

Review Article

Cryptosporidiosis: An under-recognized public health problem

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KEY WORDS

Children, *Cryptosporidium*, diarrhea, immunocompromised

ABSTRACT

Cryptosporidium spp. is under recognized as an important pathogen causing diarrhea in children and HIV-infected individuals with associated high morbidity and mortality. In endemic areas, most symptomatic infections are in childhood and in immunocompromised adults. The immune status of the host plays a critical role in determining the severity of cryptosporidiosis. Infection is self-limited in immunocompetent hosts, but can be severe and persistent in the immunocompromised such as AIDS patients or malnourished children. Cryptosporidiosis in developing countries is a major cause of acute and persistent diarrhea in children and is associated with subsequent impairment in growth, physical fitness, and cognitive function. Despite recognition of the importance of immune status, the correlates of protective immunity in cryptosporidiosis in humans are poorly understood, and treatment modalities are limited.

INTRODUCTION

Cryptosporidium spp. are obligate, intracellular, protozoan parasites belonging to the phylum Apicomplexa.^[1] Currently, there are 22 known species of *Cryptosporidium* that infect vertebrate hosts reported in the scientific literature,^[2] of which the zoonotic *Cryptosporidium parvum* and the anthroponotic *Cryptosporidium hominis* are the major causes of human cryptosporidiosis throughout the world.

First discovered by Ernest Edward Tyzzer in the year 1907 in the gastric mucosa of mice,^[3] *Cryptosporidium* remained largely unrecognized as a human pathogen until the first reported case in 1976 in an immunocompetent child.^[4]

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A review of the first 7 cases of cryptosporidiosis led to the conclusion that it was predominantly a disease of the immunocompromised,^[5] although later it was also found to be widely prevalent among immunocompetent children.^[6]

Cryptosporidium spp. is increasingly being recognized as an important pathogen causing diarrhea in children, with the highest associated morbidity and mortality, especially among children in developing countries.^[7] The highest prevalence of cryptosporidiosis has been documented in children aged 6-12 months.^[8,9] The ability of *Cryptosporidium* to cause large-scale explosive outbreaks has been well documented. It was implicated in the largest waterborne outbreak of acute gastroenteritis in the Milwaukee, Wisconsin, USA, in which an estimated 403,000 people were infected.^[10]

Cryptosporidiosis is often asymptomatic and almost always self-limiting in immunocompetent hosts, but may be severe and life-threatening in immunocompromised patients such as those with acquired immunodeficiency syndrome (AIDS) or severe malnutrition.^[11] It is a highly infectious parasite with a minimal infectious dose as low as 10 oocysts.^[12] Because of the potential for intentional contamination of water supplies, this parasite is listed by CDC and NIH as a category B pathogen for biodefense.^[13]

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Cryptosporidiosis among children in developing countries

In developing countries, *Cryptosporidium* spp. are a major cause of diarrhea in children.^[14] Cryptosporidial infections in early childhood have been reported to be associated with subsequent impairment in growth, physical fitness, and cognitive function.^[15,16] Malnourished children tend to have a higher prevalence of this parasitic infection and with more severe consequences.^[17] In studies in developing countries, even a single episode of cryptosporidiosis predicted a subsequent increased risk of diarrheal disease.^[18] Watery diarrhea, vomiting, and dehydration are the commonest symptoms,^[19] with persistent diarrhea frequently reported from developing countries.^[20]

Among children, low socio-economic status, crowded living conditions, age less than 2 years, male gender, presence of animals (pigs, cats, and dogs) in the household, storage of cooked food, diarrhea in the family, drinking non-potable water, rainy season, low-birth weight, stunting, and lack of breast feeding have all been identified as important risk factors for the acquisition of cryptosporidiosis.^[19-23]

Cryptosporidiosis among children in India

Studies conducted in both hospital and community settings have reported *Cryptosporidium* to be a leading cause of infectious diarrhea in Indian children with positivity rates ranging from 1.1-18.9%.^[24] Among southern Indian children, cryptosporidiosis was associated with prolonged diarrhea and occurred mostly in those prescribed antibiotics.^[25]

