

# Protozoan parasites (*Cryptosporidium*, *Giardia*, *Cyclospora*)<sup>1</sup>

## Description

Several species of parasitic protozoa are transmitted through water, with *Giardia intestinalis* and *Entamoeba histolytica/dispar* being among the most important intestinal parasites worldwide. Morbidity, and particularly mortality, rates for *E. histolytica/dispar* are high, especially in nonindustrialized countries. More information on *Entamoeba* can be found in Volume 2 of the WHO *Guidelines for drinking-water quality*. A wide variety of free-living amoebae occur in water, but only *Naegleria fowleri* and *Acanthamoeba* spp. have been identified as pathogenic for man. *N. fowleri* may be present in thermally polluted waters and sporadically causes fatal primary amoebic meningoencephalitis; however, only one outbreak has been related to a drinking-water supply system (Marshall et al., 1997). *Acanthamoeba* spp. can be found throughout the aquatic environment; these organisms cause sporadic cases of keratitis in wearers of contact lenses after exposure to contaminated recreational water and contact lens cleaning fluids (Marshall et al., 1997). Drinking-water taps were identified as the source of contamination when home-made lens-cleaning solutions were found to contain *Acanthamoeba*

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<sup>1</sup> This review was prepared by C.R. Fricker, Thames Water Utilities, Reading England; G.D. Medema, Kiwa NV, Nieuwegein, Netherlands; and H.V. Smith, Stobhill Hospital, Glasgow, Scotland, with contributions from M. Abbaszadegan, American Water Works Services Inc., Belleville, IL, USA; J. Bartram, Water, Sanitation and Health, World Health Organization, Geneva, Switzerland; P. Berger, United States Environmental Protection Agency, Washington, DC, USA; D. Cunliffe, Environmental Health Branch, South Australian Health Commission, Adelaide, Australia; I. Feuerpfel, Institut für Wasser-, Boden- and Lufthygiene, Bad Elster, Germany; A.H. Havelaar, Microbiological Laboratory of Health Protection, Rijksinstituut voor Milieuhygiene en Volksgezondheid, Bilthoven, Netherlands; Y. Magara, Hokkaido University, Japan; Y. Ortega, University of Arizona, Tucson, AZ, USA; E. Pozio, Istituto Superiore di Sanità, Rome, Italy; S. Regli, United States Environmental Protection Agency, Washington, DC, USA; J. Rose, University of South Florida, St Petersburg, FL, USA; S. Schaub, United States Environmental Protection Agency, Washington, DC, USA; and S. Shaw, United States Environmental Protection Agency, Washington, DC, USA.

(Seal, Hay & Kirkness, 1995). *Acanthamoeba* has also been suggested as a vehicle for environmental transmission of *Legionella* bacteria (Campbell et al., 1995).

The growth in the number of severely immunocompromised individuals—as a result of the AIDS epidemic, cancer chemotherapy, and organ transplants—has been paralleled both by the increasing prevalence of opportunistic infections and by greater recognition of the disease-causing potential of various other intestinal protozoan parasites, such as *Cryptosporidium parvum*, *Cyclospora cayetanensis*, and microsporidia, as human pathogens. The first human cases of cryptosporidiosis were reported in 1976 (Meisel et al., 1976; Nime et al., 1976). *Cryptosporidium* was initially thought to be an opportunistic pathogen of immunocompromised persons, but a number of waterborne outbreaks, plus frequent cases in immunocompetent individuals, have disproved this. Indeed, *C. parvum* is now one of the most commonly identified intestinal pathogens throughout the world. Its occurrence is dependent on factors that include season, and the age and other demographic characteristics of a population: among children aged 1–5 years with diarrhoea, *C. parvum* may be the most frequently found pathogen (Palmer, 1990).

*Cyclospora cayetanensis*—originally referred to as “cyanobacterium-like bodies”—has recently been recognized as a waterborne pathogen and reclassified (Bendall et al., 1993; Ortega et al., 1993). It has been associated with several waterborne outbreaks worldwide.

There are almost 1000 species of microsporidia, widely distributed in nature (Stewart & Osborn, 1996). Microsporidia have long been recognized as pathogens in fish, birds, and some mammals, but several species have recently been identified as the cause of disease in severely immunocompromised humans. These organisms are associated primarily with infections of the intestinal tract, but dissemination to the biliary, urinary, and respiratory tracts may occur and some species have been implicated in ocular infections in immunocompetent persons. The mode of transmission is still unclear, but a faecal–oral route is likely. The persistence of these organisms in water, their resistance to disinfection, and their small size (some as small as 1–2  $\mu\text{m}$ ) suggest that waterborne transmission must be considered possible, especially for immunocompromised individuals, although this has not yet been demonstrated.

*Toxoplasma gondii* is an intracellular coccidian parasite that has long been recognized as a human pathogen. Felines are the definitive host and are infected primarily by the consumption of infected mammals and birds, which act as secondary hosts. In secondary hosts, the parasite becomes encysted in muscle and brain tissue; only felines carry the parasite in the intestinal tract and shed oocysts that sporulate in the environment. The oocysts are 10–12  $\mu\text{m}$  in diameter and can survive in water and moist soils for long periods of time. Consumption of undercooked meats and raw milk and contact with feline faeces (in cat litter or sand boxes) are the primary sources of *Toxoplasma* infections in humans (Stewart & Osborn, 1996). One waterborne outbreak has been reported, and was believed to have resulted from contamination of water by cat faeces (Bowie et al., 1997).

Since *Cryptosporidium parvum*, *Giardia intestinalis*, and *Cyclospora cayotensis* are the parasites of primary concern in the area of drinking-water supply, and much information on waterborne transmission is available from recent research, it is on these three organisms that the remainder of this section concentrates.

### Significance of *Cryptosporidium* and *Giardia* as waterborne pathogens

Oocysts of *Cryptosporidium* and cysts of *Giardia* occur in the aquatic environment throughout the world. They have been found in most surface waters, where their concentration is related to the level of faecal pollution or human use of the water (Hansen & Ongerth, 1991; LeChevallier, Norton & Lee, 1991). The environmentally robust (oo)cysts are very persistent in water (DeReignier et al., 1989; Robertson, Campbell & Smith, 1992; Chauret et al., 1995) and extremely resistant to the disinfectants commonly used in drinking-water treatment (Hibler et al., 1987; Korich et al., 1990; Finch et al., 1993a, 1993b). These characteristics, coupled with the low numbers of (oo)cysts required for an infection (Rendtorff, 1954; Dupont et al., 1995; Okhuysen et al., 1998), place these organisms among the most critical pathogens in the production of safe drinking-water from surface water. Well protected groundwaters that are not mixed with surface water or otherwise contaminated are free of these and other enteropathogens. If abstraction, treatment, and distribution systems for these waters are properly designed and operated, the risk of faecal contamination is very low and there will be no waterborne transmission of parasitic protozoa. Groundwaters that mix with surface water or other sources of contamination (e.g. surface run-off) may contain low levels of *Cryptosporidium* and *Giardia* (Hancock, Rose & Callahan, 1997) and give rise to waterborne illness (Craun et al., 1998). Filtration of such waters is essential to the production of safe drinking-water: treatment by disinfection alone offers no protection against *Cryptosporidium* and only limited protection against *Giardia*.

Many waterborne outbreaks of giardiasis and cryptosporidiosis have been reported in industrialized countries (Craun, 1990; MacKenzie et al., 1994; Craun et al., 1998). In these outbreaks, (oo)cysts have entered the drinking-water because of surface-water treatment failure, contamination of the source water, and leakage into the distribution system. In a significant number of these outbreaks, the drinking-water implicated as the cause complied with the WHO Guidelines for *Escherichia coli* levels and turbidity (Craun 1990; Craun et al., 1998), but deviations from normal raw water quality or treatment operations recommended in the *Guidelines* were identified. However, in an outbreak in Las Vegas, USA, that was traced to drinking-water, no abnormalities in treatment operations or in the quality of raw or treated water were detected (Goldstein et al., 1996).

The fact that outbreaks occur in the absence of any warning signal from the routine water quality monitoring for coliforms points to a severe limitation of

coliform level as an indicator for microbiological safety of drinking-water. Additional means of safeguarding drinking-water are therefore imperative.

### *Cryptosporidium parvum*

#### **Taxonomy**

Members of the genus *Cryptosporidium* (Apicomplexa, Cryptosporidiidae) are small coccidian protozoan parasites that infect the microvillous region of epithelial cells in the digestive and respiratory tracts of vertebrates. Several species of *Cryptosporidium* have been described and appear to be specific for a class of vertebrates: *C. parvum*, *C. muris*, *C. felis*, and *C. wrairi* infect mammals, *C. baileyi* and *C. meleagridis* infect birds, *C. serpentis* infects reptiles, and *C. nasorum* infects tropical fish. Infections in humans are almost exclusively caused by *C. parvum*, although this species is also frequently found in infections of cattle and sheep and causes infections in many other mammal species.

#### **Life cycle**

Oocysts, the environmentally resistant transmission stage of the parasite, are shed by infected hosts with their faeces (Fayer & Ungar, 1986; Fayer, Speer & Dubey, 1997) and are immediately infectious. They may remain in the environment for very long periods without loss of infectivity: a very robust oocyst wall protects the sporozoites inside against physical and chemical damage. When an oocyst is ingested by a new host, excystation—opening of the suture in the oocyst wall—is triggered by the body temperature and the interaction with stomach acid and bile salts. Four motile sporozoites are released, which infect the epithelial cells of the small intestine, mainly in the jejunum and ileum. The parasite infects the apex of the epithelial cells, residing beneath the cell membrane but outside the cytoplasm. The sporozoites undergo several transformations in an asexual (merogony) and a sexual (gametogony) reproduction cycle; it is the latter that generates the oocysts.

Oocysts of *C. parvum* are spherical, with a diameter of 4–6  $\mu\text{m}$ , and may be either thick- or thin-walled oocysts. Thin-walled oocysts may excyst within the same host and start a new life cycle (autoinfection). This can lead to heavily infected intestinal epithelia and result in malabsorptive or secretory diarrhoea. Thick-walled oocysts are excreted with the faeces.

