Prevalence of sporozoan and parasitic enteropathogen protozoans in patients with gastroenteritis in Iran

Abstract
Parasitic gastroenteritis is one of the most common illnesses in humans worldwide. Most studies on gastroenteritis have focused on viral and bacterial infections, while parasitic enteropathogens and especially intestinal protozoan parasites may play a role that has not been well studied. The aim of this study is to identify the prevalence of enteropathogenic parasites and sporozoan protozoa in patients with gastroenteritis in selected hospitals in seven provinces of Iran.

Methods: In this cross-sectional study, randomly 4200 stool specimens were obtained from patients with gastroenteritis in selected hospitals. Primarily samples were examined directly for enteroparasites. The samples were filtered and concentrated by using the Paraseb Kit; and fixed, and stained by different methods including acid-fast staining, Auramin phenol Fluorescence staining, and Giemsa staining were observed using light microscopy under various magnification.

Results: The results revealed the overall rate of parasitic infection was 2.4% in the studied population. The highest rate of infection was observed in the 0-10 year age-group (47.2%). Among the infected patients, 59.5% were male and the rest were female patients.

In the regions studied regions Tehran and Mazandaran had the highest and the lowest frequency with 41 (97%), and 2 (47%) cases per 4200 patients respectively. Gastroenteritis was found in patients infected with Cryptosporidium, Microsporidium, Isospora and Cyclospora, 13 cases in total (0.3%) with 5 (0.12%), 4 (0.09%), 3 (0.07%) and 1 (0.02%) cases for all four parasites respectively. Parasitic enteropathogens were detected in 88 cases of all studied cases (2.09%). Among these observed parasitic enteropathogens, Giardia lamblia with 42 cases (1%) had the highest rate of infection. The observed frequency of infection for Blastocystis hominis, Entamoeba histolytica, were 21 (0.5%), and 20 (0.47%) cases respectively while for the rest was 10 cases.

Conclusion: Despite a relatively low prevalence of sporozoans group, giardia was the most prevalent agent for gastroenteritis (1%) parasitic infections in Iran, specifically in Tehran with the highest rate in children (0-10 year age-group). High frequency of Blastocystis hominis and co-infection of Giardia lamblia and Blastocystis hominis implies possible interactions.
between enteropathogens in a host, which require more studies to be clarified. The study also showed presented knowledge of personal and community health and hygiene needs to be more emphasized

**Keyword:** Cryptosporidium, Isospora, Microspidian, Cyclospora, Enteropathogens, gastroenteritis, Iran

**Introduction:**
Despite of development in health care criteria, parasitic infections have been reported frequently as one of the most common cause of diseases in developing countries (1-6). Protozoa and helminthic intestinal infections are estimated to affect 3.5 billion people worldwide, the majority being children age 7 to 10.

Geographical conditions and poor nutritional and socioeconomic status are the effective parameters that contribute on the frequency of parasitic infections, although iron deficiency anemia and physical and mental difficulties are some of other causes, besides public health problems. Most studies on gastroenteritis have focused on viral and bacterial infections, while gastroenteritis from intestinal protozoan parasites has limited studies (11-13).

The primary health care programs in Iran have played an important role in reducing the morbidity and mortality associated with parasitic disease; however, the amount of diarrheal illness in the population, particularly among young children in some part of the country remains high (14, 15).

Four species of intestinal parasitic infections with particular importance are *Giardia lamblia, Cryptosporidum parvum, Entamoeba histolytica* and *Blastocystis hominis* (16). The most identified pathogens currently in public health laboratories are *Salmonella* spp., *Shigella* spp., *Entamoeba histolytica* and *Giardia* lamblia (14, 15, 17). Cryptosporidium and Isospora, though considered a health problem, are generally not investigated in routine laboratory examinations. Because an etiologic agent is not detected for a large portion of patients with diarrhea, the possibility exists that a portion of the undiagnosed illness may be attributable to one or more of these parasites. Besides, necessity of preventive measures needs to be carefully evaluated for each province. Each region has obviously its own specific conditions
with different possible pattern for high-risk populations that needs to be identified for increasing their level of knowledge about personal and community health and hygiene.

The present study is a comprehensive survey to assess the prevalence of parasitic enteropathogens and to determine the encounter pattern of these infections in studied provinces of the country.

Materials and Methods:
A population-based prevalence study was done in randomly selected patients from June 2008 to May 2009. Four thousands and four hundreds (600 in each region) fecal specimens were selected from all admitted patients with confirmed gastroenteritis infections in the city-hospital of these seven provinces. The specimens were selected by random sampling method after calculation the required number specimens. These selected centers were Azzahra hospital in Rasht, Children’s hospital in Tabriz, Qods Children hospital in Qazvin, Tohid and Besat hospitals in Sanandaj, Bu-Ali hospital in Sari, Children Medical Center in Tehran, Sheikh and Emam Reza hospitals in Mashhad.

Macroscopic examination of stool samples was done for the presence of worms (Ascaris lumbricoides, Strongyloides stercoralis, Enterobius vermicularis, Trichuris trichiura, Taenia saginata), and intestinal sporozoa. Blood and physical characteristics such as color, appearance and odor were noted. Besides direct examination of stool samples, all specimens were examined under various magnifications (10, 100, 1000X) after being stained with three different methods, Acid Fast staining, Auramin Phenol Fluorescence staining, Giemsa staining for the presence of parasitic trophozoites, cysts eggs and ooacysts larva. Following concentration using Paraseb kit (Pasteur Institute of Iran) three smears were prepared for each sample. Statistical analysis was performed using SPSS 18.0 and Graph Pad Prism 7.0.

