CASE STUDY OF HEALTH EFFECTS OF *CRYPTOSPORIDIUM* IN DRINKING WATER

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Keywords: *Cryptosporidium*, cryptosporidiosis, coccidia, diarrhea, immunocompromised, infectivity, immunology, transmission, outbreaks, waterborne, environment, occurrence, detection, water treatment, inactivation, risk analysis, exposure, regulations.

Contents

Summary

Cryptosporidium parvum is an intracellular intestinal parasite, which can infect

numerous mammalian hosts. Infection in immunocompetent individuals is self-limiting, but in immunocompromised individuals, especially those individuals with AIDS, cryptosporidiosis can be protracted, causing severe disease which can be fatal. Cellular immunity appears important in limiting infection, but immunocompetent individuals can become re-infected. *C. parvum* has a worldwide distribution and is endemic in areas of poor hygiene. The infectious dose is thought to be small and development is intracellular, but extracytoplasmic.

The life cycle, consisting of both asexual and sexual stages, is completed within a single host and an autoinfective cycle ensures that numerous environmentally robust oocysts are excreted in feces. Up to 10^{10} oocysts can be excreted by a symptomatic individual and can contribute to the gross contamination of terrestrial and aquatic environments. There are no pathognomonic signs and symptoms.

Transmission is by any route whereby infectious oocysts are ingested by a susceptible host. Person to person, zoonotic, waterborne, foodborne and airborne transmission routes have been documented. Waterborne transmission has gained importance in the last thirteen years because of the large number of potential hosts, which can be infected from one contamination event. *C. parvum* oocysts can occur commonly in the aquatic environment and their small size enables them to penetrate through physical water treatment processes.

Oocysts are chlorine insensitive at concentrations normally used in water treatment. More than 25 waterborne and foodborne outbreaks of cryptosporidiosis have been documented, affecting more than 417,000 individuals. The sources of waterborne contamination are not always readily identified because of the low efficiency of detection methods, which rely on filtering large volumes of water through depth filters or membranes, selectively recovering oocysts from the retentate and identifying them by epifluorescence microscopy using genus specific monoclonal antibodies. Methods are tedious and time consuming and rely on the expertise of highly trained personnel microscopists for reliable identification.

Effective assessment of likely risk to a population must be based upon solid biological parameters including species identification, viability status and infectivity potential of oocysts detected in environmental concentrates. Molecular biological methods of identification permit the discrimination of *C. parvum* from other species of *Cryptosporidium* that are not infectious to humans and between genotypes that are restricted to a particular host.

Further developments in this area should focus on more sensitive methods for oocyst isolation and identification, a firmer comprehension of oocyst survival in the environment and a better understanding of those genotypes that cause infection and disease in humans. Further effort should be directed towards developing effective chemotherapy and immunotherapy for cryptosporidiosis. *Cryptosporidium* can be controlled in water treatment and regulations governing oocyst densities in treated water or removal in treatment have been laid. Both education and legislation have significant roles in limiting oocyst contamination of drinking water.

1. Introduction

In 1907, the parasitologist, Tyzzer described parasites found in the gastric crypts of an experimental laboratory mouse, which he named *Cryptosporidium muris*. In 1912 he described similar parasites in the intestinal tract of young weaned mice, which produced smaller oocysts and discovered that parasites released from these smaller oocysts caused infection in the intestinal tract of infected mice.

On the basis of these findings, he classified these parasites as a distinct species, *Cryptosporidium parvum*. The genus gained further significance when *Cryptosporidium* was recognized as a cause of morbidity and mortality in young turkeys in 1955, (*C. meleagridis*), and as a cause of scouring in calves in 1971. In 1976, two separate reports of human cryptosporidiosis were described. Since then *Cryptosporidium* has emerged as a pathogen of worldwide importance to man and as an important cause of neonatal disease in cattle and other livestock.

