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ORIGINAL ARTICLE

Current situation of *Cryptosporidium* and other enteroparasites among patients with gastroenteritis from western cities of Mazandaran province, Iran, during 2007-2008

Hossein Nahrevanian[*1], Seyedeh Atena Azarinoosh[2], Behzad Esfandiari[3], Seyed Peyman Ziapoor[3], Mohammad Shadifar[3], Galia Amirbozorgy[3], Effat Hayati[3], Javad Davoodi[3]

[1] *Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran*

[2] *Islamic Azad University, Zanjan Branch, Zanjan, Iran*

[3] *North Research Center, Mazandaran, Amol, Iran*

ABSTRACT

Aim: The aim of this study was to evaluate the prevalence rate of *Cryptosporidium* and other enteropathogen parasites among patients with gastroenteritis in western cities of Mazandaran province, northern Iran, during one year.

Background: As specific characterization, high humidity, ecological conditions, superficial water sources, municipal water supplies, domestic and industrial animal husbandry and the rate of raining made Mazandaran as a favorable province for transmission of parasitic diseases.

Patients and methods: This investigation was conducted between June 2007 to June 2008 in western cities of Mazandaran province, Northern Iran. Overall, 420 stool samples of gastroenteritis patients were collected from Chalous (194 sample), Tonekabon (187 sample) and Ramsar (39 sample), fixed and examined by Direct Method (DM) for diagnosis of enteropathogen parasites, Acid-Fast Staining (AFS) and Auramin Phenol Fluorescence (APF) for detection of *Cryptosporidium* and other sporozoan protozoa.

Results: The results confirmed the overall prevalence rate of parasitic infections to be 2.14% (9 patients) among those cities, and the highest rate of infection was observed to be among *Giardia lamblia* (1.19%, 5 patients), *Blastocystis hominis* (0.71%, 3 patients) and *Entamoeba coli* (0.24%, 1 patient) respectively. There was no *Cryptospordium* and other sporozoan infection among the test samples. Comparative prevalence rates of parasitic infections in Chalous, Tonekabon and Ramsar were 1.55% (3 patients), 2.14% (4 patients) and 2.56% (1 patient) respectively. The relative frequencies of parasitic infections among infected individuals were associated with seasons, therefore the highest and the lowest rates were observed in autumn (40%) and spring (10%), respectively.

Conclusion: Although, the current results showed a decline in the rate of parasitic infections in Mazandaran province recently in comparison with the past previous studies, the situation is always under caution for emerging and re-emerging enteropathogen parasites with the emphasize on opportunistic parasites.

Keywords: *Cryptosporidium, Enteropathogen, Mazandaran, Gastroenteritis, Iran, Parasitic infection*.
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INTRODUCTION

In spite of improvement in life style, parasitic infections are still among problems in tropical and sub-tropical areas. Opportunistic parasites including sporozoan protozoa can be considered as major threats. Cryptosporidiosis, caused by the protozoan *Cryptosporidium* is recognized as a significant enteric disease in animals and humans (1). This parasite is well known to cause severe

Received: 3 January 2010 *Accepted*: 22 February 2010
Reprint or Correspondence: Hossein Nahrevanian, PhD. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran
E-mail: mobcghn@pasteur.ac.ir

life-threatening illness in immunocompromised individuals. *Cryptosporidium* invades epithelial cells of the intestinal tract and respiratory tree of vertebrate hosts (2-4). It is a primary pathogen causing acute diarrhea and the most violent symptom of cryptosporidiosis which is watery diarrhea. Non-specific signs, such as dehydration, fever, anorexia, weakness and progressive loss of conditions may be accompanied. Diarrhea is usually self-limiting in immunocompetent humans; however, it can be life-threatening in children and immunocompromised persons (5).

Epidemiological research carried out in different countries has shown an association between the social and economical situation with different parasites. In addition, poor sanitary and environmental conditions are known to be relevant in the propagations of these infectious agents (6).