#### **Pathogenicity**

Infection studies in healthy human volunteers demonstrated a clear relationship between probability of infection and the ingested oocyst dose of a bovine *C. parvum* strain (Dupont et al., 1995). At the lowest dose (30 oocysts), the probability of infection was 20%; at a dose of 1000 oocysts, probability increased

to 100%. When the dose–response data are fitted with an exponential model, the probability of infection ( $P_i$ ) is described by:

$$P_i = 1 - e^{-r \times \text{dose}}$$

where  $r$ , the dose–response parameter, is 0.004005 (95% CI 0.00205–0.00723) for this *C. parvum* strain (Teunis et al., 1996).

This approach assumes that ingestion of even a single oocyst results in a distinct probability (0.5%) of infection. Although there was a clear dose–response relation for infection, occurrence of symptoms of intestinal illness in the volunteers was not dose-related.

### **The disease**

The average incubation period varies widely but is usually about 7 days (Ungar, 1990; Dupont et al., 1995). Watery diarrhoea is the most prominent symptom of intestinal *C. parvum* infection (Fayer & Ungar, 1986; Ungar, 1990), and the frequent and copious bowel movements can cause dehydration and weight loss (Arrowood, 1997). Other symptoms are nausea, abdominal cramps, vomiting, and mild fever. During the 1993 Milwaukee waterborne outbreak, which involved 400 000 patients, MacKenzie et al. (1994) compared clinical data from cases detected by (passive) laboratory surveillance with cases detected by (active) telephone surveys. Patients who submitted a stool sample for laboratory diagnosis suffered more serious disease, as manifested by the higher frequency of fatigue, loss of appetite, nausea, fever, chills and sweats, and vomiting.

In immunocompetent individuals, the infection is limited by the immune response that eventually clears the parasite. Infections in patients with defective cellular immune response (congenital or due to AIDS or chemotherapy) or humoral immune response (in congenital hypogammaglobulinaemia) are persistent and heavy, suggesting that both types of immune response are needed to limit and clear the infection. Several animal studies suggest that the immune response protects against reinfection (Zu et al., 1992), and protective immunity in humans is indicated by the large numbers of asymptomatic carriers in countries with a high prevalence of cryptosporidiosis. Moreover, infected volunteers who were challenged with the same strain one year after initial infection were significantly less susceptible to reinfection (Okhuysen et al., 1998); occurrence of diarrhoea was similar in both exposures, but the illness was less severe in the reinfected volunteers, which indicates some degree of protective immunity.

The duration of the infection is generally 7–14 days in immunocompetent individuals, but a median duration of 23–32 days has also been reported (van Asperen et al., 1996). The peak intensity of oocyst shedding, with an average concentration of  $10^6$ /g, coincides with the peak intensity of clinical symptoms. Oocyst shedding lasts for at least 2 weeks in 82% of infected people, 3 weeks in 42%, and 4 weeks in 21% (Baxby, Hart & Blundell, 1985). Again, there is a difference between cases under laboratory surveillance (duration 2–4 weeks) and

cases in the general population (duration typically 3–6 days). Relapses of diarrhoea are common: up to five additional episodes in 40–70% of patients have been reported in both population-based (outbreak) studies and in studies in volunteers. This phenomenon considerably increases both the mean duration of disease and its variability.

Mortality in immunocompetent patients is generally low. In immunodeficient individuals, however, the infection can be persistent and severe (Ungar, 1990), resulting in very profuse diarrhoea and consequent severe dehydration. Severe infections have been reported in patients with concurrent infections (principally AIDS, but also measles and chickenpox), people with congenital immune deficiencies, patients receiving immunosuppressive drugs (for cancer therapy, transplants, or skin lesions), and malnourished individuals (Fayer, Speer & Dubey, 1997). It is also reported that pregnancy may predispose to *Cryptosporidium* infection (Ungar, 1990).

The prevalence of cryptosporidiosis in AIDS patients in industrialized countries is around 10–20% (Current & Garcia, 1991). In the absence of an effective immune response, the infection may spread throughout the entire intestinal tract and to other parts of the body (gall bladder, pancreas, respiratory tract). No consistently effective therapeutic agent has been found (Blagburn & Soave, 1997). Immunotherapy with monoclonal antibodies or hyperimmune bovine colostrum has been reported to resolve diarrhoea, at least temporarily, in AIDS patients (Riggs, 1997), and similar findings have been reported for other chemotherapeutic agents (azithromycin, paromomycin) (Blagburn & Soave, 1997).

The severe dehydration, the spread of infection, and the lack of an effective therapy lead to high mortality in immunodeficient patients, although this has not been accurately quantified. In one study in the UK, 19% of AIDS patients with cryptosporidiosis were thought to have died from the infection (Connolly et al., 1988). Another study compiled case reports of cryptosporidiosis and found a mortality rate of 46% in AIDS patients and 29% in patients with other immunodeficiencies (Fayer & Ungar, 1986).

### **Prevalence**

In stool surveys of patients with gastroenteritis, the reported prevalence of *Cryptosporidium* is 1–4% in Europe and north America and 3–20% in Africa, Asia, Australia, and south and central America (Current & Garcia, 1991). Peaks in the prevalence in developed countries are observed in spring (Casemore, 1990) and in the late summer (van Asperen et al., 1996).

Numbers of asymptomatic carriers, as determined by stool surveys, are generally very low (<1%) in industrialized countries (Current & Garcia, 1991), although higher rates have been reported in day-care centres (Lacroix et al., 1987; Crawford & Vermund, 1988; Garcia-Rodriguez et al., 1989). Routine bile endoscopy suggests a higher prevalence of asymptomatic carriage: 13% of

non-diarrhoeic patients were shown to carry *Cryptosporidium* oocysts (Roberts et al., 1989). High rates of asymptomatic carriage (10–30%) are common in non-industrialized countries (Current & Garcia, 1991). Seroprevalence rates are generally higher than faecal carriage rates, from 25–35% in industrialized countries to 95% in south America (Casemore, Wright & Coop, 1997), increase with age (Zu et al., 1992; Kuhls et al., 1994), and are relatively high in dairy farmers (Lengerich et al., 1993) and day-care centre attendants (Kuhls et al., 1994).

### **Routes of transmission**

A major route of infection with *Cryptosporidium* is person-to-person transmission, as illustrated by outbreaks in day-care centres (Fayer & Ungar, 1986; Casemore, 1990; Cordell & Addiss, 1994) and the spread of infection within the households of children attending these centres. Sexual practices involving oro-anal contact also involve a high risk of exposure to the organism. *Cryptosporidium* can also be transmitted from mammals—especially newborn animals—to humans, and many infections have been derived from contact with infected calves and lambs (Casemore, 1990). Domestic pets can be infected with oocysts, but do not appear to be important sources of human infection (Casemore, Wright & Coop, 1997; Glaser et al., 1998). Indirect person-to-person or zoonotic transmission may occur through contaminated water used for recreation (e.g. swimming pools) or through food and drinks (raw meat and milk, farm-made apple cider) (Casemore, Wright & Coop, 1997).

Waterborne outbreaks of cryptosporidiosis have been attributed to contaminated drinking-water, from both surface-water and groundwater sources (Craun, 1990; Mackenzie et al., 1994; de Jong & Andersson, 1997), and to recreational water, including swimming pools (Joce et al., 1991; MacKenzie, Kazmierczak & Davis, 1995; van Asperen et al., 1996; Anon., 1998; Kramer et al., 1998).

Outbreaks caused by drinking-water have been attributed to contamination of the source water by heavy rainfall or snow-melt (Richardson et al., 1991; Pett, Smith & Stendahl, 1993; MacKenzie et al., 1994), to sewage contamination of wells (d'Antonio et al., 1985; Kramer et al., 1996), to inadequate treatment (Richardson et al., 1991; Craun et al., 1998) or treatment deficiencies (Badenoch, 1990; Leland et al., 1993; Craun et al., 1998), and to combinations of these factors (MacKenzie et al., 1994). Leakages and cross-connections in water-distribution systems have also caused outbreaks of cryptosporidiosis (Craun, 1990; de Jong & Andersson, 1997; Craun et al., 1998). As many as 400 000 people have been affected by a cryptosporidiosis outbreak transmitted through drinking-water.

A wide range of oocyst concentrations in drinking-water have been detected during outbreaks of disease (Haas & Rose, 1995). However, tests carried out during an outbreak are usually too late to determine the concentration that triggered the outbreak. To obtain “historical” data on the occurrence of oocysts in drinking-water, researchers have attempted to detect oocysts in ice (MacKenzie et al., 1994), in in-line filters (van Asperen et al., 1996), and in sediments of

water-storage tanks (Pozio et al., 1997). The results probably represent underestimates of the concentrations that caused the outbreaks. For the 1993 Milwaukee outbreak, however, Haas & Rose (1994) showed (with certain assumptions) that the measured concentration in drinking-water was close to the value predicted on the basis of the attack rate, water consumption, and dose–response relationship.

Low oocyst concentrations in drinking-water have also been found in situations where there was no evidence for the occurrence of an outbreak (LeChevallier, Norton & Lee, 1991; Karanis & Seitz, 1996; Rose, Lisle & LeChevallier, 1997; McClellan, 1998). Current detection methods do not allow the determination of pathogenicity of oocysts in water. Detection of oocysts in treated water should therefore, wherever possible, prompt further tests to confirm the presence of (viable) *C. parvum* oocysts. Whether or not the capacity for such tests exists, every effort should be made to thoroughly examine other water quality parameters that may point to faecal contamination and to trace both the source of contamination and the operational conditions that resulted in the presence of oocysts in the drinking-water. If the presence of *C. parvum* oocysts is confirmed, control measures (improved source protection and/or water treatment) should be instituted; ideally, an epidemiological study should also be undertaken to determine whether there has been significant waterborne transmission. Lack of capacity to confirm the pathogenicity or viability of oocysts should not delay or prevent further investigation or remedial action if a significant threat to public health is suspected.

