

Prevalence and genetic characterization of *Giardia lamblia* in relation to diarrhea in Limpopo and Gauteng provinces, South Africa

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ABSTRACT

Background: Very few studies have determined the prevalence and assemblage distribution of *Giardia lamblia* in South Africa. The present study aimed to ascertain the prevalence of *G. lamblia* infection and the spread of the various assemblages in two communities in South Africa - Giyani, Limpopo province (rural community) and Pretoria Gauteng province (urban community).

Methods: Prevalence was determined by immunological and molecular methods analyzing a total of 516 stool samples collected from patients visiting different health centres in Giyani and Pretoria. For immunological assays, samples were screened by ELISA to detect *G. lamblia* antigen. Furthermore, a semi nested PCR amplifying the triose phosphate isomerase (tpi) gene was used to differentiate between the two most common human assemblages (A and B).

Findings: Of the 516 participants, 40 (7.75%) were identified as positive by ELISA. A statistically significant correlation was observed between the stool texture and *Giardia* infection ($\chi^2 = 10.533$; $p = .005$). *G. lamblia* was significantly associated with watery stool types in females $p = .008$. Furthermore, a significant association was also noticed between the origin of samples ($\chi^2 = 9.725$; $p = .002$). No significant correlation between age and gender was noted. Regarding the age groups, most people who were infected were between 3 and 20 years. A statistically significant association was seen ($p = .001$) with the distribution of the pathogen with the stool type. The prevalence of *Giardia* infection was higher in watery stool samples (71.4%) in Giyani region (rural) whereas in Pretoria, high prevalence was found in loose stool samples (6.2%). Generally, the distribution was statistically significant in the stool type collected for the study ($p = .005$). Genotyping revealed more *G. lamblia* assemblage B (17.8%) than assemblage A (1.7%). Furthermore, 21.0% of the samples exhibited single infection while 4.2% had mixed infections. Assemblage B was more common in Giyani than in urban Pretoria.

Conclusions: The study confirms *Giardia* as an important cause of diarrhea in the concerned communities with people in rural areas more at risk compared to those in urban areas with

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higher prevalence among younger patients. Therefore, health education campaigns should target young age groups.

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1. Introduction

Giardia lamblia (also known as *Giardia duodenalis*, or *Giardia intestinalis*) is a cosmopolitan intestinal protozoan parasite of several terrestrial vertebrates, with an estimated 2 million cases per year (Hill et al., 2005). It represents the most common gastrointestinal parasitic infections of humans with a wide spectrum of symptoms ranging from asymptomatic carriage to long lasting diarrhea resulting in malabsorption (Lloyd and Harris, 2002; Kwitshana et al., 2008; Samie et al., 2009). In immunocompetent individuals, giardiasis is usually self-limiting, but can develop into persistent and life-threatening infection in immune-deficient individuals and malnourished children in developing countries (Lima et al., 2000).

The prevalence of *Giardia* infections in humans and animals, may vary among countries, probably due to differences in the management, weather, and study type (Veronesi et al., 2010). The protozoan is currently considered as a complex species, due to the existing genetic differences among isolates infecting different hosts (Monis and Thompson, 2003). This infection is regarded as a serious public health problem, as it can cause acute diarrhea, iron deficiency anemia, growth retardation in children and other physical and mental health condition (Kwitshana et al., 2008). It is well documented that in developing countries infections are associated with poor sanitary conditions, poor water quality and overcrowding (Younas et al., 2008). For example, a case study from Malamulele, Limpopo Province, indicated that pathogens can be transmitted through ingestion of vegetables irrigated by wastewater re-use (Gumbo et al., 2010). In contrast, cases in industrialized countries are usually associated with institutionalization, homosexuality, international travel and immigration (Ekdahl and Andersson, 2005). Populations at increased risk of autochthonous infection include small children in day care centers, men who have sex with men and person in custodial institutions (Yoder et al., 2007).

