

First Survey of Epidemiology and Associated Risk Factors of Human Intestinal Parasitic Infections among Gonabad Residents, Northeast of Iran

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ABSTRACT

OBJECTIVE: To investigate the prevalence of parasitic infections in the human population and identify the related risk factors of Gonabad city, southeast of Khorasan Razavi province, northeastern Iran.

METHODOLOGY: This cross-sectional study was performed from March to April 2019 in Gonabad city in Khorasan Razavi province in northeastern Iran. 1038 stool samples were examined based on parasitological methods like direct wet-mounting, formalin-ether concentration and the Gomori's trichrome staining. Moreover, the socio-demographic data of inhabitants were recorded. All statistical analyses were performed using SPSS software (version 16). Qualitative variables were reported as numbers and percentages.

RESULTS: The overall intestinal parasitic infection prevalence was 18.1 %, whereas this value for protozoan agents was 16.7% (95% CI 14.14 to 19) and 1.3% (95% CI 0.7 to 2) for helminth parasites. The highest parasitic agent's prevalence belongs to *Blastocystis sp* (12.4 %), *Entamoeba coli* (1.8%) and *Enterobius vermicularis* (1.2%), respectively. *Giardia lamblia* (1.1%) *Entamoeba histolytica /dispar* (0.3%), *Iodamoeba butschlii* (0.3%), *Chilomastix mesnili* (0.3%), *Hymenolepis nana* (0.2 %) *Dientamoeba fragilis* (0.2%) and *Trichomonas hominis* (0.3%) were other identified parasites. Multivariate logistic regression analysis revealed a significant association of parasitic intestinal infections with factors such as age, residency status and source of drinking water (P<0.05).

CONCLUSION: Our findings approved that the protozoan agents have a relatively high prevalence among the studied population. So, it could be severe enough to alarm the entities implementing healthcare services measures for parasitic intestinal infection control.

KEYWORDS: Intestinal parasites, helminth, Prevalence, Iran, protozoan

INTRODUCTION

Despite rapid progress in healthcare services performance and public hygiene status in developing countries, parasitic intestinal infection remains a significant public health concern. Annually, it is evaluated that more than three billion cases worldwide experience intestinal parasite infections, whereas 450 million of this population exhibit clinical manifestations. The annual mortality rate of parasitic intestinal infection is estimated to be nearly 16 million. Parasitic intestinal infections are commonly present with clinical symptoms such as weight loss, malnutrition, abdominal pain or cramping, diarrhoea, vomiting and anemia. These symptoms are particularly prevalent in children and older adults. Additionally, more dangerous complications such as intestinal obstruction, myocarditis, cholecystitis and appendicitis were reported¹¹.

In Iran, the intestinal parasite's prevalence varies between 4.7 and 56% in the healthy human population²². There are many reports of parasitic intestinal infections from different parts of Iran, such that *Blastocystis sp* and *Giardia lamblia* are among Iran's most common pathogenic protozoa. The oral faecal and contaminated food/water are intestinal parasitic agents' most common transmission routes²⁴. Intestinal parasitic infections caused by *Giardia lamblia*, *Blastocystis*, *Entamoeba* and *Cryptosporidium spp(s)* are the most important causes of diarrhea in developing countries²⁴. Over the past four decades, approximately 50% of the Iranian population has experienced pathogenic intestinal parasite infections³¹.

However, several efforts have been made in Iran to decrease the parasitic burden, but the determination of intestinal parasite distribution \ incidence pattern remains a significant health problem. The prevalence of parasitic intestinal infection in any region has been proven to depend on several factors: socioeconomic, personal and public hygiene, and geographic educational status¹². So, it is urgent to identify these factors and establish an efficient prevention\ control program for parasitic infections in each zone separately.

Based on our knowledge, there is no scientific report about the distribution of parasitic intestinal infections and associated risk factors in Gonabad city in Khorasan Razavi province in northeastern Iran. Therefore, the present study was designed for the first time to determine the prevalence and associated risk factors with parasitic intestinal infections in the studied human population.

