Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Distribution and risk factors associated with intestinal parasite infections among children with gastrointestinal disorders

Hamed Kiani[1, 2], Ali Haghighi[1], Roya Salehi[1], Eznollah Azargashb[3]

[1] Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Iranian Veterinary Organization. Hamadan, Iran

[3] Department of Community Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Aim: Prevalence and risk factors associated with intestinal parasites among children ≤ 12 years old in Nahavand county western Iran, was the objective of this search.

Background: Intestinal parasites (IPs) are important health problems among most societies.

Methods: This cross sectional study was carried out during 6 months from April to September 2014 in Nahavand County western Iran. Fecal samples were collected from 500 children suffering from gastrointestinal disorders (GIDs) and examined by macroscopy and microscopic (using saline and iodine wet mount, formalin-ether sedimentation, Trichrome and modified Ziehl Neelsen staining) methods. Finally, data was analyzed using Chi-square (Chi2) test and Fisher's exact test as well as logistic regression.

Results: 21.8% (109/500) of the samples were infected by one or more IPs. The most common parasites were *Blastocystis* sp. (16.2%), followed by *Cryptosporidium* spp. (2.6%), *Giardia lamblia* (1.6%), and *Entamoeba coli* (1.6%). Prevalence of intestinal parasite infections were significantly associated with age (OR= 2.280; CI 95% = 1.375-3.830; P<0.002), gender (OR= 0551; CI 95% = 0.348-0.875; P<0.011), contact with domestic animal or soil (OR= 0.492; CI 95% = 0.282-0.860; P<0.013) and seasons (OR= 2.012; CI 95% = 1.254-3.227; P<0.004). There was a significant correlation between IPs with diarrhea (OR= 3.027; CI 95% = 1.712-5.345; P<0.001) and nausea or vomiting (OR= 3.261; CI 95% = 1.281-8.175; P<0.013).

Conclusion: *Blastocystis* sp. was the most prevalent parasites among children in Nahavand County and Helminthes infection have been dramatically decreased. Our finding shown that gender, age, season and contact with domestic animals or soil polluted are main predictive factors for intestinal parasite infections among children in this region. Moreover, IPs infection among children with gastrointestinal disorders were significantly associated with diarrhea and vomiting or nausea signs.

Key words: Parasitic infection, Distribution and risk factors, Children, Western Iran.

(Please cite as: Kiani H, Haghighi A, Salehi R, Azargashb E. Distribution and risk factors associated with intestinal parasite infections among children with gastrointestinal disorders. Gastroenterol Hepatol Bed Bench 2016; 9(Suppl. 1): S80– S87).

Introduction

Intestinal parasitic infections (IPIs) can cause acute gastrointestinal disorders (AGIDs), and these organisms result in most common communicable diseases (1-3). Human parasitic infections lead to significant morbidity and mortality among different societies in the world (4,5). Globally, more than 3.5 billion individuals are infected by IPs, and of them 450 million are ill as a result of IPs, the main part of them are being children (6-8). Protozoan infections such as (Ameobiasis, Giardiasis and Cryptosporidiosis) and helminthic infections such as (Ascariasis, Enterobiasis, hookworms, and Trichuriasis) are among the most common intestinal parasitic infection in the worldwide. These organisms are also known as serious public health problems in children and complications such as iron deficiency (anemia), vitamin A deficiency, diarrheal or dysentery, malnutrition, delay growth, physical and mental health problems (7-13). Moreover, IPIs can result in serious problems in gastrointestinal disorders patients, immunocompromised patients such as HIV positive, transplanted and hemodialysis patients (2, 14, 15). The incidence and frequency rate of IPIs vary in different countries due to environmental condition, geographical factors, location, level of family education, a range of family income, health education, accessibility of

Corresponding Author: Eznollah Azargashb, PhD
Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Tel: +989125610344, +2122439936
Email: e.azargashb@sbmu.ac.ir

threatened/or unthreatened water, hand washing practices before meal and after the toilet and etc (16-19). The epidemiological data based on the frequency of IPIs is one of the basics for developing appropriate control measures. Previous epidemiological studies in Iran have demonstrated high prevalence of IPIs among different population and have shown that *Blastocystis* sp., *Entamoeba histolitica/E. dispar*, *Giardia lamblia*, *Cryptosporidium spp*., *Enterobius vermicularis* and *Ascaris lumbericoides* are the predominant IPIs (1, 2, 15, 20-25). Surprisingly, in spite of the public health importance of these infections and the potential consequences, there was no enough data about their epidemiology of IPIs among children in western Iran. The aims of this study were to identify the epidemiology and associated risk factors of IPIs in children with gastrointestinal disorders in Nahavand County, western Iran.

