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Current status of intestinal parasitic infections and associated risk factors in rural population of Guilan province, northern Iran: trichostrongyliasis is the most prevalent helminthic infection

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ABSTRACT

Aim: This study aimed to determine the distribution of enteric parasitic infections and related risk factors among rural communities of Guilan province, Northern Iran, and to compare the results with the situation in the past.

Background: Intestinal parasitic infections are still considered as a major public health concern, particularly in human communities with poor economy and sanitation.

Methods: This cross-sectional study was performed in rural areas of Masal and Shanderman district from February to December 2020. A total of 917 stool samples were collected and examined for presence of intestinal helminthes and protozoa using direct, formalin-ether and Kato-Katz techniques.

Results: A total of 156 (17%) out of 917 examined individuals were infected with intestinal parasites. The overall prevalence of protozoa, helminths and mixed infections were 11.8% (108/917), 4.5% (41/917) and 0.8% (7/917), respectively. Blastocystis was the most prevalent intestinal protozoa (9.6%) followed by Giardia lamblia (1.9%), Endolimax nana (1.1%), E. coli (0.8%) and Entamoeba hartmani (0.1%). The highest prevalence of intestinal helminths belonged to Trichostongylus spp. (3.5%) and Strongyloides stercoralis (1.3%). Statistical analysis showed significant association between giardiasis and sex ($P<0.03$). On the other hand, prevalence of enteric helminths was influenced by close contact with livestock, keeping herbivorous animals at home, job, education, and consumption of uncooked vegetables ($P<0.05$).

Conclusion: The findings indicate a decreasing trend in the prevalence of intestinal parasitic infections in Guilan province in comparison to the past few decades. Hookworm infections, which was very prevalent in the area, are now rare, while trichostrongylosis showed a high prevalence in rural residents of the study area.

Keywords: Intestinal parasites, Trichostrongyliasis, Risk factors, Guilan, Iran.

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Introduction

Parasites are responsible for hundred thousand of mortality in human populations, particularly those living in tropical and subtropical regions of the world (1). Intestinal parasitic infections (IPIs) are considered

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as a substantial globally public health problem, especially in underdeveloped and developing countries. About 1.45 billion people have been estimated to be infected with soil-transmitted helminths, resulting in 4.98 million years lived with disability (YLDs) and 5.18 million disability-adjusted life years (DALYs). The number of cases infected with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Necator americanus* and *Ancylostoma duodenale*) in 2010 was reported as 819 million, 464.6 million and 438.9 million, respectively (2). The annual number of death has also been estimated as 10,500 for A. *lumbricoides* and 65,000 for hookworms (1). The global burden reported for soil transmitted helminthes in 2017 has been estimated at 1.9 million disability-adjusted life years (DALYs) (3).

Intestinal parasites are mainly prevalent among rural population of equatorial regions with warm and humid climate, where proper sanitation facilities are lacking. In such a situation, children are more influenced by the parasites compared to general population (1, 4, 5). Iron deficiency anemia, malnutrition, poor cognitive functions and learning ability, stunting of growth, malabsorption, and weight loss are among the most important consequences of intestinal parasitic infections, particularly in children (6-8).

Untreated drinking water, low socioeconomic status, poverty, poor sanitation, close proximity with animals, ignorance, eating unwashed vegetables, and contact with contaminated soil are major risk factors predisposing rural communities to intestinal parasitic infections (4, 5, 9-11).

During past decades, intestinal helminthic infections, soil-transmitted helminths in particular, have been reported with very high prevalence from different parts of Iran. Recent epidemiological studies indicate a very sharp decrease in prevalence of IPIs in the country with prevalence rate of less than 1% for most of them (12-15). Although the prevalence of most of intestinal helminthic infections such as hookworms, *T. trichiura* and *A. lumbricoides* has significantly decreased in recent decades, but *Trichostrongylus* species and *Strongyloides stercoralis* have been frequently reported in northern provinces of Iran (13, 14). Indeed, some protozoan parasites such as *Giardia lamblia* are still a major public health problem in the country (14).