METHODOLOGY

Sample size

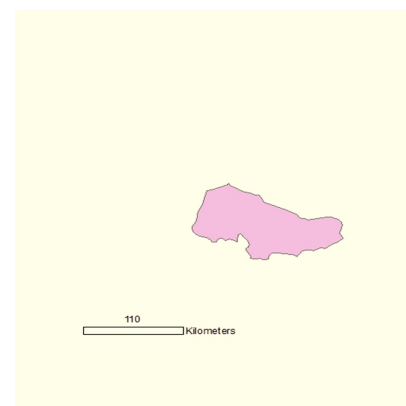
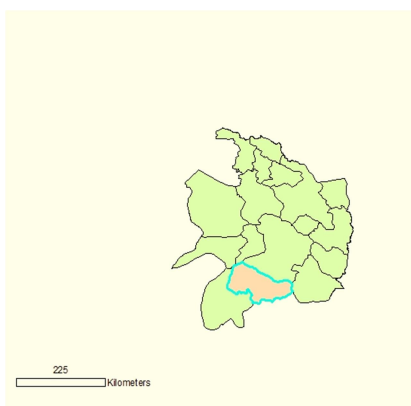
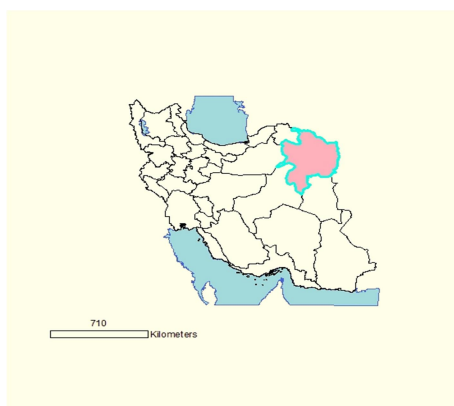
Based on the prevalence of infections in the recent study ($p=0.17$), the estimated sample size was calculated using the formula below. Considering a 95% confidence interval and 80% test power, 957 people were selected, which was increased to 1038 individuals, assuming a 10% attrition rate (Abbaszadeh Afshar et al., 2020).

$$n = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 * p(1 - p)}{d^2} = \frac{(1.96 + 0.84)^2 * (0.17 * 0.83)}{(0.2 * 0.17)^2} = 957$$

In this research, stratified random sampling was performed. Thus, comprehensive health service centres of Gonabad city were considered as classes; then, according to the number of people covered by each centre, the samples were randomly selected.

Study area

This cross-sectional study was performed from March to April 2019 in Gonabad city in Khorasan Razavi province in northeastern Iran ($34^{\circ}21'10''N$ $58^{\circ}41'01''E$). It has an area of about 2188.91 km² and over 36367 people. The city is located in a semi-arid area, with an elevation between 894 and 2774 meters above sea level and has hot summers and cool winters. Gonabad weather station data shows its rainfall and average annual temperature are 149–155 mm/year and 16.4–17.3°C, respectively. This city is famous for its unique groundwater supply, and it is named Qanats. It was added to UNESCO's list of World Heritage Sites in 2007 due to its unique features, like historical sites and ancient artefacts²³. **Figure I** shows the geographical location of Iran and Gonabad.



Iran

Razavi Khorasan province

Gonabad

Sample collection and laboratory analysis

In the first step, written informed consent was obtained from all of our study's volunteers. Next, they filled out a structured checklist containing individual socio-demographic and health data like sex, age, occupation, education, drinking water source, raw vegetable consumption and family size (Small = less than 5, Large = more than 5). Signing informed consent, delivering

stool specimens and not receiving anti-parasitic treatments in the last 21 days or during data collection were our inclusion criteria in the present examination.

