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Giardia duodenalis infection among rural communities in Yemen: A community-based assessment of the prevalence and associated risk factors

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ABSTRACT

Objective: To investigate the prevalence and risk factors of *Giardia* infection among rural communities among rural communities in Yemen.

Methods: In this cross-sectional study, a total of 605 stool samples were collected and screened for the presence of *Giardia duodenalis* (*G. duodenalis*) cysts and/or trophozoites by using three different diagnostic methods: direct smear, formalin-ether sedimentation, and trichrome staining. A pre-tested questionnaire was used to collect information on the demographic, socioeconomic, behavioural and environmental characteristics of the participants.

Results: Overall, 28.1% (170/605) of the participants were infected by *G. duodenalis*. The prevalence was significantly higher among male participants compared to female (P = 0.034); however, it was not significant among different age groups (P > 0.05). Univariate and multivariate analyses identified four variables as the significant key risk factors of *Giardia* infection among the sampled communities. These are, in addition to being of the male gender, using unsafe water sources for drinking water, not washing hands after defecation, presence of other family members infected with *Giardia*, and close contact with domestic animals.

Conclusions: The study reveals that *Giardia* infection is still prevalent among rural communities in Yemen. The provision of clean and safe drinking water, proper sanitation, and health education regarding personal hygiene practices, particularly handwashing, as well as identifying and treating infected family members is imperative and these interventions should be considered in a strategy to control intestinal parasites among these communities in order to curtail the transmission and morbidity caused by *G. duodenalis*.

1. Introduction

Giardia duodenalis (also known as *Giardia lamblia* and *Giardia intestinalis*), a flagellate binucleated protozoan parasite, is probably among the most frequently reported gastrointestinal parasites worldwide. Globally, recent estimates have revealed that about 200 million cases of *Giardia*-related diarrhoea are reported every year, with infants, young children, and young adults being the groups most at risk of infection [1]. Due to its high prevalence and significant impact on child health, *Giardia* infection (also known as giardiasis) was included in the WHO's neglected diseases initiative in 2004 [2]. In developing countries, the prevalence of *Giardia* infection ranges between 10% and 50% [3,4]. In the developed world, the prevalence ranges between 0.1% and 5%, with many waterborne outbreaks reported in countries such as the USA, Canada, the UK, and New Zealand [5–9]. Moreover, *Giardia* infection is commonly reported among travellers and children attending day-care centres [10–12].

Giardiasis is transmitted by ingesting mature cysts in contaminated food or water and person-to-person transmissions

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may also occur through direct faecal-oral contact among family members living in the same household with *Giardia*-infected siblings [13,14] and among homosexual men [15]. Once inside the small lumen, excystation occurs to produce trophozoites that multiply by binary fission. These trophozoites attach to the brush border surface of the upper part of the small intestine, which results in the shortening of the epithelial microvilli (villus atrophy) and causes malabsorption of glucose, sodium and water, and reduced disaccharidase activity [16]. Infections with *Giardia* may remain asymptomatic or cause acute diarrhoea that tends to be self-limiting in immunocompetent individuals or develops into chronic giardiasis, particularly among children and immunocompromised individuals [3,17]. In addition, a range of extra-intestinal consequences of chronic *Giardia* infection have been reported by many previous studies [3]. These include ocular pathologies [18], arthritis [19], allergies [20], protein-energy malnutrition or long-term growth retardation [21–23], and impaired cognitive function [24].

Yemen is one of the poorest countries in the world, with more than 50% of the total population (26 million people) living under the national poverty line [25]. Moreover, the country is suffering severe water depletion and only 25% of the population have access to primary healthcare services, sanitation, and safe drinking water [26,27]. In addition, the current civil war that started in March 2015 has worsened the situation; over 2 million people have been internally displaced, the prevalence of infectious diseases has increased, about 80% of the population are in need of humanitarian aid, and there are about half a million children in Yemen suffering severe malnutrition and 3.3 million children and pregnant or lactating women suffering from acute malnutrition [28].