Since, few studies have been conducted on the prevalence of IPIs in northern Iran, the present study aimed to determine the prevalence of IPIs and their risk factors in the rural residents of Masal and Shanderman district, located in Guilan province, northern Iran, to obtain a clear picture from the current status of parasitic infections in this area.

Methods

Ethics approval

This study was approved by the ethics committee of GUMS (IR.GUMS.REC.1396.270). Only the subjects with a signed consent form were included in the present study. In case of children the approval was obtained from their parents or legal guardians.

Study area

This cross-sectional study was performed from February 2018 to January 2019 in the rural community of Masal and Shanderman district (37º15′N to 37º29′N, 48º43′E to 49º11′E) (Figure 1). This district covers an area of 622 km^2 and is located in western part of Guilan province. The study area constitutes of forested mountainous, foothill and plain areas with humid climate in the plain and semi-humid in mountainous regions.

A total of 52000 people are estimated to live in this district, of which 25000 (48.1%) inhabitants reside in rural regions. Agriculture, mainly rice planting, is the main source of employment for people in plain areas and animal husbandry for those living in the mountainous regions. Mean annual temperature and rainfall of the study area are 16.1°C (-11°C to 35°C) and 1241 mm, respectively (https://en.climate-data.org/asia/iran/gilan/masal-51910/#climate-graph).

Study population

The sample size was calculated using the statistical formula, $n=z^2p(1-p)/d^2$, where n is the sample size, p is the estimated prevalence of intestinal parasites in the area, z is the standard score at a 95% confidence interval and d is the allowed relative error. The minimum sample size calculated for this study was 850 individuals. In order to compensate the anticipated loses during follow-up, the samples size was increased by 8% to final number of 917.

The rural population of Masal and Shanderman live in 7234 households in 86 villages (about 3.5 individuals

