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Minireview: Clinical cryptosporidiosis

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ABSTRACT

Cryptosporidium has emerged as an important cause of diarrhoeal illness worldwide, especially amongst young children and patients with immune deficiencies. Usually presenting as a gastro-enteritis-like syndrome, disease ranges in seriousness from mild to severe and signs and symptoms depend on the site of infection, nutritional and immune status of the host, and parasite-related factors. Sources and routes of transmission are multiple, involving both zoonotic and anthroponotic spread, and facilitated by the resistance of the parasite to many commonly used disinfectants. Prevention and control measures are important for the protection of vulnerable groups since treatment options are limited. This review covers the life cycle, pathogenesis, clinical presentations, diagnosis, prevention and management of cryptosporidiosis in humans.

1. Introduction

Cryptosporidiosis is the clinical disease, usually presenting as a gastro-enteritis-like syndrome, caused by infection with protozoan parasites of the Apicomplexan genus *Cryptosporidium*. Disease ranges in seriousness from mild to severe and signs and symptoms depend on the site of infection and nutritional and immune status of the host: evidence is also emerging that the clinical picture may vary with infecting species. At least eight of the currently identified 20 *Cryptosporidium* species (*Cryptosporidium hominis*, *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium suis*, *Cryptosporidium muris* and *Cryptosporidium andersoni*), and seven of the >40 genotypes (*C. hominis* monkey genotype, *C. parvum* mouse genotype, and the *Cryptosporidium* cervine (W4), chipmunk genotype I (W17), skunk, horse and rabbit genotypes) have been detected in humans, but some may be incidental findings (Ajjampur et al., 2007; Robinson et al., 2008a; Xiao and Ryan, 2008). Those that are currently considered to be human pathogens include *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C.canis* and the *Cryptosporidium* rabbit genotype (Xiao et al., 2001; Xiao and Ryan, 2008; Chalmers et al., in press). The pathogenicity of other species and genotypes for man is not known, but requires further investigation. The majority of human infections are with *C. parvum* and *C. hominis*, although this varies geographically, and infection with unusual species and genotypes occurs in both immune-competent and immune-compromised populations (Cama et al., 2008).

In immune-competent patients, cryptosporidiosis is a self-limiting disease of the intestinal tract, acquired following ingestion of the oocyst life cycle stage which is shed in faeces. The oocysts survive well in moist, ambient environments. While some disinfectants are efficacious at high concentrations, or after long exposure time, oocysts are resistant to many commonly used disinfectants, including chlorine, particularly at normal recommended concentrations. Acquisition of infection is mainly by ingestion of oocysts, either during direct contact with human or animal faeces, or from indirect exposure *via* a transmission vehicle such as contaminated water, food or on fomites. Respiratory infection in patients with diarrhoea or respiratory symptoms has been reported, usually with other pathogens particularly *Mycobacterium* spp., and the clinical significance and route of acquisition are unclear (Lopez-Velez et al., 1995; Clavel et al., 1996). Intestinal cryptosporidiosis occurs most commonly in young children, people who have contact with young animals or children, travellers returning from abroad, recreational water users and consumers of poor quality drinking water. In immune-compromised patients severe, chronic disease may occur and infection can be fatal.

The importance of *Cryptosporidium* is increasingly being recognised, and it was identified as a 'neglected pathogen' in the World Health Organization's Neglected Diseases Initiative 2004. Infections included in the initiative occur "in developing countries

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where climate, poverty and lack of access to services influence outcomes" and "exhibit a considerable and increasing global burden, and impair the ability of those infected to achieve their full potential, both developmentally and socio-economically" (Savioli et al., 2006). This review summarises the life cycle, pathogenesis, clinical presentations, diagnosis, prevention and management of cryptosporidiosis in humans.

2. Life cycle

The *Cryptosporidium* life cycle has been described and illustrated in detail (Chen et al., 2002a; Fayer, 2008). Excystation of ingested oocysts is triggered by conditions in the intestine and four motile sporozoites are released. These actively probe, attach, invade and become engulfed by host epithelial cells at the luminal surface, establishing an intracellular but extracytoplamsic position within a parasitopherous vacuole (PV). Intracytoplasmic invasion occurs only occasionally, at Peyer's patches (Marcial and Madara, 1986). An attachment or feeder organelle develops, and the internalised sporozoite becomes more spherical in shape. An asexual cycle follows, involving differentiation and, sequentially, trophozoite, Type I meront and merozoite production. The parasite proliferates as six or eight merozoites, structurally similar to sporozoites, are produced and released from the PV. The merozoites invade neighbouring epithelial cells and either develop into trophozoites, repeating the asexual cycle, or into Type II meronts.

