Protozoan parasites
(Cryptosporidium, Giardia, Cyclospora)

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**WATERBORNE PROTOZOAN PARASITES**

Several species of parasitic protozoa are transmitted through water. Of these, *Giardia intestinalis* and *Entamoeba histolytica/dispar* are long recognised as the most common intestinal parasites throughout the world. Morbidity and, in particular for *E.histolytica/dispar*, mortality rates are high, especially in non-industrialised countries. More information on *Entamoeba* can be found in volume 2 of the WHO Guidelines for Drinking Water Quality and in the informal consultation document on enteric protozoa (1994).

A wide variety of free-living amoebae is capable of multiplication in (drinking) water, but only few species have been identified as pathogenic for man. These are *Naegleria fowleri* and *Acanthamoeba* spp. *Naegleria fowleri* can be present in thermally polluted waters and sporadically causes lethal primary amoebic meningoencephalitis. Only one outbreak has been related to a drinking water supply system (Marshall et al., 1997). *Acanthamoeba* can be found in the entire aquatic environment. It sporadically causes keratitis in contact lens wearers 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 of home-made lens cleaning solutions containing *Acanthamoeba* (Seal et al., 1995). *Acanthamoeba* has also been suggested as a vehicle for environmental transmission of *Legionella* bacteria (Campbell et al., 1995).

The increasing population of severely immunocompromised people, due to the AIDS epidemic, cancer chemotherapy and organ transplants, has increased the prevalence of opportunistic infections and has led to the recognition of the disease causing potential of other intestinal protozoan parasites, such as *Cryptosporidium parvum*, *Cyclospora* sp. and *Microsporidia* as human pathogens.

The first human cases of cryptosporidiosis were reported in 1976 (Meisel et al., 1976; Nime et al., 1976) and *Cryptosporidium* was first thought to be an opportunistic pathogen of immunocompromised persons. The recognition of frequent cases in immunocompetent individuals and a number of waterborne outbreaks have changed this image. *C. parvum* is now recognised as one of the most commonly identified intestinal pathogens throughout the world. It’s relative occurrence is dependent on factors such as age and other demographic characteristics of the study population and season. In children at the age of 1-5 with diarrhoea, it can be the most frequently found pathogen (Palmer, 1990). *Cyclospora* sp. has recently been recognised as a waterborne pathogen. Initially, it was referred to as cyanobacterium-like bodies but is now classified as *Cyclospora* sp. (Bendall et al., 1993; Ortega et al., 1993). It has been associated with several waterborne outbreaks world-wide.

*Microsporidia* are a large group (almost 1000) of species and are widely distributed in nature (Casemore, 1996). Although they were recognised as pathogens in fish, birds and some mammals, several species have recently been identified as cause of disease in severely immunocompromised humans. These species have primarily been associated with infections of the intestinal tract, but
dissemination to the biliary, urinary and respiratory tract may occur. 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. Waterborne transmission has not been demonstrated, but their persistence in water, resistance to disinfection and small size (some as small as 1-2 µm) suggest that this must be considered possible, especially for immunocompromised individuals.

Toxoplasma gondii is a coccidian parasite that has long been recognised as human pathogen. It is an intracellular parasite with felines as the definitive host. These are infected primarily by the consumption of infected mammals and birds, that act as secondary hosts. In these secondary hosts, the parasite settles itself as tissue cysts in muscle and brain tissue. Only felines carry the parasite in their intestinal tract and shed oocysts that sporulate in the environment. The oocysts are 10-12 µm 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 (cat litter, sand boxes) are the primary sources of Toxoplasma infections in humans (Guy, 1996). Two waterborne outbreaks have been reported. Both were believed to have been derived from contamination of water by cat faeces.

This review focuses on Cryptosporidium parvum, Giardia intestinalis and Cyclospora sp., since these are the parasites of primary concern to drinking water supply and a large amount of information on waterborne transmission has accumulated from recent research.

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

Oocysts of Cryptosporidium and cysts of Giardia are ubiquitously present in the aquatic environment. They have been found in most surface waters, their concentration being related to the level of faecal pollution or human use of the surface water (Hansen & Ongerth, 1991; LeChevallier et al., 1991). The environmentally robust (oo)cysts are very persistent in water (DeReignier et al., 1989; Robertson et al., 1992; Chauret et al., 1995) and are extremely resistant to the disinfectants commonly used in drinking water treatment (Hibler et al., 1987; Korich et al., 1990; Finch et al., 1993ab). These characteristics, coupled with the low numbers of (oo)cysts required for an infection (Rendtorff, 1954; Dupont et al., 1995; Okhuysen et al., 1998) make these organisms the most critical pathogens for the production of safe drinking water from surface water with disinfection and for post-treatment contamination. Well protected groundwaters, that are not under the influence of surface water or other sources of contamination, are free of these (and other) enteropathogens. If abstraction, treatment and distribution of these waters are properly designed and operated, the risk of faecal contamination is very low and they will not be a source of waterborne transmission of parasitic protozoa. Groundwaters that are under the influence of surface water or other contamination sources (surface run-off) can be contaminated with, low levels of, Cryptosporidium and Giardia (Hancock et al., 1997) and cause waterborne illness (Craun et al., 1998). Treatment of these waters with disinfection alone offers no protection against
Cryptosporidium and only limited protection against Giardia. Hence, filtration of these waters is necessary to produce safe drinking water. Many waterborne outbreaks of giardiasis and cryptosporidiosis have been reported in industrialised 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, (increased) contamination of the source water and leakage into the distribution system. In a significant number of these outbreaks, the drinking water that was implicated as the cause of the outbreak complied with the WHO-guidelines for Escherichia coli levels and turbidity (Craun 1990; Craun et al., 1998). In most outbreaks, deviations from normal raw water quality or treatment operation could be identified. However, in a drinking waterborne outbreak in Las Vegas, no abnormalities in operation or water quality (raw or treated) were detected (Goldstein et al., 1996). The occurrence of outbreaks in the absence of a warning signal from the routine water quality monitoring for coliforms that the water may be contaminated is a severe shortcoming of the coliforms as indicator for microbiologically safe drinking water, which calls for additional means to safeguard drinking water.

THE PARASITES AND THE DISEASE

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 tract of vertebrates. Several species of Cryptosporidium have been described. These species 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 tropical fish. Infections in humans are almost exclusively caused by C. parvum. This species is also frequently found in infections of cattle and sheep and causes infections in a wide range of other mammal species.

Life-cycle
Infected hosts shed oocysts, the environmentally resistant transmission stage of the parasite, with their faeces (Fayer & Ungar, 1986, Fayer et al., 1997). These oocysts are immediately infectious and may remain in the environment for very long periods without losing their infectivity, due to a very robust oocyst wall that protects the four sporozoites against physical and chemical damage. When the oocyst is ingested by a new host, the suture in the oocyst wall opens (excystation), triggered by the body temperature and the interaction with stomach acid and bile salts. Four motile sporozoites are released that infect the epithelial cells of the small intestine, mainly in the jejunum and ileum. The parasite infects the apex of the epithelial cells and resides beneath the cell.
membrane of the epithelial cells but outside of the cytoplasm. The sporozoites transform into several life stages in an asexual (merogony) and a sexual reproduction cycle (gametogony). The oocysts are the result of the sexual reproduction cycle. Oocysts of C. parvum are spherical with a diameter of 4-6 µm. Thick- and thin-walled oocysts are formed. The thin-walled oocysts may excyst within the same host and start a new life cycle (autoinfection). This may lead to a heavily infected epithelium of the small intestine, resulting in malabsorptive or secretory diarrhoea. The thick-walled oocyst is excreted with the faeces and is environmentally robust.

