Volume 3 Datasheets – Micro-organisms

Part 1.2: Protozoa

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# *Acanthamoeba*

### Maximum Acceptable Value

No specific MAV is proposed for *Acanthamoeba* spp. but cysts or trophozoites should not be present in New Zealand drinking-waters. If *Acanthamoeba* species are detected in drinking-water or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

*Acanthamoeba* spp. are small free-living amoebae commonly found in aquatic environments and are one of the predominant free-living protozoa (FLP) found in soil. The organism exists in the environment as an amoebic trophozoite (25 to 40 μm) feeding on bacteria or as a dormant cyst stage (10 to 25 μm diameter) (Marciano-Cabral and Cabral, 2003). *Acanthamoeba* has a feeding, replicative trophozoite, which, under unfavourable conditions, such as an anaerobic environment, will develop into a dormant cyst that can withstand extremes of temperature (-20 to 56°C), disinfection and desiccation (WHO 2004).

The genus contains some 20 species, of which *A. castellanii*, *A. polyphaga* and *A. culbertsoni* are known to be human pathogens. However, the taxonomy of the genus may change substantially once evolving molecular biological knowledge is taken into consideration. *Acanthamoeba* spp have been isolated from a wide variety of water habitats including fresh, brackish, and seawater, water taps, swimming pools, sink drains, air conditioning units and emergency eye wash stations (Nwachuku and Gerba 2004). They can be found in water of cooler temperatures than *Naegleria* spp. However, the relative importance of water is unknown as soil, airborne dust and water are all likely sources.

*Acanthamoeba* spp. are one of the two main FLP groups implicated in the presence/ amplification of legionellae and mycobacteria. FLP are ubiquitous where bacteria, their main food source, are found. That they are present in distributed water is not a surprise, and it would be logical that the types and numbers of active FLP in distributed water relate directly to the quality and quantity of their food source present in infrastructure biofilms, and perhaps to a lesser extent in the bulk water. Over many years, water has been implicated as a source of opportunistic pathogens in healthcare and community disease outbreaks, particularly for the opportunistic respiratory pathogens *Legionella* spp. and *Mycobacterium* spp. Epidemiological data, along with laboratory reports of pathogens resisting digestion by amoebae, replicating inside amoebae, and dispersed by amoebae, have raised the spectre of FLP as Trojan horses delivering pathogens throughout distribution networks, and hence being real villains in the battle to provide safe drinking water. However, FLP almost certainly provide significant benefits to drinking water by removing/digesting bacteria in biofilms and bulk water. From DWI (2015).

### Health considerations

At least six species of *Acanthamoeba* are capable of causing disease in humans; pathogenic strains of *Acanthamoeba*, like those of *Naegleria*, are opportunistic pathogens not parasites (Schuster and Visvesvara 2004). *Acanthamoeba* species (eg, *A. culbertsoni)* cause a cerebral infection known as Granulomatous Amoebic Encephalitis (GAE), a rare but usually fatal disease (Marciano-Cabral and Cabral 2003). This condition has occurred in temperate and tropical regions of the world. GAE is a less acute condition than primary amoebic meningo-encephalitis (PAM: see *Naegleria fowleri*) and although rare, usually occurs in immunocompromised patients over a longish period. It can affect organs other than the central nervous system but invasion of the central nervous system finally causes death after what may be a protracted illness. The infective stage is the amoebic trophozoite. The route of GAE infection is thought to be via inhalation of amoebae through the nasal passages or respiratory tract, eg, lungs, or introduction through skin lesions rather than water consumption.

*A. castellanii* and *A. polyphaga* are associated with acanthamoebic keratitis (AK) and acanthamoebic uveitis (WHO 2004). AK transmission occurs via corneal abrasions and can induce painful vision-threatening corneal infections. AK is found in people who sustain corneal lesions before or at the time of infection and those who wear contact lenses (Morlet et al 1997). Tap water may be the origin of the infection via contact lenses and poor contact lens hygiene (Kilvington et al 2004). It is a rare disease that may lead to impaired vision, permanent blindness and loss of the eye. However, the prevalence of antibodies to *Acanthamoeba* and the detection of the organism in the upper airways of healthy persons suggest that infection may be common with few apparent symptoms in the vast majority of cases. Cleaning of contact lenses is not considered to be a normal use for tap water, and a higher-quality water may be required (WHO 2004).

### New Zealand significance

Amoebic keratitis has been recorded in New Zealand and there have been eight reported cases since 1995 in Auckland (Ellis-Pegler 2003). In contrast, there have been no recorded cases of GAE in New Zealand; four cases of GAE have been diagnosed in Australia (ADWG 2004). A survey (Brown et al 1983) has shown *Acanthamoeba* spp. to be widely spread throughout New Zealand soil and thermal pools. The organisms are able to survive and grow over a wide temperature range replicating optimally at temperatures of 25–30°C where bacterial food is available as happens with contamination of thermal pools receiving soil runoff. Trophozoites can exist and replicate in water while feeding on bacteria, yeasts and other organisms. However, whilst warm water may enhance growth particularly of thermotolerant species, swimming and other water activities have not been implicated directly as the cause of *Acanthamoeba* infections as *Acanthamoeba* has a wide distribution in the environment.

### Treatment of drinking-water

*Acanthamoeba* spp. may be found throughout the year in source waters, domestic water storage tanks and piped water supplies even when chlorine is present (Robinson and Christy 1984; Hoffmann and Michel 2001; Kilvington et al 2004). Their cysts are much more resistant than the amoebic trophozoites to chlorine and other disinfectants and are more resistant than the cysts of *Naegleria* spp., making removal difficult at generally accepted levels of disinfectant for drinking-water (Cursons et al 1980; Rodriquez-Zaragoza 1994). However, *Acanthamoeba* cysts may be as sensitive if not more to UV irradiation than parasitic protozoan (oo)cysts (Chang et al 1985; Maya et al 2003). Compared with *Giardia* and *Cryptosporidium*, *Acanthamoeb*a trophozoites and cysts are relatively large and physical treatment processes such as flocculation, sedimentation and filtration can be effective in their removal (Hoffmann and Michel 2001). Control of *Acanthamoeba* spp. may be most important in the cases of specialised uses of water such as renal dialysis or industrial eye wash solutions. The DWSNZ do not address these issues for water required for such special purposes.

### Method of detection and identification

Detection and maintenance of *Acanthamoeba* spp. from water supplies can be done with simple growth media and standard laboratory facilities. Identification to genus level can be made using morphological criteria: *Acanthamoeba* trophozoites are of a similar size to those of *Naegleria* but possess thin acanthapodia and do not possess a flagellate stage in the life cycle. Identification at the species level is more difficult and molecular methods for classification have been developed (see Marciano-Cabral and Cabral 2003). Specific investigations may require comparison with reference strains by experts in this field.

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Acanthamoeba* spp in the DWSNZ. However, *Acanthamoeba* spp would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* removal or inactivation assumes that the level of treatment selected to remove/inactivate these enteric parasitic protozoa during water treatment should also provide a high level of protection from *Acanthamoeba* trophozoites and cysts. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *Acanthamoeba* will be present in drinking-water. Nevertheless, water authorities should remain aware of the pathogenic significance of *Acanthamoeba* spp. and the possibility that these organisms might serve as vectors for bacterial infections from water sources. Both trophozoites and cysts can retain viable pathogenic bacteria such as *Vibrio cholerae* and *Legionella pneumophila*, both of which are well-recognised waterborne/water-based pathogens (Marciano-Cabral and Cabral 2003). Regular monitoring for *Acanthamoeba* spp. is not appropriate but consideration of their possible presence when planning the maintenance of eye wash stations and the use of mains water for optical hygiene use must be considered.

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# *Balantidium*

### Maximum Acceptable Value

No specific MAV is proposed for *Balantidium coli* but cysts should not be present in New Zealand drinking water. If *B. coli* is detected in drinking-water, or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

*Balantidium coli* is found in the intestines of humans and animals as a motile unicellular ciliated trophozoite (up to 200 μm length) and in the environment as a large dormant cyst, 60–70 μm in length (Sargeaunt 1971), making it the largest of the human intestinal protozoa. The infective stages, known as cysts, are environmentally robust (resistant to unfavourable environmental conditions, such as pH and temperature extremes) and are excreted in the faeces of infected hosts (usually animal hosts, particularly pigs). Drinking-water is probably not a significant route of transmission relative to other foci of infection. However, cysts have been detected in source waters, with faecal material from infected pigs contaminating the water the most likely cause. One waterborne outbreak of balantidiasis has been reported attributed to stormwater runoff containing swine faeces that contaminated a drinking-water supply after a typhoon (CDC 1972). Humans seem to be the most important host of *B. coli*, and the organism can be detected in domestic sewage. The prevalence of *B. coli* cysts in water supplies in New Zealand is not known.