Four merozoites are produced by Type II meronts, initiating the sexual cycle when they are released from the PV, invade host cells, and differentiate into either macrogamonts (ova) or microgamonts. Microgamonts become multi-nucleate and release free microgametes (sperm) into the lumen, which attach and penetrate through the PV to fertilise the macrogamete, producing a zygote. Following meiosis, the zygote differentiates into four naked sporozoites as the oocyst matures and is released into the lumen. This process of *in situ* sporulation enables autoinfection of the host, as sporozoites may be released directly into the lumen, and the life cycle perpetuates. Chronic cryptosporidiosis occurs when the host's immune system fails to eliminate the parasite. Furthermore, asexual cycling results in proliferation of the parasite, even if the sexual cycle is interrupted. The sexual cycle results in shedding of sporulated oocysts in the faeces, often in large numbers. Those detected in stools have thick walls, and are resistant to many environmental pressures. Thus the life cycle is completed in a single host (monoxenous) and the parasite is immediately infectious on shedding for the next susceptible host.

The parasite derives nutrients from the host cell, not the host's food flow, and can theoretically survive on any mucosal epithelial surface. Coupled with the ability of oocysts to excyst in warm aqueous solutions as well as being triggered by intestinal conditions, infection of extra-intestinal sites such as the gastric epithelium, biliary tract, liver, pancreas and respiratory tract is enabled.

3. Pathogenesis

The main site of infection is the small intestine, although infection may be spread throughout the gastrointestinal tract and extra-intestinal sites. In HIV patients, more proximal small intestine infections generally cause more severe diarrhoea and reduce survival rates, compared to heavy infection of the colon which, in the absence of small bowel infection, can cause intermittent diarrhoea or even asymptomatic infection (Clayto et al., 1994). Although duodenal infection was commonest in AIDS patients, more widespread infection throughout the intestinal tract was associated with the most severe diarrhoea (Lumadue et al., 1998). Gastric involvement has also been reported in this patient group (Berk et al., 1984).

Invasion of host cells is restricted to the luminal border of the enterocytes and leads to displacement of the microvillous border and loss of the surface epithelium, causing changes in the villous architecture, with villous atrophy, blunting and crypt cell hyperplasia, and mononuclear cell infiltration in the lamina propia (Meisel et al., 1976; Farthing, 2000). Osmotic, inflammatory and secretory aspects of diarrhoea have all been investigated for cryptosporidiosis, yet the causative mechanisms have not been fully elucidated. They are, however, multi-factorial, consisting of the effect of the parasite and its products on the epithelial layer and the immunological and inflammatory responses of the host, leading to impaired intestinal absorption and enhanced secretion (Farthing, 2000). The voluminous, watery nature of the diarrhoea resembles secretory diarrhoea, suggestive of an enterotoxin as a specific mechanism. This was indicated by electrolyte analysis of stools from HIV-infected children (Guarino et al., 1997), but not confirmed in case-controlled perfusion studies (Kelly et al., 1996). Despite evidence for endotoxin-like activity *in vitro*, a *Cryptosporidium* toxin has not been isolated (Guarino et al., 1994, 1995).

Cryptosporidium infection has also been noted to cause apoptosis of human intestinal epithelium in cell cultures (Griffiths et al., 1994; Laurent et al., 1997; Ojcius et al., 1999). Interestingly, although T-cells, particularly $CD4^+$ lymphocytes, are crucial to cryptosporidial immunity and clearance of the parasite, changes such as the villous atrophy and crypt hyperplasia are also characteristic of T-cell induced pathology. (McDonald et al., 2000). Increased intercellular permeability and inflammation in the lamina propia could contribute to secretory diarrhoea *via* cytokines and neuropeptides (Laurent et al., 1997; McDonald et al., 2000). However, faecal leukocytes are usually absent, although in studies of malnourished children the presence of cytokines or lactoferrin in stool is indicative of intestinal inflammation (in the absence of breastfeeding), and infection has been associated with a persistent systemic inflammatory response (Kirkpatrick et al., 2006). In HIV patients, histopathology shows evidence of gastrointestinal mucosal injury and a variable inflammatory response that was often associated with co-infection with other pathogens (Lumadue et al., 1998).

Osmotic aspects, characterised by enterocyte malfunction, have been demonstrated in a pig model by decreased Na^+ absorption caused by villous blunting and increased Cl^- secretion due to crypt cell hyperplasia (Argenzio et al., 1990). This results in overall enhanced secretion. Disruption and changes in the microvillous border also lead to the loss of membrane-bound digestive enzymes, reduction in the absorptive surface and uptake of fluids, electrolytes and nutrients (Adams et al., 1994; Griffiths et al., 1994). Diarrhoea is often associated with malabsorption, demonstrated by, for example, abnormal D-xylose tests and radiographic studies (Berk et al., 1984).

