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REVIEW

Cryptosporidiosis: environmental, therapeutic, and preventive challenges

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Abstract *Cryptosporidium* spp. are responsible for endemic and epidemic disease worldwide. Clinical manifestations may include acute, persistent, or chronic diarrhea, biliary, and pulmonary disease. Disease severity ranges from asymptomatic or mild to severe, intractable diarrhea with wasting depending on immune status, nutrition, and age. Transmission is fecal–oral with both human and animal reservoirs. Disease is often self limited in healthy individuals, but therapy remains a challenge in the immune-compromised. Prevention currently depends on appropriate hygiene and proper water management and treatment.

Introduction

Cryptosporidium spp. are a major cause of concern for potable water supplies and play a significant role in diarrheal illness worldwide. This protozoan parasite of the apicomplexan phylum was first discovered in the gastric mucosa of mice by Tyzzer in 1907 [1], but its medical significance was not recognized until the first biopsy-diagnosed human cases in 1976 [2, 3].

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Prevalence

Cryptosporidium spp. infection is prevalent worldwide. Older reports reveal rates varying from 0.26 to 22% (mean 2.2%) in immunocompetent patients with diarrhea in developed countries versus 1.4–40.9% (mean 6.1%) in developing countries. In HIV-positive patients, rates between 6 and 70% (mean 14%) were found in developed countries versus 8.7–48% (24%) in developing countries. Up to 7.5% of controls without diarrhea also harbored *Cryptosporidium* spp. [4]. These reports used stool microscopy for detection. However, recent studies suggest that these rates are likely to be higher if PCR is used for detection [5]. Seroprevalence rates, which may detect asymptomatic and non-diagnosed cases, are approximately 16.9–54% in the United States, 33–88% in Southern and Eastern Europe, and 64–94.6% in resource poor settings. Seroprevalence increases with age and depends on the quality of water sources [6–13]. However, serology is not a good diagnostic tool since it does not necessarily reflect active infection. Reported non-outbreak *Cryptosporidium* cases in the US increased from 3,411 in 2004 to almost 8,300 in 2007. There were 7 recreational water outbreaks reported in the US in 2004 versus 26 in 2007. This may be due to increased incidence, increased testing, improved surveillance, or a combination of the three [14].

Diagnosis

Oocysts can be identified by stool microscopy with modified acid fast stains, but an experienced operator is necessary to distinguish *Cryptosporidium* spp. from debris, yeast forms and other protozoa. Sensitivity using one sample has been found to be insufficient [15] and therefore

several samples should be tested when the diagnosis is strongly suspected [16]. Enzyme-linked immunosorbent assays (ELISA) generally have excellent sensitivities and specificities, but false-positives have been reported [17]. Immunomagnetic separation of oocysts can increase sensitivity in clinical samples [18]. Heterogeneous distribution of the parasite in biopsy material may result in false-negatives [19]. Other detection methods include PCR, immunofluorescence microscopy, colorimetric in situ hybridization, fluorescent in situ hybridization, and real-time quantitative PCR, but these methods are mainly used for environmental or epidemiological research purposes. Analysis of the small-subunit rRNA locus by PCR RFLP is generally used to determine species. Sub-types can be identified by PCR RFLP or sequencing of polymorphic loci, the most commonly used being the *Cpgp40/15* (also called GP60) locus [20].

Detection of oocysts in environmental samples

Analysis of environmental samples for oocysts generally involves concentration by centrifugation and filtration, purification through immunomagnetic separation, staining using fluorescently labeled monoclonal antibodies and 4′,6-diamidino-2-phenylindole (DAPI), and fluorescence and differential interference contrast (DIC) microscopy for visualization [21]. Several methods of assessing viability and infectivity of oocysts in environmental samples exist. One comparison of dye permeability assays with excystation and animal infectivity assays found that permeability patterns could predict infectivity [22]. However, other studies showed that dye permeability and excystation assays over-estimate infectivity [23]. Fluorescent in situ hybridization (FISH) targeting rRNA coupled with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody allows species specific identification and permits viability assessment [24–26]. However, overestimation of viability and infectivity is a concern [27]. Neonatal mouse infection assays and human ileo-cecal adenocarcinoma-8 (HCT-8) cell culture assays are considered the gold standard for assessing infectivity, but are resource- and time-intensive [28]. Additionally, the neonatal mouse model cannot be used to study *C. hominis*.

