8.8.5 Cryptosporidium and cryptosporidiosis 1424

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section 8 Infectious diseases 1424 Robert-Gagneux S, Balas F (2016). Molecular diagnosis of toxo- plasmosis in immunocompromised hosts. Curr Opin Infect Dis, 29, 330–9. Saeij JP, et al. (2006). Polymorphic secreted kinases are key virulence factors in toxoplasmosis. Science, 314, 1780–3. Schmidt DR, et al. (2006). Treatment of infants with congenital toxo- plasmosis: tolerability and plasma concentrations of sulfadiazine and pyrimethamine. Eur J Pediatr, 165, 19–25. Syrocot (2007). Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet, 369, 115–22. Thalib L, et al. (2005). Prediction of congenital toxoplasmosis by polymerase chain reaction analysis of amniotic fluid. BJOG, 112, 567–74. Thiebaut R, et al. (2007). Effectiveness of prenatal treatment for con- genital toxoplasmosis: a meta-analysis of individual patients' data. Lancet, 369, 115–22. 8.8.5 Cryptosporidium and cryptosporidiosis Simone M. Cacciò ESSENTIALS Parasites within the genus Cryptosporidium infect the mucosal epi- thelia of a variety of vertebrate hosts, including humans, affecting the health, survival, and economic development of millions of people and animals worldwide. Human infection is mainly caused by two species, Cryptosporidium parvum and C. hominis. The former species is also prevalent in young livestock and has a demonstrated zoonotic potential, whereas the latter species is essentially a human parasite. Direct and indirect (through contaminated water and food) transmis- sion routes exist for both species. Clinical features—infection involves either children or adults, but is a major cause of diarrhoea in children under 5 years old in both de- veloped and developing countries. A recent study demonstrated that Cryptosporidium is a significant cause of moderate to severe diarrhoea and associated mortality among very young children in Sub-Saharan and South Asia. Patients may be asymptomatic or experience acute or chronic diarrhoea, depending on their age and immune status. In the immunocompetent, infection usually results in acute self-limiting diarrhoea, whereas in immunocompromised patients (e.g. AIDS) and those with concurrent infections such as measles or chickenpox, clinical symptoms are more severe and persistent and may become chronic, leading to electrolyte imbalance, wasting, and even death. Since 2004, Cryptosporidium has been included in the World Health Organization's 'Neglected Diseases Initiative', in recognition of the importance of this infection in developing countries. Diagnosis and treatment—diagnosis is usually based on detection of oocysts in stool, often by use

of direct fluorescent-antibody tests. Detection of sol- uble Cryptosporidium antigens in faecal samples by enzyme-linked immunosorbent assay or by an immuno-chromatographic lateral flow assay is useful for the screening of large numbers of specimens. Molecular methods allow reliable identification of species and geno- types, and are therefore of paramount importance for environmental or epidemiological research purposes. Treatment of immunocompe- tent patients, when necessary, is based on nitazoxanide, a thiazolide drug with broad antiparasitic activities. Nitazoxanide is the only US Food and Drug Administration-approved drug for the treatment of cryptosporidiosis, but it is not licensed in Europe. Management of patients who are immunocompromised is difficult: aside from sup- portive care, highly active antiretroviral therapy is effective, both by immune reconstitution (in patients with HIV/AIDS) and by direct in- hibition of parasite proteases. There is little evidence for efficacy of nitazoxanide in immunocompromised individuals. Prevention—primary control is by limiting the opportunity for faecal–oral transmission, both direct and indirect, with maintenance of drinking-water quality and general hygiene (especially in hos- pitals, wards, and so on) essential for the prevention of the infection. Secondary control, when water supplies are contaminated, can be achieved by boiling or filtering water before drinking. Introduction The cryptosporidia are obligate intracellular parasites of many species from all vertebrate classes. In humans, infection is caused mainly by two species, Cryptosporidium parvum, which is also prevalent in young livestock and can be transmitted zoonotically, and C. hominis, which is essentially a human parasite. First de- scribed in laboratory mice by Tyzzer in 1912, Cryptosporidium was recognized as a cause of human infection in 1976. In the 1980s it emerged worldwide as a common cause of severe or life-threatening infection in severely immunocompromised patients, especially those with AIDS, and of acute, self-limiting gastroenteritis in other- wise healthy subjects, especially children. Biology Cryptosporidium species have been traditionally considered as members of the coccidia (phylum Apicomplexa), but recent in- vestigations have revealed a closer phylogenetic affinity with the Gregarinae, which are parasites of invertebrates. The oocyst, con- taining four sporozoites, is an environmentally robust transmissible stage and is fully sporulated and infective upon excretion with the host faeces. Cryptosporidia are monoxenous; this is, they complete their lifecycle in a single host (Fig. 8.8.5.1). C. parvum is not tissue specific but shows a predilection for the lower ileum during the primary stages of infection. Following ingestion of oocysts, the motile sporozoites are released, through a suture in the oocyst wall, in the lumen of the small bowel. Acknowledgement: The author and editors acknowledge the inclusion of material from the chapter by Dr D. P. Casemore in the 4th edition of this textbook. Plates for this chapter were kindly provided from photographs by A. Curry and D. P. Casemore.