In a recent birth cohort study from Vellore in southern India, *Cryptosporidium* was the commonest cause of parasitic diarrhea in children under the age of 3 years.^[26] Longitudinal analysis of stool samples in a subset of children from the same cohort found 40% of children had multiple infections, mostly symptomatic. Children with multiple infections had lower weight-for-height and height-for-age z-scores at 24 months of age, as compared to children with single infections. Prolonged, asymptomatic oocyst shedding before and after an episode of cryptosporidial diarrhea was also documented in 50% of the children.^[27]

High frequencies of asymptomatic cryptosporidiosis have also been noted in Indian children. Examination of fecal specimens obtained from non-diarrheic children residing in a peri-urban slum in Kolkata, India revealed an asymptomatic carriage rate of 2.3%. Children below the age of 1 year had the highest rate of infection.^[28] Similarly, in another study among children with and without cryptosporidial diarrhea in southern India, researchers could detect *Cryptosporidium* oocysts

in the stool samples of 3% of healthy children.^[29] However, other studies have failed to find evidence of asymptomatic cryptosporidiosis in children.^[30,31] Most of these studies used microscopy to detect the presence of *Cryptosporidium* oocysts in stool specimens. It has been found that the detection rates increase significantly when molecular methods are applied in addition to conventional methods.^[32]

Cryptosporidiosis in HIV-infected individuals

Cryptosporidiosis is a substantial threat to HIV-infected individuals with an estimated risk of infection of around 10% in developed countries.^[33] Patients can have chronic watery diarrhea lasting for more than 2 months and shed oocysts in stool during the entire period, and mortality.^[24] Other symptoms include abdominal cramps, anorexia, nausea, vomiting, fatigue, and low-grade fever. Cryptosporidiosis continues to remain a major risk to the immunocompromised because of the lack of effective specific therapy.^[34] *Cryptosporidium* spp is also the most commonly isolated pathogen in the biliary tract in patients with AIDS-cholangiopathy.^[35] There have been reports of other sites of infection, such as pancreas and lungs.^[24]

There have been reports from the mid 1990s on the prevalence of symptomatic cryptosporidiosis in HIV-infected adults from different parts of India, ranging from as low as 0.7% to as high as 81%.^[24] Several studies in India have showing that symptomatic cases had CD4 counts <200 cell/mm³ and asymptomatic cases had CD counts >300 cell/mm³ reinforcing the importance of CD4 T cells in mediating resistance to this pathogen.^[24,36-39]

Cryptosporidiosis in transplantation

A study on patients undergoing allogeneic bone marrow transplantation in Vellore, India identified *Cryptosporidium* spp. in 7 of 65 cases.^[40] *Cryptosporidium* was identified in 2.9% of allogeneic bone marrow transplant recipients and 1.7% of pediatric allogeneic BMTs in recent study from the same center.^[24,41]

A study on renal transplant recipients in North India identified cryptosporidial diarrhea in 16.6% of cases.^[42] A study from Turkey, which examined 115 fecal specimens from 69 renal transplant recipients and 42 fecal specimens from controls, reported 18.8% of renal transplant recipients had *Cryptosporidium* spp. in at least one fecal specimen. The rate was significantly higher in renal transplant recipients than in the control group ($P < 0.05$).^[17,43]

Cryptosporidiosis has also been found in children with liver transplantation.^[17] A study from Belgium following liver transplantation in children showed that 3 of 461 children developed diffuse cholangitis associated with

intestinal *Cryptosporidium* carriage.^[44] In a retrospective study from Pittsburgh, 4 out of 1,160 non-renal, abdominal organ transplant recipients reported cryptosporidiosis, all children.^[45] Three of 4 occurred in patients receiving liver transplants, and 1 followed a small bowel transplantation.^[17]

Molecular epidemiology of cryptosporidiosis

Although 22 species and several subtypes of *Cryptosporidium* have been identified so far, more than 90% of the human infections are caused by two species-*C. hominis* and *C. parvum*.^[46] The anthroponotic species *C. hominis* (until recently called *C. parvum* genotype I) almost exclusively infects humans and *C. parvum*, the zoonotic species (genotype II), infects humans and animals.^[47,48] Other species reported to infect humans include *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, *C. suis*, *C. andersoni* and a few other *Cryptosporidium* cervine, horse, skunk, and chipmunk I genotypes.^[46] More recently, cases of human cryptosporidiosis from a rabbit genotype, *C. cuniculus*, have also been reported.^[49,50]

