Rev. Inst. Med. Trop. Sao Paulo
2016;58:42
http://dx.doi.org/10.1590/S1678-9946201658042

ORIGINAL ARTICLE

PREVALENCE, RISK FACTORS AND SYMPTOMS ASSOCIATED TO INTESTINAL PARASITE INFECTIONS AMONG PATIENTS WITH GASTROINTESTINAL DISORDERS IN NAHAVAND, WESTERN IRAN

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SUMMARY

We studied the prevalence of intestinal parasites (IPs), their risk factors and associated symptoms among patients with gastrointestinal disorders. A total of 1,301 participants aged 22 days-90 years were enrolled in this study. We used a structured questionnaire to obtain socio-demographic and stool examination to investigate intestinal parasite infections. Data analysis was performed using $SPSS_{16}$. The overall prevalence of intestinal parasites (IPs) was 32.2% (419/1,301). Three hundred and fifty nine cases/1,301 (27.6%) were infected with a single parasite and 60/1,301 cases (4.6%) presented polyparasitism. The most common IP was *Blastocystis* sp. 350/1,301 (26.9%), followed by *Entamoeba coli* 38/1,301 (2.92%), *Giardia lamblia* 30/1,301 (2.3%) and *Cryptosporidium* spp. 17/1,301 (1.3%). Regarding the socio-demographic variables, educational status ($p = 0.001$), contact with domestic animals and soil ($p = 0.02$), age above 15 years ($p = 0.001$) and seasons ($p = 0.001$) were significantly associated to intestinal parasitic infections. Concerning clinical characteristics, the presence of IPs was significantly associated to diarrhea (OR = 1.57; CI 95% = 1.24-1.98; $p < 0.001$) and dysentery (OR = 1.94; CI 95% = 1.03-3.66; $p < 0.04$). Our findings suggest that IPs are one of the main causal agents of gastrointestinal disorders. Improving the knowledge on local risk factors such as poverty, low level of education, poor sanitation, contact with soil and contact with domestic animal is warranted.

KEYWORDS: Gastrointestinal disorders; Parasitic diseases; Intestinal parasites; Iran.

INTRODUCTION

Globally, intestinal parasitic infections (IPIs) are one of the main causes of human morbidity and mortality especially in developing countries in which public health standards are not as high as in developed countries. IPIs are associated with climate conditions, poor sanitation and economic variables, lack of access to potable water, improper food and cultural habits[1-3]. The most important protozoan etiologic agents of IPIs are *Entamoeba histolytica* (affecting 50 million people), *Giardia lamblia* (affecting 200 million people), and also *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli* and *Microsporidia* spp. in immune compromised patients[4-6].

The soil-transmitted helminths (STH) *Ascaris lumbricoides, Trichuris trichiura,* and *hookworms* are the most common causes of IPIs and affect more than 2 billion people worldwide[7]. Other common helminths in IPIs are *Enterobius vermicularies, Strongyloides stercoralis, Taenia* spp., *Schistosoma mansoni* and *Hymenolepis nana*[8].

IPIs can cause diarrhea, abdominal pain, nausea and vomiting, bloating and weight loss. In addition, the rate of disability adjusted life years (DALYs) resulting from IPIs is approximately 39 million, indicating a substantial economic burden of these infections[9]. The effects of IPIs on children are: (i) growth disorders[10-12], (ii) vitamin A deficiency[13,14], (iii) iron deficiency anemia[15,16] and (iv) poor educational performance[17,18]. In addition, IPIs can result in severe complications in immune compromised patients such as those with HIV, transplant recipients and hemodialysis patients[19,20].

Gastrointestinal disorders refer to diseases involving the gastrointestinal tract such as the ones compromising the stomach, small and large intestines and rectum. They are associated to many symptoms such as diarrhea, abdominal/stomach pain, nausea and vomiting, weight loss, indigestion/dyspepsia, bloating and constipation. It has been shown that intestinal parasites have a high prevalence in patients with gastrointestinal disorders and are related to the above mentioned symptoms[21-24].

In Iran, despite a sustained improvement of the sanitary conditions that has happened during the last three decades, IPIs are still highly

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prevalent, especially among rural communities and small towns where residents survive thanks to agriculture and pastoral culture[20].