### *Giardia intestinalis*

#### **Taxonomy**

*Giardia* is a flagellated protozoan the taxonomy and host specificity of which remain the subject of considerable debate. The organism has been found in more than 40 animal species (Meyer, 1994). Nowadays, five species of *Giardia* are established in the scientific literature, including the three species proposed by Filice (1952)—*G. muris* in rodents, birds, and reptiles, *G. intestinalis* (syn: *duodenalis*, syn: *lamblia*) in mammals (including man), rodents, reptiles, and possibly birds, *G. agilis* in amphibians—*G. ardae* in the great blue heron (Erlandsen et al., 1990), and *G. psittaci* in the budgerigar (Erlandsen & Bemrick, 1987). A morphologically distinct *Giardia* isolated from the straw-necked ibis (Forshaw, 1992) was later suggested to be a strain of *G. ardae* (McRoberts et al., 1996).

*Giardia* is thought to be predominantly asexual, and the species concept—defined on the basis of sexually reproductive compatibility—is therefore difficult to apply. The high degree of genetic heterogeneity found in human and animal isolates (Nash et al., 1985; Andrews et al., 1989; Meloni, Lymbery & Thompson, 1989; Morgan et al., 1994) makes speciation uncertain and suggests that

*Giardia* is a clonal parasite (Tibayrenc, 1994). *G. intestinalis* can be subdivided by several techniques into two groups (Homan et al., 1992, 1994). It is still uncertain how, or even whether, this heterogeneity is related to host specificity and pathogenicity of *Giardia*.

### **Life cycle**

The life cycle of *Giardia* is simple (Feely, Holberton & Erlandsen, 1990; Meyer, 1994). As with *Cryptosporidium*, the parasite is shed with the faeces as an environmentally robust cyst, which can then be transmitted to a new host. In the duodenum of the new host, the trophozoite emerges from the cyst and undergoes a mitotic division. Each of the two trophozoites produced in this way attaches to the epithelial cells by means of an adhesive disc, then feeds on the epithelial cells. The trophozoites detach from the epithelial cells, probably because of the rapid turnover (72 hours) of these cells, and undergo mitotic division in the intestinal lumen. During periods of diarrhoea, these trophozoites may be transported with the intestinal contents and excreted, but do not survive long outside the host. Some of the trophozoites encyst during the passage through the intestine and leave the host with the faeces as cysts. In formed stools, cysts are encountered more often than trophozoites.

*Giardia intestinalis* cysts are elliptical, 8–12 µm long and 7–10 µm wide. The cyst wall is 0.3–0.5 µm thick and has a fibrillous structure. Two to four nuclei are found in each cyst, together with axonemes of the flagella of the trophozoite.

### **Pathogenicity**

Studies in human volunteers revealed a dose–response relationship between the probability of infection and the ingested dose of *G. intestinalis* cysts (Rendtorff, 1954), although no data on the viability of the ingested cysts were provided. A dose of 10 cysts resulted in an infection in 100% (2/2) of the volunteers.

The probability of infection,  $P_i$ , has been described with an exponential model (Rose et al., 1991b):

$$P_i = 1 - e^{-r \times \text{dose}}$$

where  $r$ , the dose–response parameter, is 0.0199 (95% CI: 0.0044–0.0566). Although 53% of the volunteers became infected in this study, and changes in bowel motions were observed, none of the volunteers developed symptoms of giardiasis. The infection-to-illness ratio varies between isolates, as shown by the different response of volunteer subjects to two different isolates from symptomatic human infections in a study by Nash et al. (1987). Other factors, such as age, nutritional status, predisposing illness, and previous exposure, determine the outcome of an infection (Flannagan, 1992). Asymptomatic carriage appears to be the most common form of infection with *Giardia* (Farthing, 1994); 16–86% of infected individuals are asymptomatic.

The mechanism by which *Giardia* causes diarrhoea and malabsorption is still unclear. The organism could act as a physical barrier, but the area covered by trophozoites is probably too small to affect the absorption of nutrients. There is no evidence for the production of toxins (Buret, 1994). *Giardia* infections appear to affect the activity of gut enzymes (lactase, disaccharidase), damage the mucosal surface (causing shortening of crypts and villi), and give rise to overgrowth of bacteria (Tomkins et al., 1978) or yeasts (Naik et al., 1978) in the small intestine.

### **The disease**

The time between infection and the appearance of *Giardia* cysts in the stool is 12–19 days (Jokipii, Hemila & Jokipii, 1985). Symptoms appear between 1 and 75 days after infection, but generally at 6–15 days, coinciding with the appearance of *Giardia* in stool (Rendtorff, 1954; Brodsky, Spencer & Schultz, 1974). The most prominent symptoms are diarrhoea (fatty, yellowish) weakness, weight loss, abdominal pain, and—to a lesser extent—nausea, vomiting, flatulence, and fever. In most cases, the infection is acute and self-limiting, with a duration of 2–4 weeks. However, a significant proportion of the infected population (estimated at 30–50%) will then develop chronic infection with intermittent diarrhoea (Farthing, 1994); weight loss can be substantial (10–20%) in this group. The ability of *Giardia* to change the surface epitopes of the trophozoites during infection (Nash, 1992) may play a role in the occurrence of chronic infections. There is evidence that infection of children with *Giardia* causes failure to thrive by impairing the uptake of nutrients (especially fats and vitamins A and B<sub>12</sub>) (Farthing, 1994; Hall, 1994).

Excretion of cysts varies between 10<sup>6</sup> and 10<sup>8</sup> per gram of stool, as determined in positive stool samples (Tsuchiya, 1931), but a significant proportion of the stool samples do not contain detectable levels of *Giardia*. Excretion patterns vary with the host and with the isolate.

### **Prevalence**

*Giardia* infections are very common in children in developing countries (Farthing, 1994; Rabbani & Islam, 1994). In developed countries, prevalence peaks at the age of 1–4 years (Flannagan, 1992) and again in the 20–40-year age group, partly through caring for young children and partly as a result of travelling.

In developing countries, the prevalence of giardiasis in patients with diarrhoea is about 20%, (range 5–43%) (Islam 1990). The figure for developed countries varies from 3% (Hoogenboom-Verdegaal et al., 1989; Adam, 1991; Farthing, 1994; Kortbeek, van Deursen & Hoogenboom-Verdegaal, 1994) to 7% (Quinn, 1971).

Both humoral and cellular immune responses are elicited by infection with *Giardia*. Secretory IgA and IgM appear to play a role in clearance of the intestinal

infection, by reducing the mobility of trophozoites and preventing their adhesion to the mucosa (Farthing & Goka, 1987). The immune response can also be seen in the serum antibodies. The immune response provides some protection against reinfection, as indicated by lower attack rates in chronically exposed populations (Istre et al., 1984; Rabbani & Islam, 1994). This protection is limited, however, and recurrence of symptomatic infections is common, even after several infections (Gilman et al., 1988; Wolfe 1992; Hall, 1994), which may be related to the antigenic variation shown by *Giardia* (Nash, 1992).

Giardiasis can be treated with nitroimidazoles, quinacrine, and furazolidone (Boreham, 1994). For patients with persistent giardiasis several approaches are possible, such as increasing the doses and duration of treatment, or giving an alternative drug or a combination of drugs.

### **Routes of transmission**

Faecal–oral transfer of *Giardia* cysts is the major route of transmission of giardiasis, as indicated by the high prevalence in developing countries with poor standards of hygiene and sanitation, in day-care centres and nurseries (Black et al., 1977; Pickering & Engelkirk, 1990; van de Bosch, 1991), and by secondary spread within the household of those who attend day-care centres (Black et al., 1977). Foodborne outbreaks are the result of contamination of food by infected workers or household members (Osterholm et al., 1981; Islam, 1990; Thompson, Lymbery & Meloni, 1990).

The role of animals in the transmission of human giardiasis is still a matter of some speculation. Although *Giardia* commonly occurs in domestic pets, farm animals, and wild mammals, there is no unequivocal evidence that organisms from these sources have caused infections in humans (Erlandsen, 1994). *Giardia intestinalis* isolates from animals and humans may be morphologically indistinguishable (Flannagan, 1992) and this has led to many reports of animal sources of human giardiasis, including waterborne infections caused by *Giardia* cysts from beavers and muskrats (Moore et al., 1969; Dykes et al., 1980). However, the genetic diversity within and between human and animal isolates (Thompson, Meloni & Lymbery, 1988) is too high to allow definite conclusions to be drawn regarding host specificity. Cross-transmission studies have not been well controlled and the results have been contradictory (Davies & Hibler, 1979; Hewlett et al., 1982; Belosevic, Faubert & MacLean, 1984; Kirkpatrick & Green, 1985; Woo & Patterson, 1986).

Waterborne outbreaks of giardiasis have been reported for some 30 years (Moore et al., 1969; Brodsky, Spencer & Schultz, 1974; Craun, 1990). In the USA, *Giardia* is the most commonly identified pathogen in outbreak investigation, with more than 100 waterborne outbreaks, based on epidemiological evidence (Craun, 1990). Waterborne outbreaks have also been reported in Australia, Canada, New Zealand, Sweden, and the United Kingdom. These outbreaks have

been linked to consumption of untreated surface water contaminated by human sewage (Craun, 1990) or by wild rodents (Moore et al., 1969; Dykes et al., 1980), to groundwater that was contaminated by human sewage or contaminated surface water, to surface water systems treated only by disinfection (Craun, 1984; Kent et al., 1988) or by ineffective filtration (Dykes et al., 1980; Craun, 1990), and to cross-connections or damage in water-distribution systems (Craun, 1986).

### *Cyclospora cayatanensis*

#### **Taxonomy**

*Cyclospora* was first isolated by Eimer in 1870 from the intestines of moles; it is related taxonomically to other protozoan parasites such as *Cryptosporidium* and *Toxoplasma*. The first observation of this parasite as a pathogen for human beings was probably that reported by Ashford (1979). Confirmation of the coccidian identity and genus was made in 1993 (Ashford et al., 1993; Ortega et al., 1993). *Cyclospora* is a member of the subphylum Apicomplexa, class Sporozoa-sida, subclass Coccidiasina, family Eimeriidae. Molecular phylogenetic analysis suggests that the genus is closely related to the genus *Eimeria* (Relman et al., 1996). *Cyclospora* organisms have been found in snakes, insectivores, and rodents.

