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ORIGINAL ARTICLE

Giardiasis in symptomatic children from Sharkia, Egypt: genetic assemblages and associated risk factors

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Abstract *Giardia intestinalis* (*G. intestinalis*) is a common enteric protozoan parasite worldwide and in Egypt. Identification of true prevailing *Giardia* assemblages helps in identification of the sources of infection. The study's aim was to determine the true prevalence of *Giardia* assemblages in Egyptian children from Sharkia governorate presenting with gastrointestinal symptoms and to investigate their association with molecularly detected *Giardia*. A total of 617 stool specimens were collected from children presenting with gastrointestinal symptoms in Alquraeen, Sharkia governorate, Egypt for 17 months. All stool specimens were microscopically examined by wet mount smear before and after stool concentration to recover parasitic stages. *Giardia* copro-DNA was amplified from microscopically detected stool specimens using Copro-nPCR targeting the tpi gene for *Giardia,* followed by sequencing products of nPCR. The molecular prevalence of *Giardia* among symptomatic children was 9.88%, 83% of which were assemblage B and 17% were assemblages A. *Giardia* affected both sexes and all ages and was most prevalent in preschool children. Abdominal pain was the most common GIT symptom followed by diarrhoea. However, none of the patients' demographic variables (sex, age, weight and height) nor clinical symptoms showed significant association with molecular detection of Giardia. *Giardia* was common among symptomatic children from Sharkia, Egypt, with the predominance of assemblage B, which suggests the possibility of sharing common transmission source and route. *Giardia* had age, sex and clinical symptom distributions without statistical significance. The results necessitate further genomic studies targeting multiple gene targets for a better understanding of the ecology, dynamics of transmission, pathogenicity and clinical impact of *Giardia* infection, to improve its management and strategic control.

Keywords *Giardia* · Assemblage · Diarrhoea · Children · Sharkia · Egypt

Introduction

In developing countries of Asia, Africa, and Latin America, around two hundred million humans have symptomatic giardiasis (Yason and Rivera 2007). *Giardia intestinalis* (*G. intestinalis*), also known as *G. duodenalis* or *lamblia*, is the first reason for parasitic diarrhoea within developing and developed countries. Additionally, there is an association between diarrhoea/dysentery and detection of the flagellate protozoan *Giardia* and its assemblages (Haque 2007).

In giardiasis, there is an immune response in the form of infiltration of intestinal mucosa with mononuclear inflammatory cells, hypertrophy of crypts and blunting of intestinal villi. This immune response clears *Giardia*

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trophozoites, protects against reinfection and production of the disease (Gillespie and Pearson 2001).

G. intestinalis cysts are the transmissible stages. Transmission of these cysts to humans occurs mainly due to ingestion of contaminated food, intake of contaminated water and person-to-person contact, in addition to autoinfection. Clinical presentations of symptomatic *Giardia* protozoal infection are flatulence, diarrhoea, abdominal pain, epigastric tenderness and abdominal cramps. However, asymptomatic cases of giardiasis also happen (Furness and Roberts 2000; Gardner and Hill 2001).

There are six established *Giardia* species, *G. intestinalis* (synonyms, *G. doudenalis* or *G. lamblia*), *G. microti, G. muris*, *G. psittaci*, *G. ardeae* and *G. agilis* (Monis et al. 1999; Feng and Xiao 2011). Among these six species, *G. intestinalis* is the only *Giardia* species that infects humans and diverse mammals. Based on sequencing of target genes PCR products, isolates of *G. intestinalis* are classified into eight assemblages from A to H (Feng and Xiao 2011). Isolates of *Giardia* Assemblage A were divided into two subgroups, AI and AII; Isolates of *Giardia* assemblage B isolates were separated into two subgroups, BIII and BIV. Genetic *Giardia* assemblages C, D, E, F and G seem to be limited to livestock, domestic animals and wild animals (Adam 2001). In Egypt, human infections are mainly by *Giardia* assemblages A and B, with rare cases of assemblages E and C (Abdel-Moneim and Sultan 2008; Foronda et al. 2008; Helmy et al. 2009; Soliman et al. 2011; Amer 2013; Sadek et al. 2013, El-Tantawy and Taman 2014; El-Badry et al. 2017; Taha et al. 2018; Nasr et al. 2018).

Routine laboratory diagnosis of Giardiasis is done by detection of *Giardia* trophozoites and/or cysts in stool using microscopy, and *Giardia* copro-antigen using immunological methods as ELISA (Johnston et al. 2003), but these methods are lacking the ability to differentiate between the different genetic assemblages of *G. intestinalis*. Molecular assays based on polymerase chain reaction (PCR) can accurately detect copro-*G. intestinalis* DNA sequences in stool with high sensitivity and specificity (Nash et al. 1985). In addition, PCR and post-PCR based assays enable the genotyping of assemblages of *G. intestinalis* (Aydin et al. 2004).

