



DIARRHOEAL DISEASES CONTROL PROGRAMME

CRYPTOSPORIDIOSIS

1. ETIOLOGICAL AGENT

Cryptosporidium is a coccidian sporozoan parasite. It has been isolated in various parts of the world from more than 20 different species of animals, including chickens, turkeys, mice, guinea-pigs, horses, pigs, calves, sheep and rhesus monkeys. Some species, like rats, mice, guinea-pigs, turkeys and chickens, appear to be naturally resistant, while humans, calves, deer, lambs, goats and piglets are susceptible and become ill when infected, particularly at an early age.

Until early 1982, only 8 cases of cryptosporidium infection in man were reported; since then there has been a rapid increase in the number of such reports from almost all parts of the world. Although natural and experimental interspecies transmission has been described, there is sharp disagreement regarding the host-specificity of Cryptosporidium species, of which 11 have so far been identified. It is currently believed that cryptosporidiosis is a zoonosis and that the common cryptosporidia of man, cattle, mice, deer and lambs are not host-specific, though there may be differences between strains.

All the known developmental stages of the parasite (trophozoite, schizont and oocyst) occur in the brush border of the mucosal epithelium of the stomach and intestine; they may also infect the upper respiratory tract of some birds. Although by light microscopy they appear to be extracellular, electron-microscopy demonstrates that all the developmental stages of the parasite are actually intracellular but extracytoplasmic as, while developing within the brush border, they are covered by an outer membrane of host-cell origin. The infective form may be the small spherical oocysts (4-6 µm diameter), which contain four sporozoites and are excreted in faeces.

The organism is extremely resistant to common laboratory disinfectants and antimicrobial agents, including sodium hypochlorite, but is inactivated by 10% formalin, commercial bleach and exposure to temperatures of below 0°C and above 65°C for 30 minutes.

2. EPIDEMIOLOGY

Cryptosporidiosis occurs worldwide and is more common in developing than in developed countries. In the former, children below 2 years of age are at greatest risk and the asymptomatic carrier rate among older children and adults is high. The potential for zoonotic spread is high, but by no means exclusive. Transmission involves all recognized pathways of faecal-oral spread both from infected animals and man, i.e., by direct contact, fomites, water and possibly food. Unpasteurized goat and cow's milk have been suspected as a source. Outbreaks with high infection rates have been recorded in day-care centres.

The incidence appears to be greater during the warm, rainy and humid months of the year, though only a limited number of epidemiological studies have been reported; seasonal patterns have not been consistent in USA, Finland and Australia. Cryptosporidiosis has been less frequently detected in infants in rural areas, where breast-feeding is more common, than in infants in urban areas. The disease has also been noted to be more severe in urban areas.

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Among healthy adults, it has been found to be more common in animal handlers and their families; again, information is limited. It is not known which animals can act as a source of human infection, or in what circumstances, though sick calves and cats are currently considered important. The organism is also recognized as an important cause of travellers' diarrhoea. The high susceptibility to cryptosporidia of immunodeficient individuals is now widely recognized.

An indirect immunofluorescence test has been developed that can measure specific antibody in the sera of infected animals and man and has been used for limited serosurveys; it is rather demanding, however.

3. CLINICAL FEATURES

The incubation period has been found to be 3-5 days in calves and between 4-12 days in travellers. The spectrum of clinical manifestations in man appears to be closely related to the immunological competence of the host. Otherwise healthy subjects, particularly young children, usually develop a self-limited, mild illness characterized by acute diarrhoea lasting for 1-3 weeks associated with abdominal cramps relieved after defecation, low fever, anorexia, nausea and vomiting. Stools are generally profuse, watery, green and invariably described as offensive, but are free of blood, mucus and leucocytes; the number of stools may vary from 5 to 25 and a patient may lose up to 10 litres a day. The degree of dehydration therefore is very variable.

In immunodeficient individuals, including those with acquired immunodeficiency syndrome (AIDS) or receiving immunosuppressive drugs, diarrhoea is severe and protracted and is often associated with severe malabsorption, weight loss, low-grade fever, dehydration and cachexia. Cough is occasionally present, which may or may not be due to colonization of the bronchial epithelium by the parasite. Spontaneous resolution of the infection in patients with AIDS has been recorded. Withdrawal of immunosuppressive drugs has been noted to bring about the cessation of diarrhoea within 1-2 weeks.

4. PATHOGENESIS AND PATHOLOGY

The mechanism by which the parasite causes diarrhoea is not known. It is unclear whether it is mediated through metabolites or toxins liberated by the parasite, or through a hypersensitivity reaction of the mucosa to the parasitic antigen. The large number of parasites covering the mucosa could also mechanically bring about diarrhoea and malabsorption by disturbing normal intestinal function.