Molecular tools analyses of human isolates from various geographical localities revealed that assemblages A and B are commonly associated with *G. lamblia* infections (Caccio and Ryan, 2008). However, the role of these two assemblages in the outcome of infection is not yet clear (Paintlia et al., 1999). Understanding what determines the outcome of giardiasis is very important. It remains puzzling why only a subset of individuals develops symptomatic diseases while others do not (Caccio and Ryan, 2008). The outcome of such infections is dependent on several factors, among which the parasite genotypes have been described as the significant ones. Most studies have focused on prevalence of parasite, and some had indicated that the prevalence of each assemblage varies considerably from country to another, yet no strong conclusion has been drawn from this data (Cooper et al., 2007). An earlier study by Samie et al. (2009) in the Vhembe district of Limpopo province revealed the prevalence of 12.8% for *Giardia* in diarrheal samples obtained from primary school children. Another study conducted in Mthata, Eastern Cape province, South Africa showed a prevalence of 9.9% from primary school children (Nxasana et al., 2013). Unlike the present report, a major limitation of these two studies was the use of microscopy in the identification of the parasite.

The recently developed molecular methods have demonstrated considerable specificity and sensitivity compared with microscopy and antigen detection methods. Another added advantage of these tools is the direct detection and molecular characterization of the identified parasite (Verweij et al., 2004; Abe et al., 2005).

To date, not enough data on the prevalence and molecular genotyping of *G. lamblia* using nucleic acid-based approaches are available in South Africa. Thus, the present study aimed at determining the prevalence and occurrence of *G. lamblia* assemblages in relation to diarrhea in two different settings (rural and urban) in South Africa using ELISA and PCR methods.

2. Materials and methods

2.1. Ethical clearance

The study was approved by the health and ethics committee of the University of Venda, Thohoyandou, South Africa and the Medunsa ethical clearance committee, Pretoria, South Africa. Ethical Clearance was also obtained from the different hospitals where the samples were collected. The objectives of the study were clearly explained to the sample providers and they were requested to sign the consent forms before data and sample collection. The information obtained from the patients contained only a code in order to protect the identity of the patients.

2.2. Study areas

The study site was Giyani, which is situated approximately 200 km from the city of Polokwane and approximately 70 km from the town of Thohoyandou in the Limpopo province, North Eastern of South Africa (Fig. 1). This is a rural area with people of different religions, educational and socio-economic backgrounds, living in neighborhoods with distinctly low level of sanitation. Pretoria is a city in the Northern part of Gauteng Province, South Africa. In this area sanitation provision is reasonably better.

Republic of South Africa

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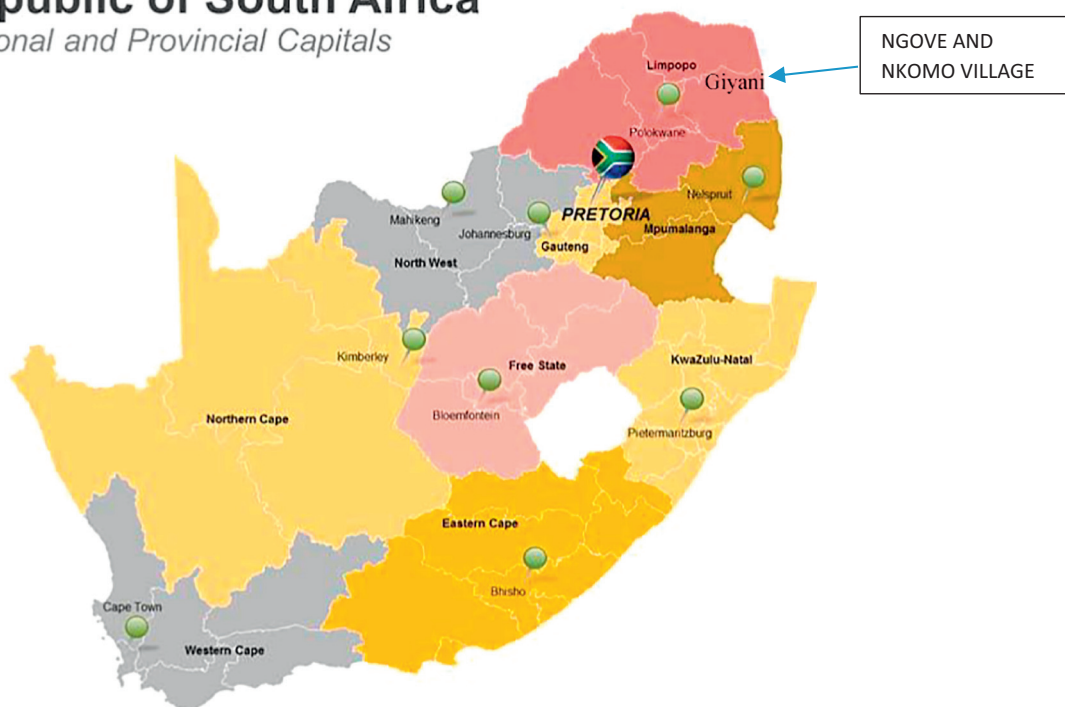