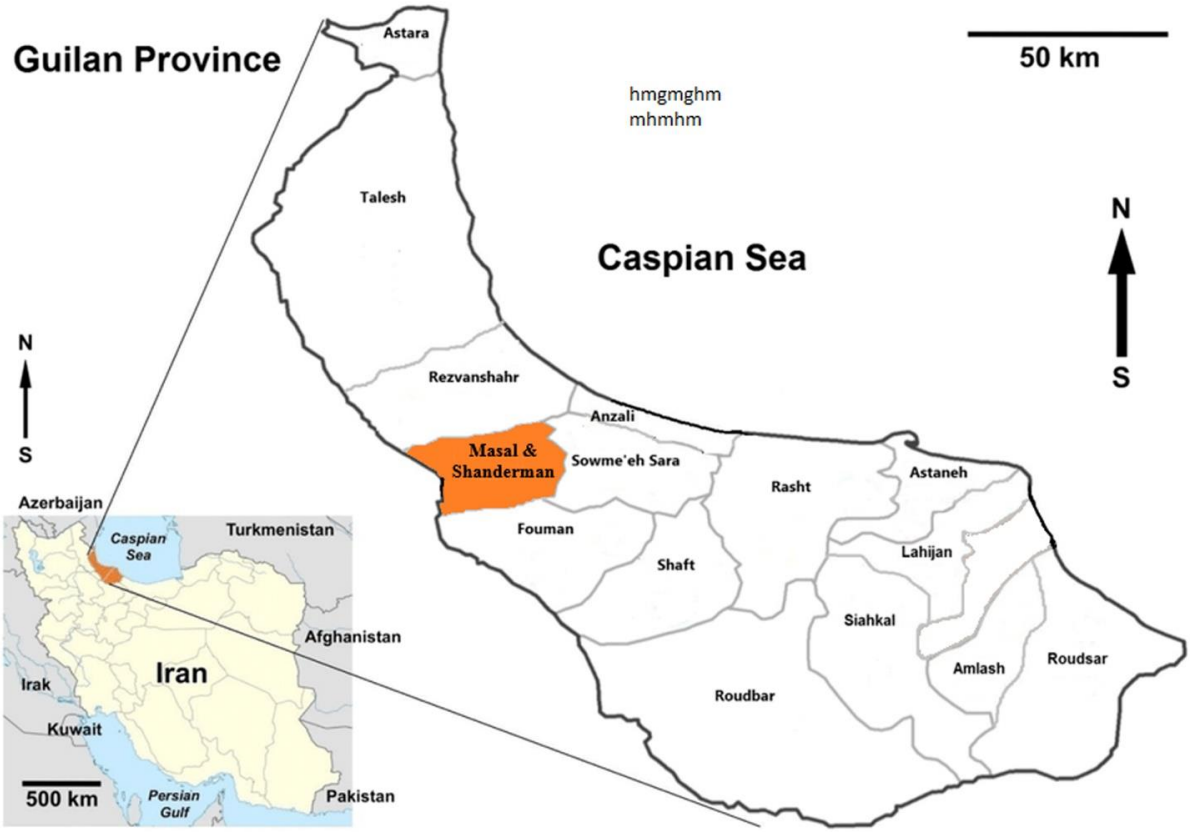

Figure 1. The study area. Masal is located in western part of Guilan province in northern Iran.

per household), so for collecting the determined samples 262 households were randomly selected.

Samples collection and processing

Following coordination with the authorities of health center of study area, we referred to the villager's houses and the protocol of the study and procedure of sample collection were clearly explained to the participants. A written consent form was provided to each person, to the parents in case of the children, and a predesigned questionnaire was administered to each participant to obtain demographic data (age, gender, occupation and level of education) and potential and personal risk factors such as eating raw vegetables, drinking untreated water, contact with contaminated soil, close proximity to domestic animals, washing hand before meals and after using toilets.

Thereafter, a pre-labeled screw capped plastic container was provided to each person and on the second day the stool samples were collected and confirmed by the participant name and number and immediately transferred to the parasitology laboratory of School of Medicine of Guilan University of Medical Sciences (GUMS). The stool samples were examined using formalin-ether concentration and Kato-Katz techniques. Direct wet smear was also used in the case of watery stool samples. One Kato-Katz smear and two 22 × 22 mm slides from formalin-ether concentrate were examined for each participant.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 22.0 was used for analyzing the collected data. The chi-square ($\chi 2$) test was used to determine whether demographic data, type of drinking water, close contact with livestock, eating raw vegetables, hygiene status, contact with contaminated soil, livestock raised at home and prevalence of pathogenic parasites were significant risk factors for intestinal parasitic infections. The differences were considered as significant when the P value was less than 0.05.

Results

A total of 917 individuals, aged 1-85 years (35.9±19.9) participated in this study, of which 45.3% (415.917) and

54.7% (502.917) were male and female, respectively. The study population was categorized in four age groups (<20, 20-39, 40-59 and >60-year-old). The numbers of participants in the age groups were 240 (26.2%), 271 (29.5%), 277 (30.2%) and 129 (14.1%) respectively. The highest prevalence rate of 27.1% (35.129) was seen in participants aged ≥60 and the lowest rate 9.7% (24.240) in individuals aged <20 years. A statistically significant difference was found between the infection with IPs and age groups of the individuals (P<0.0001)

The overall prevalence of IPIs was 17% (156.917) of which 4.5% (41.917), 11.8% (108.917) and 0.7% (7.917) belonged to helminthic, protozoan, and mixed infections, respectively. The prevalence rate was 18.1% (81/502) in females and 16.2% (75.415) in males. No statistically significant difference was found between IPIs and gender (P<0.43) (Table 1).

Considering the occupation, IPIs were most prevalent in farmers and ranchers with prevalence rate of 25% (16.64) followed by housewives 18.2% (59.325) and other occupations 15.3% (81/528). The difference between occupation and the infection rate of IPIs was not statistically significant (P<0.120).

According to the educational status, illiterate individuals had the higher prevalence rate at 21.3% (64.301) when compared to participants with higher educational level but this difference was not statistically significant (P<0.059).