### Health considerations

*Balantidium coli* belongs to the largest protozoan group, the ciliates, with about 7,200 species, of which only *B. coli* is known to infect humans. *B. coli* has worldwide distribution. It is the largest of the human intestinal protozoa but infections are relatively rare (worldwide human prevalence of 0.02–0.1 percent with rates up to 6 percent in some areas Esteban et al 1998), and most are asymptomatic (Garcia and Bruckner 1993). Following ingestion, the cysts excyst in the small intestine and the released trophozoites invade the mucosa and submucosa of the large intestine. Clinical symptoms may include acute bloody dysentery, diarrhoea, nausea, etc. Humans tend to be resistant and even when the disease does occur it is usually mild and self-limiting. *B. coli* is more commonly found in pigs (prevalence of 20–100 percent) but the parasite is harmless to that host. Transmission of *B. coli* is usually by consumption of food or water contaminated with pig faeces, or by the faecal-oral route resulting from contact with infected pigs, or from direct person-to-person contact.

### New Zealand significance

There have been no recorded instances of balantidiasis in New Zealand. The only reported waterborne outbreak of balantidiasis occurred in Micronesia in 1971 (CDC 1972).

### Treatment of drinking-water

The cysts of *B. coli* are large and thus amenable to removal by physical methods such as filtration. However, in the absence of information on the susceptibility of *Balantidium* cysts to normal disinfectant measures, it is probable that they are highly resistant to disinfection like the cysts of other enteric protozoan parasites. Protection of source waters from faecal contamination, particularly from pigs, would reduce the potential risk of cysts of *B. coli* from entering raw waters. Due to resistance to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index for the presence/absence of *B. coli* in drinking-water supplies.

### Method of detection and identification

No specific techniques are available presently for the isolation of *B. coli* cysts from water, although diagnosis of the large cysts is fairly easy in faecal preparations. Cultivation techniques are an additional and confirmatory method of identification, but not suitable for routine monitoring (Diamond and Clark 2002). Specific investigations may require comparison with reference strains by experts in this field.

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *B. coli* in the DWSNZ. However, *B. coli* would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* should ensure that the level of treatment selected to remove/inactivate these enteric parasitic protozoa during water treatment should also provide a high level of protection from *B. coli* cysts. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *Balantidium coli* will be present in drinking-water.

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# *Blastocystis*

### Maximum Acceptable Value

No specific MAV is proposed for *Blastocystis* but cysts should not be present in New Zealand drinking water. If *Blastocystis* is detected in drinking-water, or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. *Blastocystis* is included in the [plan of work of the rolling revision](http://www.who.int/entity/water_sanitation_health/gdwqrevision/en/index.html) of the WHO *Guidelines for Drinking-water Quality*. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

*Blastocystis* is a common anaerobic intestinal parasite that was first described in the early 1900s. *Blastocystis* spp. have been detected in a range of animal hosts, with isolates from humans identified as *Blastocystis hominis*. *Blastocystis hominis* lives in the colon and has several morphological forms, including a faecal cyst that is believed to be the infective form. WHO (2012, Chapter 2 in particular) summarises recent experience on the occurrence and effects of *Blastocystis*.

### Health considerations

*Blastocystis hominis* is probably the most commonly detected protozoan in human faecal samples worldwide. Infection occurs in both immunocompetent and immunocompromised individuals. Reported prevalence ranges from 2–50 percent, with the highest rates reported for developing countries with poor environmental hygiene. Infection appears to be more common in adults than in children. However, one study showed that peak infection occurs at 10 years of age and then later in life. Pathogenicity of *B. hominis* is controversial because of the nonspecific symptoms and prevalence of asymptomatic infections. Some case–control studies of individuals with and without symptoms show no difference in the prevalence of *B. hominis*. Symptoms attributed to *B. hominis* include watery or loose stools, diarrhoea, abdominal pain, anal itching, weight loss and excess gas. Duration of infection is not well known; some infections can last for weeks, months or years. In some patients, the symptoms resolve, even though *Blastocystis* can still be detected in stools. It has been suggested that *B. hominis* may be a commensal organism that becomes pathogenic when the host is immunosuppressed, is malnourished or has other infections.

The routes of transmission have not been established, but the faecal–oral route is considered to be the main mode of transmission. The source of human infectious *Blastocystis* is uncertain. *Blastocystis* occurs in many animals, including insects, reptiles, birds and mammals. Some evidence suggests that *Blastocystis* may not be host specific and that animal-to-human transmission is possible. WHO reported that a recent survey in Malaysia showed that animal handlers and abattoir workers were at greater risk of infection than a control group of high-rise city dwellers.

Molecular studies suggest that there is considerable antigenic and genetic heterogeneity within *B. hominis* and *Blastocystis* spp.

### New Zealand significance

No information available. An Australian study found no correlation between clinical symptoms and infection with *Blastocystis hominis*. There is no evidence of waterborne transmission in Australia.

### Treatment of drinking-water

The role of drinking-water as a source of *Blastocystis* infections has not been established. However, an investigation in Thailand provided evidence of waterborne transmission, and identification in sewage samples suggests potential for this to occur. There is little information on the removal and/or inactivation of *Blastocystis* by water and wastewater treatment processes. The morphology of *Blastocystis* varies over a broad range, and size estimates vary. Faecal cysts can be as small as 3–10 µm in diameter, and these are likely to be removed by conventional granular media-based filtration methods in a similar manner to *Cryptosporidium* oocysts that are 4–6 µm in diameter. It has been reported that *Blastocystis* cysts are relatively resistant to chlorine. Because of this resistance, *E. coli* (or, alternatively, thermotolerant coliforms) should not be relied upon as an index of the presence/absence of *Blastocystis* in drinking-water sources.

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Blastocystis* in the DWSNZ. However, *Blastocystis* would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* should ensure that the level of treatment selected to remove/inactivate these enteric parasitic protozoa during water treatment should also provide a high level of protection from *Blastocystis* cysts. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *Blastocystis* will be present in drinking-water.

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# *Cryptosporidium*

### Maximum Acceptable Value

No specific MAV is proposed for *Cryptosporidium* but the Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water. See Note 4 to Table 2.1 in the DWSNZ re testing for infectivity.

The *Drinking-water Standards for New Zealand* require that water leaving a treatment plant must either be treated in such a way that ensures removal or inactivation of *Cryptosporidium*, or that it is obtained from secure bore water.

If *Cryptosporidium* is detected in drinking-water or if drinking-water is suspected as a source of infection, further action may be necessary to control this organism and advice should be sought from the relevant health authority.

To demonstrate compliance with the MAV, various operational treatment requirements are used instead of routine monitoring for *Cryptosporidium*.

Microbial agents (which include *Cryptosporidium*) are included in the [plan of work of the rolling revision](http://www.who.int/entity/water_sanitation_health/gdwqrevision/en/index.html) of the WHO *Guidelines for Drinking-water Quality.*

### Sources to drinking-water

*Cryptosporidium* is a protozoan parasite that causes gastrointestinal illness. It has a complex coccidian life cycle with intracellular stages, and both sexual and asexual reproductive phases. Small thick-walled oocysts (4–6 μm diameter) shed in the host faeces are responsible for disease transmission. In 2004 there were 13 species of *Cryptosporidium* recognised that infect over 150 hosts (Fayer 2004; Xiao et al 2004). However, more recent research and gene technology has resulted in 25 species being recognised by 2014 (Caccio and Widmer 2014, Chapter 1).

