

# Sporozoan Protozoa and Enteroparasites in the Gastroenteritic Patients Referring to the Healthcare Centers of Seven Provinces of Iran

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**Background:** Sporozoan protozoa and enteroparasites cause gastroenteritis. Sporozoa are the major cause of self-limiting diarrhea in immunocompetent patients, however they cause serious diseases in patients with immunosuppression.

**Objectives:** The current study aimed to identify the prevalence of sporozoa and enteroparasites among patients with gastroenteritis referred to the healthcare centers in seven provinces of Iran.

**Patients and Methods:** In this cross-sectional study, 4200 stool samples were randomly collected from patients with gastroenteritis in the selected hospitals of Gilan, East Azerbaijan, Qazvin, Kurdistan, Mazandaran, Tehran and Khorasan-e-Razavi provinces. Primarily samples were examined directly for enteroparasites. The samples were then filtered and concentrated using Paraseb kit. The pellets were fixed, stained by different assays including acid fast staining, Auramin Phenol Fluorescence, Giemsa and light microscopy.

**Results:** The results revealed the overall rate of infection, 3.86% (163 cases), as an indicator of parasitic enteropathogens in Iran. Among the provinces, Khorasan-e-Razavi and Mazandaran with 8.83% (53 cases) and 0.34% (2 cases) showed the highest and lowest rates of infection, respectively. The frequencies of sporozoa including *Cryptosporidium*, *Microsporidium*, *Isospora* and *Cyclospora* spp. were 0.1%, 0.1%, 0.07% and 0.02% respectively. Among the parasites, *Giardia lamblia*, *Taenia saginata* and Hookworms with 1.78% and 0.02% had the highest and lowest rates of infection, respectively. Regarding the age groups, the highest and the lowest rates of infection were in 0-10 (48%) and 41 years old and above (6.7%) groups, respectively.

**Conclusions:** Despite relatively low prevalence of sporozoa, giardiasis is the most prevalent agent for gastroenteritis amongst 3.86% of parasitic infections in Iran. The current study confirmed the abundance of infection in warm and wet seasons, and more frequency of infections among children than adults. Meanwhile, geographical and agricultural conditions, seasonal rainfall, abundance of water, and animal contact are key factors affecting sporozoan infections.

**Keywords:** *Cryptosporidium*; *Isospora*; *Microspora*; *Cyclospora*; Gastroenteritis

## 1. Background

Despite the improvement of life style, parasitic infections are still one of the problems in tropical and sub-tropical areas. Opportunistic parasites including sporozoan protozoa may be major threats. Epidemiological researches carried out in different countries show an association between the social and economic status with different parasites (1). In addition, poor sanitary and environmental conditions are known to be relevant in the propagations of such infection agents (2).

Intestinal parasites are one of the most common causes of infections worldwide. Parasitic enteropathogens and sporozoan protozoa can cause different types of gastroenteritis depending on the parasite type (3). These parasites can cause a self-limiting diarrhea in immunocompetent patients, however they can cause serious diseases in patients with immunosuppression (4). The transmission

route is through infected food and water, person to person, animals to man and also through infected vegetables (5). Despite the importance and seriousness of sporozoan infection in patients with gastroenteritis, and their protection against infection, there are not enough valid data to provide a comparison between the prevalence of this sporozoan infection and other enteropathogen parasites in this group (6, 7).

## 2. Objectives

The current epidemiological study aimed to identify the prevalence of enteropathogenic parasites and sporozoan protozoa among patients with gastroenteritis referred to the selected hospitals in seven provinces of Iran including Gilan, East-Azarbayjan, Qazvin, Kurdistan, Mazandaran, Tehran, and Khorasan-e-Razavi.

### 3. Patients and Methods

#### 3.1. Patients' Samples

In the current cross-sectional study, 4200 stool samples randomly obtained from patients with gastroenteritis in the selected hospitals (Azzahra hospital in Rasht, Children hospital in Tabriz, Qods Children hospital in Qazvin, Tohid and Beasat hospitals in Sanandaj, Bu-Ali hospital in Sari, Children medical center in Tehran, Sheikh and Emam Reza hospitals in Mashhad) from June 2008 to June 2009. Sample size was calculated according to the previous year data on patients with gastroenteritis obtained from local health authorities. Primarily, samples were examined directly for enteroparasites, then filtered and concentrated using Paraseb kit. The pellets were fixed, and examined by different assays including Parasitology assay to diagnose enteropathogen parasites, stained by different assays including acid fast staining (AFS) and Giemsa, and observed by light microscopy to detect sporozoan protozoa, as previously stated (8).