Materials and Methods

Study areas & population:

This cross-sectional survey was carried out from April to September 2014 in Nahavand County, Hamadan province, western Iran. Stool samples from 500 children under 12 years old suffered from GIDs who referred to medical centers laboratories were randomly collected. The sample size was calculated using formula: $n = z^2*p(1-p)/d^2$ under assumptions as follow: the reference prevalence was considered as 35 % (20), a 95% level of confidence and 5% marginal error. The sample size required was 496, but for more accuracy 500 samples were collected.

Questionnaire

The questionnaire was prepared to elicited information on the demographic data (age, gender and location (urban & rural)), acute clinical symptoms (diarrhea and dysentery, abdominal pain, stomach pain, bloating, vomiting & nausea), environmental sanitation and living condition characteristics (season, contact with domestic animals and soil) which will be used to assess the potential risk factors for IPIs. Informed consent was obtained from each attendee prior to the sampling. Certain criteria were applied to enroll children in the study. Inclusion criteria included age ≤12 years old with no history of taken anti parasites drugs in two weeks before the test. Exclusion criteria included children suffering from GIDs (diarrhea, dysentery diarrhea, stomach pain, bloating and Nausea or vomiting) disease.

Stool Collection and Processing

From all of the participants a single fresh stool sample were collected from 12 clinical laboratories in Nahavand County. The collected specimens were transported to the parasitological laboratory of Ayatollah Alimoradian hospital, in Nahavand city for the stool analysis process. At first, Information related to patients was recorded on a daily basis in the registered office information and stool samples were observed macroscopically to consistency and the presence of worm, larva, segments, blood, and mucus were recorded.

After macroscopic analysis, direct wet mount with normal saline (0.85% NaCl solution) in all of the stool specimens in one side of the slides (for the presence of motile intestinal parasites and trophozoite) was done and lugol's iodine staining was performed to distinguish cysts of intestinal parasites in other side of the slide. In the next step formalin-ether concentration was prepared and the sediments were stained with iodine, put on a slide and covered with a cover slip to accurate detection of cyst or eggs of intestinal parasites. Moreover, slide smear was prepared in fresh stool and immediately stained with trichrome's staining to accurate differentiation of intestinal protozoa *(Entamoeba*, *Giardia*, *Blastocystis* sp. and etc). For detection of coccidian parasites (*Cryptosporidium* spp.), Modified Zeihl- Neelsen staining after concentration technique was done. All of the smears prepared were microscopically observed using 100x, 400x, and1000 x magnification (26).

Statistical analysis

For statistical analysis the data was exported to Statistical Package for the Social Sciences software version16 (SPSS, Chicago, IL, USA). The proportion percentage was used to describe the characteristics of the participants, including the frequency of IPIs according to age, sex and etc. A Pearson's Chi-square (Chi2) test and Fisher's exact test were used for differences in the proportions of IPIs between different variables and odds ratios (OR) and 95% confidence intervals (CI) were used for associations of variables. The t-test wasused to compare the mean age. Logistic regression was used to detect risk factors of parasite infection. The *P* value <0.05 was considered statistically significant.

Ethical clearance

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

Results

Sociodemographic characteristic

Out of 500 children with GIDs, 278 boys and 222 girls, aged 22 days-12 years were enrolled in this study. 246 children lived in rural areas and 254 subjects lived in urban area.149 of 500 participants had frequently contact with domestic animal or soil and 351 child no contact. The proportion of children according to age groups were ≤ 3years (276 patients), 4 – 6 years (135 children) and 7 – 12 years (89 isolates). These specimens were taken during spring (224

Table 1. Frequency of IPIs and poly-parasitism among children with GIDs in Nahavand County, western Iran (N = 500).

