HUMAN CRYPTOSPORIDIOSIS: CLINICAL ASPECTS, EPIDEMIOLOGY AND CONTROL

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Cryptosporidium was first described almost a century ago but remained obscure until *Cryptosporidium parvum* was recognised as a cause of acute enteric infection, in calves in the early 1970s, and subsequently in humans in whom it was assumed to be a zoonosis. It subsequently emerged worldwide as a cause of severe, protracted and often life-threatening infection in the severely immunocompromised, especially those with AIDS, and as a common cause of acute, self-limiting but often protracted gastroenteritis in otherwise healthy subjects, especially children. In the 1980s it became clear that waterborne transmission represented a serious public health threat, resulting in the UK in the introduction of regulations that are intended to limit this. More recently, molecular studies have added considerably to our understanding of the natural history of cryptosporidiosis, including confirming a non-zoonotic cycle of infection in humans. Although effective specific therapy remains elusive, this infection in AIDS patients can have its severity diminished.

BIOLOGY

Host range and development

Cryptosporidia are obligate intracellular, enteric, coccidian parasites infecting a wide variety of vertebrate host species. It includes at least ten separate species and can be found in mammals, birds, reptiles and fish.[1–3] The species infective for man, *C. parvum*, is generally described as readily cross-transmissible to a variety of mammalian host species and cryptosporidiosis has generally been regarded as a zoonosis.[3,4] Recent molecular studies (see below) show, however, that the situation is considerably more complex.[5,6] The parasite has a complex life cycle (see Figure 1), which is completed within an individual host animal (monoxenous).[1,2,7,8] Life cycle stages include oocysts (the transmissive stage) containing four motile sporozoites (the infective stage), and asexual and sexual endogenous (tissue) stages. Following ingestion of oocysts, sporozoites are released and quickly attach superficially to host cells, initially in the brush border of enterocytes in the small bowel. The parasite is, however, capable of infecting mucosal cells of the entire enteric tract and other epithelial tissues such as those of the respiratory tract. The endogenous stages develop within a parasitophorous vacuole, situated on the luminal surface of the cytoplasm, the outer layer of which is derived from the host's cell membrane. The parasite is thus, uniquely, intracellular but extracytoplasmic. Oocysts are usually fully sporulated and infective when excreted.