Considerable regional diversity in the distribution of species of *Cryptosporidium* has been noticed. For example, *C. parvum* is the dominant human species in the Middle-East, whereas in European countries, *C. parvum* and *C. hominis* are both observed in almost equal proportions. In developing countries, however, *C. hominis* is considered to be the predominant species and is responsible for a majority of human infections.^[46] In India too, *C. hominis* has been identified as the commonest species isolated from the stool samples of children with cryptosporidial diarrhea as well as in asymptomatic children.^[27,51]

Several reports have demonstrated differences in clinical manifestations between the different species of *Cryptosporidium*. Infection with *C. hominis*, but not *C. parvum*, was associated with an increased risk of non-intestinal sequelae among immunocompetent patients in England and Wales.^[52] Similarly, Kuwaiti children with *C. hominis* infection suffered from diarrheal episodes of longer duration and presented with more severe and diverse clinical manifestations than those with *C. parvum* infection.^[53] Comparison of cases of *C. hominis* and *C. parvum* infection with unusual genotypes among the UK residents showed that patients with unusual infections were older, immunocompromised and more likely to travel abroad.^[54] Additionally, the intensity and duration of oocyst shedding was more in Peruvian children with *C. hominis* infection.^[55]

Cryptosporidium have been further classified by molecular techniques based on a range of genes encoding specific proteins. Among these, there is an increasing use of sub-genotyping using the *gp60* (also called *cpgp40/15*)

gene. Association between *gp60* sub-genotypes, and transmission and disease has also been documented. These include an increased virulence and waterborne transmission of the *C. hominis* subgenotype Ib.^[56]

Seasonality of cryptosporidiosis

The incidence of cryptosporidiosis seems to exhibit strong seasonality-low endemic levels followed by pronounced seasonal outbursts. However, the seasonal patterns tend to vary with location. In tropical climates, an increase in incidence is observed during the warm, rainy season,^[9,20] whereas in temperate climates, cryptosporidiosis peaks during spring and fall.^[57,58]

In a multisite study of cryptosporidiosis among hospitalized children in India, the incidence of cryptosporidial diarrhea among children residing in the more temperate northern part of India correlated positively with temperature and negatively with humidity. However, no such correlation was observed for children residing in the more tropical southern region.^[59] In another study from North-Eastern India, the highest prevalence of cryptosporidiosis was observed during the rainy months.^[60] Among children in Kolkata, cases of symptomatic as well as asymptomatic cryptosporidiosis increased with increasing rainfall.^[51]

In a recent meta-analysis on the seasonality of cryptosporidiosis, increases in temperature and precipitation were associated with an increase in the incidence of cryptosporidiosis. Precipitation was found to be a strong seasonal driver for cryptosporidiosis in moist tropical climates. On the other hand, in temperate climates, the incidence of cryptosporidiosis peaked with an increase in temperature.^[61]

Transmission of *Cryptosporidium*

Cryptosporidium spp. is ubiquitous in the environment and has been isolated from a wide range of host species as well as from treated and untreated drinking water supplies, groundwater, surface water bodies, untreated and secondarily treated wastewater, estuarine and marine waters, and soil surfaces.^[1,62] In developed countries, cryptosporidiosis frequently occurs as water-related outbreaks associated with breakdown of water treatment facilities or increased contamination of drinking water sources by animal fecal material or sewage.^[63,64] Even in endemic settings, consumption of unsafe water is associated with an increased risk of acquisition of cryptosporidial infections.^[65]

There are certain important biological characteristics of *Cryptosporidium* spp. that favor its transmissibility. The infective form – oocyst can survive in the environment for months under suitable conditions. The relatively small size of the oocysts prevents their rapid settlement

on the ground, thereby allowing them to be transported over long distances in air. Also, the low infective dose results in easy acquisition of infection, facilitating the rapid spread of the organism. They are hardy, resistant to most chemical disinfectants, and can sustain a wide range of environmental pressures.^[14,63]

Most chemical methods of water purification, including chlorination, have little or no effect on the viability of *Cryptosporidium* oocysts. On the other hand, conventional techniques of drinking water treatment such as coagulation, sedimentation, and filtration can reduce the oocyst load by 99% and greater. Using multiple disinfectants has been shown to be more efficacious in reducing *Cryptosporidium* oocysts than exposure to a single chemical.^[62,66]

