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Prevalence and Risk Factors of *Giardia lamblia* and *Blastocystis hominis* Infections in Children Under Ten Years Old, Hamadan, Iran

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Background: Nowadays, parasitic infections are a major health problem throughout the world, particularly in the developing countries.
Objectives: Considering the high susceptibility of children against parasitic infections, the current study aimed to determine the prevalence and associated risk factors of intestinal parasitic infections among children less than 10 years old in urban and rural areas of Hamadan district.
Patients and Methods: The current study was conducted on 395 children (214 males and 181 females), referred to urban and rural health centers in Hamadan district in 2013. Stool samples were examined by formalin-ether concentration technique, and trichrome and modified Ziehl-Neelsen staining methods. The results were analyzed by chi-square test.
Results: Of the 395 studied children, 112 (28.4%) were infected with intestinal parasites. *Blastocystis hominis* was the most frequently detected parasite with the prevalence of 18.5%, followed by *Giardia lamblia* (10.9%), *Entamoeba coli* (2.8%), *Dientamoeba fragilis (0.8%), Iodamoeba buetschlii (0.8%), Chilomastix mesnili (0.5%), Cryptosporidium* spp. (0.5%), *Endolimax nana* (0.3%) and *Entamoeba hartmanni* (0.3%). No cases of infection with helminth parasites were found.
Conclusions: The results of the study showed a high prevalence of *Giardia lamblia* and *Blastocystis hominis* in rural areas compared to urban regions. Therefore it is necessary to promote the public health awareness in the rural population, in order to reduce the frequency of parasitic infections.

Keywords: Prevalence; Risk factors; *Giardia lamblia*; *Blastocystis hominis*; Child; Iran

1. Background

Currently, in spite of hygiene and technology development, parasitic infections are one of the most common and important hygiene problems worldwide, particularly in the developing countries because of uncontrolled growth of population, weather variety, low level of knowledge, and poor nutrition (1).

In addition to pathogenic intestinal parasites, humans are infected with commensal protozoa that are living and feeding in their gastrointestinal tract. Generally, pathogenesis, complications and mortality of intestinal parasites in an area depend on the parasite species, immunologic, physiologic and demographic factors as well as socioeconomic and cultural situations. These infections can cause a wide range of clinical symptoms such as acute watery diarrhea, abdominal cramps, dehydration, fever, nausea, and vomiting, which are more common among children and may cause failure to thrive (2). According to the World Health Organization (WHO) reports, nearly two thirds of people worldwide are infected with parasites, among which *Giardia lamblia* and *Ascaris lumbricoides* are the most common ones (3).

G. lamblia, a flagellated protozoan, is colonized in upper segment of small intestine and humans are infected through ingestion of parasite cysts. *Giardia* cysts are relatively resistant to environmental conditions, particularly drinking water chlorine. Giardiasis is the disease of all ages; however, its prevalence is higher in children (4). Clinical picture in newborns and children varies from an asymptomatic infection to an acute infection or a chronic disease with sudden attack of watery diarrhea, abdominal cramps, malabsorption and weight loss. The prevalence is as high as 10% - 50% in the developing countries (5).

Blastocystis hominis, a common protozoan of large intestine, has two forms of cyst and trophozoite in its life cycle. The cyst is the transmissible stage, which is transmitted directly or indirectly via the fecal-oral route. The symptoms are diarrhea, vomiting, abdominal cramps and bloating. Its prevalence is 1.6% - 16% and up to 60% in the developed and developing countries, respectively (1).

Since children are more susceptible to intestinal protozoan infections and the physical and psychological damages are more predominant among them, related epidemiologic studies in each area should be conducted in order to control the disease (6-8).

2. Objectives

The current study aimed to determine the prevalence and risk factors of protozoan infections, with more emphasize on *G. lamblia* and *B. hominis*, in children less than 10 years old in Hamadan district in 2013.

