OriginalArticle

Epidemiological Study of Intestinal Parasites in Referred Individuals to the Medical Centers' Laboratories of Haji-Abad City, Hormozgan Province, Iran, 2015

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Abstract

Background:intestinal parasitic infection is one of the most prevalent health problems in developing countries. This study was conducted to determine the prevalence of intestinal parasitic infection and its correlation with socio-demographic parameters in Haji-abad, 2015.

MaterialsandMethods: This cross-sectional descriptive study was conducted on 635 samples. After completing questionnaires, stool samples were assessed macroscopically, and microscopically using direct slide smear with saline and lugol, formalin-ether concentration, Ziehl-Neelsen staining to track *Cryptosporidium* species and Trichrome staining for the samples suspected to amoeba and other indeterminate cases. PCR using specific primers was conducted for *Entamoebahistolytica/E. dispar* suspected samples. The results were analyzed using $SPSS_{ver.16}$ software.

Results: Of total 635 samples, 198 cases (31.2%) were infected by at least one intestinal parasite. The most common parasites in this area were: *Blastocystis sp.* (105, 16.5%), *Endolimax nana* (43, 6.8%), *Entamoeba coli* (32, 5.0%), *Giardia lamblia* (31, 4.9%), and *Iodamoeba butschlii* (11, 1.7%). *Enterobius vermicularis* (1, 0.2%) was the only detected helminthic infection. Regarding socio-demographic variables, age, residence, sampling month, and job showed a significant correlation with IPIs (p-value=0.031, 0.019, 0.014, 0.012; respectively). None of nine microscopically suspected *E. histolytica/E. dispar* cases were confirmed by molecular investigations (PCR method) and were considered as *E. coli*.

Conclusion: In agreement with previous studies, helminthes infections show a dramatic decline compare to protozoa in this study. The relatively high incidence of intestinal protozoan infections in studies performed in Iran supports strategies for preventing the transmission and expansion of these parasites as a priority.

Keywords: Intestinal parasites, Prevalence, Haji-abad, Iran

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Introduction

Intestinal parasitic infection is a major public and economic problem with a worldwide distribution, especially in developing countries[1-3]. It is estimated that about 3.5 billion people were infected by protozoa and/or helminthes in the world annually, approximately 450 million are ill as a result of these infections[4], which about 58 million of them are children[5]. Diversity and prevalence of each parasitic infection is influenced by many factors, including social, cultural, economic, environmental factors, and life cycle of the parasites. Availability of clean water, personal and public health situations, temperature, humidity and survival of environmental stage of parasite are critical factors in transmission and spread of intestinal parasites[6].

In spite of a great development in health care during the last years, according to the studies performed in Iran[1,7-12], intestinal parasitic infections is a public health burden yet. On the other hand, in order to design an efficient infection control strategy in the endemic areas, a precise epidemiological data is a prerequisite[13].

The pattern and frequency of intestinal parasitic infections varies over the time, due to a change in behavior and population's life style. So a periodic epidemiological study is required. Recently, several epidemiologic study have been conducted in Iran, which shows a significant decline in overall intestinal parasitic infections, especially human helminthes infections[14,15].

The aim of this study was to determine the frequency of intestinal parasitic infections in population referred to the health centers in Haji-abad city, Hormozgan province, southern of Iran.

Methods

Study area, population, studied variables, and ethical issues: This cross-sectional study was carried out from April to November 2015 in Haji-abad city, (55.54N; 28.18E), southern Iran. This region has a dry and warm climate. The average annual rainfall in this area is 124 mm and the average temperature ranges from 15 to 31.5 °C. Haji-abad city is located at the most northern part of Hormozgan province (Figure 1), between Bandar abbas and Sirjan cities, with a population of 65,889; including 26,404 urban and 39,485 rural populations. Based on a reference prevalence of 48.8% in a previous study[7], a 5% level of confidence, and 4% of marginal error, the calculated sample size was 600 cases; but for more accuracy 635 cases were included in this study.