Pathogenicity
Infection studies with healthy human volunteers show a very good relation 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%. This probability increased to 100% at 1000 oocysts. 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 of infection (of 0.5%). Although there was a clear dose-response relation for infection, occurrence of symptoms of intestinal illness in the volunteers was not dose related. Recent studies indicate that the relation between oocyst dose and probability of infection and illness varies between C. parvum strains (Chappell, pers. comm).

The disease
The average incubation period is around 7 days, but shows a strong variation (Ungar, 1990; Dupont et al., 1995). Watery diarrhoea is the most prominent symptom of an intestinal infection with C. parvum (Fayer & Ungar, 1986; Ungar, 1990). Frequent and voluminous bowel movements can cause dehydration and weight loss (Arrowood, 1997). Other symptoms are nausea, abdominal cramps, vomiting and mild fever. MacKenzie et al. (1994) compared clinical data from cases detected by (passive) laboratory surveillance with cases detected by (active) telephone surveys during the 1993 Milwaukee waterborne outbreak, which involved 400 000 patients. Patients who submitted a stool sample for laboratory diagnosis suffered more serious disease, as manifest from the higher prevalence of the following complaints in these patients: fatigue, loss of appetite, nausea, fever, chills and sweats, and vomiting. In immunocompetent persons, the infection is limited by the immune response that eventually clears the host of the parasite. The occurrence of persistent and heavy infections in patients with deficiencies in the cellular (AIDS, chemotherapy, congenital) or humoral (congenital hypogammaglobulinaemia) immune responses suggests that both types of immune response are needed to limit and clear the infection. Several animal studies suggest that the immune response results in protection against re-infection (Zu et al., 1992). Protective immunity in humans is suggested by the high rates of asymptomatic carriage in
countries with a high prevalence of cryptosporidiosis. Also, infected volunteers
that were rechallenged with the same strain one year after the initial infection
were significantly less sensitive to (re)infection (Okhuysen et al., 1998).
However, the rates of diarrhoea were similar in both exposures, but the illness
was less intense in the re-infected volunteers, which indicates some degree of
protective immunity.

The duration of the infection is generally 7-14 days for the immunocompetent,
but also 23-32 days have been reported as median duration of the infection (van
Asperen et al., 1996). The peak intensity of oocysts 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 the infected persons, 42%
shed oocysts for at least 3 weeks and 21% for at least 4 weeks (Baxby et al.,
1985). Again, there is a difference between cases from laboratory surveillance
duration 2-4 weeks) and cases in the general population (duration typically 3-6
days). Relapses of diarrhoea are commonly seen, both population based
(outbreak) studies and in volunteer experiments report 1-5 additional episodes in
40-70% of patients. This phenomenon considerably increases the mean duration
of disease and its variability.

The mortality in immunocompetent patients is generally low. In immunodeficient
persons however, the infection can be persistent and severe (Ungar, 1990)
resulting in very profuse diarrhoea that leads to severe dehydration. Severe
infections have been reported in patients with concurrent infections (AIDS, but
also measles, chicken pox), persons with congenital immune deficiencies,
persons receiving immunosuppressive drugs (for cancer therapy, transplants or
skin lesions) and malnourished persons (Fayer et al., 1997). Also, pregnancy
may predispose to Cryptosporidium infection (Ungar, 1990). The prevalence of
cryptosporidiosis in AIDS patients in industrialised 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). Despite extensive effort, no
consistently effective therapeutic agent has been found (Blagburn & Soave,
1997). Immunotherapy with monoclonal antibodies or hyperimmune bovine
colostrum have been reported to resolve diarrhoea in AIDS patients at least
temporarily (Riggs, 1997). Similar findings were reported for several
chemotherapeutic agents (azithromycin, paromomycin) (Blagburn & Soave,
1997).

The severe dehydration, the spread of the infection and the lack of an effective
therapy lead to a high mortality in immunodeficient patients. Accurate data are
lacking. In one study in the UK, 19% of the AIDS patients with cryptosporidiosis
were suspected to have died from the infection (Connolly et al., 1988). A
compilation of case reports of cryptosporidiosis resulted in 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 gastro-enteritis, the reported prevalence of Cryptosporidium is 1-4% in Europe and North America and 3-20% in Africa, Asia, Australia, South and Central America (Current & Garcia, 1991). Peaks in the prevalence in developed countries are observed in the late summer (van Asperen et al., 1996) and in spring (Casemore, 1990). Asymptomatic carriage, as determined by stool surveys, generally occurs at very low rates in industrialised countries (<1%) (Current & Garcia, 1991), although in day care centres higher rates have been reported (Lacroix et al., 1987; Crawford & Vermund, 1988; Garcia-Rodriguez et al., 1989). Routine bile endoscopy suggests a higher asymptomatic prevalence: 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-industrialised countries (Current & Garcia, 1991). Seroprevalence rates are generally higher than faecal carriage rates, from 25-35% in industrialised countries up to 95% in South America (Casemore et al., 1997). Seroprevalence rates increase with increasing 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 exposure 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 these outbreaks in the households of the attending children. Also sexual practices that imply oro-anal contact yield a high risk for exposure to Cryptosporidium. Transmission from animals (mammals) to man occurs, especially from newborn animals. Many infections have been derived from contact with infected calves and lambs (Casemore, 1990). Also pet animals can be infected with oocysts, but appear to be no important source of human infection (Casemore et al., 1997; Glaser et al., 1998). Indirect person-to-person or zoonotic transmission may occur by contamination of water used for recreation (swimming pools) or drinking or by food (raw milk and meat, farm-made apple cider) (Casemore et al., 1997).

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

Drinking-waterborne outbreaks have been caused by contamination of the source water due to heavy rainfall or melting snow (Richardson et al., 1991; Pett et al., 1993; MacKenzie et al., 1994) or to sewage contamination of wells (d’Antonio et al., 1985; Kramer et al., 1996), inadequate treatment practices (Richardson et al., 1991; Craun et al., 1998) or treatment deficiencies (Anon., 1990; Leland et al., 1993; Craun et al., 1998) or combinations of these factors (MacKenzie et al., 1994). Also, leakage or cross-connections in the distribution system have caused outbreaks of cryptosporidiosis (Craun, 1990; de Jong & Andersson, 1997; Craun et al., 1998). The number of people affected by a
Cryptosporidiosis outbreak through drinking water ranges from several up to 400,000. During several of these outbreaks, oocysts were detected in the drinking water in a wide range of concentrations (Haas & Rose, 1995). Examination of drinking water during outbreaks is usually too late to determine the concentrations 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 detected concentrations are probably an underestimation of the concentrations that led to the outbreak, although Haas & Rose (1994) showed for the Milwaukee outbreak that, with some assumptions, the measured concentration in drinking water was close to the predicted concentration on the basis of the attack rate, water consumption and dose-response relation.

