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Review

A Review of the Prevalence and Diagnostic Points of Cryptosporidium Species in Immunocompromised and Healthy Human Samples in Iran

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Abstract

Cryptosporidium species are important intestinal pathogens with widespread distribution in humans and other hosts. Whereas the parasite causes acute and self-limiting gastroenteritis in people with healthy immune systems, many reports on this infection around the world are limited to people with defective or suppressed immune systems who suffer from a persistent and deadly infection. Using laboratory-serological and molecular methods for the detection of *Cryptosporidium* species in immunocompromised and healthy human samples, recent studies in Iran indicated that the prevalence of *Cryptosporidium* species in different samples varied between 0 to 14%. The samples in Iranian studies included human fecal and diarrheic samples from diarrheic children, patients with gastroenteritis, immunocompromised individuals, and people in contact with livestock. Furthermore, some species were reported based on molecular studies including *Cryptosporidium parvum* and *Cryptosporidium hominis*. Some studies have also reported *Cryptosporidium meleagridis*. In this review study, data were collected regarding the prevalence of cryptosporidiosis in high-risk individuals such as children and immunocompromised individuals. The results revealed that the higher prevalence of *C. parvum* in Iranian studies in the last 10 years may be attributed to the transmission of infection from animal sources.

Keywords: *Cryptosporidium* species, Health human samples, Immunocompromised individual, Diarrheic children, Iran

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Introduction

Cryptosporidium is a protozoan parasite of the phylum Apicomplexa that infects the margins of the gastrointestinal tract epithelial microvilli in a wide range of vertebrate hosts including humans (1). *Cryptosporidium* infection (cryptosporidiosis) is recognized as acute and self-limiting gastroenteritis in people with healthy immune systems, while it is regarded as a persistent and life-threatening infection in people with defective immune systems (2). It is estimated that millions of cases occur annually in developing and underdeveloped countries. This parasite is a critical factor in endemic infections, traveler's diarrhea, and epidemics (3). Frequent patterns of the disease are described based on age, season, geographical location, and routes of transmission (4).

Cryptosporidiosis is more dominant among young children in developing countries that causes severe diarrhea similar to cholera (5, 6). Different species of *Cryptosporidium* can infect humans and a wide range of animals. In addition, the widespread occurrence of parasitic oocysts in the environment facilitates the acquisition of human infection in different ways (6). Person-to-person transmission may play a major role in the spread of *Cryptosporidium* infection in children and elderly populations, especially in kindergartens and nursing homes (7).

Cryptosporidium is transmitted feco-orally via ingestion of water or food contaminated by parasite oocysts (8)

which are physically and chemically resistant, can survive in the environment for a long time, and are insensitive to water chlorination (9-10).

Cryptosporidium Species Isolated From Humans

Cryptosporidium parvum

Cryptosporidium parvum is one of the most frequently reported species among mammals and was initially identified in mice. The oocyst of this species is smaller than that of *Cryptosporidium muris* and, its habitat is usually the small intestine. More than 150 mammalian species have been identified as hosts of *C. parvum* and other similar parasites. Molecular characterization of the parasite has shown the occurrence of various genotypes with different base sequences and infectivity. DNA of some of the genotypes is now known as a distinct species such as *C. hominis* and *C. parvum* as human class II and I genotypes, respectively (11).

Other genotypes of the parasite are found in mice, pigs, marsupials, monkeys, mice, and mink. Currently, most researchers suggest that a species isolated from mammals is called *C. parvum* (11).

Cryptosporidium hominis

Although *Cryptosporidium* species that infect humans have previously been referred to as *C. parvum*, a human genotype (genotype 1) or genotype H has recently been identified as well. Thus, in recent years, studies conducted on biological and molecular differences, have categorized the human *Cryptosporidium* under a separate species called *C. hominis* (12, 13). These studies have not only demonstrated wide biological genetic differences between *C. hominis* and *C. parvum* (bovine genotype or genotype II) but also evidenced that the human species are antigenically more stable than the bovine species (13).

Cryptosporidium meleagridis

Stages of parasite growth were observed on the epithelial cells of the distal third of the small intestine of turkeys (14). A child with diarrhea in Mazandaran province of Iran was identified to be infected by *C. meleagridis*. In this study, it was found that the last two species (i.e., *C. parvum* and *C. meleagridis*) potentially had a shared transmission between humans and animals (15).