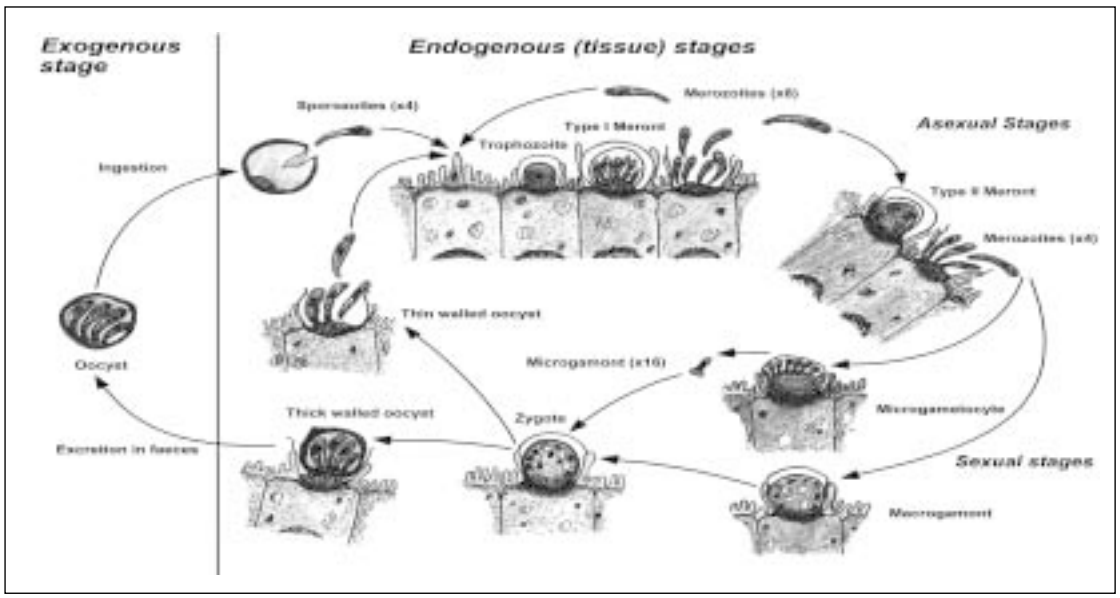

FIGURE 1

Diagrammatic representation of the life cycle of *Cryptosporidium parvum*. Infection starts with ingestion of an oocyst (5 μm) containing four motile sporozoites. In response to stimulation by increased temperature, pH changes and exposure to digestive fluids, sporozoites are released in the small intestine. They attach to enterocytes and take up a pseudo-external location on the surface of the cytoplasm. They are then enveloped by the outer cell membrane, within a parasitophorous vacuole, thus becoming intracellular but extracytoplasmic. The parasite develops into a fixed uninucleate meront (trophozoite). As this matures, it undergoes schizogony (asexual multiple budding) to produce, in the first generation, eight motile merozoites that are released to form new meronts. The next generation produces four merozoites that develop as gamonts (sexual stages). Microgametocytes (male equivalent forms) produce 16 small motile microgametes that, on release, fertilise the macrogamont (macrogameteocyte) to produce a zygote. The zygote matures *in-situ* to form either (i) a thick-walled oocyst, which sporulates prior to excretion, or (ii) a thin-walled oocyst that releases its sporozoites internally.

Molecular Biology

Genotypic studies have largely confirmed the different species described and have differentiated various *C. parvum* isolates. *C. parvum* seems to be genetically complex and can be subdivided into a number of sub-types or lineages, some of which may represent host-adapted variants or cryptic species.[5, 6, 9–11] The type commonly found in livestock (genotype 2 or C) can also be found in humans (i.e. a zoonotic type), while some isolates from humans appear not to be transmissible to most other species (genotype 1 or H), supporting the view that cryptosporidiosis is not always zoonotic.[3, 5] Some isolates of *Cryptosporidium* sp. from cats and dogs are distinct from genotypes 1 and 2 and may represent either new genotypes or separate species.[11] They have only been reported in a small number of humans who have AIDS and may thus be less infectious for humans. In several large waterborne outbreaks the predominant genotype has been type 1 and therefore most likely to have been derived from sewage contamination.[10] These findings have far-reaching implications for the water industry and public health.[12, 13]

PATHOLOGY

Histopathology

Published histological studies are based primarily on biopsy and *post-mortem* material from cases among immuno-compromised subjects, particularly those with AIDS (see below). However, tissues taken from immunologically normal subjects and veterinary studies suggest that the changes seen are typical. There is involvement of the small bowel mucosa, other parts of the gastroenteric tract, and sometimes beyond.[2, 7, 8] Abnormalities of villous architecture occur; there may be evidence of mild inflammation, with cellular infiltration into the lamina propria; apoptosis may be seen. Rectal biopsy may reveal mild, non-specific proctitis. The infection may sometimes be more extensive, and persistent involvement of the biliary tract and even the respiratory tract is found in patients with AIDS.[2, 7, 8, 14–7] *C. parvum* in tissues appears, as inconspicuous, small (2–8 μm) bodies, apparently superficially attached to the brush border, unevenly distributed over the apical cells and within the crypts, and sometimes on cells in other tissues; there may be marked lengthening of microvilli adjacent to parasites (Figure 2).[7, 8] There is surprisingly little intracellular change at the ultrastructural level beyond the attachment zone of the parasite.