Several epidemiological studies have been carried out in Yemen to investigate the prevalence of intestinal parasitic infections [29–37], with only one [35] focussing on the prevalence and distribution of *Giardia* infection. Nevertheless, information on the risk factors of *Giardia* infection among Yemeni communities is lacking. Moreover, there is a scarcity of information on the prevalence of the infection among the adult population as the majority of previous studies concentrated on children. Hence, this study aims to determine the prevalence of *G. duodenalis* infection and to investigate its risk factors among all age groups in the rural communities in Yemen.

2. Materials and methods

2.1. Ethical declaration

The protocol of the study was approved by the Medical Ethics Committee of the University of Malaya Medical Centre, Kuala Lumpur. In Yemen, the study was approved by the Hodeidah University and health offices in the respective governorates. Before starting data collection, important information on the aims and procedures of the study was provided to the targeted populations. They were invited to take part in the study and were informed that their participation was voluntary and that they could withdraw from the study at any time without notice or giving any reason whatsoever. Then, written and signed or thumb-printed informed consents were obtained from adult individuals regarding their own participation. For children, the consents were collected from their parents/guardians, a procedure that was also approved by the ethics committee.

2.2. Study design

Community-based cross-sectional surveys were conducted in rural communities in Yemen between January and July 2012 and between February and April 2014. The study was designed to assess the burden of intestinal parasitic infections as well as urogenital and intestinal schistosomiasis in different governorates of Yemen. The study involved house-to-house questionnaire surveys as well as collection of faecal samples. In each governorate, four villages were selected randomly in collaboration with health officers from the governorate/district health offices. The number of individuals per household was recorded and all of them were invited to take part in the study after being informed about the study objectives and procedures. Unique reference codes were assigned to each household and each participant was given an identity code accordingly.

2.3. Study area

The study was carried out in four governorates in Yemen: Taiz, Hodeidah, Sana'a, and Dhamar. In each governorate, two rural districts were randomly selected from the available official district lists, and then two villages were randomly selected from the provided lists according to the following inclusion criteria: located in a rural area, more than 50 households or ≥300 inhabitants, and there have been no threats of war or kidnapping in the district and village.

Taiz governorate (44.01°E, 13.41°N) is located 256 km south of Sana'a City (the capital) and covers an area of 10 677 km^2 at an altitude of about 1 400 m above sea level. It is the most heavily populated governorate with a total population of about three million, representing 12.2% of the country's population, and it has the richest farmland in the country [38]. It has a diverse geography from coastal areas on the Red Sea to mountainous areas in the interior. Hodeidah governorate (43.15°E, 14.46°N) is located 226 km east of Sana'a City and is a coastal area of 17 509 km^2 along the Red Sea with a total population of about 2.5 million [38]. The climate of Taiz and Hodeidah is characterized by occasional rains in summer and dry weather in winter and two rainy seasons (February–April and July–September) with a mean rainfall of 200 mm/year and an average temperature of 29.5 °C. There are many valleys in both governorates and streams are considered the main source of water for both drinking and domestic purposes.

On the other hand, Sana'a governorate (44.12°E, 15.21°N) is a highland area located in the central part of Yemen of 15 052 km^2 with a total population of 1.4 million [38]. Dhamar governorate (43.56°E, 15.40°N) is located 90 km to the south of Sana'a City, covers an area of 9 495 km^2, and has a total population of 1.6 million [38]. At altitudes of more than 2 000 m above sea level, both Sana'a and Dhamar are considered the highlands of Yemen. The climate in these highlands is moderate in summer and dry and cold in winter, with average annual temperature of 22 °C. The average annual rainfall ranges between 400 and 800 mm/year and numerous dams and traditional small-to-medium uncovered cisterns have been constructed to collect water for drinking and domestic purposes. Moreover, a huge number of artesian wells are being drilled illegally into natural groundwater aquifers that are scattered across these two governorates and this is contributing to a severe water depletion crisis in Yemen. Furthermore, since 2010, Yemen has been devastated by a civil war that has had devastating humanitarian consequences and shattered the economy.