Type of parasites	Mono parasites n (%)	Mixed infections n (%)	Total n (%)
Total infected patients	97 (19.4)	12 (2.4)	109 (21.8)
Protozoa			
Blastocystis sp.	70 (14)	11 (2.2)	81 (16.2)
Giardia lamblia	7 (1.4)	1 (0.2)	8 (1.6)
Cryptosporidium spp.	7 (1.4)	6 (1.2)	13 (2.6)
Entamoeba histolytica/E. dispar	0	1 (02)	1 (0.2)
Entamoeba coli	4 (0.8)	4 (0.8)	8 (1.6)
Endolimax nana	1 (0.2)	1 (0.2)	2 (0.4)
Iodamoeba bucheli	1 (0.2)	0	1 (0.2)
Entamoeba hartmanni	3 (0.6)	0	3 (0.6)
Trichomonas hominis	2 (0.4)	0	2 (0.4)
Chilomastix mesnilii	1 (0.2)	1 (0.2)	2 (0.4)
Helminthes			
Enterobius vermicularis	1 (0.2)	0	1 (0.2)
Children infected with protozoa	96 (19.2)	12 (2.4)	108 (21.6) *
Children infected with helminthes	1 (0.2)	0	1 (0.2)

n: number; * The frequency of intestinal protozoa was significantly higher than intestinal helminthes.

Table2. Frequency of IPIs by socio-demographic and clinical features in children with GIDs in Nahavand County, western Iran (n = 500).

Variables	Positive n %	Negative n %	Totl n %	OR	CI 95%		*P-value*
					Lower	*Uper*	
Gender							0.011
Male	71 (25.5)	207 (74.5)	278 (100)	Reference			
Female	38 (17.1)	184 (82.9)	222 (100)	0.551	0.348	0.873	
Age (Year)							0.008
≤3	44 (15.9)	232 (84.1)	276 (100)	Reference			
4-6	44 (32.6)	91 (67.4)	135 (100)	2.280	1.357	3.830	0.002
7-12	21 (23.6)	68 (76.4)	89 (100)	1.484	0.800	2.755	0.211
Residence							0.606
Urban	51 (20.1)	203 (79.9)	254 (100)	Reference			
Rural	58 (23.6)	188 (76.4)	246 (100)	1.147	0.680	1.936	
Contact with domestic animal & soil							0.013
Yes	49 (32.9)	100 (67.1)	149 (100)	Reference			
No	60 (17.1)	291 (82.9)	351 (100)	0.492	0.282	0.860	
Seasons							0.004
Spring	35 (15.6)	189 (84.4)	224 (100)	Reference			
Summer	74 (26.8)	202 (73.2)	276 (100)	2.012	1.254	3.227	

n: number; OR: odds ratio; Reference: The top group is considered as baseline. *Chi 2 test and univariate Logistic regression was used

Table 3. Clinical features associated with IPIs among children with GIDs in Nahavand County, western Iran (n = 500).

Characteristics	Total examined	Infected N (%)	Non infected N (%)	OR	CI 95%		*P.* value
					Lower	*Uper*	
Abdominal pain							0.371
Yes	368	79 (21.5)	289 (78.5)	Reference			
No	132	30 (22.7)	102(77.3)	1.265	0.756	2.119	
Nausea or vomiting							0.013
Yes	21	9 (42.9)	12(57.1)	3.236	1.281	8.175	
No	479	100 (20.9)	379(79.9)	Reference			
Stomach pain							0.041
Yes	185	30 (16.2)	155(83.8)	0.586	0.352	0.979	
No	315	78 (24.8)	237(75.2)	Reference			
Bloating							0.241
Yes	57	13 (22.8)	44(77.2)	1.522	0.755	3.067	
No	443	96 (21.7)	347(78.3)	Reference			
Type of stool							0.002
Formed stool	145	17 (11.7)	128 (88.3)	Reference			
Soft stool	49	10 (20.4)	39 (79.6)	1.931	0.817	4.560	0.134
Diarrheal stool	272	78 (28.7)	149(71.3)	3.027	1.712	5.354	0.001
Dysenteric diarrhea stool	11	2 (18.2)	9 (81.8)	1.673	0.333	8.400	0.532
Hard stool	23	2 (8.7)	21 (91.3)	0.717	0.154	3.332	0.671

OR: odds ratio; n: Number; Reference: The top group is considered as baseline; *Chi 2 test as well as Fisher exact test and univariate Logistic regression for type of stool analysis are used.

patients) and summer season (276 patients). According to macroscopic analysis and consistency of stools 272 (54.4%) child have diarrheal stool and frequency of patients with formed, soft, dysenteric diarrhea and hard stools was 145 (29%), 49 (9.8%), 11 (2.2%) and (4.6%) respectively.