C. meleagridis is spherical and measures 4.6 by 5.2 µm with sporozoites, but it is not easily detectable inside the oocyst. The disease is associated with diarrhea, but the mortality rate is low. The parasite completes its entire life cycle on the surface of the intestinal epithelium and does not seem to invade host tissues (16).

Further studies have indicated that, in addition to turkeys, other birds such as chickens and parrots are susceptible to infection induced by *C. meleagridis*. Likewise, molecular studies have shown that *C. meleagridis* is a species distinct from other species and is the third most common *Cryptosporidium* parasite found in humans (17, 18).

Important Predisposing Factors for Cryptosporidiosis

Age

In tropical and temperate countries, *Cryptosporidium* is an important agent causing diarrhea in children. The effect of age may be related to the immune status of the young host and the infectivity of the *Cryptosporidium* species. Epidemiological studies have shown that cryptosporidiosis reaches the peak at two extremes of age. The first is under five years old and the second one occurs in adulthood (twenty to forty years old), which can be due to their occupational contacts (19).

Individual Immune System

As previously stated, cryptosporidiosis is a self-limiting gastrointestinal disorder in people with healthy immunity, while it causes persistent and life-threatening diarrhea in people with immunodeficiency (primary or secondary). Suppression of the immune system for any reason will increase the likelihood of cryptosporidiosis (20).

Other predisposing factors associated with cryptosporidiosis include weather, season, travel, occupation, contact with the animals, nutritional status, and personal health status (21).

Importance of Diagnostic Points

Similar to most intestinal parasites, *Cryptosporidium* is detected by microscopic examination of the stool. Stool samples are usually stored in 10% formalin. Fresh samples are also used for direct examination, but bear an increased risk of infecting the examiner (22).

Care should be taken when using stool preservatives, for example, polyvinyl alcohol interferes with staining techniques and is usually not recommended. Frozen stool samples and 2.5% potassium dichromate can be used for enzyme-linked immunosorbent assay (ELISA) and for preserving this parasite in stool samples, respectively (23).

Direct diagnosis of this parasite using a microscope is extremely challenging due to its wide spectrum of hosts and discrepancies in its oocyst size compared to other parasites transmitted feco-orally (24).

Cryptosporidium can be found elsewhere in the body that is suitable for parasite development, such as respiratory epithelium. However, the main site of the parasite is the surface of the small intestinal epithelium, causing recurrent and chronic diarrhea as well as diarrhea interspersed with constipation. Therefore, stool and sputum examination are the more preferred ways to diagnose the parasite**.** However, factors such as small size, transparency, and similarity of the parasite oocyst with other similar parasites make it impossible for direct examination to be considered as a gold standard test (25, 26).

Diagnostic Methods

Direct Stool Test Method

In this method, the parasite oocyst, which has been obtained by reliable methods of concentration (e.g., Shitter), is detected by a phase-contrast microscope or bright background (27).

Concentration Methods

Floatation

This method includes the Shitter method or flotation by sugar-water and the use of various salts (i.e., chloride, ZnSo4, and NaCl), which are less sensitive and specific than the Shitter method. Further, they prevent the oocyst wall from rapid crystallization during the direct detection and diagnosis of parasites (28).

Sedimentation

Some references consider the formalin-ether deposition method to be a better option than the shitter flotation method (29). Nevertheless, Pal et al assume that the use of formalin-ethylacetate can reduce the number of oocysts, and the sediment obtained in this method due to having additional materials must be painted by appropriate painting techniques (30).

Smear Staining Methods

Two points are important to be considered in this method: First, the type of sample used (direct or concentrated), and second, the type of smear staining. In the case of the samples, direct, concentrated, or deposited samples can be used depending on the type of work.

- Kinyoun's acid-fast
- Modified acid-fast (Figure 1)
- Fluorescent acid-fast stains such as auramine-rhodamine: This method is faster with higher sensitivity than acid-fast (31).

Histopathological Method

This was the first test to detect the *Cryptosporidium* parasite in intestinal tissues. The cell forms of the parasite turn into purple with hematoxylin-eosin staining. Tissues become available only after invasive procedures, and organisms are not always detected on biopsies (32).