Prior to the sample collection, all of the participants were informed about the procedures. After taking a written consent, a personal information questionnaire including age, sex, socio-demographic data (education, occupation), chief complaint, signs and symptoms, such as abdominal pain, tenesmus, bloating, diarrhea, dysentery, constipation, and possible infection resource (water resources and animal contact) was filled for each participant.

Fecal sample collection and analysis: A total of 635 individuals were referred to medical laboratories for checkup or gastrointestinal complaints during April to November 2015 were studied. Stool samples (hard, soft, loose, watery, and dysenteric) were collected randomly and the samples were sent immediately to the Fateme Zahra hospital medical laboratory. Specimens were tested macroscopically and microscopically for protozoan and helminthic infections by direct smear (saline wet mount and lugol stain) and formalin-ether concentration methods. Prepared smears were studied by using an optical microscope under magnification of 10x and 40x.

All specimens were stained by modified Ziehl-Nelson technique for detection of *Cryptosporidium spp.*[16] and also, trichrome staining were done for undetermined, suspicious parasites and especially for more accurate detection of amoeba parasites[17]; and finally, stained samples were studied with magnification of 100x. In order to increase the sensitivity for detection of *Entamoebahistolytica/E. dispar*,Liquefied serum media (Horse serum, ringer, starch media; HSr+S) were also used in all suspected specimens for ameobic infection, based on macroscopic (i.e. dysentric, mucoid, bloody samples) and microscopic (i.e. morphologically suspected amoebae) findings[18].

In order to confirm the diagnosis of *E. histolytica/E. disapr*in suspected microscopic cases, a PCR program was also conducted[19]. Two sets of oligonucleotide primers, Hsp1 (GAGTTCTCTTTTTATACTTTTATATGTT) and Hsp2 (ATTAACAATAAAGAGGGAGGT) for *E. histolytica* and Dsp1 (TTGAAGAGTTCACTTTTTATACTATA) and Dsp2 (TAACAATAAAGGGGAGGG) for *E. dispar* were used for PCR amplification. These primers amplify a region of about 340 bps and 470 bps of locus D-A for *E. histolytica* and *E. dispar*, respectively. Amplification consisted of initial denaturing 94°C for 5 minutes (min),

Figure1. Map of Homozgan province and Haji-abad city.

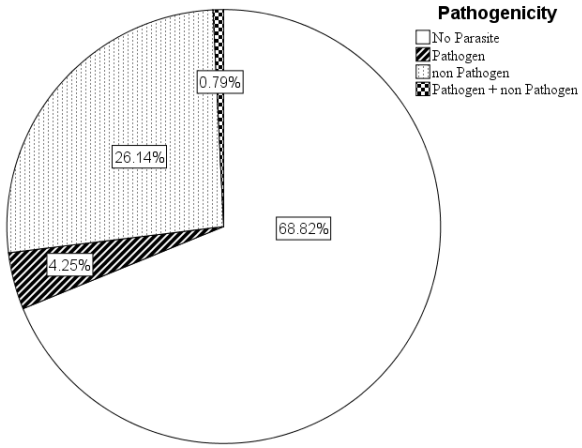

Figure2. Non infected, pathogen, non-pathogen, and poly-parasitism in 635 individuals referred to the medical health centers laboratories in Haji-abad city.

35 cycles of denaturation at 94°C for 30 seconds (s), annealing at 55 °C for 45 s and extension at 72°C for 60 s, with a final extension of 5 min at 72°C. Amplified products were visualized by electrophoresis using 1.5% agarose gels (Fermentas, # R0491) in Tris-boric acid-EDTA buffer containing ethidium bromide

Statistical analysis: Obtained data were analyzed using SPSS ver.16 to show the frequency and prevalence of various parasitic infections. In addition to, descriptive statistics, Pearson-chi square, t-test and forward logistic regression are also used for analysis and interpretation of studied variables.