2.4. Sample size and study population

The minimum sample size required for the current study was estimated according to the formula provided by Lwanga and Lemeshow [39]. Based on a 5% level of significance, a 95% confidence level, and design effect of 2, the minimum sample size was estimated as 576 for the main study on intestinal parasitic infections and schistosomiasis. This number was also judged applicable for the current study based on the assumption that the average prevalence of *Giardia* infection in rural Yemen stands at 30%.

Overall, 800 eligible individuals were invited to participate in the current study. Proper containers were distributed to these participants so that they could provide a faecal sample. Altogether, 605 individuals delivered stool samples for examination and were involved in the study while 195 subjects (mostly adult females) were excluded from the study as they did not deliver the samples.

Six hundred and five participants (387 males, 218 females) aged between 1 and 80 years with mean age of 11 years (IQR = 8–18) were recruited for this study. The general demographic and socioeconomic characteristics of these participants are presented in Table 1. Generally, poverty prevails in the communities under study. About 2/3 (68.4%) of the participants had a low monthly family income (<YER20 000 equivalent to US$93) and 77.5% of them were not working. Moreover, illiteracy is very high as 77.7% of the participants, particularly females were non-educated. Most of the houses were traditionally constructed of stone and mud with mud-type floor while some were built of bricks and concrete with cement floor. At the studied communities, different kinds of domestic animals were observed including cows, sheep, goats, donkeys, camels, dogs, cats, and chickens in barns and sheds within the immediate vicinity of the house or inside the house in some communities. In general, housing conditions in terms of home appliances and furniture were very poor in all the studied communities. Moreover, the majority of the houses (74.0%) had no access to a safe piped water supply and 74.9% had no electricity while almost half of the houses had no toilet facilities.

Table 1

General demographic and socioeconomic characteristics of the participants (n = 605).

Characteristics	n (%)
Age (years)	11 (8, 18)[a]
Age groups (years)	
<5	28 (4.6)
5–10	246 (40.7)
11–17	158 (26.1)
18–40	136 (22.5)
≥41	37 (6.1)
Gender	
Males	387 (64.0)
Females	218 (36.0)
Address (governorate)	
Taiz	159 (26.3)
Hodeidah	147 (24.3)
Sana'a	149 (24.6)
Dhamar	150 (24.8)
Socioeconomic status	
Non-educated	470 (77.7)
Not working	469 (77.5)
Low family monthly income (<YER 20 000)	414 (68.4)
Large family size (>7 members)	317 (52.4)
Absence of safe water supply	448 (74.0)
Absence of toilet facilities in house	304 (50.2)
Absence of electricity supply	459 (75.9)
Presence of domestic animals	402 (66.4)

[a] Median (interquartile range). All values are number (%). YER, Yemen Rial; (US$1 = YER216).

2.5. Stool examination

A 60-mL clean, labelled, wide-mouth, screw-cap container was distributed to each participant to enable them to provide a faecal sample at the time of the visit of the research team member to their household or the next morning when the research team member returned. The samples were collected and transported within 5 h in suitable cool boxes (4–6 °C) to the nearest health centre for examination according to prior arrangements. The samples were processed immediately by direct smear for the detection of cysts and/or trophozoites of *Giardia*. Then, each sample was divided into three portions. The first portion was processed at the immediate stations by using the formalin–ether sedimentation technique according to Cheesbrough [40] and examined for the presence of *Giardia* cysts as well as the diagnostic stages of other intestinal parasites. The second portion (about 5 g) was mixed thoroughly into 3 mL of polyvinyl alcohol (PVA) in a 15-mL screw-cap centrifuge tube for further examination by trichrome staining according to the WHO procedure [41]. The third portion (about 1 g) was preserved in 70% ethanol (DNA-friendly) for genomic DNA extraction and molecular analysis for other intestinal parasites of interest [42].