By close proximity to livestock and/or livestock raised at home, the prevalence of intestinal infections was more frequent among participants with livestock contact at 19.1% (105.551) in comparison with those who denied livestock contact 13.9% (51.366). Statistical analysis verified the relationship between livestock contact and/or livestock raised at home with IPIs (P<0.043)

Concerning the type of drinking water, a higher prevalence of intestinal parasites was observed in people who drink untreated water from wells, springs and streams at 22.8% (18.79) compare to those who drink pipe water 16.5% (138.838). This difference was not statistically significant (P<0.153). Considering raw vegetable consumption, no significant differences were seen in relationship with IPIs (P<0.882) (Table 1).

Trichostrongylus spp. was the most frequently detected helminth with infection rate of 3.5% (32.917)

Table 1. Prevalence of intestinal parasitic infections in rural areas of Guilan province, northern Iran, by demographic factors.

Variables	Positive N (%)	Negative N (%)	*P*-value
Age group (yr)			0.001
<20	24 (9.7)	216 (90.3)	
20-39	43 (16.3)	228 (84.1)	
40-59	54 (19.5)	223 (80.5)	
≥60	35 (27.1)	94 (72.9)	
Gender			0.437
Male	75 (18.1)	340 (81.9)	
Female	81 (16.1)	421 (83.9)	
Educational status			0.059
Illiterate	64 (21.3)	237 (78.7)	
Under diploma	56 (15.8)	298 (84.2)	
diploma	26 (12.4)	183 (87.6)	
postgraduate	10 (18.9)	43 (81.1)	
Occupation			0.120
Farmer and Shepherd	16 (25)	48 (75)	
Housekeeper	59 (18.2)	266 (81.8)	
Other	81 (15.3)	447 (84.7)	
Raw vegetable consumption			0.882
Daily	32 (18)	146 (82)	
Sometimes	115 (16.9)	565 (83.1)	
Never	9 (16.3)	50 (84.7)	
Water supply status			0.153
Untreated (well, mineral spring)	18 (22.8)	61 (77.2)	
Treated pipe water	138 (16.5)	700 (83.5)	
Livestock proximity			0.043
No	51(13.9)	315(86.1)	
Yes	105(19.1)	446(80.9)	

Table 2. Prevalence of different species of intestinal parasites in 917 inhabitants of rural areas of Guilan province, northern Iran.

Parasite	Number	Percentage (%)
Giardia lamblia	17	1.9
Entamoeba coli	8	0.9
Entamoeba hartmani	1	0.1
Endolimax nana	10	1.1
Blastocystis sp	88	9.6
Trichostrongylus spp	32	3.5
Strongyloides stercoralis	12	1.3
Enterobius vermicularis	4	0.4
Taenia sp.	1	0.1
Total	173	18.9

in study population, followed by *S. stercoralis* at 1.3% (12.917). We also found 4 cases (0.4%) of *Enterobius vermicularis* and 1 case (0.1%) of *Taenia saginata*. The most prevalent protozoa were identified as *Blastocystis* 9.6% (88.917) and *G. lamblia* 1.9% (17.917). Three species of non-pathogenic protozoa were also identified including *Endolimax nana* 1.1% (10.917), *Entamoeba coli* 0.9% (8.917) and *Entamoeba hartmani* 0.1% (1.917) (Table 2).

Risk factors associated with intestinal helminthic infections

Trichostrongyliasis

Thirty-two among 917 participants (3.5%) were found to be infected with *Trichostrongylus* spp. As far as we know this is the highest rate of infection in recent two decades in the country. Bivariate (chi-square) statistical analysis indicated that the demographic variables, age and gender were significantly associated with trichostrongylosis (P<0.002 and P<0.02, respectively). Close proximity with livestock and/or raising livestock at home was also significantly associated with *Trichostrongylus* infection (P<0.01). No statistically significant associations were observed between trichostrongylosis and eating fresh vegetables and education (Table 3).

Strongyloidiasis

S. stercoralis was the second prevalent intestinal helminth among study population (1.3%; 12.917). Age, gender and occupation were identified as risk factors associated with *strongyloidiasis* using bivariate (chi-square) statistical analysis (P<0.0001, P<0.03 and P<0.04 respectively). No significant association was observed between *S. stercoralis* infection and education, source of drinking water, eating fresh vegetables, and close contact with livestock (Table 4).