Ever since the first reported case of cryptosporidiosis in a child brought up on a cattle rearing farm, a large number of cryptosporidial outbreaks arising from human-animal contact have been reported.^[67] Direct contact with pre-weaned calves, lambs or animal feces and inadequate hand washing facilities were considered to be contributory factors.^[68]

Apart from outbreaks caused by contaminated water and infected animals, foodborne outbreaks of cryptosporidiosis have also been reported,^[69-71] although such reports are less frequent. Contamination of food items by ill food handlers has been implicated as the cause of foodborne outbreaks of cryptosporidiosis.^[69,71] Fresh fruits, vegetables and raw or undercooked shellfish have also been identified to be an emerging etiological source of *Cryptosporidium* spp.^[1,72] *Cryptosporidium* spp. can also be transmitted directly from person-to-person.^[73] Although contaminated fomites and environmental surfaces were implicated in a few instances, evidence of direct person-to-person spread has also been documented.^[74,75] Other modes of transmission include mechanical transport through soil and insects such as cockroaches and houseflies.^[14] *Cryptosporidium* spp. have also been detected in sputum and aspirated bronchial materials in HIV patients as well as in non-HIV infected children, suggestive of a possible airborne mode of transmission.^[76,77]

Clinical features

In immunocompetent patients, infection is frequently asymptomatic, particularly in adults. In symptomatic cases, symptoms appear 2-10 days after infection and result in either acute diarrhea or a persistent form that can last for a few weeks. Symptoms appear from 2 to 10 days after infection and last for up to 2 weeks. The diarrhea is usually watery with mucus, sometimes associated with cramps, nausea, vomiting, and a low-grade fever. Dehydration can result from large

volume watery diarrhea, particularly in young children, and prolonged infection can result in malabsorption. In immunocompromised individuals, infection can be asymptomatic or acute, but frequently results in chronic diarrhea, lasting for several months.^[5,7]

Life cycle and pathogenesis

The spherical thick walled, environmentally hardy oocysts (3-6 µm in diameter), shed in the fecal material of the infected host, are immediately infectious, unlike other coccidian parasites.^[24] The life cycle of *Cryptosporidium* spp. is completed within the luminal surface of small intestine and colon of the host where it remains intracellular but extracytoplasmic.^[78] Infective dose is few as 10 oocysts (ID₅₀ 132 oocysts) as evidenced in healthy adult volunteer studies.^[12] Ingestion follows release of motile sporozoites in the intestine, which invade the epithelial cells involving Gal/GalNAc epitopes of sporozoite surface glycoproteins^[79] and recruitment of the host actin cytoskeleton to form a parasitophorous vacuole.^[78] A feeder organelle at the site of attachment is believed to function as a portal to allow nutrients from the host cell to the parasite. Parasite surface glycoproteins including gp900, gp60, and circumsporozoite surface ligand (CSL) has been documented to be involved in the invasion and attachment process.^[80,81] Asexual (merogony) and sexual reproduction of the parasite occurs within the extracytoplasmic vacuole, resulting in merozoites that infect adjacent epithelial cells and the production of sporulated thin-walled and thick-walled oocysts. Thin-walled oocysts can excyst endogenously, resulting in autoinfection, which, along with repeated first-generation merogony, helps to maintain persistent infections in AIDS patients.^[24]

The alterations in the intestinal structure and physiology lead to the pathogenesis of cryptosporidiosis. Infection leads to rapid loss of the microvillus border, shortening and fusion of the villi, and lengthening of the crypts resulting in malabsorption due to loss of membrane-bound digestive enzymes, decreased absorption, reduced glucose-NaCl absorption, and increased chloride anion secretion. Production of proinflammatory cytokines, specifically IFNγ and TNFα, also contribute to the pathogenesis of cryptosporidiosis by increasing production of prostaglandins, neural peptides and reactive nitrogen intermediates, disruption of the epithelial barrier leading to a leaky and dysfunctional epithelium and alteration of solute transport leading to osmotic diarrhea.^[24,81]

Diagnosis

Demonstration of 4-6 µm size *Cryptosporidium parvum* oocysts in stool using modified Kinyoun's acid-fast stain is the most commonly used method for the diagnosis of cryptosporidiosis. Hot Safranin stain and fluorescent

dyes such as auramine/carbol fuchsin fluorescence method have also been used. Multiple stool samples (at least 3) and prior concentration of stool, such as by formol ethyl acetate centrifugation, maximize recovery of oocysts.^[82] Direct immunofluorescent-monoclonal antibody staining increases detection rate over acid-fast staining^[83] although acid-fast staining has been shown to have 83.7% sensitivity and 98.9% specificity compared to PCR.^[84]

