





# Cryptosporidiosis in HIV-Positive Patients, Bandar Abbas, Iran: A Cross-Sectional Study

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## Abstract

**Background:** Cryptosporidiosis is a parasitic disease caused by a small protozoan of the genus coccidian. It is important in the medical and veterinary area, since it is a zoonotic parasite. Transmission of fecal-oral infections occurs through direct and indirect contact with food and contaminated drinks. Many HIV-positive individuals live in the areas where intestinal parasites are hyper-endemic.

**Objectives:** Given the significance of the study associated with opportunistic infectious diseases in this group of patients, we decided to study the prevalence of the parasite in those individuals.

**Methods:** In this descriptive cross-sectional study, we collected a single fecal specimen of 133 HIV-positive patients seeking treatment from the center for Behavioral Disease Counseling of Bandar Abbas, Iran. Stool samples examined by direct, formalin-ether concentration method and modified Ziehl-Neelsen stain for *Cryptosporidium* spp.

**Results:** The mean age of the patients was 42.15 (ranged 12 - 82 years). No oocyst of *cryptosporidium* spp. or other intestinal coccidian was found in any of the samples. Only four species including *Giardia lamblia*, *Hymenolepis nana*, and both *Entamoeba coli* and *Blastocystis hominis* were found in three patients, which indicated less diversity and low frequency of parasitic infection in this study.

**Conclusions:** Monitoring and evaluating the therapeutic process of the patients lead to less infectious diseases, especially those of opportunistic parasites.

**Keywords:** Cryptosporidiosis, HIV, Iran

## 1. Background

*Cryptosporidium* is a small, obligate intracellular coccidian parasite causing severe, prolonged and cholera-like diarrhea and sometimes results in death with untreated human immunodeficiency virus (HIV), the cause of acquired immunodeficiency disease syndrome (AIDS) (1-3). *Cryptosporidium* can infect the microvilli borders of the vertebrate host of the digestive tract epithelium (4). *Cryptosporidium* species infect human and many vertebrate animals. In healthy humans, it is self-limiting and may clear the infection in less than a month; however, individuals who have suppressed immune systems, especially AIDS, transplant, and cancer patients who are at a higher risk for long and potentially fatal diarrhea. Waterborne, foodborne, and zoonotic routes are the ways of the transmission of *Cryptosporidium* (5-7). There have been many stud-

ies on prevalence and epidemiology of *Cryptosporidium* in patients with AIDS. The results of those studies show the prevalence of cryptosporidiosis in HIV-positive patients is broad. It varies in those with immunodeficiency syndrome, children in developing countries, as well as outbreak cases among immunocompetent individuals from 0 to 100 with a mean of 32% (8). In a meta-analysis study, the prevalence of this parasite in people with AIDS was 14% (9). Medical diagnostic laboratories of Bandar Abbas run routine procedures for the detection of intestinal parasites, however, they do not use the proper method for detection of this parasite, unless it is requested by the physician.

## 2. Objectives

The aim of this study was to evaluate the prevalence of this parasite in people with HIV-positive patients in Bandar

Abbas, Hormozgan province, Iran.

### 3. Methods

In this descriptive cross-sectional study, a single fecal specimen collected from HIV-positive patients seeking treatment from the center for Behavioral Disease Counseling (BDC) of Bandar Abbas, Iran, from December 2018 to June 2019. This city is located in southern Iran; a tropical region, attached to the Persian Gulf with high humidity (20% - 100%) and warm climate (10). BDC is the largest center in the Hormozgan province and provides care and treatment for 294 HIV-infected cases. Clinical and laboratory investigations including CD4<sup>+</sup> T-cell counts and biochemical assessments at enrolment and follow-up visits help determine patient's eligibility for early initiation of antiretroviral therapy as well as their responses to treatment.

All patients were supposed to be examined, where 133 patients cooperated with us in providing the stool specimens. The Ethics Committee of Hormozgan University of Medical Sciences (HUMS) approved the study protocol (HEC-91-5-4). The aim of the study was described to the patients or their parents and informed consent was obtained from all enrolled cases. After obtaining the written consent, the researcher administered a comprehensive questionnaire to each patient. The checklist included items on patient variables including age, sex, education level, and drug addiction. Clinical characteristics including diarrhea, nausea, vomiting, cramp, flatulence, the history of diarrhea, and laboratory characteristics, including the CD4<sup>+</sup> T-cell counts and the type of stools, were recorded by the physician of the center. Stool samples were obtained from each patient and examined by direct, formalin-ether concentration method and modified Ziehl-Neelsen stain for *Cryptosporidium* spp. The samples were examined first by a senior expert and then by a specialist.

The collected data were analyzed using SPSS software (version 20, Chicago, IL, USA). The prevalence of intestinal parasites were evaluated with descriptive statistics, while the relationship between the variables and presence of intestinal parasites were assessed by the chi-square test. The level of significance was 0.05.

### 4. Results

Out of the 133 patients, 80 were males (60.2) and 53 females (39.8%). The mean age of the patients was 42.15, of which the youngest was 12 and the oldest 82. The tests showed no oocyst of *cryptosporidium* spp. or other intestinal coccidian in any of the samples. Only four species of parasites were found in three patients including *Giardia*

*lamblia*, *Hymenolepis nana*, and both *Entamoeba coli* and *Blastocystis hominis*, which showed less diversity and low frequency of parasitic infection in this study (Table 1).