Distribution of Intestinal Parasitic Infections

According to this study, 21.8 % (109/500) of the children were infected with one or more IPs infection, among them 97 (19.4%) and 12 (2.4%) of individuals had mono and poly parasitism respectively. The most common IPs among children was *Blastocystis sp.* (81 case/16.2%), followed by *Cryptosporidium spp.* (13 case/2.6%), *Giardia lamblia* (8 case/1.6%) and *Entamoeba coli* (8 case/1.6%). The prevalence of other IPs are shown in (Table 1). Among the all children 21.6% (108/500) were infected with protozoan parasites and 0.2% (1/500) were infected with helminthes infection. Frequency of intestinal helminthes among this children was significantly lower than intestinal protozoa ($P < 0.001$).

Poly Parasitic Infection

Generally 2.4% (12 patients) were diagnosed with more than one parasite. More frequency of co-infection was *Criptosporidium* spp. with *Blastocystis* sp. in 1 % (5/500 cases) and *Entamoeba coli* with *Blastocystis* sp. In 0.8% (4/500). Other co-infection was *Entamoeba histolytica/E. dispar* with *Chilomastix mesnili* and *Giardia lamblia* with *Blastocystis* sp. Triple infection was also observed in one cases *Blastocystis hominis* with *Endolimax nana & Cryptosporidium* spp. According to this results high frequency of poly parasitic infections observed in *Blastocystis* sp. 11 case (2.2%) and *Cryptosporidium* spp. 6 case (1.2%).

Risk factors and Clinical features associated with (IPIs)

Logistic regression by forward method detected the risk factors associated with IPIs among children with GIDs and socio-demographic, environmental and personal hygiene factors evaluated in this study(Table 2). Prevalence of IPIs had significantly different by gender (girls 17.1% was lower than boys 25.5%), (OR= 0.551; CI $_{95\%}$= 0.348-0.875; P<0.011), age groups (children 4-6 years 32.6% higher than other age group) (OR= 2.280; CI $_{95\%}$= 1.375-3.830; P<0.002), children had no contact with domestic animal or soil (17.1%) was lower than children with contact (32.9%) (OR= 0.492; CI $_{95\%}$= 0.282-0.860; P<0.013) and summer

Table 4. Frequency of *Cryptosporidium* spp., *Giardia lamblia* and *Blastocystis* sp. by socio-demographic and clinical features in children with GIDs in Nahavand County, western Iran (n = 500).

Variables	Parasites									
		Cryptosporidium spp.			*Giardia lamblia*			*Blastocystis* sp.		
	N*Total*	Positive (%)	Negative (%)	*P-value*	Positive (%)	Negative (%)	*P-value*	Positive (%)	Negative (%)	*P-value*
Age (Year)				0.023*			0.001*			0.009*
≤3	276	4 (1.44)	272(98.55)		0	276 (100)		35 (12.68)	241 (87.31)	
4-6	135	3 (2.22)	132 (97.77)		5 (3.7)	130 (96.3)		33 (24.44)	102 (75.55)	
7-12	89	6 (6.74)	83 (93.25)		3 (3.37)	86 (96.62)		13 (14.6)	76 (85.39)	
Location				0.021*			0.170			0.194
Rural	246	11 (4.47)	235 (95.52)		6 (2.439)	240 (97.56)		34 (13.82)	212(88.17)	
Urban	254	2 (0.787)	252 (99.21)		2 (0.787)	252 (99.21)		47 (18.50)	207(81.49)	
Contact with domestic …				0.001*			0.010*			0.155
Yes	149	12 (8.05)	137 (91.94)		6 (4.026)	143 (95.97)		30 (20.13)	119 (97.86)	
No	351	1 (0.285)	350 (99.71)		2 (0.569)	349 (99.43)		51 (14.52)	300 (85.47)	
Seasons				0.045*			0.080			0.017*
Spring	224	2 (0.892)	222 (99.1)		1 (0.446)	223 (99.55)		26 (11.6)	198 (88.39)	
Summer	276	11(3.98)	265 (96.01)		7 (2.53)	269 (97.46)		55 (19.92)	221(80.08)	
Vomiting & nausea				0.001*			1.000			0.506
Yes	21	8 (38)	13 (62)		0	21 (100)		5 (23.8)	16	
No	479	5 (1.045)	474(98.95)		8 (1.67)	471 (98.33)		75 (15.65)	403	
Diarrhea				0.031*			0.725			0.007*
Yes	256	11(4.29)	245 (95.7)		5 (1.95)	251 (98.04)		53 (20.7)	203 (79.29)	
No	244	2 (0.81)	242 (99.18)		3 (1.22)	241 (98.77)		28 (11.47)	216 (8.52)	