Low oocyst concentrations in drinking water have also been found in situations where no evidence for the occurrence an outbreak was present (LeChevallier et al., 1991; Karanis & Seitz, 1996; Rose et al., 1997; McClellan, 1998). Current detection methods do not allow the determination of pathogenicity of oocysts in water, which makes it difficult to determine the significance of low oocyst levels in drinking water. Given this uncertainty, detection of oocysts in treated water should always lead to the use of additional tests to confirm the presence of (viable) C. parvum oocysts and a thorough examination of other water quality parameters that may indicate a faecal contamination event. If these additional tests indicate the presence of C. parvum oocysts, this should lead to an epidemiological study to determine if significant waterborne transmission occurs and careful examination of the source of the contamination and the installation of control measures (improved source protection and/or water treatment).

**Giardia intestinalis**

**Taxonomy**

Giardia is a flagellated protozoan. The taxonomy and host specificity of this organism have been and still are matter of much debate. Giardia 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 in 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). Recently, a morphologically distinct Giardia was isolated from the straw-necked ibis (Forshaw et al., 1992), that was later suggested to be a distinct strain of G. ardae (McRoberts et al., 1996).

Giardia is thought to be predominantly asexual, which makes the species concept difficult to apply.
A high degree of genetic heterogeneity is found in human and animal isolates (Nash et al., 1985; Andrews et al., 1989; Meloni et al., 1989; Morgan et al., 1994) which makes speciation uncertain and suggests that it 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 if and how this heterogeneity is related to host specificity and pathogenicity of Giardia.

**Life-cycle**

Giardia has a simple life cycle (Feely et al., 1990; Meyer, 1994). As with Cryptosporidium, the parasite is shed with the faeces as environmentally robust cyst, that is transmitted to a new host. In the duodenum of the new host, the trophozoite emerges from the cysts and completes a mitotic division to produce two trophozoites that attach to the epithelial cells by their adhesive disc and feed on the epithelial cell. The trophozoites detach from the epithelial cells, probably because these cells have a rapid turnover (72 hours) and undergo mitotic division in the intestinal lumen. During periods of diarrhoea, these trophozoites may be transported with the intestinal contents and are excreted. The trophozoites do not survive long outside the host. During the passage through the intestine, part of the trophozoites begin to encyst and leave the host with the faeces as cysts. Cysts are more often encountered in formed stools. Giardia intestinalis cysts are elliptical and 8-12 µm long and 7-10 µm wide. The cyst wall is 0.3-0.5 µm thick and has a fibrillous structure. In the cyst, two to four nuclei can be found together with axonemes of the flagella of the trophozoite.

**Pathogenicity**

Human feeding studies with G. intestinalis cysts produced a dose response relation between the probability of infection and the ingested cyst dose (Rendtorff, 1954). 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 dose-infection relation could be described with an exponential model (Rose et al., 1991b): \( P_i = 1 - e^{-0.0199\times\text{dose}} \) (95% CI of r: 0.0044-0.0566). Although overall 53% of the volunteers became infected in this feeding study, and changes in bowel motions were observed, none of the volunteers developed symptoms of giardiasis. The infection-to-illness ratio varies between different isolates, as shown by the different response to two different isolates from symptomatic human infections in the volunteer study of Nash et al. (1987). Also host factors (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), ranging from 16-86% of the infected individuals. The mechanism by which Giardia causes diarrhoea and malabsorption is still unclear. Giardia could act as physical barrier, but the area covered by trophozoites is probably too small for affecting the absorption of nutrients. No evidence has been found for the production of toxins (Buret, 1994). Giardia infections appears to affect gut enzyme (lactase, disaccharidase) activities and damage the mucosal surface (shortening of crypts...
and villi) and give rise to overgrowth of the small intestine by bacteria (Tomkins et al., 1978) or yeasts (Naik et al., 1978).

**The disease**
The time between infection and the occurrence of *Giardia* cysts in the stool is 12 to 19 days (Jokipii et al, 1985). The incubation period for the occurrence of symptoms varies between 1-75 days, but is generally between 6-15 days, and coincides with the occurrence of *Giardia* in stool (Rendtorff, 1954; Brodsky et al., 1974). The most prominent symptoms are diarrhoea (fatty, yellowish) weakness, weight loss and abdominal pain and to a lesser extent nausea, vomiting, flatulence and fever. In the majority of cases, the infection is acute and self-limiting within 2-4 weeks. A significant proportion of the infected population will go on to have chronic infection with intermittent diarrhoea (estimated at 30-50%) (Farthing, 1994). Weight loss can be profound (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 with *Giardia* results in ‘failure to thrive’ in children, by impairment of the uptake of nutrients (especially fats and vitamin A and B12) (Farthing, 1994; Hall, 1994). Excretion of cysts varies between $10^6$-$10^8$ per gram of stool, as determined in positive stool samples (Tsuchiya, 1931), but a significant proportion of the stool samples does not show *Giardia* in detectable levels. Excretion patterns vary between hosts and isolates.

**Prevalence**
*Giardia* infections are very common in children in developing countries (Rabbani & Islam, 1994; Farthing, 1994). Also in developed countries, the prevalence peaks at the age of 1-4 years (Flannagan, 1992); a second peak is observed at the 20-40 age group, partly due to the care for the young children and partly due to travelling. In developing countries, the prevalence of giardiasis in patients with diarrhoea lies around 20%, ranging from 5-43% (Islam 1990). In developed countries, this prevalence varies from 3% (Hoogenboom-Verdegaal et al, 1989; Adam, 1991; Farthing, 1994; Kortbeek et al., 1994) to 7% (Quinn, 1971). As a reaction to infection with *Giardia*, both a humoral and cellular immune response is generated by the host. 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 and Goka, 1987). The immune response can also be seen in the serum antibodies. The immune response can give some degree of 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, recurrence of symptomatic infections, even after several infections, is common (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 can
be taken, such as increasing duration and dose of drug admission, administering an alternate drug or a combination of drugs.

**Routes of transmission**
Person-to-person faecal-oral transfer of Giardia cysts is the major route of transmission of giardiasis, as indicated by the high prevalence in situations with poor hygienic conditions in developing countries, in day-care centres and nurseries (Black et al., 1977; Pickering & Engelkirk, 1990; van de Bosch, 1991) and secondary spread to the house-hold in day care centre outbreaks (Black et al., 1977). Foodborne outbreaks have been the result of contamination of food by infected workers or household members (Osterholm et al., 1981; Islam, 1990; Thompson et al., 1990).

The role of animals in the transmission of human giardiasis is still controversial. Although Giardia commonly occurs in pet, farm and wild mammals, there is no unequivocal evidence that these Giardia have caused infections in humans (Erlendsen, 1994). Giardia intestinalis isolates from animals and man may be morphologically indistinguishable (Flannagan, 1992) and this has led to many reports on animal sources of human giardiasis, including waterborne cases 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 et al., 1988) is too high to draw definite conclusions regarding host specificity. Cross-transmission studies have not been well controlled and the results have been contradicting (Davies & Hibler, 1979; Hewlett et al., 1982; Belosevic et al., 1984; Kirkpatrick & Green, 1985; Woo & Patterson, 1986).

Waterborne outbreaks of giardiasis have been reported for almost 30 years (Moore et al., 1969; Brodsky et al., 1974; Craun, 1990). In the US, Giardia is the most commonly identified pathogen with more than 100 waterborne outbreaks, based on epidemiological evidence (Craun, 1990). Waterborne outbreaks have also been reported from Canada, Australia, New Zealand, United Kingdom and Sweden. These outbreaks have been linked to consumption of untreated surface water that was contaminated by human sewage (Craun, 1990) or by wild rodents (Moore et al., 1969; Dykes et al., 1980), to ground water that was contaminated by human sewage or contaminated surface water, to surface water systems receiving only disinfection (Craun, 1984; Kent et al., 1988) or ineffective filtration (Dykes et al., 1980; Craun, 1990) and by cross-connections or damage in the distribution system (Craun, 1986).