Results:
Parasitic infection was found in 101 cases (2.4%) of 4200 specimens after analysis of all applied methods, although directed examination showed the highest sensitivity (Figure 1). Analyzed results revealed the highest rate of infection were observed in the 0-10 year age-group with both sporozoan and other parasitic enteropathogens with 59 cases out of a total of
Among the infected patients 58.5% were male and 41.5% were female.

Among the provinces, Tehran with 0.90% (38 cases) had the highest rates of infection followed by Kordestan with 6.83% (24 cases), Eastern Azarbayejn 0.30% (13 cases), Khorasan Razavi, Gilan, Qazvin and Mazandaran with 11 (0.26%), 10 (0.23%), 3 (0.07%) and 2 (0.04%) cases respectively (Figure 2).

In this study the frequency of Sporozoan protozoa including Cryptosporidium, Microsporidium, Isospora and Cyclospora in patients with gastroenteritis in selected provinces were 5 (0.12%), 4 (0.09%), 3 (0.07%) and 1 (0.02%) respectively and 0.3% totally.

Among parasitic enteropathogens, *Giardia lamblia* with 1% (42 cases) had the highest rate of infection, frequency of other parasitic enteropathogens were 0.47% (20 cases) for *Entamoeba histolitica*, 0.5% (21 cases) for *Blastocystis* spp, 0.07% (3 cases) for *Hymenolepis nana*, 0.02% (1 case) for each of *Taenia saginata*, *Endolimax nana*, *Taenia saginata* and hook worm (Table 1).

**Discussion:**

Despite of development in health conditions and standard criteria, parasitic infections have remained as one of the most common cause of diseases in the developing countries. In the present study, various enteropathogens were detected in all studied regions. Analysis of the result showed the highest rate of infection was in children less than 10 years with *Giardia lamblia* among the highest all detected parasites.

Several microscopy and PCR based studies have underlined the presence and the rate of intestinal protozoan parasitic infection from 0.5 to 30% in Iran (15, 17, 18). In another study on 802 gastroenteritis patients in Babol and Babolsar cities, a northern Iran, the prevalence rate of enteropathogenic parasites was 3.4% (19). Analyses the results showed only 2 positive cases in the Mazandaran region among 600 examined specimens (0.33%) that was the lowest rate in all studied regions. The lower rate determined in our study may be due to improving the level of health care conditions in Mazandaran, although other parameters should be
considered such as the season of the study, and the specific selected region of the Mazandaran province.

Recently Haghighi (20) has studied 427 patients infected with one or more intestinal parasites. Their prevalence of detected protozoa were reported as follows: *Giardia lamblia* (10.1%), *Blastocystis hominis* (2.2%), *Chilomastix mesnili* (1.7%), *Trichomonas hominis* (0.7%), and *E. histolytica/E. dispar* (0.51%) (20). In our study the most frequent parasites detected were *G. lamblia*, *Blastocystis*, and *E.histolitica*, that is nearly similar to Haghighi’s report. Haghighi’s report cannot be compared to selected regions in our study because of the specific geographical situation in the Zahedan region in their study. The selected region of this study, "Baluchestan", has a lot of immigration from Pakistan and Afghanistan. The frequency of parasitic infection is relatively lower than among other Middle Eastern countries. Studies of children with diarrhoea in Egypt, revealed the prevalence of parasitic infections rate was 46% (21). In another report it was showed that 38.5% of school children in northern Iraq were infected with *G. lamblia* (22).

**Conclusion:** Despite the relatively low prevalence of Sporozoan, *Giardia lamblia* is the most prevalent agent for gastroenteritis, 3.86% of the parasitic infection in studied region of Iran. High frequency of *Blastocystis hominis* and co-infection of *Giardia lamblia* and *Blastocystis hominis* implies possible interactions between enteropathogens in a host, which require more studies to be clarified. The study also showed presented knowledge of personal and community health and hygiene needs to be more emphasized.
Table 1: Frequency of parasitic infections in various age groups

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Sporozoan protozoa</th>
<th>parasitic enteropathogens</th>
<th>Total</th>
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<tbody>
<tr>
<td>0-10</td>
<td>11</td>
<td>48</td>
<td>59</td>
</tr>
<tr>
<td>11-20</td>
<td>1</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>21-30</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>31-40</td>
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<td>6</td>
<td>6</td>
</tr>
<tr>
<td>41-50</td>
<td>1</td>
<td>7</td>
<td>8</td>
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<tr>
<td>Over51</td>
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<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>88</td>
<td>101</td>
</tr>
</tbody>
</table>

Table 2: Frequency of various parasites in the regions of study

<table>
<thead>
<tr>
<th>Region</th>
<th>Khorasan R.</th>
<th>Tehran</th>
<th>Kordestan</th>
<th>E. Azarbayjan</th>
<th>Gilan</th>
<th>Qazvin</th>
<th>Mazandaran</th>
<th>Total</th>
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<tr>
<td>Cryptosporidium parvum</td>
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<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
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<td>Microsporidium SPP</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cyclospora</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>9</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>42</td>
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<tr>
<td>Entamoeba histolytica</td>
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<td>2</td>
<td>15</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td>3</td>
<td>14</td>
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<tr>
<td>Hymenolepis nana</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>38</td>
<td>24</td>
<td>12</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>101</td>
</tr>
</tbody>
</table>
Figure 1: Frequency of identified positive cases in all applied methods.

Figure 2: Number of parasitic infection cases in studied provinces

References:


