



Original Article

Assessment of *Cryptosporidium* in Patients With Gastroenteritis by Modified Ziehl-Neelsen Staining Method in East Azerbaijan Province of Iran During 2018-2019

Alireza Javidmehr¹ , Yagoob Garedaghi^{2*} , Amir Babak Sioufi³

¹Department of Food and Agriculture, Sacramento, California, USA

²Department of Parasitology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

³Department of Pathobiology, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Introduction: Cryptosporidiosis is a worldwide zoonotic disease that is caused by *Cryptosporidium* species and leads to acute or chronic diarrhea and vomiting in patients. Due to the suitable conditions of East Azerbaijan province for parasite transmission, the present study was performed to evaluate the prevalence of *Cryptosporidium* infection in patients with gastroenteritis referred to hospitals and reference laboratories of East Azerbaijan province during 2018-2019.

Methods: A descriptive cross-sectional study was performed on patients with diarrhea who referred to hospitals and reference laboratories of East Azerbaijan province during 2018-2019. In this study, 180 fecal samples were collected from patients with gastroenteritis by cluster-random sampling. Stool samples were stored in 10% formalin and transferred to a parasitology laboratory and examined for parasite contamination by modified acid-fast staining (Modified Ziehl-Neelsen) method. Data were analyzed by chi-square test using SPSS.

Results: Out of 180 samples, the highest number of diarrhea samples belonged to the age group over 40 years (35.5%) and the lowest belonged to the age group of 10-20 years (7.7%). Additionally, 56% of subjects were male and 44% were female. Of these patients, 57.7% lived in urban areas and 42.3% in rural areas. *Cryptosporidium* was observed in 1.6% (3 cases) of patients with gastroenteritis. There was a statistically significant relationship between *Cryptosporidium* infection and the age of patients with diarrhea ($P < 0.05$).

Conclusion: In the present study, a lower prevalence of cryptosporidiosis has been determined in comparison with previous studies in East Azerbaijan province. However, *Cryptosporidium* and other parasitic infections in the feces of patients with diarrhea referred to hospitals or reference laboratories need to be carefully diagnosed by appropriate parasitological methods.

Keywords: *Cryptosporidium*, Modified Ziehl-Neelsen staining, Gastroenteritis patients, East Azerbaijan province, Iran

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Introduction

Today, in most countries of the world, gastroenteritis and acute diarrhea are among the most important medical conditions. According to the World Health Organization, 4.5 to 6 million deaths from diarrhea occur in Asia, Africa, and Latin America annually, with more than 10 000 reported in the United States (1,2). In the last two decades, several different genera of intestinal protozoa have been introduced as new human pathogens that can also cause diarrhea in people with normal immune systems in addition to people with defective immune systems (3,4). One of these new pathogens is *Cryptosporidium parvum*, which is currently one of the four most important intestinal pathogens associated with diarrhea in humans (5). Cryptosporidiosis is a parasitic

infection caused by *Cryptosporidium* that occurs in a wide range of vertebrates, including mammals, fish, reptiles, and birds (6,7). Cryptosporidiosis is a gastrointestinal infection caused by *Cryptosporidium* that is associated with diarrhea. It can occur at any age group but it is more common in children under 5 years of age in developed countries (8-10).

The disease is a serious public health problem that is now widely regarded as one of the causes of acute infectious gastroenteritis which is considered to be lethal in immunocompromised individuals (11). The severity of the disease depends mostly on the immune status of the host; therefore, in immunocompromised individuals, it may be seen as short-term diarrhea that resolves spontaneously. However, in immunocompromised



patients, especially those with AIDS, it presents as a life-threatening, prolonged, cholera-like illness (12-14). The parasite causes disease by different mechanisms. Most studies of this disease have been performed on people with diarrhea and immunodeficiency; however, in this study, we examined seemingly asymptomatic and healthy people (15,16). Due to the fact that the number of studies conducted in Iran on the prevalence of this parasite in humans is limited and most studies have been performed on people with immunodeficiency or diarrhea in the hospital area, the results of this study, along with other studies, can be helpful for future planning to control and prevent this parasitic disease.

Materials and Methods

This descriptive cross-sectional study was conducted on gastroenteritis patients referred to medical centers and reference laboratories of medical sciences during 2018-2019 in Tabriz, the capital of East Azerbaijan province of Iran. In this study, 180 fecal samples were collected from patients with diarrhea by cluster-random sampling method. The samples were then stored in 10% formalin and transferred to a parasitology research laboratory. Stool samples in the laboratory, after preparation by parasitological methods, were examined for the presence of cysts, trophozoites, and oocysts of parasites using concentration method and light microscopy (magnification: ×10 and ×40). In this study, after filling out a questionnaire by patients with gastroenteritis, fecal samples were examined in the Parasitology Department in terms of physical condition and consistency of the feces and their characteristics were recorded. Then, 5 to 10 g of feces were placed in the tubes containing 3.5 mL of commercial kit for parasite testing and the resulting suspension was centrifuged at 2000 rpm for 2 minutes. The supernatant was discarded and the precipitate was expanded on the slide. After preparing the required samples, the remaining sediment was stored in the Eppendorf tube in the refrigerator at 4°C for later use. Smear was prepared for all samples and dried well at laboratory temperature. Then, by pouring a few drops of 96% methanol onto them, the samples were fixed on the slide. From the slides in the early stages, the sample was detected either positive or suspected. The prepared extensions were then stained with acid-fast stain. The slides were then examined for *Cryptosporidium* by light microscopy. In the acid-fast staining method, samples and slides are examined for *Cryptosporidium* using a light microscope with ×40 and ×100 lenses. In this method, *Cryptosporidium* oocysts are seen as round pink-red objects on a light green background (17,18). Stained cysts are different in color depending on the age and condition of the cysts.

Data Analysis

Data were analyzed by chi-square test ($P < 0.05$) using

SPSS.

Results

In this study, which was performed during one year from 2018 to 2019, a total of 180 diarrheal fecal samples from patients with gastroenteritis referred to hospitals and reference laboratories of East Azerbaijan province were examined for *Cryptosporidium* infection. Out of 180 samples, the highest number of diarrhea samples belonged to the age group over 40 years (35.5%) and the lowest belonged to the age group of 10-20 years (7.7%) (Table 1).

Moreover, 56% of the subjects were male and 44% were female. Of these patients, 57.7% lived in the city and 42.3% in the village. Patients were asked questions regarding their clinical symptoms, and the highest number of clinical symptoms following diarrhea included nausea (22.1%), dehydration (18.4%), and fever (15%) and the lowest number of clinical symptoms belonged to abdominal pain (12%).

The rate of *Cryptosporidium* infection was 1.6% (3 cases) among patients with gastroenteritis using acid-fast staining. The study of *Cryptosporidium* infection in patients with diarrhea by age showed a difference in infection rate at different ages in this study. The prevalence of *Cryptosporidium* infection in the age groups of 10-19 years, 20-29 years, and over 40 years was determined to be 0.5%. There is a statistically significant relationship between *Cryptosporidium* infection and age (Table 2).

Additionally, the study of the relationship between *Cryptosporidium* infection and place of residence of patients with gastroenteritis showed that 1.1% lived in urban areas and 0.5% in rural areas. There was no statistically significant relationship between the rate of *Cryptosporidium* infection and place of residence (city/village) (Table 3) ($P > 0.05$).

Discussion

Cryptosporidium is an intestinal protozoan that causes self-limiting diarrheal disease. It can cause severe disease in people with AIDS or other forms of immunodeficiency. This protozoan is the causative agent of cryptosporidiosis,

Table 1. Frequency Distribution of Patients With Diarrhea Referred to Hospital and Laboratory Centers in East Azerbaijan Province by Age Groups During 2018-2019

| Patients A (year) | No. (%) |
|-------------------|-----------|
| <5 | 29 (16.1) |
| 5-10 | 16 (8.8) |
| 10-20 | 14 (7.7) |
| 20-30 | 22 (12.2) |
| 30-40 | 35 (19.4) |
| >40 | 64 (35.5) |
| Total | 180 (100) |

Table 2. Frequency Distribution of *Cryptosporidium* Infection in Patients With Gastroenteritis Referred to Hospital and Laboratory Centers in East Azerbaijan Province by Age During 2018-2019

| Parasitic infection | Patients' Age (y) | | | | | | Total |
|---------------------|-------------------|----------|----------|-----------|-----------|-----------|------------|
| | <5 | 5-9 | 10-19 | 20-29 | 30-39 | >40 | |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | |
| Infected | 0 (0) | 0 (0) | 1 (0.5) | 1 (0.5) | 0 (0) | 1 (0.5) | 3 (1.6) |
| Non-infected | 29 (16.1) | 16 (8.8) | 13 (7.2) | 2 (11.6) | 35 (19.4) | 63 (35) | 177 (98.4) |
| Total | 29 (16.1) | 16 (8.8) | 14 (7.7) | 22 (12.2) | 35 (19.4) | 64 (35.5) | 180 (100) |

Table 3. Frequency Distribution of *Cryptosporidium* Infection in Patients with Gastroenteritis in Patients Referred to Hospital and Laboratory Centers in East Azerbaijan Province by Place of Residence During 2018-2019

| Parasitic infection | Urban | Rural | Total |
|---------------------|------------|-----------|------------|
| | No. (%) | No. (%) | No. (%) |
| Infected | 2 (1.1) | 1 (0.5) | 3 (1.6) |
| Non-infected | 102 (56.6) | 75 (41.8) | 177 (98.4) |
| Total | 104 (57.7) | 76 (42.3) | 180 (100) |

which is one of the zoonotic diseases of humans and animals. The disease manifests itself in two clinical forms: acute self-limiting gastroenteritis in immunocompromised individuals and chronic diarrhea in immunodeficiency individuals and the main transmission route of infection is oral (19,20). The parasite may be transmitted directly by contact with an infected human or indirectly from a contaminated environment such as water and food (21). In this study, in 180 samples obtained from patients with gastroenteritis in the hospital and laboratory center of East Azerbaijan province, the prevalence of *Cryptosporidium* was 1.6%. The prevalence of this parasite in recent studies in Iran in gastroenteritis patients, AIDS patients, and school children has been reported to vary from 0.1% to 7.7%. The prevalence of *Cryptosporidium* is estimated to be between 1 and 3% in Europe and North America, about 5% in Asia, and 10% in Africa. Protozoans are also the cause of diarrhea in 10 to 20% of people with AIDS living in industrialized countries, reaching up to 50% in developing countries (22-26). There are several reports of the prevalence of these protozoans in Iran in recent years. In a study conducted by researchers at Iran University of Medical Sciences in 1993, the rates of infection in patients with gastroenteritis, Hodgkin's disease, lymphoblastic leukemia, and renal failure were 2.97%, 12.5%, 6.75%, and 4.5%, respectively (27,28). East Azerbaijan Province due to its traditional and industrial livestock production, as well as climatic conditions, high humidity, nutritional culture, and abundance of surface water has the potential for causing zoonotic diseases on a large scale and it seems that zoonotic parasites are of great importance in this province. As a result, the diagnosis of *Cryptosporidium* infection in patients with gastroenteritis is important at the level of hospital and laboratory centers in East Azerbaijan province, considering that patients refer to these centers from all over the province. Therefore, the

prevalence of *Cryptosporidium* infection in our country is less than 5%, and it is 1.6% in the present study. Since East Azerbaijan province has a temperate climate suitable for parasite survival, the low prevalence can be due to the improved health status of the study population, the number of samples, and less contact with animals, especially domestic animals. The reported results of studies in other parts of the world showed a difference in the prevalence of *Cryptosporidium* ranging from 3.8% to 17% (29,30).

The number of studies conducted in the world on the prevalence of *Cryptosporidium* infection is somewhat higher compared to Iran (31). Probably one of the reasons for the low prevalence of *Cryptosporidium* in human societies of East Azerbaijan is the low level of infection in animals, which unfortunately has not been sufficiently studied. In addition, the increased level of literacy, improved public health, widespread use of treated water, and the implementation of large-scale health-related projects recommended by World Health Organization in recent years in Iran such as family physicians throughout the country at a high level could be considered the reasons for the decrease in the prevalence of parasitic infections. Therefore, the geographical realms, how to cultivate roses, livestock breeding, and most importantly the flow of streams from contaminated areas and beds following the seasonal rains are effective factors in increasing the prevalence of *Cryptosporidium* and other intestinal parasites in the region.

Conclusion

Identification of parasite species by parasitological and molecular methods in future studies in all treatment centers should be considered. It is also suggested that by introducing this parasite to general and specialist physicians and laboratory staff and holding retraining courses, as well as encouraging medical diagnostic laboratories to use modified Ziehl-Neelsen staining method, which is an easy method for the identification of this pathogen, more efforts should be made for the diagnosis of this parasite in the requested tests in patients with gastroenteritis. On the other hand, *Cryptosporidium* is known as an important cause of diarrhea, especially in patients with AIDS and weakened immune system. Although there is no known effective treatment for parasitic infections, especially in those with AIDS, mortality from cryptosporidiosis in

immunocompromised patients, especially AIDS, should be studied. Since cryptosporidiosis is a common disease between humans and animals, the high prevalence of infection in animals, especially domestic ruminants, results in reduced growth, general weakness, and thinness of the animal, which is considered in terms of loss and economic importance.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Issues

In this research, ethical considerations have been fully observed.

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Authors' Contributions

YG did the writing of the manuscript. ABS did the editing of the manuscript. AJ did data collection and statistical analysis.

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