For quality control, duplicate slides were prepared from 20% of the samples, which were selected randomly for each diagnostic technique, and the slides were re-examined by another microscopist to confirm the results. A sample was recorded as positive if cysts and/or trophozoites of *Giardia* were detected by any of the three techniques, *i.e.*, direct smear, formalin-ether sedimentation, or trichrome staining.

2.6. Data analysis

Data was collected and entered into an Excel data spreadsheet. Then, the data was reviewed and double-checked by two different researchers before and after a single data set was created using the SPSS data template. Only the participants who had a full data record including faecal examination results by the different methods, biodata and complete questionnaire were considered in the data analysis. Data analysis was performed by using IBM SPSS Statistics, version 20 (IBM Corporation, NY, USA). In the descriptive analysis, the prevalence of *Giardia* infection was expressed as a percentage while the median (interquartile range; IQR) was used to present the age of the participants, the only quantitative data. The association of *Giardia* infection as the dependent variable with some demographic, socioeconomic, behavioural, and environmental factors as the independent variables was assessed by Pearson's Chi Square test. The variables were coded as binary dummy variables (*i.e.*, 0 and 1). For example, *Giardia* infection (positive = 1, negative = 0); gender (male = 1, female = 0); age group (≤12 = 1, >12 = 0); washing vegetables before consumption (no = 1, yes = 0); and family size (>7 members = 1, ≤7 members = 0). Age was categorized into two groups (≤12 and > 12) and family size (number of people living in the same household) was categorized into two groups (>7 and ≤ 7 members) in line with previous studies conducted in Yemen [34,43].

In order to identify the risk factors of *Giardia* infection, multiple logistic regression analysis was applied to all the variables that showed significant associations in the univariate analysis. The odds ratios (*OR*) and the corresponding 95% confidence intervals (*CI*) were calculated by using univariate and multiple logistic regression. In addition, the population attributable risk fraction (PARF) was calculated to quantify the contribution of each of the identified risk factors [44]. A *P* value of <0.05 was considered as the level of statistical significance for all tests.

3. Results

3.1. Prevalence and distribution of Giardia infection

Out of the 605 participants, 28.1% (170/605) were found positive for *Giardia* infection. The prevalence of infection was significantly higher among male participants than female (31.0% vs 22.9%; $\chi^2 = 6.712$; $P = 0.034$). On the other hand, the prevalence was the highest among young children aged 5 years and below (33.3%) while the lowest prevalence was among those aged over 40 years (13.5%); however, the difference was not statistically significant among the different age groups ($\chi^2 = 4.749$; $P = 0.314$). With regards to the governorates, the prevalence of infection was the highest among the participants from Hodeidah (34.0%) followed by those from Sana'a (29.5%) while the participants from Taiz had the lowest prevalence rate (22.0%). However, the differences between governorates were not statistically significant ($\chi^2 = 5.655$; $P = 0.130$). Table 2 shows the prevalence of *Giardia* infection according to age, gender, and geographical location.

3.2. Risk factors of Giardia infection

Table 3 shows the associations between the prevalence of *Giardia* infection and some demographic, socioeconomic, behavioural, and environmental factors. The results revealed a significant association between *Giardia* infection and gender with males having a significantly higher prevalence rate compared to females (31.0% *vs.* 22.9%; $P = 0.034$). Interestingly, the prevalence was significantly higher among participants who had other family members infected with *Giardia* compared to their peers ($P = 0.001$). However, those who lived in houses with more than seven family members had a lower prevalence compared to those from smaller families with more than seven members (24.3% *vs.* 32.3%; $P = 0.029$). Moreover, the participants who used unsafe sources such as rain, wells and streams for drinking water had a significantly higher prevalence of *Giardia* infection compared to those who used safe piped water (34.2% *vs.* 10.8%; $P < 0.001$). Similarly, there was a significantly higher prevalence among those who lived in houses without functioning toilets (32.6% *vs.* 23.6%; $P = 0.014$) and those who did not wash their hands after defecation (41.5% *vs.* 19.9%; $P < 0.001$) when compared to those who lived in houses with proper toilet facilities and those who always washed their hands after defecation. In addition, *Giardia* infection was found to be significantly associated with monthly family income and the presence of domestic animals with higher prevalence rates among those who had a monthly income below YER20 000 (31.2% *vs.* 21.5%; $P = 0.014$) and those who had close contact with domestic animals (30.8% *vs.* 22.7%; $P = 0.034$) compared to their counterparts.