A correlation between genetic *Giardia* assemblages and susceptible age group, sex, infectivity, transmission, pathogenicity, symptomatology or potential of involvement of animal were studied in many geographical areas worldwide, including Egypt (Homan and Mank 2001; Read et al. 2002; Stuart et al. 2003; Sackey et al. 2003; Ghieth et al. 2016). Although *Giardia* assemblages were studied in many geographical areas in Egypt, it had not yet been done in Alquraeen, Sharkia. The present study aimed to determine the molecular prevalence of *Giardia* and the prevailing *Giardia* assemblages in symptomatic Egyptian children from Alquraeen, Sharkia governorate, Egypt and to explore the possibility of an association between molecularly detected *Giardia* and presenting gastrointestinal symptoms (Fig. 1).

Material and methods

The study was a cross-sectional, it was conducted over 17 months, from January 2016 to May 2017. Stool specimens were obtained from children suffering from gastrointestinal symptoms in Alquraeen, Sharkia governorate, Egypt after informed consent was obtained from all children's parents or guardians. This study was approved ethically by the Ethical Board of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt. All related demographic and clinical data were collected using standard questionnaires that were completed by the children's parents/guardians.

Stool specimens were obtained in 100 ml sterile screw-capped containers labelled with each child's name and number. Immediately after specimen collection, fresh faecal specimens were microscopically examined for detection of gastrointestinal parasites by direct wet mount smear stained with Lugol's iodine, both before and after stool concentration. Based on the results of microscopic examination of stool specimens, cases were divided into the following two groups.

Group A: positive for *Giardia*.
Group B: negative for *Giardia*.

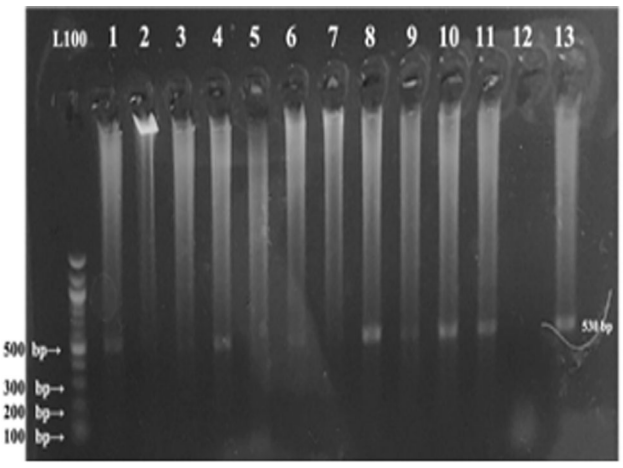

Fig. 1 Showing agarose gel electrophoresis for the products of the nested PCR targeting *TPI* gene of *G. intestinalis* at 530 bp. Lane L100: 100 bp DNA M.W marker "ladder". Lanes 1, 4, 6, 8–11: represent positive samples. Lanes 2, 3, 5, 7: represent negative samples. Lane 12: represent positive control. Lane 13: represent negative control

Part of each stool sample in group A were put in one and a half ml Eppendorf tube and stored frozen at − 20 °C without using preservatives for further molecular assays. All stool samples of group A were processed for DNA extraction preceded by ten cycles of thermal shock of freeze-thawing using liquid nitrogen for five minutes then water bath (95 °C) for five minutes. Copro-DNA from all group A stool samples was extracted using Favor Prep stool DNA isolation Kit (Favorgen Biotech corporation ping-Tung 908, Taiwan, Cat. No. FASTI001) as indicated by the kit instructions. Nested PCR (nPCR) was done using two sets of primers targeting tpi gene: AL3543: 50-AAA-TIATGCCT GCTCGTCG-30 and the reverse primer AL3546: 50-CAAA CCTTITCCGCAAACC-30 for the primary reaction to amplify 605 bp DNA sequence and a fragment of 530 bp for the secondary reaction using AL3544: 50-CCCTTCATCGGI GGTAACTT-30 and the reverse primer AL3545: 50-GTGG CCACCA-CICCCGTGCC-30 (Sulaiman et al. 2003). The reaction conditions and mixture were done following Sulaiman et al. (2003). The amplified products of nPCR were electrophoresed with 1.5% agarose gel following ethidium bromide staining.

To assess the genotypic assemblages of *Giardia* isolates from symptomatic children amplified by nPCR, nPCR products using marker tpi genes were sequenced in both directions using BigDye® Terminator v3.1 Ready Reaction Cycle Sequencing Kit on an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA) following the methodology mentioned earlier in Ghieth et al. (2016) and El-Badry et al. (2017). The obtained DNA sequences were blasted using BLAST search in NCBI website for similarities with GenBank sequences.