In animals with disease, the mucosal changes are very dramatic and include a pronounced inflammatory reaction often unrelated to the number of organisms attached to the surface. In man, however, ileal biopsy shows marked villous atrophy with crypt hyperplasia, a moderate degree of infiltration of the lamina propria by mononuclear cells, and numerous minute spherical organisms present on the epithelium lining the villi and crypts. The lesions may be focal in distribution. Though the organisms may infect the whole bowel, they are more numerous in the lower small bowel.

The relationship between immunosuppression and cryptosporidiosis may not be a simple one, as sheep and monkeys with cryptosporidia treated with large doses of immunosuppressive drugs develop no ill effects.

5. IMMUNITY

Epidemiological data from developing countries strongly suggest that protective immunity to illness develops with repeated exposures in early life. Both humoral and cell-mediated immunity appear to be important. Immunocompetent individuals clear the parasite spontaneously, but immunodeficient persons fail to do so and suffer from persistent illness.

The prolonged course of illness in hypogammaglobulinaemic patients with normal T-cell function indicates the importance of humoral immunity. Measles, which is known to depress cell-mediated immunity, has been found to predispose to cryptosporidiosis.

6. DIAGNOSIS

Specific diagnosis of cryptosporidiosis-associated diarrhoea is possible only by laboratory techniques. Examination of biopsy specimens from the lower small intestine by light and/or electronmicroscopy is the most reliable technique, but it is obviously unpractical. As oocysts are discharged in faeces by infected individuals, a number of laboratory techniques have recently been developed for their detection in stool, but there is no agreement as yet on the simplest, most sensitive, reliable and rapid ones. Indirect immunofluorescence with monoclonal antibody has been found to be very sensitive and specific but it is not widely available.

The oocysts are minute, usually spherical bodies of about 4-6 μm in diameter*; they do not stain with iodine and are acid-fast. They are excreted intermittently and in small numbers by asymptomatic persons; during diarrhoea the number of oocysts in the faeces is usually high ($> 10^5/\text{ml}$). Oocyst excretion may persist for 1-2 weeks, or rarely up to 3 months after the resolution of symptoms. Examination of the usual iodine wet-mount is sometimes practised either alone or in combination with other methods; in iodine preparations, the oocysts appear as unstained, colourless structures, while the yeasts, blastocysts and other faecal materials are stained dark brown. However, since yeast cells may remain unstained, this technique when used alone has been found less reliable than those that stain the parasites positively.

Concentration of stools prior to the preparation of faecal smears may be helpful for identifying the parasite in the stools of asymptomatic persons and of convalescents, and when examination of an unconcentrated specimen from a suspected case fails to show oocysts. For concentration, both the zinc sulfate flotation technique and the formol-ether sedimentation technique can be used, but modification of the latter by using 10% formalin in distilled water instead of in saline, and centrifugation at 3000 instead of at 2000 r/min^{**} , has been found to be more effective. Sheather's sugar coverslip flotation technique for direct microscopy, using a solution containing 500 g of sucrose and 6.5 g of phenol in 320 ml of water, has been claimed to be even better for concentration than the classical formol-ether technique.

Difficulty is often encountered in fixing the faecal material on a slide when preparing smears for staining; 96% methanol is generally used for 2-5 minutes with or without flaming.

* Caution is needed in differentiating cryptosporidium oocysts from similar bodies which may also be present in the faeces. These include: (1) yeast cells, which are generally oval, 5-8 μm in size, and often have buds; (2) Blastocystis hominis (5-20 μm), which are usually round but have angular or irregular edges; a large refractile vacuole takes up almost the whole of the cell while the cytoplasm contains one or more nuclei compressed to form a peripheral rim; (3) Isospora belli and Isospora hominis (15 x 30 μm), which are usually elliptical or oval in shape containing, when mature, four banana-shaped sporozoites and coarsely granular, round, residual bodies; (4) leucocytes (10-20 μm) which are round or irregular in shape with a granular cytoplasm containing nuclei at different stages of degeneration.

** To convert r/min into g_{17} measure the radius (R) of the rotor arm and use the formula: $\text{g} = \text{R} \times (\text{r}/\text{min})^2 \times 118 \times 10^{-7}$.

