and environmental AII sequences. Assemblage B formed a cluster with AF069561 (Monis *et al.*, 1999) obtained reference sequences from the GenBank.

DISCUSSION

Waste management, which includes the processes of collecting, transporting, and disposing of solid waste, plays an important role in public and environmental health status (Porta *et al.*, 2009). It is estimated that annually more than 2 billion tons of waste are generated globally (Giusti, 2009). In a majority of developing countries, lack of political will and poor education have made waste management a low priority (Giusti, 2009). Furthermore, workers might be infected through ingestion of infective agents by dirty hands during eating at work (Alvarado-Esquivel *et al.*, 2008).

Although several studies have investigated the viral diseases in waste solid workers (Ansari-Moghaddam *et al.*, 2016; Mol *et al.*, 2015; Rachiotis *et al.*, 2012), available data on parasitic diseases have been restricted to a few studies (Alvarado-Esquivel *et al.*, 2008; Eassa *et al.*, 2016; Mba, 2004).

In Iran, like other developing countries, intestinal parasitic diseases are considered a public health problem. In a study by Sharif *et al*. in 2015, a prevalence of 53.9% was reported for *G. duodenalis* in food handlers in Sari, northern Iran (Sharif *et al.*, 2015). In another study from western Iran, *G. duodenalis* was observed in 2.9% of food handlers (Kheirandish *et al.*, 2014). Previous microscopic studies have been focused only on a special worker group, food handlers; and the only study on municipal waste workers in Iran has been conducted by microscopy methods (Molavi *et al.*, 2007). In Khuzestan Province, no comprehensive data are available on the prevalence of *G. duodenalis*, and only a few studies have been conducted in recent years (Kasaei *et al.*, 2018; Rafiei *et al.*, 2013).

In the present study, the positive samples were confirmed by the *SSU* rRNA gene, and then typing was performed at the *tpi* gene. Because of the multi-copy nature of the *SSU* rRNA gene, it often only used for confirmation of microscopic positive samples, while the single-copy genes, such as *tpi*, *gdh*, and *β*-giardin genes are used to differentiate species and sub-genotypes of *G. duodenalis* (Thompson & Ash, 2016). In addition, associated risk factors such as age, educational status, and clinical symptoms were investigated. In the present study, 16 (4.0%) of the municipal waste workers were infected with *G. duodenalis* by microscopy showing a significant decrease in the *Giardia* frequency compared to a previous study carried out in Esfahan (Molavi *et al.*, 2007). The difference in the prevalence might be attributed to several factors, including the sample size, studies conducted in different periods, diagnostic methods, and geographic and climatic conditions of the regions. In a similar study carried out in Egypt, 346 municipal waste workers were investigated microscopically for intestinal parasitic infections. The results indicated that 2.9% of the workers were infected with *G. duodenalis* (Eassa *et al.*, 2016). Although age is an important factor in intestinal parasitic infections (Faria *et al.*, 2017), no significant association was found between age and *Giardia* infection.

Educational status and awareness are the other effective factors associated with intestinal parasitic infections, which can reduce the chance of infection (Ostan *et al.*, 2007). However, our findings revealed a higher infection among workers with high school educational level. It seems that, the educational status did not have an effect on the prevention of intestinal parasites infection among workers. Regarding *Giardia* infection in different districts of the county, a notable point is that approximately one third (5/16; 31.2%) of the infections was observed in District 6, which includes northern and west northern parts of Ahvaz. Compared to other districts, people living in this district are in poor hygiene conditions. Moreover, due to keeping domestic animals at home, they could be more at risk of zoonotic infections (Table 1).

A noteworthy finding in our study was that all infected workers (100.0%) were asymptomatic and unaware about their disease. Since asymptomatic patients are cyst passer, therefore, they can play an important role in the transmission of *Giardia*. Clinical symptoms in giardiasis vary from mild diarrhea to severe malabsorption syndrome. It seems that in giardiasis, symptoms are associated with various host and parasite related factors (Homan and Mank, 2001). The possible correlation of clinical symptoms with parasite strain is not completely clear. However, according to previous studies, assemblage A is more frequent in symptomatic subjects (Cardona *et al.*, 2011; Haque *et al.*, 2009; Sahagun *et al.*, 2008).

Interestingly, in contrast with previous studies (Cardona *et al.*, 2011; Haque *et al.*, 2009; Sahagun *et al.*, 2008), our findings revealed the predominance of assemblage A in asymptomatic subjects. Assemblage A, sub-assemblage AII was the most common identified assemblage with a frequency of

56.2% (9/16), and assemblage B, sub-assemblage BIII was found in 6.2% (1/16) of the isolates. However, the worrying thing is that asymptomatic workers could be a potential source of infection to others (Cardona *et al.*, 2011; Haque *et al.*, 2009; Sahagun *et al.*, 2008). Our findings are in line with a recent study from central Iran in which assemblage A, sub-assemblage AII was reported in 24 (54.5%), and assemblage B, sub-assemblages BII and BIV were identified in seven (15.9%) and two (4.6%) examined isolates, respectively. In the study, co-infections of assemblages A and B were also observed in 11 (25.0%) isolates (Hooshyar *et al.*, 2017). In contrast to our study, Rafiei et al. observed sub-assemblages BIII and AII in 74.0% and 36.0% of the asymptomatic subjects, respectively (Rafiei *et al.*, 2013). When looking at the studies available, it can be claimed that assemblage AII is the most common sub-assemblage in Iranian population.