 They quickly attach superficially to cells, rounding up to form fixed trophozoites (meronts). The initial site of infection is the brush border of enterocytes in the small bowel, but the parasite is able to infect other epithelial and parenchymal cells. The complex life cycle includes both asexual and sexual stages of replication (Figs. 8.8.5.1 and 8.8.5.2). The endogenous (tissue) stages develop within a parasitophorous vacuole, the outer layer of which is derived from the host cell’s outer mem- branes, in a unique intracellular but extracytoplasmic location. Molecular biology The sequences of the genome of both C. parvum and C. hominis have been described and have revealed many peculiar characteristics. The two genomes are remarkably similar, displaying 95–97% DNA sequence identity and c.30% GC content, and are organized in eight chromosomes that are apparently completely collinear. The compacted nature of the genome, which is comprised of only c.9.2 Mbp, is reflected in its high coding capacity

(about 4000 genes that account for two-thirds of the genome). The highly streamlined metabolic pathways imply that Cryptosporidium heavily relies on scavenging nutrients from the host, salvage rather than de novo biosynthesis, and glycolysis or substrate-level phosphorylation for energy production. The increasing availability of whole Cryptosporidium genome sequences and functional genomics and metabolomics data will assist in the identification of new drug targets. Moreover, a recent study showed the possibility of genetically engineering the parasite, Sporozoite Ingested Exit host Thick-walled oocyst (sporulated) Thin-walled (sporulated) Microgamont Type II meront Merozoite Macrogamont Zygote Auto infection Merozoite Type I meront Trophozoite Fig. 8.8.5.1 Diagrammatic representation of the lifecycle of C. parvum. Following ingestion of oocysts, the motile sporozoites are released, attach to cells, and develop into fixed trophozoites (uninucleate meronts) in an intracellular but extracytoplasmic location. These undergo schizogony (asexual multiple budding), the first-stage meronts producing eight merozoites, some of which recycle to form further type I meronts. Type II meronts produce four merozoites, which form gamonts (sexual stages) that mature as either macrogametes or as microgamonts containing 16 motile microgametes. Most of the zygotes formed after fertilization develop into thick-walled, environmentally resistant, transmissible oocysts, which then sporulate, usually by the time they are excreted. Some have only a thin unit membrane, which ruptures to release the sporozoites in situ to produce an autoinfective cycle. Adapted from a drawing by Kip Carter, University of Georgia, and shown by courtesy of W I Current and CRC Press, Inc., Boca Raton, FL.

