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Intestinal parasitic infections frequency in referred patients to a large teaching hospital, Khuzestan, Southwest, Iran, 2017

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Abstract. Intestinal parasitic infections (IPIs) are among the most important infectious diseases in Iran. A cross sectional study was designed to determine frequency of intestinal parasites among referrals to a large teaching hospital in Khuzestan, Southwest of Iran, 2017. A total number of 5613 stool samples were examined through direct smear and formalin-ether concentration methods to detect possible parasitic infections. Samples consisted of 2643 (47.09%) male and 2970 (52.91%) female. A total of 1468 (26.15%) samples were positive (13.11% male and 13.4% female) and 4145 (73.85%) were negative. The results also showed that 255 of samples had more than one type of parasite (mix infections). Counting single and mix parasite infections, the total number of positive cases reached to 1723. Helminthes parasites were present in 12 (0.7%) cases, while intestinal protozoan parasites were in 1711 (99.3%) cases. Almost equally, pathogenic and non-pathogenic parasites infected 860 (49.91%) and 863 (50.09%) of patients, respectively. The frequency for helminthes was determined at 0.52% with *Hymenolepis nana* and *Enterobius vermicularis* however, *Giardia lamblia* in 38.54% and *Entamoeba histolytica/dispar* at 10.68% were concluded as protozoa elements. The IPIs frequency was recorded in female and male patients at 49.16% and 50.14%, respectively. According to the current results the infection rate of intestinal parasites has been significantly reduced especially for helminths infections in this region possibly due to public attention to health issues such as; increased awareness of people, improvement of sanitation, seasonal variations, health education and personal hygiene.

INTRODUCTION

The factors of low levels of environmental sanitation, cultural practices, low socio-economic status, and frequent contact with contaminated water favour the transmission and wide distribution of intestinal parasitic infections (IPIs) (Abadi *et al.*, 2017) . Parasites are the most common concern in connection to growth deficiencies and cognitive development; especially in children (Degarege and Erko, 2013). Among parasites, IPIs proved to have a high prevalence and it is estimated that more than three billion people are infected in the world today (Fallahi *et al.*, 2016). Almost 450 million individuals suffer from parasitic infections and approximately half this number are children (Sadeghi and Borji, 2015; Alum *et al.*, 2010). The majority of IPIs cases are asymptomatic, however, they can be the cause of dehydration, mucoid or watery diarrhoea, nausea, vomiting, abdominal pains, fever, growth retardation in children, vitamin deficiencies, iron deficiency anaemia and physical and mental health disorders (Alum *et al.*, 2010; Shobha *et al.*, 2013). Lack of health care, poverty, illiteracy, tropical wet climate and no access to safe drinking water are the main sources of transmission of various microorganisms including parasitic agents (Feiz Haddad *et al.*, 2016). Existing estimates indicate that more than one-quarter of the world's population are chronically infected with IPIs and that most of these infected people live in developing countries (Alli *et al.*, 2011) with a lack of standard public health principles, economic instability, and social marginalization (Feiz Haddad *et al.*, 2013).

The IPIs have massive consequences on individual health and determined preventive efforts were in place to control enteric infections. Yet, parasites such as *Giardia intestinalis*, *Entamoeba* spp., *Cryptosporidium* spp., *Isospora belli*, *Microsporidia* spp. and *Cyclospora* spp., pose unique epidemiological constraints as they are ubiquitous in nature and treatment is still not available for some; others are highly resistant to chlorination and other antiseptic processes (Mariam *et al.*, 208). However, as a result of changes in human behaviour and life styles, parasitic infection patterns' dissemination in the population may alter in the course of time. Numerous studies have been recently conducted to divulge the intestinal parasites prevalence in different parts of Iran and demonstrate a sharp decline in human parasites prevalence, especially helminthes infections (Rasti *et al.*, 2017; Meamar *et al.*, 2007). Therefore, intermittent epidemiological studies will provide more precise understanding for parasitic infection frequency. Within this context, the present study was conducted to provide comprehensive data of the current status of IPIs frequency among referred patients of a large teaching hospital from Dezful County in Khuzestan, Southwest Iran, August 2016 to March, 2017 to evaluate the determinant factors in referred contributors. The establishing of such information should be constructive for public health services in order to justify and facilitate reassessment of control strategies and policies in terms of reducing the IPIs and improving strategies for future intestinal parasite prevention and control (Fallahizadeh *et al.*, 2017). Dezful County, the second largest city of Khuzestan Province is situated in Northeast of the province and the teaching hospital is the largest and most important academic medical centre where many patients from Dezful County are referred to this health centre.