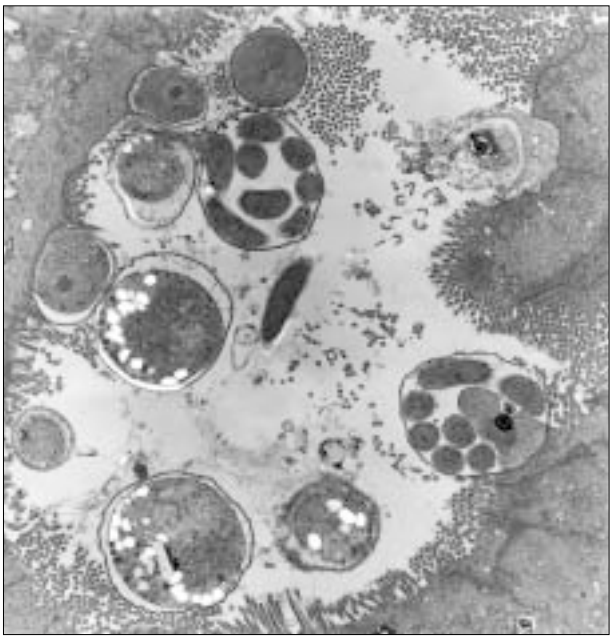

FIGURE 2

Electron micrograph of a crypt in the gut tissue of a mouse infected with *Cryptosporidium parvum*. The section shows various developmental forms: uninucleate meronts with prominent nucleolus, surrounded by a host-derived outer membrane; type 1 meronts (schizonts) showing sections of eight merozoites; a free merozoite; and macrogametes showing unstained polysaccharide granules, pale stained lipid bodies, and dark-staining wall-forming granules. Some of the parasites appear to be free in the lumen because the point of attachment is not in the plane of sectioning. Characteristic lengthening of microvilli can be seen on some infected cells; there is little other intra-cellular reaction (although apoptosis can sometimes be seen).[8]

Possible pathogenetic mechanisms

Watery diarrhoea is characteristic of non-inflammatory infection of the small bowel as seen with viruses and toxin-producing organisms.[2, 7, 8, 18–20] Toxin-like activity has been described but has not so far been fully characterised; such activity may be derived from either the host or the parasite but it is not clear which of these is the more important.[8, 21–3]

There is evidence to suggest that the pathogenetic mechanisms for the diarrhoea involved may include:

- reduction in absorptive capacity for water and electrolytes
- increased secretory capacity due to crypt hypertrophy
- toxin-induced secretion
- loss of brush-border enzymes resulting in malabsorption of sugars
- osmotic effects from microbial fermentation of sugars in the colon (consequent fatty acids may contribute to the characteristic malodour)
- immunological factors, e.g. cytokine activity

Clinical malabsorption has been described and may significantly add to the effect of the disease on normal development, and infant and child weight gain, especially in developing countries.[8, 24–6] It contributes to weight loss in AIDS patients, and this may be a marker for intensity of infection.[27–32]

Immunological response

Both humoral and cellular factors have a role in limiting or controlling infection although the latter are probably the more important.[2, 14, 18–20, 33–5] T cells, especially CD4 and possibly CD8, are of particular importance; other factors such as interferon gamma may also have a role: the data are derived mainly from animal studies and their involvement in human infection is uncertain or controversial. In AIDS patients, CD4 cell counts of fewer than 200 cells/mm^3 indicate the need to take special care to avoid exposure to *Cryptosporidium;* there is generally a poor prognosis if infection occurs with <100 CD4 cells/mm^3.

An immune response has been demonstrated but is generally of little diagnostic value for individual cases. Recent studies on human volunteer infection suggest, however, that

excretory faecal IgA is a reliable marker for current infection.[36] Some studies show a specific protective effect from breast-feeding although protection from the environment is also likely to be a factor.[4, 37] Possible immunotherapeutic approaches have been investigated, including the use of passive hyperimmune bovine globulin, but have not generally been adopted.[2, 14, 38, 39]

Serological and sero-epidemiological studies have added considerably to our understanding of the natural history of the infection and in identifying risk factors.[4, 40–4]

CLINICAL PRESENTATION IN OTHERWISE HEALTHY (IMMUNOCOMPETENT) PEOPLE

Cryptosporidiosis in the immunocompetent is a self-limiting but often protracted, acute gastroenteritis with a variety of presenting symptoms.[1–3, 7–8, 45] The incubation period is about five to ten days (range two to fourteen days). There may be a prodrome of one to a few days, with malaise, abdominal pain, nausea, and loss of appetite. Gastroenteric symptoms usually start suddenly, the stools often being described as watery, greenish with mucus, and 'very offensive'. Blood and pus in the stool are not usually found and if present may indicate mixed infection, for example with *Campylobacter* or *Giardia*. Patients usually open their bowels three to six times a day but sometimes much more frequently.[8] There may be colicky, abdominal pain, especially after meals, anorexia, nausea, vomiting, abdominal distension, and marked weight loss (up to 10%); systemic symptoms may include malaise, headache, myalgia, fever. Cough occurs in some cases.