Life cycle

The life cycle begins with ingestion of viable, infective oocysts by a susceptible host. Excystation of the oocyst results in liberation of four sporozoites that invade and are engulfed by the apical surface of the intestinal epithelium after recognition and attachment to surface receptors. A dense matrix of cytoskeletal elements isolates the host cytoplasm from the parasite, resulting in a supra-cytoplasmic parasitophorous vacuole. Within parasitophorous vacuoles, sporozoites differentiate into trophozoites that undergo asexual merogony resulting in infection of adjacent cells by released merozoites. Alternatively, merozoites may differentiate into type II meronts producing either micro- or macrogametocytes that engage in a sexual cycle. The zygotes thus formed undergo meiosis to produce oocysts with four sporozoites. Thin-walled oocysts are implicated in autoinfection, while immediately infectious thick walled oocysts are excreted into the environment to restart the cycle [29, 30].

Transmission

Transmission of *Cryptosporidium* spp. is fecal–oral and often linked to contaminated drinking water, but person to person , recreational water, food-borne [31], and zoonotic [32] transmission also occur. Aspiration and hematogenous spread have been proposed to explain respiratory symptoms associated with isolation of *Cryptosporidium* spp. in the airways [33]. Airborne transmission has also been postulated [34]. Mechanical transport of infectious oocysts by flies and other insects has been experimentally proved [35].

Reservoir characteristics and environmental factors influence the epidemiology and transmissibility of *Cryptosporidium* spp. *Cryptosporidium* spp. are immediately infectious when excreted from reservoir species into the environment. However, oocysts are sensitive to inactivation by several adverse environmental conditions before a suitable new host is found.

Animal husbandry involving cattle, and possibly goats and sheep, has been implicated in human outbreaks [36–38]. An epidemic of foot and mouth disease in cattle in 2001 in the UK leading to culling operations and restrictions in animal movement resulted in a significant decrease in human *Cryptosporidium* cases [39]. Young calves have been found to be a greater reservoir for *C. parvum* than older calves and adult cattle [40].

Temperature is a critical parameter in the survival and infectivity of oocysts shed into the environment. Higher temperatures increase metabolic activity, exhausting carbohydrate energy stores, and decrease autonomous survival and infectivity [41]. Oocysts are less infective with age [19] and are very susceptible to desiccation [42].

Exposure to sunlight for 10 h renders oocysts entirely non-infective in mouse models [43]. Using batch process solar disinfection (SODIS) and vital dye tests, 12 h of strong sunlight led to a reduction in oocyst viability from 98% to 0.3%. Isolated UV light, particularly UV-B and artificial UV-C, is rapidly germicidal for *Cryptosporidium*

spp. [44–46]. *C. parvum* loses infectivity in a mouse model after 150 min of exposure to UV light [47]. Despite DNA repair genes, oocysts do not regain infectivity after exposure to UV light [48]. *C. hominis* is as sensitive to UV light as *C. parvum* [49]. The effect of solar light is not limited to its UV component as exposure of oocysts to UV-shielded autumnal solar radiation reduced mean infectivity by 52% [45].

Vegetation cover can effectively reduce the number of *Cryptosporidium* spp. transferred from stool on land to water. Vegetated buffer zones of 1.1 to 2.1 m between sources of oocysts and water bodies were found to result in a 3.2 to 8.8 log reduction of oocysts, depending on residual dry vegetation matter, land slope, rainfall, and runoff conditions [50].