 opening avenues for investigating the basis of drug susceptibility by gene knockout experiments. Protocols based on nucleic acid amplification of specific markers are available to differentiate Cryptosporidium species and genotypes in both clinical and environmental samples. Epidemiology C. parvum occurs worldwide and is common in humans and in young livestock animals, especially lambs and calves, and has been reported in goats, horses, pigs, and farmed deer as well as in mammalian wild- life. Prevalence in humans varies both geographically and temporally. Because of the diversity of host species that can infect humans, the epidemiology of the infection is complex and involves both direct and indirect routes of transmission from animals to man (zoonotic trans- mission) and from person-to-person (urban cycle). A recent study has demonstrated that respiratory involvement commonly occurs in HIV-seronegative children with intestinal cryptosporidiosis and cough, sug- gesting the potential for respiratory transmission of the infection. Zoonotic transmission Transmission from livestock is common, particularly in children, including those from urban homes and schools visiting educational farms and rural activity centres. Companion animals have long been considered potential sources for human cryptosporidiosis. However, they appear to be most commonly infected with host-specific and nonzoonotic Cryptosporidium species; they are, therefore, not con-sidered important reservoirs of infection. Cryptosporidiosis is rarely seen in adults in rural areas, presumably as a result of frequent ex- posure and the development of immunity. Zoonotic transmission of other species, such as C. meleagridis, a parasite of turkeys and other birds, has been recently demonstrated. Human-to-human transmission Cases of human-to-human transmission have been reported be- tween family members, sexual partners, children in daycare centres, and hospital patients and staff. Outbreaks in daycare centres have been reported in the United Kingdom and the United States of America, mainly as a result of direct (person-to-person) faecal–oral transmission, although the infection may be introduced in the first instance through zoonotic contact. Affected adults may acquire in- fection from young children in the home or occupationally. Infection may be transmitted sexually where this involves faecal exposure. In

developing countries, the high prevalence of C. hominis and of anthroponotic subtypes of C. parvum, particularly in children, has been interpreted as an indication of the importance of person-to- person transmission. Cryptosporidium is a cause of traveller's diar- rhoea, although apparently not as frequently as Giardia. A new species, Cryptosporidium viatorum, has been identified as a cause of infection among travellers, and appears to be restricted to humans. Waterborne transmission In the United Kingdom, the United States of America, and elsewhere, there have been numerous well-documented outbreaks resulting from contamination of public drinking-water supplies. Outbreaks, which can be massive, have been associated with C. hominis, which indicates contamination of the supply by human sewage, or with C. parvum, which suggests an animal source of contamination. Recently, another species, C. cuniculus, which infects rabbits, has been associated with a waterborne outbreak in the United Kingdom. Isolates from endemic (sporadic) cases, some of which will be water- borne, fall into both categories. Oocysts have been demonstrated widely in both raw and treated water and legislation has been intro- duced in the United Kingdom and the United States of America in an attempt to limit the latter. Cryptosporidium is also one of the most commonly recognized causes of recreational waterborne disease. Most outbreaks are the re- sult of faecal accident or cross-connection in swimming pools. Faecal contamination coupled with oocyst resistance to chlorine, low in- fectious dose, and high bather densities facilitate transmission. The potential for intentional contamination of water supplies has led to in- clusion of Cryptosporidium as a Category B pathogen for biodefence. Food-borne transmission Food-borne transmission is probably underestimated, because the long incubation period (3–7 days or more) makes the relationship between cryptosporidiosis and a possibly contaminated food item difficult to establish. Cryptosporidiosis has been attributed to in- gestion of contaminated apple juice, chicken salad, milk, and food items prepared by sick food handlers. A large outbreak occurred Fig. 8.8.5.2 Electron micrograph of a transverse section of small bowel of a mouse infected with C. parvum. The section shows numerous developmental stages: uninucleate meronts (trophozoites); type I meronts (schizonts) containing merozoites in which may be seen the darker granules of the apical complex organelles; the degenerate remains of a schizont and a free-swimming merozoite within the
lumen; and macrogamonts showing dark wall-forming granules and electron-lucent amylopectin (polysaccharide) food-storage granules.
The parasitophorous vacuole can be clearly seen surrounding the parasite stages. Some of the intracellular stages appear to be free within the lumen because of the plane of sectioning.

 in 2012 across England and Scotland, and was strongly associ- ated with the consumption of pre-cut mixed salad leaves sold by a single retailer. Typing revealed the outbreak strain to be C. parvum. Methods for the detection of oocysts in fruits and vegetables have been developed and validated. Nosocomial transmission Transmission has been reported between healthcare staff and pa- tients and between patients, particularly the immunocomprom- ised. Large numbers of oocysts may be present in patients' stools and in vomit; transmission via fomites occurs, although this route is limited by the susceptibility of oocysts to desiccation. Poor hand- washing practice has been identified as an important risk factor. In an outbreak with high mortality in a ward of immunocompromised patients in Denmark, transmission was probably by patients' hands via a ward ice-making machine. Demography Age and sex distribution In the United Kingdom, approximately two-thirds of Cryptosporidium- positive samples are from children between 1 and 10 years of age, with a secondary peak in adults under 45 years; the infection is uncommon in infants less than 1 year old and in older people. Distribution appears