3. Patients and Methods

This cross sectional study was conducted on 395 apparently healthy children less than 10 years old referred to health care centers of Hamadan district from April to November 2013. The subjects were selected by two-stage sampling: in the first step, two urban and seven rural health centers were randomly selected and in the second phase, patients were selected consecutively. Stool samples were collected after receiving written informed consent from the parents and filling out the questionnaire. The samples were transferred to the Parasitology Laboratory of Hamadan Medical School. Polyvinyl alcohol (PVA) was used to transmit the watery samples. After evaluation of physical appearance of samples, they were examined by formalin-ether concentration technique, and trichrome and modified Ziehl-Neelsen staining methods. In formalin-ether method, 0.5 gr of each stool sample was emulsified in 7 mL of 10% formalin and strained through two layers of wet gauze in centrifuge tube. Then 3 mL of ether was added, mixed well and centrifuged at 2000 rpm for one minute. The resulting sediment was studied by light microscope. For Ziehl-Neelsen staining, fecal smears were fixed with methanol and then stained by Carbol Fuchsine for 15 minutes, washed and decolorized by 1% acid alcohol. Then slides re-stained with 4% Methylene Blue for 30 seconds and after the final washing, were studied for intestinal coccidia. Another smear was studied with trichrome stating. The results were analyzed with SPSS, version 20, and chi-square (X^2) test.

4. Results

Out of the 395 children 214 (54.2%) were male and 181 (45.8%) female. The majority of referrals were from rural areas with 327 cases (82.8%) and the rest were related to urban areas with 68 (17.2%). Totally, 112 subjects (28.4%) were infected with protozoa, 57 (50.9%) male and 55 (49.1%) female. Of these, 73 (18.5%) stool samples were infected with *B. hominis* and 43 (10.9%) with *G. lamblia*. The distribution frequency of the parasites by type is shown in Table 1.

Concomitant infection with two or more protozoa was also studied; 12 (3%) were infected with *G. lamblia* and *B. hominis*, 2 (0.5%) with *E. coli* and *B. hominis*, 2 (0.5%) with *E. coli* and *G. lamblia* and 2 (0.5%) with *G. lamblia*, *B. hominis* and *E. coli*.

Interestingly, no sample was infected with helminthic parasites. *G. lamblia* infection was significantly higher in rural (12.8%) than urban (1.5%) children (P = 0.004). *B hominis* was also more prevalent in rural (22%) than urban (1.5%) population (P < 0.001). *G. lamblia* and *B. hominis* were respectively found in 27 (11.4%), and 55 (23.2%) of the total 237 subjects in close contact with animals. Totally, 33.3% of all protozoan infections were observed in children in close contact with animals (P = 0.007). The highest frequency of protozoan infections was observed in children of eight to nine years old (41.7%). There was a significant relationship between protozoan infection and age (P < 0.001). Protozoan infections were found in 108 (33%) cases of the rural subjects and it was significantly higher than that of the urban area (four cases, 5.9%) (P < 0.001) (Table 2). As shown in Figure 1, the highest number of infections was found in children whose mothers had primary school education (P = 0.006). In the current study, there was no significant relationship between protozoan infections and gender, kind of drinking water (Figure 2) and breast-feeding (P > 0.05).

Table 1. The Frequency of Protozoan Infections in Children, According to the Place of Residence [a]

Parasite	Urban	Rural	Total
Blastocystis hominis	1 (1.5)	72 (22.1)	73 (18.5)
Giardia lamblia	1 (1.5)	42 (12.9)	43 (10.9)
Other protozoa			
Entamoeba coli	0 (0.0)	11 (3.4)	11 (2.8)
Dientamoeba fragilis	0 (0.0)	3 (0.9)	3 (0.8)
Iodamoeba butschlii	1 (1.5)	2 (0.6)	3 (0.8)
Chilomastix mesnili	0 (0.0)	2 (0.6)	2 (0.5)
Cryptosporidium spp.	0 (0.0)	2 (0.6)	2 (0.5)
Endolimax nana	0 (0.0)	1 (0.3)	1 (0.3)
Entamoeba hartmanni	1 (1.5)	0 (0.0)	1 (0.3)

[a] All of the values are presented as No. (%).

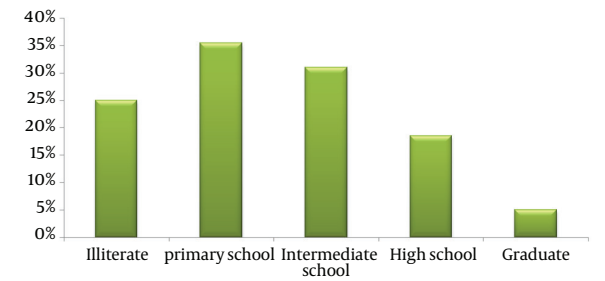

Figure 1. Frequency of Protozoan Infections According to Mothers' Education Level