Fig. 1. Map of South Africa showing the study site.

Most people have access to a hygienic toilet, and most sewage is treated before discharge to the environment in a controlled manner. However, a significant minority of the city's population still lacks adequate sanitation.

2.3. Study design and sample collection

The study design was a cross sectional survey of *Giardia* among patients presenting at the clinic or hospital with gastroenteritis with or without diarrhea. All patients were invited to participate in the study and those who agreed and sign a consent form were requested to provide a stool sample. A total of 516 stool samples were collected between March and September 2016 from patients attending primary health care centers at Nkomo and Makhuva clinics in Giyani, Limpopo province and Pretoria, Gauteng province and labeled with the patient's code, sex, age and the collection date. The samples were taken to the University of Venda laboratory and South African Medical Research Council (SAMRC) Diarrhoeal Pathogens Research Unit in cooler boxes with ice within 4 h of collection for analysis.

2.4. Detection of *Giardia lamblia* in stool samples by ELISA

All stool samples were tested for *Giardia lamblia* using a commercial Enzyme linked immunosorbent assay (ELISA) kit manufactured by TECHLAB (Blacksburg, VA 24060, U.S only, 1-800-TECHLAB) following manufacturer's instructions. The optical densities (OD) were read at 450 nm with a Spectronic ELISA reader (Spectra Max 340, Molecular Devices Co., Sunnyvale, CA). Any sample with an OD >0.150 was considered positive.

2.5. DNA extraction and molecular detection of *Giardia lamblia* by PCR

Genomic DNA was purified from all stool samples using Zymo research (quick miniprep) DNA kit (Inqaba, South Africa). The extraction procedure was performed following the manufacturer's instruction. The DNA samples were kept frozen at -20°C until needed for further analysis. The initial PCR was performed using specific primers to amplify the *tpi* gene of *Giardia lamblia* (Amar et al., 2002). Primer set TPIA Fi and TPIAR with product size 576 bp was used to amplify *tpi* genotype A, while primer set TPIB Fi and TPIBR with product size of 208 bp was used to amplify *tpi* genotype B. The amplification was performed in 25 μL PCR reaction mixture containing 0.2 μM of each primer, 0.2 μL Taq DNA polymerase, 1.5 mM MgCl_2 , 0.5 μL BSA, 2.5 μL of PCR buffer. For cycling condition all reactions involved an initial denaturation step at 94°C for 30 s, 52°C for 30 s, 72°C for 1 min and final extension of 72°C for 10 min. A nested PCR was performed using initial PCR products (3 μL) as template, primers TPIA-Fi and TPIA-R which amplify 476 bp fragments of genotype A and primers TPIB-Fi and TPIB-R were used to amplify 140 bp fragments of genotype B.

2.6. Statistical analysis

The analysis was conducted using the statistical package for social sciences (SPSS) program, version 22.0 with the fisher chi square test and the difference between two variables was considered significant if the p value was <0.05 .