CONCLUSIONS

The current study provided new data on the prevalence and genotypic diversity of *G. duodenalis* among municipal waste workers in Ahvaz, southwestern Iran. Our findings revealed that Assemblage A, sub-assemblage AII was the predominant assemblage. Furthermore, we found that transmission of human giardiasis in Ahvaz County is mainly of anthroponotic origin. Given that all infected participants were asymptomatic, it is suggested that health educational programs along with periodic health examinations be implemented to prevent and control intestinal parasitic diseases.

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CONFLICT OF INTEREST
The authors have no conict of interest.

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REFERENCES

Alvarado-Esquivel, C., Liesenfeld, O., Marquez-Conde, J.A., Cisneros-Camacho, A., Estrada-Martinez, S., Martinez-Garcia, S.A., Gonzalez-Herrera, A. & Garcia-Corral, N. (2008). "Sero-epidemiology of infection with *Toxo-plasma gondii* in waste pickers and waste workers in Durango, Mexico." *Zoonoses and Public Health* **55**: 306-12.

Ansari-Moghaddam, A., Ansari, H., Khosravi, S., Sanei-Moghaddam, E., Mohammadi, M., Alavian, S.M. & Poustchi, H. (2016). "The Prevalence of Hepatitis B Virus Among Municipal Solid Waste Workers: Necessity for Immunization of At-Risk Groups." *Hepatitis Monthly* **16**: e30887.

Athanasiou, M., Makrynos, G. & Dounias, G. (2010). "Respiratory health of municipal solid waste workers." *Occupational Medicine (Oxford, England)* **60**: 618-23.

Baruch, A.C., Isaac-Renton, J. & Adam, R.D. (1996). "The molecular epidemiology of *Giardia lamblia*: a sequence-based approach." *Journal of Infectious Diseases* **174**: 233-6.

Cardona, G.A., Carabin, H., Goni, P., Arriola, L., Robinson, G., Fernandez-Crespo, J.C., Clavel, A., Chalmers, R.M. & Carmena, D. (2011). "Identification and molecular characterization of *Cryptosporidium* and *Giardia* in children and cattle populations from the province of Alava, North of Spain." *Science of the Total Environment* **412-413**: 101-8.

Eassa, S.M., El-Wahab, E.W.A., Lotfi, S.E., El Masry, S.A., Shatat, H.Z. & Kotkat, A.M. (2016). "Risk Factors associated with parasitic infection among municipality solid-waste workers in an Egyptian community." *Journal of Parasitology* **102**: 214-221.

Faria, C.P., Zanini, G.M., Dias, G.S., da Silva, S., de Freitas, M.B., Almendra, R., Santana, P. & Sousa, M.D.C. (2017). "Geospatial distribution of intestinal parasitic infections in Rio de Janeiro (Brazil) and its association with social determinants." *PLoS Neglected Tropical Diseases* **11**: e0005445.

Feng, Y. & Xiao, L. (2011). "Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis." *Clinical Microbiology Reviews* **24**: 110-40.

Fletcher, S.M., Stark, D., Harkness, J. & Ellis, J. (2012). "Enteric protozoa in the developed world: a public health perspective." *Clinical Microbiology Reviews* **25**: 420-49.

Gillhuber, J., Pallant, L., Ash, A., Thompson, R.C., Pfister, K. & Scheuerle, M.C. (2013). "Molecular identification of zoonotic and livestock-specific *Giardia*-species in faecal samples of calves in Southern Germany." *Parasit Vectors* **6**: 346.

Giusti, L. (2009). "A review of waste management practices and their impact on human health." *Waste Management* **29**: 2227-2239.

Haque, R., Mondal, D., Karim, A., Molla, I.H., Rahim, A., Faruque, A.S., Ahmad, N., Kirkpatrick, B.D., Houpt, E., Snider, C. & Petri, W.A. Jr. (2009). "Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh." *Clinical Infectious Diseases* **48**: 1191-7.

Hatam-Nahavandi, K., Mohebali, M., Mahvi, A.-H., Keshavarz, H., Mirjalali, H., Rezaei, S., Meamar, A.-R. & Rezaeian, M. (2017). "Subtype analysis of *Giardia duodenalis* isolates from municipal and domestic raw wastewaters in Iran." *Environmental Science and Pollution Research International* **24**: 12740-12747.

Hazrati Tappeh, K., 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." *Iran J Parasitol* **9**: 541-7.

Homan, W.L. & Mank, T.G. (2001). "Human giardiasis: genotype linked differences in clinical symptomatology." *International Journal for Parasitology* **31**: 822-6.

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**: 83.

Kasaei, R., Carmena, D., Jelowdar, A. & Beiromvand, M. (2018). "Molecular genotyping of *Giardia duodenalis* in children from Behbahan, southwestern Iran." *Parasitology Research* **117**: 1425-1431.