The main purpose of this study was to assess the prevalence and clinical manifestations of IPIs in patients with gastrointestinal disorders in Nahavand County, Western Iran, and investigate the association of IPIs with socio-demographic factors.

MATERIAL AND METHODS

Study areas

This cross sectional study was carried out from April to September 2014 in Nahavand County, (34°14'N, 48°14'E), Western Iran. The average annual rainfall in the area is 376 mm and the average temperature ranges from -0.5 to 25.6 ℃.

Population, studied variables and ethical issues

The Nahavand County has 196 villages and the population is estimated at 181,711 people in 112,297 families[25]. The majority of inhabitants are farmers and ranchers especially those living in rural areas and small towns around the Nahavand city.

The study population was composed of individuals with gastrointestinal disorders who presented one or more symptoms (abdominal pain, diarrhea, dysenteric diarrhea, crumping, nausea and vomiting, constipation) and were referred to medical laboratories in the Nahavand County. In order to calculate the sample size, we used a reference prevalence of 27% in patients with gastrointestinal disorders, a 95% level of confidence and 5% of marginal error[21]. The sample size required was 1,038 people, but for more accuracy 1,301 samples were collected.

A written consent was obtained from each participant, and then the participants were asked to respond to a questionnaire containing basic individual information (sex, age, residence), socio-demographic data (education, occupation), health status (water supply, contact with domestic animal and soil) and clinical symptoms such as abdominal pain (defined as a diffuse pain in the abdominal cavity), bloating (any abnormal general swelling or fullness), diarrhea (at least three loose or liquid stools per day), stomach pain (cramps or a dull abdominal ache), nausea and vomiting (the sensation or act of forcible and frequent emptying of the stomach) was filled by each participant. The study has enrolled individuals with gastrointestinal disorders that did not use any anti-parasitic drugs for one week prior to the study, and agreed to participate.

All the procedures were approved by the Ethics Committee of the Shahid Beheshti University of Medical Science (SBMU), Iran, before the beginning of the study. All the study participants were informed about the procedures and written informed consents were obtained from all of them prior to the sample collection.

Fecal sample collection and analysis

In this study, a simple random sampling technique was used. All the participants were given a clean and dry plastic collector, pre-labeled with their name and an identification number. Collected samples were sent to the medical laboratory located at the Ayatollah Alimoradian hospital, as soon as possible. All the samples were physically examined and screened for intestinal parasites by the following methods: I. Direct smear using the normal saline (0.85% NaCl solution), II. Lugol's iodine staining, III. Formalin-ether concentration technique, IV. Modified Ziehl-Neelsen acid-fast technique for the detection of *Cryptosporidium* spp. oocysts, V. Trichrome staining technique for the detection of intestinal protozoa such as *Giardia lamblia* and *Entamoeba histolytica*[26,27]. Prepared slides were examined under light microscopy (Zeiss, Germany) with 10×, 40× and 100× magnification.

Statistical analysis

Data analysis was carried out using the Statistical Package for the Social Sciences software version$_{16}$ (SPSS, Chicago, IL, USA). For the descriptive data, frequencies and percentages were used to describe the characteristics of the participants, including the prevalence of IPIs according to the residence, education, occupation, age and gender. The Pearson's Chi-square (Chi2) test and the Fisher's exact test were used for comparison of proportions of IPIs between different subgroups and odds ratios (OR) and 95% confidence intervals (CI) were used to associate the variables. A p value < 0.05 was considered statistically significant. A logistic regression was used to identify a potential contribution of each of the variables for the acquisition of intestinal parasite infections.

RESULTS

Socio-demographic characteristics

A total of 1,301 participants (52.4% male, 47.6% female) aged 22 days-90 years with a mean age of 26.31 ± 22.22 were enrolled in this study. Six hundred and eighty-three subjects (52.5%) lived in rural areas and 618 subjects (47.5%) lived in urban areas. Six hundred and eighteen (47.5%) had access to clean treated potable water and 683 (52.5%) used untreated water (river, well, rain). Four hundred and eighty-five participants (37.3%) reported a frequent contact with domestic animals or soil. The percentage of participants categorized as government employees, farmer, student, house wife, other and children < 17 years old were 7.5%, 11.4%, 8.2%, 20.1%, 12.8% and 40%, respectively.