Based on the animal hosts from which they were isolated, more than forty "species" of *Cryptosporidium* have been described in the literature. However, cross transmission studies have demonstrated that *Cryptosporidium parvum* is not host specific, and that classification of species simply on the basis of isolation from a particular mammalian host is erroneous.

2. The Genus Cryptosporidium

Cryptosporidium is a member of the phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eucoccidiorida, suborder Eimeriorina, family Cryptosporidiidae. The genus name describes the transmissive stage (the oocyst), which contains four sporozoites that are not contained within sporocysts, hence *Cryptosporidium*. *Cryptosporidium spp*. are classified as coccidians and they make up the order Eucoccidiorida with four other genera, *Cyclospora*, *Isospora*, *Sarcocystis* and *Toxoplasma*, all of which cause disease in humans.

The genus *Cryptosporidium* differs from other coccidia whose oocysts require a period of maturation (sporulation) outside the host in order to become infectious for the next host. *Cryptosporidium* oocysts are fully sporulated, hence are infectious for another susceptible host, when excreted.

Currently, eight species are recognized: *C. parvum* (primary pathogen in humans and ruminants), *C. muris* (rodents and ruminants), *C. meleagridis* (turkeys) and *C. baileyi* (primarily gallinaceous birds), *C. serpentis* (reptiles), *C. nasorum* (fish), *C. felis* (cats) and *C. wrairi* (guinea pigs).

The current classification is based upon a variety of parameters including host preference and cross transmissibility, morphological differences, sites of infection, etc., and, increasingly, molecular taxonomic methods will assist in assigning species and genus status. *C. nasorum*, which infects fish may be reclassified into a new genus *Piscicryptosporidium*, on the basis of ultrastructural and development differences in the parasites.

Species of *Cryptosporidium*	**Dimensions of oocysts (µm)**	**Site of infection**	**Host**	**Molecular typing**
Cryptosporidium parvum	4.5 x 5.5	small intestine	mammals	PCR-RFLP of 18S rRNA gene
Cryptosporidium muris	5.6 x 7.4	stomach	mammals	PCR-RFLP of 18S rRNA gene
Cryptosporidium felis	4.5 x 5.0	small intestine	felids	PCR-RFLP of ITS1 region of 18S rRNA gene
Cryptosporidium wrairi	4.0-5.0 x 4.8-5.6	small intestine	guinea pigs	PCR-RFLP of COWP gene
Cryptosporidium baileyi	4.6 x 6.2	trachea, bursa of Fabricius, cloaca	gallinaceous birds	PCR-RFLP of 18S rRNA gene
Cryptosporidium meleagridis	4.5-4.0 x 4.6-5.2	intestine	turkeys	not known
Cryptosporidium serpentis	4.8-5.6 x 5.6-6.6	stomach	snakes	not known
Cryptosporidium saurophilum	4.2-5.2 x 4.4-5.6	intestinal and cloacal mucosa	lizards	not known
Cryptosporidium nasorum	3.6 x 3.6	intestine	fish	not known
Cryptosporidium sp.	4.5-6.0 x 3.6-5.6	small intestine	bobwhite quail	not known
Cryptosporidium sp.	5.8-5.0 x 8.0-5.6	? intestine	snakes, reptiles	not known

Table 1. Some differences between some species within the genus Cryptosporidium

Host species, site of development and oocyst morphometry (size and shape) are used to determine *Cryptosporidium* species (Table 1). Latterly, molecular methods for species identification have also been described and should be used in conjunction with more conventional parameters. Oocysts of various *Cryptosporidium* species vary in shape and size (Table 1) although overlap in size can occur.

Isolates from humans are named *C. parvum* or *Cryptosporidium sp*. Current molecular evidence suggests that within the parasites excreted by human beings, there are two genotypes (human (H); calf (C)) with different mammalian host ranges. Both genotypes (H and C) can infect humans, genotype C isolates can infect humans and other mammalian hosts, whereas genotype H does not appear to infect non-human (calf and mouse) hosts.