Kheirandish, F., Tarahi, M.J. & Ezatpour, B. (2014). "Prevalence of intestinal parasites among food handlers in Western Iran." *Revista do Instituto de Medicina Tropical de Sao Paulo* **56**: 111-4.

Mba, O.O. (2004). "Intestinal parasites among waste-handlers in jos metropolitan area of plateau state, Nigeria." *Sahel Med J* **7**: 13-17.

Mol, M.P., Greco, D.B., Cairncross, S. & Heller, L. (2015). "Hepatitis B and C in household and health services solid waste workers." *Cadernos de Saúde Publica* **31 Suppl 1**: 295-300.

Molavi, G., Masoud, J., Moubedi, I. & Hassanpour, G. (2007). "Prevalence of intestinal parasites in Esfahan municipal workers." *J Sch Public Health Inst Public Health Res* **5**: 43-50.

Monis, P.T., Andrews, R.H., Mayrhofer, G. & Ey, P.L. (1999). "Molecular systematics of the parasitic protozoan *Giardia intestinalis*." *Molecular Biology and Evolution* **16**: 1135-44.

Ostan, I., Kilimcioglu, A.A., Girginkardesler, N., Ozyurt, B.C., Limoncu, M.E. & Ok, U.Z. (2007). "Health inequities: lower socio-economic conditions and higher incidences of intestinal parasites." *BMC Public Health* **7**: 342.

Porta, D., Milani, S., Lazzarino, A.I., Perucci, C.A. & Forastiere, F. (2009). "Systematic review of epidemiological studies on health effects associated with management of solid waste." *Environmental Health: A Global Access Science Source* **8**: 60.

Rachiotis, G., Papagiannis, D., Markas, D., Thanasias, E., Dounias, G. & Hadjichristodoulou, C. (2012). "Hepatitis B virus infection and waste collection: prevalence, risk factors, and infection pathway." *American Journal of Industrial Medicine* **55**: 650-5.

Rafiei, A., Roointan, E.S., Samarbafzadeh, A.R., Shayesteh, A.A., 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**: 389-95.

Ramirez, J.D., Heredia, R.D., Hernandez, C., Leon, C.M., Moncada, L.I., Reyes, P., Pinilla, A.E. & Lopez, M.C. (2015). "Molecular diagnosis and genotype analysis of *Giardia duodenalis* in asymptomatic children from a rural area in central Colombia." *Infection Genetics and Evolution* **32**: 208-13.

Rayani, M., Unyah, N.Z. & Hatam, G. (2014). "Molecular identification of *Giardia duodenalis* isolates from Fars province, Iran." *Iran J Parasitol* **9**: 70.

Robertson, L.J., Hanevik, K., Escobedo, A.A., Mørch, K. & Langeland, N. (2010). "Giardiasis – why do the symptoms sometimes never stop?" *Trends in Parasitology* **26**: 75-82.

Roointan, E.S., Rafiei, A., Samarbaf-Zadeh, A.R., Shayesteh, A.A., Shamsizadeh, A. Borujeni, M.P. (2013). "Genotype Analysis of *Giardia lamblia* Isolated From Children in Ahvaz, Southwest of Iran." *Jundishapur J Microbiol* **6**: 279-283.

Sahagun, J., Clavel, A., Goni, P., Seral, C., Llorente, M.T., Castillo, F.J., Capilla, S., Arias, A. & Gomez-Lus, R. (2008). "Correlation between the presence of symptoms and the Giardia duodenalis genotype." *European Journal of Clinical Microbiology and Infectious Diseases* **27**: 81-3.

Savioli, L., Smith, H. & Thompson, A. (2006). "*Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'." *Trends in Parasitology* **22**: 203-208.

Sharif, M., Daryani, A., Kia, E., Rezaei, F., Nasiri, M. & Nasrolahei, M. (2015). "Prevalence of intestinal parasites among food handlers of Sari, Northern Iran." *Revista do Instituto de Medicina Tropical de Sao Paulo* **57**: 139-44.

Sprong, H., Caccio, S.M. & van der Giessen, J.W. (2009). "Identification of zoonotic genotypes of *Giardia duodenalis*." *PLoS Neglected Tropical Diseases* **3**: e558.

Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H., Trout, J.M., Schantz, P.M., Das, P., Lal, A.A. & Xiao, L. (2003). "Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*." *Emerging Infectious Diseases* **9**: 1444.

Thompson, R. & Ash, A. (2016). "Molecular epidemiology of Giardia and Cryptosporidium infections." *Infection, Genetics and Evolution* **40**: 315-323.

Thompson, R.A. (2000). "Giardiasis as a re-emerging infectious disease and its zoonotic potential." *Int J Parasitol* **30**: 1259-1267.

Zarasvandi, A., Carranza, E.J.M., Moore, F. & Rastmanesh, F. (2011). "Spatio-temporal occurrences and mineralogical-geochemical characteristics of airborne dusts in Khuzestan Province (southwestern Iran)." *J Geochem Explor* **111**: 138-151.