to be the same in both sexes. Other EU countries reported a similar age and sex distribution of cryptosporidiosis cases. A relative increase in adult cases is often seen in waterborne outbreaks. In developing coun- tries, infection is common in infants less than 1 year old and asymp- tomatic infection is common in older subjects. Temporal distribution Data from the European Centre for Disease Control, and especially the United Kingdom, indicate that cryptosporidiosis exhibits a strong sea- sonality in Europe, with low endemic levels followed by pronounced seasonal increases, particularly during late spring and late summer to early autumn. Springtime cases are more often due to C. parvum, and are likely the result of an increased exposure to oocysts shed by young animals, as this coincides with the calving/lambing season. In recent years, the spring peak has reduced, largely due to improved drinking- water supplies. On the other hand, the late summer-early autumn peak is mainly due to C. hominis, and has not reduced in recent years; it is likely linked to increased travel and exposure to recreational water at this time of year. In the United States of America, the peak onset of cryptosporidiosis occurs annually from early summer to early au- tumn. This might reflect the increased use of communal swimming venues, particularly by susceptible hosts like young children. Frequency of occurrence Laboratory rates of detection in immunocompetent subjects average about 2% in developed countries (range <1–5%) and about 8% in developing countries (range 2–30%), and Cryptosporidium is about fourth in the list of pathogens detected in stools submitted to the laboratory. In the United Kingdom, about 5000 to 6000 confirmed cases are reported annually; it is generally somewhat less frequent than giardiasis. Among young children in the United Kingdom, cryptosporidiosis is more common than salmonellosis and detec- tion rates may exceed 20% during peak periods. Cryptosporidiosis is one of the most common causes of diarrhoea in patients with AIDS and in some studies prevalence has exceeded 50%. The infection rate in patients with AIDS in industrialized countries has been falling in recent years, due to infection control advice and the use of antiretroviral therapy. Infection rates are not generally increased for most other immunocompromised groups. Clinical aspects Pathology Histopathology There is mucosal involvement of the small bowel, other parts of the gastrointestinal tract, and sometimes beyond. Moderate to severe abnormalities of villous architecture occur, with stunting and fusion of villi and lengthening of crypts. There may be evidence of mild in-flammation, with some cellular infiltration into the lamina propria. The endogenous stages of the parasite in the luminal surface are generally inconspicuous and appear as small (2–8 μm) bodies, apparently superficially attached to the brush border, unevenly distributed over the apical cells, and within the crypts of the villi (Figs. 8.8.5.1 and 8.8.5.2). Peaking and apoptosis of infected cells have been reported. There is usually little intracellular change at the ultrastructural level beyond the attachment zone of the parasite. Rectal biopsy might reveal mild nonspecific proctitis. Extensive and chronic involvement of the bile duct and gallbladder can occur in some patients with AIDS. Immunological response T cells play a crucial role in the elimination of cryptosporidial in-fections. In humans, T-cell immunosuppression caused by other infection or chemotherapy increases susceptibility to infection. Moreover, severe cryptosporidiosis has been reported in individ- uals with mutations affecting the costimulatory CD40 or CD40L re- quired for T-cell activation. In particular, CD4+ T cells are necessary to control infection and achieve sterile immunity in adults, whereas the role of CD8+ T cells is not fully established. In agreement with these findings, low levels of CD4+ T cells counts (<100 cells/μl) in- dicate a poor prognosis if infection occurs. The most important cytokine in resistance to Cryptosporidium is interferon-γ and the principal sources are CD4+ T cells. Therefore, it appears that a Th1 immune response is involved in the clearance of the infection. IL12, produced by dendritic cells and macrophages upon exposure to antigens, plays an important role in the activation of interferon-γ production by T cells.