**Cyclospora sp.**

**Taxonomy**
Cyclospora was first described by Eimer in 1870 from the intestines of moles, and is related taxonomically to other protozoan parasites such as Cryptosporidium and Toxoplasma. The first likely observation of this parasite as a pathogen for human beings was 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 Sporozoasida, subclass Coccidiasina, family Eimeriidae. Organisms of the genus Cyclospora have an oocyst with two sporocysts, each of which contains two sporozoites (Levine, 1973) and have been found in snakes, insectivores and rodents. Molecular phylogenetic analysis suggests that the genus Cyclospora is closely related to the genus Eimeria (Relman et al., 1996).

**Life-cycle**

Many of the details of the life cycle of Cyclospora in human beings are not yet known. Cyclospora completes its life cycle within one host (monoxenous). Ortega et al (1993) proposed that Cyclospora that are infective to human beings should be designated Cyclospora cayetanensis on the basis of the development of the oocyst in vitro. However, Ashford et al., (1993) question the species name and Bendall et al (1993) preferred the use of the term CLB (denoting Cyclospora-like body) until further information is forthcoming regarding the biology of this coccidian parasite. In this review, Cyclospora sp. will be the nomenclature used to describe those organisms infective to man. The endogenous stages of Cyclospora sp. are intra-cytoplasmic 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 unsporulated 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 whilst sporulated oocysts contain two ovoid sporocysts. Within each sporocyst reside two sporozoites. Each sporozoite measures 1.2 x 9 µm.

**Pathogenicity**

Cyclospora sp. infects 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). However, 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 less than 100 sporulated oocysts may lead to a high probability of infection.

**The disease**

Symptoms include watery diarrhoea, fatigue, abdominal cramping, anorexia, weight loss, vomiting, low-grade fever and nausea which can last 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 60 days or more. 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), whereas in immunocompromised individuals diarrhoea may be prolonged.

**Prevalence**

Cyclospora sp. oocysts have been isolated from the stools of children, immunocompetent and immunocompromised adults. Oocysts have been described in the stools of residents in, and travellers returning from, developing nations, and in association with diarrhoeal illness in individuals from North, Central and South America, the Caribbean, the Indian sub-continent, Southeast Asia, Australia and Europe. Outbreaks of cyclosporiasis have been reported from Nepal and North and South America. In North America and Europe cyclosporiasis is associated with overseas travel and travellers’ diarrhoea. Point source outbreaks have been reported in the USA and Nepal. In 1996, a total of 1465 cases were reported in the USA and Canada, with around half of them occurring following events at which raspberries had been served (Anon., 1996; Herwaldt et al., 1997). Most cases occurred during spring and summer. Sporadic cases of cyclosporiasis have been reported from many countries and Cyclospora sp. oocysts are increasingly being identified in stools from immunocompetent individuals without foreign travel histories. Studies in Nepal, Peru and Tanzania seek to address Cyclospora sp. epidemiology, life cycle and pathology. Cyclospora sp. oocysts were detected in faecal samples from 11% of Haitians with chronic diarrhoea who were seropositive for human immunodeficiency virus (HIV) (Pape et al., 1994). Apart from HIV, Cyclospora sp. oocysts were the sole pathogen identified in many of these patients. Whilst clinical disease can resolve without treatment, trimethoprim-sulphamethoxazole (TMP-SMZ) is the drug of choice.

**Routes of transmission**

Epidemiology indicates that Cyclospora sp. is transmitted by water and food (Hoge et al., 1993; Anon., 1996; Herwaldt et al., 1997). An outbreak amongst house staff and employees in a hospital dormitory in Chicago occurred 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 sp. oocysts were detected in the stools of 11 of 21 persons who developed diarrhoea (Anon, 1991; Wurtz, 1994). An outbreak occurred amongst British soldiers and dependants stationed in a small military detachment in Nepal and 12 of 14 persons developed diarrhoea. Cyclospora sp. 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).

**DETECTION METHODS**
Protozoan parasites

**Cryptosporidium and Giardia**
The methodology required for the detection of Cryptosporidium oocysts and Giardia cysts in water is completely different from that traditionally used in the water industry. The methods that are currently available are at best tentative because of their low and variable recovery and the inability to differentiate viable oocysts of strains that are infectious to humans. The overall procedure consists of several stages, namely: 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 effect on the overall recovery efficiency and thus it is almost impossible to compare the effectiveness of two methods that have been performed in different laboratories unless these factors are standardised. Furthermore, there is considerable interest in determining if (oo)cysts are viable and potentially infectious. Thus methods have been and are currently being developed to assess the viability of (oo)cysts in the environment.

**Quality assurance**
**Microscope counts**
Care must be taken to ensure that the particles being counted are (oo)cysts, whether or not they contain sporozoites, and that algae and yeast cells are excluded from any counts that are made. The criteria used for determining that a particle is in fact a Cryptosporidium oocyst or 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 that a particle is a cyst or oocyst, whilst others will additionally use differential interference contrast microscopy or nucleic acid stains to determine if the particles that are 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, experience and alertness of the counting technician, fluorescence intensity after staining with the monoclonal antibody and the quality of the microscope. QA protocols should define how these factors are addressed.

**Recovery efficiency**
Given the low and variable recovery efficiency of the methods that are used for environmental monitoring for Cryptosporidium and Giardia, it is essential that laboratories collect their own data on the recovery efficiency of their method 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 which percentage of these (oo)cysts is recovered by the total protocol for sampling, processing and counting of environmental samples. This assay is influenced by the number, age and storage conditions of the (oo)cysts used for seeding. These have to be standardised (at least within a laboratory) to collect meaningful recovery data. The recovery efficiency should be assessed sufficiently frequent to be able to determine how the variation in
the recovery efficiency influences the uncertainty of the monitoring data. This is essential for the interpretation of environmental monitoring.

**Concentration techniques**

**Cartridge filtration**
The initial methodology to detect *Giardia* and *Cryptosporidium* in water used polypropylene cartridge filters with a nominal pore size of 1 µm, through which large volumes of water (100-1000 litres) are passed at a flow rate of 1-5 litres per minute. Trapped material is then eluted by cutting the filter open and washing either by hand or by stomaching using a dilute detergent solution. The resulting washings from these cartridges sometimes totals three or four litres and they must then be further concentrated by centrifugation. The ability to recover *Cryptosporidium* oocysts by this technique was originally reported to be in the range of 14-44% (Musial et al., 1987) although lower recovery efficiencies (<1-30%) have often been reported since (Ongerth & Stibbs, 1987; Clancy et al., 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**
A method described by Ongerth & Stibbs (1987) utilised large (142 or 293 mm diameter) 2 µm 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, whilst with low turbidity water, it is relatively easy to filter 10-40 litres, with some high turbidity waters, it is only possible to filter 1-2 litres. As with cartridge filtration, a range of recovery efficiencies has been reported for flat bed membranes. Nieminski et al. (1995) reported an average recovery of 9% for *Cryptosporidium* and 49% for *Giardia*. In a study of the efficiencies of several different membranes for recovering both *Cryptosporidium* oocysts and *Giardia* cysts, Shepherd & Wyn-Jones (1996) suggested that 1.2 µm cellulose acetate membranes gave higher recovery (30-40% and 50-67% respectively) than the 2 µm polycarbonate membranes (22-36% and 41-49% respectively) preferred by Ongerth & Stibbs (1987).