Results

Socio-demographic characteristics: Out of 635 admitted individuals, 320 males (50.4%) and 315 females (49.6%), aged between 1 to 84 years old (mean 21.9±17.9) with majority aged less than 11 years (42.5%, 270/635) were studied. Of this population, 414 cases (65.2%) were resident of urban areas, and 221 cases (34.8%) of rural areas. Twenty two out of 635 cases (3.5%) mentioned a history of untreated water usage or soil and frequent animal contact. The participants over 17 years old, were categorized in five groups based on occupation, as follows: government employee (68 cases, 20.2%), farmers and ranchers (16 cases, 4.8%), house wife (84 cases, 25.0%), student (5 cases, 1.5%), and the others (163cases, 48.5%). The most frequent cause of referral was checkup (387 cases, 60.9%), and the most frequent chief complaints were abdominal pain, diarrhea, and tenesmus (16.1%, 16.1%, and 9.0%; respectively).

Prevalence and distribution of intestinal parasitic infections: In this study, 198 participants (31.2%) were infected by one or more intestinal parasites, including 33 cases (5.2%) with co-infection; in which, 183 cases were detected on direct smears (with saline and logul) and 7 cases detected after performing formalin-ether sedimentation technique (with a statistically significant difference, p-value <0.001). One hundred sixty six cases (26.14%) were infected by non-pathogen protozoa, 27 cases (4.25%) by pathogen parasites, and 5 cases (0.78) had pathogen and non-pathogen co-infection (Figure 2).

The distribution of IPIs among studied populations, are shown in table 1. 105 *Blastocystis*sp (16.33%), 43*Endolimax nana* (6.77%),32 *Entamoeba coli* (5.03%), *and* 31 *Giardia lamblia*(4,88%) were the most frequent observed protozoan parasites, respectively. Only one of samples (0.16%) was infected with *Entrobiusvermicularis eggs.* None of nine microscopically suspected *E. histolytica/E. dispar* cases were confirmed by molecular investigations (PCR method) and were considered as *E. coli* (the second differential diagnosis). All of obtained samples were stained by modified Zheil-Nelson technique, but no positive *cryptosporidium* spp. was observed.

Table 1: Distribution of parasites in people referred to Haji-abad's health centers laboratories.

Type of parasites	Mono infection n (%)	Mixed infection n (%)	Total n (%)
Protozoa			
Blastocystis sp.	77 (12.12)	28 (4.41)	105 (16.53)
Endolimax nana	36 (5.67)	7 (1.10)	43 (6.77)
Entamoeba coli	17 (2.66)	15 (2.35)	32 (5.03)
Giardia lamblia	26 (4.09)	5 (0.78)	31 (4.88)
Iodomoebabutschilii	2 (0.31)	9 (1.42)	11 (1.73)
Chilomastixmesnilii	4 (0.63)	2 (0.31)	6 (0.94)
Trichomonashominis	2 (0.31)	1 (0.16)	3 (0.47)
Entamoebahartmanni	0 (0.0)	2 (0.31)	2 (0.31)
Total protozoa	164 (25.82)	33* (5.19)	197 (31.02)
Helminthes			
Entrobiusvermicularis	1 (0.16)	0 (0.0)	1 (0.16)
Total helminthes	1 (0.16)	0 (0.0)	1 (0.16)
Total protozoa & helminthes	165 (25.98)	33 (5.19)	198 (31.18)

* Total cases with poly parasitism

Sixty four cases with doubtful, unrecognizable parasite were stained using trichrome technique, and all of the cases (100%) showed at least one detectable parasite. Cultivation in HSr+S culture media were done in 45 cases with mono and/or co-infection; in which, in 42 cases an agreement with microscopy diagnosis were seen. In one case with triple infection (*Blastocystis + E. coli + I. butschilii*), and two cases with co-infection (a double infection with *E. coli* plus *G. lamblia*, and a triple infection with *Blastocystis*+ *E. coli + G. lamblia*), *Iodomoebabutschilii*and *Giardia lamblia*were not grown.