#### **Life cycle**

*Cyclospora* completes its life cycle within one host (monoxenous), but many details remain to be elucidated. Ortega et al. (1993) proposed that *Cyclospora* that are infective to human beings should be designated *Cyclospora cayatanensis* on the basis of the development of the oocyst in vitro. However, use of this species name was questioned by Ashford, Warhurst & Reid (1993), and Bendall et al. (1993) prefer the term CLB (for *Cyclospora*-like body) until further information is available on the biology of this coccidian parasite. For the purposes of this review, however, the term "*Cyclospora* spp" is used to describe organisms of this genus that are infective to humans.

The endogenous stages of *Cyclospora* sp. are intracytoplasmic and contained within a vacuole (Bendall et al., 1993), and the transmissive stage, the oocyst, is excreted in the stool. The life cycle of *Cyclospora* sp. may complete within enterocytes (Sun et al., 1996). *Cyclospora* sp. oocysts are spherical, measuring 8–10 µm in diameter; they are excreted in the stool and sporulate to infectivity in the environment. Unsporulated oocysts contain a central morula-like structure consisting of a variable number of inclusions. Sporulated oocysts contain two ovoid sporocysts, within each of which there are two sporozoites (Levine, 1973). Each sporozoite measures  $1.2 \times 9 \mu\text{m}$ .

### **Pathogenicity**

*Cyclospora* sp. infect enterocytes of the small bowel and can produce disease (Bendall et al., 1993). Both symptomatic and asymptomatic states have been described. A moderate to marked erythema of the distal duodenum can occur with varying degrees of villous atrophy and crypt hyperplasia (Connor et al., 1993), but little is known of the pathogenic mechanisms. As yet, no virulence factors have been described for *Cyclospora* sp. No animal or human feeding studies have been undertaken. As for *Giardia* and *Cryptosporidium*, it is assumed that the organisms are highly infectious, and that doses lower than 100 sporulated oocysts carry a high probability of infection.

### **The disease**

Symptoms of infection include watery diarrhoea, fatigue, abdominal cramping, anorexia, weight loss, vomiting, low-grade fever, and nausea; these can last for several weeks, with bouts of remittance and relapse. The incubation period is between 2 and 11 days (Soave, 1996) with moderate numbers of unsporulated oocysts being excreted for up to about 60 days. Illness may last for weeks and episodes of watery diarrhoea may alternate with constipation (Soave, 1996). In immunocompetent individuals the symptoms are self-limiting and oocyst excretion is associated with clinical illness (Shlim et al., 1991); in immunocompromised patients, diarrhoea may be prolonged.

### **Prevalence**

Oocysts of *Cyclospora* sp. have been isolated from the stools of children and from both immunocompetent and immunocompromised adults. They have been described in the stools of residents of, and travellers returning from, developing countries, and in association with diarrhoeal illness in individuals from north, central, and south America, south-east Asia, Australia, the Caribbean, Europe, and the Indian subcontinent. Outbreaks of cyclosporiasis have been reported from north and south America and from Nepal. In north America and Europe cyclosporiasis is associated with overseas travel and travellers' diarrhoea. Point-source outbreaks have been reported in Nepal and the USA. In 1996, a total of 1465 cases were reported in Canada and the USA; most cases occurred during spring and summer, and about half occurred following events at which raspberries had been served (Centers for Disease Control and Prevention, 1996; Herwaldt & Ackers, 1997). Sporadic cases of cyclosporiasis have been reported from many countries and *Cyclospora* sp. oocysts are increasingly being identified in stools from immunocompetent individuals with no history of foreign travel.

*Cyclospora* sp. oocysts were detected in faecal samples from 11% of Haitians with chronic diarrhoea who were HIV-seropositive (Pape et al., 1994); they were the only pathogens, other than HIV, identified in many of these patients. Clin-

ical disease may resolve without treatment, but trimethoprim–sulfamethoxazole is the treatment drug of choice.

### **Routes of transmission**

Epidemiological data indicate that *Cyclospora* spp are transmitted by water and food (Hoge et al., 1993; Centers for Disease Control and Prevention, 1996; Herwaldt & Ackers, 1997). An outbreak occurred among house staff and employees in a hospital dormitory in Chicago following the failure of the dormitory's water pump. Illness was associated with the ingestion of water in the 24 hours after the pump failure, and *Cyclospora* spp oocysts were detected in the stools of 11 of the 21 individuals who developed diarrhoea (Centers for Disease Control, 1991; Wurtz, 1994).

In an outbreak that occurred among British soldiers and their dependants stationed in a small detachment in Nepal, 12 people out of 14 developed diarrhoea. *Cyclospora* oocysts were detected in stool samples from 6 of 8 patients. Oocysts were also detected microscopically in a concentrate from a 2-litre water sample. Drinking-water for the camp consisted of a mixture of river water and chlorinated municipal water. Chlorine residuals of 0.3 to 0.8 ppm were measured before and during the outbreak. No coliforms were detected in the drinking-water (Rabold et al., 1994).

## **Monitoring and assessment**

### *Cryptosporidium* and *Giardia*

The methodology for the detection of *Cryptosporidium* oocysts and *Giardia* cysts in water is completely different from that traditionally used for quantification of faecal indicator bacteria in the water industry. Currently available methods are at best tentative—recovery is low and variable, and it is not possible to differentiate viable oocysts of strains that are infectious to humans. The procedure consists of several stages: sample collection and concentration, separation of (oo)cysts from contaminating debris, and detection of (oo)cysts. Many factors, such as water quality and age of the (oo)cysts, can have significant effects on the overall efficiency of recovery, and it is almost impossible to compare the effectiveness of methods used in different laboratories unless these factors are standardized.

There is considerable interest in determining whether (oo)cysts recovered from the environment are viable and potentially infectious.

### **Quality assurance**

*Microscope counts.* Care must be taken to ensure that the particles being counted are (oo)cysts, to determine whether or not they contain sporozoites, and to exclude algae and yeast cells from any counts that are made. The criteria used for

determining that a particle is in fact a *Cryptosporidium* oocyst or a *Giardia* cyst vary between laboratories. Some workers use only the fact that (oo)cysts fluoresce when labelled with a fluorescein isothiocyanate-tagged anti-*Cryptosporidium* or anti-*Giardia* monoclonal antibody and that it is in the proper size range for a cyst or oocyst. Others will additionally use differential interference contrast microscopy or nucleic acid stains to ascertain that the particles counted are indeed (oo)cysts. This more detailed analysis allows the confirmation of the counted particles as presumptive (oo)cysts.

Many factors influence the microscope counts: the amount of background debris and background fluorescence, the experience and alertness of the technician who performs the count, the intensity of fluorescence after staining with the monoclonal antibody, and the quality of the microscope. Quality assurance protocols should define how these factors are addressed.

*Recovery efficiency.* In view of the low and variable efficiency of recovery in the methods used for monitoring *Cryptosporidium* and *Giardia*, it is essential that laboratories collect their own data on recovery efficiency in the different water types they monitor. This can be achieved by seeding a second water sample with a known number of cysts and oocysts and determine the percentage of these recovered by the total protocol for sampling, processing, and counting of environmental samples. However, this assay is influenced by the number, age, and storage conditions of the seeding (oo)cysts, all of which should be standardized (at least within a particular laboratory) if recovery data are to be meaningful. The recovery efficiency should be assessed sufficiently often to reveal how its variation influences the uncertainty of the monitoring data. This is essential for the interpretation of environmental monitoring data.

*Cartridge filtration.* The first reported method for detection of *Giardia* and *Cryptosporidium* in water used polypropylene cartridge filters, with a nominal pore size of 1  $\mu\text{m}$ , through which large volumes of water (100–1000 litres) were passed at a flow rate of 1–5 litres/minute. Trapped material was then eluted by cutting open the filter and washing it either by hand or by stomaching using a dilute detergent solution. The resulting washings from these cartridges sometimes totalled 3 or 4 litres and required further concentration by centrifugation. The recovery of *Cryptosporidium* oocysts by this technique was originally reported to be 14–44% (Musial et al., 1987), although lower efficiencies (<1–30%) have often been reported since then (Ongerth & Stibbs, 1987; Clancy, Gollnitz & Tabib, 1994; Shepherd & Wyn-Jones, 1996). Differences in reported recovery rates may be due to a number of factors including water quality, laboratory efficiency, and oocyst age.

*Membrane filtration.* Ongerth & Stibbs (1987) described a method using large (diameter 142 or 293 mm), 2  $\mu\text{m}$  absolute, flat-bed membranes for the concentration of oocysts from water samples, and many workers have now adopted

this procedure. Water is pumped through the membranes and the concentrated materials are recovered by “scraping” the surface of the membrane together with washing with dilute detergent, followed by further concentration using centrifugation. However, while it is relatively easy to filter 10–40 litres of low-turbidity water, with some high-turbidity waters it is possible to filter only 1–2 litres. As with cartridge filtration, a range of recovery efficiencies has been reported for flat-bed membranes. Nieminski, Schaeffer & Ongerth (1995) reported an average recovery of 9% for *Cryptosporidium* and 49% for *Giardia*. In a study of the recovery efficiency of several different membranes, Shepherd & Wyn-Jones (1996) suggested that 1.2- $\mu\text{m}$  cellulose-acetate membranes gave higher recovery (30–40% and 50–67%, respectively, for *Cryptosporidium* and *Giardia*) than the 2- $\mu\text{m}$  polycarbonate membranes (22–36% and 41–49% respectively) preferred by Ongerth & Stibbs (1987).