Here, 1038 stool samples were collected from Gonabad inhabitants. Stool specimens were collected in sterile plastic containers with numbered labels and promptly transferred to the Department of Parasitology and Mycology, Gonabad University of Medical Sciences.

All collected samples were initially examined through macroscopic observation regarding consistency, the existence of blood or mucus, color and adult forms of helminth parasites. For watery/bloody stool samples, fresh smears were prepared and examined with normal saline (0.85% NaCl solution) and Lugol's Iodine solution. All stool samples were stored in buffered formalin (10 %) for further parasitological techniques. According to the previously described protocol, besides wet mount, concentration techniques using formalin-ethyl acetate and Merthiolate-iodine-formaldehyde solution (MIF) were applied²⁰. The same expert observed the prepared slides (blinded to participants' information) through different microscope magnifications. If parasite forms like eggs, cysts, larvae, and/or trophozoites were seen in at least one of the three techniques, that sample would be considered positive, and then the permanent trichrome staining technique would be used to confirm detection.

Statistical analysis

All statistical analyses were performed using SPSS software (version 16). Qualitative variables were reported as numbers and percentages.

The logistic regression model was adopted to determine the factors related to parasitic intestinal infections. Therefore, the variables that had $P < 0.2$ in simple logistic regression were entered into the multiple logistic regression model, and their relationship was assessed in the presence of other variables following the parasitic intestinal infections. The p -values less than 0.05 were considered statistically significant.

In the logistic regression model, positive/negative parasitic infection was the dependent variable. In contrast, other examined variables like sex, age, occupation, education, drinking water source, raw vegetable consumption, and family size were independent or predictive variables.

RESULTS

Our research collected 1038 stool samples from the Gonabad participants, including 569 (54.8%) males and 469 (45.2%) females. Nearly 31 (3%) of the studied population were illiterate, 180 (17.3%) had primary education, 153 (14.7%) belonged to middle school education level, 452 (43.5%) had completed a diploma, and the rest of the people attained an academic level. Our data showed that 585 (56.4%), 386 (37.2%), and 67 (6.5%) of the studied participants consumed tap water, purified water, and groundwater as water resources, respectively (**Table I**).

Table I: Socio-demographic data of participants

Characteristics	Levels	Number (1038)	Percent	Number of infections (187)	Percentage of infection
Sex	Male	569	54.8	87	18.6
	Female	469	45.2	100	17.6
Age	≤ 9	28	2.7	11	39.3
	10 to 19	179	17.2	35	19.6
	20 to 29	334	32.2	54	16.2
	30 to 39	339	32.7	63	18.6
	40 to 49	123	11.8	21	17.1
	≥50	35	3.4	3	8.6
Occupation	Student University	198	19.1	27	13.6
	Student	175	16.9	38	21.7
	Employed	42	4	5	11.9
	Housewife	51	4.9	7	13.7
	Free-Farmer-Livestock	53	5.1	14	26.4
	Labor	466	44.9	89	19.1
	Unemployed	40	3.9	3	7.5
	children	13	1.2	4	30.8
Education	Illiterate	31	3	6	19.4
	Primary	180	17.3	31	17.2
	guidance	153	14.8	31	20.3
	Diploma	452	43.5	81	17.9
	Super Diploma	53	5.1	9	17
	Bachelor	84	8.1	17	20.2
	MA-PHD	85	8.2	12	14.1
Residency	Urban	254	24.5	29	11.4
	Village	784	75.5	158	20.2
Source of drinking water	Tap water	585	56.4	109	18.6
	Purified water	386	37.2	55	14.2
	Ground water(qanat)	67	6.5	23	34.3
Raw\Unwashed vegetables consumption	No	754	72.6	126	16.7
	Yes	284	27.4	61	21.5
Family Size	Small	350	33.7	62	33.2
	Large	688	66.3	125	66.8

Table II demonstrates the prevalence of intestinal parasites detected by the formalin ether sedimentation method. Nearly 18.1% of the total examined stool samples were positive for at least one species of the parasitic agent. Out of ten parasitic agents detected in current research.

