

Cryptosporidiosis among immunocompetent patients with gastroenteritis in Iran: a comparison with other enteropathogenic parasites

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Background and Purpose: *Cryptosporidium* is a protozoan parasite that reproduces within the epithelial cells of several organs of vertebrate hosts. The manifestation of the disease is either self-limiting acute diarrhea in immunocompetent patients, or fatal chronic diarrhea in immunocompromised patients. Common clinical symptoms include watery diarrhea, abdominal pain, and weight loss.

Methods: This randomized pilot study conducted in Tehran, Iran, included 104 children and adult patients with gastroenteritis referred to the Children's Hospital Centre and Pasteur Institute of Iran. Control samples from healthy individuals (36 children and adults) were also collected; the entire test group had diarrhea and the control group had formed stool consistency. Stool samples were primarily examined by the direct method, then fixed and tested by 3 assays including acid-fast staining, auramine phenol fluorescence, and direct fluorescence using monoclonal antibody.

Results: The study revealed that 2.9% of the patients were infected by *Cryptosporidium* spp. Other parasites observed included *Giardia lamblia* (5.8%), *Ascaris lumbricoides* (1.9%), and *Entamoeba histolytica* (0.96%). Formed stool samples showed no oocysts of *Cryptosporidia*.

Conclusions: In addition to common enteropathogenic organisms, *Cryptosporidium* is indicated as a key causative agent of diarrhea in humans. Although cryptosporidiosis may, in many cases, be terminated by self-limiting mechanisms, it could cause pathologies requiring preventive and therapeutic policies.

Key words: Cryptosporidiosis; *Cryptosporidium*; Diarrhea; Gastroenteritis; Iran

Introduction

The genus *Cryptosporidium* identifies protozoan parasites that grow and reproduce within the epithelial cells of digestive organs of vertebrate hosts [1]. The human host range is broad and includes children in developing countries, people with immunodeficiency, and immunocompetent individuals [2]. The most common clinical feature of cryptosporidiosis is profuse and watery diarrhea that may contain mucus, but rarely blood and leukocytes with weight loss. Other less common clinical features include abdominal pain, nausea and

vomiting, and low-grade fever [3]. Infected individuals show a wide spectrum of clinical presentations; the pathogenicity of *Cryptosporidium* varies with the parasite spp. involved and the type, age, and immune status of the host [4]. In addition to susceptible humans, it is a frequent cause of diarrheal disease in some domesticated animals [3]. In developing countries, *Cryptosporidium* infections occur mostly in children younger than 5 years of age, with peak occurrence of diarrhea in children younger than 2 years of age [4].

Cryptosporidiosis probably has several reservoirs and routes of transmission in both rural and urban communities [5]. Human infection with *Cryptosporidium* spp. has been described across 6 continents, in developed and less developed countries, and in urban and rural areas [1]. The data are variable even within the same

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geographic location. Direct comparison of the results is often difficult because different study populations, stool sampling, and oocyst detection procedures were used [3]. The mode of spread of the infection is from person to person, from animals, and via food and water [6]. The diagnosis of cryptosporidiosis rests on the identification of the spherical oocysts in stool or biopsy specimens of human gastrointestinal mucosa [2]. Auramine phenol fluorescence (APF) screening of stool sediment smears followed by modified acid-fast staining (AFS) is a sensitive and specific approach for the identification of oocysts in stools [2]. Currently, a database is being compiled from which a limited understanding of the geographic distribution and prevalence of human cryptosporidiosis is beginning to emerge [3]. This randomized pilot study describes the medical importance of *Cryptosporidium* in comparison with other enteropathogenic parasites in Tehran, Iran.

Methods

Patients and samples

This randomized pilot study conducted in Tehran included 104 patients with gastroenteritis (93 children below 10 years of age and 11 adults) referred to the Children's Hospital Centre and Pasteur Institute of Iran. Control samples from healthy individuals (36 children and adults) with formed stool consistency were also included. All personal information, stage of disease, clinical symptoms, and stool consistency were recorded.

Fixation and smear preparations

Stool samples were primarily examined morphologically and microscopically for consistency and other parasites. Stool samples (25 g) were mixed with 10 mL of fixation buffer (10 mL phosphate-buffered saline, 20 mL formaldehyde, 100 mL glycerine with distilled water to a final volume of 1000 mL [all materials from Sigma Chemical Co., Deisenhofen, Germany]) and incubated for 1 h in order to be fixed and inactivated. The suspension was filtered through 4 layers of netting cotton and centrifuged at 2000 rpm for 5 min. Three smears were made from the pellet, air dried, fixed with methanol or acetone then examined by 3 different assays including AFS, APF, and direct fluorescence using monoclonal antibody (DF×mAb).

AFS

Fixed smears were stained with carbol fuchsin 5% and heat (2-5 min using a candle flame) until evaporation, rinsed with tap water, destained with acid-alcohol 3%,

restained for background color with malachite green 0.5% (5 min). The smears were then rinsed with tap water, dried at room temperature, and observed under a light microscope (all materials from Sigma) [7].

APF

Fixed smears were stained with auramine O 0.1% (15 min), rinsed with tap water, destained with acid-alcohol 3%, and restained for background color with potassium permanganate 0.5% (3 min). The smears were then rinsed with tap water, air dried, and observed under a fluorescence microscope (all materials from Sigma) [7].