2.1. Life cycle

C. *parvum* is an obligate intracellular parasite and is monoxenous, completing its life cycle in a single host. All stages of the life cycle occur either in epithelial cells lining the intestine or, less frequently, the respiratory tract, within parasitophorous vacuoles situated in the brush border between the plasma membrane and the cytoplasm, or in the lumen. The oocyst of *C. parvum* is spherical or sub-spherical, smooth walled, measures 4.5 - 5.5 microns in diameter and contains four naked sporozoites. Upon ingestion by a susceptible host, exposure of oocysts to the acidic environment of the stomach and the alkaline environment of the small intestine causes the four crescent-shaped sporozoites to excyst and initiate the endogenous stages of asexual development in the brush border

of the intestinal epithelium. Several cycles of asexual reproduction (merogony, schizogony) initiated by merozoites occur, augmenting the number of parasites. Some merozoites transform into microgametocytes and macrogametocytes to initiate the sexual component of the life cycle which, after fusion of the gametes to form the zygote, transforms into a thick walled oocyst. The newly formed oocyst undergoes sporogony and is excreted in the feces as a fully sporulated, environmentally robust, thick walled oocyst. A small proportion of zygotes (~20%) are believed to form thin walled, autoinfective oocysts, whose sporozoites excyst during passage through the intestinal tract to augment infection.

3. Human cryptosporidiosis

3.1. Clinical aspects

There are no pathognomonic signs. Cryptosporidiosis is associated with profuse watery diarrhea, rapid weight loss, dehydration and abdominal cramps. Less frequent symptoms may also include low grade fever, nausea, vomiting, anorexia and general fatigue. The incubation period, the time from ingestion of organisms to the manifestation of symptoms, ranges from 5 to 10 days but can be as much as 28 days. In immunocompetent individuals, both duration and severity of disease can vary, but the diarrhea is self-limiting and the infection is limited to the small and large intestine. In immunocompromised individuals, especially those with acquired immunodeficiency syndrome (AIDS), infection may lead to dehydration, electrolyte imbalance, and eventually death.

Infection in such patients may spread along the gastrointestinal tract to the esophagus, stomach, gall bladder, common bile duct, rectum, appendix and into the respiratory tract. Immunocompromised individuals include those with AIDS, primary immune deficiencies, other acquired abnormalities of T lymphocytes, hypo- and agammaglobulinaemia, X-linked hyperimmunoglobulin M syndrome, severe combined immunodeficiency syndrome, leukemia (especially during aplastic crises), those receiving immunosuppressive drugs for transplantation and chemotherapy and those with severe malnutrition where infection may be associated with measles.

Young children are more susceptible to infections and may have more severe clinical signs due to an immature immune system and poor hygiene habits. Breast fed babies are protected from a variety of intestinal infections including *Cryptosporidium*. This protection arises from various specific and non-specific mechanisms including antibodies colostrum and milk, and the production of organic acids from the metabolism of intestinal anaerobes (especially bifidobacteria) stimulated by human milk substances. After weaning, the acquisition of infection is frequently due to exposure to contaminated individuals, fluids and food.

Asymptomatic infections appear to be more prevalent in children, young adults and AIDS patients in endemic areas of disease. Children infected with *Cryptosporidium* suffer retarded growth. In a study that measured the weight of 1064 children with cryptosporidiosis from Guinea-Bissau, a loss of 392 g in boys and 294 g in girls at two years of age was observed compared to uninfected controls. Weight loss was not

compensated by time, and the study suggested that cryptosporidiosis in infancy has a permanent deleterious effect on growth. Asymptomatic infections are reported to be more common than symptomatic infections and to cause weight loss in young Peruvian children. Of 207 children (aged 0 to 3 months) studied, 45% became infected over the following two years of life. Fifty seven infected children were assessed, of which 63% were asymptomatic.