Loss of intestinal barrier function, attributable to the *Cryptosporidium*-induced disruption of epithelial permeability (Savioli et al., 2006), is similar to changes seen in a variety of intestinal disorders, including the inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis (Fiocchi, 1998). It has been suggested that previously stable Crohn's disease and ulcerative colitis might be re-activated by *Cryptosporidium* infection (Manthey et al., 1997), and the development of IBD-like lesions in germfree T-cell receptor-alpha-deficient mice was found to be associated with *C. parvum* (Waters and Harp, 1996; Waters et al., 1997; Sacco et al., 1998). However, a study of intestinal mucosal biopsies and serology from human patients with IBD did not support a major role of *C. parvum* in the pathogenesis of these diseases (Chen et al., 2001) and the relationship between IBD and *Cryptosporidium* remains unclear.

Interestingly, cryptosporidiosis in healthy subjects is also associated with a seronegative reactive arthritis, similar to that seen

with certain bacterial gastrointestinal pathogens. As with other infective causes of reactive arthritis, the pathogenesis remains uncertain, and may have an autoimmune basis, or reflect deposition of antigen in the synovium; however, there have been no studies to investigate these aspects in *Cryptosporidium* infection specifically.

Cryptosporidiosis of extra-gastrointestinal sites has been reported in the immune-competent (Hawkins et al., 1987; Norby et al., 1998; Westrope and Acharya, 2001) but occurs mainly in immune-compromised patients. Biliary and pancreatic cryptosporidiosis have been reported following gastric or intestinal infection (Bonacini, 1992; Benhamou et al., 1993; Manabe et al., 1998), but not exclusively (McLauchlin et al., 2003). *Cryptosporidium* causes apoptosis of human biliary epithelial cell lines (Chen et al., 1998, 1999), an observation that may be relevant in the pathogenesis of sclerosing cholangitis, a well-described complication of biliary cryptosporidiosis (Davis et al., 1987; Dowsett et al., 1988; Cello, 1989; Chen and LaRusso, 2002; McLauchlin et al., 2003). Tracheo-bronchial involvement and even sinusitis have been described in the severely immune-compromised (Clavel et al., 1996; Dunand et al., 1997). The precise clinical significance of this is uncertain as most cases have concomitant infection with an identified respiratory pathogen (Clavel et al., 1996). The mode of entry to the respiratory tract is also unknown. Aspiration from the gastrointestinal tract seems most likely, but in birds cryptosporidiosis is transmitted by inhalation and so the possibility of entry by this route cannot be excluded.

4. Clinical features in immune-competent people

Cryptosporidiosis is an acute self-limiting gastoenteritis in immune-competent humans. It occurs worldwide, and in all age groups, although children especially those under 2 years old are most frequently and severely affected. Clinical features are summarised in Table 1. Diarrhoea can be of sudden onset and is generally watery and voluminous; between three and six stools (but sometimes many more) may be passed each day, which are sometimes offensive and may contain mucus (Casemore, 2000). Other acute symptoms are abdominal pain, nausea or vomiting, pyrexia, anorexia, malaise and fatigue (Fayer and Ungar, 1986). Weight loss can be considerable. Bloating and gas production may be reported. Cough has also been reported in some cases but is not explained. Pus, blood and faecal leukocytes are not typically present in the stool. Symptoms usually last up to three weeks, and are resolved through stimulation of immune responses. Some patients experience chronic diarrhoea of a month or longer. Oocysts may continue to be shed for a mean period of 7 days (range 1–15 days) after symptoms have ceased, although exceptionally for up to 2 months (Jokipii and Jokipii, 1986).

4.1. Natural infection in immune-competent people

Clinical cryptosporidiosis arising from natural infection has been studied either in cases presenting to medical attention or in prospective studies of enrolled cohorts of selected populations. Data from the former are biased towards the more severe cases and those shedding detectable numbers of oocysts or *Cryptosporidium* antigens in their faeces (see Diagnosis, below). However, they underpin disease surveillance based on laboratory reporting and are useful in describing cryptosporidiosis in the general population presenting to medical attention. Most cases are detected in children under 5 years of age, and mainly in those <2 years of age, in both developing (e.g. Khan et al., 2004) and developed countries (e.g Palmer and Biffin, 1990). The epidemiology and molecular epidemiology of cryptosporidiosis is presented by Xiao in this volume (Xiao, 2009).