#### 3.2. Fixation and Smear Preparation

Stool samples were morphologically and microscopically examined for consistency and trophozoites of protozoa and larvae of helminthes, before fixing. Twenty-five grams of each stool sample was mixed with 10 mL fixation buffer and incubated for one hour for fixing and inactivation. The suspension was passed through Paraseb kit (Dis Sys Co. UK) and centrifuged at 2,000 rpm for 5 minutes. Three smears were made from the obtained pellet, air-dried, fixed with methanol, and then examined by Giemsa and acid-fast staining methods, and auramin phenol fluorescence (APF) (8).

#### 3.3. Giemsa Staining

This stain is used on fixed specimens to identify some sporozoan protozoa including *Microsporidium* and *Cyclospora* spp. with excellent results. Other intestinal protozoa are difficult or impossible to identify. For Giemsa stain, sample preparation was done by spreading the specimen on a slide to obtain a thin smear. Slide was allowed to air dry, fixed in 100% methanol for one minute, allowed to air dry, placed in 10% Giemsa solution for 30 minutes, washed well with tap water, final air dried and after putting immersion oil, the stained slides were examined at high power (100x) of light microscope (9).

#### 3.4. Acid-Fast Staining

The fixed smear was stained with carbol fuchsin, rinsed with tap water, destained with 3% acid-alcohol, restained for background color with 0.5% malachite green (5 minutes), rinsed with tap water, dried at room temperature and observed under light microscope (all materials from Sigma, Co., Germany) (8).

#### 3.5. Auramin Phenol Fluorescence

For APF, the fixed smear was stained with auramine-O (15 minutes), rinsed with tap water, destained with 3% acid-alcohol, restained for background color with 0.5% potassium permanganate (3 minutes), rinsed with tap water, dried at room temperature and observed under fluorescence microscope (all materials from Sigma, Co., Germany) (8).

#### 3.6. Statistical Analysis

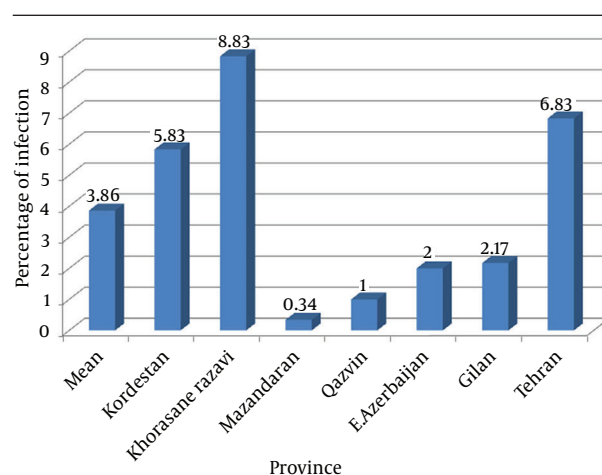
The data were evaluated by the statistical package of Excel Microsoft and SPSS softwares. Sample size for the precision of 5% and confidence interval of 95% was calculated.

### 4. Results

The results revealed the overall rate of infection, 3.86% (163 cases), as an indicator of parasitic enteropathogens in Iran. Among the provinces, Khorasan-e-Razavi with 8.83% (53 cases) had the highest rate of infection to enteropathogenic parasites and sporozoan protozoa followed by Tehran 6.83% (41 cases), Kurdistan 5.83% (35 cases), Gilan 2.17% (13 cases), East Azerbaijan 2% (12 cases), Qazvin 1% (6 cases), and Mazandaran 0.34% (2 cases) (Figure 1).

In the current study the frequencies of sporozoan protozoa including *Cryptosporidium*, *Microsporidium*, *Isospora*, and *Cyclospora* spp. in patients with gastroenteritis in the selected provinces were 0.1%, 0.1%, 0.07% and 0.02%, respectively (Figure 2).