Symptomatic cases lost an average of 342 g during the first month of infection whereas asymptomatic children lost an average of 162 g, compared to uninfected controls. Since asymptomatic infections were more common in the population studied, they may have accounted for retarded growth. Asymptomatic infections appeared to be common (39.7%) in a rural population of southern Indians with *Cryptosporidium* being one of the commonest parasites found in this highly parasitized population (97.4% of individuals infected, with 74.3% having multiple intestinal parasites). In 377 individuals studied, asymptomatic *Cryptosporidium* infections in 5 to 19 year olds of the northern Bolivian Altiplano were frequent (31.6%).

3.2. Pathogenesis

Histopathology of intestinal tissue reveals loss of villus height, villus edema and an inflammatory reaction. The loss of microvilli and a decrease in the levels of microvillar disaccharidases might interfere with absorption and contribute to malnutrition. Local cellular infiltrates are usually of plasma cells and neutrophils, but also of sub-epithelial macrophages and lymphocytes and in moderate to severe infections, intraepithelial neutrophils are present. *Cryptosporidium* infected piglets demonstrate an increase in lamina propria macrophages producing tumor necrosis factor (TNF). Peyer's patches appear reactive.

The mechanism by which *Cryptosporidium* infections cause severe diarrhea is not well understood and an intensive search for an enterotoxin has been inconclusive. The secretory diarrhea has also been attributed to a prostaglandin-dependent effect however, experiments in infected piglets and calves, designed to measure the electrical resistance of the intestinal epithelium *in vivo*, demonstrate little change in permeability. This is supported by the observation that cell disruption is not usually seen as it is believed that the epithelium is repaired by the host.

The loss of epithelial barrier integrity has been observed in cell lines infected with sporozoites *in vitro*, yet pathological studies on the intestines of infected animals and humans indicate that diarrhea is the consequence of malabsorption, possibly due to a reduction of lactase activity. The neutrophil attracting chemokines, interleukin eight (IL8) and GROα, are upregulated in *C. parvum* infected monolayers of intestinal epithelial cells (HCT8, Caco 2) and basolateral secretion of IL8 into the underlying mucosa provides a mechanism for neutrophil accumulation at the site of infection.

3.3. Immune response

Both immunocompetent and immunocompromised infected individuals can mount antibody (humoral) responses to *Cryptosporidium* antigens. Infected individuals

respond to infection by producing IgM, IgG and IgA antibody isotypes which persist after infection thus, seropositive uninfected individuals reflect the fact that they have been exposed / infected, or both, previously. Exposure to *Cryptosporidium* can be common in some communities depending on a variety of factors including the level of endemic disease, the availability of transmission routes and hygiene.

A complete understanding of the significance of the humoral immune response of an individual to eradicate *Cryptosporidium* infections is still unclear. Chronic cryptosporidiosis can occur in hypogammaglobulinemic individuals in the absence of detectable antibodies. Chronically infected AIDS patients produce higher serum and salivary IgA antibody titers than non-chronically infected AIDS patients or uninfected immunocompetent controls, however they are unable to eradicate infections, indicating that other factors are involved in an effective protective immune response.

Crude, water soluble sporulated oocyst extracts, which contain a complex array of antigens have been used predominantly for serodiagnosis. In electro-immuno-transfer-blotting (Western blots, WB) using crude oocyst antigen, IgG antibody reacts primarily with 27, 17 and 15-kDa antigens whereas IgM can show modulation by reacting with the 27-kDa antigen and the IgA with the 17-kDa antigen.

The serological response of human volunteers to experimental infection was also assessed by WB. Volunteers with IgG antibody to the 27-kDa antigen moiety prior to exposure excreted fewer oocysts than volunteers without this antibody specificity. When sera from individuals involved in a waterborne outbreak in Talent, Oregon, USA were tested, both enzyme linked immunosorbent assay (ELISA) and WB detected an increase in antibody titre in individuals that reported consuming oocyst contaminated Talent water close to the time of the waterborne outbreak however, statistical significance was only observed with the WB results.