**Table 1.** Demographic and Clinical Characteristics of the HIV-Positive Patients, Bandar Abbas, Iran, 2019

Variable	Number (%)
<b>Age</b>	
Mean ± SD	42.15 ± 10.01
Range	12 - 82
<b>Sex</b>	
Male	80 (60.2)
Female	53 (39.8)
<b>Education</b>	
Illiterate	19 (14.3)
Elementary	48 (36.1)
High school	37 (27.8)
≥ Diploma	29 (21.8)
<b>CD4<sup>+</sup> T-cell counts</b>	
Mean ± SD	502.9 ± 302.5
Range	19 - 1650
<b>Nausea</b>	
Yes	1 (0.8)
No	132 (99.2)
<b>Vomiting</b>	
Yes	2 (1.5)
No	131 (98.5)
<b>Cramp</b>	
Yes	2 (1.5)
No	131 (98.5)
<b>Diarrhea</b>	
Yes	0
No	133 (100)
<b>Flatulence</b>	
Yes	1 (0.8)
No	132 (99.2)
<b>History of diarrhea</b>	
Yes	27 (20.3)
No	106 (79.7)
<b>Stool type</b>	
Formed	111 (83.5)
Loose	6 (4.5)
Hard	16 (12.0)
<b>Addict</b>	
Yes	61 (45.9)
No	72 (54.1)
<b>Parasite</b>	
<i>Giardia lamblia</i>	1 (0.8)
<i>Hymenolepis nana</i>	1 (0.8)
<i>Entamoeba coli</i> and <i>Blastocystis hominis</i>	1 (0.8)

## 5. Discussion

This is the first study on cryptosporidiosis in HIV-positive patients in Bandar Abbas, Iran. The present study indicates no oocyst of *Cryptosporidium* species in any of the samples. There have been similar studies by Zali et al., in Tehran and Kermanshah, as well as the study of Kiani et al., in Nahavand, western Iran 1.5%, Memar et al., in Tehran 0.9%, and 0.5% in Taiwanese patients (11-14). This prevalence is very low compared to the studies carried out in other regions of Iran and the world (1, 2, 15). This disparity may be attributed to the ongoing care and health surveillance of these patients at this center and could also be due to an insufficient number of stool samples taken from each patient, while, WHO recommends the collection of three stool specimens every other day (16). The last reason is the laboratory methods, where other researchers employed molecular or serological methods to diagnose such parasite as the ELISA technique (11, 17). It is worth noting that a minimum threshold of 50,000 oocysts/mL of the stool sample is required for the detection of cryptosporidiosis through microscopic methods (18).

Contrary to the study of Izadi et al., in Isfahan 14.9% (6), and Nahrevanian and Assmar (4) in Tehran 8.7%, cryptosporidiosis in HIV-positive patients of Bandar Abbas was very low. In addition, most studies outside of Iran, particularly in the developing countries, show a relatively high prevalence of this parasite (19). The study of Shimelis et al., and Girma et al., among HIV-positive individuals in southern Ethiopia (16, 20) showed a high level of infection 13.2% and 34.3%, respectively. This may be due to the geographical location or the study population, due to the fact that we considered patients in BDC, whereas they studied hospitalized diarrheic patients. It is worth noting that some of the patients in our study had a history of diarrhea but showed no complication at the time of sample collection (Table 1), most of whom even had constipation. This is in contrast to the study of Taherkhani et al., in Kermanshah (15), which showed that although some of the patients had no diarrhea and even with constipation, where parasite was seen. *Cryptosporidium* is dangerous and deadly in people with immune deficiencies, even in those without diarrhea. Therefore, specific diagnostic procedures for this parasite should be performed in medical diagnostic laboratories. Geographical locations and the climate of the different regions affect the prevalence of intestinal parasites, in particular *Cryptosporidium*. The prevalence of the parasite was 9.1% in Ahvaz, one of the tropical regions of Iran (21). They performed stool exams by ELISA method, perhaps the prevalence of the parasite would have been lower if they had used the conventional diagnostic method.

In contrast to the study of Vignesh et al., and Kulkarni

et al., in India, *Isospora belli* or *Cyclospora cayetanensis*, the other coccidian parasites, were not detected in any of the samples (22, 23). Contrary to the study of Mohandas et al., Vanathy et al., and Mathur et al. (18, 24, 25), the prevalence of the other intestinal parasites was also very low, which could be due to the regular follow-up and administration of multiple drugs such as co-trimoxazole to control the outcomes. The low frequency of intestinal parasites was found in those patients who refer to the BDC for routine tests and other screening exams. It is suggested that the stool exam, along with other various tests were performed for the patients who first refer to the BDC.

One of the limitations of the study was the lack of cooperation of the patients for sampling as well as obtaining three samples from each patient to increase the chance of detection the parasite.

### 5.1. Conclusions

The relatively low parasite diversity in the studied samples would be likely due to continuous monitoring and appropriate treatment. We recommend that the infection with *Cryptosporidium* spp. in HIV infected people with chronic diarrhea, weight loss, and vomiting in the first enrollment is considered. Detection of etiologic pathogens might contribute to the appropriate decisions made for appropriate management strategies.

### Supplementary Material

Supplementary material(s) is available [here](#) [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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### Footnotes

**Authors' Contribution:** Majid Najafi-Asl contributed in sampling and laboratory work. Khojasteh Sharifi-Sarasiabi designed the study, contributed in the laboratory work and wrote the manuscript. Saeed Hosseini Teshnizi helped with analysis of the data and also helped with the writing of the manuscript. Parivash Davoodian contributed in writing proposal, the data gathering and also helped with the manuscript.

**Conflict of Interests:** The authors declare that there is no conflict of interests.

**Ethical Approval:** The Ethics Committee of Hormozgan University of Medical Sciences (HUMS) approved the study protocol (HEC-91-5-4).

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**Informed Consent:** The aim of the study was described to patients or their parents and informed consent was obtained from all the enrolled cases.

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