Gastrointestinal symptoms usually last about three to fourteen days, but weakness, lethargy, mild intermittent abdominal pain (sometimes associated with biochemical evidence of pancreatitis) and occasional loose stools may persist.[8] Rare sequelae include toxic megacolon and reactive arthritis.[7, 8] Duration of oocyst excretion and oocyst numbers vary and may fluctuate with time; they may generally be detected for two to three weeks after the disappearance of symptoms by conventional staining methods.[8, 46–8]

Those who have some immunity resulting from a previous infection can have very mild symptoms limited to a few loose stools and perhaps some nausea and loss of appetite, or may be asymptomatic.[8, 47] Apparently asymptomatic infections, i.e. stool-positive in the absence of acute gastro-enteric symptoms, are common in children in developing countries, reflecting repeated exposure due to poor environmental hygienic conditions.[2, 4, 8, 24–6, 37] These may be associated with increased enteropathy and malnutrition. Symptomatic infection is not normally seen in those with frequent risk exposures in developed countries, such as rural adults, probably because of immunity resulting from recurrent exposure.[4]

Transplacental transmission has not been documented but symptomatic infection during late pregnancy may cause metabolic disturbances in the mother, leading to failure to thrive in the infant.[8] Some older infants and children may have persistent infection and enteropathy even in developed countries.[8] In developing countries, infection in apparently otherwise well children may have significant morbidity, particularly in terms of enteropathy and malnutrition, and increased mortality.[24–6]

CLINICAL PRESENTATION IN IMMUNOCOMPROMISED PATIENTS

Susceptibility to cryptosporidiosis and disease severity may be increased in subjects immunocompromised by AIDS, hypo- or agammaglobulinaemia, severe combined immunodeficiency, leukaemia, malignant disease, bullous pemphigoid, and immunosuppressive treatment (cyclophosphamide, corticosteroids etc.), e.g. in leukaemia or for bone marrow transplantation (BMT) in CD40 ligand deficiency.[1, 2, 7, 8, 14, 17, 27, 38, 48–50] Children who are immunosuppressed by measles and chickenpox, especially where there is concomitant malnutrition, may also have more severe or persistent infection.[4, 8] Infection in acute leukaemic patients may be unusually severe when associated with aplastic crises, and may then require modification of chemotherapy for resolution of the infection.[7, 8]

In AIDS patients, diarrhoea may be frequent, profuse, and watery.[2, 7, 16–20, 27–9, 35, 50] Symptoms often develop insidiously although several different patterns are seen – transient, persistent, cholera-like, and relapsing. Associated symptoms are generally similar to those described above but generally more severe. The entire enteric tract and its associated organs may be involved, often in association with other pathogens. Biliary tract involvement with right upper-quadrant abdominal pain, cholecystitis, sclerosing cholangitis, pancreatitis, and hepatitis commonly occur. Infection of the respiratory tract may occur, possibly as a result of aspiration of vomit.[8, 51]

While cryptosporidiosis was one of the earliest recognised AIDS-defining infections, the fulminant form of the disease with severe diarrhoea, persistent nausea and vomiting is now largely seen in end-stage AIDS and indicates a poor prognosis. The symptoms may then prove intractable and extremely distressing, and persist until death from some other cause or because of severe dehydration, acid-base or electrolyte imbalance, and cachexia associated with the cryptosporidiosis. Examination may reveal other features of HIV infection, including mixed enteric infections, particularly with cytomegalovirus (CMV) and *Isospora belli*, as well as direct pathogenic effect of HIV on the cells of the gut, which may have an exacerbating effect.[7, 16, 17, 29]

