

## The prevalence of *Cryptosporidium Parvum* infection in adults presenting with acute diarrhea to diagnostic medical laboratories in Jahrom in 2014

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### Abstract

#### Introduction:

*Cryptosporidium parvum* is an Apicomplexan protozoan that can cause acute diarrhea in humans and animals. The present study was conducted to determine the prevalence of this infection in adults with acute diarrhea presenting to diagnostic medical laboratories in Jahrom, Iran, in 2014.

#### Materials & Methods:

The present descriptive study was conducted on 117 adults with acute diarrhea presenting to diagnostic medical laboratories in Jahrom. The patients' epidemiological data were collected using a questionnaire. After they were condensed with Formalin-Ether and stained with modified Ziehl-Neelsen, the patients' stool samples were examined for *cryptosporidium parvum* oocysts both under the microscope and using the Nested-PCR method. The data obtained were analyzed in SPSS-16.

#### Results:

Of the total of 117 adults presenting with acute diarrhea, only one (0.9%) was found to be *Cryptosporidium parvum* positive both through the Ziehl-Neelsen staining and the Nested-PCR method.

#### Conclusion:

The results of the examinations with microscopic and molecular methods showed a very low percentage of *cryptosporidium* infection in adults. Given the higher cost of the molecular method compared to the microscopic method, the modified acid-fast staining method can be used as an alternative, provided that it is performed by technicians who are experienced in detecting this parasite.

**Keywords:** *Cryptosporidium Parvum*, Adult, Prevalence

### Introduction

Cryptosporidiosis is a disease caused by protozoa of the genus *cryptosporidium* belonging to the **apicomplexa- group**, and clinically appears as a gastroenteritis-like syndrome (1). *Cryptosporidium* is a 2-6

micron - obligate intracellular protozoan that mainly infects gastrointestinal epithelium and –sometimes the respiratory system in vertebrates (2). This parasite was first identified in the gastric epithelium of

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mice by Ernest Edward Tyzzer in 1907, who named it Morris *Cryptosporidium* (3). At least eight of the 29 known species of this parasite cause diseases (4). Due to the similarity - - between human and bovine *cryptosporidium* and - their cross transmission-, *parvum* -has been introduced ----- as the main cause of the disease in - immunodeficient - patients. Accordingly, it is essential to know how this protozoan is transmitted to humans. Thus, human *cryptosporidium* that often infects people is named *cryptosporidium parvum* or genotype 1 (5), and bovine *cryptosporidium parvum* that not only infects humans, but also other mammals, is named genotype 2. Because of the difference in sequence and also in electrophoretic pattern after the digestion of the gene by restriction enzymes, these two species are considered as two distinct genetic species (6). This parasite spreads universally and infects humans through drinking water and eating food contaminated with spored oocysts (1). This disease can - range from - moderate to severe depending on the severity of symptoms, patient's immune system, infected site in the body, and one's nutritional status (4). In an immunocompetent host, *cryptosporidium* infection usually appears as spontaneously limiting intestinal symptoms (7). In - immunodeficient patients, this infection may be intestinal or - extraintestinal. Regardless of the patient's immune system, the most common clinical symptom is diarrhea (8). The prevalence of *cryptosporidium* varies according to climate- and - the minimum of 5% to 12.7% has been reported in developing countries (8). Studies conducted in Iran - show the prevalence of infection with this parasite as 1.5% in adults with HIV and 7% in children (9-14). In different parts of Iran, the prevalence varies from 2.2% in the south, 4.1% in the west, and 7% in southeast to 7.7% in northwest (15). In a study conducted by Khalili et al. in 2008, in Shahrkord, on 146 stool samples from

hospitalized adults with diarrhea, five cases of *cryptosporidium* oocysts were identified and the prevalence was reported as 3.5% (16). - There is an increase in the prevalence of this parasite in developing countries - like Iran. Due to the specialized detection method, the contamination assessment - is not usually - possible in medical laboratories. The stool sample-assessment in terms of contamination with *cryptosporidium* is not routinely performed in - the laboratories across the country, and it is only performed - if deemed necessary by the doctor. Specific methods are required for the detection of *cryptosporidium* oocysts in stool such as Ziehl-Neelsen staining-, or other methods such as auramine-rhodamine staining-, - monoclonal antibodies, or molecular - techniques such as PCR (17). The majority of studies - conducted so far have been concerned with frequency and clinical symptoms of contamination with this parasite in children and -immunodeficient patients, and very few studies have been conducted on adults. Thus, the present study was conducted with the aim to investigate the frequency of infection with *cryptosporidium parvum* in adults presenting with diarrhea to medical laboratories in Jahroom.