During infection, antigen-specific antibodies can be detected in serum, including IgG, IgA, and IgM. If the in- fection is brought under control, the IgM titre declines very soon, whereas IgG may persist for several months. Experimental studies in the murine model and data from studies involving AIDS patients have shown that, although antibodies may contribute to the pro- tective immune response against the parasite, they are not normally essential for establishing host resistance. Possible pathogenic mechanisms The watery diarrhoea is characteristic of noninflammatory infection of the small bowel, especially that associated with toxin-producing

section 8 Infectious diseases 1428 organisms and enteric viruses. Several mechanisms have been sug- gested to explain the symptoms: reduction in absorptive capacity, particularly for water and electrolytes; increase in secretory capacity from crypt hypertrophy; osmotic effects from loss of brush-border enzymes (e.g. disaccharidases) resulting in malabsorption of sugars, increased osmolality of chyme, and subsequent microbial fermenta- tion of sugars in the colon (which may account for the characteristic offensive smell); and toxic activity. Clinical presentation in otherwise healthy (immunocompetent) people Cryptosporidiosis in the immunocompetent person is a self-limiting, acute gastroenteritis with a variety of presenting symptoms. In cases where the time of exposure has been known, the incuba- tion period was about 5 to 7 days (range probably 2–14 days; wider limits have been suggested but are unlikely). There may be a pro- drome of 1 day to a few days, with malaise, abdominal pain, nausea, and loss of appetite. Gastrointestinal symptoms start suddenly, the stools being described as watery, greenish with mucus in some cases, without blood or pus, and very offensive. Patients may open their bowels more than 20 times a day but more usually 3 to 6 times. Other symptoms include colicky, abdominal pain, especially after meals, anorexia, nausea, and vomiting, abdominal distension, and marked weight loss. Influenza-like systemic effects, including malaise, head- ache, myalgias, and fever, commonly occur. Gastrointestinal symp- toms usually last about 7 to 14 days (average 12 days), but weakness, lethargy, mild abdominal pain, and intermittent loose bowels some- times persist for up to a further month. There is no evidence of transplacental transmission but infec- tion during late pregnancy may cause metabolic disturbances in the mother, leading to the infant’s failure to thrive. Failure to thrive has also been observed in older infants and children and may be associated with persistent infection and enteropathy, especially in developing countries. Reported sequelae include pancreatitis (associated with se- vere abdominal pain), toxic megacolon, and reactive arthritis. In immunocompetent patients, deaths are rarely attributable to cryptosporidiosis. Recent studied in the United Kingdom and Sweden have fur- ther demonstrated that the impact of cryptosporidiosis on public health extends beyond that of acute diarrhoeal illness. Notably, an increased risk of extra-intestinal sequelae (joint pain, eye pains, recurrent headache, and fatigue) is associated with infection with C. hominis but not with C. parvum. A follow-up study after two large waterborne outbreaks in Sweden found that outbreak cases were more likely to report diarrhoea, watery diarrhoea, abdominal pain, joint pain, fatigue, and nausea compared to noncases. Therefore, these studies showed a significant burden of illness even after out- breaks are over. Clinical presentation in immunocompromised patients Susceptibility to cryptosporidiosis and the severity of the disease is increased in patients who are immunocompromised because of AIDS, hypo- or agammaglobulinaemia, severe combined immuno- deficiency, leukaemia, malignant disease, and bullous pemphigoid. Disease susceptibility and severity are also increased during im-munosuppressive treatment with cyclophosphamide and cortico- steroids, as in patients undergoing bone marrow transplantation, and in children immunosuppressed by measles and chickenpox, especially where there is associated malnutrition. Infection in pa- tients with leukaemia