*Flocculation.* Another established method for concentrating (oo)cysts is the calcium carbonate flocculation procedure developed by Vesey et al. (1993b). A fine precipitate of calcium carbonate ( $\text{CaCO}_3$ ) is formed in a water sample by adding calcium chloride and sodium bicarbonate and adjusting the pH to 10.0 with sodium hydroxide. After the precipitate has settled, the supernatant fluid is removed by aspiration, the calcium carbonate is dissolved with sulfamic acid, and the sedimented material is resuspended. Recovery efficiencies using this method have been reported to be as high as 70% for both *Cryptosporidium* and *Giardia* (Vesey et al., 1993b; Campbell et al., 1994; Vesey et al., 1994; Shepherd & Wyn-Jones, 1996). More recent work has demonstrated that this is the upper limit of the detection efficiency and that recoveries are usually lower. Using aged oocysts for seeding experiments and leaving the oocysts in contact with water for a few days before analysis normally produces recovery rates of 30–40%. The viability of the oocysts is affected by this concentration (Campbell et al., 1995). Flocculation with aluminium sulfate ( $\text{Al}_2(\text{SO}_4)_3$ ) does not affect the viability of oocysts, while the recovery efficiency is comparable to that achieved with  $\text{CaCO}_3$  flocculation (Schwartzbrod, personal communication).

*New methods.* The search continues for new methods of concentrating water samples to detect the presence of protozoan parasites, and many techniques have been evaluated, including cross-flow filtration, continuous-flow centrifugation, and vortex-flow filtration (Whitmore, 1994) as well as a number of proprietary systems.

There continues to be much debate over which method is most appropriate. Realistically no single method is suitable for all situations. The choice of method should be made with due regard to a number of factors, including the purpose of sampling, the water quality, and the facilities in the laboratory that will perform the analysis. Ideally, the method chosen should efficiently concentrate as large a sample as possible and yield a concentrate that can be examined easily. Many workers prefer to concentrate only a small volume of water and to examine the entire concentrate, while others take large samples and examine only a

fraction of the final concentrate. Both approaches are valid, but the methods used to concentrate small volumes (e.g. 10–20 litres) tend to be easier to perform and generally have a higher recovery efficiency. It is therefore often preferable to take a large number of low-volume samples and examine all of the concentrate. Other factors that may affect the choice of concentration method include the site of sample collection and the distance over which samples must be transported.

### **Separation techniques**

Since the concentration of *Cryptosporidium* oocysts and *Giardia* cysts is based almost exclusively on particle size, the techniques are not specific and a large amount of extraneous material is concentrated as well. This material may interfere with the successful detection of (oo)cysts, either by increasing the total volume of material that needs to be examined, or by obscuring or mimicking (oo)cysts during examination. Some form of separation technology is therefore normally required to reduce the time taken to examine a sample and to prevent (oo)cysts being missed.

*Density centrifugation.* Density centrifugation is often used to separate (oo)cysts from background debris and thus reduce the amount of material to be examined. Several workers use sucrose density centrifugation to separate parasites from faecal material in clinical samples, and this basic technique has also been adopted for use with environmental samples. Whatever flotation method is used, several groups have demonstrated that this procedure is inefficient for detecting protozoan parasites in water concentrates. Of particular interest was the finding of Bukhari & Smith (1996) that sucrose density centrifugation selectively concentrated viable, intact *Cryptosporidium* oocysts. Fricker (1995) demonstrated that the recovery of oocysts from water samples could be affected by the length of time that they were in contact with the water concentrate but only when sucrose flotation was used. Spiked samples examined directly, without density centrifugation, gave similar recovery efficiencies, whether they were examined immediately after seeding or after 48 hours' contact with the concentrate. When sucrose flotation was used, however, the recovery of (oo)cysts in raw water fell from a mean of 55% to 18% after the same period of contact. This reduction in recovery efficiency also occurred with concentrates of reservoir water (67% to 23%) and fully treated water (80% to 52%).

*Immunomagnetic separation.* Autofluorescing algae, which may not be completely removed by density gradient centrifugation, can cause severe problems when slides are examined for protozoa by epifluorescence microscopy. More efficient methods for separation of (oo)cysts from other particulates have been sought, and many workers have tried immunomagnetic separation. The principles underlying this technique are attachment of specific antibodies to magnetizable

particles and efficient mixing of the particles in the sample. The (oo)cysts attach to the magnetizable particles and are isolated from this debris with a strong magnet. The technique is very simple, but there are several sources of failure, including the quality and specificity data of the available monoclonal antibodies. Most of the commercially available monoclonal antibodies to *Cryptosporidium* or *Giardia* are of the IgM type, and are therefore of low affinity since they have not undergone affinity maturation or isotype switching. When immunomagnetic separation is used and beads are mixed with water concentrates, the immunoglobulin-(oo)cyst bonds are subjected to shear forces; the stronger the bond, the more likely the bead is to remain in contact with the (oo)cyst. The way in which the antibody is attached to the bead may also have an effect on recovery efficiency: if the attachment is weak, the antibody may detach and the oocyst will not be recovered.

The turbidity of the water concentrate appears to be the most critical factor associated with the recovery efficiency of immunomagnetic separation. Oocysts seeded into relatively clean suspensions are recovered efficiently—Campbell & Smith (1997) and Campbell, Gron & Johnsen (1997) have reported recoveries in excess of 90%. However, the real benefit of a good separation technique is seen with samples that have yielded a highly turbid concentrate; in these samples immunomagnetic separation seems to perform less efficiently. The use of antibodies of higher affinity may improve the recovery efficiency of oocysts from high turbidity samples. Although the technique is also able to separate *Giardia* cysts, little effort has been devoted to testing the recovery efficiency of these cysts by immunomagnetic separation.

*Flow cytometry.* Flow cytometry has been attempted with environmental samples to detect *Cryptosporidium* oocysts, but it was found that the sensitivity of the instruments was insufficient to distinguish oocysts from background noise (Vesey et al., 1991). However, incorporation of a cell-sorting facility enabled oocysts to be sorted efficiently from background material (Vesey et al., 1993a). This technique seems to work equally well for *Giardia* cysts (Vesey, Slade & Fricker, 1994; Medema et al., 1998a). Water concentrates are stained in suspension with fluorescein isothiocyanate-labelled (FITC-labelled) antibodies and passed through the fluorescence-activated cell sorter (FACS). Particles with the fluorescence and light-scattering characteristics of (oo)cysts are sorted from the sample stream and collected on a microscope slide or membrane filter, which is then examined by epifluorescence microscopy to confirm the presence of (oo)cysts. The FACS procedure is not sufficiently specific or sensitive for the count of sorted particles to give a definitive indication of the number of (oo)cysts present. Other organisms and particles of similar size may cross-react with the monoclonal antibody and have similar fluorescence characteristics. Moreover, some water samples contain high numbers of autofluorescent algae which may also mimic (oo)cysts and lead to incorrect conclusions if the FACS is used directly to produce (oo)cyst counts. However, the confirmation by epifluorescence microscopy can be performed

much more easily and reliably than direct microscopy of non-sorted samples. Several researchers from France, the Netherlands, and the United States have confirmed the benefits of FACS for examining water samples for the presence of (oo)cysts (Danielson, Cooper & Riggs, 1995; Compagnon et al., 1997; Medema et al., 1998a). FACS is widely used in the United Kingdom for water analysis and is becoming more widely adopted in other parts of Europe, in Australia, and in South Africa.

### **Detection**

*Immunofluorescence microscopy.* Detection of *Cryptosporidium* oocysts and *Giardia* cysts relies on epifluorescence microscopy, which may be used to examine material deposited on multi-well slides or membrane filters. The (oo)cysts are specifically stained with monoclonal antibodies which have been either labelled directly with FITC or labelled during staining with an FITC-labelled anti-mouse antibody. There have been no definitive studies to compare the efficiency of these procedures, but the tendency now is towards staining with a directly labelled antibody, which seems to produce less nonspecific binding and can make preparations easier to examine. Several anti-*Cryptosporidium* and anti-*Giardia* antibodies are commercially available; most workers have their own preferences, and there does not appear to be a single antibody that is preferred for all purposes. A failing of some antibodies, including commercially available antibodies, is that they apparently cross-react with other members of the genera and therefore cannot be used to specifically identify *C. parvum* or *G. intestinalis*.

A number of other detection techniques have been tried in an effort to improve the ease of identification of both *Cryptosporidium* oocysts and *Giardia* cysts.

*Fluorescence in-situ hybridization (FISH).* FISH has been suggested as a tool for the specific detection of *Cryptosporidium parvum* (Lindquist, 1997; Vesey et al., 1997). Vesey et al. (1997) also showed that the stainability of oocysts with the FISH method correlated with excystation. The FISH method could be combined with the immunofluorescent assay (IFA) method. However, the FISH-fluorescence signal is relatively weak, which makes microscopic interpretation difficult.

*Polymerase chain reaction (PCR).* One of the most extensively tested procedures is the polymerase chain reaction for detection of specific sequences of nucleic acids that may be species- or genus-specific. Clearly, the ability to distinguish between *C. parvum* and other morphologically similar members of the genus is useful and nucleic-acid-based techniques may prove useful for this.

Despite the specificity and sensitivity offered by PCR, however, difficulties have been experienced with application of the technique to water concentrates, largely because of inhibition of the DNA amplification process. PCR is sensitive to the concentration of many compounds within the reaction mixture; those of

particular concern to researchers working with water concentrates are divalent cations and humic and fulvic acids, which are frequently found in water and can cause a high degree of inhibition. Nonetheless many workers have described protocols for the detection of *Cryptosporidium* oocysts by PCR and a wide variety of primers have been described. These primers have been designed from various regions of the genome. Primers with apparent specificity include those from regions coding for the 18S rRNA (Johnson et al., 1995), or mRNA coding for the *Cryptosporidium* heat shock protein *Hsp70* (Stinear et al., 1996, Kaucner & Stinear, 1998), in combination with cell culture (Rochelle et al., 1996; Rochelle, 1997).

Abbaszadegan et al. (1997) first reported the use of PCR primers from gene sequences coding for inducible heat shock proteins to specifically detect *Giardia* cysts. The sensitivity of the standard PCR was reported to be one cyst in water samples. These workers also reported that amplification of heat-shock-induced mRNA using the same HSP primers was indicative of viable *Giardia* cysts.