Among the parasitic enteropathogens, *Giardia lamblia* with 1.78% had the highest infection rate followed by the other parasitic enteropathogens *Entamoeba histolytica* and *Blastocystis* spp. 0.47%, *Entamoeba coli* 0.38%, *Endolimax nana* 0.14%, *Iodamoeba bustschlii* 0.14%, *Hymenolepis nana* 0.07%, *Chilomastix mesnili* 0.04%, *Taenia saginata* 0.02%, Hook worms 0.02%, respectively. No other enteroparasite cases were observed (Figure 3).



**Figure 1.** Prevalence of Enteroparasites in the Patients With Gastroenteritis Referring to the Healthcare Centers of Seven Provinces of Iran

Among the infected patients, 59.5% were male and 40.5% female. Regarding different age groups, the highest and lowest rates of infection were among 0 - 10 (48%) and above 41 years old groups (6.7%) (Figure 4).

The association between rate of sporozoan protozoa infection and other parasitic enteropathogens and contact with animals was the highest in Tehran province with 1.19% infection, followed by Khorasan-e-Razavi (0.5%), Gilan (0.07%) and Qazvin (0.04%). No cases were detected in Kurdistan, East Azerbaijan and Mazandaran (Figure 5).

According to the reliability of laboratory detection method, the most frequent enteroparasites was recognized by direct parasitology method and the majority of sporozoan protozoa were detected by acid fast staining (Figure 6).

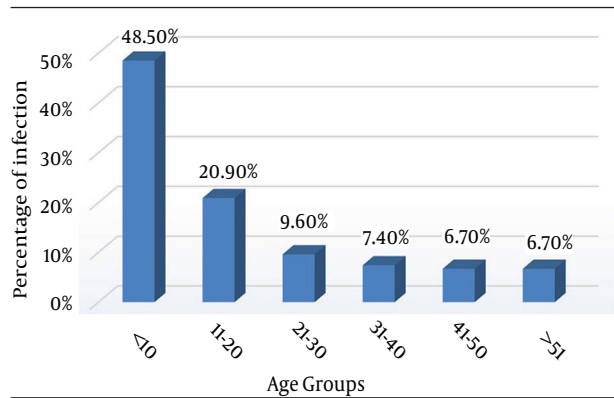


Figure 4. Association of Enteroparasites Prevalence With Age Groups in the Patients With Gastroenteritis in the Seven Provinces of Iran

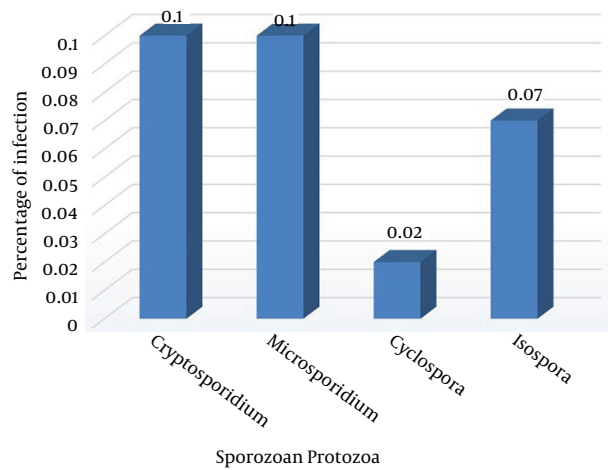


Figure 2. Prevalence of Sporozoan Protozoa in the Patients With Gastroenteritis Referring to the Healthcare Centers of Seven Provinces of Iran

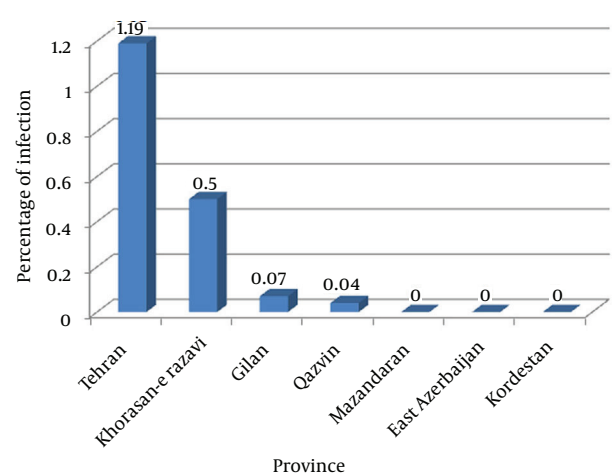


Figure 5. Association of Enteroparasites Prevalence With Animal Contacts of the Patients With Gastroenteritis in the Seven Provinces of Iran