For staining, Giemsa-stain has sometimes been used and claimed to be reliable; however, it can be difficult to read as both yeast cells and cryptosporidia stain purple and some developmental stage may remain unstained or faintly stained. Various modifications of the Ziehl-Neelsen technique have been used most widely, as with this technique acid-fast oocysts are easily differentiated from non-acid-fast, similar-looking bodies. A modified technique has been claimed to perform better than the classical hot Ziehl-Neelsen or the Kinyoun cold acid-fast (see below) methods: if the specimen is mucoid, vortex it after mixing with 10 drops of 10% KOH until homogenous, rinse with 10% formalin, and centrifuge at 300 x g for 2 minutes; use a drop from the upper layer of the sediment to make a thin smear on a clean slide and heat fix at 70°C for 10 minutes; flood the slide with usual carbol fuchsin (0.3-1%), heat to steaming from below and allow to stain for 5 minutes, add more stain without heating if the slide begins to dry up, rinse in tap water, decolourize with 5% H₂SO₄ for 30 seconds (longer for thick smears), rinse in tap water, counterstain with methylene blue for 1 minute, wash, drain, air dry, and examine.

The original Kinyoun cold acid-fast technique has also been modified in different ways. One modification uses carbol-fuchsin (Harleco, Gibbstown, NJ) for 1 minute and decolourizes with 10% H₂SO₄. The following modification has also worked well and may be practical: Dry the smear at room temperature, fix in 96% methanol for 2-5 minutes, dry at room temperature, and fix briefly in flame. Stain with concentrated carbol-fuchsin (about 4%) for 20-30 minutes without heating and rinse in tap water; decolourize with 0.25-10% H₂SO₄ for 20-60 seconds, and rinse in tap water. Counterstain with 5% malachite green for 5 minutes and rinse in tap water. Dry at room temperature and mount.

With both methods of staining, the oocysts usually appear as densely stained red bodies which may contain dark blue or brownish internal bodies when examined at 200X or 400X magnification.

The main reason for the different modifications of these staining techniques seems to be the variation in the properties of carbol-fuchsin dye. The concentration of carbol-fuchsin, the time taken to stain, the strength of H₂SO₄ and the time taken to decolourize depend on the quality of the carbol-fuchsin dye and also on the thickness of the smear. It is therefore advisable for each laboratory to standardize the technique of smear preparation and its own reagents using known positive specimens.

Staining with 1% aqueous safranin has also been reported to be simple, inexpensive, rapid and even more sensitive than the classical Ziehl-Neelsen technique. The following procedure has been reported to be satisfactory: Emulsify 2 g of faeces with 5 ml of 10% formalin in distilled water and filter through 2 layers of gauze; after adding 4 ml of solvent ether to the filtrate, thoroughly shake the mixture, centrifuge it at 1500 x g for 2 minutes and decant the supernatant. Add 1 or 2 drops of saline to the precipitate to make a thick suspension and use this to make a thin smear on a slide. Air dry the slides, pass them over a flame, and fix in 3% HCl in 100% methanol for 3-5 minutes. Dry, cover the smear with 1% safranin in water and heat for about 1 minute from below until bubbles appear in the solution. After a further 3 minutes, wash the slide in tap water, counterstain with 1% aqueous methylene blue for 0.5 minute, wash, blot, dry and mount with a cover slip.

The oocysts appear bright orange in colour with safranin, usually with a clear halo. They may appear like a doughnut with either a lighter-stained centre or a dark centre and light periphery depending on their internal structure. Though yeast cells are also stained by safranin, they stain evenly, show a thick rim of counterstain, have no clear halo, and may have buds. Smears can be screened at 200X or 400X magnification; oil immersion may not be necessary.

Detection of other pathogens along with cryptosporidium oocysts is common in developing countries and makes interpretation of the results difficult. The presence during diarrhoea of a large number of oocysts in unconcentrated faeces indicates a causal relationship.

7. TREATMENT

Clinical management of cryptosporidiosis-associated diarrhoea in otherwise healthy children consists of rehydration - oral or intravenous depending on the severity of dehydration - and a proper diet, as most episodes end spontaneously within 7-15 days.

There is a need for a safe and effective drug for the treatment of cryptosporidiosis in severe cases, and particularly in immunodeficient subjects. Almost all the common antimicrobials, including coccidiostats, anti-protozoals, broad-spectrum antibiotics and antihelminthic drugs have been tried in man and/or in animals, without success. Recently, symptomatic and microbiological improvements were reported in uncontrolled trials of spiramycin in immunodeficient patients; controlled trials are in progress. Treatment with several other agents is also under investigation.

8. PREVENTION AND CONTROL

There are no specific means of preventing and controlling cryptosporidiosis. General measures for prevention of the faecal-oral transmission of other enteric infections should be effective, except that chemical purification of water without filtration may not be adequate to prevent the transmission of this parasite, as is the case with Giardia and amoeba. Health education, emphasizing in particular the risk of acquiring infection through contact with sick animals (calves, cats, etc.), should be helpful.

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