Study area

The study was designed to examine stool samples of referred individuals to a large teaching hospital from Dezful city and in addition, many of the referred patients are from cities of Shooshtar, Andimeshk, Shoosh, Shawoor, Gotvand, Lali, Dasht Abbas, Dehloran, Abdanan and Mormoori. The reason for choosing of this center is the geographical location and more facilities of this center than others in Northern Province of Khuzestan, which has been the main reference for many people of cities and villages surrounding this center (Maniey *et al.*, 2012). Furthermore, this study can indicate the condition of these infections in the northern region of Khuzestan province and part of Ilam province. Dezful is located in Northern Province Khuzestan, 140 km away from the provincial capital of Ahvaz, 721 kilometres away from the national capital of Tehran and 300 kilometres away from the Persian Gulf. According to Population and Housing Census of Iran's Statistics Centre; City Population in 2011 was 420,000. The area is at an altitude of 143 meters above sea level with a hot semi-arid climate with extremely hot summers and mild winters. Rainfall is higher than most of southern Iran, but is almost exclusively confined to the period from

November to April, though on occasions it can exceed 250 millimetres (9.8 in) per month or 600 millimetres (24 in) per year.

Study strategy and sampling

A cross-sectional intestinal parasite investigation was directed on referrals patients from August 2016 till March 2017. In addition, a retrospective study of intestinal parasites was also carried out by reviewing previous laboratory records. The study sample size was assessed by means of single population proportion formula, considering prevalenceof the previous study with a confidence level of 95% and a 5% margin forerror. A sample size of 2795 was assigned, however, after multiplying this digit by two to account for design effect the final figure was planned to be 5590 cases. Subjects for the study were intended to be selected from referrals of all ages and both sexes. The single stage sampling technique was applied based on; 1) data collection methods, 2) applied examination techniques (direct saline smear and formalin ether concentration technique), and 3) contributor's profiles study (studied area residents, age and gender). At the beginning of study, an oral briefing explaining of the study was given to the participants and a voluntary written informed consent was taken. The privacy rights of referrals' subjects were observed at all times.

Data collection and laboratory processing

A demographic profile study was planned and related data were collected via patient by patient interview. In addition, data connected to single stool specimen was recorded and processed based on each participant.

Pre-labelled wide mouth screw capped containers with patient's code and names were distributed. The contributors were given a scoop to provide a thumb size faecal sample and asked to ensure the sample was not contaminated with urine. The samples were processed and examined for the presence of parasites by the formalin ether concentration technique (Allen & Ridley, 1970). Briefly, 1 to 2g of faecal sample was mixed with 7 ml of formalin and 3 ml ethyl acetate, centrifuged, stained with 0.85% iodine and examined under light microscope. To avoid losing fragile parasites, direct saline smears method was also applied. The patients' extracted data including, stool examination information and species of isolated intestinal parasites was recorded. During the study, the individuals who were found to be infected with intestinal parasites were referred to GPs for treatment based on the national instructions protocols.

Non-pathogenic and pathogenic parasites

Non-pathogenic intestinal parasites in this study were single-celled protozoa which commonly found in the gastrointestinal tract but never related with illness even in individuals with weak immune systems. Symptomatic persons who had indicated these protozoa in their stool samples should be scrutinized for other reasons of their symptoms. The included non-pathogenic intestinal protozoa in this study were;

E. d= Entamoeba dispar
E. c= Entamoeba coli
B. h= Blastocystis hominis
I. b= Iodamoeba butchelii
T. h= Trichomonas hominis
C. m= Chilomastix mesnili
E. n= Endolimax nana

Parasites that caused a disease in patients were considered as pathogenic parasites for this study. The pathogenic intestinal parasites include:

G. l= Giardia lamblia
E. h= Entamoeba histolytica
H. n= Hymenolepis nana
E. v= Enterobius vermicularis

Ethical Consent

Ethical authorization and ethical clearance for this study were obtained from the Ethics Committee of the Dezful University of Medical Sciences (Ref. No. IR.DUMS.REC. 1396.18). In addition, informed agreement was obtained from studied contributors and filed for each patient confidentially, prior to data assortment. The contributors were informed that the used procedures were safe and did not pose any potential risk to them; also that their identities and personal particulars would be kept strictly confidential. The contributors were additionally informed that their contribution was voluntary and they could withdraw at any time throughout the study without giving any reason.