DIAGNOSTIC INVESTIGATION

Laboratory detection and diagnosis

Characteristic endogenous (tissue) stages may be found in histological sections (where they may readily be overlooked) but diagnosis is usually by detection of oocysts in stools.[2, 7, 8, 46, 48] Stool consistency varies according to the time elapsed since onset; it should not be used as a criterion for examination. Age-banded selection criteria have been recommended in the UK to improve standardisation of approach in screening and reporting for epidemiological purposes.[52] A single stool examination will usually suffice in acute cases, but more may be required in delayed investigation and in more chronic or relapsing infection. Concentration of stool specimens is not usually required for diagnosis in acute cases, although oocyst excretion does fluctuate and then decline as the infection progresses; detection of low-level oocyst excretion by conventional microscopy is difficult. Usually, the stool from patients with cryptosporidiosis does not contain blood, pus, cells, or Charcot-Leyden crystals and their identification may indicate a mixed infection. Oocysts of *C. parvum* (4 to 6 μm) are difficult to identify in standard wet faecal

preparations and the sporozoites within are difficult to distinguish even with special optical systems. Oocysts can be detected microscopically in fixed smears using, for example, the modified Ziehl-Neelsen method or phenol-auramine fluorescent stain.[46] Immunofluorescent antibody (IFAT) and enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies, are available.[46] Molecular techniques have been developed for detection and characterisation, but are not generally used for routine diagnostic purposes.[6]

Peripheral leucocytosis and eosinophilia are found only rarely. Serum electrolyte abnormalities develop in patients who become severely dehydrated. In immunocompromised patients with cryptosporidial cholecystitis, serum alkaline phosphatase and γ-glutamyl transpeptidase levels are raised, while amino-transferases and bilirubin may remain normal.[7]

Radiographic abnormalities may include dilatation of the small bowel with mucosal thickening, prominent mucosal folds and abnormal motility, and in the biliary system, dilated distal biliary ducts, stenosis with an irregular lumen, and other changes which are reminiscent of primary sclerosing cholangitis, particularly in AIDS cases.[7]

Differential diagnosis

In immunocompetent patients, cryptosporidiosis should be considered in any acute diarrhoeal illness associated with abdominal pain and other gastrointestinal symptoms, which may resemble those of acute giardiasis, cyclosporiasis or isosporiasis.[7] However, bloating and malabsorption are commoner with these infections, which also generally respond readily to specific treatment. The diagnosis is particularly likely in patients with traveller's diarrhoea, those who have been in contact with farm animals, in children from day-care centres, and in health care personnel.[3, 4] In immunocompromised patients in particular, especially those with AIDS, the effects of multiple infections may confuse clinical presentation.

Treatment

In immunocompetent patients, cryptosporidiosis is self-limiting, but they may become dehydrated and require intravenous fluids, electrolytes, and symptomatic treatment for vomiting and diarrhoea.[2, 7]

Immunocompromised patients with persistent severe diarrhoea, malabsorption, and other complications may require prolonged palliative treatment. They should avoid excess milk, as lactose intolerance may develop. Parenteral feeding and fluid, electrolyte, and nutrient replacement may be needed. Anti-peristaltic agents such as loperamide, diphenoxylate or opiates may increase abdominal pain and bloating; anti-emetics may be needed but are sometimes poorly effective. Temporary relief of biliary obstruction has been achieved by endoscopic papillotomy, and of cholecystitis by cholecystectomy.[7]

Few antimicrobials have an effect on *Cryptosporidium*, although some reports suggest some activity, either *in vitro* or in clinical trails, for paromomycin (Humatin), letrazuril/diclazuril, somatostatin, azidothymidine, diloxanide furoate, furazolidone, amprolium, the macrolides, roxithromycin and nitazoxanide, either alone or in combination; early reports of apparent activity of spiramycin have not been confirmed.[2, 7, 14, 53–6] In some cases, there may be amelioration of symptoms without eradication of the parasite, some of which may be attributable to eradication of co-infecting agents.[7, 17]