### Materials and Methods

In the present descriptive study, sample size was calculated as 117, which were collected by convenient sampling from 117 adults presenting with acute diarrhea to medical laboratories in Jahroom from mid-June 2014 to late March 2015. The samples were transferred to the laboratory within an hour. The samples belonged to people from Jahroom and its six surrounding villages (Heidarabad, Chahrtaqi, Mobarakabad, Hakan, qotbabad, and simakan). Data including age, gender, drinking water conditions, contact with livestock, and place of residence (urban, rural) were collected from their parents in the course of sampling. Patients who had taken anti-

parasitic medication or antibiotics were excluded from the study. Samples were condensed using formalin-ether technique, in which one gram of stool sample was mixed with 10 ml of formalin 10% and filtered into the centrifuge tube through two layers of gauze. Three milliliters of diethyl-ether was added to each tube and then vigorously stirred until fully mixed. The mixture obtained was then centrifuged. Contents of each tube were emptied and the sediment was spread onto a glass slide and - air-dried (19). Smears were stained using the modified acid-fast - staining method. Alkaline fuchsin solution was poured on slides and they were gently heated over the Bunsen burner such that they do not boil. Slides were then incubated at room temperature for 5 minutes, rinsed with tap water and de-stained with sulfuric acid 2% for 1-2 minutes, and rinsed again with tap water and dried at room temperature. They were then stained with methylene blue for one minute, rinsed with tap water, and dried at room temperature. Then using emersion oil, they were examined for cryptosporidium under an optical microscope (magnification 100×) (20). Figure 1 shows 4-6 micron cryptosporidium oocytes in light red in a blue background. To perform molecular tests, the DNA was first extracted using Qiagen stool DNA extraction kit (Germany) using the manufacturer's instructions, and - then the nested PCR test was performed on all samples. In the first stage, 1056bp piece was proliferated using primer pairs (PF: 5-CCACATCTAAGGAAGGCAGC-3) and (PR: 5-ATGGATGCATCAGTGTAGCG-

3), and in the second stage, 434bp piece was proliferated using primer pairs (PF: 5-AAGCTCGTAGTTGGATTTCTG-3) and (PR: 5-TAAGGTGCTGAAGGAGTAAGG-3) (18). Then, the PCR product underwent electrophoresis and was observed using the Gel Document (Gel Doc) device.

### Results

Of 117 adults of mean age  $45.83 \pm 19.72$  years, 64 (54.7%) were male and 53 (45.3%) were female, of whom, 95 (81.2%) lived in the city and 22 (18.8%) in villages. Modified acid-fast staining technique showed positive results in terms of cryptosporidium contamination in a 28-year-old urban man (0.9%). Of all participants, 104 (88.9%) had no contact with livestock, 4 (3.4%) had constant contact and 9 (7.7%) had little contact with livestock. The person infected had little contact with livestock. City pipe water was available to 108 (92.3%) participants, and rural pipe- water to 9 (7.7%). Cryptosporidium infected person had access to city pipe water. The - nestedPCR assessment of all participants produced the same results as those of acid-fast staining technique. Out of 117 participants, the same infected person in staining and microscopic assessment was also found infected with cryptosporidium parvum in the molecular method. Figure 1 shows the one positive sample and a number of negative electrophoresed samples. In the present study, the frequency and the frequency distribution of infection with cryptosporidium were determined based on the study variables.

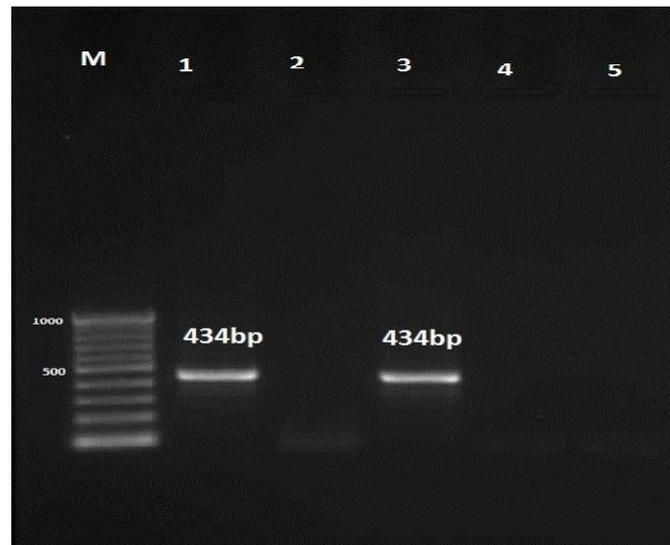


Figure 1: Detection of *cryptosporidium parvum* in Nested PCR technique

Column 1: Positive control of *cryptosporidium parvum*

Column 2: Negative control

Column 3: Sample from the patient infected with *cryptosporidium parvum*

Column 4-5: Samples from patients not infected with *cryptosporidium parvum*

## Discussion

The present study results - confirmed the infection with *cryptosporidium parvum* in adults with acute diarrhea in the city of Jahrom for the first time. *Cryptosporidium* infection was observed in 0.9% out of 117 patients with gastroenteritis studied using modified acid-fast staining and Nested PCR techniques. In a study conducted by Khalili et al. in Chaharmahal-Bakhtiari, 3.5% of adults were infected with *cryptosporidium* (3). In - a study, conducted by Dalimi et al, on 1128 stool samples from patients attending Aliasghar, Mofid, and Imam Khomeini hospitals in Tehran, *cryptosporidium parvum* was detected in 10 samples (0.8%), which is very close to the present study results (21). Similarly, in a study - conducted, in Jahrom by Kargar et al. (2014), on children with acute diarrhea using the staining technique, the infection rate of 1.9% with this parasite was reported - which is also close to the present study results (22). In a- study conducted in Qazvin, Ghoreishi et al. reported the prevalence of this infection in children with gastroenteritis to be 0.3% (23). In another study by Khalili et al., the prevalence of the infection with