**Flocculation**
Another established procedure for concentrating (oo)cysts is the calcium carbonate flocculation procedure developed by Vesey et al. (1993b). A fine precipitate of calcium carbonate (CaCO₃) is formed in a water sample by the addition of calcium chloride and sodium bicarbonate, followed by adjusting the pH to 10.0 with sodium hydroxide. After allowing the precipitate to settle, the supernatant fluid is aspirated off and the sedimented material resuspended after dissolving the calcium carbonate with sulphamic acid. Recovery efficiencies using this method have been reported to be as high as 70% for both *Cryptosporidium* and *Giardia* (Campbell et al., 1994; Vesey et al., 1993b; 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 lower recoveries are usually encountered. Use of aged oocysts for seeding experiments together with leaving the oocysts in contact with water for a few days prior to analysis showed that recovery rates of 30-40% were more normally seen. The viability of the oocysts is affected by this concentration (Campbell et al., 1995). Flocculation with aluminium sulphate \((\text{Al}_2\text{(SO}_4)_3)\) did not affect the viability of oocysts, while the recovery efficiency was comparable to the CaCO\(_3\) flocculation (Schwartzbrod, pers. comm.).

**New methods**

The search for new methods for concentrating water samples to detect the presence of protozoan parasites continues and many methods have been evaluated, including cross-flow filtration, continuous flow centrifugation and vortex flow filtration (Whitmore, 1994). Methods which are currently receiving attention include vortex flow filtration (Fricker et al., 1997), the Gelman envirochek filters (Clancy et al., 1997) and the Genera filter system (Sartory, pers. comm.), amongst others.

There continues to be much debate over which method is most appropriate. Realistically there is no one single method which is most 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 which will perform the analysis. Ideally, the method chosen should efficiently concentrate as large a sample as possible and yield a concentrate which can be examined easily. Many workers prefer to concentrate only a small volume of water initially and to examine the entire concentrate, whilst others take large samples and examine only a fraction of the final concentrate. Either approach is defensible, but the methods used to concentrate small volumes (e.g. 10-20 l) tend to be easier to perform and generally have a higher recovery efficiency and so it is often preferable to take a large number of low volume samples and to examine all of the concentrate. Other factors which may affect the choice of concentration method include the site of sample collection and the distance 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 which 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 used by many workers 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. This basic technique has been adopted for use with environmental samples, although some workers prefer to use Percoll-sucrose or Percoll-percoll gradients. Whatever flotation method is used, several groups have demonstrated that this is an inefficient procedure when trying to detect 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 that this was only the case when sucrose flotation was performed. Spiked samples which are examined directly without density centrifugation gave similar recovery efficiencies, irrespective of whether they were examined immediately after seeding or after 48 hrs contact with the concentrate. However, when sucrose flotation was used, 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 was also seen 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 the density gradient centrifugation, can cause severe problems when examining slides for protozoa by epifluorescence microscopy. More efficient methods for separation of (oo)cysts from other particulates have been sought. Many workers have attempted the use of immunomagnetic separation (IMS). The principles behind this technology involve the attachment of specific antibodies to magnetisable particles and efficient mixing of the particles in the sample. The (oo)cysts attach to the magnetisable particles and are isolated from this debris with a strong magnet. The technique is very simple, but there are several sources of failure. An important source is the quality and specificity data of the monoclonal antibodies which are available. 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 IMS is used and beads are mixed with water concentrates, the immunoglobulin-(oo)cyst-bonds are subjected to shear forces and therefore 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, since if the attachment between the bead and the antibody is not strong, 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 IMS. Oocysts seeded into relatively clean suspensions are recovered efficiently, with recoveries of over 90% being reported (Campbell et al., 1997a,b). However, the real benefit of a good separation technique is with samples which have yielded a highly turbid
concentrate and it is in these samples that IMS does not appear to perform as efficiently. The use of antibodies of higher affinity may serve to improve the recovery efficiency of oocysts from high turbidity samples. Although this technique is also able to separate Giardia cysts, not much effort has been put into testing the recovery efficiency of these cysts by IMS.

**Flow cytometry**

Workers in the United Kingdom attempted to use flow cytometry with environmental samples in order to detect Cryptosporidium oocysts, but found that the sensitivity of these instruments was not high enough to distinguish oocysts from background noise (Vesey et al., 1991). Incorporation of a cell sorting facility onto flow cytometers enabled oocysts to be sorted efficiently from background material (Vesey et al., 1993). This technique is shown to work equally efficient for Giardia cysts (Vesey et al., 1994; Medema et al., 1998). Water concentrates are stained in suspension with FITC-labelled antibodies and passed through the fluorescence activated cell sorter (FACS). Particles with the fluorescence and light scatter characteristics of (oo)cysts are sorted from the sample stream and collected on a microscope slide or membrane filter, that is examined by epifluorescence microscopy to confirm the presence of (oo)cysts. The FACS procedure is not specific and sensitive enough to enable the count of sorted particles as a definitive number of (oo)cysts present, since other organisms/particles of similar size may cross-react with the monoclonal antibody and have similar fluorescence characteristics. In addition, some water samples contain high numbers of autofluorescent algae which may also mimic (oo)cysts and therefore 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 easier and more reliably than direct microscopy of non-sorted samples. Several researchers from the United States, France and the Netherlands have confirmed the benefits of FACS when examining water samples for the presence of (oo)cysts (Danielson et al., 1995; Compagnon et al., 1997; Medema et al., 1998). FACS is widely used in the United Kingdom for water analysis and is becoming more and more adopted in other parts of Europe, in Australia and in South-Africa.

**Detection**

Immunofluorescence microscopy

Routine detection of Cryptosporidium oocysts and Giardia cysts relies on the use of epifluorescence microscopy which may be applied to examine material deposited on multiwell slides or membrane filters. The (oo)cysts are specifically stained with monoclonal antibodies which have been labelled directly with FITC or are 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. This tends to give less non-specific binding and can make preparations easier to examine. Several anti-Cryptosporidium antibodies and anti-Giardia antibodies are commercially available and whilst most workers have their preferences, there does not appear to be a single antibody which is preferred for all purposes. One
specific failing of the commercially available antibodies is that they all 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 by various workers in
order to improve the ease of identification of both Cryptosporidium oocysts and
Giardia cysts.