Zydovudine (Retrovir) therapy alone or in combined anti-retroviral therapy (HAART) may also decrease symptoms, probably as a result of improved immune function, although HIV may itself cause or exacerbate gastrointestinal symptoms.[14, 50, 53, 57, 58] Immunotherapy (e.g. with hyper-immune bovine colostrum or immunoglobulin and transfer factor, and interleukin 2) has been attempted, also with variable results.[2, 14, 33, 38, 39] The falling incidence among some AIDS populations might possibly reflect earlier detection of HIV infection and improved case management, including the effects of triple therapy but may also reflect widespread advice not to consume unboiled water.[58] This continuing decline in the incidence of severe cryptosporidiosis and the change of AIDS management in developed countries to a mainly out-patient programme makes controlled drug trials increasingly problematic (B.Gazzard, pers comm).

EPIDEMIOLOGY

C. parvum has been reported worldwide and is common in man and in livestock animals.[1–4] Other cryptosporidial species and some sub-types of *C. parvum* do not seem to be readily transmissible to man or may be less virulent. The epidemiology is complex, involving both direct and indirect routes of transmission, from animals to man and from person to person. Infection is common in children attending day-care centres, where initial cases may be zoonotic in origin (for example, following visits to educational farms by class members or their siblings) or be part of the urban cycle of transmission.[3, 4, 59] Domestic pets are an uncommon source of infection. Well-documented outbreaks have resulted from contamination of public drinking-water supplies, some involving thousands of cases, often amplified by transmission from person to person.[1–4, 13, 42, 60–2] Such outbreaks may involve both human and animal types, although in the outbreaks studied so far one or the other tends to predominate.[5, 6, 10, 42] Thus outbreaks may be attributed to either agricultural or sewage contamination of drinking water supplies. Outbreaks have been associated with consumption of, or recreational exposure to, surface waters and have been increasingly recognised in association with swimming pools.[3, 60–2] Foodborne transmission has been described, although not frequently, particularly in association with the consumption of raw milk, raw (non-fermented) sausage, salad and fresh pressed apple juice.[3, 4, 37, 63, 64] *Cryptosporidium* is a common cause of traveller's diarrhoea and may reflect a variety of exposures; travel-associated transmission includes that within the home country as well as overseas.[3, 4] Cryptosporidiosis in male homosexual AIDS patients can be attributed to any of the above, but also to sexual practices leading to increased faecal exposure.[44, 65, 66]

DEMOGRAPHY

Distribution

In some developing countries, infection is common in infants aged less than one year.[3, 4, 8, 24–6, 37] In developed countries, infection is most common in children aged from one to five years, with a secondary peak in young adults. Infection is uncommon in adults over 45 years and is uncommon in older children and adults inrural populations, probably reflecting immunity from frequent exposure.[3, 4, 8, 45] A relative increase in urban adult cases is often seen in waterborne outbreaks.[4, 61] Distribution of cases appears to be the same in both sexes in developed countries but there is evidence for increased prevalence in males in some

developing countries, which is unexplained.[37, 45]

Seasonal peaks are seen although these may vary from year to year and from district to district, and may coincide with lambing and calving and with periods of maximal rainfall.[3, 4] This emphasises the importance of livestock as reservoirs of infection and water as a vehicle of transmission. Increases in late summer may be attributable in part to traveller's diarrhoea.

Frequency of occurrence

Laboratory rates of detection in faeces from non-immunocompromised subjects in developed countries average about 1-2% of samples examined and, as expected, is more prevalent in developing countries.[2, 4, 8, 24–6, 45, 67] Marked increases in the detection rate locally in developed countries may indicate outbreaks likely to be associated with waterborne infection.[4, 61] In such an event, it is important to monitor the positivity rate as increased numbers of samples may be submitted, leading to increased ascertainment.[61]

Cryptosporidiosis has been one of the most common causes of diarrhoea in AIDS patients, in whom prevalence has sometimes exceeded 50%, with high mortality.[4] In some areas the prevalence is falling among such patients, possibly reflecting earlier detection of HIV infection and subsequent care and HAART (see above). Infection rates are not generally increased for other immunocompromised groups although severity may be increased.