Intestinal parasitic infection (IPIs) risk factors: In order to determine the risk factors for intestinal parasitic infection, some socio-demograhic factors, such as age, sex, educational status, sampling time, location, contact with contamination resources (i.e. untreated water, and frequent contact with animals), and job were analyzed.

As shown in Table 2; there was statistically significant relationship between age groups, residency location (urban or rural), sampling month, job, and prevalence of intestinal infection (p values=0.031, 0.019, 0.014, 0.012; respectively). Nevertheless, there was not found any relationship between sex, level of education, contact with contamination resources, and the prevalence of intestinal parasite (p values=0.970, 0.841, 0.141; respectively).

As shown in Table 2 there is an increase in prevalence of parasitic infection from April to June 2015, and a dramatic decline in parasitic infection in July.

In order to determine the predictive factors for IPIs, a forward logistic regression analysis were conducted, and parameters including sex, age group, residency, education status, job, history of contact with contamination resources, and date of sampling were added to the model (Table 2). This model showed that date of sampling (OR=1.52, $CI_{95\%}$=0.76-3.04, p value=0.005), age group (OR=2.47, $CI_{95\%}$=1.24-4.91, p value=0.021), and residence (OR=1.48, $CI_{95\%}$=1.03-2.14, p value=0.034) were the major determinant of prevalence of intestinal parasitic infection.

Table 2: Frequency and percentage of intestinal parasites based on socio-demographic variables in subjects referred to medical centers laboratories of Haji-abad, 2015.

Variables	Positive n (%)	Negative n (%)	OR	CI 95%		p-value
				Lower	Upper	
Gender						0.970
Male	100 (31.2)	220 (68.8)	1.006	0.719	1.408	0.970
Female	98 (31.1)	217 (68.9)	Reference			0.970
Age (Year)						0.031
<7	48 (23.6)	155 (76.4)	Reference			0.033
7-12	22 (27.2)	59 (72.8)	0.418	0.215	0.811	0.010
13-18	5 (26.3)	14 (73.7)	0.503	0.236	1.074	0.076
19-30	48 (35.8)	86 (64.2)	0.482	0.149	1.559	0.223
31-50	55 (36.4)	96 (63.6)	0.753	0.383	1.484	0.413
>50	20 (42.6)	27 (57.4)	0.773	0.397	1.506	0.450
Educational status						0.841

Intestinal parasitic infections (IPIs) and clinical manifestations: Regarding to the clinical manifestations and chief complaint in participants and the frequency of IPIs, there was no statistically significant relationship between them (p value=0.761), except for dysentery (OR=23.191, CI95%=2.948-182.457, p value<0.001)

Table 3: Chief complaint of referred individuals to themedical labs of Haji-abad, 2015.

Chief complaint	Positive n (%)	OR	$CI_{95\%}$		p-value
			Lower	Upper	
Abdominal pain					0.780
Yes	33 (32.4)	1.067			
No	165 (31.0)	Reference	0.678	1.679	
Tenesmus					0.505
Yes	20 (35.1)	0.823	0.465	1.459	
No	178 (30.8)	Reference			
Bloating					0.704
Yes	8 (34.8)	0.844	0.352	2.025	
No	190 (31.0)	Reference			
Diarrhea					0.780
Yes	33 (32.4)	1.067	0.678	1.679	
No	165 (31.0)	Reference			
Dysentery					<0.001
Yes	10 (90.9)	23.191	2.948	182.457	
No	188 (30.1)	Reference			
Constipation					0.222
Yes	3 (17.6)	0.465	0.132	1.636	
No	195 (31.6)	Reference			
N.P.I.D.*					0.970
Yes	6 (31.6)	0.981	0.367	2.620	
No	192 (31.2)	Reference			
N.I.D.**					0.429
Yes	6 (24.0)	1.455	0.572	3.700	
No	192 (31.5)	Reference			
Checkup					0.241
Yes	114 (29.5)	1.227	0.871	1.727	
No	84 (33.9)	Reference			

* Non parasitic infectious disease

** Non infectious disease

(Table 3).