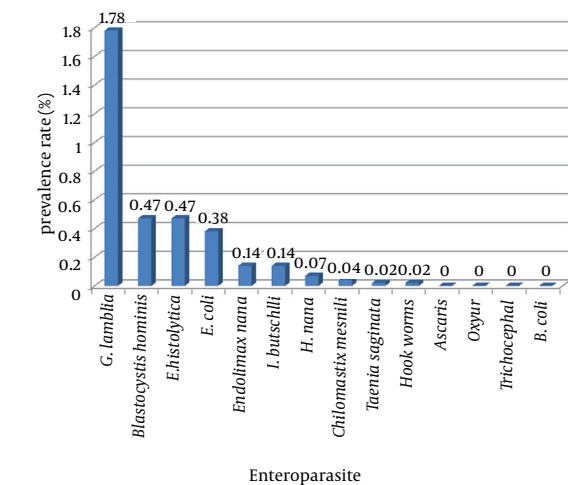


Figure 3. Prevalence of Other Parasitic Enteropathogens in Patients With Gastroenteritis in the Seven Provinces of Iran

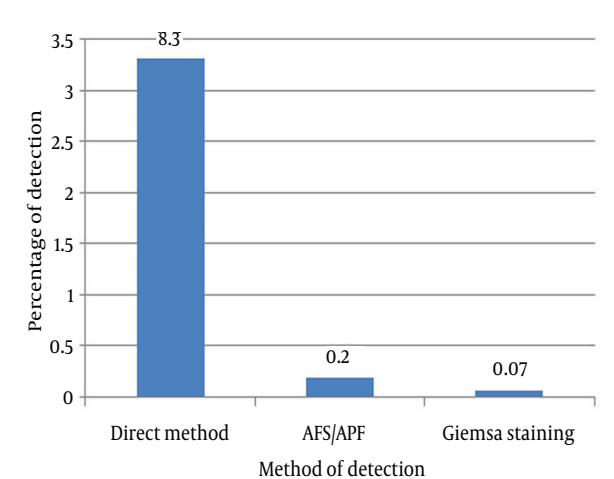


Figure 6. Association of Laboratory Methods to Detect Enteroparasites and Sporozoan Protozoa in the Patients With Gastroenteritis in the Seven Provinces of Iran

## 5. Discussion

The final results of the current research indicated that giardiasis was the most prevalent parasitic infection in the gastrointestinal tract and also the most important parasitic enteropathogenes in Iran (10). Despite, relatively low prevalence of sporozoan group, Giardiasis is still the most prevalent agent for gastroenteritis (11) amongst 3.88% of parasitic infections; therefore, it was indicated that *Giardia lamblia* with 1.78% rate of infection, is the most prevalent enteropathogenic parasite in Iran.

Among enteropathogenic parasites, the most and least prevalent ones were *G. lamblia* and sporozoan parasites, respectively. More studies are needed to evaluate the pathogenesis of *Blastocystis* sp. (12). Co-infection of two or more parasites especially *G. lamblia* and *Blastocystis* sp. implies the possible interaction of these pathogens, which requires more researches. High frequency of *Blastocystis* sp. and co-infection of *Giardia* and *Blastocystis* spp. imply possible interactions of enteropathogens in the hosts, which needs more studies to clarify (13, 14).

Although, some enteropathogenic parasites including *E. histolytica*, *E. coli*, *Endolimax nana*, *Iodamoeba butschlii*, *Hymenolepis nana*, *Chilomastix mesnili*, and *Taenia saginata* also cause lower infection rate, other parasites including Hookworms, *Strongyloides* spp., *Balantidium coli*, *Ascaris* spp., *Oxyur* and *Trichuris* and *Fasciola* spp. were not detected in the current study, which was consistent with the recent publications data (15-18).