*Cryptosporidium* spp. are found in humans, wild and domestic animals, livestock, birds, reptiles etc. Species infecting humans are predominantly *C. hominis*[[1]](#footnote-1)and *C. parvum*.[[2]](#footnote-2) *C. parvum* also causes diarrhoea in calves are considered a significant source of human infectious *C. parvum* oocysts in pastoral landscapes common in New Zealand. Older livestock do not show clinical signs of infection, but nevertheless can act as a reservoir of infection. Excretion of *Cryptosporidium* oocysts by infected hosts (eg, calves) can be in excess of 1010 with up to 107 per gram of faeces (Smith and Grimason 2003). Large numbers of oocysts have also been found in human wastes, pasture runoff and downstream surface waters (Hansen and Ongerth 1991). See also Victorian Department of Health (2011).

Cow pats can become very warm in the sun. An inactivation rate of 3.3 log/day in cow faeces has been reported for daily temperature cycles typical of spring/autumn in a Mediterranean climate. In winter the inactivation rates are much slower, with 0.2 log/day when the internal faecal matrix temperature was 30°C and 0.03 log/day when the matrix was at 20°C (Li et al2010). Desiccation, predation, sunlight, and ammonia (from urine) can enhance inactivation.

*Cryptosporidium* does not replicate outside the intestines of hosts.

*Cryptosporidium* spp. oocysts have been detected in a wide variety of water sources including lakes, rivers, springs, streams, groundwaters, roof-collected rainwater and they are found frequently in pasture run-off. In New Zealand, oocysts have been detected in
5–13 percent of natural surface waters tested nationwide especially in areas of intensive livestock farming and concentrations of more than 100 oocyst/100 L have been reported (Ionas et al 1998; McBride et al 2002). Agricultural livestock wastes can be a significant source of *Cryptosporidium* and runoff following rainfall events can increase oocyst concentration in unprotected water supply sources. Concentrations of oocysts as high as 14,000 per litre for raw sewage and 5,800 per litre for surface water have been reported (WHO 2004/2011). The oocysts can survive for many months in fresh water without losing their infectivity.

Numerous outbreaks of cryptosporidiosis worldwide have been associated with contaminated recreational water and drinking-water, demonstrating that water can be a significant route of transmission for *Cryptosporidium* (Rose et al 2002). Oocysts have been found in 7–40 percent of treated water samples analysed in the UK and USA with concentrations ranging from 0.7 to 140/100 L (Smith and Grimason 2003). The largest waterborne outbreak of cryptosporidiosis to date was linked to contaminated drinking-water supplies in Milwaukee, USA when more than 400,000 people were infected (MacKenzie et al 1994). The total cost of illness associated with the 1993 outbreak in Milwaukee, USA, has been estimated at US$96.2 million. Runoff from dairy farms was suspected but later analysis of stored faecal samples indicated human-derived sources probably resulting from wastewater discharges into the water source. The largest outbreak in Europe occurred in Östersund (Sweden) in 2010, and was caused by *C. hominis* subtype IbA10G2 (Widerström et al 2014). This is also the commonest sub-type of *C. hominis* found in New Zealand.

Contaminated recreational water, swimming pools and poor quality drinking-water have also been associated with outbreaks and higher reported rates of cryptosporidiosis in New Zealand (Duncanson 2000; Taylor and Ball 2004).

FWR (2011) summarises current knowledge.

### Health considerations

*Cryptosporidium* is a small protozoan parasite that infects the microvillous region of epithelial cells in the digestive and respiratory tract of vertebrates. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish. It requires its host to multiply. Environmentally robust oocysts are shed by infected hosts into the environment. These oocysts can survive the adverse conditions of the environment for months until ingested by a new suitable host. In the new host, the life cycle starts again and multiplication occurs, using resources of the host. Oocysts also survive well in estuarine waters (over 12 weeks at 20°C and a salinity of 10), but less in seawater (four weeks at salinity of 30 ppm) (from WHO 2009).

Oocysts remain infective in water at (MPI 2010):

67.5°C for 1 minute

-5°C for up to 8 weeks

-10°C for up to 7 days

-15°C for up to 24 hours

-20°C for up to 5 hours.

The parasite was first described in mice in 1907 but was not recognised as a causative agent for human illness until 1976. It was first associated with disease in severely immune-compromised individuals, especially AIDS patients with low CD4-counts, but is now also recognised as widespread, general pathogen of immune-competent humans. The infectivity of oocysts is high. Extrapolation of the dose-response data indicates that ingestion of a single oocyst gives a discrete probability of infection. The occurrence of waterborne outbreaks with high attack rates substantiates this (WHO 2009). In theÖstersund (Sweden), nearly 50 percent of the 60,000 inhabitants were infected (Widerström et al 2014).

*Cryptosporidium hominis* and *C. parvum* are responsible for most human infections. In various studies around the world, these two species usually account for at least 95 percent of infections (Caccio and Widmer 2014, Chapter 12). *Cryptosporidium hominis* is found primarily in humans, whereas *Cryptosporidium parvum* infects humans and mainly ruminants so is a typical zoonotic disease. Species of *C. meleagridis*, *C. baileyi* (birds), *C. felis* (cat), *C. canis* (dog) and *C. muris* (rodent) have also caused sporadic infections in humans, usually as opportunistic infections in immuno-compromised hosts (Xiao et al 2004).

*Cryptosporidium* is transmitted faecal – orally by a number of routes including person-to-person contact, direct contact with infected farm animals or domestic pets, and from contaminated food and water. The incubation period for the illness is usually about one week. The potential for transmission is great as the infective oocysts can be excreted in very large quantities for many weeks after disease symptoms have ended, and ingestion of fewer than 10 oocysts can lead to infection (Okhuysen et al 1999). Oocysts excyst after ingestion, releasing four motile sporozoites that invade and parasitise epithelial cells, mainly in the intestine. Infection of humans results in diarrhoea, which in immunocompetent individuals is usually self-limiting after a few days, sometimes up to a week. The elderly, infants, and immuno-compromised patients are at greatest risk from *Cryptosporidium* infections as symptoms are of greater severity and infections can be life threatening.

During acute infection, oocysts can be found in high numbers in the faeces of the host. At the peak of the infection, infected humans shed up to 105 to 107 per gram faeces. Various studies have reported oocyst numbers in raw sewage in the range of 1,000 to 12,000 per litre, and 50 to 1,000 per litre in the effluent.

*Cryptosporidium* was discovered to infect humans in 1976, and waterborne transmission was confirmed for the first time in 1984. Outbreaks in the UK in the early 1990s resulted in extensive reports with recommendations from Badenoch, followed by Bouchier (1998). A large outbreak (242 cases) of cryptosporidiosis in Galway (Ireland) was detected in 2007. The cause was traced back to two of the three water treatment plants where UV disinfection was subsequently installed (EPA 2007).

An outbreak in northwest Wales in 2005 resulted in 218 confirmed cases of *Cryptosporidium hominis*. Oocyst counts in final treated water at the treatment plant and at different points in the distribution system were consistently very low, maximum count in continuous monitoring 0.08 oocysts per 10 litres. Data from continuous monitoring and the epidemic curve was consistent with the hypothesis that low numbers of oocysts of *C. hominis* were present in treated water continuously during the outbreak and these were of sufficient infectivity to cause illness (Mason et al 2010).

WHO (2009) discusses many other outbreaks.

### New Zealand significance

Cryptosporidiosis has been a notifiable disease in New Zealand since July 1996. Regional rates of *Cryptosporidium* infection can vary significantly, ranging between 3–141 cases per 100,000 people during 2003–2004. The national rate of infection in New Zealand is one of the highest worldwide (Duncanson et al 2000) with 21.9 and 16.3 cases per 100,000 reported for 2003 and 2004 respectively (ESR 2004), averaging 23.6 per 100,000 from 2000 to 2005. Cryptosporidiosis can also be prevalent in New Zealand livestock (Learmonth et al 2003).

The following chart summarises the *Cryptosporidium* species, relative abundance, and hosts found by Massey University in their New Zealand studies during 2003–2016. Data are from human and animal sources where samples were PCR positive for *Cryptosporidium*.