Global situation of Cryptosporidiosis is varied. The disease is occurred at an overall rate of 6 per 100,000 populations per year in Canada, although a large outbreak occurred in the second half of the summer of 2001 (7). In Guinea Bissau, *Cryptosporidium sp*. had a prevalence of 7.7% and was the second most common parasite with a marked seasonal variations; peak prevalence found consistently at the beginning of or just before the rainy season from May to July (2-8). There were also discrepancies in different surveys about the prevalence rate of *Cryptosporidium sp*. which were done in Iran (9-10). A prevalence rate of 21.4% of Cryptosporidiosis was reported in children under 15 years old from South Eastern Iran (11). Among acquired immunodeficiency groups in Iran and those with diarrhea, the infection with *Cryptosporidium*, were reported to be 1.4% and 6.3% respectively (5).

A variety of diagnostic options are available for the detection of *Cryptosporidium* in clinical stool samples. Auramin phenol fluorescence (APF) screening followed by modified acid fast staining (AFS) is a sensitive and specific approach for the identification of *Cryptosporidium* oocysts in stools (5-8, 11-12).

Despite the importance of the seriousness of *Cryptosporidium* infection in gasteroenteritic patients, and their protection from infection, there are no enough valid data to provide a comparison of the prevalence of this sporozoan infection with other enteropathogen parasites in this group. This epidemiological study was carried out to analyze the current situation of enteropathogenic parasites in the Western cities of Mazandaran, Northern Province of Iran, during 2007-2008.

PATIENTS and METHODS

This descriptive study was conducted between June 2007 to June 2008 in Chalous, Tonekabon and Ramsar cities which are located in western part of Mazandaran province, north Iran. Sample size was calculated according to data of gastroenteritis patients of previous year, which was obtained from local health authorities. Overall, 420 stool samples of gastroenteritis patients were collected from Chalous (194 sample), Tonekabon (187 sample) and Ramsar (39 sample), fixed and examined by different assays including direct method (DM) for diagnosis of enteropathogen parasites, acid-fast staining (AFS) and auramin phenol flourescence (APF) for detection of *Cryptosporidium* and other sporozoan protozoa.

Stool samples were examined morphologically and microscopically for consistency and parasites. Twenty-five grams of stool samples were mixed with 10 ml fixation buffer [10 ml PBS, 20 ml formaldehyde, 100 ml glycerin and enough distilled water to make a final volume of 1,000 ml (all materials from Sigma) and incubated for 1 h] for fixing and inactivation. The suspension was passed through Paraseb Kit (Dis Sys Co. UK) and centrifuged at 2,000 rpm for 5 min. Two smears were made from the pellet obtained, air-dried,

fixed with methanol and then examined by AFS and APF (12).

For acid-fast staining (AFS), the fixed smear was stained with carbol fucshin, rinsed with tap water, destained with 3% acid-alchohol, restained for background color with 0.5% malachite green (5 min), rinsed with tap water, dried at room temperature and observed under light microscope (all material from Sigma) (12-13).

For auramin phenol fluorescence (APF), the fixed smear was stained with auramine-O (15 min), rinsed with tap water, destained with 3% acid-alchohol, restained for background color with 0.5% potassium permangenate (3 min), rinsed with tap water, dried at room temperature and observed under fluorescence microscope (all material from Sigma) (12-13).

The data was evaluated by Spearman's correlation test using the statistical package of Excel Microsoft and SPSS softwares. A sample size for the precision of 5% and confidence interval of 95% was calculated.

RESULTS

Amongst patients participated in the study, 39.4% were female and 60.6% were male. Attitude was not significantly correlated with knowledge confidence 95%.

The results confirmed the overall prevalence rate of parasitic infections which was 2.14% (9 patients) among those cities, and the highest rate of infection was observed to be among *Giardia lamblia* (1.19%, 5 patients), *Blastocystis hominis* (0.71%, 3 patients) and *Entamoeba coli* (0.24%, 1 patient) respectively. There was no *Cryptospordium* infection among the test samples (Figure 1).