RESULTS

A participant demographic characteristic study was considered for a total of 5613 contributors with 2643 (47.09%) male and 2970 (52.91%) female. Figure 1 shows the distribution of referred patients according to month and gender.

From the 5613 participants, 1468 (26.15%) were positive at least for one parasite species, while mixed infection patients amounted to 255 (4.54%). *Hymenolepis nana* and *Enterobius vermicularis* were the only helminth parasites found in 9 (0.53%) and 3 (0.17%) of contributors, respectively. However, the most common protozoan parasites were *G. lamblia* in 664 (38.54%), *B. hominis* in 489 (28.38%) and *E. histolytica/dispar* in 184 (10.68%) of the contributors.Of the positive infected cases, pathogenic and non-pathogenic parasites were counted in 860 (49.91%) and 863 (50.09%) cases, respectively (Figure 2). The mixed parasite infections were detected at 49.86% of females and 50.14% of males (Table 1). The proportional frequency of males (47.9%; 2643/5613) was slightly lower than that of females (52.91%; 2970/5613). The females were less infected than males by all protozoa except *E. histolytica/dispar* (Table 2). The other intestinal protozoa frequencies were 307 (17.82%) for *E. coli*, 53 (3.08%) for *I. butchelii*, 4 (0.23%) for *T. hominis*, *C. mesnili* 8 (0.46) and *E. nana* 2 (0.12%). According to the statistics from Table 3 the most and the least common protozoa parasitic infections were 38.54% and 0.12% for *G. lamblia* and *E. nana*, respectively. Of 5613 contributors, 1723 were detected (either single or in mixed combinations) as having parasitic infections in which protozoa parasites were predominantly identified in 1711 (99.3%) of cases, while helminths were marginally existed only in 12 (0.7%) cases. Table 3 summarizes the results for parasitological examination in contributors, August 2016 to March, 2017.

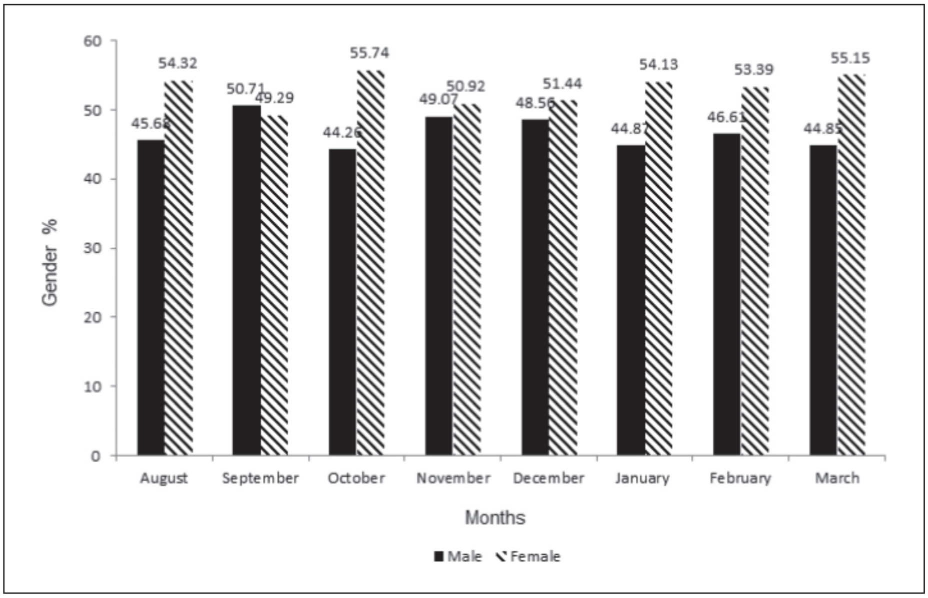

Figure 1. Distribution of contributors by month and gender, August 2016-March 2017.

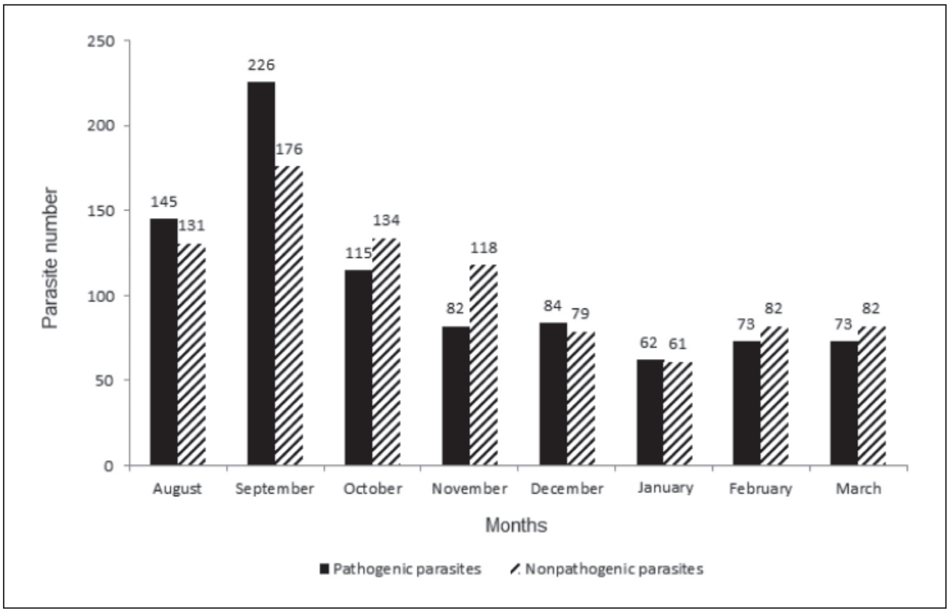

Figure 2. Distribution of positive IPIs in contributors by month, pathogenic and non-pathogenic parasites, August 2016-March 2017.

Table 1. Distribution of IPIs in contributors by month and gender, August 2016–March 2017

Month	Cases examined	Positive %	Negative %	Positive in males	Negative in males	Positive in females	Negative in females
Aug.	821	28.99	71.01	117	258	121	325
Sept.	1120	29.65	70.35	173	395	159	393
Oct.	784	27.29	72.71	96	251	118	919
Nov.	591	27.56	72.44	87	205	77	226
Dec.	628	22.61	77.39	77	228	65	258
Jan.	624	19.55	80.45	62	218	60	284
Feb.	517	25.72	74.28	72	169	61	215
Mar.	524	23.47	76.53	52	183	71	218
Total	5613	26.15	73.85	736	1907	732	2238

Table 2. Distribution of IPIs in contributors by types, gender and months, August 2016–March 2017

Parasites	G. l		E. h/E. d		E. c		B. h		I. b		T. h		C. m		H. n		E. n		E. v		Positive
Month	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Aug.	67	53	9	16	28	30	26	40	3	3	1	-	-	-	-	-	-	-	-	-	276
Sept.	96	83	25	22	30	28	58	51	4	4	-	1	-	-	-	-	-	-	-	-	402
Oct.	41	45	12	14	23	27	31	41	7	4	1	-	-	-	-	3	-	-	-	-	249
Nov.	29	31	12	8	18	19	42	33	2	4	-	-	-	-	1	1	-	-	-	-	200
Dec.	32	25	12	14	10	12	22	24	7	1	1	-	1	-	-	1	-	1	-	-	163
Jan.	18	28	5	7	11	9	21	17	1	1	-	-	-	-	1	-	-	1	2	1	133
Feb.	35	17	8	12	13	17	26	17	2	4	-	-	2	1	1	-	-	-	-	-	155
Mar.	27	37	5	3	14	18	13	27	1	5	-	-	1	3	-	1	-	-	-	-	155
Total	664		184		307		489		53		4		8		9		2		3		1723