OR: odds ratio; n: Number; Reference: The top group is considered as baseline; *Chi 2 as well as Fisher exact test are used. Also, univariate Logistic regression was used for age group analysis *p< 0.05

seasons (26.8%) higher than spring (15.6%) (OR= 2.012; CI $_{95\%}$= 1.254-3.227; P<0.004). The frequency rate among rural children (58/23.6%) was slightly (no statistically significant) more than urban residence (51/ 20.1%). Moreover, monthly prevalence of IPIs in July (32.1%) and August (29.3%) was meaningfully more than April (9.3%), May (16.5%), June (17.6%) and September (16%) (*P*=0.003). Regarding to clinical symptoms among children with GIDs, frequency of IPIs was significantly correlated with diarrhea (OR= 3.027; CI $_{95\%}$= 1.712-5.345; P<0.001) and nausea or vomiting (OR= 3.261; CI $_{95\%}$= 1.281-8.175; P<0.013). Frequency of IPIs in patients with stomach pain were statistically less than patients without stomach pain signs (OR= 0.586; CI $_{95\%}$= 0.352-0.979; P<0.041). The frequencies of IPIs in other clinical symptoms have not significant differences (Table 3).

Discussion

Epidemiological studies based on frequency of IPs infection in different society, have primary objective to identify high-

risk groups and formulate appropriate interventions. The present study attempted to assess the accurate data about the prevalence rate, associated risk factors and symptoms results from IPs among children with GIDs.

One hundred and nine patients (21.8%) were infected with IPs. This results is equal to previous study among patients ≥15 years old (24.3%) (21), and is very higher than checkup individuals under 15 years old (9.3%) in Nahavand laboratories (21). 21.6% of children infected by one or more protozoan parasites and only one children infected by helminthes (*Enterobius vermicularis*) infection. However prevalence of helminthes infection may be increased if cellophane tape, The Baerman techniques and agar plate culture were used (27). However, our study and recently reported studies in different part of Iran (28), Isfahan (29), Hamadan (30), Nahavand (21) represent a dramatic decrease of helminthic infections (*Ascariasis*, hook worms, *Entrobiosis* and etc.) and protozoan infections (Ameobiasis, Giardiasis, Cryptosporidiosis and etc.). It may be results from increasing public and individual hygienic, increase awareness of the prevention and control of parasitic infections and access to the safe food and water sources. Also, variations in prevalence of IPs may be due to differences in climatic conditions, environmental hygiene, economic and educational status and study subjects, and previous control efforts.

The most common IPs in our study was *Blastocystis* sp. with 81 cases (16.2%). It is similar to previous studied in Nahavand County. This study and other study in Tehran and Isfahan show that prevalence of *Blastocystis* sp. recently increased in Iran (21, 29, 31). But the pathogenicity of *Blastocystis* sp. is still controversial, some studies suggest that it is a pathogen (32, 33), whereas other studies believe it is a non-pathogen or commensal (33, 34). Recently, discussion on pathogenesis and accurate diagnosis from *Blastocystis* sp. can be cause of increase reported of this parasite. In our study *Blastocystis* sp. are related with diarrhea and season. Also previous clinical studies by Tan et al. and Moosavi et al. presented that abdominal pain and diarrhea are two the major signs among *Blastocystis*-positive patients (31,32). Regardless of *Blastocystis* sp., *Cryptosporidium* spp. *and Giardia lamblia* with the frequency of 13 (2.6%) and 8 (1.6%) respectivelly was the most common intestinal pathogenic protozoan. Because, staining method for *Cryptosporidium* spp. and concenteration methods are not usulally use in laboratories , Modified Zeihl-Neelsen staining after concentration technique specifically in children with GIDs is required in this region and other same regions.