Notified cases in New Zealand commonly show a distinct seasonal pattern with peaks during spring and autumn. Genotyping studies have found a higher incidence of *C. parvum* causing infections around September to October, with the second peak around March dominated by *C. hominis* infections. This seasonal shift suggests two transmission cycles occurring in New Zealand for human *Cryptosporidium* infections. The zoonotic cycle involves *C. parvum* infections and plays a major role in spring possibly associated with calving and a high environmental load.[[3]](#footnote-3) The anthroponotic cycle occurring in autumn involves *C. hominis* (Learmonth et al2004). UK studies report similar *C. parvum* dominant infections in spring and *C. hominis* infections in late summer/autumn reportedly associated with contact with farm animals and foreign travel respectively (Xiao et al 2004). Geographical differences in genotype distribution have also been found in New Zealand. *C. parvum* infections tend to predominate in rural areas compared with urban areas, where *C. hominis* is prevalent. Differences in distribution tend to reflect land use particularly in districts where farming is intensive. Dairy cattle can be an important reservoir for infection and monthly cumulative rates of *Cryptosporidium* infection for these districts (eg, Waikato DHB) are often higher than the national rate.

Risk factor information collected from cases (reported 2005) includes; 342/593 (57.7 percent) had farm animal contact, 171/492 (35 percent) had consumed untreated water, 95/475 (20 percent) had contact with sick animals, 130/383 (33.9 percent) consumed food from retail premises, 178/557 (32 percent) had recreational water contact (147/557 of which was swimming pools), 146/543 had faecal contact and 137/543 (25.2 percent) had contact with other symptomatic people during the incubation period.

Over the 10-year period 1997 to 2006, the average annual rate of notified cryptosporidiosis was 22.0 cases per 100,000 population. The number of hospitalisations was equivalent to 3.6 percent of the notified cases. There was only one reported fatality. The annual incidence of infection appeared fairly stable, but showed marked seasonality with a peak rate in spring. The highest rates were among Europeans, children 0–9 years of age, and those living in low deprivation areas. Notification rates showed large geographic variations, with rates in rural areas 2.8 times higher than in urban areas, and with rural areas also experiencing the most pronounced spring peak. At the territorial authority level, rates were also correlated with farm animal density (Snel et al 2009). The authors concluded that contact with farm animals was the most commonly reported risk factor (59.4 percent) followed by attending school or childcare (43.4 percent) and drinking or using untreated drinking water (38.7 percent). Overseas travel during the incubation period was relatively uncommon (5.7 percent).

Since 2000 there have been seven outbreaks of *Cryptosporidium* in New Zealand where water has been implicated as a mode of transmission (ESR 2005 unpublished data). However, the significance of waterborne transmission of cryptosporidiosis in New Zealand is still not clear. Although oocysts of *Cryptosporidium* spp. are found in New Zealand surface waters, recent studies elsewhere indicate that many *Cryptosporidium* species found in water do not have a high potential for infecting humans (Xiao et al 2004). The high prevalence of *C. hominis* in urban areas and the seasonal shift from *C. parvum* to *C. hominis* dominated infections in rural areas during late summer/autumn implies human-derived sources of oocysts and possibly direct faecal contact are also important. Nevertheless, for reported cases, water contact or water consumption is often an identified risk factor (ESR 2004). Without genotyping and viability testing of oocysts detected in water, the actual source of contamination and risk to human health from waterborne transmission in New Zealand remains unclear.

The Massey University Protozoa Research Unit is conducting a study for the Ministry of Health, from September 2009 to March 2018, 8.25 years and 660 samples to date. *Cryptosporidium* oocysts were found in 43 percent of the 139 lowland river samples, but in only about 1 percent of samples collected from intermediate rivers and bush catchments. Most *Cryptosporidium* oocysts in the lowland river samples were found in autumn and spring. All 160 bore (shallow, non-secure) samples have been negative so far. A sample collected from Brookvale Road bore 1 during the August 2017 Havelock North outbreak contained *E. coli* and *Campylobacter*, but *Giardia* and *Cryptosporidium* were not found in that sample or any other water sample collected at the time.

This research is developing a database archive and genotyping library of characterised strains of *Cryptosporidium* and *Giardia* from isolates obtained from range of host species and geographical locations in New Zealand. The count of faecal samples that have been screened for *Cryptosporidium* and *Giardia* within their surveillance program since 2009 is 5,338. There have been 1,395 faecal samples that were tested for *Cryptosporidium* and 3,346 for *Giardia*. For *Cryptosporidium,* 401 isolates were sequenced at the 18S locus, 1,395 at the gp60 locus and 278 at the MS1 locus. *Giardia* assemblages have been completed for 3,346 isolates using the *gdh* locus. There were 50 human faecal samples that were positive for both protozoa.

The research includes studies to assess the differences in infectivity between *Cryptosporidium* sub-genotypes found in New Zealand.

### Treatment of drinking-water

The small oocysts of *Cryptosporidium* are extremely difficult to remove during water treatment and have been found in treated water, even when compliance with standard operating procedures and current microbial standards have been met (Goldstein et al 1996). Due to their relatively small size, removal of oocysts by physical treatment processes represents a challenge. The oocysts are also extremely resistant to disinfection. A substantial percentage of *Cryptosporidium* oocysts have been shown to be able to survive 24-hour exposure to 1,000 mg/L of chlorine (Smith et al 1989). Alternative disinfectants including chlorine dioxide, ozone and UV can be more effective.

The water treatment plant that was breached in Östersund (Sweden) comprised pre-ozonation, flocculation and sedimentation, followed by rapid sand filtering and chloramination. To the knowledge of the authors, no post-treatment contamination or extensive failures in the treatment processes had occurred, and routine tests of the drinking water showed no increased levels of faecal indicator bacteria. The first indication was from workplaces reporting 10–20 percent of employees with gastroenteritis. Raw water *E. coli* levels were about 10 times greater than the average level on three occasions a few weeks before the outbreak. During the outbreak, the average oocyst density in drinking water was 0.32 per 10 L in WTP samples and 0.20 per 10 L in samples from the distribution network. Densities in raw water samples were generally higher: 0.2–3.1 oocysts/10 L (Widerström et al 2014).

The DWSNZ require that water leaving a treatment plant must either be treated in such a way as to ensure removal and/or inactivation of *Cryptosporidium,* or is obtained from secure bore water. The level of treatment required for non-secure waters is selected based on the assessed risk. This is determined for supplies serving at least 10,000 people or more, on the concentration of *Cryptosporidium* detected in the raw water. The higher the oocyst concentration, the higher the level of treatment required (expressed as log removal credits). For supplies serving <10,000 people, the protozoal log credit removal requirement is based on the result of a Catchment Risk Categorisation Survey (Appendix 3, DWSNZ 2008). A minimum of 3 and maximum of 5 log removal credits is required for all non-secure surface sources.

Routine monitoring for *Cryptosporidium* is not recommended (see section below). Instead, the DWSNZ require that various operational treatment criteria are used to demonstrate compliance with the MAV. The operational requirements include turbidity monitoring or particle counting (for filtration processes), direct integrity testing (for membrane filtration plants), indirect integrity testing (for membranes, bags and cartridges), pressure differential (for bag and cartridge filtration), UV intensity sensors, and C.t[[4]](#footnote-4) values (for ozone and chlorine dioxide disinfection).[[5]](#footnote-5)

### Method of detection and identification

Oocysts typically occur at low densities in natural waters so methods enabling their recovery from large volumes (>100 L) are required. Methods available for enumerating *Cryptosporidium* and oocyst viability/infectivity are not yet suitable for routine monitoring particularly for smaller supplies. Concentration by filtration followed by immuno-based methods using monoclonal antibodies for separation (Immuno Magnetic Separation, IMS) and detection (Immuno Fluorescent Assay, IFA) with confirmation through vital dye staining (DAPI) and differential interference contrast (DIC) microscopy are regarded as the most effective methods for isolating and enumerating waterborne oocysts (Quintero-Betancourt et al 2002). Oocysts are identified based on definitive criteria of fluorescence, morphometry and morphology. Detection of *Cryptosporidium* spp. can be done individually or in conjunction with *Giardia* spp. as described in the USEPA Method 1623 where combination monoclonal antibody reagents are available. However, most monoclonal antibodies are only specific at genus level or may cross-react with non-*parvum* species. Method recovery success is usually <50 percent, and may not be sensitive enough to detect low numbers of oocysts found in water supplies and there is no reliable method for determining infectivity other than by using animal models.

Molecular-based methods show promise in differentiating between human infective *Cryptosporidium* species and genotypes from animals in environmental waters. Studies underway in New Zealand are investigating the suitability of a small double-stranded RNA sequence to identify variants within Cryptosporidium genotypes and the suitability of mRNA heat shock protein 70 as a method for determining the viability of Cryptosporidium oocysts (Ionas personal communication 2005).