Table 4 shows the results of the multivariate analysis for significant risk factors that were retained in the multiple logistic regression analysis. The Hosmer–Lemeshow test, which was used for the inferential goodness-of-fit test, showed that the model fitted to the data well ($\chi^2 = 5.903$; $P = 0.658$). The results showed that using unsafe sources for drinking water and not washing hands after defecation increased the individual's odds of having *Giardia* infection by 3.45 and 3.41 times, respectively. Similarly, participants who lived with other family members infected with *Giardia* and those who had close contact with domestic animals were found to have 2 times higher the odds of having a *Giardia* infection when compared to their counterparts. Moreover, males had 1.92 times (95% *CI* = 1.15, 3.20) the odds of having a *Giardia* infection compared to females.

Interestingly, the PARF analysis showed that the prevalence of *Giardia* infection among the study population could be reduced by 61.5% if they had safe drinking water. Moreover, 29.0% of the cases could be prevented if the participants washed their hands after defecation. Furthermore, 19.4%, 18.4%, and 17.6% of the cases could be avoided by focussing control and

Table 2

Prevalence and distribution of *Giardia* infection among the participants according to age, gender and location ($n = 605$).

Variables	No. examined	No. *Giardia*-positive	Prevalence (%)	χ^2 (*P*)
Age groups (years)				4.749 (0.314)
≤5	48	16	33.3	
6–10	226	63	27.9	
11–17	158	46	29.1	
18–40	136	40	29.4	
≥41	37	5	13.5	
Gender				4.497 (0.034)[a]
Male	387	120	31.0	
Female	218	50	22.9	
Location				5.655 (0.130)
Taiz	159	35	22.0	
Hodeidah	147	50	34.0	
Sana'a	149	44	29.5	
Dhamar	150	41	27.3	
Total	605	170	28.1	

All values are number (%). χ^2 Chi-square test statistic. [a] Significant difference between the groups ($P < 0.05$).

Table 3

Univariate analysis of factors associated with *Giardia* infection among participants in rural Yemen (n = 605).