In recent years, prevalence of enteropathogenic parasites declined compared to the data from decades ago. Sharif et al. reported 26.2% infection of intestinal protozoans (19) and Daryani et al. reported 33.3% infection rate (20) in the North of Iran and Assmar et al. (1999) reported that the rate of parasitic infection in 6252 pupils in Mazandaran province was 57.1%, which may be associated with poor sanitation, low education and drinking water contamination (21-23). A recent study by Kousha et al. (2011) in Tabriz, Northwest of Iran, reported 44% infection by one or more intestinal parasites in school children (18). The lower rate of parasitic infections may be the result of health training, increased awareness and improvement of environmental conditions.

The infection rate of sporozoan parasites including *Cryptosporidium* sp., *Microspora* sp., *Isospora* sp., and *Cyclospora* sp. among patients in the studied provinces was consistent with those of some recent studies (9, 13). The prevalence of *Cryptosporidium* sp. in the Eastern and Central Mazandaran is reported 0.1% and 0.12% respectively (14, 24). The results of current study was also approved by reports of Jafari et al. (2012) in Hamadan district, Iran (25). Nahrevanian et al. reported no *Cryptosporidium* infection in the Western Mazandaran (8). Some studies reported higher prevalence of *Cryptosporidium* infection in different parts of Iran including 14.5% by Heidarnegadi et al. in rural areas of Shushtar in Khuzestan province, South-West of Iran, 7.7% by Nouri et al. in Azerbaijan (16), 2.9% by Nahrevanian

et al. among immunocompetent patients with gastroenteritis in Tehran (6), 4.1% by Fallah and Haghghi in children with diarrhea in Hamadan (west of Iran) (17) and 2.4% by Taghipour et al. (2011) in children with diarrhea in Tehran (26). In previous studies low prevalence of sporozoan parasites was reported, which were in harmony with that of the current study (6, 27).

In the current study, the age group less than 10 years old had the highest infection rate indicating more sensitivity of children than adults to infection. In fact, children were in higher risk of parasitic infections compared to adults, which was similar to the results of previous studies (28). The current study results indicated 59.5% infection in males and 40.5% infection in females. Although the rate of infection was different between males and females the difference was not statistically significant. This result was confirmed by some studies (29). It seems that because of outdoor activities the males are more exposed to infection agents.

The role of drinking water in infection was very important. Filtration drinking water may reduce or even in some species delete the parasite cysts or oocytes (30). Drinking untreated water has proven this fact (21). In the current study the association between the source of drinking water and rate of infection was not statistically significant, which is not supporting the results of the study by Shojaei et al. (22). There are very few reports on the prevalence of intestinal parasites among HIV-positive patients in Iran. To determine the prevalence of intestinal parasites among such individuals, Zali et al. reported that the overall prevalence of intestinal parasites was 18.4% among them (31). In a surprising study, Meamar et al. (2007) reported no significant difference of intestinal parasitic infections between two groups of HIV/AIDS patients and non-HIV individuals from 2003 to 2005 in Iran (32). In another study, Meamar et al. (2009) indicated chronic severe watery diarrhea due to *Isospora belli* in another patient with immunodeficiency diagnosed with mediastinal thymoma (33).

This result suggested that more infection was observed in hot and humid seasons, which was confirmed by recent studies (5, 34). The results of the current study was consistent with those of most studies in this field based on abundance of infection in warm and wet seasons, and more frequency of infection among children than adults. Meanwhile, geographical and agricultural conditions, seasonal rainfall, abundance of water, and animal contact are key factors affecting sporozoan infections. It is suggested that health education, improvement of sanitation, treatment of drinking water, sewage disposal and accessibility to anti-parasitic drugs are the most important factors in reduction of parasitic enteropathogen infection. Although, the current results showed a decline in the rate of parasitic infections in seven provinces of Iran compared with those of the previous studies, the situation is always under caution for emerging and re-emerging opportunistic enteropathogen parasites.

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## Authors' Contributions

Hossein Nahrevanian and Mehdi. Assmar conceptualized and designed the study. Seyed Mohsen Zahraei and Moharram Mafi conducted the monitoring of project and laboratory facilities in seven provinces. Hossein Masoumi Asl involved other official co-ordination and consultancy. Fatemeh Sadat Ghasemi prepared figures and proof-read the manuscript.

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