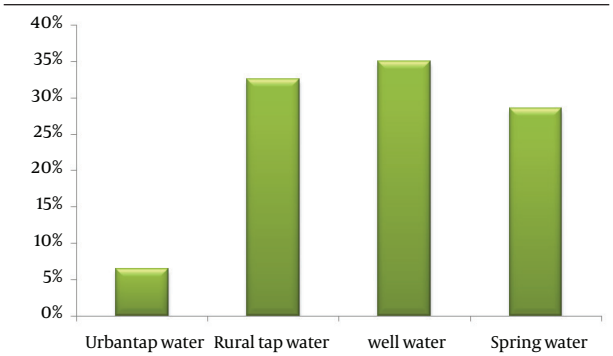

Figure 2. Frequency of Protozoan Infections According to Type of Drinking Water

Table 2. The Frequency of Protozoan Infections According to Some Demographic Features [a]

Demographic Feature	Number of Examined Children	*Giardia lamblia*	*Blastocystis hominis*	Total Protozoan Infections [b]	Chi-square Test [c]
Gender					P = 0.434
Male	214	19 (8.87)	42 (19.62)	57 (26.63)	
Female	181	24 (13.25)	31 (17.12)	55 (30.38)	
Age, y					P < 0.001
0-1	101	3 (2.97)	3 (2.97)	7 (6.93)	
2-3	47	6 (12.76)	8 (17.02)	12 (25.53)	
4-5	50	10 (20.00)	11 (22.00)	19 (38.00)	
6-7	70	8 (11.42)	15 (21.42)	21 (30.00)	
8-9	127	16 (12.59)	36 (28.34)	53 (41.73)	
Place					P < 0.001
Town	68	1 (1.47)	1 (1.47)	4 (5.88)	
Village	327	42 (12.84)	72 (22.01)	108 (33.02)	
Animal Contact					P = 0.007
Yes	237	27 (11.39)	55 (23.20)	79 (33.33)	
No	158	16 (10.12)	18 (11.39)	33 (20.88)	

[a] All of the values are presented as No. (%).
[b] All protozoan infections including *Giardia lamblia and Blastocystis hominis.*
[c] P values are related to total protozoan infections.

5. Discussion

Results of the current study showed that the prevalence of protozoan infections in children was 28.4% in Hamadan, and in comparison with South Khorasan (47.7%), Sari (33.3%) and Urmia (42.5%) it was considered moderate to low contamination (9-11). In the current study, *G. lamblia* and *B. hominis* were the most common infections. Giardiasis prevalence in the developing countries is 20% - 30% (5); its prevalence in the current study was 10.9%, similar to those of the studies in Sari (10.6%) and Golestan (9.9%) (10, 12), less than those of the studies in Urmia (20.5%) and south of Tehran (18.1%) (11, 13) and more than that of Mazandaran (4.1%) (14).

The prevalence of infection among females and males was almost the same, a finding in accordance with Tonekabon (6), Sari (10), Golestan (12) and South Khorasan (9) studies; however it was significantly different in Tehran (15). But the prevalence of infection was higher in rural areas rather that urban region, which can be explained by low level of sanitation knowledge, lack of healthy drinking water reservoir in some villages, more contact with soil, unpleasant environmental condition and large number of family members. The relationship between the prevalence of infection and the place of residence was statistically significant, like the study in South Khorasan (9).

The prevalence of infection was significantly higher among children of eight to nine years old (41.73%). It may be explained by school attendance and as a result of peer connection. This result was the same as Urmia (11) and Tehran (15) reports. It can also be concluded that the prevalence of infection increases as children get older (Table 2). According to Urmia (11) and South Khorasan (9) surveys, there was a significant relationship between infection and mothers' education level. The prevalence was higher among children whose mothers had primary school education, likely because of low hygiene knowledge. In the present study, 33.33% of protozoan infections were among children who had direct contact with animals, which was a significant relationship. In the current study, there was no significant relationship between breastfeeding and protozoan infection in infants, unlike the study conducted in Semnan (16).

In the present study most of the positive stool samples were among children using rural piping water, and well water, which can be due to lack of healthy drinking water, lack of proper wasting system and low health facilities in villages. However, higher frequency of positive stool samples in children using rural piping water and well water was not statistically significant, contrary to the study in Tonekabon (6).