Data from cases where the date of exposure is known, for example, during point source outbreaks, show that symptoms occur up to 14 days (mean 7 days) after the ingestion of oocysts (Jokipii and Jokipii, 1986; MacKenzie et al., 1995). However, in most sporadic cases the source of infection is difficult to ascertain as so many risk factors are common in everyday life. Public health investigations of cases need to ask about exposures in the two weeks prior to the onset of illness in trawling questionnaires.

A series of patients seeking medical assistance with laboratory confirmed cryptosporidiosis in the UK showed that symptoms lasted for 1–90 days (mean 9 days, median 7 days) (Palmer and Biffin, 1990). For similarly recruited patients in Melbourne, Australia symptoms lasted longer, from 1 to 100 days (mean 22 days) and in Adelaide from 2 to 120 days (mean 19 days) (Robertson et al., 2002). A second case series from the UK showed duration as mean 13 days, median 11 days, with no significant difference in median duration between illness caused by the main causative species, *C. parvum* and *C. hominis*, although *C. hominis* duration was more variable (Hunter et al., 2004a). This was similar to the 12 days reported by Jokipii and Jokipii (1986) and by Kahn and colleagues who studied children <5 years of age in Bangladesh (Khan et al., 2004).

Clinical details of diarrhoeic patients with laboratory confirmed cryptosporidiosis in the UK show that abdominal pain is nearly always experienced (96% patients), and vomiting is frequent (65% patients), fever was reported by 59% while bloody diarrhoea was surprisingly reported in 11% cases (Hunter et al., 2004a). However, this may have been due to co-infections which were not identified in the study. Fourteen percent of patients were hospitalised for a range of 1–9 days (median 3 days). Children in Bangladesh had a similar clinical presentation, with persistent diarrhoea developing in 41% cases (Khan et al., 2004).

Recurrence of gastrointestinal symptoms is frequently reported, for example, in the Milwaukee outbreak in 1993 by over 30% cases (MacKenzie et al., 1995) and by 40% sporadic cases in the UK study (Hunter et al., 2004b).

Less is known about the long term health effects of *Cryptosporidium* infection. As with bacterial gastrointestinal pathogens, a seronegative reactive arthritis, has been reported in adults (Hay et al., 1987; Ozgul et al., 1999) and children (Shepherd et al., 1989; Cron and Sherry, 1995) including one report of Reiter's syndrome (arthritis, conjunctivitis and urethritis) (Cron and Sherry, 1995). Reported joint pain was of longer duration in cryptosporidiosis cases than healthy controls in a UK study, conducted 2 months after initial *Cryptosporidium* diagnosis (Hunter et al., 2004b). That study also identified that joint pain, eye pains, recurrent headache, dizzy spells, and fatigue were significantly more common in *C. hominis* but not *C. parvum* cases than in well controls (Hunter et al., 2004b).

It is of great concern that in non-industrialised countries cryptosporidiosis is associated with significant morbidity and infant mortality (Mølbak et al., 1993), and with malnutrition in children (Sarabia-Arce et al., 1990; Lima et al., 1992; Sallon et al., 1988). In Guinea-Bissau, malnourished children under 3 years at infection suffered significant weight loss and impaired growth and did not catch up with their peers (Mølbak et al., 1997). However, in these studies it is not possible to tell whether the malnutrition was a causative factor in *Cryptosporidium* infection or *vice versa*. In a shanty town in Peru, infection in infants was linked to growth faltering and stunting (Checkley et al., 1998). In another study in the same area, normally nourished children with symptomatic cryptosporidiosis tended to grow less than their peers, an effect also seen in children with asymptomatic infection (Checkley et al., 1997). Although this effect was not as strong as that for children with clinical cryptosporidiosis, asymptomatic infection occurred more frequently and is of concern in endemic areas. Thus the term "asymptomatic" for

Table 1
Clinical features of cryptosporidiosis in different groups.

	Immune-competent Laboratory diagnosed patients seeking medical assistance in UK	Immune-compromised patients AIDS, haematological malignancy, primary T-cell deficiency	Immune-competent Children in prospective studies in non-industrialised countries
Length/severity of illness	Self limiting (mean 13 days, median 11 days)[a]	Severe or chronic or intractable increased morbidity and mortality	Acute or chronic disease or chronic carriage
Clinical features	Gastrointestinal: Diarrhoea (98%)[b] Watery (81%)[b] Loose (17%)[b] Bloody (11%)[a] Relapsing (40%)[a] Abdominal pain (60–96%)[b,a] Vomiting (49–65%)[b,a] Fever (36–59%)[b,a] Nausea (35%)[b]	Gastrointestinalt[c,d]: Diarrhoea Transient Relapsing Chronic Cholera-like Abdominal pain Vomiting Fever Nausea Severe weight loss Biliary tract involvement[d]: Cholangitis Pancreatitis Sclerosing cholangitis Liver cirrhosis Respiratory involvement[d]: Cough Sinusitis	Gastrointestinal[e]: Watery diarrhoea (96%) Vomiting (57%) Fever (37%) and/or: Malnutrition[f,g,h,i] Failure to thrive[f,k] Weight loss[f] Impaired cognitive function[j]