The use of PCR for the detection of (oo)cysts in water concentrates offers some advantages over direct microscopic examination, since the procedure can be largely automated, allowing several samples to be handled simultaneously. Furthermore, the technique is, theoretically, sensitive down to the level of a single (oo)cyst, and recent developments have suggested that it may be possible to distinguish viable from non-viable (oo)cysts. Some workers claim to be able to detect a single oocyst in a water concentrate by using a procedure involving reverse transcription (RT-PCR) where the target sequence codes for the *Cryptosporidium* heat shock protein *Hsp 70* (Stinear et al., 1996). The data presented showed that a single viable oocyst could be detected even in the presence of PCR inhibitors. Such a method would be of considerable value to the water industry, facilitating rapid screening of samples; as yet, though, the method is not quantitative and thus may be of limited value in some circumstances.

The use of RT-PCR against induced mRNA, a nucleic acid with a short half-life, overcomes the concern that false-positive results could be obtained either from non-viable oocysts or from free DNA. Many researchers still favour a holistic approach, where the intact organism can be viewed directly. A combined approach might be possible, with molecular techniques being used as a screening tool on a portion of a water concentrate, followed by microscopic examination when positive results are obtained.

### **Methods for determining oocyst viability**

The significance of finding oocysts in treated and, to a lesser extent, raw waters is not always clear, since some of the organisms that are detected may be non-viable and thus pose no threat to public health. Consequently, there has been considerable interest in developing in vitro methods capable of determining oocyst viability.

*Excystation.* The most widely accepted in vitro procedure for determining oocyst viability, excystation, has not been used with the IFA method, because it is difficult to incorporate into the IFA protocol. However, it has been used in combination with PCR to detect the presence of viable *Cryptosporidium* oocysts (Filkorn, Wiedenmann & Botzenhart, 1994; Wiedenmann et al., 1997). The sensitivity of this method in environmental samples needs further research. Excystation has also been used in survival and disinfection studies; in the latter, the technique appears to yield a lower inactivation rate than the neonatal mouse infectivity assay (Finch et al., 1993a; Clancy et al., 1998).

*Vital dyes.* The ability of *Giardia* cysts to stain with the vital exclusion dye propidium iodide (PI) has been shown by various workers to correlate with their inability to excyst or infect animals (Schupp & Erlandsen, 1987; Smith & Smith, 1989). It is therefore possible to use PI as an indicator of cell death for *Giardia* cysts.

Campbell, Robertson & Smith (1992) developed a procedure for *Cryptosporidium* oocysts based on the exclusion of PI; they used 4'-diamidino-2-phenyl indole (DAPI) as supporting stain, which gave a good correlation with in vitro excystation. Four classes of oocysts can be identified using the assay: those that are viable and include DAPI but exclude PI, those that are non-viable and include both DAPI and PI, and two classes that include neither DAPI nor PI—those with internal contents (sporozoites) and therefore potentially viable, and those without and therefore non-viable, as determined by differential interference contrast (DIC) microscopy. The DAPI/PI procedure is simple to perform and, despite some workers' reservations about its applicability, can be used for routine environmental work. The incorporation of DAPI into the nucleic acid acts as a further criterion for determining whether a particle is an oocyst or not.

An alternative to the DAPI/PI approach for determining viability has been suggested by Belosevic & Finch,<sup>1</sup> who used new nucleic acid stains to differentiate between viable and non-viable oocysts. Two new stains have been identified. With SYTO9, non-viable oocysts stain green or bright yellow; a viable oocyst has a green halo but its interior remains unstained. MPR71059 stains non-viable oocysts red while viable oocysts remain unstained. These methods have not been widely tested, although Belosevic & Finch (1997) demonstrated that the results obtained with the dyes correlate well with mouse infectivity using an outbred CD-1 neonatal mouse model. Since these vital-stain assays are apparently simple and quick to perform, they may be suitable for incorporation into the methods for the detection of oocysts in water samples—but this has yet to be proven.

*Cell culture.* Attempts have been made to develop in vitro models of infectivity using tissue culture (Upton, Tilley & Brillhart, 1994; Rochelle et al., 1996; Slifko,

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<sup>1</sup> Presented at the International Symposium on Waterborne *Cryptosporidium*, March 1997, Newport Beach, CA, USA.

1997). For these assays, water samples are concentrated by normal procedures and bacteria may be removed by exposure of the concentrate to chlorine at levels that are lethal to bacterial cells but that are thought not to affect oocysts. The concentrates are then inoculated onto the tissue-culture monolayer and left in contact for a period to allow potentially infectious oocysts to infect cells before the remaining debris is washed away. The monolayer is then left for 24–48 hours before being examined for the presence of intracellular parasite antigen or nucleic acid. Immunofluorescent techniques have been used to identify cells that have become infected. This method offers a means of quantifying infection, although it is not clear whether the presence of a single infectious oocyst will lead to one or more infected cells. In theory, an oocyst that excysts successfully would be expected to produce 2–4 infected tissue culture cells (for *Giardia* and *Cryptosporidium* respectively), but initial results have not demonstrated that this can be consistently achieved.

Some workers (Rochelle et al., 1996) have adopted a somewhat different approach, detecting the presence of *Cryptosporidium* nucleic acids using PCR. While the cell culture method cannot be used to directly enumerate the oocysts present in any given sample, it can be applied in a “most probable number” format to give an estimate of the number of oocysts present in a water concentrate.

*Molecular methods.* The RT-PCR methods that amplify induced mRNA coding for heat shock proteins can also be used to indicate viability of *Giardia* cysts (Abbaszadegan et al., 1997) and *Cryptosporidium* oocysts (Stinear et al., 1996; Kaucner & Stinear, 1998). In combination with the reported sensitivity and specificity (see p. 89), these methods may in the future prove to be very valuable for the water industry.

### **Typing methods**

With the current detection techniques, it is not possible to identify the origin of (oo)cysts in a water sample. Several typing methods are available for both *Cryptosporidium* and *Giardia* and will discriminate between human and animal *C. parvum* strains (Ogunkolade et al., 1993; Bonnin et al., 1996; Deng & Cliver, 1998); however, these methods are not yet applicable to surface-water samples.

### *Cyclospora*

#### **Detection methods for stool samples**

No methods have been developed for the detection of *Cyclospora* in environmental samples; the information provided in this section on detection of the parasite in stool samples is therefore intended for guidance only.

Identification of *Cyclospora* in stool samples is based on the appearance of the oocyst in either direct or concentrated wet films. Concentration either by the formalin–ether (formalin–ethyl acetate) method or by sucrose flotation is effective. Oocysts have also been reported from jejunal aspirates (Bendall et al., 1993). Organisms seen in stool samples are normally the unsporulated oocysts of *Cyclospora* sp. By bright-field microscopy of wet mounts, oocyst walls appear as well-defined, non-refractile spheres of remarkably uniform size (diameter 8–10 µm) (Ashford, 1979; Long et al., 1991); within each oocyst is a central morula-like structure containing a variable number of inclusions. At higher (×400) magnification, the inclusions appear refractile, exhibiting a greenish tinge. Oocysts that are empty or that have collapsed into crescents are occasionally seen. Under UV illumination (330–380 nm) the oocyst wall autofluoresces so that the organisms appear as blue circles.

The organisms do not stain with Lugol's iodine. Staining of air-dried faecal smears with acid-fast stains can aid identification, and, according to Wurtz (1994), the rapid dimethyl sulfoxide-modified acid-fast staining method is more effective than either the Kinyouin or the modified Ziehl-Neelsen method. Oocysts stain variably with acid-fast stains, from no staining through to deep red. A modified safranin method (microwaving followed by safranin staining) stains oocysts a brilliant reddish orange (Visvesvara et al., 1997).

Sporulated oocysts contain two sporocysts and each sporocyst contains two crescent-shaped sporozoites. In instances where excystation has been achieved in vitro, by exposure of oocysts/sporocysts to an excystation medium at 37°C for up to 40 minutes, two sporozoites emerge from each sporocyst.

### **Concentration techniques for environmental samples**

No method has been developed specifically for the detection of *Cyclospora* sp. in environmental samples. However, *Cyclospora* sp. oocysts are larger than *C. parvum* oocysts and smaller than *G. intestinalis* cysts, and it is therefore assumed that methods developed for *Cryptosporidium* and *Giardia* will prove effective for sampling and recovering *Cyclospora* sp. oocysts from water concentrates.

### **Detection techniques for use in environmental samples**

There are no in vitro culture methods for increasing the numbers of *Cyclospora* sp. oocysts nor have any in vivo amplification models been described. A proportion of oocysts stored in faeces, water, or 2.5% potassium dichromate at temperatures between 22°C and 37°C for up to 14 days in the laboratory will sporulate (Ortega et al., 1993; Smith et al., 1997). Currently there is no commercially available polyclonal or monoclonal antibody with specificity to exposed epitopes on *Cyclospora* sp. oocysts; the autofluorescent properties of the oocyst wall under UV illumination have therefore been used in an attempt to detect

oocysts in a variety of food and water concentrates. The primers identified by Relman et al. (1996), which amplify the small subunit rRNA coding region, have been used to amplify the *Cyclospora*-specific sequence from nucleic acid liberated from the berries (strawberries and raspberries) implicated in a series of outbreaks in the USA in 1996. To date, however, no positive results have been reported.

## Control

*Cryptosporidium* and *Giardia* are ubiquitous in surface waters worldwide. Reported concentrations are generally in the range 0.01–100 per litre; these values are not corrected for the (low) recovery of the detection method, so actual concentrations may be more than 10-fold higher. Higher concentrations are found in urban or agricultural waters than in pristine waters (LeChevallier, Norton & Lee, 1991; Rose, Gerba & Jakubowski, 1991).

Sources of surface-water contamination are the discharge of untreated and treated sewage, manure, run-off from grazing land, and wildlife. The relative significance of these sources may differ between watersheds. Large rivers and lakes often receive both agricultural run-off and treated and untreated domestic wastewater, and their relative contribution has not been quantified. Contamination by wildlife may be important in pristine watersheds and has been implicated as a source of waterborne giardiasis, although this is still a matter of much controversy.