Prevalence and distribution of intestinal parasitic infections

Overall, 32.2% (419/1,301) of the participants were infected with at least one intestinal parasite species. The prevalence rate among males (231/682 cases; 33.9%) was slightly higher than in females (188/619 cases; 30.4%). The most common IP was *Blastocystis* sp. (350 participants, 26.9%), followed by *Entamoeba coli* (38 participants, 2.92%), *Giardia lamblia* (30 participants, 2.3%) and *Cryptosporidium* spp. (17 participants, 1.3%). The prevalence of other IPs is presented in Table 1.

Three hundred and fifty nine participants (27.6%) were infected with a single parasite and in 60 cases (4.6%) polyparasitism was observed. The prevalence of intestinal helminths in this study (5 cases, 0.38%) was significantly lower than intestinal protozoa (416 cases, 32%) (p < 0.001).

Table 1
Prevalence of intestinal parasites (IPs) and poly-parasitism among individuals with gastrointestinal disorders in Nahavand County, western Iran (n = 1301)

Type of parasites	Mono parasites n (%)	Mixed infections n (%)	Total n (%)
Protozoa			
Blastocystis hominis	291 (22.4)	59 (4.5)	350 (26.9)
Giardia lamblia	24 (1.8)	6 (0.5)	30 (2.3)
Cryptosporidium spp.	8 (0.6)	9 (0.7)	17 (1.3)
Entamoeba histolytica/E. dispar	0	4 (0.3)	4 (0.3)
Entamoeba coli	14 (1.1)	24 (1.84)	38 (2.92)
Endolimax nana	5 (0.4)	12 (0.9)	17 (1.3)
Iodamoeba bucheli	4 (0.3)	5 (0.4)	9 (0.69)
Entamoeba hartmanni	6 (0.5)	3 (0.2)	9 (0.69)
Trichomonas hominis	3 (0.2)	2 (0.15)	5 (0.38)
Chilomastix mesnili	1 (0.07)	2 (0.15)	3 (0.23)
Total protozoa	356 (27.3)	60 (4.61)	416 (32.0)
Helminthes			
Enterobius vermicularis	2 (0.15)	1 (0.07)	3 (0.23)
Taenia spp.	0	1 (0.07)	1 (0.7)
Hymenolepis nana	1 (0.07)	0	1 (0.07)
Total helminthes	3 (0.2)	2 (0.15)	5 (0.38)
[*] **Total protozoa & helminthes**	**359 (27.59)**	**60 (4.61)**	**419 (32.2)**

n: number; [*] The prevalence of intestinal helminthes was significantly lower than intestinal protozoa ($p < 0.001$). Chi 2 test was used

Risk factors for intestinal parasitic infections (IPIs)

The results of the univariate analysis to evaluate the risk factors associated to IPIs and socio-demographic, environmental and personal hygiene factors are presented in Table 2. Among the selected socio-demographic variables, some were significantly associated to IPIs: educational status ($p = 0.001$), contact with domestic animals and soil ($p = 0.02$), age above 15 years ($p = 0.001$) and seasons ($p = 0.001$). However, regarding other socio-demographic characteristics such as sex, residence, water supply, and occupation, they did not show any association with the finding of parasites (Table 2). Moreover, according to the period of the year (season), it was observed that the prevalence of IPIs in July, August and September was significantly higher than in April, May and June ($p = 0.001$).

Using a binary logistic regression analysis, variables such as age, sex, residence, educational level, occupation, contact with domestic animals and soil, as well as the water supply status were added to the model. Then, we used a forward logistic regression to perform the analysis. Among the added variables, only the age (OR = 2.07; $CI_{95\%} = 1.4\text{-}2.8$; $p < 0.001$) and the seasons (OR = 1.16; $CI_{95\%} = 1.28\text{-}2.13$; $p < 0.001$) were identified as major socio-demographic determinants of IPIs.