Incidence of *Cryptosporidium* spp. infections exhibit seasonal variations. Oocyst concentrations in agricultural runoff have been found to be highest at the beginning of storm events and storm seasons [36]. Some investigators, however, have not found any consistent relationship between intensity of rainfall and oocyst contamination of watercourses [51]. In Massachusetts, USA, *Cryptosporidium* spp. incidence peaks approximately 40 days after temperature peaks [52]. A recent meta-analysis revealed a seasonal relationship between cryptosporidiosis, temperature, and the first annual peak in precipitation in moist tropical climates. The meta-analysis of colder temperate climates revealed a slight seasonal peak of cryptosporidiosis in the fall, associated with relatively higher precipitation [53]. Peak human infections in East Africa coincide with the end of the rainy season and an increase in *Cryptosporidium* spp.-contaminated surface waters in Meru, Kenya [54].

Clinical manifestations

Clinical manifestations vary considerably according to immune status. The infectious dose varies from 9 to 2066 oocysts depending on the strain [55–59], with the infectious dose necessary to infect 50% of subjects (ID50) estimated to be 10–83 for *C. hominis* [57] and 132 for *C. parvum* [58]. Subsequent infections in otherwise healthy hosts result in decreased oocyst shedding and clinical severity as well as 20-fold higher ID50s [60, 61]. The pre-patent period, the period between ingestion and excretion of oocysts has been experimentally found to be 5 days on average with a range of 4–9 days using various *C. parvum* isolates [55, 59]. Symptoms may begin before the passage of oocysts [55]. In immune competent individuals, symptoms are variable, but typically include self-limited watery or mucoid diarrhea. Asymptomatic carriage also occurs. The median duration of symptoms was 9 days (1–51 days) in the 1993 Milwaukee outbreak [62]. In contrast, *Cryptosporidium* spp. is a common cause of persistent (>14 days) diarrhea in malnourished children in the developing world [63, 64]. Stool leukocytes or blood are unusual and should prompt a search for co-infection or an underlying pathology. Possible accompanying symptoms include abdominal cramps, nausea, vomiting, and fever [62, 65, 66]. Asymptomatic oocyst passage has been observed up to 2 months after resolution of symptoms [15]. Infections after apparent resolution are not infrequent, occurring in up to 40% of cases; however, it is not clear whether these are relapses or re-infections [62, 67]. In immunodeficient individuals, such as HIV/AIDS patients with CD4 counts <50, the course can be chronic, severe, or even fulminant leading to wasting and death. Extra-intestinal manifestations are frequently seen in this group. Biliary pathology including sclerosing cholangitis, acalculous cholecystitis, and/or pancreatitis correlates with shortened survival [68]. Respiratory involvement ranging from no symptoms to bilateral infiltrates with dyspnea can occur [69]. The incidence of severe HIV-associated *Cryptosporidium* spp. infection has decreased in the developed world with the generalization of effective anti-retroviral therapy [70, 71].

Children in resource-poor settings are particularly at risk, not only with an increased incidence of *Cryptosporidium* spp. infection, but also with increased acute and long-lasting morbidity. Psychomotor developmental stunting may occur following infection, especially in children under 1 year of age, and its effects are still measurable many years after infection [72, 73]. Malnutrition is both a contributing factor to and a result of *Cryptosporidium* spp. infection [74, 75]. In this environment, malnutrition, immune immaturity, and HIV often combine to multiply the impact of *Cryptosporidium* spp. infection. Not surprisingly, cryptosporidiosis has been found to be a major independent risk factor for childhood mortality in the developing world [76].

Species and subtypes

Zoonotic potential and inter-host infectivity of specific species are important in formulating public health measures. Identification at the species level permits preferential tracking of species known to have human infectivity in water sources [77]. *Cryptosporidium* hominis [78] and *C. parvum* are the species most often implicated in human disease. *C. hominis* was initially thought to be infectious only for humans and gnotobiotic piglets [78]; however, more recent studies indicate that this species may also infect other animals [79]. In the US and in many developing countries, *C. hominis* has been shown to be the dominant species involved in human infection. In developing nations, *C. parvum* is predominantly anthro-

ponotic, while zoonotic transmission may occur more frequently in developed countries. Other species that less commonly cause zoonotic transmission of *Cryptosporidium* to humans and possibly anthroponotic disease include *C. andersoni* (cattle), *C. canis* (dogs), *C. felis* (cats), *C. meleagridis* (birds), *C. suis* (pigs), and *C. muris* (mice). Cervine (deer), skunk, chipmunk I, horse, and rabbit genotypes may be unusual human pathogens [20].