Discussion

Intestinal parasites are one of the important health problems in almost all communities, especially in tropical and subtropical areas. The distribution and pattern of intestinal parasites are strongly depended on human behavior and life style, overcrowding, poverty, and seasonal variations, such as temperature, rainfall and humidity[20-22].

In this study, 31.2% of people of this region were infected by one or more intestinal parasites. Based on this study, almost one third of studied individuals are infected by a parasitic infection, as in kiani et al[24] (32.2%), and shahdoost et al[27] (27.1%) nearly same results were reported. The prevalence of protozoan infection was 31.0%. This finding shows a dramatic progressive decline in the prevalence of protozoan infection regarding to the previous studies were done in Bandar Abbas by Sheiban et.al[23] and in rural areas of Bandar Abbas by Bairami et.al[7], from 80.4% in 1981 to 48.8% in 2011. This could be, to some extent, due to more suitable basic infrastructures and educations, improved health facilities, and availability of clean water regarding to the past; and on the other hand, the lower humidity and climate differences with the other regions of Hormozgan province because of higher altitude and distance from the sea. Nevertheless, this is significantly higher than overall prevalence of intestinal parasite infection in Iran determined by Zebardast etal in 2012-2013 (10.06%); which represents very low hygienic situations in this area regarding to the overall health level of Iran[22].

This study showed that the prevalence of protozoan infection (31.2%) is higher than helminthes infection (0.16%). This finding is correlated with previous studies in Iran (with the same ratio, 9.7% to 0.3%, and a higher magnification, attributable to the lower socio-economic conditions), which could be attributed to the more readily infection by protozoan cyst through contaminated water and food resources, which makes their acquisition very easier[22,24,27].

At the present study, *Blastocystis*sp. was the most common intestinal parasite, followed by *Endolimax nana*, *Entamoeba coli*, and *Giardia lamblia*. As previous studies, *Blastocystissp*, and *Giardia lambelia* were the most common detected non-pathogenic and pathogenic protozoa, respectively[22,24,25]. As in kiani et al, study[24], we found a statistically significant relationship between *Blastocystis*sp and *Entamoeba coli* co-infection (p value=0.001); this could be an accidentally finding due to high prevalence of this two protozoa, or as a consequence of similar life cycle and distribution, or due to transmission situations similarities.

As in studies performed by Badparva et al. in 2014[26], and Kiani et al, in 2014[24], there was no significant relationship between sex and intestinal parasitic infection. But in contrast to Kiani et al research, we didn't find any statistically significant difference between level of education, and a history of contaminated resources contact, possibly due to difference in case selection and studied groups.

Our study showed a strong correlation between date of sampling and the prevalence of intestinal parasitic infection. This finding emphasize on the rule of temperature and humidity on the prevalence of intestinal parasites. In contrast to other studies in Nahavand county[24], Qazvin province[20], and Tehran[28] which showed a higher prevalence of parasitic infection in summer, our study showed a higher prevalence of parasitic infection in last spring; which could be attributed to a longer duration of heat and lower humidity and rain fall in this region. Our findings also indicated that date of sampling, and age groups are good predictors of the prevalence of intestinal parasitic infections[21].

By microscopy methods differentiation of *E. histolytica from E. dispar* or from *E. coli* and *Entamoebahartmanni* is difficult. All the 9 suspected isolates for *E. histolytica/E. dispar* were checked by PCR. This method confirmed that the tested samples were negative for the *E. histolytica or E. dispar* infection.

Conclusion

In agreement with previous studies, helminthes infections show a dramatic decline compare to protozoan infection in this study. The relatively high incidence of intestinal protozoan infections in studies performed in Iran supports strategies for preventing the transmission and expansion of these parasites as a priority. Adequate knowledge and periodic surveillance of the prevalence of parasites and the demographic variables are the prerequisites for this disease control program planning.

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