The SPSS program was used to analyze the study data. The qualitative and quantitative data were presented, the chi-squared test and Fisher's exact test used to compare groups when applicable. The associations between *Giardia* positive cases using PCR and independent variables were tested for statistical significance. The statistical significance was defined as $P < 0.05$.

Results

The overall prevalence of parasitic infection was 22.2%, among them, 0.97% of samples showed multiple infections. The prevalence of each parasite as single or multiple infections is shown in Table 1. *G. intestinalis* showed the highest infection rate (9.88%), it was detected in 61 cases, among which three cases showed mixed parasitic infections. *Entamoeba histolytica* complex was detected in 39 cases (6.32%), among which three cases had mixed parasitic infections. *Enterobius vermicularis* was detected in 38 cases (6.15%), among which two cases had mixed parasitic infections. *Entamoeba coli* were only detected in 2 cases (0.32%), among which one case with mixed parasitic infections. The molecular prevalence of *Giardia* was 9.88% among symptomatic children, 83% of which were Assemblage B. Among the 61 cases in group A microscopically positive for *G. intestinalis* cyst/trophozoite, only 37 were positive by *Giardia* nPCR targeting tpi gene (60.66%) (Table 2).

Table 1 Prevalence of intestinal parasitic infections among patients

Parasite	No. infected	%
Single		
G. intestinalis	58	9.40
Entamoeba histolytica complex	36	5.83
Enterobius vermicularis	36	5.83
Entamoeba coli	1	0.16
Total	131	21.22
Multiple		
Enterobius vermicularis + *Entamoeba histolytica* complex	3	0.48
G. intestinalis + *Enterobius vermicularis*	2	0.32
G. intestinalis + *Entamoeba coli*	1	0.16
Total	6	0.96
Total	**137**	**22.2**

The range and mean of age, weight and height of positive and negative *Giardia* cases using nPCR are shown in Tables 3, 4 and 5. Sex and clinical symptom distribution of *Giardia* nPCR positive and negative cases and their association are presented in Table 6. There was no association of statistical significance between Giardia positive by nPCR and both studied patients' demographics data (sex, age, weight and height) and clinical symptoms (diarrhea, abdominal pain, vomiting and anorexia).

Sequencing of tpi PCR products revealed that 83% of the detected infections were due to assemblage B, and 17% of infections were due to Assemblage A of *G. intestinalis*.

Discussion

In this study, we found that the overall prevalence of parasitic infection was 22.2%. Less than 1% of our study populations showed multiple parasitic infections. *Entamoeba histolytica* complex was the second most common enteric parasite (6.32%), followed by *Entrobius vermicularis* (6.15%). Higher parasitic infection rates, up to 60.9%, were reported in other areas of Egypt (Shalaby et al. 1986; El-Gammal et al. 1995; El-Masry et al. 2007; Bakr et al. 2009; Mousa et al. 2010).

Table 2 Results of microscopy and nPCR to detect *Giardia* infection

	Microscopy				
	Positive			Negative	Total
	nPCR negative	nPCR positive	Total		
N	24	37	61	556	617
%	3.89 %	5.99 %	9.88 %	90.12 %	100 %

G. intestinalis was the most common enteric protozoa and the most frequent parasitic agent (9.88%) in the current study of children with gastrointestinal symptoms. Similar findings were reported in Egypt and worldwide, especially in developing countries (Núñez et al. 2003; Escobedo et al. 2008; Foronda et al. 2008; Babiker et al. 2009; Helmy et al. 2009; Lalle et al. 2009; Cañete et al. 2012; Sadek et al. 2013; Puebla et al. 2014). In other studies, lower prevalence rates of *G. intestinalis* have been reported (Norhayati et al. 2003; Natividad et al. 2008). The differences in prevalence of enteric parasites, including *Giardia,* may be due to many factors, including geographical, epidemiological, socioeconomic level, environmental sanitation, hygienic measures and water supply.

The present study indicates that 60.66% (37/61) of microscopically positive samples were identified by nPCR. Similar false-negative results were reported by using different *Giardia* genes, GDH gene, tpi gene, B giardin and rRNA genes (Amar et al. 2004). The false-negative results could be due to a low DNA level, mismatch of the used primers, or existence of a robust wall that inhibits the release of DNA from the cysts (Lalle et al. 2009; Ghieth et al. 2016).