In this study, *Cryptosporidium* spp*., Giardia lamblia* and *Blastocystis* sp. have the most prevalent IPIs in children with GIDs. Frequency of *Cryptosporidium* spp., *Giardia lamblia* and *Blastocystis* sp. with Socio-demographic and clinical features in children with GIDs are shown in Table 4. There was significant relationship between cryptosporidiosis by age (p = 0.023), location (p= 0.021), contact with domestic animal and soil polluted by animal feces (p= 0.001), season (p= 0.045), vomiting/ nausea (p= 0.001) and diarrhea (p= 0.031). Significantly different observed among Giardiasis by age (p= 0.001) and contact with domestic animal or soil (p= 0.010).There was significant correlation among Blastocystis sp. by age (p= 0.009), season (p= 0.017) and diarrhea sign (p= 0.007).

Blastocystis sp.,*Cryptosporidium* spp. and *Giardia lamblia* are zoonotic protozoa that are a great threat to public health and can be transmitted through contaminated water and food. Water and food contaminated with animal wastes and farming practice are important source of transmission. Intestinal parasitic infections, especially *Cryptosporidium* spp. and *Giardia lamblia* were significantly associated with contact with domestic animals and soil. Thus it is necessary that animals be kept away from water and food source that are used by human. Animal husbandry and agriculture are common in Nahavand County, in the warm season livestock going to pastures and contamination of water sources and vegetables by livestock fecal can increase pollution. Also frequency of IPs (especially *Cryptosporidium* spp. and *Blastocystis* sp.) in warm season (summer) is higher than cold season (spring).

According to age groups significant different was observed among children 6-12 years old by Cryptosporidiosis and giardiasis. It may be due to contact children with polluted source such as vegetable in farms or soil polluted by animal feces during play in soil land and farms especially in rural region or direct contact (person to person) during meal food or etc. together.

In relation to poly parasitism, our results are similar to previous study in Isfahan, Hamadan and previous study in Nahavand County that poly parasitism detected. The observed co-infection could be clarified by the facts that many species of protozoa (*Cryptosporidium* spp., *E. histolytica/E. dispar, Blastosystis* sp*., Entamoeba coli, Giardia lamblia* and etc.) have the same mode of transmission and that hygiene is poor in these areas (21,29,30).

Infection happen by IPs may be consequences such as gastrointestinal disorders (diarrhea or dysentery and etc., anemia, malabsorption, delay growth in children and physical complications, therefore, it was seen as the main health problem (31,35,36). According to clinical signs in our study there was significant correlation among IPs and cryptosporidiosis in GIDs children with diarrhea, and vomiting/ or nausea. Similar to our results various studies in other countries and in severe acute malnutrition related diarrhoea have shown intestinal parasites as risk factors of diarrhoea (37,38). Our results show that there are significant relationship between *Blastocystis* sp. by diarrhea sign and in previous study in Iranian patients moosavi et al.(*Blastocystis* sp), keshavarz et al. (*Cryptosporidium* spp.) and haghighi et al. (*Entamoeba histolitica/E. dispar*) are significantly

associated with diarrhea (2, 31 ,39). Also in turkey the most common complaint related with IPIs was intestinal dysmotility, nausea/ vomiting and abdominal distention (8).

There were some limitations in our study.

1. Stool samples were collected only once, It is likely that the analysis of three consecutive stool samples could have increased the diagnostic sensitivity.

2. Sample collected was done during two warm season and there is no data about autumn and winter.

3. Due to the lack of facilities and financial funding we could not use all the laboratory available techniques, such as the Baerman technique to find larvae of helminth including *Strongyloides stercoralis* or cellophane tape to detect *Enterobius vermicularies* and agar plate culture to find accurate detection of helminthes larva.

4. Also, it is likely that the prevalence of some protozoa such as *Entamoeba* and *Blastocystis* sp. could be even higher if culture techniques had been used.

Based on the present study *Blastocystis* sp. is the prevalent parasites in Nahavand County. Especially pathogenic parasites such as *Cryptosporidium* spp. and *Giardia lamblia* among children with GIDs, still are prevalent in western Iran, and helminthes infection have been dramatically decreased during the past decades. Thus, Modified Zeihl-Neelsen staining after concentration technique is recommended for *Cryptosporidium* spp. identification in this area. Also, our finding suggests the risk factors such as gender, season and contact with domestic animals and soil polluted are increase susceptibility of children with IPs infection. IPs infection among children with GIDs are associated with diarrhea and vomiting/or nausea in this area and the role of parasites in children's should be considered. Thus, health interventions to children and prevent children from contact with contaminated resources such as land and livestock waste in this area is necessary.