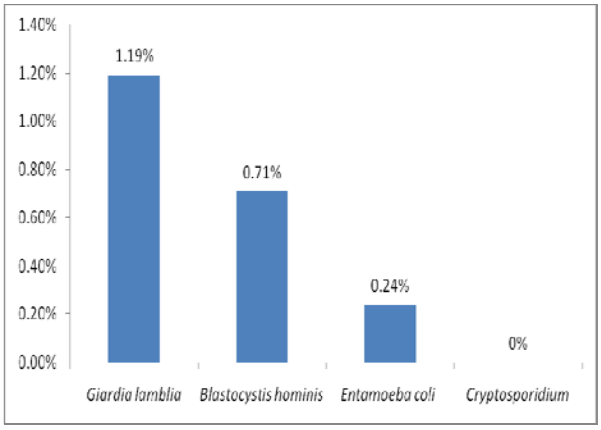

Figure 1. A comparison of the prevalence rate of different parasites among patients with gastroenteritis in three western cities of Mazandaran province, Iran, during 2007-2008

Out of patients with gastroenteritis in western cities of Mazandaran province, only 40% of them used continuous clean drinking water and the majority (60%) of them used occasionally non-qualified water supplies. Attitude was significantly correlated with knowledge ($P< 0.05$) (Figure 2).

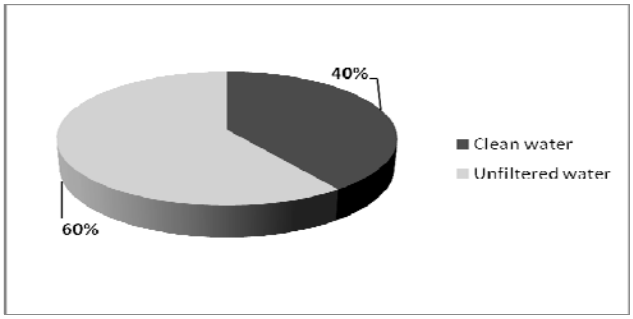

Figure 2. Related frequency about type of drinking water among patients with gastroenteritis in three western cities of Mazandaran province, Iran, during 2007-2008

Comparative prevalence rates of parasitic infections in Chalous, Tonekabon and Ramsar cities were 1.55% (3 patients), 2.14% (4 patients) and 2.56% (1 patient) respectively. Attitude was not significantly correlated with knowledge confidence 95% (Figure 3).

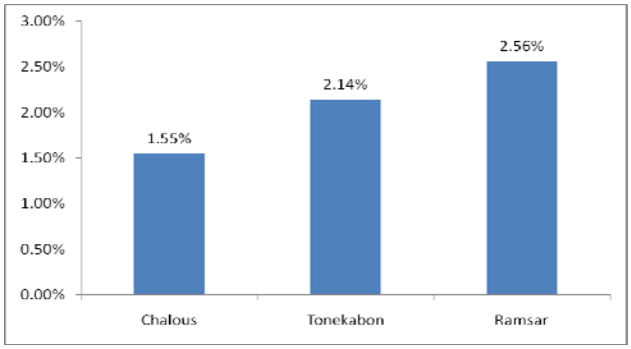

Figure 3. A comparison of prevalence rate of positive parasitic cases among patients with gastroenteritis in three western cities of Mazandaran province, Iran, during 2007-2008 according to the residential city

The relative frequencies of parasitic infections among infected individuals were associated with seasons, therefore the highest and the lowest rates were observed in autumn (40%) and spring (10%) respectively. Attitude was not significantly correlated with knowledge confidence 95% (Figure 4).