may be unusually severe and has sometimes proved fatal, particularly when associated with aplastic crisis, and may then require modification of chemotherapy to control the infection. Symptoms of cryptosporidiosis are generally similar to those in immunocompetent patients but often develop insidiously. In those with late-stage AIDS and very low CD4 cell counts, or in some other profound deficiency states, diarrhoea may be frequent, profuse, and watery, like cholera. Patients may open their bowels frequently, passing up to 20 litres of infected fluid stool per day; persistent nausea and vomiting is usually associated with severe diarrhoea and suggest a poor prognosis. Associated symptoms include colicky, abdom- inal pain often associated with meals, severe weight loss, weakness, malaise, anorexia, and low-grade fever. Infection in immunocom- promised patients can spread to the pharynx, oesophagus, stomach, duodenum, jejunum, ileum, appendix, colon, rectum, gallbladder, bile duct, pancreatic duct, and the bronchial tree. Cryptosporidial cholecystitis (presenting with severe right upper quadrant ab- dominal pain), sclerosing cholangitis, pancreatitis, hepatitis, and respiratory-tract symptoms may occur, with or without diarrhoea. The clinical picture might include other features of HIV infection and there is often coinfection with other pathogens such as cyto- megalovirus, Pneumocystis jiroveci, and Toxoplasma gondii. Patients with less severe impairment of immunity can experience resolution or a more chronic course, with less profuse diarrhoea, sometimes with remission and then recurrence, possibly associ- ated with biliary tract involvement. Except in those patients whose immune suppression can be relieved by stopping immunosuppres- sant drugs, or, in the case of HIV, intensifying antiretroviral therapy, severe symptoms may persist until the patient dies. This is either because of dehydration, acid–base or electrolyte disturbances and cachexia, from some other opportunistic infection or malignant dis- ease, or a combination of these. Recently, a role of C. parvum infection in the development of cancer in the digestive tract has been suggested. A zoonotic C. par vum isolate, that caused a fulminant cryptosporidiosis in a trans- planted patient, was able to induce invasive gastrointestinal and biliary adenocarcinoma in severe combined immune deficiency (SCID) mice. The hypothesis that cryptosporidiosis increases the risk for some types of gastrointestinal cancer is of interest, even though experimental evidence for this correlation is limited to animal models with immunodeficiency. Laboratory investigations In early acute cases, the stools are usually watery, greenish, some- times with mucus but without blood or pus. Peripheral leucocytosis and eosinophilia are found rarely. Serum electrolyte abnormalities will develop in patients who become severely dehydrated. In im- munocompromised patients with cryptosporidial cholecystitis, serum alkaline phosphatase and γ-glutamyl transpeptidase levels increase, while aminotransferase and bilirubin levels may remain normal. In patients with AIDS, cytomegalovirus and Cystoisospora belli are commonly associated with Cryptosporidium; in immunocompetent

 patients, mixed infection with Campylobacter, Giardia, and Cyclospora species may be found. In the bowel mucosa, there is histological evidence of entero- cyte damage, villous blunting, and inflammatory-cell infiltration of the lamina propria; cell peaking and apoptosis have been reported. Histopathological appearances of the affected biliary tract resemble primary sclerosing cholangitis. Radiographic abnormalities in- clude dilatation of the small bowel, mucosal thickening, prominent mucosal folds, and abnormal motility. In the biliary system, ab- normalities include dilated distal biliary ducts, stenosis with an ir- regular lumen, and other changes reminiscent of primary sclerosing cholangitis. Differential diagnosis The absence of blood, pus cells, or Charcot–Leyden crystals may distinguish cryptosporidiosis from some acute bacterial diarrhoeas, and that associated with amoebiasis and cystoisosporiasis. In im-