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

PCR
Perhaps one of the most extensively tested procedures is the use of the
polymerase chain reaction (PCR) to detect specific sequences of nucleic acids
which 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.
However, despite the exquisite specificity and sensitivity which PCR can offer,
difficulties have been experienced with the application of PCR to water
concentrates. This has largely been due to inhibition of the DNA amplification
process. PCR is sensitive to the concentration of many compounds within the
reaction mixture and those of particular concern to researchers working with
water concentrates are divalent cations and humic and fulvic acids, which are
compounds frequently found in water and which 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 and some which have apparent specificity include those from regions
coding for the 18 S rRNA (Johnson et al., 1995), or mRNA coding for the
Cryptosporidium heat shock protein Hsp70 (Stinear et al., 1996, Kaunser &
Abbaszadegan et al. (1997) first reported the application 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. They also reported that amplification of heat shock-induced
mRNA utilising the same HSP primers was indicative of viable Giardia cysts.
The use of PCR for the detection of cysts and oocysts in water concentrates
offers some advantages over that of direct microscopical examination, since the
process can largely be automated and thus several samples can be processed
simultaneously. Furthermore, the technique is theoretically sensitive down to a
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., 1997). The data presented showed that a single viable oocyst could be detected by this procedure, even in the presence of PCR inhibitors. Such a method would be of considerable value to the water industry, facilitating rapid screening of samples although as yet it 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 may be used whereby molecular techniques are used as a screening tool on a portion of a water concentrate and that where positive results are generated, other approaches which involve microscopical examination are used.

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 which are detected may be non-viable and thus pose no threat to public health. Therefore, there has been considerable interest in developing in vitro methods which can determine oocyst viability.

Excystation
The most widely accepted in vitro procedure for determining oocyst viability, excystation, has not been used in combination with the IFA method, because excystation is difficult to incorporate in the IFA protocol. Excystation has been used in combination with PCR to detect the presence of viable Cryptosporidium oocysts (Filkorn et al., 1994; Wiedenmann et al., 1997). The sensitivity of this method in environmental samples needs further research. Excystation has been used in survival and disinfection studies. In the latter, this technique appears to yield a lower inactivation rate than the neonatal mouse infectivity assay (Finch et al., 1993; 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 the inability to excyst or infect animals (Schupp & Erlandsen, 1987; Smith & Smith, 1989). PI can therefore be used as indicator of cell death for Giardia cysts.

Campbell et al. (1992) developed a procedure based on the exclusion of PI for Cryptosporidium oocysts, using 4′6-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 which are viable and include DAPI but exclude PI, those which are non-viable and include both DAPI and PI and two classes which include neither DAPI or PI, those with internal contents (sporozoites) and therefore potentially viable, and those without and
therefore non-viable, as determined by DIC microscopy. The DAPI/PI procedure is simple to perform and whilst some workers have expressed some reservations over its’ applicability, it can be used for routine environmental work. The incorporation of DAPI into the nucleic acid acts as a further criterion for determining if a particle is an oocyst or not. An alternative to the DAPI/PI approach to determine viability has been suggested by Belosevic and Finch (1997) who have used new nucleic acid stains to differentiate between viable and non-viable oocysts. Two new stains have been identified, SYTO9 which stains non-viable oocysts green or bright yellow, while viable oocysts have a green halo surrounding the cell whilst the interior remains unstained and MPR71059 which stains non-viable oocysts red whilst viable oocysts remain unstained. These approaches have not been widely tested although Belosevic and Finch (1997) have demonstrated that the results obtained with these 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 et al, 1994, Rochelle et al, 1996; Slifko et al., 1997). For these assays, water samples are concentrated by normal procedures and bacteria may be removed by exposure of the concentrate to concentrations of chlorine which are lethal to bacterial cells but which are thought not to effect oocysts. The concentrates are then inoculated onto the tissue culture monolayer, 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 which have become infected. This offers a way in which infection may potentially be quantified. However, it is not clear if the presence of a single infectious oocyst will lead to one or more infected cells. In theory one might expect that an oocyst which excysts successfully would produce four infected tissue culture cells, but initial results have not demonstrated that this can be consistently achieved. Other workers (Rochelle et al., 1996) have adopted a somewhat different approach whereby they detect the presence of Cryptosporidium nucleic acids using PCR. Whilst 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 estimation of the number of oocysts present in a water concentrate.

Molecular methods

The RT-PCR methods that amplify induced mRNA that codes for heat shock proteins also indicate viability of Giardia cysts (Abbaszadegan et al., 1997) and Cryptosporidium oocysts (Stinear et al., 1997; Kaucner & Stinear, 1998). In
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combination with the reported sensitivity and specificity (see Detection), these methods may 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 these are able to discriminate between human and animal C. parvum strains (Ogunkadale et al., 1993; Bonnin et al., 1996; Deng & Cliver, 1998), but these 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. Therefore, the information on detection of this parasite in stool samples is given as guidance.
Identification of Cyclospora in stool samples is based upon the appearance of the oocyst either in direct or concentrated wet films. Concentration either by the formalin-ether (formalin-ethyl acetate) method or 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. In wet mounts, oocyst walls appear as well-defined non-refractile spheres measuring 8-10 µm in diameter by bright field microscopy, and within an oocyst is a central morula-like structure containing a variable number of inclusions. At higher (x 400) magnification, the inclusions appear refractile, exhibiting a greenish tinge. Oocysts are remarkably uniform in size (Ashford, 1979; Long et al., 1991). Occasionally, oocysts which either have collapsed into crescents or are empty are encountered. Under UV illumination (330-380 nm) the oocyst wall autofluoresces causing the organisms to appear as blue circles. 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 sulphoxide-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 ranging from deep red to unstained. 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 crescentic sporozoites. In instances where excystation in vitro have been successful, exposure of oocysts/sporocysts to an excystation medium at 37°C for up to 40 minutes causes the emergence of two crescentic sporozoites from each sporocyst.

**Concentration techniques for environmental samples**
As mentioned earlier, no method has been developed specifically for the detection of Cyclospora sp. in environmental samples, but because Cyclospora
sp. oocysts are larger than C. parvum oocysts and smaller that G. intestinalis cysts, it is 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). No commercially available polyclonal or monoclonal antibody with specificity to exposed epitopes on Cyclospora sp. oocysts is available currently. Therefore, the autofluorescent properties of the oocyst wall under UV illumination have 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 berries (strawberries and raspberries) implicated in a series of outbreaks in the USA in 1996. However, to date, no positive results have been reported.

**CONTROL OF WATERBORNE TRANSMISSION**

Cryptosporidium and Giardia are ubiquitous in surface waters throughout the world. Reported concentrations generally range from 0.01-100 per litre. These concentration data are not corrected for the (low) recovery of the detection method, so the actual concentrations may be more than tenfold higher. Higher concentrations are found in urbanised or agricultural waters than in pristine waters (LeChevallier et al., 1991; Rose et al., 1991). Sources of surface water contamination are the discharge of untreated and treated sewage, run-off of manure 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. Wildlife may be an important contamination source in pristine watersheds and has been implicated as the source of waterborne giardiasis, although this is still a matter of much controversy.

Oocysts and cysts can survive for months in surface water (DeRegnier et al., 1989; Robertson et al., 1992; Chauret et al., 1995; Medema et al., 1997). Under natural conditions, the die-off rate of Cryptosporidium oocysts in water is 0.005-0.037 10 log-units per day. For Giardia, the die-off rate is higher and (more) temperature dependant: from 0.015 10 log units per day at 1°C to 0.28 10 log-units per day at 23°C (DeRegnier et al., 1989). Although the state in which (oo)cysts occur in water (suspended or attached to particles) is relevant for water treatment (sedimentation, filtration), and cysts and oocysts readily attach to particles (Medema et al., 1998), little information
is available as yet on the significance of these factors in the environmental ecology of (oo)cysts. Recent information shows that overall 12% of groundwater supplies in the US were contaminated with Cryptosporidium and/or Giardia (Hancock et al., 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 to contamination with protozoa and the installation of adequate treatment coupled with verification that the treatment works effectively by monitoring of water quality and operational parameters.