*Clostridium perfringens* is fairly resistant to lower doses of chlorine, so it has been suggested as an alternative indicator organism for protozoa; spores of *Clostridium perfringens* showed the strongest correlation (r = 0.76) with *Cryptosporidium* in a study on the River Meuse, a stronger correlation than thermotolerant coliforms or turbidity (WHO 2003).

### Derivation of Maximum Acceptable Value

*Cryptosporidium* is considered as a Priority 1 determinand in the DWSNZ given its public health importance in New Zealand and because coliforms, faecal coliforms, and *E. coli* have been shown to be poor indicators of the presence of pathogenic protozoa in drinking-water. The DWSNZ have a MAV of <1 infectious (oo)cysts per 100 L for total pathogenic protozoa in treated water.

The principle behind the MAV is that treatment technology is used to control the presence of this protozoan parasite in treated water along with other protozoan pathogens that may be present. Treatment processes are selected based on their efficacy of removing and/or inactivating *Cryptosporidium.* *Cryptosporidium* is used as an indicator for other pathogenic protozoa because it is extremely robust, small, very infective, and is considered the most resistant and difficult to remove for which water treatment data is available on removal and/or inactivation. The approach taken in the DWSNZ is that treatment that can remove and/or inactivate *Cryptosporidium* will also be effective in removing and/or inactivating other pathogenic protozoa. The MAV is based on the premise that treatment processes will have removed or inactivated *Cryptosporidium* spp. and *Giardia* spp (oo)cysts from the raw water source. To demonstrate compliance with the MAV, various operational treatment criteria are monitored instead of routine testing for *Cryptosporidium.* If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria are satisfied, then the DWSNZ considers it unlikely that *Cryptosporidium* will be present in drinking-water.

A multiple barrier approach including protection of catchments from contamination by humans and animals, and safe storage is recommended. Water from unprotected catchments is subject to potentially greater contamination with animal-derived sources of (oo)cysts from direct deposition or rainfall runoff events likely to contain *Cryptosporidium* spp. oocysts, whilst exposed storage systems allow potential recontamination by animals and birds.

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# *Cyclospora*

### Maximum Acceptable Value

No specific MAVs are proposed for *Cyclospora* but oocysts and spores should not be present in New Zealand drinking-water. If *Cyclospora* are detected in drinking-water or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

Microbial agents (which include *Cyclospora*) are included in the [plan of work of the rolling revision](http://www.who.int/entity/water_sanitation_health/gdwqrevision/en/index.html) of the WHO *Guidelines for Drinking-water Quality*.

### Sources to drinking-water

*Cyclospora cayetanensis* is a single-celled, obligate, intracellular, coccidian protozoan parasite, which belongs to the family Eimeriidae. Parasitic protozoa such as *Cyclospora* are recognised as emerging waterborne pathogens (Curry and Smith 1998). *Cyclospora* produces thick walled oocysts (8–10 μm), which are excreted as immature unsporulated oocysts. These oocysts mature into infective sporulated oocysts within 7–12 days in the environment. In an infected host, new spores are produced and released in faeces, urine, respiratory secretions or other body fluids depending on the species type and site of infection. The oocysts of *Cyclospora* are likely to survive for many months in water environments.

Several outbreaks of *Cyclospora* infection have been associated with waterborne transmission. Drinking-water has also been implicated as an important route of transmission since *Cyclospora* oocysts have been found in source waters and chlorinated municipal supplies (Rabold et al 1994; Dowd et al 2003).

### Health considerations

*Cyclospora* is an obligate protozoal parasite related to *Cryptosporidium*. The primary routes of exposure are contaminated water and food. The initial source of organisms in foodborne outbreaks has generally not been established, but contaminated water has been implicated in several cases. Drinking-water has also been implicated as a cause of outbreaks. The first report was among staff of a hospital in Chicago, USA, in 1990. The infections were associated with a chlorinated supply from a drinking tap water that had possibly been contaminated with stagnant water from a rooftop storage reservoir. Another outbreak was reported from Nepal, where drinking-water consisting of a mixture of river and municipal water was associated with infections in 12 of 14 soldiers (WHO 2004).

The unsporulated oocysts pass into the external environment with faeces and undergo sporulation, which is complete in 7–12 days, depending on environmental conditions. Only the sporulated oocysts are infectious. Symptoms of cyclosporiasis begin an average of seven days (range, 2 days to >2 weeks) after ingestion of sporulated oocysts. Owing to the lack of a quantification technique, there is limited information on the prevalence of *Cyclospora* in water environments. However, *Cyclospora* has been detected in sewage and water sources.

Humans are the only host identified for this parasite. Cyclosporiasis is not considered to be a zoonotic disease. The only species of *Cyclospora* identified in humans is *C. cayetanensis*.

*C. cayetanensis* is transmitted by the faecal-oral route with primary routes of exposure via contaminated food and water. Person-to-person transmission is virtually impossible because the oocysts must sporulate outside the host to become infectious. Sporozoites are released from the oocysts when ingested and penetrate epithelial cells in the small intestine of susceptible individuals. Clinical symptoms associated with infections by *Cyclospora* are similar to that of *Cryptosporidium* with diarrhoea, abdominal cramps, weight loss, anorexia and occasionally vomiting and/or fever. Relapsing illness often occurs. Symptoms are more pronounced in immunocompromised hosts. Some people with *Cyclospora* infection experience no symptoms at all, particularly persons living in areas where the disease is endemic. Most people who have healthy immune systems will recover without treatment, although if not treated, symptoms can last for several weeks to a month or more.

### New Zealand significance

*Cyclospora cayetanensis* occurs worldwide but infections are most common in tropical and subtropical areas. Prevalence of *Cyclospora* is generally very low in developed countries with rates of <0.5 percent to 2 percent reported in the general population or immunocompromised hosts (Goodgame 1996; Herwaldt 2000). Cases of *Cyclospora* infections in New Zealand have been reported in travellers returning from areas where *C. cayetanensis* is present (Ockelford et al 1997).

Two people in the Auckland region without overseas travel history were diagnosed with cyclosporiasis in 2012. ARPHS linked the cases to a private water supply. Although the incidence of cyclosporiasis is low in New Zealand, it is probably under-diagnosed. Cyclosporiasis is notifiable in New Zealand if it causes a gastrointestinal outbreak.

### Treatment of drinking-water

There is very limited information regarding the effectiveness of water treatment processes on the removal of *Cyclospora*. *Cyclospora* oocysts are resistant to chlorination. However, oocysts of *Cyclospora* are considerably larger than those of *Cryptosporidium* so may be more easily removed by flocculation and filtration treatment processes (Herwaldt 2000).

Owing to the resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *Cyclospora* in drinking-water supplies.

### Method of detection and identification

Oocysts of *Cyclospora* are shed typically in relatively low numbers. Acid-fast staining used for clinical diagnosis from faecal samples is not recommended for environmental samples due to variability in stain uptake. Methods used to detect *Cryptosporidium* have been used successfully to detect *Cyclospora* in drinking-water. The development of new PCR-based molecular methods shows promise for the detection and identification of species and genotypes of *Cyclospora* in water so that sources of contamination can be distinguished. However, the sensitivity of these methods to reliably detect low levels of contamination for routine monitoring of water sources is as yet uncertain. Robust methods are also needed to assess virulence and to determine viability/infectivity of human infectious oocysts and spores (Quintero-Betancourt et al 2002).

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Cyclospora cayetanensis* in the DWSNZ. However, it would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* compliance should ensure that the level of treatment selected to remove/inactivate *Giardia* and *Cryptosporidium* (oo)cysts during water treatment of non-secure water sources are likely to also provide a high level of protection from *Cyclospora*. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *Cyclospora* will be present in drinking-water. Authorities should remain aware of the pathogenic significance of *Cyclospora* and precautions taken to protect sourced water from human and animal faecal contamination and protection of distributed supplies.

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# *Entamoeba*

### Maximum Acceptable Value

No specific MAV is proposed for *Entamoeba histolytica* but cysts should not be present in drinking-water. If *E. histolytica* is detected in drinking-water, or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

*Entamoeba histolytica* is the most prevalent enteric protozoan parasite found worldwide and belongs to the superclass Rhizopoda in the subphylum Sarcodina. It has two stages to its life cycle, an amoeboid feeding replicative trophozoite stage (10–60 μm) which under unfavourable conditions, will develop into a dormant cyst stage (10–20 μm). Humans are the reservoir of infection, and there would not appear to be other meaningful animal reservoirs of *E. histolytica*.