Clinical features associated to IPIs

Concerning clinical characteristics, the presence of IPIs was significantly associated to diarrhea (OR = 1.57; $CI_{95\%} = 1.24\text{-}1.98$; $p < 0.001$), dysentery (OR = 1.94; $CI_{95\%} = 1.03\text{-}3.6$; $p < 0.04$) and constipation (OR= 0.298; CI 95%= 0.141-0.633; $p < 0.001$). The frequencies of other clinical characteristics including the presence of abdominal pain, nausea and vomiting, stomach pain and bloating did not show significant differences between parasite-infected and non-infected participants (Table 3). According to these data diarrheic disease may result from IPs in patients with gastrointestinal disorders.

DISCUSSION

The results of the present study have indicated that approximately one third of patients with gastrointestinal disorders were infected with one or more IPs. This rate is slightly higher than the results of a previous study conducted in similar populations in Zahedan, where the prevalence of IPIs was 27.3%[21].

Climate differences and thriving in agriculture and animal husbandry in Nahavand County might be responsible for the higher prevalence of IPs in this region compared to Zahedan. However, our subsequent study in a different region of Iran has shown a prevalence of 10% for IPs in patients with gastrointestinal disorders[28]. The differences of results can be attributed to peculiarities of the studied regions. Zahedan and Nahavand have poorer hygiene conditions and lower socio-economic status compared to the areas studied by Zebardast *et al*[28]. It is well established that providing a basic infrastructure and suitable education are effective tools to decrease the prevalence of IPIs[29]. In Iran, some areas located at the western, southern and eastern regions of the country are less developed in comparison with areas close to the capital and people live in traditional-built houses, although using untreated water from wells or rivers.

The findings of our study showed that protozoan infections (32%)

Table 2
Univariate analysis of risk factors associated with intestinal parasitic infection among individuals with gastrointestinal disorders in Nahavand County, western Iran (n = 1301)

Variables	Positive n (%)	Negative n (%)	OR	$CI_{95\%}$		*p*-value
				Lower	Upper	
Gender						0.177
Male	231 (33.9)	451 (66.1)	1.174	0.93	1.483	
Female	188 (30.4)	431 (69.6)	Reference			
Age (Year)						< 0.001
≤15	120 (22.7)	408 (77.3)	Reference			
16-30	99 (38.5)	158 (61.7)	2.13	1.542	2.944	< 0.001
31-45	85 (37.9)	139 (63.1)	2.079	1.483	2.915	< 0.001
46-60	74 (39.2)	115 (60.8)	2.188	1.532	3.123	< 0.001
>60	41 (39.8)	62 (60.2)	2.248	1.442	3.505	< 0.001
Educational Status						<0.001
Pre school	87 (21.2)	316 (78.4)	Reference			
Illiterate	54 (38.8)	85 (61.2)	2.354	1.552	3.572	<0.001
Primary school	101 (35.1)	187 (64.9)	2.002	1.424	2.814	<0.001
Secondary school	111 (36.9)	190 (63.1)	2.165	1.549	3.027	<0.001
Collage and above	66 (38.8)	104 (61.2)	2.352	1.591	3.475	<0.001
Residence						0.474
Rural	225 (32.9)	458 (67.1)	Reference			
urban	194 (31.4)	424 (68.6)	0.918	0.727	1.159	
Occupation (>17 years old)						0.791
Gov't employer	33 (34)	64 (66)	Reference			
Farmer & shepherd	62 (41.9)	86 (58.1)	1.398	0.821	2.38	0.217
Student	41 (38.3)	66 (61.7)	1.205	0.679	2.137	0.524
House wife	103 (39.3)	159 (60.7)	1.256	0.771	2.046	0.359
Other	62 (37.3)	104 (62.7)	1.156	0.684	1.954	0.588
Contact with domestic animal & soil						0.021
yes	175 (36.1)	310 (63.9)	1.323	1.043	1.68	
No	244 (29.9)	572 (70.1)	Reference			
Water supply status						0.590
Untreated (river, well, rain water)	225 (32.9)	458 (67.1)	1.067	0.842	1.352	
Treated pipe water	194 (31.4)	424 (68.6)	Reference			
Seasons						< 0.001
spring	139 (25.8)	399 (74.2)	Reference			
summer	280 (36.7)	483 (63.3)	1.664	1.305	2.121	