**Watershed protection**

The major sources of surface water contamination with Cryptosporidium and Giardia are discharges of treated or untreated sewage (stormwater overflows), run-off or discharges of manure from agricultural lands and, in more pristine waters, wildlife. One of the most important aspects of watershed protection is the recognition of the local sources of contamination with Cryptosporidium and Giardia and to control the contamination as much as possible, by diversion or treatment of discharges, reduction of direct input of faeces, especially in otherwise pristine waters, by man, farm animals, wildlife or manure. Treatment of sewage in activated sludge systems or waste stabilisation ponds is an important barrier against environmental transmission. Both types of processes remove 90-99.7% of the cysts and oocysts (Sykora et al., 1991; Grimason et al., 1992). Treatment of agricultural wastes before land application 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 also composting of bedding reduces the viability of oocysts. Storm runoff and snowmelt from unprotected watersheds have been implicated as source of peak contamination of source water (Stewart et al., 1997; Atherholt et al., 1998), and may result in a 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. The importance of this is illustrated by the fact that the intake of the southern plant of Milwaukee in Lake Michigan 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 in the Milwaukee drinking water leading to the massive outbreak (MacKenzie et al., 1994). Installation of pretreatment storage reservoirs 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.
Since the protozoa are typically related to faecal contamination of surface water, several studies have tried to determine the use of indicator bacteria to predict high protozoa levels. No consistent relation is observed, however, between indicator bacteria (thermotolerant coliform) levels and concentration of Giardia or Cryptosporidium. The low and varying recovery of the protozoa detection methods may be an important confounder in detecting these relationships. As (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 bacteria (spores of Clostridium perfringens) may prove useful indicators for these persistent protozoa (Payment & Franco, 1993; Hijnen et al., 1997). Since no valid surrogates are available, watershed monitoring to determine local sources of contamination and to define the amount of treatment necessary should therefore include monitoring for protozoa.

Development of transport and fate models for predicting the (oo)cyst concentrations based on data on the sources may help identify important sources or environmental events that determine protozoa levels at abstraction points (Medema et al., 1997b).

Currently, neither the number of species of Cyclospora infective to human beings is known nor is it known whether human-derived oocysts are infectious to non-human hosts. However, the primary sources of pollution will be human faeces contaminated with oocysts. As Cyclospora sp. oocysts are larger than C. parvum oocysts but smaller than G. intestinalis cysts, it is likely that they will be discharged with final effluents from waste stabilisation ponds and sewage treatment works. Oocysts take up to 14 days to mature (sporulate) in the laboratory, sporulating more rapidly at higher (up to 37°C) temperatures. Sporulation time in the environment will depend upon ambient temperature and sporulated oocysts may be found distant from the pollution source in the aquatic environment. Sources of pollution with unsporulated oocysts are likely to be effluent discharges from sewage treatment and waste stabilisation 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. Cyclospora sp. oocysts stored 4°C do not appear to sporulate (Smith et al., 1997). A proportion of oocysts stored at 4°C for up to 2 months 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 these resistant protozoa is physical removal by filtration. The smaller size of Cryptosporidium oocysts makes them more difficult to remove than Giardia cysts. Rapid sand filtration is a common treatment process used to remove particles and when operating efficiently is theoretically capable of 3 log removal of Cryptosporidium oocysts (Ives, 1990). Other investigations
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have given a range of removal rates including 91% (Rose et al., 1986) and greater than 99.999% (Hall et al., 1994) with the higher removal rates being achievable when coagulant dosing has been applied to the water prior to filtration.

Diatomaceous earth filtration has been reported to achieve > 99% removal of Giardia (Jakubowski, 1990) and even 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 the (oo)cysts, when properly designed and operated (LeChevallier et al., 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 sulphate, polyaluminium chloride, ferric sulphate and ferric chloride in removing oocysts and similarly sized particles (Ives, 1990).

If filters are backwashed, the backwash water may contain high levels of (oo)cysts (Richardson et al., 1991). If this backwash water is recycled, treatment with coagulation and sedimentation or microfiltration will reduce re-contamination of the water with (oo)cysts. If this is not feasible, it is recommended that the recycled water is returned at a constant, low rate (Rose et al., 1997).

Slow sand filtration can efficiently remove (oo)cysts, but the efficiency reduces 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 where the removal efficiencies were generally good. Hall et al. (1994) demonstrated removals of greater than 99.95%. In another study using surface water, heat-inactivated oocysts were added at a concentration of 4000 per litre and 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 et al., 1995).

Micro- and ultrafiltration can remove over 99.99% (Jacangelo et al., 1991; Adham et al., 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 composition of the soil (Mawdsley et al., 1996).

Pretreatment reservoirs
Storage in reservoirs with a residence time of 5 months can reduce the (oo)cyst concentration 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., 1998).

Disinfection
Disinfection with chlorine has always been an important barrier for waterborne pathogens. The high resistance of especially Cryptosporidium oocysts against
chlorine disinfection (Korich et al., 1990; Smith et al., 1990; Ransome et al., 1993) renders this process ineffective for oocyst inactivation in drinking water treatment. Chlorine dioxide is slightly more effective, but still requires a high CT product (measure of disinfectant dose: $(\text{residual}) \times \text{contact time}$) of 78 mg.min l$^{-1}$ for 90% inactivation of oocysts (Korich et al., 1990). Giardia is less resistant against chlorine: 99.99% reduction can be achieved with a CT of 180-530, depending on temperature and pH of the water (Hibler et al., 1987). Chlorine dioxide gives 99% reduction at CT values of 4.7-28 (Leahy et al., 1987; Rubin, 1988).

Ozone is the most potent (oo)cysticide: at 20°C, the CT for 99% inactivation of C. parvum oocysts is 3.5 mg.min.l$^{-1}$ (Finch et al., 1993a) and for G. intestinalis cysts 0.6 mg.min.l$^{-1}$ (Finch et al., 1993b). The effectiveness of ozone reduces at lower temperatures. Peeters et al.(1989) found that 0.4 mg.l$^{-1}$ residual ozone for six minutes was sufficient to kill 10 000 oocysts ml$^{-1}$ whilst Korich et al. (1990) demonstrated that 1 mg.l$^{-1}$ for ten minutes at 25°C would result in a reduction in viability of 99%. Parker et. al. (1993) found that 3 mg.l$^{-1}$ for ten minutes was required to kill all oocysts and similar high figures were quoted by Ransome et al. (1993). Hence, the CT values required for inactivation of cysts and oocysts are high. CT values are limited, however, since high CT’s can give rise to formation of high concentrations of (geno)toxic by-products. Exposure of Cryptosporidium oocysts to multiple disinfectants has been shown to be more effective than was to be expected from both disinfectants alone (Finch et al., 1994; Liyanage et al., 1997) and synergism between environmental stress during sand filtration has also been observed (Parker et al., 1993). The multiple stresses that (oo)cysts encounter 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. UV doses of 110-120 mJ/cm$^2$ result in 99% inactivation of C. parvum oocysts (Ransome et al., 1993), as assayed with in vitro viability methods and 97% of G. intestinalis cysts (Rice & Hoff, 1981). A recent study of Clancy et al. (1998), using animal infectivity, 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$^2$. 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 against 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 et al., 1993). Moreover, the design and operation of full-scale treatment systems will, in general, be less optimal for inactivation than the laboratory setting.