Oocysts and cysts can survive for months in surface water (DeReignier et al., 1989; Robertson, Campbell & Smith, 1992; Chauret et al., 1995; Medema, Bahar & Schets, 1997). Under natural conditions, the die-off rate of *Cryptosporidium* oocysts in water is 0.005–0.037 log<sub>10</sub>-units per day. For *Giardia*, the die-off rate is higher and more temperature-dependent, varying between 0.015 log<sub>10</sub>-units per day at 1 °C and 0.28 log<sub>10</sub>-units per day at 23 °C (DeReignier et al., 1989). Little information is yet available on the significance to the environmental ecology of the state in which (oo)cysts occur in water, i.e. suspended or attached to particles (although this is relevant for water treatment by sedimentation and filtration), or the fact that they readily attach to particles (Medema et al., 1998b).

Recent work showed that, overall, 12% of groundwater supplies in the US were contaminated with *Cryptosporidium* and/or *Giardia* (Hancock, Rose & Callahan, 1997), mostly in infiltration galleries and horizontal wells. No data on the level of protection and travel time and distance of these groundwater sources were given.

Prevention of the transmission of protozoan parasites through drinking-water requires a multiple barrier approach: protection of watersheds used for drinking-water production against contamination with protozoa, plus adequate treatment of water—and verification by monitoring of water quality and operational parameters that the treatment is effective.

## Watershed protection

One of the most important aspects of watershed protection is the recognition of local sources of contamination with *Cryptosporidium* and *Giardia* and the control of that contamination by diversion or treatment of discharges and reduction of direct input of faeces, especially in otherwise pristine waters, by people, farm animals, and wildlife or from manure storage. Treatment of sewage in activated sludge systems or waste stabilization ponds is an important barrier against environmental transmission: both processes remove 90–99.7% of the cysts and oocysts (Sykora et al., 1991; Grimason et al., 1992).

Treatment of agricultural wastes before their application to the land also reduces the number and viability of *Cryptosporidium* oocysts: aerobic treatment of cattle slurry at increased temperatures and ammonia concentrations rapidly inactivates oocyst (Svoboda et al., 1997) and composting of bedding reduces the viability of oocysts.

Storm run-off and snowmelt from unprotected watersheds have been implicated as sources of peak contamination of source water (Stewart et al., 1997; Atherholt et al., 1998), and may result in treatment overload and the contamination of drinking-water with (oo)cysts. Knowledge of the characteristics of the plume of contamination from watershed sources can be used to locate and design abstraction points. An illustration of the importance of this is provided by the intake of the southern plant of Milwaukee in Lake Michigan, which proved to be exactly in the plume of the Milwaukee river. The turbidity in the raw water peaked and this coincided with treatment failure, resulting in the breakthrough of turbidity and oocysts into Milwaukee drinking-water and a consequent massive outbreak of disease (MacKenzie et al., 1994). Installation of pretreatment storage reservoirs flattens peak contaminations (Ketelaars et al., 1995) and the storage capacity makes it possible to stop the intake of surface water temporarily during high contamination events (see “Pretreatment reservoirs”, page 96).

Since the protozoa are typically related to faecal contamination of surface water, several studies have investigated the use of indicator bacteria to predict high levels of protozoa. No consistent relationship has been observed, however, between indicator bacteria (thermotolerant coliform) levels and concentrations of *Giardia* or *Cryptosporidium*. The low and varying recovery rates of the protozoa detection methods may be an important confounder in detecting these relationships. Since (oo)cysts are much more persistent than coliforms and enterococci in water, it is likely that these bacteria are not valid indicators, especially if the contamination source is distant. More persistent bacterial indicators (spores of *Clostridium perfringens*) may prove useful indicators for these protozoa (Payment & Franco, 1993; Hijnen et al., 1997). In the absence of valid surrogates, watershed assessment to determine local sources of contamination and define the amount of treatment necessary should include monitoring for protozoa.

Development of transport and fate models for predicting (oo)cyst concentrations based on data on the sources may help in identifying important sources

or environmental events that determine protozoa levels at abstraction points (Medema et al., 1997).

The number of species of *Cyclospora* that are infective to human beings is not currently known, nor is it known whether human-derived oocysts are infectious to non-human hosts. However, the primary sources of contamination will be human faeces containing oocysts. Because the oocysts of *Cyclospora* sp. are larger than those of *Cryptosporidium parvum* but smaller than *G. intestinalis* cysts, it is likely that they will be discharged with final effluents from waste stabilization ponds and sewage treatment works. Oocysts take up to 14 days to mature (sporulate) in the laboratory, but sporulate more rapidly at higher (up to 37°C) temperatures. Sporulation time in the environment will thus depend upon ambient temperature, and sporulated oocysts may be found distant from the pollution source in the aquatic environment. Sources of unsporulated oocysts are likely to be effluent discharges from sewage treatment and waste stabilization ponds with detention times of less than 1 week.

Like *C. parvum* oocysts and *G. intestinalis* cysts, oocysts of *Cyclospora* sp. are likely to survive longer at lower temperatures when suspended in water. Oocysts stored at 4°C do not appear to sporulate (Smith et al., 1997); however, a proportion of oocysts stored for up to 2 months at 4°C will sporulate when subsequently incubated at temperatures between 22°C and 37°C. No data are available regarding survival and transport in soil.

## Adequate treatment

### **Filtration**

The principal barrier for protozoa is physical removal by filtration. *Cryptosporidium* oocysts are relatively small, making them more difficult to remove than *Giardia* cysts. Rapid sand filtration, a common treatment process used to remove particles, is theoretically capable of 3 log removal of *Cryptosporidium* oocysts (Ives, 1990). Other investigators have published a range of removal rates, from 91% (Rose et al., 1986) to greater than 99.999% (Hall, Pressdee & Carington, 1994); the higher removal rates were achieved when coagulant dosing was applied to the water before filtration.

Diatomaceous earth filtration has been reported to remove more than 99% of *Giardia* cysts (Jakubowski, 1990) and up to 4–6 log-units for *Cryptosporidium* under laboratory conditions (Ongerth & Hutton, 1997).

Conventional treatment (coagulation, sedimentation, filtration), direct filtration (with chemical pretreatment), and high-rate filtration can remove 99% of (oo)cysts when systems are properly designed and operated (LeChevallier, Norton & Lee, 1991; Nieminski, 1994; West et al., 1994). Typically the chemicals used are ferric or aluminium salts and there appears to be no real difference in the effectiveness of aluminium sulfate, polyaluminium chloride, ferric sulphate, and ferric chloride in removing oocysts and other particles of similar size (Ives, 1990).

If filters are backwashed, the backwash water may contain high levels of (oo)cysts (Richardson et al., 1991). Ideally, backwash water should be discarded, but in many circumstances this is uneconomical. If it is recycled, treatment with coagulation and sedimentation or microfiltration will reduce recontamination of the water with (oo)cysts. Where such treatment is not feasible, it is recommended that the recycled water is returned at a constant, low rate (Rose, Lisle & LeChevallier, 1997).

Slow sand filtration will efficiently remove (oo)cysts, but efficiency is reduced at lower temperatures. No data are available for removal of oocysts in full-scale plants but a number of pilot-scale studies have been completed in which the removal efficiencies were generally good. Hall, Pressdee & Carrington (1994) achieved removal of better than 99.95%. In another study, heat-inactivated oocysts were added to surface water at a concentration of 4000/litre before filtration; no oocysts were found in the filtrate. At the end of the study, intact oocysts were found only in the upper 2.5 cm of the sand filter (Timms, Slade & Fricker, 1995).

Micro- and ultrafiltration can remove over 99.99% (Jacangelo et al., 1991; Adham, Jacangelo & Laine, 1994; Drozd & Schwartzbrod, 1997) as long as the integrity of the system is maintained.

### **Soil passage**

Soil passage, used in bank filtration and infiltration, is probably an effective physical barrier against (oo)cysts. Its effectiveness depends on travel time and distance and on the composition of the soil (Mawdsley, Brooks & Merry, 1996).

### **Pretreatment reservoirs**

Storage in reservoirs with a residence time of 5 months can reduce (oo)cyst concentrations by 99% (Ketelaars et al., 1995). Experimental evidence suggests that sedimentation of *Cryptosporidium* oocysts and *Giardia* cysts is unlikely to have a significant effect on their removal from a body of water unless they are attached to other particles (Medema et al., 1998b). Installation of pretreatment storage reservoirs also flattens peak contaminations (Ketelaars et al., 1995) and, because of the storage capacity, it is possible to stop the intake of surface water temporarily during high contamination events.

### **Disinfection**

Disinfection with chlorine has always been an important means of preventing transmission of waterborne pathogens. High resistance to chlorine disinfection, especially of *Cryptosporidium* oocysts (Korich et al., 1990; Smith et al., 1990; Ransome, Whitmore & Carrington, 1993), however, makes the process ineffective for oocyst inactivation in drinking-water. Chlorine dioxide is slightly more effective, but still requires a high *CT* value (concentration (residual) of disinfectant *C*

× contact time  $T$ ) of 78 mg·min/litre for 90% inactivation of oocysts (Korich et al., 1990). *Giardia* is less resistant to chlorine: 99.99% reduction can be achieved with a  $CT$  of 180–530 mg·min/litre, depending on the temperature and pH of the water (Hibler et al., 1987). At  $CT$  values of 4.7–28 mg·min/litre chlorine dioxide reduces *Giardia* by 99% (Leahy, Rubin & Sproul, 1987; Rubin, 1988).

Ozone is the most potent agent against (oo)cysts: at 20 °C, the  $CT$  for 99% inactivation of *C. parvum* oocysts is 3.5 mg·min/litre (Finch et al., 1993a) and for *G. intestinalis* cysts 0.6 mg·min/litre (Finch et al., 1993b). The effectiveness of ozone decreases at lower temperatures. Peeters et al. (1989) found that a residual ozone concentration of 0.4 mg/litre for 6 minutes was sufficient to kill 10 000 oocysts/ml, while Korich et al. (1990) demonstrated that 1 mg/litre for 10 minutes at 25 °C would result in a reduction in viability of 99%. Parker, Greaves & Smith (1993) reported that 3 mg/litre for 10 minutes killed all oocysts, and similar results were quoted by Ransome, Whitmore & Carrington (1993). Hence, the  $CT$  values required for inactivation of cysts and oocysts are high. Exposure of *Cryptosporidium* oocysts to multiple disinfectants has been shown to be more effective than was to be expected from any single disinfectant (Finch, Kathleen & Gyurek, 1994; Liyanage et al., 1997) and synergism between environmental stresses during sand filtration has also been observed (Parker, Greaves & Smith, 1993). The multiple stresses encountered by (oo)cysts in the environment and during treatment might limit the infectivity of (oo)cysts.