n: number; OR: odds ratio; Reference: The subgroup is considered as baseline

were more common compared to helminth ones (0.38%). In recent years, there has been a decrease in the incidence of intestinal helminth infections such as ascariasis, strongyloidiasis and hookworm infections in Iran[20,30]. This decline is probably due to general improvements in sanitation in Iran. However, some helminth parasites with direct fecal-oral transmission, such as *Hymenolepis nana* and *Enterobius vermicularis* remain common in many parts of the country[30]. In addition, among the five cases of helminth infections that were found in this study, *Hymenolepis nana* and *Enterobius vermicularis* were observed in one and three cases, respectively. Moreover, other authors in Iran and other countries have also found higher frequencies of protozoan compared to helminth infections[31-33].

The findings of the present study showed that *Blastocystis* sp. was the most common IP. This finding is in agreement with a previous study carried out in a similar population[22]. The pathogenicity of *Blastocystis* is still under debate. Some studies have strongly suggested that it is pathogenic and can be associated to several disorders[34,35], whereas other studies have reported that it is a commensal organism rather than a pathogenic one, and it is probably not responsible for clinical symptoms[35,36], The Centers for Disease Control and Prevention (CDC) suggests that *Blastocystis* treatment is necessary when there are no other causes for the occurrence of diarrhea or gastrointestinal disorders[37]. Previous clinical studies have shown that abdominal pain

Table 3
Clinical features based on questionnaires associated with IPIs among individuals with gastrointestinal disorders in Nahavand County, western Iran (n = 1301)

Clinical features	Examined individuals n	Infected with intestinal parasites n (%)	OR	$CI_{95\%}$		*p*-value*
				Lower	Upper	
Abdominal pain						0.409
Yes	980	322 (32.9)	1.130	0.860	1.485	
No	321	97 (30.2)	Reference			
Nausea or vomiting						0.250
Yes	58	23 (39.7)	1.406	0.820	2.411	
No	1243	396 (31.9)	Reference			
Stomach pain						0.752
Yes	522	165 (31.6)	0.959	0.756	1.217	
No	779	253 (32.6)	Reference			
Bloating						1.000
Yes	168	54 (32.1)	0.997	0.705	1.410	
No	1133	365 (32.2)	Reference			
Diarrhea						<0.001
Yes	585	221 (37.8%)	1.573	1.245	1.988	
No	716	198 (27.7%)	Reference			
Dysentery						0.04
Yes	40	19 (47.5%)	1.948	1.035	3.663	
No	1261	400 (31.7%)	Reference			
Constipation						< 0.001
Yes	62	8 (12.9%)	0.298	0.141	0.633	
No	1239	411 (33.2%)	Reference			

OR: odds ratio; n: Number; Reference: The subgroup is considered as baseline; *Chi 2 test was used

and diarrhea are two of the main symptoms among *Blastocystis*-positive patients[22,34]. Excepting for *Blastocystis*, *Giardia lamblia* (2.3%) and *Cryptosporidium* spp. (1.3%) were the most pathogenic protozoan parasites. In a recently published study that has been carried out in Iran, 4.73%, 2.30% and 0.06% of the studied population had *Blastocystis, Giardia lamblia* and *Cryptosporidium* spp., respectively[28]. These zoonotic protozoa are a great threat to public health in Iran. They can be transmitted through contaminated water and food. Animal wastes and farming practices are important sources of water and food contamination. Thus it is necessary to keep animals away from water and food sources that are used by humans.

Considering the symptoms, the results of our study have shown that there was a significant association between the presence of IPs and the finding of diarrheal stools. Diarrhea is one of the most reported symptoms due to IPs. *Entamoeba histolytica* can be the cause of severe dysentery, colitis, constipation, tenesmus and extra-intestinal complications[38]. *Giardia lamblia* is responsible for acute diarrhea, steatorrhea and malabsorption[39]. *Cryptosporidium* spp. infections has been increasingly recognized as a cause of diarrhea in both immune competent and immune compromised patients, as well as in children[19,20]. Although in our study, most of referred participant were infected with nonpathogenic protozoan parasites.