Although rarely used for diagnosis, a combination of Jenner and Giemsa stains can be used to show endogenous stages in gut mucosa. *Cryptosporidium* oocysts stain blue to azure, often with a crescentic pattern, about 4 to 6 red or purple dots may be seen. A clear halo surrounding the oocyst resulting from shrinkage can be demonstrated.^[85]

Demonstration of cryptosporidial antigen in stool using ELISA is useful for screening large numbers of specimens while immunofluorescence assay offers a high combination of sensitivity and specificity and is considered the gold standard by many laboratories. PCR is more sensitive and easier to interpret but required more hands-on time to perform and is more expensive than microscopy. An important advantage of the PCR is the ability to differentiate between different *Cryptosporidium* genotypes, which can assist in determining the source of cryptosporidial outbreaks and in epidemiology.^[84]

Immunity to *Cryptosporidium* infection

Immune responses to infection with this parasite are not well understood. Evidence from immunodeficient animal models have shown that a Th₁ response involving primarily TCR $\alpha\beta$ + CD4 + lymphocytes, IFN γ , and IL-12 play a major role.^[86] Experimental infection studies with mice and calves show that immunity is dependent on the number of CD4 T cells increasing within the intestinal intraepithelial lymphocytes and generating gamma interferon. Interleukin-12 may play a role, possibly through its ability to induce gamma interferon production.^[17] The susceptibility of HIV/AIDS patients to this pathogen and resolution of cryptosporidiosis following immune restitution underscores the importance of CD4 + T cells.^[80] Serological response to cryptosporidial antigens is coincident with resolution of symptoms. This has been associated with some protection, and pre-existing antibodies have found to be associated with decreased severity and duration of infection, but these antibody responses may only be markers of other cell-mediated protective responses.^[24] Studies on mannose binding lectin in Haitian children with cryptosporidiosis,^[87] toll-like receptors mediating response to infection via IL-8 and NF κ B in epithelial cell lines,^[88] IL-15-mediated elimination of parasites by

NK cells,^[89] and IL-18 potentially mediating secretion of anti-microbial peptides all indicate the importance of an initial innate response.^[90]

Treatment

There have been a large number of studies aimed at developing satisfactory therapy for cryptosporidiosis, particularly in patients with AIDS.^[17] Therapeutic approaches tried for treatment of cryptosporidiosis in past have included macrolide antibiotics, aminoglycoside paromomycin, ionophores such as maduramycin, rifaximin, octreotide, and immunotherapy.^[17,24] Although nitazoxanide is licensed for use in immunocompetent patients, a recent meta-analysis found it to be ineffective in HIV. In patients with HIV, anti-retroviral therapy greatly influences the outcome of cryptosporidiosis.^[24] Anti-retroviral therapy most likely acts indirectly by immune restitution and increase in CD4 counts and also by the direct effect of protease inhibitors on oocyst shedding, resulting in a sustained therapeutic effect after follow up.^[24]

CONCLUSION

Cryptosporidium spp. is increasingly recognized as an important protozoan parasite, causing diarrhea in children and immunocompromised individuals. Early childhood cryptosporidiosis has been associated with subsequent impairment in growth, physical fitness, and cognitive function. *Cryptosporidium* spp. has a complex lifecycle with multiple modes of transmission. The oocysts are hardy, can survive in the environment for a very long time and are resistant to most chemical disinfectants, which makes its removal from drinking water difficult. Studies on immunity to this parasite suggest cell-mediated immunity, especially CD4 + T cells along with IFN γ and IL-12 plays the key role in protection. Despite recognition of the importance of immune status, the correlates of protective immunity in cryptosporidiosis in humans are poorly understood, and treatment modalities are limited.

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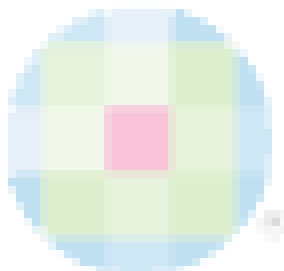
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