Table 3. Prevalence of infection with Trichostrongylus spp. in rural areas of Guilan province, northern Iran by demographic factors.

Variables	Positive N (%)	Negative N (%)	*P*-value
Age group (yr)			0.002
<20	0 (0)	248 (100)	
20-39	13 (4.9)	258 (95.1)	
40-59	10 (3.6)	267 (96.4)	
≥60	9 (7)	120 (93)	
Gender			0.020
Male	11 (2.7)	404(97.3)	
Female	21 (4.2)	481 (95.8)	
Occupation			0.211
Farmer and Shepherd	2 (3.1)	62 (96.9)	
Housekeeper	16 (4.9)	309 (95.1)	
Other	14 (2.7)	514 (97.3)	
Raw vegetable consumption			0.135
Yes	32 (3.7)	827 (96.3)	
No	0 (0)	58 (100)	
Livestock close contact			0.013
No	6 (1.6)	360 (98.4)	
Yes	26 (4.7)	525 (95.3)	

Table 4. Prevalence of infection with Strongyloides stercoralis in rural areas of Guilan province, northern Iran, according to the demographic factors.

Variables	Positive N (%)	Negative N (%)	*P*-value
Age group (yr)			0.001
<20	0 (0)	248 (100)	
20-39	2 (0.7)	269 (99.3)	
40-59	1 (0.4)	276 (99.6)	
≥60	9 (7)	120 (93)	
Gender			0.037
Male	9 (2.2)	406 (97.8)	
Female	3 (0.6)	499 (99.4)	
Occupation			0.046
Farmer and Shepherd	3 (4.7)	61 (95.3)	
Housekeeper	3 (0.9)	322 (99.1)	
Other	6 (1.1)	522 (98.9)	
Educational status			0.050
Illiterate	8 (2.7)	293 (97.3)	
Under diploma	4 (1.9	358 (98.9)	
diploma	0 (0.0)	209 (100)	
postgraduate	0 (0.0)	53 (100)	

Risk factors associated with intestinal protozoan infections

Giardiasis

G. lamblia infection was the second intestinal protozoan infection (1.9%; 17.917) next to *B. hominis* (9.6%; 88.917). Among demographic variables sex was the only risk factor associated with giardiasis by using chi-square statistical analysis (P<0.03). No statistically significant associations were observed between giardiasis and age, source of drinking water, eating fresh vegetables, personal hygiene and, close proximity to livestock (Table 5).

Discussion

It has been estimated that 1.45 billion people were globally infected only with soil-transmitted helminthes in 2010. Despite the significant reduction in the number of people infected with intestinal parasitic infections, in particular infections caused by soil-transmitted helminthes, the prevalence of these infections has remained highly endemic in tropical and subtropical areas of the world (2, 3). This reflects the fact that IPIs still have significant public health effects on human populations, so it is important to monitor the situation of these infections in order to determine the

Table 5. Prevalence of infection with Giardia lamblia in rural areas of Guilan province, northern Iran by demographic factors.

Variables	Positive N (%)	Negative N (%)	*P*-value
Age group (yr)			0.235
<20	5 (2.0)	243 (98.0)	
20-39	4 (1.5)	259 (98.5)	
40-59	8 (2.9)	269 (97.1)	
>60	0 (0)	129 (100)	
Gender			0.034
Male	12 (2.9)	403 (97.1)	
Female	5 (1)	497 (99)	
Occupation			0.493
Farmer and Shepherd	2 (3.1)	62 (96.9)	
Housekeeper	4 (1.2)	321 (98.8)	
Other	11 (2.1)	517 (97.9)	
Raw vegetable consumption			0.940
Yes	16 (1.9)	843 (98.1)	
No	1 (1.7)	57 (98.3)	
Water supply status			0.180
Untreated (well, mineral spring)	3 (3.8)	76 (96.2)	
Treated pipe water	14 (1.7)	824 (98.3)	

predisposing factors and implement the control strategies in endemic regions.