3.4. Seroprevalence

Thirty two percent of adult US Peace Corps volunteers had anti-*Cryptosporidium* serum IgG prior to traveling to developing countries. Of 803 individuals, aged 6 months to 21 years from Oklahoma, USA, who were assessed for antibody to *Cryptosporidium*, 13% of children under five years of age had antibodies: this rate was higher for both those in day-care centers and those with a recent history of diarrhea. Antibody titer increased with age and remained constant with two peaks, one at five years (>30%) and the other at 14 years (58%) of age. These seropositivity rates are high compared to previous studies in the USA. A high antibody prevalence to *Cryptosporidium* (36%) has been associated with farming and milking in a Wisconsin (USA) farming community. In developing countries, seroprevalence rates can be extremely high: 95% of adults and children up to five years old were seropositive in a community in Fortaleza, Brazil; 64% of children and adults from Peru and Venezuela were seropositive, while 91% of Thai children from an orphanage had IgG antibodies. This rate contrasts sharply with reported incidence in countries such as Germany. Analysis of 495 sera from individuals of all age groups demonstrated 15.4% with anti-*Cryptosporidium* antibodies, yet considering that only 2% of cases of diarrhea were attributed to *Cryptosporidium* in Germany, this seroprevalence level is higher than anticipated. This discrepancy between

serology and oocyst detection in feces could be due to asymptomatic infection, failure to report cryptosporidiosis or to a lower sensitivity of the coprodiagnostic test.

Both the inability to demonstrate effective protection with antibodies and to demonstrate that T cell immunocompromised patients contract chronic cryptosporidiosis indicates that involvement of the cellular immune response is necessary for the resolution of infection. Studies in humans support the concept that athymic or $CD4^+$ cell-depleted mice cannot terminate *Cryptosporidium* infections. Correlation between $CD4^+$ cell densities and the development of clinical symptoms was studied in a waterborne outbreak among 1731 members of a drug rehabilitation community in Italy. In this community, 19.6% of individuals were HIV-positive and the attack rate in this group was 30.7% compared to 13.6% in the HIV-negative individuals. Amongst HIV-positive cases, the $CD4^+$ cell densities determined the severity of clinical symptoms and their duration, with chronic disease (>30 days) occurring in 16 individuals (15.4%) with less than 150 $CD4^+$ cells per mm^3 at the onset of the infection.

3.5. Laboratory detection

Laboratory detection of human cryptosporidiosis is by identification of oocysts in feces by microscopy following staining with modified Ziehl Neelsen (mZN), auramine phenol (AP) or immunofluorescence using a commercially available, genus specific, fluorescein isothiocyanate-labeled monoclonal antibody (FITC-mAb). Fresh or preserved stools can be concentrated to increase the yield of oocysts by sedimentation using the formol-ether or formol-ethyl acetate techniques or by flotation using sucrose, salt or zinc sulphate solutions. The sensitivity of detection is low using these methods: a detection limit of 10^6 oocysts mL^{-1} of feces, in unconcentrated smears, was reported using the Kinyoun mZN (KmZN). Following formol-ethyl acetate concentration, between 1 x 10^4 and 5 x 10^4 oocysts g^{-1} are necessary to obtain a 100% detection efficiency using either KmZN or FITC-mAb. Oocysts are more readily detected in concentrates made from watery, diarrheal specimens than from formed stool specimens. The threshold for 100% detection efficiency using AP or FITC-mAb is 1 x 10^3 oocysts g^{-1} for bovine feces. Fecal antigen detection immunoassays offer no increase in sensitivity over microscopy. The polymerase chain reaction (PCR) is more sensitive than conventional methods for detecting infections being able to identify *C. parvum* specific DNA from extracts of less than 100 oocysts. PCR will be useful for identifying asymptomatic carriers, and fomites, food and water contaminated with small numbers of oocysts. Rapid detection of carriers could limit clinical sequelae in 'at risk' groups and reduce environmental spread.

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