3. Results

3.1. Prevalence and distribution of *Giardia lamblia* in the study population using ELISA

The highest number of samples were collected from Pretoria 289/516 (56.0%) compared to Giyani 227/516 (44.0%). Females made up 58.1% of the study participant while males constituted (41.8%). The majority of the study participants age ranged between 3 and 20 years followed by 21–45 years with the least number of samples from patients aged between 46 and 72 years. In terms of stool type, more loose stool samples 311/516 (60.0%) were collected followed by formed 33.5% and watery stool 6.2% (Table 1). ELISA results revealed 40/516 (7.75%) positive samples. A statistically significant correlation was seen between the stool texture/type and *Giardia* infection ($\chi^2 = 10.533$; $p = .005$). The prevalence of *Giardia* infection was highest in watery stool samples 18.8% while loose and formed stool samples had a lower prevalence of 9.0% and 3.5%, respectively. Furthermore, a significant association was noted between the origin of samples ($\chi^2 = 9.725$; $p = .002$). Prevalence was highest in Giyani 11.9% compared to Pretoria 4.5%. No significant correlation between age and gender was noted. Regarding the age groups, most people who were infected were between 3 and 20 years, followed by age group 21–45 years (8.8%) whereas the age group 46–72 years were not infected by *Giardia* infection. However, the differences were not significant ($p = .162$). (Table 1).

3.2. Impact of gender and stool type on *Giardia* infection

The occurrence of *Giardia* infection was high in watery stool samples (23.5%) from females than males (13.3%), whereas loose and formed stool samples in female had lower prevalence but higher in males. *G. lamblia* was significantly associated with watery stool types in females $p = .008$ (Table 2). Higher prevalence was found in females of age group between 3 and 20 years than males. There was no difference from the male group aged between 3 and 20 years and 21–45 years and female age group of 21–45 had lower prevalence of *Giardia* infection of 8.3%. Both male and female of age group 46–72 years showed no infection of *Giardia*. The differences from the different age groups and sexes were not statistically significant (Table 2).

3.3. Distribution of *Giardia lamblia* in relation to place of origin, stool type and gender

The prevalence of *Giardia* infection was higher in watery stool samples (71.4%) in Giyani region followed by loose stool samples (12.1%) and the least from formed stool samples (5.6%). There was a statistically significant difference in the distribution of the pathogen in the stool type in Giyani ($p = .001$) whereas in Pretoria, high prevalence was found in loose stool samples (6.2%) followed by watery and formed with 4.0% and 2.0%, respectively. Overall, the distribution was statistically significant in the stool type collected for the study ($p = .005$) (Table 3). Males from Giyani were more infected with the prevalence of 12.6% than males from Pretoria (4.7%) and also females from Giyani were more infected (10.9%) than females from Pretoria (4.3%) (Table 3).

3.4. Genotype distribution and classification of *Giardia* infection in relation with host gender, stool type and place of origin

Of all the samples tested by PCR, *Giardia lamblia* assemblages comprising A and B were detected using type specific primers. It was found that 21.0% had single infection while 4.2% had mixed infections. Assemblage B was found to be most common compared to assemblage A (Table 4). The relationship of both assemblages A and B to host gender, stool type and place of origin revealed that assemblage B was more common in Giyani compared to Pretoria. On the other hand, no assemblage A was found

Table 1
Prevalence and distribution of *Giardia* detected using ELISA.

Characteristics		Positive for <i>Giardia</i> ELISA (%)	Total (%)	P-value
Gender	Male	17 (7.9)	216	$\chi^2 = 0.066$; $p = .797$
	Female	23 (7.6)	300	
Stool type	Formed	6 (3.5)	173 (33.53)	$\chi^2 = 10.533$; $p = .005$
	Loose	28 (9)	311 (60.27)	
	Watery	6 (18.8)	32 (6.20)	
Age groups	3–20 years	26 (13.0)	200 (38.75)	$\chi^2 = 3.644$; $p = .162$
	21–45 years	14 (8.8)	159 (30.81)	
	46–72 years	0.0 (0)	157 (30.42)	
Origin	Giyani	27(11.9)	227(44.0)	$\chi^2 = 9.725$; $p = .002$
	Pretoria	13(4.5)	289(56.0)	
Total		40(7.75)	516	

Table 2
Impact of gender, age group and stool type on *Giardia* infection.