[a] Hunter et al. (2004a).
[b] Palmer and Biffin (1990).
[c] Manabe et al. (1998).
[d] Hunter and Nichols (2002).
[e] Khan et al. (2004).
[f] Mølbak et al. (1997).
[g] Sallon et al., (1988).
[h] Sarabia-Arce et al. (1990).
[i] Lima et al. (1992).
[j] Guerrant et al. (1999).
[k] Checkley et al. (1997).

infections that do not result in gastrointestinal symptoms is probably mis-leading since they might be associated with clinical sequelae. Other effects of cryptosporidiosis have also been estimated. In a pilot study in Brazil, reduced physical fitness and impaired cognitive function correlated with early childhood *Cryptosporidium* infection when measured 4–7 years later (Guerrant et al., 1999).

Evidence for resistance to infection has been found in the low incidence of cryptosporidiosis in adults in developing countries, and in rural areas, who probably experience frequent exposure to low numbers of oocysts (Casemore, 1990). Although clinical cryptosporidiosis is rarely reported in the elderly, sero-prevalence rates are high and asymptomatic infection or mild disease may be present. During the Milwaukee outbreak, hospitalisation and emergency room attendance by patients over 64 years of age increased compared with before the outbreak and elderly cryptosporidiosis patients experienced shorter incubation periods (5–6 days compared with 8–9 days) (Naumova et al., 2003). The elderly may be at risk of secondary spread in care homes and other institutions.

The clinical presentation may vary with infecting species. Oocysts are shed for longer and the number of oocysts detected in stools is higher in *C. hominis* infections than *C. parvum* (McLauchlin et al., 1999; Xiao et al., 2001; Bushen et al., 2007; Cama et al., 2008). Although there was no difference between acute clinical details of patients seeking medical assistance with *C. parvum* and *C. hominis* in the UK, (Hunter et al., 2004a), clinical correlates of different *Cryptosporidium* species have been observed elsewhere. Children in a birth cohort in Brazil with *C. hominis* infection had increased faecal lactoferrin and delayed growth compared with those with *C. parvum* (Bushen et al., 2007). In a longitudinal study of children in Lima, Peru, *C. hominis* was associated with diarrhoea, nausea, vomiting and general malaise while *C. parvum*, *C. meleagridis*, *C. canis* and *C. felis* were associated with diarrhoea only (Cama et al., 2008). In a prospective survey of stool submissions from childhood diarrhoea cases (<5 years of age) in Kenya, there was a statistically significant association between *Cryptosporidium*, most of which was *C. hominis*, and vomiting (Gatei et al., 2006) At the subtype level, based on analysis of the GP60 gene, all the *C. hominis* families detected (Ia, Ib, Id and Ie) were associated with diarrhoea while Ib was also associated with nausea, vomiting and general malaise (Cama et al., 2008). *C. hominis* family Ib is the most commonly detected subtype in sporadic cases of cryptosporidiosis identified from laboratory surveillance in the UK (Chalmers et al., 2008), representing the more seriously affected patients. However, it has also been identified in an asymptomatic child in a study of carriage rates among children in daycare centres (Davies et al., in press).

4.2. Experimental human infection

To investigate infectivity and virulence, healthy adult volunteers have been subjects of experimental infection with four *C. parvum* (IOWA, TAMU, UCP and Moredun) and one *C. hominis* (TU502) isolate to date (Table 2). The infectious dose has been measured using both clinical outcomes and microbiological detection of oocysts or antigens in faeces, and can be compared between isolates by calculating the ID_{50} (the number of oocysts required to cause infection in 50% subjects). The ID_{50} varies between *Cryptosporidium* species and isolates when measured clinically or microbiologically (Table 2). The clinical attack rate (proportion of people with symptoms) varies between isolates. For *C. hominis*, there was a dose-response relationship for diarrhoea, but not for *C. parvum* isolates although increased dose of IOWA was linked to increased enteric symptoms.

The calculation of both the pre-patent period (i.e. the time from ingestion to the appearance of oocysts in stools) and ID_{50}

by microbiological criteria are subject to the analytical sensitivity of the methods used. Direct immunofluorescent antibody microscopy has a limit of 10^4 oocysts per gram faeces and so some subjects may have been shedding oocysts below this threshold (Valdez et al., 1997). Incubation period between the ingestion of oocysts and development of symptoms tended to be longer at lower doses.