Variables	*Giardia* infection		*OR* (95% *CI*)	*P*-value
	No. examined	Infected (%)		
Age (years)				
<12	320	28.4	1.04 (0.73–1.48)	0.844
≥12	285	27.7	1.00	
Gender				
Male	387	31.0	1.51 (1.03–2.21)	0.034*
Female	218	22.9	1.00	
Location				
Coastal/foothill areas	306	27.8	0.97 (0.68–1.38)	0.859
Highlands	299	28.4	1.00	
Education level				
Non educated	470	28.5	1.10 (0.71–1.69)	0.674
Educated	135	26.7	1.00	
Occupational status				
Not working	469	29.2	1.29 (0.83–2.00)	0.259
Working	136	24.3	1.00	
Family size				
>7	317	24.3	0.67 (0.47–0.96)	0.029*
≤7	288	32.3	1.00	
Family monthly income				
<YER20 000	414	31.2	1.66 (1.11–2.48)	0.014*
≥YER20 000	191	21.5	1.00	
Presence of other family members infected with *Giardia*				
Yes	251	35.1	1.79 (1.25–2.56)	0.001*
No	354	23.2	1.00	
Source of drinking water				
Unsafe (well, streams, rain, dams)	448	34.2	4.27 (2.49–7.33)	<0.001*
Safe (piped water)	157	10.8	1.00	
Presence of toilet in house				
No	304	32.6	1.56 (1.09–2.24)	0.014*
Yes	301	23.6	1.00	
Washing hands before eating				
No	244	27.0	0.92 (0.64–1.32)	0.637
Yes	361	28.8	1.00	
Washing hands after playing with soil or gardening				
No	254	28.3	1.02 (0.71–1.46)	0.908
Yes	351	27.9	1.00	
Washing hands after defecation				
No	229	41.5	2.85 (1.98–4.10)	<0.001*
Yes	376	19.9	1.00	
Cutting nails periodically				
No	352	30.7	1.36 (0.95–1.97)	0.096
Yes	253	24.5	1.00	
Close contact with domestic animals				
Yes	402	30.8	1.52 (1.03–2.25)	0.034*
No	203	22.7	1.00	
Indiscriminate defecation				
Yes	332	26.5	0.84 (0.59–1.20)	0.336
No	273	30.0	1.00	
Washing vegetables before consumption				
No	212	27.8	0.98 (0.68–1.42)	0.914
Yes	393	28.2	1.00	
Washing fruits before consumption				
No	182	25.3	0.82 (0.55–1.21)	0.311
Yes	423	29.3	1.00	
Wearing shoes when go outside				
No	192	25.5	0.83 (0.56–1.22)	0.336
Yes	413	29.3	1.00	
Boiling drinking water				
No	554	27.8	0.84 (0.45–1.57)	0.587
Yes	51	31.4	1.00	

YER = Yemen Rial; (US$1 = YER216). Reference group marked as *OR* = 1.00; *OR* = Odds ratio; *CI* = Confidence interval. *Significant association ($P < 0.05$).

Table 4

Multivariate analysis of factors associated with *Giardia* infection among participants in rural Yemen (n = 605).

Variables	*Giardia* infection		
	Adjusted *OR*	95% *CI*	*P*
Gender (male)	1.92	1.15–3.20	0.013*
Family size (>7 members)	0.94	0.59–1.50	0.793
Family monthly income (<YER20 000)	1.10	0.63–1.91	0.749
Presence of other family members infected with *Giardia* (yes)	2.14	1.35–3.38	0.001*
Source of drinking water (unsafe)	3.45	1.85–6.46	<0.001*
Presence of toilet in house (no)	1.38	0.81–2.33	0.236
Washing hands after defecation (no)	3.41	2.15–5.41	<0.001*
Close contact with domestic animals (yes)	2.01	1.21–3.34	0.007*

OR, Odds ratio. *CI*, Confidence interval. *Significant key risk factors (P < 0.05).

preventive efforts on males, those who had domestic animals within the vicinity of the house, and diagnosing and treating all infected family members, respectively.

4. Discussion

The current study showed that the prevalence of *Giardia* infection among the studied rural communities was 28.1%. This prevalence is in accordance with that reported by some previous studies conducted in Yemen; 32.7% among 352 children in Hadhramout governorate [31] and 23.6% among 258 schoolchildren from Ibb governorate [37]. However, the majority of the previous studies reported lower prevalence rates; 16.8% among 292 orphans aged 4–20 years in Sana'a City [32], 16.7% among 2 477 children presenting to the Paediatric Centre in Sana'a City [30], 17.7% among 503 participants aged between 1 and 80 years old in Sana'a City [34], 12.7% among 330 outpatients at different health centres in Taiz City [36], and 5.2% among 500 restaurant workers in Hadhramout [45]. By contrast, a previous study found a prevalence of 52% among schoolchildren from urban areas in Sana'a and Al-Mahweet governorates [29]. Similarly, a prevalence of 35.3% was reported among healthy restaurant workers aged between 12 and 70 years old in Sana'a City [33].