munocompetent patients, the symptoms of cryptosporidiosis re- semble those of giardiasis or cyclosporiasis. Intense abdominal pain and cramps are generally more common in cryptosporidiosis, but bloating and weakness less common. In immunocompromised pa- tients, especially in those with AIDS, cystoisosporiasis is clinically indistinguishable, but can be diagnosed by finding the organisms in the stool, where Charcot–Leyden crystals may also be found. This infection responds to treatment with co-trimoxazole, as does cyclosporiasis. Treatment of cryptosporidiosis Several groups may benefit from an effective treatment, particularly patients with HIV/AIDS, transplant recipients, patients undergoing cancer chemotherapy, those with severe malnutrition, and older people. However, existing therapeutics for other apicomplexan dis- eases are largely ineffective against Cryptosporidium infection, prob- ably because of the unique intracellular, extracytoplasmic location of cryptosporidia, and limited understanding of the host–parasite interaction. Hundreds of drugs have been tested in the laboratory, but results have suggested that only paromomycin, azithromycin, spiramycin, and albendazole are partially effective. The failure to develop effective therapy for cryptosporidiosis is also related to the limited attempts undertaken by health agencies and the pri- vate sector, mostly because of a perceived limited market for such drugs in developed countries. Recent developments, which include the sequencing of the genomes of C. parvum and C. hominis and the possibility of genetic engineering of the parasite, will help the identification of new molecular targets for drug development. The availability of a substantial number of chemical libraries for drug discovery should also facilitate screening for effective drugs. Today, the therapy of choice is nitazoxanide (2-acetyloloxy-N- (5-nitro-2-thiazolyl) benzamide), a synthetic agent that has a dem- onstrated activity against a broad range of parasites as well as some bacteria. In vitro studies showed inhibition of growth at concen- trations of less than 10 µg/ml, and studies in adults have shown that single doses of up to 4 g are well tolerated without important adverse effects. Nitazoxanide has been approved by the US Food and Drug Administration (FDA) for the treatment of cryptospor- idiosis in immunocompetent patients in the United States. There is little evidence for efficacy of nitazoxanide in immunocompromised individuals. Immunocompromised patients with persistent severe diarrhoea, malabsorption, and other complications may require prolonged palliative treatment. They should avoid excess milk, as lactose in- tolerance may develop. Parenteral feeding and fluid, electrolyte, and nutrient replacement may be needed. Antiperistaltic agents such as loperamide, diphenoxylate, or opiates may increase abdominal pain and bloating. Antiemetics may be needed for symptomatic re- lief. Temporary relief of biliary obstruction has been achieved by endoscopic papillotomy and of cholecystitis by cholecystectomy. Diarrhoea and vomiting, however, may prove intractable. Antiretroviral therapy (ART) is the treatment of choice for cryptosporidiosis in immunocompromised patients with HIV ART is effective against cryptosporidiosis and acts both by immune re- constitution and direct inhibition of parasite proteases. Laboratory detection and diagnosis The characteristic endogenous stages (Figs. 8.8.5.1 and 8.8.5.2) may be found in histological sections, using light and electron mi- croscopy, but diagnosis is usually by detection of oocysts in stools. Oocysts have also been found in vomit and sputum in some cases, especially those associated with AIDS. The oocysts of C. parvum and C. hominis are spherical or slightly ovoid, about 4–6 µm, and appear refractile in wet faecal preparations with a highly refractile inner body, the cytoplasmic residuum; the four sporozoites within the oocyst may be distinguished with difficulty using special optical systems (Figs. 8.8.5.3–8.8.5.12). Several conventional stains have been adapted for diagnostic purposes, such as the modified Ziehl–Neelsen method and phenol-auramine fluorescent stain. Fig. 8.8.5.3 Modified Giemsa-stained faecal smear showing oocysts of C. parvum, examined with × 100 oil-immersion objective lens. The uniformity of size (4.5–5 µm)

but variability of staining of oocysts can be seen. The eosinophilic nuclei and basophilic bodies of the sporozoites can be clearly seen within the oocysts that have taken up the stain.

section 8 Infectious diseases 1430 Fig. 8.8.5.4 Modified Ziehl-Neelsenstained faecal smear showing oocysts of C. parvum examined with × 100 oil-immersion objective lens. The uniformity of size (4.5–5 µm) but variability of staining of oocysts can be seen. Fig. 8.8.5.5 Modified Ziehl-Neelsen-stained faecal smear showing oocysts of C. parvum. The uniformity of size (4.5–5 µm) is apparent but the oocysts in this preparation show a definite increase in refractility and marked failure to take up the stain (identity confirmed by immunofluorescence and electron microscopy). Fig. 8.8.5.6 Modified Ziehl-Neelsen-stained faecal smear showing oocyst-like bodies (mushroom spores) examined with × 100 oil-immersion objective lens (from specimen submitted to Reference Unit for identification). Fig. 8.8.5.7 Modified Ziehl-Neelsen-stained faecal smear showing oocyst-like bodies (mould spores) examined with × 100 oil-immersion objective lens. The spores are uniform in size but a little smaller (4.0 µm) than oocysts of C. parvum. They are generally more uniform in their acid-fast staining (identity confirmed by mycological culture and electron microscopy). Fig. 8.8.5.8 Phenol-auramine/carbol fuchsin-stained faecal smear showing oocysts of C. parvum, examined with × 720 dry objective lens (screening magnification) on a fluorescence microscope. Fig. 8.8.5.9 Phenol-auramine/carbol fuchsin-stained faecal smear showing oocysts of C. parvum, examined with × 100 oil-immersion objective lens on a fluorescence microscope.