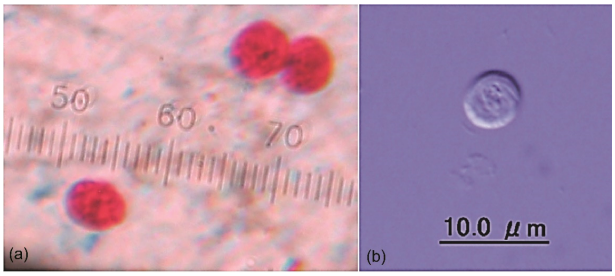

Figure 1. *Cryptosporidium parvum* Oocyte Size (4.5 × 4.2 µm) by the Modified Acid-fast Staining Method and **(b)** *Cryptosporidium hominis* (6.5 × 4.7). *Note*. The size of oocysts varies in different species of parasites.

Serological Method

This method employs immunofluorescent tests based on monoclonal antibodies (copro-antigen-copro antibodies) that are specific to *Cryptosporidium* oocysts. The sensitivity of this method is 10 times greater than that of acid-fast dyeing methods. The direct immunofluorescence method using monoclonal antibodies is currently regarded as the gold standard test for fecal samples. The rate of parasite antigen detection using commercial kits in the form of ELISA and immunochromatographic tests is increasing. The ELISA tests are designed with high sensitivity and specificity)25).

Cryptosporidium Culture

Technically, the cultivation of *Cryptosporidium* faced some challenges in the past, but significant progress has been made in recent years. Today, the asexual stages of the parasite can be produced in antimicrobial systems (33).

Molecular Methods

The inability to identify and differentiate *Cryptosporidium* at the species and subspecies level delimits our knowledge of natural history, epidemiology, and the risk of zoonosis. Assessing the risks of water and food contamination by oocysts for public health is considered compounded (31). Given the new findings from the *Cryptosporidium* strains and their apparent similarities, this point becomes more evident and even more important when discussing *Cryptosporidium*. It is required to identify this parasite in terms of species, genotype, and its relationship with the age of the host via molecular pathways (34). Furthermore, the small size of oocysts in different species of *Cryptosporidium* with an extremely thin line demarcating their characteristics makes us realize the importance of using molecular methods (34, 35).

Currently, the use of such molecular methods as polymerase chain reaction (PCR), PCR- Restriction fragment length polymorphism (RFLP), nested-PCR, and DNA hybridization to detect *Cryptosporidium* parasites is increasing. These methods have higher sensitivity and specificity than the microscopic methods (36, 37).

The application of these methods is especially important in diagnosing clinical and environmental samples with low parasite numbers, detecting species differences and the genetic relationship between *Cryptosporidium* parasites, and examining the relationship between *Cryptosporidium* and other Apicomplexa. These methods are also used in epidemiological studies and geographical diversity of parasites (38), The details of which are provided in Table 1 (39-60).

Discussion

The aim of this study was to examine the prevalence and diagnostic points of *Cryptosporidium* species in immunocompromised and healthy human samples