In recent decades a sharp decline was observed in the prevalence of intestinal parasitic infections particularly soil-transmitted helminthes in Iran (Table 6). This reduction is mainly due to the considerable improvement in economic and living conditions, health facilities, and personal hygiene, access to safe drinking water and health services and significant increase in the level of education of the Iranian people.

According to the results of the present work 17% of the study populations were infected with intestinal parasites of which 4.5% belonged to helminthic, 11.8% to protozoan and 0.7% to mixed infections. In comparison to the past decades, the prevalence of some IPIs including ascariasis, trichuriasis and hook worm infections has sharply decreased in Guilan province, so that these infections are very rarely encountered. However, *S. stercoralis* and *Trichostrongylus* spp. are still prevalent in northern part of the country particularly in the study area (13, 14). It appears that the reduction in the prevalence of *A. lumbricoides*, *T. trichiura* and hook worms is related to the facts that (i) these parasites are mainly anthroponotic and animals have no critical role in their transmission (ii) mechanization of agriculture has decreased the human contact with soil (iii) chemical fertilizers are more attractive for villagers than night soil which was frequently used in the past (iv) whole rural population have access to health services (v) the majority of the villagers have a higher level of education, personal hygiene, economic condition and life facilities.

Despite the significant decrease in the prevalence of ascariasis, trichuriasis and hookworms, trichostrongyliasis and strongyloidiasis are still prevalent in Guilan province. Thirty-two among 917 participants (3.5%) were found to be infected with *Trichostrongylus* spp. and twelve (1.3%) with *S. stercoralis*. The prevalence of trichostrongyliasis in present study was the highest rate in Iran in last two decades. Based on literature, the highest rate of infection (2.1% and 2.6%) reported from other provinces before our study belonged to neighboring province of Mazandaran, located at eastern part of Guilan (16). The prevalence rate of the present study is also higher than those reported from Brazil (1.2%) (17) and southern Sudan (2.5%) (18), while significantly lower than that of Laos (36.9%) (19).

Up to the moment ten valid species of *Trichostrongylus* have been reported from livestock all over the country, nine of which have also been reported from humans (13, 20). Recent studies have indicated the

Table 6. Comparative prevalence (%) of some intestinal helminthes in different provinces of Iran based on community-based studies in 1970s, 2005 and 2020s.

Province	Intestinal helminthes						Refs
	Ascaris	Hookworms	*Trichuris*	*Strongyloides*	*Trichostrongylus*	*Hymenolepis*	
				1970s			
Azerbaijan	56-93	-	10-76	-	-	0-17	(62)
Khorasan	23-95	-	0-57	-	-	0-22	(62)
Khorasan	15-74	-	2-12	-	-	4-8	(63)
Isfahan	62-98	5	29-32	-	69.5	4	(63)
Khuzestan	27-76	25-75	9-13	-	59-69	7-13	(63)
Mazandaran	14-58	2-76	6-78	-	2-7.5	3-13	(63)
Kermanshah	59-86	-	32-80	-	2-5	-	(64)
Guilan	4-38	29-63	19-57	5-9	3-20	-	(65)
				2005			
Iran	1.5	<0.1	0.1	-	0.2	-	(66)
				2020s			
Khorasan	0.55	-	-	-	-	6.7	(67)
Golestan	0.5	0.4	-	-	-	1.5	(68)
Mazandaran	-	-	-	1.6	2.1	-	(16)
Mazandaran	-	1.7	0.1	1.1	2.6	0.6	(69)
Kerman	0.2	-	-	-	0.1	1.1	(40)
Azerbaijan	0.09	-	-	-	ND	0.03	(70)
Guilan	-	0.07	0.07	1.5	3.13	-	(14)
Khuzestan	-	0.1	-	-	0.5	0.4	(71)
Hamadan	-	-	0.4	-	-	0.9	(72)

zoonotic transmission of *T. colubriformis*, *T. vitrinus*, *T. axei* and *T. longispicularis* in Guilan province (13, 21). The high prevalence of trichostrongyliasis in the study area might be due to the close proximity of rural residents to their livestock and the potential for zoonotic transmission of *Trichostrongylus* spp. Bivariate (chi-square) statistical analysis identified a positive association between close proximity to livestock and trichostrongyliasis (P<0.003).