Susceptibility to re-infection and illness following primary infection has been investigated experimentally by re-challenging volunteers from a previous study (DuPont et al., 1995) with the homologous isolate 1 year later (Okhuysen et al., 1999). After the second inoculum, fewer subjects shed oocysts, and there was a decrease in the severity of disease, although the rates of diarrhoea were similar, onset and duration of illness were similar indicating that protection against disease is not afforded by single prior exposure (Okhuysen et al., 1999). Sero-conversion was absent after primary exposure but occurred in 33% subjects after second exposure, but the serum antibody response did not correlate with evidence for infection. Further studies were also undertaken to investigate infectivity of the *C. parvum* IOWA isolate in a new group of volunteers with pre-existing anti-*Cryptosporidium* serum IgG (Chappell et al., 1999). The ID_{50} was increased by over 20 fold and fewer subjects shed oocysts, indicating that prior exposure may provide protection from illness after exposure to low oocyst numbers. Where pre-infection IgG was high, this did not change but in subjects with lower initial IgG there was evidence for post-challenge increase.

In summary, these experiments have shown that as few as 9 oocysts can cause infection in healthy adults and that isolates differ in infectivity and virulence. The course of disease and immune response in adults has been described, and prior infection confers some reduction in illness in subsequent infections.

5. Clinical features in immune-compromised people

While otherwise healthy, well nourished, immune-competent patients will usually spontaneously recover from cryptosporidiosis, albeit after sometimes lengthy illness, some groups of immune-compromised patients can suffer prolonged, chronic disease, sometimes with devastating effects. Patients with T-cell immune deficiency are at most risk, including those with haematological malignancies (particularly children), primary T-cell deficiencies such as SCID and CD40 ligand deficiency (hyper IgM syndrome), and HIV patients with $CD4^+$ lymphocyte counts of <50/mm^3. Hunter and Nichols (2002) reviewed the published literature and found that that the severe disease seen in bone marrow transplant patients usually appeared to depend on and reflect the underlying diagnosis for which the transplant was performed. In solid organ recipients and cancer patients (other than those with haematological malignancies) cryptosporidiosis, whilst certainly described, does not appear to be as problematic as it is in haematological malignancies (Hunter and Nichols, 2002).

These groups of high-risk patients frequently experience chronic or intractable disease. In such patients, in addition to typical but severe intestinal disease, atypical and extra-intestinal disease can also develop. The whole gastrointestinal tract including the gall bladder, pancreatic duct and even the bronchial tree can be affected. Ultrasonic examination of AIDS patients with biliary cryptosporidiosis has revealed a generalised dilation of the bile duct and gall bladder (Chen et al., 2002), an increase in the presence of pericholecystic fluid, and a thickening of the epithelium (Dolmatch et al., 1987; Teixidor et al., 1991; Chen et al., 2002), and involvement of the pancreato-biliary system may lead to pancreatitis, cholecystitis and sclerosing cholangitis (Davis et al., 1987; Dowsett et al., 1988; Cello, 1989; Chen and LaRusso, 2002; McLauchlin et al., 2003). Papillitis, terminal bile duct stenosis and rarely, subsequent biliary cirrhosis have all been described. Infection of the biliary tree also represents a reservoir from which intestinal cryptosporidiosis may relapse and allows the organisms to avoid luminal anti-parasitic agents such as paromomycin; drugs with biliary excretion (such as nitazoxanide) should be used (Baishanbo et al., 2006). Tracheo-bronchial involvement is uncommon but can occur and sinusitis has also been described (Dunand et al., 1997). Cryptosporidiosis in advanced HIV may occasionally be associated with pneumatosis cystoides intestinalis in which cysts containing gas occur in the gut wall and may rupture, resulting in pneumoretroperitoneum and pneumomediastinum (see Hunter and Nichols, 2002).

There is a wealth of information about severe diarrhoeal cryptosporidiosis in HIV patients (see Hunter and Nichols, 2002) and the effect of reduced $CD4^+$ lymphocyte count has been well documented. With $CD4^+$ counts >180/mm^3 patients were more likely to have transient or self-limiting disease (Flanigan et al., 1992). In a study in London, UK, transient infection was also more common in patients with higher $CD4^+$ counts, and fulminant disease (marked by the passing of >2 L stool per day) only occurred in patients with $CD4^+$ counts <50/mm^3 (Blanshard et al., 1992). A study in Atlanta, USA, found that lower $CD4^+$ counts were predictive of chronic diarrhoea (Navin et al., 1999). Another study in the USA identified four distinct clinical syndromes of cryptosporidiosis in AIDS patients with $CD4^+$ counts <200/mm^3: transient diarrhoea, relapsing illness, chronic diarrhoea, and cholera-like illness (Manabe et al., 1998). In this patient group, chronic diarrhoea and cholera-like illness with severe weight loss predominated, but there was no relationship between intensity of infection and clinical syndrome or nature and intensity of inflammatory response, although there was a trend towards acute inflammation in patients with cholera-like illness. Although cryptosporidiosis significantly influenced survival rates, this was not linked to individual cryptosporidiosis clinical syndromes. Candidal oesophagitis was more common in this study in AIDS patients with cryptosporidiosis and may mark mucosal changes or altered intestinal immunity. Likewise cytomegalovirus was a common co-infection, often at the same site, and one may predispose to infection with the other (Manabe et al., 1998).