Table II: Prevalence of intestinal parasites in the studied population

Characteristic	Levels	Number	Percent	95% Confidence Interval	
				Lower	Upper
Infection					
	<i>Blastocystis hominis</i>	129	12.4	10.4	14.6
	<i>Entamoeba coli</i>	19	1.8	1.1	2.6
	<i>Giardia lamblia</i>	11	1.1	0.5	1.8
	<i>Entamoeba.histolytica/ dispar</i>	3	0.3	0.0	0.7
	<i>Dientamoeba fragilis</i>	2	0.2	0.0	0.5
	<i>Trichomonas hominis</i>	3	0.3	0.0	0.7
	<i>Iodamoeba butschlii</i>	3	0.3	0.0	0.7
	<i>chilomastix mesnili</i>	3	0.3	0.0	0.7
	<i>Enterobius vermicularis</i>	12	1.2	0.6	1.9
	<i>Hymenolepis nana</i>	2	0.2	0.0	0.7
	Negative	851	82	80.9	85.4

Our findings indicated that the prevalence of the protozoan parasites (16.7%,95% CI 14.14 to 19) was more than that of helminthic parasites (1.3%95% CI 0.7 to 2). The most prevalent detected parasites were *Blastocystis sp* (12.4%, 95% CI 10.4 to 14.6), *Entamoeba coli* (1.8%, 95% CI 1.1 to 2.6) and *Enterobius vermicularis* (1.2%,95% CI 0.6 to 1.9). Moreover, as shown in Table.2 *Giardia lamblia* (1.1%), *Entamoeba histolytica /dispar* (0.3%), *Iodamoeba butschlii* (0.3%), *Chilomastix mesnili* (0.3%), *Hymenolepis nana* (0.2 %) *Dientamoeba fragilis* (0.2%) and *Trichomonas hominis* (0.3%) were other observed intestinal parasitic agents. We provided images of trichrome-stained *Entamoeba coli*, *Giardia lamblia* and *Entamoeba histolytica /dispar* in **Figure II**.

Figure II: Microscopic picture of detected parasites from Stool samples stained with trichrome staining (magnification 1000x). A. *Giardia lamblia* trophozoite B. *Entamoeba histolytica /dispar* cyst. C. *Entamoeba coli* cyst

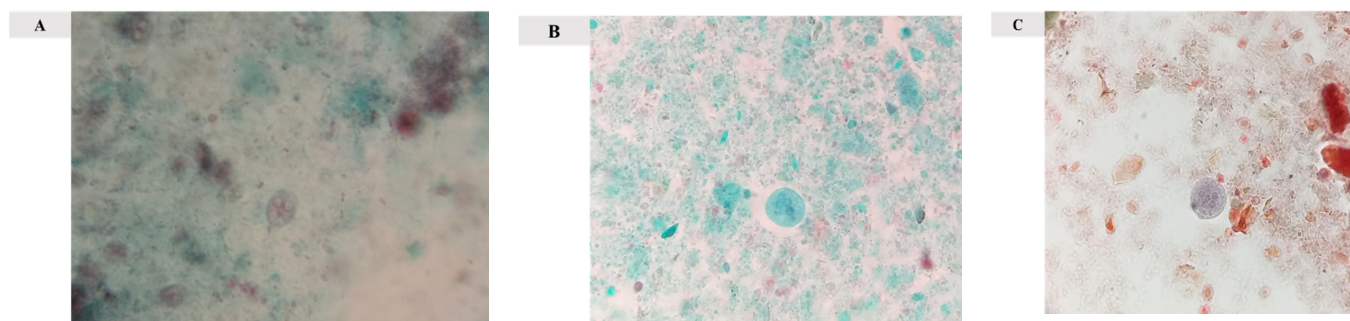


Table III exhibited the analyzed data by unadjusted and adjusted logistic regression of the socio-demographic correlates with parasitic intestinal infections among the participants. Our findings indicated that among possible risk factors investigated in this study, age, drinking water source and residency (rural/urban) status have a significant association with parasitic intestinal infections (p-value < 0.05). In contrast, sex, occupation, education level, raw/unwashed vegetable consumption and the number of family members showed no effects on the rate of parasitic intestinal infections (p-value >0.05)