Acknowledgments

This study was part of the MSc. thesis of Hamed Kiani (Grant. No. 13/1285) and was financially supported by *Shahid Beheshti University of Medical Sciences* (SBMU). The author's likes to express their gratitude to administrators and stuffs of Ayatollah Alimoradian hospital, authorities and personnel of Nahavand Health Care Network and centers, medical laboratories and study participants for their kind cooperation during sample collection.

Conflict of interest

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

References

1. Fallah M, Haghighi A. Cryptosporidiosis in children with diarrhea submitted to health centers in the west of iran (hamedan). MJIRI 1996; 9: 315-7.
2. Haghighi A, Khorashad AS, Mojarad EN, Kazemi B, Rostami Nejad M, Rasti S. Frequency of enteric protozoan parasites among patients with gastrointestinal complaints in medical centers of Zahedan, Iran. Trans R Soc Trop Med Hyg 2009; 103: 452-4.
3. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clin Invest 2008; 118: 1311-21.
4. Legesse M, Erko B. Prevalence of intestinal parasites among schoolchildren in a rural area close to the southeast of Lake Langano, Ethiopia. Ethiop J Health Dev 2004; 18: 116-20.
5. Omorodion A, Nmorsi O, Isaac C, Umukoro D, Akhile A. Distribution of intestinal parasites among school-age children in Delta and Edo States of Nigeria. Parasitol Union J 2012; 5: 1-6.
6. Ayalew A, Debebe T, Worku A. Prevalence and risk factors of intestinal parasites among Delgi school children, North Gondar, Ethiopia. J Parasitol Vector Biology 2011; 3: 75-81.
7. Mengistu A, Gebre-Selassie S, Kassa T. Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethiopia. Ethiop J Health Dev 2007; 21: 12-7.
8. Okyay P, Ertug S, Gultekin B, Onen O, Beser E. Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. BMC Pub Health 2004; 4: 64.
9. Al-Mekhlafi HM, Surin J, Sallam AA, Abdullah AW, Mahdy M. Giardiasis and poor vitamin A status among aboriginal school children in rural Malaysia. Am J Trop Med Hyg 2010; 83: 523-7.
10. Banke ROK, Omudu E A, Ikenwa D, Feese E. Prevalence of gastro-intestinal parasites in relation to availability of sanitary facilities among schooling children in makurdi, Nigeria. Animal Res Int 2006; 3: 489-93.
11. Gutierrez-Jimenez J, Torres-Sanchez MG, Fajardo-Martinez LP, Schlie-Guzman MA, Luna-Cazares LM, Gonzalez-Esquinca AR, et al. Malnutrition and the presence of intestinal parasites in children from the poorest municipalities of Mexico. J Infect Dev Ctries 2013; 7: 741-7.
12. Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L, Tielsch JM. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0-to 5-y old Zanzibari children and these relationships change with age. J Nutr 2008; 130: 1724-33.
13. Yami A, Mamo Y, Kebede S. Prevalence and predictors of intestinal helminthiasis among school children in jimma zone; a cross-sectional study. Ethiop J Health Sci 2011; 21: 167-74.
14. Huang DB, Chappell C, Okhuysen PC. Cryptosporidiosis in children. Semin Pediatr Infect Dis 2004; 15: 253-9.
15. Omrani VF, Fallahi S, Rostami A, Siyadatpanah A, Barzgarpour G, Mehravar S, et al. Prevalence of intestinal parasite infections and associated clinical symptoms among patients with end-stage renal disease undergoing hemodialysis. Infection 2015; 43: 537-44.
16. Asemahagn M A. Parasitic infection and associated factors among the primary school children in Motta town, western Amhara, Ethiopia. Am J Pub Health 2014; 2: 248-54.
17. Auta T, Kogi E, Oricha KA. Studies on the intestinal helminths infestation among primary school children in Gwagwada, Kaduna, North Western Nigeria. J Biology Agriculture Healthcare 2013; 3: 48-53.
18. Committee WE. Prevention and control of schistosomiasis and

soil-transmitted helminthiasis. World Health Organ Tech Rep Ser. 2002; 912:i-vi, 1-57, back cover.