NOSOCOMIAL INFECTION

Outbreaks have been reported involving transmission of *Cryptosporidium* between staff and immunocompromised patients, and between patients, for example in bone-marrow transplant units and between leukaemic children.[4, 68] Poor hand-washing practice and contaminated naso-gastric feeding tubes have been implicated as possible vehicles. AIDS patients may have profuse, watery diarrhoea and intractable vomiting, which, together with other problems including dementia, may lead to significant environmental contamination. In one such outbreak in Denmark, with several deaths, transmission via a ward ice-making machine was suspected.

INFECTIVITY, RESISTANCE, AND CONTROL

Infectivity

In one study using monkeys the infective dose of *C. parvum* was fewer than ten oocysts while in gnotobiotic lambs the minimum infective dose of a lamb adapted isolate was one to five oocysts.[69] Human volunteer studies in the US suggest that the infectious dose is variable, with an infective dose varying from less than ten to more than 1,000 oocysts, according to the isolate.[70, 71] This variability is one of the factors that makes the establishment of an action or trigger value for oocysts in drinking water problematic.

Resistance and disinfection

Oocyst can survive for many months in a cool, moist environment, but are susceptible to desiccation, prolonged freezing, and moderate heat (e.g. pasteurisation temperatures).[1–3, 72] They are remarkably resistant to many disinfectants, particularly those used in the treatment of drinking water; moderate levels of ozone, and medium or high pressure UV may be effective.[72, 73] Many disinfectants in hospital use, including glutaraldehyde, may be ineffective although it has been suggested that Nu-Cidex™ may be effective.[72, 74, 75] Some disinfectants may be more effective if used at elevated temperature (≥35°C). Oocysts are sensitive to 10 vol. (3%) hydrogen peroxide.[72]

CONTROL OF TRANSMISSION

The multiple reservoirs and routes of transmission of *Cryptosporidium*, the heavy output of oocysts from infected hosts, and the low infectious dose can make control difficult. Primary control is by limiting the opportunity for direct and indirect faecal-oral transmission.[3, 76] Symptom-free subjects not in contact with immunocompromised patients can normally be permitted to work or return to school if they use good hygiene. Contamination of water supplies and swimming pools, and open farm visits are the main sources of outbreaks and of an indeterminate number of sporadic cases.

New legislation has been introduced in the UK to limit transmission by means of treated drinking water (see http://www.dwi.detr.gov.uk).[12] The combined approach of structured risk assessments and monitoring, together with other measures introduced such as catchment control and improved water treatment, should ensure improvement in water quality generally. However, it cannot totally remove the risk of transmission given the complexity of the dynamics of transmission and difficulty in ensuring adequate water treatment at all times in relation to this parasite, even for ground water supplies previously thought to be safe.[3, 13, 61] Those particularly at risk need to be educated to avoid or limit these exposures as awareness is often lacking.[77] Bottled water is unlikely to contain parasites but may carry an increased bacterial load, the health significance of which is uncertain for AIDS cases and other profoundly immuno-compromised patients. They should be advised not to drink water that has not been boiled (raising it just to the boil is sufficient). Hospitals and other institutions having large numbers of susceptible individuals should consider the use of appropriate point-of-entry or point-of-use water treatment units (filters or UV) rather than be subject to the problems of responding to a boil water advisory notice, in the event of contamination of the public water supply. This will also protect against other waterborne potential pathogens. Such water treatment units, whether in institutions or the home, need careful maintenance; care needs to be taken in handling used filter units, as these will contain an increased microbial load, including potential pathogens.

Spread via fomites is limited by the effect of desiccation on oocysts. Reduction of faecal contamination, and thus of oocyst numbers, by physical cleaning and thorough drying is essential for control. The adequate disinfection of instruments, such as endoscopes, presents particular difficulties. Staff of infectious disease units and those of other wards to which seriously immuno-compromised patients may be admitted, need to be particularly vigilant in the management of patients with cryptosporidiosis. Staff should report even minor gastrointestinal symptoms and be investigated to minimise risk of spread.[68]

The impact of cryptosporidiosis, even on otherwise well subjects, but particularly on the immunocompromised and on children in the developing world, and the continuing therapeutic challenge, makes control of this infection an important clinical and public health goal.

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