The infective stage is the cysts which are excreted in the faeces of infected hosts; up to 1.5 x 107 cysts can be excreted daily by an infected individual. Not surprisingly, high densities of cysts, up to 5,000 cysts/litre, have been detected in sewage (Garcia and Bruckner 1993) and in contaminated source waters (Bakir et al2003). Cysts are environmentally robust and can remain viable for several months in water at low temperatures (Garcia and Bruckner 1993).

Transmission of *E. histolytica* has been associated with contaminated drinking-water and several waterborne outbreaks of amoebiasis have been reported, although cysts were not looked for or found in the treated water supplies (Chen et al 2001; Barwick et al 2002). Factors contributing to waterborne transmission included faecal contamination of source waters by sewage and storm sewer overflow, inadequate filtration and/or disinfection of water, interruption of water supply, damaged distribution pipes and increased water consumption.

### Health considerations

Six species of amoebae are common in man, the most important is *Entamoeba histolytica* which causes amoebic dysentery (amoebiasis), with an incubation period of 1–14 weeks. Recent studies with RNA and DNA probes demonstrated genetic differences between pathogenic and non-pathogenic *E. histolytica*; the latter has been separated and reclassified as *E. dispar*.

Worldwide surveys have indicated a prevalence of 0.8–50 percent for *E. histolytica* infections, and carrier rates during epidemics have been estimated up to 63 percent. Global estimates indicate that *E. histolytica* causes up to 50 million symptomatic infections each year. Transmission of infection is usually by person-to-person contact, consumption of contaminated food, or the ingestion of contaminated water. Although about
85–95 percent of human infections with *E. histolytica* are mild or asymptomatic, severe cases can occur with metastatic complication causing death. The usual clinical signs are gastroenteritis with symptoms ranging from mild diarrhoea to fulminating bloody dysentery. Approximately 10 percent of infected individuals present with dysentery or colitis. In amoebic dysentery the amoebic trophozoite (feeding stage) living in the lower small intestine or colon start to invade the mucosa causing ulcers, then they may enter the blood stream and be transported to other sites in the body such as the liver causing further amoebic abscesses, sometimes with fatal outcome. Pathogenicity depends on strain virulence and host factors, including diet.

Individuals suffering from an attack of dysentery caused by *E. histolytica* excrete the non-infective trophozoites along with blood and mucus. The trophozoites, which are susceptible to desiccation, survive only a short time in the environment. In chronic cases and asymptomatic carriers, the trophozoites multiply in the intestine and form characteristic four nucleated cysts which when excreted are a major source of infection.

### New Zealand significance

The carrier rate for *E. histolytica* in temperate regions is generally regarded to be less than 10 percent compared with tropical areas where prevalence of infection may exceed 50 percent. *E. histolytica* is not endemic in New Zealand and amoebiasis is not a notifiable disease. However, intermittent cases of amoebiasis have been reported by Auckland hospitals, generally in travellers returning from overseas (Lane and Nicholson 1984), 54 in 1991, 101 in 1992 (Hollis personal communication). Low prevalence of amoebiasis in the New Zealand population means that cysts from human sources of infection are unlikely to be present in source waters.

### Treatment of drinking-water

*E. histolytica* cysts are resistant to chlorination and standard chemical disinfection of drinking-waters is unlikely to effectively remove/inactivate *E. histolytica.* There is no available information on the effect of ozone and UV disinfection but it is likely that cysts of *E. histolytica* would respond similarly to those of *Giardia*. Also, as an organism with similar sized cysts to *Giardia,* physical treatment methods are likely to be relatively effective in removing *E. histolytica*. Owing to the resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *E. histolytica* in drinking-water supplies.

### Method of detection and identification

Detection of *E. histolytica* from water supplies is not standardised. Reported cases are diagnosed from faecal samples and detection is by wet preparation staining methods and morphological identification. Monoclonal antibodies and molecular methods need to be used to distinguish between pathogenic and non-pathogenic strains such as *E. dispar* (Huston et al1999).

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *E. histolytica* in the DWSNZ because of the uncertainty of the source of infection and the intermittent nature of New Zealand infections. However, they would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 mL of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* compliance should also ensure that the level of treatment selected to remove/inactivate these enteric parasitic protozoa during water treatment should also provide a high level of protection from *E. histolytica*. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *Entamoeba histolytica* will be present in drinking-water. Water authorities should remain aware of the pathogenic significance of this enteric protozoan parasite.

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# *Giardia*

### Maximum Acceptable Value

No specific MAV is proposed for *Giardia* but the Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water. See Note 4 to Table 2.1 in the DWSNZ re testing for infectivity.

If *Giardia* is detected in drinking-water or if drinking-water is suspected as a source of infection, further action may be necessary to control this organism and advice should be sought from the relevant health authority.

Giardiasis is endemic in all populations studied (FWR 2012).

### Sources to drinking-water

*Giardia* are zoonotic, waterborne, flagellated protozoa. It is a parasite of many animals and birds, and one of the most common sources of human intestinal infection in the developed world. *G. intestinalis* is the species that infects humans; it is also known as *G. lamblia* or *G. duodenalis*. It has been known as a human parasite for over 200 years but has only been regarded as pathogenic since 1965, after the Aspen (Colorado) outbreak. In the US giardiasis is sometimes called campers’ disease, or beaver fever.

The life cycle of *Giardia* spp. consists of a motile flagellate trophozoite stage (15 x 9 μm) that multiplies in the gastrointestinal tract, and a thick-walled cyst (12 x 8 μm) stage, which is the infectious form. Cysts are shed intermittently in the host’s faeces. High numbers of cysts can be shed (eg, up to 107 per g of faeces, Smith and Grimason, 2003) and large numbers have been found in human and animal derived wastewaters and in contaminated surface waters. *Giardia* cysts have been reported from 10,000 to 100,000 per litre in untreated sewage, and 10 or “a few” cysts per L in treated sewage (FWR 2012). The organism does not grow outside of the animal reservoir so controls designed to restrict the growth of bacteria will be ineffective.

*Giardia* is the most frequently identified agent of waterborne disease worldwide. *Giardia* spp cysts have been detected in a wide variety of water sources. The cysts survive better at low temperatures so can remain viable for many months in cold water lakes, reservoirs, rivers etc. In New Zealand, cysts have been detected in 8 to 23 percent of natural surface waters tested nationwide (Ionas et al 1998; McBride et al 2002). It has not been detected in roof-collected drinking-water in New Zealand, unlike *Cryptosporidium* spp, but this may have been due to a low recovery rate; *Giardia* spp cysts have been found in roof-collected rainwater elsewhere at concentrations of 4 cysts/100 L (Crabtree et al 1996).

Greatest prevalence of contamination in New Zealand has been associated with water from areas of intensive stock farming with concentrations of *Giardia* cysts reaching 84 to 375 cysts/100 L. However, water from reservoirs in protected bush catchments has also been found to contain *Giardia* cysts at 13 cysts/100 L. A number of animals in New Zealand have been found to be infected with *Giardia,* eg, possums, rabbits, birds and in particular, livestock, rats and mice are known to carry *Giardia intestinalis* (Tonks et al 1991; Marino et al 1992;Chilvers et al 1998; Learmonth et al 2003).

The survival of *Giardia* cysts in the environment is significantly affected by temperature; survivability decreases as the temperature increases. A small fraction of cysts can withstand a single freeze-thaw cycle. Cysts can survive for 2–3 months in water temperatures of less than 10°C, and at 21°C, cysts have remained viable for almost one month. Cysts are killed in 10 minutes at a water temperature of 54°C. Raising the water temperature to boiling immediately kills cysts.