*cryptosporidium* in children under 5 years of age attending -Hajar hospital in Shahrkord was reported as 2% (19). In a study by Dabirzadeh et al. - in Zahedan, - in children under five years of age admitted to the pediatric hospital, the infection with this parasite was 4.7% (24). In a study conducted by Postchian et al. in Isfahan on all age groups, the prevalence of this infection was reported as 3.9% (25). In a- study conducted in Tehran on children under 10,- the prevalence of this infection was 2.4% (26). This was reported 4.7% in Khoramabad (27), 4.04% in Ardebil (28), and 3.26% in Semnan (29). The contradictory results -- related to the prevalence of *cryptosporidium* across the world may be due to various factors such as one's lifestyle, cultural and economic status, -living in urban or rural areas, geographical climate and -their health knowledge. In the present study, the frequency of infection with this protozoon in male participants was 0.9%, which was different from 0% in the female participants- and disagrees with similar domestic (29-31) and foreign (32-35) studies with similar odds for infection. These results are similar to those obtained in studies conducted in Korea and

Slovenia that showed a higher prevalence among male samples - (36-37). In a study conducted by Dabirzadeh et al. in Zahedan, the infection level was higher in boys than in girls, but not statistically significantly, which agrees with the present study (38). In a study conducted in Shahrekord on 504 diarrhea samples, 2% infection with cryptosporidium parasite - was detected in 12 samples, which is - close to the present study figures, but - higher in initial sample size (39). Given the above studies, since the immune system improves with aging, the infection with this parasite decreases. In the present study, the person found infected was in contact with livestock. In the study conducted in Semnan, the infection was observed in 3.26% of people that were in contact with livestock, and this relationship was statistically significant (29). In the present study, the molecular technique was also used to detect infection with cryptosporidium, which produced the same result as that of modified acid-fast method (0.9%). In their study conducted in 1998 in Australia, Morgan et al. compared PCR and staining methods used for detection of cryptosporidium in diarrheic human stool samples. Of 511 stool samples, 36 samples were found positive using the PCR technique. - Only 29 samples could be diagnosed using modified acid-fast staining method, equivalent of 7 false-negative samples. Moreover, positive samples in PCR showed 5 false positive results in modified acid-fast method (40). In a study conducted in Tehran by Salehi et al. on 2500 samples using both methods, 30 positive samples were detected in modified acid-fast staining and no false positive cases, and all were reported positive in PCR technique. Of negative cases, -two positive ones - were detected using the PCR technique, which suggested false negative caused by the staining method. The results obtained by Morgan et al. disagree with those of the present study that showed no false positive or negative

cases in modified acid-fast method, which may have been due to the difference in test method (condensing), modified acid-fast staining and magnification  $\times 1000$  that eliminated false positive cases and more accurately detected actual cryptosporidium oocytes. In Germany, Bylak et al. considered the specificity of immunological methods sufficient for screening stool samples for cryptosporidium, and reported that the PCR method does not enhance the accuracy of detection of oocytes (29), which agrees with the present study that showed no greater accuracy with molecular method (without considering the staining method).

For the early treatment of this disease and prevention of its spread, its detection is essential, especially in infection specialist pediatric hospitals, where oocytes of cryptosporidium parvum should be differentiated from larger yeast cyclospora oocytes (8-10 microns) using ocular micrometer. Hence, the correct detection of this parasite requires experience and morphological knowledge of cryptosporidium parvum oocytes (41). Given the high cost and unavailability of molecular tests in conventional laboratories, it is recommended that smears obtained from sedimentation in formalin-ether method be replaced by PCR for detecting cryptosporidium oocytes in stool samples by experts. Previous studies have proposed PCR as a useful method for differentiation of pathogenic genotypes of this protozoan (40). Given the negligible percentage of infection cases found in the present study, it is recommended that larger sample sizes -be used for the determination of the relationship between the percentage of this infection and other variables affecting this clinical disease.

### Conclusion

The present study results showed a very low percentage of infection with cryptosporidium in adults using microscopic and molecular methods.

Given that percentage of infection was the same in both methods and also the high cost of molecular tests for detecting cryptosporidium, modified acid-fast method on the condition that it is performed in the presence of an expert for microscopic detection of this parasite can be beneficial.

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### Conflicts of Interest

The authors did not express any conflicts of interest.

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