In this study, the described clinical symptoms are common complications induced by IPIs as well as by bacterial, viral or fungal agents. All the enrolled participants were examined by a physician, but we did not have access to their previous laboratory and medical records that would allow a more accurate interpretation on whether the symptoms were due to IPIs or to bacterial infections and/or malignancies.

Worldwide, previous studies have shown that there are several risk factors associated to the high prevalence and incidence of IPIs including untreated water supply, age, poor sanitation, low family income, low level of education, low level of parental education, eating unpeeled/unwashed vegetables and fruits, contact with livestock or pets and poor personal hygiene[40-44]. In our study, variables such as age above 15 years, low level of education, contact with soil and livestock, as well as the season were considered significant risk factors of IPIs. Results of the current study have shown that adult patients (aged ≥ 15 years) are almost twice as likely to be infected with IPs. Outdoor activities practiced by adults and a higher exposure to sources of IPs may explain the significantly higher prevalence of IPIs among these participants. Moreover, another reason for this finding is the higher prevalence of *Blastocystis* infections according to the increasing age[23,24]. In our study, the prevalence of IPIs was slightly higher in males (33.9%) than in females (30.4%), although there was no statistically significant difference ($p = 0.197$). This result is in agreement with previous studies in Iran[21,44]. Our findings have indicated that the season is a significant predictor of IPIs. The Nahavand County is located in a mountainous

area (far from the sea) and its weather in summer (July, August and September) is little to moderately warm (20-35 °C), whereas in spring (April, May and June) the weather is colder (10-18 °C). Another explanation to the higher prevalence of IPs during the summer could be the existence of more agricultural practices. This finding is in agreement with previous studies that have indicated that endemic regions have seasonal variations in the prevalence of IPIs[45-47].

There were some limitations in this study. Firstly, stool samples were collected only once, so that it is likely that the analysis of three consecutive stool samples could have increased the diagnostic sensitivity. Secondly, there is a possibility that some of the participants have decided to take antiparasitic drugs prior to the stool sampling, considering that metronidazole, mebendazole and albendazole can be purchased without prescription in Iran. Thirdly, due to the lack of facilities and financial funding we could not use all the laboratory available techniques, such as the Baerman technique that is used to find larvae of worms including *strongyloides* or adhesive tape to detect *Enterobius vermicularies*. The lack of these procedures might explain the absence of helminth infections in this study. In addition, it is likely that the prevalence of some protozoa such as *Entamoeba histolytica* and *Blastocystis* could be even higher if culture techniques had been used.

In conclusion, the findings of this study have indicated that IPIs are important causes of gastrointestinal disorders. Moreover, despite a significant reduction in the prevalence of intestinal helminths, the prevalence and incidence of intestinal protozoa are still a major public health concern in Iran. Thus, effective control programs to reduce the prevalence and incidence of IPIs should be considered in public health policies. Considering that the incidence of IPIs are significantly associated to poverty, lower levels of education, poor environmental sanitation, contact with soil and animals, there is an urgent need that these factors are effectively addressed.

ACKNOWLEDGMENTS

This study was part of the M.Sc. thesis of Hamed Kiani and was financially supported by Shahid Beheshti University of Medical Sciences (grant. nº 13/1285). The corresponding author would like to express his gratitude to the administrators and the staff of the Ayatollah Alimoradian hospital, authorities and personnel of Nahavand Health Care Network and centers, medical laboratories and the research participants for their kind cooperation during the sample collection.

CONFLICT OF INTEREST

The authors do not have any conflict of interest to report regarding this manuscript.

AUTHORS' CONTRIBUTION

H. Kiani and A. Haghighi designed the study, performed the research, analyzed data and, evaluated clinical records. They also collaborated to the manuscript writing. A. Rostami analyzed data and collaborated to the manuscript writing and revision. S. J. Seyyed Tabaei and N. Zebardast performed the laboratory assays and evaluated the clinical records. A. Solgi helped to collect samples and clinical data. All the statistical analysis was performed by E. Azargashb.

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Received: 07 July 2015
Accepted: 30 November 2015