The variation in the prevalence rates could be attributed to the differences in the study populations and study areas, as well as the different diagnostic methods used for stool examination [46]. The current study involved rural participants from a wide geographical areas and stool examination was done by three methods, direct smear, formalin-ether sedimentation (the concentration method of choice for intestinal parasites) and trichrome staining (the gold standard method for intestinal protozoa). Hence, this may explain the higher prevalence rate found by the current study compared to that reported by the majority of previous studies. Moreover, sample collection for the current study was conducted during a period of unrest that affected primary care facilities and all related living and environmental aspects including sanitation, drinking water, and fuel facilities.

Recent studies have reported the presence of *G. duodenalis* in other countries in the Middle East region; in Iraq (45.5%) [47], Egypt (14.8%) [48], Saudi Arabia (11.5%) [49], and Iran (17.5%) [50]. Moreover, a prevalence rate of 21.9% and 2.3% was reported among immigrant workers in Saudi Arabia and Qatar, respectively [51,52]. Interestingly, nested PCR amplification of the small subunit ribosomal RNA gene identified a prevalence rate of 60.4% (67/111) among expatriates residing in Sharjah, United Arab Emirates [53]. Faecal examination for intestinal parasites is a part of a medical check-up that is mandatory for immigrant workers before travelling to Qatar but not to Saudi Arabia or the United Arab Emirates [52].

It is well documented that young children are more prone to *Giardia* infection than other age groups and this could be due to their lower standards of personal hygiene and sanitary behaviours when compared to adults and older children [4,14,54–57]. However, the current study found no significant difference in the prevalence and distribution of infection across different age groups. This finding is in agreement with previous studies on Malaysia, Yemen, and Libya [23,34,36,58]. This may indicate a similar exposure to sources of infection in these communities. With regards to gender, the current study showed that there was a significantly higher prevalence among males compared to females, and this could be attributed to the greater mobility of and the more outdoor activities performed by male children and adults when compared to females in the studied communities. This finding is in accordance with previous studies on Yemen and elsewhere [14,37,59]. By contrast, a higher prevalence of *Giardia* infection among females than males was found in Ethiopia and this was explained by the higher exposure of females to contaminated sources of water compared to males [60].

The current study is the first community-based study to provide information about the risk factors of *Giardia* infection in Yemen. Beside gender (male), the results revealed that the presence of other family members infected with *Giardia*, using unsafe drinking water, not washing hands after defecation, and close contact with domestic animals were the significant key risk factors of *Giardia* infection among the studied population. There is a scarcity of studies that report on the association of the presence of other family members infected with *G. duodenalis* as a risk factor for giardiasis. A previous study among the aboriginal population in Peninsular Malaysia found that the presence of other family members infected with *Giardia* is the only significant risk factor of infection among two Orang Asli tribes while it is among four risk factors for a third tribe [14]. A similar finding was reported by a previous study on the prevalence of intestinal polyparasitism among children in Terengganu, Malaysia [61]. This may indicate a high level of transmission of infection occurring horizontally within the household and infected family members serving as a source of infection. In this situation, *Giardia* parasites are probably transmitted directly through person-to-person contact; however, transmission through food or water prepared by the infected individuals could also be a possibility. A previous study in Auckland, New Zealand revealed that nappy changing (usually by mothers or maids) of young children infected with *Giardia* is associated with a fourfold increased risk of infection [62]. Thus, in *Giardia*-endemic communities, all family members should be screened and treated.

The current study showed that individuals who used unsafe sources for drinking water had 3.45 times higher odds of having *Giardia* infection when compared with those who used piped water supplies. This finding is consistent with previous studies on Yemen and other developing countries as well as some developed countries as waterborne outbreaks of *Giardia* and other intestinal parasitic infections are commonly reported [9,34,36,63]. Yemen, particularly in its rural areas, is suffering severe water depletion and people tend to collect drinking water from uncovered sources such as streams, wells, dams, cisterns and other natural or artificial sources. Interestingly, previous studies on Yemen and Malaysia found a significant association between *Giardia* infection and using piped drinking water [37,55]. However, the association in these cases could be attributed to contaminated hands or contaminated utensils and containers used to store drinking water.