Gender	Age group	Positive for <i>Giardia</i> ELISA (%)	Total	P-values
Male	3 to 20 years	12(12.63)	95	$\chi^2 = 1.292; p = .524$
	21 to 45 years	5(9.8)	51	
	46 to 72 years	0(0)	72	
	Total	17(7.9)	216	
Female	3 to 20 years	14(13.3)	105	$\chi^2 = 1.777; p = .411$
	21 to 45 years	9(8.3)	108	
	46 to 72 years	0(0)	85	
	Total	23(7.6)	300	

Gender	Stool type	Positive for <i>Giardia</i> ELISA	Total	P-values
Male	Formed	3(4.28)	70	$\chi^2 = 2.202; p = .332$
	Loose	12(9.23)	130	
	Watery	2(12.5)	16	
	Total	17(7.9)	216	
Female	Formed	3(2.9)	102	$\chi^2 = 9.700; p = .008$
	Loose	15(8.3)	181	
	Watery	4(23.5)	17	
	Total	22(7.3)	300	

circulating in Pretoria communities. It also showed that more male patients were infected by assemblage B than female patients. Assemblage B was less common in watery stool samples and high in loose and formed samples (Table 4).

4. Discussion

Giardia lamblia has been regarded as a serious public health problem as it can cause iron deficiency anemia, growth retardation in children and other physical and mental health problem. It has been identified as one of the most common cause of gastrointestinal illness in both human and animals (Ayele-Kumi et al., 2009). Prevalence of *G. lamblia* is said to be higher in areas of poor sanitation, insufficient water treatment, day-care centers and in institutions with children who are not toilet trained (Al-Saeed and Issa, 2006).

Prevalence may vary among countries probably due to differences in the management, weather and type of study conducted to diagnose the infections (Veronesi et al., 2010). Different prevalence rates have been described throughout the world. In the present study, *G. lamblia* was detected 7.75% of the study population using ELISA. Similar results in the Vhembe district, South Africa revealed the prevalence of *Giardia* at a prevalence rate of between 1 and 30% however, the study was restricted to children (Samie et al., 2009).

Table 3
Distribution of *Giardia lamblia* in relation to place of origin, stool type and gender.

Origin	Stool type	Positive for <i>Giardia</i> ELISA	Total	P-value
Giyani	Formed	4(5.6)	71	$\chi^2 = 26.335; p = .000$
	Loose	18(12.1)	149	
	Watery	5(71.4)	7	
	Total	27(11.9)	227	
Pretoria	Formed	2(2.0)	102	$\chi^2 = 2.601; p = .272$
	Loose	10(6.2)	162	
	Watery	1(4.0)	25	
	Total	13(4.5)	289	
Total	Formed	6(3.5)	173	$\chi^2 = 10.533; p = .005$
	Loose	28(9.0)	311	
	Watery	6(18.8)	32	
	Total	40(7.8)	516	

Origin	Gender	Positive for <i>Giardia</i> ELISA	Total	P-value
Giyani	Male	11(12.35)	89	$\chi^2 = 0.164; p = .685$
	Female	15(10.9)	138	
	Total	26(11.6)	225	
Pretoria	Male	6(4.7)	127	$\chi^2 = 0.027; p = .870$
	Female	7(4.3)	162	
	Total	13(4.5)	289	
Total	Male	17(7.9)	216	$\chi^2 = 0.066; p = .797$
	Female	22(7.3)	300	
	Total	39(7.6)	516	

Table 4

Genotype classification in relation with host gender, stool type and place of origin.

Characteristics		Assemblage B		Assemblage A	
		Frequency	Percentage	Frequency	Percentage
Gender	Male	43	19.9%	6	2.7%
	Female	49	16.3%	3	1%
	Total	92	17.8%	9	1.7%
Stool type	Formed	22	4.2%	4	0.7%
	Loose	63	12.2%	4	0.7%
	Watery	8	1.5%	1	0.1%
	Total	93	18.02%	9	1.7%
Origin	Giyani	78	34.4%	9	3.9%
	Pretoria	14	4.8%	0	0%
	Total	92	17.8%	9	3.9%

The occurrence and prevalence of *Giardia* infection varies with age. In a study carried out among indigenous communities in rural parts of Malaysia, Choy et al. (2014) explained that giardiasis affects persons in all age groups, the number of reported cases was highest among children aged ≥ 12 years. Data for younger age groups are consistent with reports published previously, documenting higher rates of giardiasis among younger children. In the present study, it was observed that people who fall between the age group of 3–20 years were mostly infected followed by the age group between 21 and 45 years. This may be due to the increasing activities in younger people, for example, being at school and playgrounds and also the lack of personal hygiene. These findings are in accordance with previous studies which reported that the highest risk was seen in children than in adults (Younas et al., 2008).