Conventional UV systems have a limited effect on *Cryptosporidium* and *Giardia* viability: doses of 110–120 mJ/cm<sup>2</sup> result in 99% inactivation of *C. parvum* oocysts (Ransome, Whitmore & Carrington, 1993), as assayed by in vitro viability methods, and 97% of *G. intestinalis* cysts (Rice & Hoff, 1981). In a recent animal infectivity study, Clancy et al. (1998) showed that pulsed and advanced UV are much more effective against *Cryptosporidium*; they obtained 99.98% inactivation at UV doses as low as 19 mJ/cm<sup>2</sup>.

The results of laboratory disinfection experiments should be translated with caution to the full-scale treatment of environmental (oo)cysts. In surface-water treatment, (oo)cysts may be protected from the disinfectant because they are attached to colloids. On the other hand, (oo)cysts that have been exposed to environmental stressors may be more susceptible to disinfectants (Parker, Greaves & Smith, 1993). Moreover, the design and operation of full-scale treatment systems will, in general, be such that lower rates of inactivation are obtained than in the laboratory setting.

The removal of *Cryptosporidium* oocysts and *Giardia* cysts by various well designed and properly maintained and operated treatment processes is summarized in Table 6.

Little information is available regarding the ability of water-treatment processes to remove or inactivate oocysts of *Cyclospora* sp. At 8–10 µm diameter these are larger than *C. parvum* oocysts but smaller than *G. intestinalis* cysts, and it is likely that physical removal will be similar to that achieved with *Giardia* and *Cryptosporidium*. In an outbreak in Nepal, filtration and chlorination did not

**Table 6. Removal of *Cryptosporidium* oocysts and *Giardia* cysts by treatment processes**

Type of process	Removal efficiency (log <sub>10</sub> -units)		Most important efficiency- determining factors
	<i>Cryptosporidium</i>	<i>Giardia</i>	
<i>Disinfection processes</i>			
Chlorine	0	0–2	Dose, contact time, installation design, disinfectant demand, temperature, pH (especially for chlorine), formation of toxic by-products, synergism of multiple disinfectants.
Chloramines	0	0–2	
Chlorine dioxide	0	0–2	
Ozone	0–2	1–4	
Ultraviolet light	0–4	0–4	Dose at 254 nm, turbidity, solutes, system
<i>Filtration processes</i>			
Rapid sand	0–1	0–1	Filtration rate, recycling of backwash water
Slow sand	1.2–>3.7	1.2–>3.7	Presence of "Schmutzdecke", filter depth
Diatomaceous earth	2–6	2–6	Filtration rate, filter depth, pore size, precoat thickness, filter integrity
Membrane	2–>4	2–>4	System integrity, membrane type
Coagulation/filtration	2–2.5	2–2.5	Coagulant dose, pH, temperature, installation
<i>Other processes</i>			
Soil passage	>2–>5	>2–>5	Soil composition, residence time, travel distance, presence of sediment
Reservoir storage	0.5–2	0.5–2	Residence time, reservoir design, addition of coagulants, temperature

affect the integrity of the oocysts (Rabold et al., 1994): although chlorine residuals remained at acceptable levels (0.3–0.8 ppm) and no coliform indicator bacteria were detected, *Cyclospora* sp. oocysts were found in the drinking-water supply. Little is known about survival of these oocysts in different environments or about treatments that will effectively inactivate them.

### Risk assessment for the design of adequate treatment

One of the key issues in water treatment is to determine the level of treatment that is adequate, and this requires that the maximum acceptable concentrations in drinking-water of the pathogen(s) concerned be established. These could be derived from the maximum acceptable risk and the dose–response relationship for the parasites. An infection risk of 10<sup>-4</sup> per year has been suggested as acceptable for pathogens in drinking-water (Regli et al., 1991). The maximum concentrations of viable (oo)cysts in drinking-water to meet this risk level are very

low (Rose, Haas & Regli, 1991; Rose, Lisle & LeChevallier, 1997). Since current techniques do not allow an evaluation of compliance with these concentrations to be evaluated, safeguarding of drinking-water should ideally involve a quantitative description, or assumed values of the protozoa concentrations in the source water and knowledge of the removal efficiency of the treatment steps.

Surface-water utilities, and groundwater utilities that may be contaminated by surface water or from other sources, should ensure that treatment processes adequate to achieve effective removal are in place. Additionally, the rate of protozoa removal and inactivation achieved in the treatment plant should be determined wherever possible, in order to establish whether acceptable concentrations of protozoa in drinking-water have been achieved. Effective protection of public health also requires control of recontamination in distribution systems and in households and management of backwash water and first flow water after RSF backwashing and after SSF skimming, which are critical control points.

### Verification of efficiency of parasite removal

For routine monitoring, verification of treatment performance requires water-quality and process parameters. Several parameters have been suggested as surrogates for (oo)cyst removal by filtration processes: turbidity, particle counts (LeChevallier & Norton., 1992; Hall & Croll, 1997), clostridial spores (Payment & Franco, 1993; Hijnen et al, 1997) or aerobic spores (Niemiński, 1997), and particulate matter (USEPA, 1997). Although turbidity or particle counts of filtered water depend both on the levels in raw water and on filter performance, turbidity of 0.1–0.5 NTU or counts of fewer than 50/ml for particles larger than 3 µm below are indicative of good quality water. Direct and continuous information on (individual) filter performance is provided by on-line monitoring of turbidity or particle counts, making these very valuable tools for optimizing treatment efficiency for (oo)cyst removal.

Critical points in the filtration cycle are just after backwash or, in the case of slow sand filtration, scraping of the clogged top-layer from the filterbed. A slow increase in filtration rate or filtering-to-waste minimizes the risk of (oo)cyst breakthrough.

Disinfectant dose, contact time, residual disinfectant concentration at the end of the contact time, pH, and temperature are commonly used to monitor the performance of disinfection processes. The most critical conditions for disinfection processes are low temperatures and high turbidity in the water to be treated.

## Conclusions and recommendations

### Health risk assessment

The number and extent of outbreaks of waterborne disease in developed countries show that transmission of *Giardia* and *Cryptosporidium* by drinking-water

is a significant risk. In the case of *Cryptosporidium*, the absence of an adequate cure for immunocompromised patients increases the problem. Although the outbreaks in developed countries receive most attention, both outbreaks and low-level transmission of protozoa through drinking-water are likely in developed countries and developing countries alike (Fraser & Cooke, 1991; Isaac-Renton, Moorehead & Ross, 1996). Cysts and oocysts are regularly found in drinking-water (Isaac-Renton, Moorehead & Ross, 1996; Karanis & Seitz, 1996; Rose, Lisle & LeChevallier, 1997), although only a small proportion may be viable and infectious to man. A major drawback for the determination of the health significance of (oo)cysts in (drinking) water is that there are no detection methods that are both sensitive and specific for infectious (oo)cysts, and that provide a consistently high recovery.

### Risk management

The protozoa, and to a lesser extent the viruses, have initiated a change in philosophy towards safeguarding of drinking-water from monitoring of the “end-product” drinking-water to monitoring of raw water and of treatment efficiency. The extreme resistance of some protozoa implies that a “zero risk” is no longer achievable. Measures should be designed to reduce (oo)cyst concentrations in raw water as far as possible, and treatment should preferably include filtration step(s). This implies that information on parasite concentrations in the raw water is necessary, as well as information on the removal efficiency of treatment processes. Quantitative risk assessment provides a tool for combining information on raw water quality (concentrations detected, recovery of the detection method, viability) and treatment efficiency (removal by different steps in the treatment) (Teunis et al., 1997).

The definition of maximum acceptable concentrations of pathogens in drinking-water based on a maximum acceptable (infection) risk level has become possible with data from studies in volunteers and from dose–response models (Haas, 1983; Dupont et al., 1995; Teunis et al., 1996). An annual infection risk level of  $10^{-4}$ , as proposed by the United States Environmental Protection Agency, is currently used in Canada (Wallis et al., 1995), the Netherlands (Medema et al., 1995), and the USA (Rose, Lisle & LeChevallier, 1997) as the basis for determining the appropriate removal efficiency of surface-water treatment systems.

Current detection methods are generally sufficiently sensitive to determine the concentrations of *Cryptosporidium* and *Giardia* in surface water, but are often insufficiently sensitive for an accurate description of removal efficiency, which may require additional data from laboratory studies and (seeded) pilot plant studies. An alternative approach is to determine whether an adequate surrogate parameter can be found to describe removal efficiency for *Cryptosporidium*. (*Giardia* is easier to eliminate by both disinfection and filtration, and the description of treatment efficiency should therefore be targeted on *Cryptosporidium*.) Several parameters—aerobic spores, clostridial spores, particles, and algae—have

been evaluated on a limited scale as surrogates for protozoa removal, but a broader evaluation is necessary to determine their value.

*Cryptosporidium* poses a serious health risk to immunocompromised individuals, especially AIDS patients. All at-risk individuals should be made aware of the dangers and informed about means of avoiding exposure to (potentially) contaminated water. Boiling of tap water, use of mineral or bottled water, and not swimming in surface water or pools are some of the options for preventing exposure. Local considerations play a major role and public health authorities are encouraged to provide guidance on the safety of drinking-water for the immunocompromised and on appropriate means of reducing exposure (Anon, 1995; Juranek, 1995).

When an outbreak occurs, its size and source should be rapidly investigated and control measures implemented to prevent further transmission. Useful guidance on management of waterborne outbreaks can be found in the report of the UK group of experts (Badenoch, 1990), the CDC Guidance Manual (Juranek, 1995), and a workshop report (Anon, 1995).

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