Table III: Univariate and multivariate analysis of parasitic intestinal infections and potential risk factors in the participants

Characteristics	Levels	OR ¹	CI ¹ (95%)	p-value	OR ²	CI ² (95%)	p-value
Sex	Female				-		
	Male	1.06	0.77-1.46	0.68		-	
Age	≤ 9				-		
	10 to 19	2.66	1.14-6.18	0.023	2.57	1.06-6.23	0.035
	20 to 29	3.35	1.48-7.56	0.003	2.80	1.19-6.59	0.018
	30 to 39	2.83	1.26-6.34	0.011	2.45	1.05-5.69	0.037
	40 to 49	3.14	1.28-7.66	0.012	2.97	1.17-7.52	0.022
	≥50	6.90	1.69-28.14	0.007	6.12	1.46-25.55	0.13
Occupation	Student University				-		
	Student	0.56	0.33-0.97	0.15			
	Employed	1.16	0.42-3.23	0.76			
	Housewife	0.99	0.40-2.42	0.98			
	Free	0.44	0.21-0.91	0.28		-	
	Labor	0.66	0.41-1.06	0.19			
	Unemployed	1.94	0.56-6.76	0.29			
	children	0.35	0.10-1.23	0.16			
Education	Illiterate				-		
	Primary	1.15	0.43-3.04	0.77			
	guidance	0.94	0.35-2.50	0.90			
	Diploma	1.09	0.43-2.76	0.84			
	Super Diploma	1.17	0.37-3.68	0.78		-	
	Bachelor	0.94	0.33-2.67	0.91			
MA - PHD	1.46	0.49-4.30	0.49				
Residency	Village				-		
	Urban	1.98	1.28-2.99	0.002	2.78	1.73-4.46	<0.001
Source of drinking water	Tap water				-		
	purified water	0.43	0.96-1.96	0.045	0.34	0.18-0.63	0.001
	Ground water (qanat)	1.37	0.25-0.75	0.003	1.53	1.06-2.20	0.021
Raw\Unwashed vegetables consumption	No				-		
	Yes	1.36	0.96-1.91	0.075	1.31	0.92-1.88	0.129
Family Size	Small				-		
	Large	0.97	0.69-1.35	0.85		-	

DISCUSSION

Intestinal parasitic infections are still a significant threat to developing countries. Investigating different localities is crucial to determining high-risk communities and designing appropriate intervention mechanisms. Based on this view, we attempt to assess the prevalence of parasitic intestinal infections and associated risk factors among the human population of Gonabad city, southeast of Khorasan Razavi province, northeastern Iran.