The clinical manifestation of *G. lamblia* infection ranges from asymptomatic to symptomatic, among which diarrhea is one of the common symptoms. Diarrhea is among the major life-threatening infectious diseases and one of the major preventable gastrointestinal health problems. Risk factors for *Giardia* include poor personal hygiene, environmental sanitation problems, unhygienic food preparation and improper sewage disposal (Younas et al., 2008; Ekdahl and Andersson, 2005; Addy et al., 2004). The present study revealed a higher prevalence of *Giardia* infection in watery stool samples (18.8%) compared to loose (9%) and formed (3.5%) stool samples. These findings are in line with the ones reported in the Vhembe district by Samie et al. (2009) where a prevalence of 12.8% was obtained in diarrheal samples obtained from the primary school children and the difference was highly significant ($p = .005$). The high prevalence of *Giardia* infection found in watery stool samples in this study may be due to lack of personal hygiene and environmental sanitation.

In Giyani area a high prevalence was found in watery stool samples (71.4%), whilst in Pretoria a high prevalence was found in loose stool samples (5.6%), the difference was statistically significant ($p = .001$). Watery diarrheal stool samples are major symptom of *Giardia* infections, therefore, these results show that most patients from Giyani had symptomatic infections while patients from Pretoria had asymptomatic infections. These results support the fact that *Giardia* infection may be symptomatic or asymptomatic. However, it still remains unclear as to why only a subset of individuals develops symptomatic diseases while others do not. Literature explains that in developed countries *Giardia* is referred to as a re-emerging infectious agent because of its increasing role in outbreaks of diarrhea in day – care centers and water and foodborne outbreaks affecting general population. However, in developing countries approximately 200 million people per year experience symptomatic giardiasis (Thompson et al., 2000).

In the present study, there was no notable difference between male and female patients. These results are different from other studies worldwide which indicated that male patients are mostly infected mainly due to their higher activity and more contact with the outdoors environment compared to females (Hill et al., 2005). A non-significant statistical difference was obtained when comparing the place of origin and gender. In Giyani the prevalence was high in males than females whereas in Pretoria no difference between males and females was observed ($p = .685$). These results are in line with other previous studies showing a high prevalence of *Giardia* infection in males than females (El-Ammari and Nair, 2003).

The prevalence of *Giardia* has been estimated at 2 to 5% in industrialized areas and 20–30% in developing areas (Hill et al., 2005; Wongjindanon et al., 2005). Patients from the rural areas of Giyani were more infected by *Giardia* (11.9%) compared to those who are from urbanized areas in Pretoria ($P = .002$). This is generally the case because people who reside in rural or under developed areas are more prone to the ingestion of infective parasites as they are either not well informed or due to circumstances of poor environmental sanitation and hygiene, as compared to those who live in urban/suburban or well developed areas where sanitation is presumably better, hence possess a lower chance of infection. Water supplied in urbanized areas is obviously cleaner because there is correct monitoring as it goes through treatment processes, which in turn reduces the chance of contamination. However, in rural areas the nature of everyday activities brings people, especially children, into close contact with natural sources of soil and water, therefore increasing their risk of ingestion as well as the penetration of infective stage parasites (Addy et al., 2004).

After the stools were confirmed to contain *Giardia* antigen by ELISA method, the PCR assays was performed for *Giardia* antigen diagnosis. *Giardia lamblia* was detected in 7.2% of DNA samples. The PCR based method in this study detected 21.0% of *Giardia* infection as compared to ELISA method. In this study, it was observed that the molecular analysis was more discriminatory than the ELISA assay. Most samples which were identified as positive by ELISA assay were found to be negative when PCR was

used, as *Giardia* DNA was not detected. Similarly, most samples which were identified as negative by ELISA were found to be positive by PCR. Several reports in the past had supported that molecular identification is usually more sensitive and specific than ELISA (Amar et al., 2002; Pestehchian et al., 2012). The findings of this study are in agreement with most of the studies conducted worldwide, which indicates that PCR is still a leading technique in detection of microbial pathogens causing disease in human (Verweij et al., 2004; Abe et al., 2005; Alyousefi et al., 2013; Anuar et al., 2014).