Table 1. Detection of *Cryptosporidium* spp. in Humans in Iran During 2010-2020

Diagnostic Method (s)	Sample Size and its Characteristics	Positive Samples (%) Species *Cryptosporidium*	Area of Study	Reference
ELISA method	176 immunocompromised patients (children and adults with malignancy, kidney recipients, and HIV+)	9 (5.1%) *Cryptosporidium* spp.	In the south-west of Iran	(39)
Modified acid-fast staining method	Diarrheic children	23/184 (12.5%) *Cryptosporidium* spp.	Isfahan	(40)
Direct method, acid-fast Staining, and auramine phenol fluorescence	420 stool samples from gastroenteritis patients	0 (0%) *Cryptosporidium* spp.	Western cities of Mazandaran province, Northern Iran	(41, 42)
Microscopic by MZN staining, PCR, and nested-PCR (18S ribosomal RNA)	850 human fecal samples	29 (3.41%) *C. parvum and C. hominis*	Rural area in the south of Iran	(43)
MZN technique and GP60 PCR-sequencing	794 diarrheic children	19 (2.40%) 17 (*C. parvum*) 2 (*C. hominis*)	Pediatrics hospital in Tehran, Iran	(44)
ZN acid-fast staining and nested PCR-RFLP (TRAP-C2 gene)	469 children less than 12 years	12 (2.5%) 10 (*C. parvum)* 1 (*C. hominis*) 1 (mix infection)	Pediatrics medical centers in Gazvin provinces	(45)
MZN staining technique	36 humans in the horse farms	2 (5.5%) *Cryptosporidium* spp.	Tabriz area	(46)
MZN staining	62 humans	9/62 (14.5%) *Cryptosporidium* spp.	Rural areas in Khuzestan, Southwest of Iran	(47)
Cold MZN staining method	237 humans (in contact with livestock)	6 (2.5%) *Cryptosporidium* spp.	Hamadan District	(48)
MZN method and PCR	2510 stool samples from children with diarrhea	30 (1.19%) *Cryptosporidium* spp.	Pediatric hospitals in Tehran	(49)
Acid-fast staining, auramine phenol fluorescence, and PCR-RFLP by SSU rRNA gene	348 patients with gastroenteritis	8 (2.3%) *C. parvum* or *C. hominis*	Mazandaran Province, Northern Iran	(50)
Modified acid-fast staining and PCR-RFLP	390 fecal samples (immunocompromised individuals and children)	16 cases (4.1%) 11 *(C. parvum)* 4 *(C. hominis)* 1 *(C. meleagridis*)	Southwest of Iran	(51)
Ziehl-Neelsen acid-fast and sequence analysis (gp60) gene	547 diarrheic children	27 (4.94%) 27 (*C. parvum)*	Gonbad Kavoos, Iran	(52)
PCR-RFLP (18S rRNA, SSU-rRNA gene)	113 diarrheic children	2 (1.76%) *C. parvum*	Hospitalized in Tabriz Pediatric Hospital	(53)
MZN, direct fluorescent-antibody, and nested-PCR assay	2,510 fecal samples from diarrheic children (under 12 years old)	30 (1.19%) *Cryptosporidium* spp.	Pediatric hospitals in Tehran, Iran	(54)
MZN	200 fecal samples from children	18 (9.7%) *Cryptosporidium* spp.	Hospitals in Zabol, Southeast Iran	(55)
MZN staining, PCR, and sequenced for phylogenetic analysis (SSU rRNA (18S gene)	132 children with cancer undergoing chemotherapy	5 (3.8%) *C. parvum*	Hospital of Tabriz University of Medical Sciences, Northwest of Iran	(56)
Acid-fast method, the UDG-LAMP assay	120 AIDS patients (volunteering APs)	13 (10.83%) *Cryptosporidium* spp.	Communicable Disease Control Center of Khorramabad, Iran	(57)
nested-PCR-RFLP (18S rRNA gene)	250 HIV/AIDS patients	27 (10.8%) *Cryptosporidium* spp. (70.38%) *C. parvum* (25.92%) *C. hominis* (3.7%) *C. meleagridis*	Southwest of Iran	(58)
Microscopic examination, acid-fast staining, nested-PCR-RFLP, and sequences analysis (18S RNA gene)	764 children aged<10 years with diarrheal disease	7 (0.91%) microscopic examination and acid-fast staining 2 (0.26%) sequences analysis *C. parvum*	Zahedan, Iran	(59)
Acid-fast (AF), PCR, and ELISA	221 diarrheal stool samples of children.	7 (3.2%) *C. parvum*	Urmia, northwest of Iran.	(60)

Note. ELISA: Enzyme-linked immunosorbent assay; HIV: Human immunodeficiency virus; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; MZN: Modified Ziehl-Neelsen; AIDS: Acquired immunodeficiency syndrome; UDG-LAMP: Uracil DNA glycosylase-*supplemented* loop-mediated isothermal amplification.

in Iran, and studies conducted in the last decade were reviewed accordingly.

The existence of different methods of transmission and the intricate zoonotic relationship of this parasite makes the epidemiology of cryptosporidiosis a complex entity. Investigating the prevalence of cryptosporidiosis through molecular tools helps in better understanding the risk factors, sources of the infection, epidemiology, transmission, and determination of specific host-species relationships (61).

In most of the above-mentioned studies, stool samples were first examined by the formalin-ether concentration method, and then, after microscopic examination, the modified acid-fast staining would be performed if the test was likely to be positive. Preparation of direct smears from stool or use of formalin-ether precipitate to prepare modified Ziehl-Neelsen staining smear is a common protocol for the microscopic detection of *Cryptosporidium* oocytes. However, this method requires a skilled technician to distinguish *Cryptosporidium* oocytes from yeasts and other cyclospora. Further, this method requires a considerable number of oocytes in the stool for better sensitivity. Therefore, there is a need to use a combination of the modified Ziehl-Neelsen staining method and molecular methods to get the best results (49).