In the current study, only two assemblages, A and B, were detected, with a predominance of *Giardia* assemblage B (83%). In Egypt, the predominance of assemblages B in 80–95% of patients was reported in Cairo, Sharkia, Ismailia, Kafr El-Shiekh, and Dakahlia governorates (Soliman et al. 2011; Amer 2013; El-Tantawy and Taman 2014; El-Badry et al. 2017; Taha et al. 2018; Nasr et al. 2018). However, a higher infection rate of assemblage A than assemblage B was also reported in Egypt (Abdel-Moneim and Sultan 2008; Helmy et al. 2009, Sadek et al. 2013). Worldwide and in Egypt, there are variations in *Giardia* assemblages' distribution among infected cases. This genetic variability of *Giardia* assemblages in different geographical locations may be due to the role of zoonosis as well as geographical differences. Though identification of *G. intestinalis* assemblages can be easily achieved, the clinical and epidemiologic importance of infection by different *Giardia* assemblages is insufficiently understood (Guy et al. 2004; Cedillo-Rivera et al. 2003).

In the current study, *Giardia* affected both males and females of all ages and was most prevalent in preschool children. However, none of the demographic patients'

Table 3 Children' age for positive and negative *Giardia* cases using nPCR and its association with *Giardia* infection

Group	Age (years)		T-test	
	Range	Mean ± SD	t	*P* value
Positive	0.11–11.00	5.333 ± 2.567	− 0.161	0.872
Negative	0.20–15.00	5.399 ± 3.111		

Table 4 Children' weight for positive and negative *Giardia* cases using nPCR and its association with *Giardia* infection

Group	Weight (kg)		T-test	
	Range	Mean ± SD	t	*P* value
nPCR positive	13.00–30.00	19.208 ± 5.150	− 0.951	0.345
nPCR negative	13.00–37.00	20.730 ± 6.640		

Table 5 Children' height for positive and negative *Giardia* cases using nPCR and its association with *Giardia* infection

Group	Height (m)		T-test	
	Range	Mean ± SD	t	*P* value
nPCR Positive	87.00–133.00	109.583 ± 13.377	− 0.815	0.418
nPCR Negative	86.00–143.00	112.676 ± 15.124		

Table 6 Children' sex and GIT symptoms for positive and negative *Giardia* cases using nPCR and its association with *Giardia* infection

				Giardia (nPCR assays)		Total	*P*- value*
				Negative	positive		
Gender		**Female**	N	18	16	34	**0.013**
			%	75.0 %	43.2 %	55.7 %	
		Male	N	6	21	27	
			%	25.0 %	56.8 %	44.3 %	
GIT symptoms	Diarrhoea	**No**	N	14	15	29	**0.173**
			%	58.3 %	40.5 %	47.5 %	
		Yes	N	10	22	32	
			%	41.7 %	59.5 %	52.5 %	
	Vomiting	**No**	N	20	28	48	**0.47**
			%	83.3 %	75.7 %	78.7 %	
		Yes	N	4	9	13	
			%	16.7 %	24.3 %	21.3 %	
	Abdominal Pain	**No**	N	4	7	11	**0.822**
			%	16.7 %	18.9 %	18.0 %	
		Yes	N	20	30	50	
			%	83.3 %	81.1 %	82.0 %	
	Anorexia	**No**	N	12	22	34	**0.468**
			%	50.0 %	59.5 %	55.7 %	
		Yes	N	12	15	27	
			%	50.0 %	40.5 %	44.3 %	
Total			**N**	**24**	**37**	**61**	
			%	**100 %**	**100 %**	**100 %**	

P-value is statistically significant if at or less than 0.05.

P value is statistically significant if at or less than 0.05

variables (sex, age, weight and height) showed a significant association with molecular detection of *Giardia*.

In the present study, abdominal pain (30/37, 81.1%) and diarrhoea (22/37, 59.5%) were the predominant symptoms, however, there was no statistical significance between *Giardia* assemblages and clinical symptoms in the studied cases. Occurrence of abdominal pain was detected in 50–80% of *Giardia* infected patients in previous studies (Hill and Nash 2006; Nasr et al. 2018). Similar findings were reported in many other studies, while other studies showed a correlation between infection with assemblage B in humans and demographic data and the presence of symptoms. There continues to be a missing explanation for these contradictory findings (Hill and Nash 2006; Nasr et al. 2018).

Conclusions

Giardia was common among symptomatic children from Sharkia, Egypt, with the predominance of assemblage B, which suggests the possibility of sharing a common transmission source and route. Abdominal pain is the predominant clinical presentation, however, none of the patients' gastrointestinal symptoms significantly correlated with molecular detection of *Giardia*. Further genetic

studies of different assemblages would enhance the understanding of the ecology, dynamics of transmission, pathogenicity and clinical impact of *Giardia* infection, to improve its management and strategic control.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical approval The ethical consideration was obtained from the Ethical Board of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt, consent was obtained from all children's parents or guardians.

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