### Health considerations

Several species of *Giardia* have been described, but *G. intestinalis* is the species found in humans (as well as other mammals). Another common species *G*. *muris* is found in rodents. Transmission of *G. intestinalis* occurs via person-to-person contact, contact with infected animals, and by contaminated food and/or water. Infection occurs when the cyst is ingested, with infection able to be initiated by ingestion of less than 10 viable cysts (Rendtorff 1954). The incubation period is usually one to three weeks but it can be longer. It is on average 7–10 days. Excystation then occurs in the upper intestine to produce two trophozoites that multiply asexually by binary fission. The trophozoites attach to the intestinal wall by a ventral sucking disc and by lining the upper intestine they reduce the absorption of nutrients, especially fats. Diarrhoea (sometimes described as explosive), bloating or flatulence, and abdominal cramps of varying severity may be caused. In the immunocompromised, children, and the elderly, severe symptoms of diarrhoea and sickness can be persistent and even become life threatening. However, in the majority of cases, the disease is self-limiting, although there are reports of it lasting more than one year, even in otherwise healthy people. Asymptomatic carriers are common; for example, in day care centres, as many as 20 percent of children may carry *Giardia* and excrete cysts without clinical symptoms.

*G. intestinalis* consists of eight assemblages (genotypes) designated A to H. Human infectious isolates are referred to as Assemblage A and B, and both have subgroups. Assemblage AI is found in humans, livestock, dogs, cats, beavers and other animals; assemblage AII is found only in humans. Assemblage BIII is found in humans, dogs, beavers, rats and other animals; assemblage BIV appears to be human specific.[[6]](#footnote-6) The zoonotic potential of assemblages A (particularly AI) and B are of interest, particularly for determining the source and importance of zoonotic waterborne transmission of *Giardia* to humans from livestock and wildlife. Assemblages C to H appear to be host specific.

Between 1992 and 2003 in England and Wales 89 reported waterborne outbreaks were reviewed; *Cryptosporidium* was implicated in 62 outbreaks, *Giardia* in just two (FWR 2012).

### New Zealand significance

Giardiasis has been a notifiable disease in New Zealand since July 1996 (see Chapter 1: Introduction, Section 1.1.3 for statistics related to giardiasis). Since then, *Giardia* has become the third most commonly notified disease. The national rate of infection in New Zealand in 2003 and 2004 was 42 and 40.5 cases per 100,000 people (ESR 2004), which are high incidence rates compared with other developed countries. Highest reported rates nationally are frequently for children under five years and show significant seasonal variation, with peak periods of giardiasis in late summer/autumn and winter. This is suggestive, respectively, of recreational exposure to contaminated water and person-to-person transmission (Hoque et al 2004). *Giardia* cases tend to be sporadic and usually involve a high incidence of person-to-person transmission. However, there have been several significant waterborne outbreaks of giardiasis in New Zealand including: Linton 1989, Kakanui 1990, and Paekakariki 1993 (also involved *Cryptosporidium parvum)*. Drinking-water has been implicated in many more cases, with infections of *Giardia* typically associated with poor quality (usually unfiltered) water (Taylor and Ball 2004). Isolated cases of giardiasis appear to be prevalent in rural regions where untreated water supplies are used. In urban areas, the risk of *Giardia* infection has been found to be higher among users of non-mains water supplies, particularly with those using roof-collected rainwater (Hoque 2002). Since, 2000, there have been 33 outbreaks of *Giardia* where water has been implied as a mode of transmission (ESR 2005 personal communication).

Animals known to carry *Giardia* spp. in New Zealand include rats, mice, dogs, cats, sheep, cattle, possums, hens, birds and others (Marino 1993). The significance of infected animals as zoonotic sources of infection and water contamination remains unresolved. New Zealand studies have found a prevalence of 41 percent of *G. intestinalis* in calves (Hunt et al 2000). Genotyping studies in Australia and Canada showed that 80 percent of isolates of *G. intestinalis* from infected calves were of the livestock type (assemblage E), ie, non-zoonotic) but around 20 percent of isolates were of the common human genotype (assemblage A). Since infected calves can excrete 105 to 106 cysts/g of faeces, even a small proportion of calves carrying human infectious assemblage A genotype presents a potentially important reservoir for human infection as cysts can easily enter watercourses through farm and field runoff (O’Handley et al 2000).

Although *Giardia* cysts are widespread in New Zealand waters, the significance of waterborne transmission of giardiasis in New Zealand is still not clear. The widespread distribution and prevalence of *Giardia* infection in livestock, domestic and wild animals in New Zealand suggests a significant reservoir for zoonotic transmission. However, work is needed to differentiate genotypes of human and animal infections and to distinguish between genotypes found in contaminated waters. Genotyping *G. intestinalis* is necessary in order to assess sources of zoonotic infections and to clarify the relative importance of zoonotic infection and waterborne transmission of giardiasis of zoonotic origin. In low level infections, ie, non-outbreak situations, the most likely route of *Giardia* transmission may be by direct person-to-person contact.

Over the 10-year period 1997 to 2006 the average annual rate of notified giardiasis was 44.1 cases per 100,000 population. The number of hospitalisations was equivalent to 1.7 percent of the notified cases. There were two reported fatalities. The annual incidence of notified cases declined over this period whereas hospitalisations remained fairly constant. Giardiasis showed little seasonality. The highest rates were among children 0–9 years old, those 30–39 years old, Europeans, and those living in low deprivation areas. Notification rates were slightly higher in rural areas. The correlation between giardiasis and farm animal density was not significant at the TA level (Snel et al 2009). The authors concluded that the public health importance of giardiasis to New Zealand mainly comes from its relatively high rates in this country. The distribution of cases is consistent with largely anthroponotic (human) reservoirs, with a relatively small contribution from zoonotic sources in rural environments and a modest contribution from overseas travel. Prevention efforts could include continuing efforts to improve hand washing, nappy handling, and other hygiene measures, and travel health advice relating to enteric infections. Giardiasis by self-reported risk factors: faecal matter and vomit (40.0 percent), drinking untreated drinking water (35.3 percent), and contact with other symptomatic cases (34.9 percent) were the most common self-reported positive exposures. Overseas travel during the incubation period was reported far more commonly (19.1 percent) than would be expected.

The Massey University Protozoa Research Unit is conducting a study for the Ministry of Health, from September 2009 to March 2018, 8.25 years and 660 samples so far. *Giardia* cysts were found in 59 percent of the 159 lowland river samples, but in only about
2–3 percent of samples collected from intermediate rivers and bush catchments. All 160 bore (shallow, non-secure) samples have been negative so far. A sample collected from Brookvale Road bore 1 during the August 2017 Havelock North outbreak contained *E. coli* and *Campylobacter*; *Giardia* and *Cryptosporidium* were not found in that sample or any other water sample collected at the time.

This research is developing a database archive and genotyping library of characterised strains of *Cryptosporidium* and *Giardia* from isolates obtained from range of host species and geographical locations in New Zealand. The count of faecal samples that have been screened for *Cryptosporidium* and *Giardia* within their surveillance program since 2009 is 5,338. There have been 1,395 faecal samples that were tested for *Cryptosporidium* and 3,346 for *Giardia*. For *Cryptosporidium,* 401 isolates were sequenced at the 18S locus, 1,395 at the gp60 locus and 278 at the MS1 locus. *Giardia* assemblages have been completed for 3,346 isolates using the *gdh* locus. There were 50 human faecal samples that were positive for both protozoa.

### Treatment of drinking-water

*Giardia* cysts are not killed readily by normal levels of chlorination unless the contact time is long (see DWSNZ 1995), but other disinfectants such as ozone and UV are more effective at standard dose rates (Craik et al 2000). Cysts appear to be removed slightly more efficiently by filtration processes than *Cryptosporidium* oocysts, which may in part be due to *Giardia*’s slightly larger size (Betancourt and Rose 2004). The DWSNZ require that water leaving a treatment plant must either be treated in such a way as to ensure removal and/or inactivation of *Cryptosporidium* (if that is achieved, then *Giardia* are assumed to present no problem)*,* or obtained from a secure groundwater source. The level of treatment required for non-secure waters is selected based on the assessed risk of the raw water or is derived from the concentration of *Cryptosporidium*, see section 5.2.1 of the DWSNZ.

Routine monitoring for *Giardia* is not recommended (see section below). Instead, the DWSNZ require that various operational treatment criteria are used to demonstrate compliance with the MAV. The operational requirements include turbidity monitoring (or particle counting) for filtration processes, direct integrity testing (for membrane filtration plants), indirect integrity testing (for membranes, bags and cartridges), pressure differential for bag and cartridge filtration, UV intensity sensors, and C.t[[7]](#footnote-7) values for ozone and chlorine dioxide disinfection. Owing to the resistance of the cysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *Giardia* in drinking-water supplies.