In the developed countries, the prevalence of *B. hominis* is reported 1.6% to 16%, while in the developing countries it can be as high as 60% (1). In the present study this rate was 18.5%, which was higher than those of the studies in Urmia (13.3%), Sari (13.5%), Northern Iran (5.4%) and Mazandaran (1.8%) (10, 11, 14, 17). In a study conducted in 1994, a survey on protozoan infections among rural students of primary and middle schools of Hamadan, the prevalence

Table 3. The Frequency of Clinical Symptoms in People with Positive Stool Sample [a]

Clinical Symptoms	*Giardia lamblia*	*Blastocystis hominis*	Mixed Infection With *Giardia lamblia* and *Blastocystis hominis*
Fever	9 (29.03)	22 (36.06)	4 (33.33)
Diarrhea	12 (38.70)	28 (45.90)	2 (16.66)
Weight loss	12 (38.70)	21 (34.42)	3 (25.00)
Headache	6 (19.35)	10 (16.39)	1 (8.33)
Abdominal pain	22 (70.96)	44 (72.13)	11 (91.66)
Cachexia	21 (67.74)	48 (78.68)	9 (75.00)
Nausea	5 (16.12)	16 (26.22)	2 (16.66)
Vomiting	7 (22.58)	14 (22.95)	2 (16.66)
Anorexia	13 (41.93)	23 (37.70)	4 (33.33)
Bloating	6 (19.35)	15 (24.59)	3 (25.00)

[a] All of the values are presented as No. (%).

was reported 86%, including 20.5% for *G. lamblia*, 18.1% for *B. hominis* and 39.6% for *Ascaris* worm and the highest infection rate was among 9 - 12 year old children (52.9%) (18).

In the other studies in Hamadan from 2004 to 2005, out of 274 patients who referred to the Parasitology Research Lab of Hamadan Medical School, 20.4% were infected with *G. lamblia* and 21% with *B. hominis,* which the figure was higher than those of the present study. Abdominal pain was the most common symptom of *G. lamblia* (46.5%) and *B. hominis* (84.5%) infections. *G. lamblia* and *B. hominis* were more common in 6-10- and 1-10-year old children, respectively, consistent with the current study (19, 20). Another study in Hamadan was conducted from 1998 to 1999 and the prevalence of protozoan infections was reported 22.9%, which for *G. lamblia* it was 19.3% (21). Another study in 2009 in the same region indicated the prevalence of *G. lamblia* as 10.6% (22). Therefore, it can be concluded that the protozoan infections in recent years have a descending pattern. It can be due to the increased hygiene and health knowledge and available anti-parasite drugs, particularly in the rural areas. The most common clinical symptoms for *G. lamblia* and *B. hominis* were abdominal pain and cachexia in children; headache, nausea, vomiting and bloating were less common (Table 3). But since the pathogenicity of different *B. hominis* genotypes is different and many people with giardiasis are just healthy carrier and asymptomatic, these symptoms cannot be definitely attributed to these two protozoa. On the other hand, symptoms were present on the day of sampling, and it may be due to other causes including concomitant viral or bacterial infections.

The current study found concomitant infection with *G. lamblia* and *B. hominis* in 12 cases (3%), which is almost similar to Tonekabon results (2.65%) (6). A variety of pathogenic or non-pathogenic protozoa were found. Although, non-pathogenic parasites do not threaten the health, they can be a very good hygiene index and represent fecal-oral transmission in the infected families.

In conclusion, since the protozoan infections were more common among rural children and people using rural piping water, it can be concluded that hygiene level and health facilities in some villages are not efficiently available, which can result in physical, psychological, and economic damages. In rural children with obscure digestive symptoms and inappropriate weight gain, protozoan infections should always be considered. Also educational system should pay more attention to health knowledge improvement, especially in villages and mothers should be more educated by health care centers.

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Authors' Contributions

Study concept and design: Marzieh Asadi and Amir Hossein Maghsood, Acquisition of data: Marzieh Asadi, Analysis and interpretation of data: Marzieh Asadi and Amir Hossein Maghsood, Drafting of the manuscript: Marzieh Asadi and Mehrnaz Olfat, Critical revision of the manuscript for important intellectual content: Amir Hossein Maghsood and Iraj Sedighi, Statistical analysis: Marzieh Asadi, Administrative, technical, and material support: Amir Hossein Maghsood and Iraj Sedighi, Study supervision: Amir Hossein Maghsood and Iraj Sedighi.

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