A study by Roointan et al. (2012) in Ahfaz, Southwest of Iran, indicated that 86% (50/58) microscopic positive samples with low numbers of cysts were negative after semi nested PCR; in a study conducted by Amar et al. (2002) the *tpi* gene was amplified by semi nested PCR and the study also indicated a 6% of false negative results. Although there is no clear explanation for such false negative results. Değeri et al. (2012) explained that DNA obtained from trophozoites rather than cysts can be amplified successfully and used in the molecular diagnosis of *Giardia* and the reason for the failure of observed cyst DNA amplification for the relatively longer or shorter DNA segment is unclear. The failure in the amplification would derive from low quality of DNA of the samples, either due to their degrading in time or because of chemical modification caused by several substance, formalin among them (Dowd et al., 1998). Furthermore, these failures could be the result of the fact that the proportion of DNA in the feces samples was not enough to counteract the effect of inhibitors that would have co-purified with nucleic acids or the presence of local genotypic variants of the *tpi* gene not detected with the primers used. However, the failure of DNA amplification in the present study in which only ELISA method was used as a screening method is unclear.

A previous study Ryan and Cacciò (2013) indicates that assemblage B has a higher prevalence than assemblage A in different region of the world (Europe, Africa, America, Asia, Australia, and Oceania). This proportion does not change when data either from developed or from developing countries are analyzed, although the prevalence of mixed infections is higher in the latter (5.2%) than former (3.2%). Our observation showed that the majority of giardiasis isolates were assemblage B (21.8%) This corresponds to the findings of several studies conducted in Australia, Canada, Bangladesh and Argentina (Read et al., 2004; Guy et al., 2004; Ng et al., 2005; Minvielle et al., 2008). A limited number of mixed assemblage infections have also been reported (El-Shazly et al., 2004; Haque et al., 2005; ElBakri et al., 2014). The occurrence of mixed infections has been reported in molecular-based surveys performed in Australia, United Kingdom, India and Italy (Amar et al., 2002; Traub et al., 2004; Lalle et al., 2005). The percentage of mixed infection ranged from 2.0% to 21.0% and was higher in less economically developed countries. The rate of mixed infection in Bangladesh, India, Nepal and Egypt was reported to be 5.9%, 5.9, 5.7%, and 5%, respectively. These results are in line with those in our study where a 4.2% rate of infection was observed. The occurrence of mixed infection by several assemblages of *G. lamblia* reflects the complex circulation of the parasite in the environment and the exposure of humans to multiple sources. Understanding the epidemiology of *G. lamblia* requires an insight of the relative roles of anthroponotic, zoonotic and environmental transmission (Smith et al., 2006).

The correlation between clinical symptoms of *Giardia* and assemblages is controversial. Few studies have found a connection between symptomatic infection with assemblage B and asymptomatic infection with assemblage A (Read et al., 2004; Aydin et al., 2004; Al-Mohammed, 2011) while some reports describe severe, actual persistent diarrhea with assemblage B and a strong correlation of the mild, intermittent type of diarrhea with *Giardia* assemblage A (Homan and Mank, 2001). In the present work, the study population was grouped into diarrheal and non-diarrheal groups. A correlation between the presence of symptomatic sign/diarrhea and infection with assemblage A was observed. These findings are in agreement with the results reported by Read et al. (2002) in children in Australia and in the case-control study in Bangladesh (Haque et al., 2005), yet contrasting other study of general population survey in the Netherlands (Homan and Mank, 2001). Our study showed that *Giardia* is an important cause of diarrhea in the concerned community with people in rural areas more at risk compared to those in urban areas. The prevalence was higher among young individuals thus health education campaigns should target young age groups. Assemblage B was found to be common genotype circulating in the study populations whereas assemblage A was the cause of diarrhea in urban area (Pretoria).

Declaration of competing interest

The authors declare no conflict of interest.

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