In the severely affected immune-compromised patient, drug treatment is of uncertain and probably limited efficacy, and the infection responds best to an improvement in the host's immune status. Conversely, relapses can occur following deterioration in immune function, if infection has been suppressed but not completely cleared, as may happen. The introduction of highly active antiretroviral treatment (HAART) for immune reconstitution has dramatically reduced the incidence of cryptosporidiosis in HIV/AIDS patients. In fact, protease inhibitors have been found to inhibit parasite development and reduce invasion of host cells by *C. parvum* sporozoites *in vitro*, an effect enhanced by the addition of paromomycin (Hommer et al., 2003), and use of HAART has been found to aid parasite clearance even when patients responded poorly in terms of $CD4^+$ count to HAART (Maggi et al., 2001). It is thus considered that the use of protease inhibitors in the HAART regimen may be specifically beneficial, over and above the effect of immune reconstitution. However, HAART is still not widely available in many non-industrialised countries where cryptosporidiosis remains an important disease. Drug treatment is discussed in detail in elsewhere in this issue (Rossignol, 2009).

The distribution of *Cryptosporidium* species/genotypes in immune-compromised and immune-competent patients does not appear to differ greatly where both populations have been studied (Cama et al., 2007, 2008). However, there may be some differences in clinical manifestations, although these differ between the two host populations anyway.

Table 2
Cryptosporidium infection and clinical features in experimentally infected volunteers without evidence of pre-existing *Cryptosporidium* antibodies.

	Study reference					
	DuPont et al. (1995)	Okhuysen et al. (1999)	Okhuysen et al. (1999)	Okhuysen et al. (1999)	Okhuysen et al. (2002)	Chappell et al. (2006)
Study isolate and source	*C. parvum* IOWA Calf isolate, propagated in calves in Arizona	*C. parvum* IOWA Calf isolate, propagated in calves in Arizona	*C. parvum* TAMU Foal isolate, propagated *via* a human in calves in Arizona	*C. parvum* UCP Calf isolate, propagated in calves in Portland	*C. parvum* Moredun Deer isolate propagated in sheep in Scotland then calves in Arizona	*C. hominis* TU502 Human isolate, propogated in piglets
Infectious dose ID50 (clinical[a]; microbiological[b])	132 (clinical definition with microbiological confirmation)	87; 74.5	9; 125	1042; 2788	300; 375	10; 83
Clinical attack rate (proportion of subjects developing symptoms)	⩾1000 oocysts: 100% ⩾300 oocysts: 88% 30 oocysts: 20%	52%	86%	59%	69%	62% 500 oocysts: 75% 100 oocysts: 71% 30 oocysts: 60% 10 oocysts: 40%
Asymptomatic shedding of oocysts	Yes	Yes	Yes	Yes	No	No
Prepatent period (microbiological) mean (days)	⩾1000 oocysts: 6 300–500 oocysts: 9 30–100 oocysts: 10	7.7	4	7	5 (4–9)	
Incubation period (clinical) mean; median (days)	9; 6.5	9; 7	5; 5	11; 6	Median only 3	5.4; 4
Patent period (duration of shedding) (days)	⩾1000 oocysts: 12 300–500 oocysts: 10 30–100 oocysts 2	8.4	3.4	3.3	5 (1–11)	
Duration of diarrhoea (h)	74	64.2 (6–223)	94.5 (6–195)	81.6 (6–193)	Median 169 (6–360)	1373 (49–518)
Severity (mean number of unformed stools)	12.7	7	9	8	19	
Relapse rate (%)		18	58	46		

[a] Clinical = symptoms reported.
[b] Microbiological = oocysts detected in stools.