In urban slums in Brazil, *C. hominis* infections are more common and associated with higher disease burden and greater growth faltering than *C. parvum* infections [80]. *C. hominis* has also been found to be associated with greater severity of diarrhea [81]. Additionally, *C. hominis*, but not *C. parvum* infection, was linked to extra-intestinal sequelae such as arthralgias, ocular pain, recurrent headaches, dizziness, and fatigue [67]. A longitudinal birth cohort study in Peru revealed that first infections with all isolated *Cryptosporidium* spp. were associated with diarrhea, but only *C. hominis* subtype Ib was also associated with nausea, vomiting, and malaise [82]. In contrast, HIV-positive patients in India were more likely to have fever when infected with zoonotic species than with *C. hominis* [83]. In HIV patients in Peru, species and subtypes associated with diarrhea included *C. hominis* subtype Id, and *C. felis* or *canis*; however, only *C. parvum* was significantly associated with chronic diarrhea and vomiting. In the same study, *C. meleagridis* infection was not associated with symptoms [84]. Further studies are needed to clarify clinical associations of infection with different species and subtypes.

Immunity

Iterative infection confers immunity in individuals with an intact immune system [60]. The exact mechanisms of protective immunity are not well understood and likely involve innate and adaptive as well as cell-mediated and humoral responses. It is clear that T-cell malfunction such as in advanced AIDS predisposes to severe disease. Resolution of infection with T-cell reconstitution further illustrates the critical role of cellular immunity [85–87]. Furthermore, interferon gamma appears to be necessary for an effective immune response [88] and TNF-α, IL-4, IL-8, and IL-15 are also probably important [89–91]. Other situations of immune compromise that predispose to severe *Cryptosporidium* spp. infection include primary immune deficiencies, in particular x-linked immunodeficiency with hyper Ig M, hematological malignancies, and transplants with chemotherapeutic immune suppression [92]. Deficient serum mannose-binding lectin (MBL) levels were associated with MBL2 gene promotor and structural region polymorphisms, specific MBL2 haplotypes, and increased symptomatic and recurrent *Cryptosporidium* infections in young children [93].

Evidence to support an active role of humoral immunity in protection from and clearance of infection is not conclusive. Indeed, although studies have shown that sero-conversion coincides with resolution of infection, subsequent infections are less severe after sero-conversion [61, 87, 94] and breast feeding may be associated with protection [95, 96], a specific causal link has not been proved. Moreover, other studies show that breast-feeding is either not protective [65] or results in greater incidence of infection [97]. A high level of specific antibodies in breast milk was actually found to be associated with a shorter time to first infection than low levels of specific antibodies [98].

Therapy and prevention

Therapy for *Cryptosporidium* spp. depends largely on immune status. The most important therapy is hydroelectrolytic resuscitation, by mouth when practical or intravenous as necessary. Restoration of immune status using anti-retroviral therapy is important in HIV/AIDS patients [99]. Protease inhibitors such as ritonavir, indinavir, and saquinavir have been found to have in vitro and in vivo anti-cryptosporidial activity and can be specifically considered in these patients [100]. In cases of chemotherapeutic immune depression (e.g., hematological malignancies and transplants), a decrease in the depth of immunosuppression may be helpful. When co-infection with a dysenteric pathogen is not present, antimotility agents permit absorption of water, electrolytes, and medications such as anti-retrovirals. Biliary involvement is treated with immune reconstitution and hepato-bilio-pancreatic procedures (e.g., cholecystectomy, endoscopic retrograde cholangiopancreatography (ERCP) with sphincterotomy and stent placement). Although a number of therapeutic agents have been evaluated for anti-cryptosporidial activity, only nitazoxanide has been approved by the US Food and Drug Administration (FDA). This drug has been shown to be beneficial in reducing duration and severity of symptoms in immune competent patients (adults and children) and mildly to moderately T-cell-deficient AIDS patients in six clinical trials involving 436 patients [99, 101]. The results have been less conclusive in profoundly immunodeficient patients, in particular HIV/AIDS patients with CD4 counts <50, and longer treatment courses have been thought to be warranted [99]. However, a recent double-blind, randomized, placebo-controlled trial in pediatric HIV patients in Zambia with 28 days of high-dose nitazoxanide failed to show a benefit in clinical or parasitological response [102]. Initial studies and experimental data looking at paromomycin in HIV patients showed some benefit [99].