M= male F= female
G. l= Giardia lamblia *E. h/E. d= Entamoeba histolytica/dispar* *E. c= Entamoeba coli*
B. h= Blastocystis hominis *I. b= Iodamoeba butchelii* *T.h= Trichomonas hominis*
C. m= Chilomastix mesnili *H. n= Hymenolepis nana* *E. n= Endolimax nana*
E. v= Enterobius vermicularis

Table 3. Distribution of IPIs in contributors, August 2016–March, 2017

Month	G. l	%	E. h/E.d	%	E. c	%	B. h	%	I. b	%	T. h	%	C. m	%	H. n	%	E. n	%	E. v	%	Positive
Aug	120	43.48	25	9.06	58	21.01	66	23.92	6	2.17	1	0.36	-	-	-	-	-	-	-	-	276
Sept	179	44.53	47	11.69	58	14.43	109	27.11	8	1.99	1	0.25	-	-	-	-	-	-	-	-	402
Oct.	86	34.54	26	10.44	50	20.08	72	28.92	11	4.42	1	0.40	-	-	3	1.20	-	-	-	-	294
Nov.	60	30	20	10	37	18.50	75	37.50	6	3	-	-	-	-	2	1	-	-	-	-	200
Dec.	57	34.97	26	15.95	22	13.50	46	28.23	8	4.91	1	0.61	1	0.61	1	0.61		0.61	-	-	163
Jan.	46	37.40	12	9.76	20	16.26	38	30.89	2	1.63	-	-	-	-	1	0.81	1	0.81	3	2.44	123
Feb.	52	33.55	20	12.91	30	19.35	43	27.75	6	3.87	-	-	3	-	1	0.64	-	-	-	-	155
Mar.	64	21.29	8	5.16	32	20.65	40	25.81	6	3.87	-	-	4	-	1	0.64	-	-	-	-	155
Total	664	38.54	184	10.68	307	17.82	489	28.38	63	3.08	4	0.23	8	0.46	9	0.52	2	0.12	3	0.17	1723

G. l= Giardia lamblia *E. h/E. d= Entamoeba histolytica/dispar* *E. c= Entamoeba coli*
B. h= Blastocystis hominis *I. b= Iodamoeba butchelii* *T. h= Trichomonas hominis*
C. m= Chilomastix mesnili *H. n= Hymenolepis nana* *E. n= Endolimax nana*
E. v= Enterobius vermicularis

This study showed a prevalence of 24% (1349/5,613) for pathogenic intestinal infections (protozoa and helminths). Pathogenic parasitic infection varied across the test period; the most and the least common frequencies were found in September (335 cases) and January (100 cases), respectively. Figure 3 indicates infection frequency for protozoa and helminths inpatients regardless of single or mixed infection. Table 3 also shows that *H. nana* and *E. vermicularis* were the only helminths parasitic infections found in this study. In addition, the most common detected protozoa were *G. lamblia* followed by *B. hominis* (a parasite which still has a controversial pathogenic role). The protozoan parasites accounted for the vast

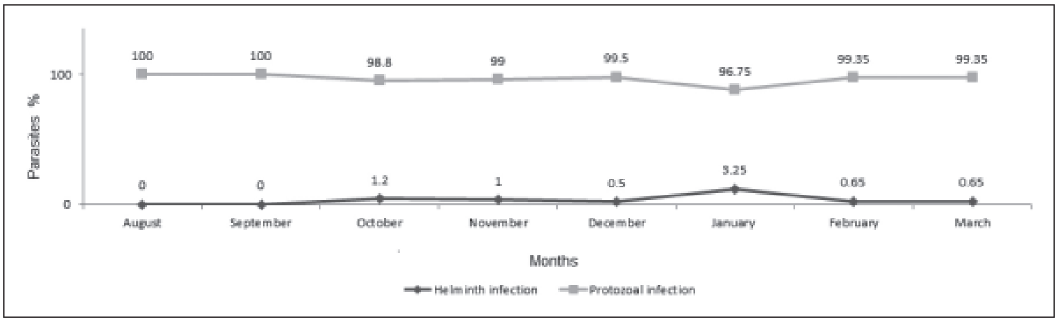

Figure 3. Distribution of positive IPIs in contributors by helminths and protozoa, August 2016-March 2017.