In the present study, the overall prevalence of intestinal parasitic infection was 18.1% among the studied population. This finding more or fewer concurs with several previously Iranian studies conducted in different areas like Jiroft city, Khuzestan, Mazandaran, Kohgiluyeh and Boyer-Ahmad, Lorestan and Hamadan provenances with a prevalence 28%, 25.4% %, 25%, 37.5%, 32.5 % and 35.1% respectively^{3,5,14-16,19,22,27}. We detect that the protozoan infection rate is higher than the helminthic infection. Similar findings were reported from studies conducted in different parts of Iran and the world^{2,5,7,8,21,25}. These variations could be due to population density, climate changes, sanitary facilities, host-parasite interactions and even study methodology⁹. If we review the status of parasitic prevalence in the last four decades, we can see that Iran's overall frequency of parasitic intestinal infections has demonstrated a descending pattern. Nevertheless, our research and studies mentioned above show that despite progress in medicine and public health, there still needs to be more strategies for controlling these parasitic agents, particularly protozoans, which are major public health issues even nowadays. This problem's importance is reinforced when most previous reports regarding intestinal parasitic prevalence's actual value are generally more than the obtained values. This difference is generally related to non-standard stool sampling (one stool specimen versus three sequential specimens in three alternate days)²². Another probable reason for this difference is the failure to use specific diagnostic methods for intestinal parasites. Similar to recent studies in Iran, in our survey, *Blastocystis sp*, *Entamoeba coli* and *Enterobius vermicularis* were most prevalent among ten types detected parasitic agents, with a prevalence of 12.4 %, 1.8% and 1.2%, respectively^{5,16,18,19}. The transmission of these parasites through the fecal-oral route indicates poor sanitation practices in Gonabad. Whereas the mean prevalence of *Blastocystis sp* in Iran's total population ranged from 7.5% to 28.4 %, this value in our report was 12.4 % (95% CI 10.4 to 14.6)^{14,19}. Since pathogenicity, zoonotic status, transmission route, and host-parasite adaption pattern of *Blastocystis sp* have been controversial, further reliable evidence is required to evaluate the associated risk factors related to this infection. Our data demonstrated that 1.8% of parasitic infections belong to *Entamoeba coli* (95% CI 1.1 to 2.6). Recent surveys on apparently healthy Iranian people estimated that up to 18.1 % of cases were infected with *Entamoeba coli*¹⁵, which reflects a remarkable downregulated prevalence in Iran over the last decade. However, non-pathogenic parasite agents like *Entamoeba coli* do not cause clinical manifestation infection and are generally transmitted through the faecal-oral route but are regarded as reliable public hygiene indicators. In the present survey, the *Giardia* prevalence was 1.1 % (95% CI 0.5 to 1.8). Other Iranian population-based surveys on *Giardia* frequency rate have stated relatively similar values like 10.2% in 2008 (Kia et al., 2008), 28.2 and 8.9% in 2009^{3,22}, 17.2% in 2011⁵, 2.2% in 2014¹⁵, 17.4 and 7.8% in 2016^{18,27}, and 1.2% in 2017¹⁴; Whereas remarkably high prevalence (91%) reported from Iran highlighted the zoonotic aspect of this protozoan parasite¹⁰. However, a declining tendency in *Giardia* prevalence has been documented over the last decade in Iran, but it seems relatively high depending on the target population. The decreasing prevalence pattern of human helminth parasites has also been approved in recent decades in Iran. Nevertheless, some parasites, especially those transmitted through direct fecal-oral routes, like *Enterobius vermicularis* and

Hymenolepis nana, are still considered prevalent helminthic agents in Iran¹⁷. Here, the prevalence of *Hymenolepis nana* was 0.2 % (95 % CI 0 to 0.7). Although it has been approved that *Hymenolepis nana* prevalence in the human population has declined since 1970, this parasitic agent continues to be common in Iran's tribal and rural regions^{18,19,22}. Our data about *Enterobius vermicularis* with a 1.2 % prevalence is relatively similar to recent reports from Tehran and Kerman provinces that this value was 0.2 % and 0.3 %, respectively^{1,14}. In the present investigation, we evaluated several possible risk factors associated with parasitic intestinal infections. In this regard, our analyzed data confirmed that age, drinking water source, and residency (rural/urban) status are predictors for the prevalence of these infective agents among our studied community.