The removal of Cryptosporidium oocysts and Giardia cysts by well designed, maintained and operated treatment processes are summarised in Table 1.

There is little information available regarding the ability of water treatment processes to remove or inactivate Cyclospora sp. oocysts. As Cyclospora sp. oocysts (8-10 µm diameter) are larger than C. parvum oocysts but smaller than G. intestinalis cysts, it is likely that physical removal will be similar to that
obtained with Giardia and Cryptosporidium. In an outbreak in Nepal, filtration and chlorination did not 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 the oocysts in different environments or what treatment can effectively inactivate the oocysts.

**Risk assessment to design adequate treatment**

One of the key issues in treatment is to determine what level of treatment is adequate. This requires maximum acceptable concentrations in drinking water. In analogy to toxic compounds, these could be derived from a maximum acceptable risk and the dose response relation of these parasites. An infection risk of $10^{-4}$
Table 1. Removal of Cryptosporidium oocysts and Giardia cysts by treatment processes.

<table>
<thead>
<tr>
<th>Type of process</th>
<th>Removal efficiency ($10^{\log}$-units)</th>
<th>Most important efficiency-determining parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cryptosporidium</td>
<td>Giardia</td>
</tr>
<tr>
<td>Disinfection processes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>0</td>
<td>0 - 2</td>
</tr>
<tr>
<td>Chloramines</td>
<td>0</td>
<td>0 - 2</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>0</td>
<td>0 - 2</td>
</tr>
<tr>
<td>Ozone</td>
<td>0 - 2</td>
<td>1 - 4</td>
</tr>
<tr>
<td>UV</td>
<td>0 - 4</td>
<td>0 - 4?</td>
</tr>
<tr>
<td>Filtration processes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid sand filtration</td>
<td>0 - 1</td>
<td>0 - 1</td>
</tr>
<tr>
<td>Slow sand filtration</td>
<td>1.2 -&gt; 3.7</td>
<td>1.2 -&gt; 3.7</td>
</tr>
<tr>
<td>Diatomaceous earth</td>
<td>2 - 6</td>
<td>2 - 6</td>
</tr>
<tr>
<td>Membrane filtration</td>
<td>2 - &gt; 4</td>
<td>2 - &gt; 4</td>
</tr>
<tr>
<td>Coagulation/filtration</td>
<td>2 - 2.5</td>
<td>2 - 2.5</td>
</tr>
<tr>
<td>Other processes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil passage</td>
<td>&gt; 2 - &gt; 5</td>
<td>&gt; 2 - &gt; 5</td>
</tr>
<tr>
<td>Reservoir storage</td>
<td>0.5 - 2</td>
<td>0.5 - 2</td>
</tr>
</tbody>
</table>

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 et al., 1991b; 1997). Current techniques do not allow an evaluation of compliance with these concentrations. Therefore, safe-guarding of drinking water with respect to protozoan parasites should be done by a quantitative description of the protozoa concentrations in the source water and the removal efficiency of the treatment steps. Surface
water utilities and groundwater utilities that may be influenced by surface water or other sources of contamination should monitor their source water for protozoa and determine the rate of protozoa removal and inactivation achieved in the treatment plant, in order to determine if acceptable concentrations of protozoa in drinking water have been achieved.

**Verification of efficiency of parasite removal**

For routine monitoring, water quality and process parameters are required to verify treatment performance. Several parameters have been suggested as a surrogate 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 (Nieminski, 1997) and particulate matter (Anon., 1997). Although turbidity or particle counts of filtered water depend on both the levels in raw water and filter performance, in general, a turbidity of 0.1 to 0.5 NTU or counts of particles > 3 µm below 50 per ml are indicative of good quality water. On-line monitoring of turbidity or particle counts gives direct and continuous information on (individual) filter performance and are very valuable tools for optimising treatment efficiency for (oo)cyst removal.

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

For disinfection processes, disinfectant dose, contact time, residual disinfectant concentration after this contact time, pH and temperature are commonly used to monitor the disinfection performance. The most critical conditions for disinfection processes are low temperatures and high turbidity of the water that is to be disinfected.

**CONCLUSIONS AND RECOMMENDATIONS**

**Health risk assessment**

The abundance and size of drinking waterborne outbreaks 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 receive most attention, low-level transmission of these protozoa through drinking water is very likely to occur in developed countries and in developing countries alike (Fraser & Cooke, 1991; Isaac-Renton et al., 1996). Cysts and oocysts are regularly found in drinking water (Isaac-Renton et al., 1996; Karanis & Seitz, 1996; Rose et al, 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 methods for a sensitive and specific detection of infectious (oo)cysts, with a consistently high recovery are not available.
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**Risk management**

Cryptosporidium poses a serious health risk to immunocompromised persons, especially AIDS patients. An important step forward in the reduction of the consequences of waterborne outbreaks and other cases of cryptosporidiosis would be the definition of an adequate therapy for the immunocompromised patients. Currently, prevention of exposure to (potentially) contaminated water is a means to reduce the risk. Especially the immunocompromised population should be informed about the risk and means to prevent exposure. Boiling of tap water, use of mineral or bottled water, not swimming in surface water or pools are some of the options. 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 applicable means to reduce exposure (Anon., 1995a, b; Juranek, 1995). In outbreak situations, rapid investigation of size and source of the outbreak and installation of control measures to prevent further transmission is required. Useful guidance on management of waterborne outbreaks can be found in the report of the UK group of experts (Anon., 1990) and the CDC Guidance Manual (Juranek, 1995) and a workshop report (Anon., 1995).

The protozoa, and to a lesser extent the viruses, have changed the philosophy in the developed countries towards safe-guarding of drinking water from monitoring of the ‘end-product’ drinking water to monitoring raw water and the efficiency of the treatment. Furthermore, the extreme resistance of these organisms implies that a “zero-risk” is no longer achievable. Treatments should be designed to reduce the (oo)cyst concentrations in the raw water as far as possible and preferably include filtration step(s). This implies that information on the parasite concentrations in the raw water is necessary, as well as information on the removal efficiency of the treatment. Quantitative risk assessment provides a tool for the combination of 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 current detection methods are generally sensitive enough to determine the concentrations of Cryptosporidium and Giardia in surface water, but are in many cases not sensitive enough for an accurate description of removal efficiency. For the latter description, data from laboratory studies and (seeded) pilot plant studies may provide additional information. Another approach is to determine if an adequate surrogate parameter can be found for the description of removal efficiency for Cryptosporidium (as Giardia is easier to eliminate both with disinfection and filtration, the description of treatment efficiency should be targeted on Cryptosporidium). Several parameters have been evaluated on a limited scale as surrogates for protozoa removal: aerobic spores, clostridial spores, particles and algae, but a broader evaluation is necessary to determine the value of these parameters. The definition of maximum acceptable concentrations of pathogens in drinking water based on a maximum acceptable (infection) risk level has become possible by the availability of volunteer data and 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 US EPA, is currently used in the US (Rose et al., 1997), Canada (Wallis et al., 1995) and the Netherlands (Medema et al., 1995) as the basis to determine the appropriate removal efficiency of surface water treatment systems. Although there are still questions about the significance of (oo)cyst occurrence in drinking water, this is the way forward. The implementation of guideline levels is still hampered, however, by the difficulty to determine source water quality and treatment efficiency accurately.

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