However, the largest prospective, randomized, double-blind, placebo-controlled trial before the widespread introduction of highly active antiretroviral therapy found paromomycin to be no better than placebo [103]. The use of bovine dialyzable leukocyte extract [104] over 8 weeks or bovine hyperimmune colostrum over 10 days [105] versus placebo in HIV/AIDS patients with *Cryptosporidium* spp. showed no significant difference in stool frequency, consistency, volume, or parasitological response. A 2007 meta-analysis concluded that the sum of evidence did not support a role for chemotherapy in the management of cryptosporidiosis in immunocompromised patients [106].

Two studies looking at HIV patients receiving *Mycobacterium avium* complex (MAC) prophylaxis or treatment revealed that rifabutin, and possibly clarithromycin, were effective for cryptosporidiosis chemoprophylaxis [107, 108]. However, azithromycin used for MAC did not afford protection against *Cryptosporidium* spp. [108]. Although bovine hyperimmune anti-*Cryptosporidium* colostrum immunoglobulin decreases the intensity of infection in vitro and in animal models [109, 110], its prophylactic use in a small number of *Cryptosporidium parvum*-challenged healthy volunteers showed no difference in onset, intensity or duration of diarrhea versus non-fat milk placebo [111].

There is currently no vaccine to prevent *Cryptosporidium* spp. infection. The search for effective vaccine targets is ongoing.

Prevention of infection from water sources

The particularly hardy nature of *Cryptosporidium* spp. is such that conventional water treatment involving coagulation/flocculation, sedimentation, filtration, and disinfection may not be sufficient to clear contamination. Chlorination has been found to be insufficient to clear *Cryptosporidium* spp. [112]. Their small size (4–6 μm) make oocysts more difficult to filter than larger protozoa such as *Giardia* and *Cyclospora*. The pliability and plasticity of oocysts is such that pore sizes less than 1 μm are necessary for effective filtration. Solar and UV-light disinfection are effective in reducing oocyst viability and infectivity [113, 114]. Solar photocatalytic disinfection (SPCDIS) using titanium oxide-coated plastic inserts improves oocyst inactivation in water under overcast conditions [113]. When achievable, segregation of possible animal reservoirs from the water supply is desirable to reduce oocyst loads.

Constructed wetlands are a cost-effective way to treat wastewater as well as runoff from confined animal feeding operations (CAFO). These wetlands remove and biodegrade pathogens like *Cryptosporidium* spp. through sedimentation, filtration, attachment to plant roots and biofilms, predation, UV inactivation, and time-dependent die-off [115].

Improvements in water treatment and management are effective provided that there is no secondary contamination during transport, storage, and terminal use. In developing countries, hand washing, education, and point of use water treatment significantly improve rates of diarrhea [116]. Water quality interventions can be effective without concomitant improved sanitation or more reliable water supplies [117].

In conclusion, *Cryptosporidium* spp. are significant pathogens worldwide; however, water treatment and good water management practices coupled with good nutrition and access to quality healthcare have mitigated its impact in the developed world. Effective specific treatment in immunocompromised individuals remains elusive. The full burden of *Cryptosporidium* spp. is still felt in the developing world.

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Conflict of interest None.

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