8.8.5 Cryptosporidium and cryptosporidiosis 1431 Direct fluorescent-antibody tests, which detect intact organisms using monoclonal antibodies that label the oocyst wall, are widely used due to their excellent sensitivity and specificity. Detection of Cryptosporidium soluble antigens in faecal samples by an enzyme-linked immunosorbent assay (ELISA) or by an immuno- chromatographic lateral flow assays is very easy to perform and particularly useful for the screening of large numbers of speci- mens, albeit its specificity is limited by cross-reactions with other antigens of parasitic and nonparasitic origin that can generate false positives. None of the abovementioned methods can differen- tiate Cryptosporidium species and genotypes. Therefore, molecular methods, including conventional and real-time polymerase chain reaction, are increasingly used for environmental or epidemiological research purposes. The high specificity and sensitivity of PCR-based methods and the possibility of detecting multiple gastrointestinal pathogens in a single reaction, suggest that these methods may find application in routine diagnostics in the close future. Standardization of approach to screening and to reporting is essential for epidemiological purposes. Ideally, all stool samples from cases of diarrhoea should be screened; restriction, where un- avoidable, should be based on age group (see demography) and not on factors such as stool consistency. Concentration of stool speci- mens is not usually required for diagnosis in acute cases. Fungal spores, yeasts, cysts of Balantidium, sporocysts of Cystoisospora, and oocysts of Cyclospora might be mistaken for oocysts of Cryptosporidium. Infectivity, resistance, and control Infectivity In studies using monkeys and lambs, the infective dose for C. parvum was fewer than 10 oocysts. In human volunteer studies in the United States of America, the minimum infective dose for C. parvum and C. hominis appeared to be similar (ID50 was 132 and 83, respectively). In contrast to C. parvum, however, C. hominis elicted a serum IgG response in most infected persons. A recent study has demonstrated the infectivity of Cryptosporidium melea gridis in healthy adult volunteers, albeit the minimum infective dose was not determined. Resistance and disinfection Oocysts can survive for several months in a cool, moist environ- ment but are highly susceptible to desiccation, prolonged freezing, and moderate heat (pasteurization temperatures). They are re- markably resistant to most disinfectants and antiseptics, including chlorine at concentrations far greater than

those used in water treatment and even to glutaraldehyde under normal use conditions. Some disinfectants may be more effective if used at elevated tem- perature (37°C or higher). Oocysts are sensitive to 10 volume (3%) hydrogen peroxide, to appropriate levels of ozone, and to medium or high-pressure ultraviolet. In hospitals, adequate disinfection of faecal contamination or of endoscopes is difficult. If such instruments have been used for patients with cryptosporidiosis, prolonged immersion in glutaraldehyde at a temperature higher than 37°C, or in hydrogen peroxide, after careful cleaning, may be required to ensure safety. Fig. 8.8.5.10 Fluorescent dye-tagged monoclonal antibody-stained faecal smear showing oocysts of C. parvum, examined with × 50 oil- immersion objective lens (screening magnification) on a fluorescence microscope. The suture or associated surface cleft or fold, through which the sporozoites are released, can be seen. Fig. 8.8.5.11 Modified Ziehl-Neelsen-stained sputum smear from an AIDS patient with respiratory involvement (examined with × 100 oil- immersion objective lens). The C. parvum bodies present may include endogenous (tissue) stages attached to exfoliated cells. For this reason, oocyst wall-specific indirect immunofluorescence may show a poor reaction. There may also be less uniformity of size and differences in the staining appearance of the internal structures. Fig. 8.8.5.12 Toluidine blue-stained semithin section of human rectal biopsy tissue of an AIDS patient with cryptosporidiosis. The apparent pseudo-external location of the parasite can be seen, the true location being intracellular but extracytoplasmic. Plates for this chapter were kindly provided from photographs by A. Curry and
D. P. Casemore.

Revision #1
Created 2026-01-22 16:46:09 UTC by Omar Ayman
Updated 2026-01-22 16:46:09 UTC by Omar Ayman