DF×mAb

MonoFluo® kit *Cryptosporidium* (Diagnostics Pasteur, Marnes-la-Coquette, France) was used for the DF×mAb assay. Smears were first fixed with acetone, then 20 µL of fluorescein isothiocyanate-mAb was placed on the samples, incubated in a humid chamber at 37°C for 30 min, rinsed with distilled water, air dried, mounted with buffered glycerine and examined on a fluorescence microscope according to the manufacturer's instructions.

Results

Clinical symptoms recorded included diarrhea, nausea, vomiting, fever, abdominal pains, flatulence, and weight loss. Among symptomatic patients, 3 cases of cryptosporidiosis were detected using all 3 assays; positive cases were under 10 years old (Fig. 1). The prevalence of *Cryptosporidium* infection in patients with gastroenteritis was 2.9%. Formed stool samples of the control group showed no oocysts of *Cryptosporidia*. Moreover, other enteropathogenic parasitic infections were observed among gastroenteritic samples including 6 cases of giardiasis (5.8%), 2 cases of ascariasis (1.9%), and 1 case of amoebiasis (0.96%) [Table 1].

Discussion

This pilot study revealed that cryptosporidiosis is the second parasitic cause of diarrhea after giardiasis in patients with gastroenteritis in Tehran, Iran. This finding is in agreement with the prevalence of cryptosporidiosis reported in surveys from Europe (1%-2%) and North America (0.6%-4.3%), but lower than those reported from Asia, Australia, Africa, and Central and South America (3%-20%) [3]. *Cryptosporidium*, in addition to *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli*,

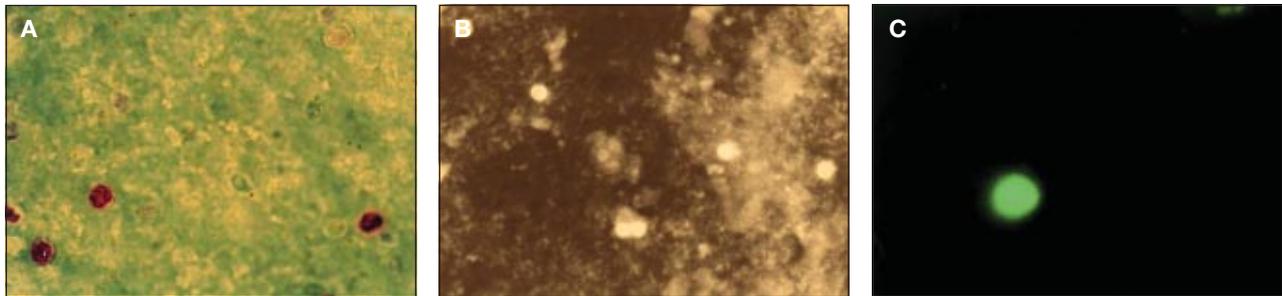


Fig. 1. Detection of oocysts of *Cryptosporidium* in stool samples of gastroenteritic patients using 3 assays: (A) acid-fast staining (original magnification, x 400), (B) auramine phenol fluorescence (original magnification, x 400), and (C) direct fluorescence using monoclonal antibody (original magnification, x 1000).

Rotavirus, *Giardia lamblia*, and *Entamoeba histolytica* are considered as key causative agents of diarrhea in humans. It is now well known that people with gastroenteritis are at high risk for *Cryptosporidium* infection and that carriage of the parasite is associated with diarrheal disease in most cases [6]. Furthermore, there was usually a higher prevalence of *Cryptosporidium* infection in children than in adults, and infections were often seasonal, with a higher prevalence during warmer, wetter months [3]. The incidence of *Cryptosporidium* in gastroenteritic subjects makes it imperative to pursue epidemiological and clinical studies. This will promote the assessment of the public health importance of various agents of gastroenteritis and allow researchers to better understand the transmission rate, risk factors, and reservoir hosts, and to establish preventive measures [4]. A review of large-scale surveys of selected populations demonstrates that the prevalence of cryptosporidiosis as a diarrheal illness is highest in poorly developed regions of the world [3]. This emphasizes the role of water contamination by oocysts of *Cryptosporidium* spp., which needs more consideration by the water industry [4].

Based on our data, both AFS and APF techniques appear to be suitable for *Cryptosporidium* diagnosis. Thus, we agree that the AFS method continues to be a useful tool for screening tests of gastroenteritis in

Table 1. Comparison of the relative frequency of cryptosporidiosis with other enteropathogenic parasites in gastroenteritis^a

Parasitic diseases	Gastroenteritis (%)	Healthy control (%)
Cryptosporidiosis	2.90	0
Giardiasis	5.80	0
Ascariasis	1.90	0
Amoebiasis	0.96	0
Total	11.56	0

^aParasites were observed among gastroenteritic samples including *Giardia lamblia*, *Ascaris lumbricoides*, and *Entamoeba histolytica* in comparison with *Cryptosporidium* spp.

clinical laboratories. Although the cost of the DFxAb assay is higher than those of AFS and APF, this new method allows cryptosporidial diagnosis, even when the parasite's integrity is compromised.

In the current study, solid immunity helped patients resolve infection using a self-limiting mechanism. The duration of disease in infected patients was up to 2 weeks, after which the stool changed to normal consistency. In conclusion, clinicians must focus on the importance of *Cryptosporidium* infection in gastroenteritis, and application of specific diagnosis assays for detection of such oocysts in parasitological laboratories is essential.

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