It is well documented that handwashing is the most essential hygienic practice for controlling pathogen transmission including bacterial, viral, fungal, and parasitic agents [64–66]. In this regard, the current study revealed that not practising hand washing after defecation was associated with about a threefold higher risk of acquiring *G. duodenalis* and this finding is consistent with a recent study in Taiz City [36]. Unlike soil-transmitted helminths and some intestinal trematodes and cestodes that need soil or intermediate hosts for the development of infective stages, *Giardia* cysts are immediately infectious when excreted in the stool or shortly afterwards. Hence, cysts on the contaminated hands of infected individuals who do not practise handwashing after defecation may infect other individuals (person-to-person via the faecal–oral route) or spread over surroundings such as door bells and handles which then become sources of infection. In fact, previous studies in different parts of the world have revealed the vital role of improving the quality of water, sanitation, and hygiene in controlling intestinal parasitic infections [66–68]. Interestingly, the PARF results of the current study showed that providing safe drinking water and promoting good standards of personal hygiene, particularly handwashing after defecation, will help in reducing the majority (about 90%) of *Giardia* infection cases in the studied population.

In the same vein, the current study showed that close contact with domestic animals was a significant risk factor for *Giardia* infection and was associated with a twofold higher risk of infection among the study population. This is in accordance with other studies that identified the presence of domestic animals as a risk factor for *Entamoeba* and other intestinal parasitic infections in Sana'a and Taiz cities [34,36]. The finding could be indirectly attributed to not practising handwashing after handling domestic animals including cats, dogs, cattle, goats, and donkeys [4]. Infective cysts in a contaminated environment are easily picked-up on the fur of animals and then transmitted to humans when handling these animals. Moreover, the zoonotic transmission of *Giardia* infection is still under debate [69], while some previous studies have suggested that transmission from human to animals or vice versa may occur [70–73], other studies have found no evidence of such transmission [74–76]. Based on molecular and phylogenetic evidence, eight distinct genetic groups (called assemblages) for *G. duodenalis* (A–H) have been identified, with two assemblages (A and B) found in humans and a wide range of mammals, and these might be involved in the proposed zoonotic transmission. The remaining six assemblages (C to H) show more restricted host specificity and do not infect humans [77]. The only molecular study available for Yemen showed that among 65 *Giardia* isolates from Sana'a City, 42 isolates (65%) were identified as assemblage A and 23 isolates (35%) as assemblage B [35]. Alyousefi and her colleagues reported a significantly higher proportion of assemblage A among participants who had close contact with domestic animals as well as among those who collect grass for animals when compared to assemblage B [35]. In the current study, the molecular characterization of the detected *Giardia* parasites as well as the occurrence of *Giardia* in samples from domestic animals or in drinking water were not investigated in the studied communities; hence a causal relationship between the identified risk factors and *Giardia* infection cannot be confirmed.

In conclusion, the current study reveals that *Giardia* infection is highly prevalent in rural Yemen. Gender, presence of another family member infected with *Giardia*, unsafe drinking water, existence of domestic animals and not handwashing after defecation are the key risk factors of infection among the studied communities. Thus, the provision of safe, potable water and adequate sanitation together with health education pertaining good personal hygiene practices should be considered by control programmes against intestinal parasites in Yemen. Moreover, other family members should be screened and treated for *Giardia* infection. Overall, the epidemiological characteristics are almost similar among Yemeni communities, particularly in rural areas, thus these findings can be generalized to other rural communities. However, further studies in other governorates using robust molecular approaches are required to confirm this conjecture and to enable a better understanding of *Giardia* epidemiology and transmission dynamics in the country.

Conflict of interest statement

The author declares no conflict of interest.

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