6. Diagnosis

The symptoms of clinical cryptosporidiosis are not unique, and the routine differential diagnosis is usually of other causes of infectious gastroenteritis such as *Giardia*, *Cyclospora*, *Isospora*, microsporidia, norovirus, rotavirus, *Campylobacter*, *Salmonella*, *Shigella* and enterohaemorrhagic *Escherichia coli* such as *E. coli* O157. Confirmation of the diagnosis is helpful to the attending clinician, so that patients or their carers can be informed that the symptoms may persist for longer than those from other common causes of acute gastroenteritis, such as viral illness. In diarrhoeal cryptosporidiosis, diagnosis is generally undertaken by testing stools for the presence of oocysts by tinctorial (acid-fast), fluorescent (auramine phenol) or immunofluorescent stains and microscopic examination or for the presence of oocyst wall antigens by enzyme immunoassays and immunochromatological tests. Analytical sensitivity in excess of 10^4 oocysts per gram maybe improved by immunofluorescent stains (Anusz et al., 1990; Weber et al., 1991; Casemore, 1993; Arrowood, 1997; Valdez et al., 1997). Microbiological examination of a single sample from a patient cannot exclude *Cryptosporidium* since oocysts may fall below detectable numbers even during symptomatic episodes (Jokipii and Jokipii, 1986). ELISA tests in a multi-well format facilitate testing of large numbers of samples which may strain resources for microscopical examination. A triple faeces test approach (based on 3 consecutive daily samples with fixation and concentration prior to permanent staining) significantly increases detection rates (van Gool et al., 2003). Clinical laboratories have varying criteria for selecting stools for testing (Chalmers et al., 2002), for instance some perform the test only on stools from young children and on immunocompromised patients. It must be noted that testing for *Cryptosporidium* is not part of an "ova, cysts and parasites" request, since the oocysts are not readily observed in wet preparation microscopy. In tissue sections, endogenous stages can be visualised by normal H&E staining.

Improved analytical sensitivity of oocyst detection can be provided by immunomagnetic staining which can record as few as 2 oocysts per gram stool, and may be appropriate in managing severely affected patients (Robinson et al., 2008b). PCR is applicable for the testing of samples where infection with shedding of few oocysts is suspected (McLauchlin et al., 2003). PCR can be applied to stools, tissue and other specimen types (e.g. bile) taken during endoscopic examination. PCR can offer improved sensitivity of detection, and underpins typing methods to differentiate *Cryptosporidium* species and genotypes.

7. Prevention and management

Since treatment modalities (discussed by Rossignol, this volume) (Rossignol, 2009) for *Cryptosporidium* are limited, prevention and risk reduction are the most important interventions. Cryptosporidiosis is highly infectious person-to-person, as large numbers of oocysts are excreted and the infectious dose is low, so scrupulous personal hygiene is required. Guidelines for the prevention of person-to-person spread should be followed (for example, see Anon, 2004). These include frequent handwashing, particularly

after using or cleaning the toilet, changing diapers, caring for a person with diarrhoea, and proper disposal of excreta and washing of soiled materials such as clothing or bedding.

People with cryptosporidiosis should be excluded from the work place, school or other institutional settings until 48 h after the last diarrhoeal episode, particularly food handlers and staff of healthcare facilities (Anon, 2004). Since swimming pool treatment may not adequately treat or remove oocysts, and oocysts can be shed in stools after they return to normal, exclusion from swimming is desirable for 2 weeks, but such exclusion is difficult to enforce. General precautions against infection with *Cryptosporidium* include handwashing prior to eating or preparing food and after contact with animals, adequate treatment of non-potable water, and thorough washing of fruit and vegetables prior to consumption. Suitable handwashing facilities should be provided and used at open farms.

Cryptosporidiosis in the immune-competent, though unpleasant and debilitating, is self-limiting. Rehydration salts may be required. In the high-risk immune-suppressed groups outlined earlier, infection can be severe and is difficult to manage. The aim of treatment is to improve symptoms, but complete clearance of the parasite is unlikely unless the underlying immune deficiency can be corrected. For some groups of patients, permanent advice such as that provided by the Department of Health in England (CMO's update 23, 1999), to boil all water for drinking may be warranted (Hunter and Nichols, 2002). Alternative measures include the use of appropriate point-of-use filters for the specific removal of *Cryptosporidium* oocysts (CDC/EPA, 1999). However, where good quality mains drinking water is available the risk is much reduced. Unfortunately, for much of the world's population, including those most vulnerable to cryptosporidiosis, drinking water quality remains poor.

8. Conclusion

The WHO's recognition of *Cryptosporidium* as a globally important pathogen has brought into sharp relief the impact of this little-understood parasite. Its implications for child health in terms of nutrition and development in the developing world, the continuing epidemic of HIV infection and the increasing numbers of immunocompromised individuals in the developed world, coupled with the limited treatment options, mean that the rise to prominence of *Cryptosporidium* can be expected to continue still further in the future.

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