majority of infections for 1711 (99.3%) of patients. Among those, *G. lamblia* was the most common protozoa with 664 (38.54%) cases, although not at significant level, more males appeared to be infected than females. *B. hominis* was the second most common species with an overall prevalence of 489 (28.38%) in subjects. Similar to the *G. lamblia*, *B. hominis* was also detected slightly more in males than females**.**

E. histolytica/dispar was the third most common protozoa species isolated in 184 (10.68%) patients, contrary to the other protozoa this was detected slightly more in females.

From a helminthes point of view, *E. vermicularis* was identified through stool examination as the only nematode in 3 patients. However, *H. nana* was the only Platyhelminthes; detected in 9 patients; 6 females and 3males.

DISCUSSION

It is estimated that 3.5 billion people, approximately half of the world's population, are infected with intestinal parasites causing nearly 450 million illnesses, annually. The intestinal parasites are particularly serious in endemic regions with poor hygiene; however, they could potentially become more common in non-endemic regions due to increased immigration and travel (WHO, 2004). The present study was carried out on parasite; protozoa and helminthes infection, using stool samples which were examined and found to contain at least one parasite species over an 8-month period (August-March) in referred patients to a large teaching hospital in 2017. As shown in the current study, intestinal parasitic infections (IPIs) are still a public health problem revealing an overall prevalence of 30.7% (1723/5613) among the individuals from the studied area. The present study found a significant difference between protozoan frequency compared with helminthes. The frequency of helminthes was 0.21% (12/5613), however, the statistics showed that protozoa frequency was substantially higher at 30.5% (1711/5613). Since, in most cases, the protozoa are directly transmitted it is expected that the protozoa frequency be higher than the helminthes.

In general, with the exceptional of *E. histolytica/dispar*, intestinal parasites were detected more in males than females. *G. lamblia* was the most common pathogenic parasite in 38.54% (664/1723) of the patients. Giardiasis as a universal flagellated protozoan has a significant impact on public health, often causing major epidemics with considerable effects on children's growth and intellectual retardation (Feng & Xiao, 2011). *G. lamblia* mostly causes a self-limited infection categorized by diarrhoea, bloating, abdominal cramps, weight loss and mal-absorption. However, asymptomatic infections are also common, especially in developing countries (Thompson, 2000). Contrary to the current study, the *Giardia* rate in industrialized countries was low in a study from Italy ranging from 0.4% to 6.2% (Crotti *et al.*, 2005). In addition, another study by Masucci L. *et al.*, in patients from

Italy confirmed the *Giardia* prevalence for 1.3% (69/5351) (Masucci *et al.*, 2011). *B. hominis* was the second most common species in the present study at 28.38% (489/1723) consistent with a study from south Tehran (capital of Iran), that discovered *B. hominis* and *G. lamblia* were the most frequent intestinal parasites (Arani *et al.*, 2008). *B. hominis*is an enteric protozoan infecting many animals and human beings. A worldwide distribution was reported and it is frequently the most commonly isolated organism in parasitological studies (Aguiar *et al.*, 2007; Tan, 2008). Studies (epidemiological, *in vitro* and *in vivo*) strongly suggest that *Blastocystis*is a pathogenic organism (Shlim *et al.*, 1995) with several genotypes in nature indicating numerous zoonotic genotypes to infect humans. In addition, both pathogenic and non-pathogenic genotypes originated from genetic diversity within the species (Tan, 2008).