In some studies that used molecular methods, after confirmation by staining, the parasite DNA was extracted from the stool samples using a kit for molecular tests. Most molecular studies performed in Iran used PCR, nested-PCR-RFLP, and sequences analysis methods. These studies applied ribosomal 18SsRNA genes, GP60, and TRAP-C2. Most of these studies used the nested-PCR method and the ribosomal 18SsRNA gene sequence methods, which can detect low-count parasites in samples. These methods are recommended to be used in cryptosporidiosis epidemiological studies, environmental studies, and water resources testing (43).

Sharbatkhori et al used the gp60 gene to determine the significant genetic heterogeneity between *C. parvum* and C. *hominis* in this gene and found different families in both species (52).

Some of the studies in Iran used ELISA and direct fluorescent-antibody serology methods and evidenced that molecular methods are more sensitive and specific compared to staining and serology methods. Moreover, one study in Iran used the uracil DNA glycosylase-supplemented loop-mediated isothermal amplification technique based on the target gene S-adenosyl methionine synthetase from *Cryptosporidium* gene to detect *Cryptosporidium species* in human immunodeficiency virus (HIV)-positive patients. This study tried to eliminate false-positive results following contamination (57).

The obtained results from various studies indicated that *C. parvum* and *C. hominis* accounted for 90% of human infections in most areas, and some studies reported *C. meleagridis* (62).

The prevalence of *Cryptosporidium species* varies around the world, and the distribution of the species may also vary within a country. Studies on human samples demonstrated the predominance of *C. parvum, C. hominis*, and *C. meleagridis,* suggesting that their cycle frequency and routes of disease transmission are higher than that of other species. Therefore, it is possible to target appropriate routes for the transmission and spread of the parasite and control and prevent the spread of this parasite using appropriate strategies specifically aimed at sources of infection (63).

In most Iranian studies, children and immunocompromised adults were most susceptible to the parasites which can be mainly attributed to poor hygiene and weakened immune systems. Subsequently, the growing size of these sub-populations indicated an increased risk of infection in the community. Therefore, it is recommended that safety points, as well as healthcare and hygiene principles be well observed in dealing with sources of infection, especially in children. Further, the lack of effective treatment for this parasite necessitates focusing attention on the principles of parasite prevention and control as well as sources of the infection. Some important points highlighted by most of these studies include the health status of the community and sources of infection (e.g., consumable water) as factors affecting the prevalence (64).

No definitive treatment and proper treatment protocol have yet been proven for the disease. Thus, prevention seems to be the main factor to control this disease, especially in immunocompromised individuals since the development and clinical manifestations of cryptosporidiosis are affected by the individual's level of immunity (65).

Cryptosporidiosis appears with severe and, sometimes, fatal symptoms in children and immunocompromised individuals (66). In some reports, immunodeficiency is the most common risk factor for cryptosporidiosis in humans (39). However, the ability of many protozoan parasites to invade and cause disease in children and immunocompromised individuals, as well as the difficulty to distinguish *Cryptosporidium* parasites from other pathogenic protozoans via direct microscopic examination are the factors that pronounce the importance of molecular methods, especially parasite genotyping, in parasite identification (67).

Molecular research-based epidemiological studies allow researchers to gain a better understanding of the parasite transmissibility and pathogenicity pathogenic in humans, animals, and environmental samples (68).

Moreover, the use of molecular methods is highly useful in evaluating the common potential of humans and animals for infection by different species of *Cryptosporidium* (63).

More than 35 *Cryptosporidium* genotypes have so far been identified, some of which have recently been recognized as new species. Human infection by several species of *Cryptosporidium* has also been reported. Different species of *Cryptosporidium* are morphologically indistinguishable from each other while being genomically heterogeneous. Therefore, using molecular methods, especially genotyping, is essential to find out the origin of human or animal infections (69).

The results of this study indicated that due to the importance of cryptosporidiosis in people, especially in HIV patients, the need for conducting more studies has arisen in this field. In addition, it is important to identify and select other high-risk individuals who have defective immune systems.

Conclusion

In this review article data were collected regarding the prevalence of cryptosporidiosis in high-risk individuals such as children and immunocompromised individuals. The results revealed that the higher prevalence of *C. parvum* in Iranian studies in the last 10 years may indicate the transmission of infection from animal sources.

Conflict of Interest Disclosures

The authors declare no conflict of interests.

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Ethical Statement

Ethical standards were observed for the review article.

Authors' Contributions

SE contributed to the study design, concept, and edition of the manuscript. OR and VR wrote the manuscript and collected the data, and finally MIG and HH edited the manuscript.

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