Both northern provinces of Iran (Guilan and Mazandaran), located at the southern littoral of Caspian Sea, are well recognized endemic regions for *S. stercoralis* in Iran. This dangerous parasite was the second most prevalent helminth in the study area (1.3%). This rate of infection is higher than the rate reported from Khuzestan province (0.47%) (22) and lower than that of Mazandaran province (1.6%) (16). Comparing to other parts of the world, the rate of infection in present study was higher than those reported from rural areas of Brazil (0.7%) (23); Cuba (0.7%) (24); Kenya (0.5%) (25) and Southern Thailand (0.9%) (26), while lower than the rates reported from Combodia (21%) (27), (44.7) (28), (48.6%) (29); China (11.7%) (30); Thailand (5%) (31); Brazil (5%) (32) and Ethiopia (20.7%) (33).

The auto-infection phenomena and presence of free-living cycle are considered as the main factors which lead to the survival of the parasite in human populations. This accompanied with soil contact, mainly through agricultural activities, expose local population to the infection caused by this pathogenic helminth. In addition, the zoonotic aspect of strongyloidiasis has recently been documented and transmission of *S. stercoralis* from dogs to humans been indicated using molecular genetic analyses (34, 35). On the other hand, dogs might be considered as a reservoir for human infection by *S. stercoralis* and this might be another factor for parasite survival in the nature. Bivariate statistical analysis indicated occupation and personal hygiene as the risk factors associated with strongyloidiasis (P<0.04 and P<0.002 respectively). Accordingly, the farmers have a higher risk for *S. stercoralis* infection through contact with contaminated soil.

As already mentioned, 12.5% of the studied population were infected by pathogenic and none pathogenic intestinal protozoa. *B. hominis* (9.6%) and *G. lamblia* (1.9%) were the most prevalent protozoa in the study area. The prevalence rate of *G. lamblia* in this study was higher than those reported in Ilam (0.5%) (36); Tehran (1.2%) (37) and Qazvin (1.6%) (38) Provinces and lower than the rates reported from Mazandaran (10.6%) (16); Chaharmahal & Bakhtiari (28.2%) (39); Kerman (7.8%) (40); Kordistan (4.1%) (41); Hamadan (10.9%) (42); Khuzestan (7.7%) (22) and Kohgiluyeh & Boyer-Ahmad (17.5%) (43) Provinces. The prevalence of this pathogenic protozoa in other countries, neighboring countries in particular, was very higher than that reported in the present study; Iraq (37%) (44), (30.9%) (45), (26.1%) (46); Turkey (18.1%) (47), (47.9%) (48); Pakistan (28.9%) (49); Afghanistan (41.9%) (50); Yemen (16.1%) (51), Saudi Arabia (11.5%) (52), India (14.4%) (53); Tajikestan (26.4%) (54); Brazil (11%) (55); Argentina (46.4%) (56); Peru (15.1%) (57); Ethiopia (13.2%) (58); Kenya (6%) (59); Sudan (9.7%) (60); and South Africa (9.9%) (61).

Conclusion

It appears that the current status of intestinal parasitic infections in Iran is quite different in comparison to the past. While the prevalence of soil-transmitted helminthes and intestinal protozoan infections has sharply decreased in almost all areas, human fascioliasis, strongyloidosis, and trichostrongylosis are frequently reporting from northern provinces of Guilan and Mazandaran. It appears that the significant reduction in the prevalence of intestinal parasitic infections in Iran might be associated with improvement in health facilities, personal hygiene, economic and living conditions, people access to safe drinking water and health services, using chemical fertilizer instead of night soil, presence of appropriate waste disposal systems, agricultural mechanization, significant increase in the level of education of the Iranian people and increasing public health awareness.

Conflict of interests

The authors report no conflicts of interest in this work.

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