19. Haque R. Human intestinal parasites. J Health Popul Nutr 2007; 25 (4): 387-91.
20. Daryani A, Sharif M, Nasrolahei M, Khalilian A, Mohammadi A, Barzegar G. Epidemiological survey of the prevalence of intestinal parasites among schoolchildren in Sari, northern Iran. Trans R Soc Trop Med Hyg 2012; 106: 455-9.
21. Kiani H, Haghighi A, Azargashb E, Solgi A. Frequency of intestinal parasitic infections among individuals referred to the medical center laboratories in Nahavand City, Hamadan Province, western Iran. Novel Biomed 2015; 3: 124-30.
22. Kheirandish F, Tarahi M, Haghighi A, Nazemalhosseini-Mojarad E, Kheirandish M. Prevalence of intestinal parasites in bakery workers in Khorramabad, Lorestan Iran. Iran J Parasitol 2011; 6: 76-83.
23. Niyyati M, Rezaeian M, Zahabion F, Hajarzadeh R, Kia E. A survey on intestinal parasitic infections in patients referred to a hospital in Tehran. Pak J Med Sci 2009; 25: 87-90.
24. Rokni M. The present status of human helminthic diseases in Iran. Ann Trop Med Parasitol 2008; 102: 283-95.
25. Tappe KH, Mohammadzadeh H, Khashaveh S, Rezapour B, Barazesh A. Prevalence of intestinal parasitic infections among primary school attending students in Barandooz-Chay rural region of Urmia, West Azerbaijan province, Iran in 2008. Afr J Microbiol Res 2011; 5: 788-91.
26. Garcia LS. Laboratory identification of the microsporidia. J Clin Microbiol 2002; 40: 1892-901.
27. Keiser PB, Nutman TB. *Strongyloides stercoralis* in the immunocompromised population. Clin Microbiol Rev 2004; 17: 208-17.
28. Zebardast N, Gharavi MJ, Abadi A, Tabaei SJS, Yeganeh F, Khazan H, et al. Frequency of intestinal parasites in patients with gastrointestinal disorders, in different parts of Iran during 2012-2013. Int J Enteric Pathog 2015; 3: e22682.
29. Jafari R, Sharifi F, Bagherpour B, Safari M. Prevalence of intestinal parasites in Isfahan city, central Iran, 2014. J Parasit Dis 2016; 40: 679-82.
30. Jafari R, Fallah M, Yousofi Darani H, Yousefi HA, Mohaghegh M A, Latifi M, et al. Prevalence of intestinal parasitic infections among rural inhabitants of Hamadan city, Iran, 2012. Avicenna J Clin Microbiol Infect 2014; 1: e21445.
31. Moosavi A, Haghighi A, Mojarad EN, Zayeri F, Alebouyeh M, Khazan H, et al. Genetic variability of *Blastocystis* sp. isolated from symptomatic and asymptomatic individuals in Iran. Parasitol Res 2012; 111: 2311-5.
32. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* sp. Clin Microbiol Rev 2008; 21: 639-65.
33. Tan K S, Mirza H, Teo JD, Wu B, MacAry PA. Current views on the clinical relevance of *Blastocystis* sp. Curr Infect Dis Rep 2010; 12: 28-35.
34. Scanlan P D, Stensvold C R. Blastocystis: getting to grips with our guileful guest. Trends Parasitol 2013; 29: 523-9.
35. Mehraj V, Hatcher J, Akhtar S, Rafique G, Beg MA. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. PLoS One 2008; 3: e3680.
36. Nematian J, Nematian E, Gholamrezanezhad A, Asgari AA. Prevalence of intestinal parasitic infections and their relation with socio-economic factors and hygienic habits in Tehran primary school students. Acta Tropica 2004; 92: 179-86.
37. Buzigi E. Prevalence of intestinal parasites, and its association with severe acute malnutrition related diarrhoea. J Biology Agriculture Healthcare 2015; 5: 81-91.
38. Tinuade O, John O, Saheed O, Oyeku O, Fidelis N, Olabisi D. Parasitic etiology of childhood diarrhea. Indian J Pediatr 2006; 73: 1081-4.
39. Keshavarz A, Athari A, Haghighi A, Kazami B, Abadi A, Mojarad E N, et al. Genetic characterization of *Cryptosporidium* spp. among children with diarrhea in Tehran and Qazvin provinces, Iran. Iran J Parasitol 2008; 3: 30-6.