It was observed that most infected participants belong to age groups ≤ 9 and 10-19, with 39.3 % and 19.6% prevalence rates. It could be assumed that the risk of acquiring infection also increases with aging. People between 40 and 49 and over 50 were approximately three times and nearly seven times, respectively, more likely to be infected than those under nine years old. Similar to our result, an association between intestinal parasitic infections and age has been reported in different parts of Iran¹⁷; this could result from a better understanding and application of hygiene measures. In contrast, this conclusion was not seen in other provenances like Kerman, Tehran and Mazandaran^{1,14,28}. Here, the logistic regression test demonstrated no association between occupation status and intestinal parasitic infection (p -value >0.05). Most infected people were Free-Farmer-Livestock (26.4%). Similar findings were documented from different parts of Iran, such as the Kerman, Tehran, Mazandaran and Isfahan provinces^{1,14,16,18,19}. We found no significant association between educational background and infection with intestinal parasites (p -value >0.05). This subject differs from another study from Kurdistan province that showed literacy level is a protective factor against infection⁴. This variation in educated people could be due to variations in awareness about the mode of parasitic agent transmission and applying the necessary measures to avoid it. In contrast, we did not see this pattern in our examination. The most identified parasites belong to the group with a diploma educational level, which could result from a participant ratio at this academic level. Regarding residence, people who came from rural areas had a higher parasitic infection rate (20.2 %) and were approximately three times more at risk (OR = 2.78, 95% CI 4.46-1.73, $p <0.001$) of getting infected with parasitic agents than urban dwellers. Several similar findings have been approved in different parts of Iran. It was proposed that limited access to adequate sanitation, inadequate healthcare services coverage, low socioeconomic status, and hygienic living conditions are the most probable factors that increase the risk of infection in rural people^{4,5,30}. The kind of source of drinking water is a leading factor in the prevalence, incidence and dissemination of pathogen agents, particularly waterborne infections. Interestingly, we found that consuming purified water protects against infection, while underground water consumption increases the risk of infection. In recent years, Gonabad has experienced severe drought and faces major problems in water resources due to several causes, such as population burden, industrial activities, climate changes, and increasing demand²⁹. Nearly all drinking water resources in arid regions like Gonabad City depend on groundwater supplies⁶ (Bakarman et al., 2019). Additionally, based on traditional belief, underground water resources like qanat have therapeutic benefits. Although the different aspects of Gonabad underground water (like heavy metal content) have been investigated several times, their safety is controversial. Despite that, these water supplies do not seem suitable for drinking water. Comparable results have been regarding the importance of drinking water sources^{14,18}. Previous studies imply that contamination of vegetables with parasitic agents could be the

primary cause of infection spread in different parts of Iran. However, our analyzed data does not support this assertion¹⁷.

As a unique characteristic of this research, we found no association between family size and parasitic intestinal infection, unlike two other studies in Ethiopia. This difference may be due to sample size and residency status³².

Limitations

There were several limitations in the present study. The most remarkable one is that we could not apply swabs (cellophane-tape) tests to comment on the *actual prevalence of Enterobius vermicular* due to the large sample size and required condition. Moreover, we cannot apply the molecular approach to detect parasitic agents due to the monetary burden. Moreover, only one stool specimen was used to identify parasites; this approach may lead to underestimating the actual prevalence of intestinal parasitic agents compared to three sequential specimens collected in three alternate days.

CONCLUSION

The present report provides a snapshot of the status of intestinal parasitic prevalence and related factors in Gonabad. Although there has been a decreasing trend in prevalence in Iran, the parasite burden rate of 18.1% in Gonabad is concerning. The report identifies age, residency status, source of drinking water and raw/unwashed vegetable consumption as potential risk factors for parasitic intestinal infections. The study's findings could help inform policies to improve public health through lifestyle changes, promoting public awareness, and infection prevention and control. The report also recommends periodic assessment and treatment of human cases of parasitic infections.

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AUTHOR CONTRIBUTION

Salehi M:	Methodology creation and writing
Razavi S:	Manuscript writing, review and editing
Khajavian N:	Statistical analysis
Ghasemzadeh-moghaddam H:	Original draft manuscript preparation and writing
Minooeian Haghghi MH:	Funding acquisition and writing
Firouzeh N:	Manuscript writing, editing and review

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