E. histolytica/dispar was the third most common pathogenic species, notable in 10.68% (184/1723) of patients. *Entamoeba* spp. can cause harmless intestine colonization or severe contamination characterized by invasion of colon wall and damage to other host tissues, *e.g.* amoebiasis in the liver, lung, and brain. A clinical diagnosis of amoebiasis, in most cases, can be confirmed microbiologically; generally, through light microscopic examination of parasites in a wet smear or stained sample. This technique is simple and economical, however, it has a number of limitations. The most significant restriction is that this technique cannot differentiate between the cysts and trophozoites of *E. histolytica* (the disease-causing species), *E. dispar* (which is non-pathogenic), and *E. moshkovskii* (the amphizoic amoeba) that occasionally contaminate human beings. Furthermore, multiple specimens often have to be examined and the diagnosis can potentially be even tougher when there are different cysts of the *Entamoeba* species, *i.e. Iodamoeba*, or *Endolimax* (Gonzalez-Ruiz *et al.*, 1994). Since reported cases of human infection with *E. moshkovskii* have been sporadic, discriminating between these three species has increasingly become essential for diagnosis and epidemiological studies (Clark Diamond, 1991). Recent findings indicate that *E. moshkovskii* species was highly dominant in young children from Bangladesh, where it is often associated with *E. histolytica* and *E. dispar* (Ali *et al.*, 2003). In the present study, *E. histolytica* and/or *E. dispar* were detected by microscopic examination in 184 samples. However, it is suggested that these cases are analysed further with molecular techniques to confirm *Entamoeba* species.

The results from the current study also showed that the referrals were infected with other non-pathogenic intestinal parasites including *E. coli* for 17.82% (n=307), *I. butcheli* 3.08% (n=53), *T. hominis* 0.23% (n=4), *C. mesnili* 0.46% (n=8) and *E. nana* 0.12% (n=2).

A decreasing pattern in the parasitic diseases frequency has been observed, especially for helminthes in recent years. The helminthes were surprisingly found to be only in 0.7% (12/1723) of contributors. A significant decrease for helminthes prevalence's was proved by other studies in recent years (Rezaeian & Saraei, 1992; Escobedo, 2015; Daryani *et al.*, 2017; Kiani *et al.*, 2016; Shahdoust *et al.*, 2016; Barkhori-Mahni *et al.*, 2016; Sarkari *et al.*, 2016; Fasihi-Karami *et al.*, 2017). The low, rare and sporadic observed cases of intestinal worms' infection can be due to lack of watering vegetables and farms and orchards with human and urban sewage, increased level of health care and people's information along with increased veterinary supervisions and the supply of healthy meat are effective factors in this issue. Since intestinal protozoans have simple life cycles and a direct transmission way, it is expected they will have a wider distribution than helminthes. Furthermore, the cysts of Protozoa could pass through the household filters' systems through the tap water and remain infective for a while; the prevalence rate is high and could be controlled by improving drinking water systems (Feiz Haddad *et al.*, 2016). These findings were in agreement with previous studies (Ahsan *et al.*, 2005; Al-Braikan, 2008). The *G. lamblia*, *B. hominis* and *E. histolytica/dispar* were found to be the most common protozoan parasites (Table 3), and this was consistent with studies of (Arani *et al.*, 2008; Akhlaghi *et al.*, 2009; Kia *et al.*, 2008).

The present study has certain restrictions that must be admitted. The analysis was based on a single sample examination and the studies were carried out at a particular university teaching hospital. Therefore, the results are not necessarily applicable to other states. However, the limitations are to some extent compensated by the large sample size of the studied subjects and the suitably large variety of recognized parasites. In addition, the infection rates in this study have been consistently demonstrated in other studies. For instance, references of 32-39 showed a reduction of intestinal parasitic infections in recent years indicating hygiene promotion by the individuals and society's backing (Rezaeian & Saraei, 1992; Escobedo, 2015; Daryani *et al.*, 2017; Kiani *et al.*, 2016; Shahdoust *et al.*, 2016; Barkhori-Mahni *et al.*, 2016; Sarkari *et al.*, 2016; Fasihi-Karami *et al.*, 2017).

In conclusion, awareness of organisms being responsible for intestinal infections is a crucial step towards the application of proper control measures and effective patient care. The present results prove that intestinal parasites must be well considered in the differential diagnosis of gastrointestinal diseases, even in better hygiene areas. Inspection of multiple stool samples per patient is still the recommended approach for unintentionally missing parasitic infection (Branda *et al.*, 2006). The stool examination for parasites can be clinically useful for populations with increased infection risk to prevent the distribution of IPIs. In this context, close collaboration between clinical parasitologists and physicians could produce significant positive effects.

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Conflict of interest
The authors declare that they have no conflict of interest.

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