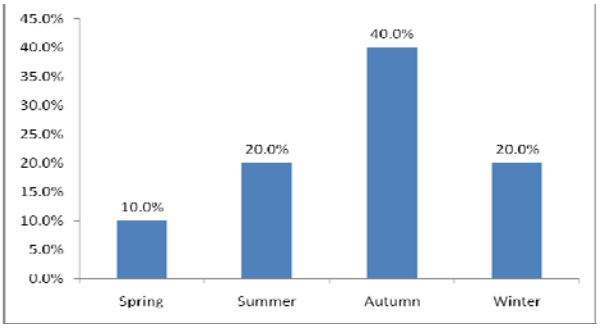

Figure 4. Prevalence rate of positive parasitic cases among patients with gastroenteritis in three western cities of Mazandaran province, Iran, during 2007-2008 according to season of infection

DISCUSSION

At a glance, the prevalence rate of parasitic infections in Mazandaran province reported to be varied depending on study group, age, type and size of sample, year, immune system, season and location. Although, Assmar et al. (1998) reported a high prevalence rate (57.1%) of different parasitic infections among primary school children in Mazandaran province in 1999 (14), recent studies by Soleimanpor et al. (2006) reported 9.1% rate of infection in the eastern parts of Mazandaran province (15); Ghorbannia Delavar et al. (2008) reported 3.4% prevalence rate of infections in central part of the province (16) and now we are reporting in current publication a prevalence rate of 2.14% in western part of Mazandaran province, Iran.

Data of current investigation, in addition to the previous studies revealed an overall prevalence rate of 10.7% infections to various enteropathogen parasites in entire parts of Mazandaran province, which may be associated with some gastroenteritis. Publication reviews and the data resulting from this study indicating, *Cryptosporidium* infection was observed in 0.08% of patients and the prevalence rates of other parasites including *Blastocystis huminis* were 5.1%, *Giardia lamblia* (2.8%), *Entamoeba coli* (1.2%), *Entamoeba histolytica* (0.4%), *Hymenolepis nana* (0.3%), *Strongyloides stercoralis* (0.2%), *Entrobius vermicularis* (0.08%), *Chilomastix mesnili* (0.03%), Nematoda (0.03%) and mixed infections of *Giardia* and *Blastocystis* (0.5%), *Giardia* and *Hymenolepis nana* (0.03%).

In addition to common enteropathogen parasites, *Cryptosporidium* is indicated as a gastroenteritis agent in Mazandaran province. Prevalence of *Cryptosporidium* in Iran reported variable rates in many publications. In Azerbaijan, Nouri et al. (1991) reported a prevalence rate of 7.7% among human diarrhea patients (17). In Tehran, Nahrevanian et al. (2007) reported a prevalence of 2.9% among immunocompetent patients (9) and in Hamedan, Fallah and Haghighi (1996) observed 4.1% prevalence rate among children with diarrhea (18). The prevalence rates of *Cryptosporidum* were also different in the world, depending on many parameters including,

study group, immune system, age, type and size of sample, country, year and season. In the Republic of Korea, the prevalence of cryptosporidiosis varied according to the localities. Lee et al. (2005) reported a prevalence of 1% among non-HIV patients (19), whereas Yu et al. (2004) published 3.3% rate among the villagers in several rural areas (20). Moreover, in Tanzania, Houpt et al. (2005) described a prevalence of 17.3% amongst HIV patients (21). In March 1993, a cryptosporidiosis outbreak occurred in Milwaukee, USA, when the municipal water supply was contaminated with C*ryptosporidium* (22) and more than 4000 people were hospitalized and it was reports that cryptosporidiosis contributed to estimated 104 deaths (23).

Although, detection of Sporozoan protozoa including *Cryptosporidium sp., Microsporidium sp., Isospora sp.* and *Cyclospora sp.* is uncommon in routine stool examination and it may be terminated in many cases, it could cause pathologies especially in immunocompromised patients, a matter that needs diagnostic, preventive and therapeutic strategies (10).

In conclusion, contaminated water is still an important cause of diarrhea among children. Waterborne protozoa including *Cryptosporidium sp., Giardia sp., Entamoeba sp., Naegleria sp. and Acanthamoeba sp.* are highly emphasized here. Although, the current results showed a decline in the rate of parasitic infections in Mazandaran province recently in comparison with the previous studies, which indicated improvement of health education, water treatment, environmental sanitation and public knowledge, however the situation is always under observation for emerging and re-emerging opportunistic enteropathogen parasites.

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