To achieve 3-log inactivation of *Giardia* at 25°C Health Canada (2008) reports C.t values of 0.48 for ozone, 11 for chlorine dioxide, 97 for chlorine and 750 for chloramine. Well operated coagulation, sedimentation, filtration plants can remove 99.99 percent (4-log) of *Giardia* cysts.

### Method of detection and identification

Detection of *Giardia* cysts involves the filtration of large volumes of water. Methods available for enumerating *Giardia* and cyst viability/infectivity are not yet suitable for routine monitoring particularly for smaller supplies. As for *Cryptosporidium,* concentration by filtration followed by immuno-based methods using monoclonal antibodies for separation (Immuno Magnetic Separation, IMS) and detection (Immuno Fluorescent Assay, IFA) with confirmation through vital dye staining (DAPI) and differential interference contrast (DIC) microscopy are regarded as the most effective methods for isolating and enumerating waterborne *Giardia* cysts (Quintero-Betancourt et al 2002). Detection of *Giardia* spp. can be done in conjunction with *Cryptosporidium* spp. as described in the USEPA Method 1623 where combination monoclonal antibody reagents are available. However, the recovery success can be variable (<60 percent), monoclonals may cross-react with other animal species, and the methods are costly. Small numbers of cysts can often be found in water supplies (Smith and Grimason 2003) and since infection in humans can be initiated by very few cysts, detection methods and viability assays must be very sensitive. There are currently no reliable methods for determining infectivity other than using animal models. Routine monitoring for *Giardia* therefore is not recommended as methods do not reliably identify strains that are infective to humans nor determine if those that are detected are infective.

Molecular methods are being developed in New Zealand to differentiate between human and animal isolates of *Giardia* *intestinalis*. Molecular typing in overseas studies indicates that most animal isolates are not infective to humans but they may be present in source waters.

### Derivation of Maximum Acceptable Value

*Giardia* is considered as a Priority 1 determinand in the DWSNZ given its public health importance in New Zealand and because coliforms, faecal coliforms, and *E. coli* have been shown to be poor indicators of the presence of pathogenic protozoa in drinking-water. The DWSNZ have a MAV of <1 infectious (oo)cysts per 100 L for total infectious pathogenic protozoa in treated water.

The principle behind the *Giardia* (and *Cryptosporidium*) MAV is that treatment technology is used to control the presence of this protozoal parasite in treated water and also other protozoa pathogens that may be present. Treatment processes are selected based on their efficacy of removing and/or inactivating *Cryptosporidium.* *Cryptosporidium* is used as an indicator for other pathogenic protozoa because it is extremely robust, small, is very infective, and is considered the most resistant and difficult to remove protozoa for which water treatment data is available on removal and/or inactivation. The approach taken by DWSNZ is that treatment that can remove and/or inactivate *Cryptosporidium* will also be effective in removing and/or inactivating other pathogenic protozoa. The MAV is based on the premise that treatment processes will have removed or inactivated *Cryptosporidium* spp. and *Giardia* spp (oo)cysts in the raw water source. To demonstrate compliance with the MAV, various operational treatment criteria are monitored instead of routine testing for *Giardia.* If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria are satisfied, then the DWSNZ considers it unlikely that *Giardia* will be present in drinking water.

A multiple barrier approach including protection of catchments from contamination by humans and animals and safe storage is recommended. Water from unprotected catchments is subject to potentially greater contamination with animal-derived sources of cysts from direct deposition or rainfall runoff events likely to contain *Giardia* cysts, whilst exposed storage systems allow potential recontamination by animals.

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# *Hartmannella (Vermamoeba)*

### Maximum Acceptable Value

No specific MAVs are proposed for *Hartmannella* but cysts should not be present in New Zealand drinking-water. If *Hartmannella* is detected in drinking-water or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

*Hartmannella* is a free living thermophilic amoeba (FLA) and is often found in similar environments as *Naegleria*, eg, geothermal waters. It is widespread in nature and has been isolated from soil, compost, freshwater, air, and a variety of engineered water systems.

*Hartmannella* and *Acanthamoeba* comprise the two major FLA groups associated with legionellae and mycobacteria. This is significant for human health as the bacteria may multiply to threatening numbers within the amoeba and then come into contact with typically immuno-compromised people through inhalation (eg, shower aerosol) (DWI 2015).

A study reported in DWI (2015) found FLA in all types of water tested. Regarding water supply, *H. vermiformis* is the most important member of the *Hartmannella* genus. There was some debate about its taxonomy – *Hartmannella vermiformis* is said to be not closely related to the other members of the genus so in 2011 was renamed as [***Vermamoeba vermiformis***](https://en.wikipedia.org/w/index.php?title=Vermamoeba_vermiformis&action=edit&redlink=1) (see Fouque et al 2014).

Kuiper et al (2006) state that *H. vermiformis* was observed in 75 percent of samples (200 mL) from freshwater environments used for water supply and cooling purposes in The Netherlands. In contrast, only 2 percent of 330 samples (50 mL) collected from the James River in Virginia were *Hartmannella* positive, and the organism was mainly found in samples collected in late summer and autumn. For the present study, all samples were taken in late autumn (water temperature 11.7°C ± 1.3°C). The difference in detection rate can be attributed partly to the high sensitivity of the method used in this study, with a detection limit of 5 cells/L. The amoebae of the genus *Hartmannella* observed in the James River were more associated with the sediment than with the water column. Hence, analysis of water samples only, as done in our survey, may give an underestimation of positive sites. *H. vermiformis* has also frequently been found in samples collected from natural waters (rivers: 36.4 percent; lakes: 16.7 percent) and from man-made environments (artificial lake: 20 percent; swimming pools: 6.3 percent) in Bulgaria, and in water at different stages of water treatment in Germany. Furthermore, *H. vermiformis* has been observed as the predominant amoeba in warm tap water. These reports and our observations indicate that this amoeba is a common component of natural freshwater environments and water installations. The majority of the surface water types included in this study serve as sources for drinking water production in The Netherlands. Cysts may survive and proliferate during different steps of water treatment, may enter the distributing system, and subsequently may multiply in biofilms attached to the pipe walls.

### Health considerations

Two distinct life cycle forms are known for *H. vermiformis*, viz. the trophozoite, an active feeding cell that also multiplies, and cysts, which are inactive (with reduced metabolism) dormant cells. Encystment occurs when environmental conditions become unfavourable such as nutrient starvation or osmotic stress.

*H. vermiformis* has direct and indirect public health significance. The organism has been isolated from the cerebrospinal fluid of a patient with meningoencephalitis and bronchopneumonia. The indirect public health significance of the organism is related to its role as a host for *Legionella pneumophila*, the causative agent of Legionnaires’ disease. (From Kuiper MW et al 2006.)

For a photo of *H. vermiformis* entrapping a *Legionella pneumophila* cell, see <http://www.microbeworld.org/index.php?option=com_jlibrary&view=article&id=6553>

### New Zealand significance

No information.

### Treatment of drinking-water

Kuchta et al (1993) found both cysts and trophozoites were sensitive to chlorine concentrations between 2.0 and 4.0 ppm and above (trophozoites somewhat more so than cysts), and 10.0 ppm was lethal to both forms. Hartmannellae treated with chlorine up to a concentration of 4.0 ppm supported the growth of legionellae. To determine whether heat would be an effective addendum to chlorine treatment of amoebae, hartmannellae were subjected to temperatures of 55 and 60°C for 30 min and alternatively to 50°C followed by treatment with chlorine at a concentration of 2 ppm. Fewer than 0.05 percent of the amoebae survived treatment at 55°C, and there were no survivors at 60°C. Pretreatment at 50°C appeared to make hartmannella cysts more susceptible to chlorine but did not further reduce the concentration of trophozoites.

*H. vermiformis* showed some sensitivity to free chlorine (DWI 2015).

Fouque et al (2015) found cysts were fully inactivated by 15 mg/L of chlorine for 10 minutes (C.t = 150).

### Method of detection and identification

Kuiper MW et al (2006).

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Hartmannella* in the DWSNZ. However, it would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* compliance should ensure that the level of treatment selected to remove/inactivate *Giardia* and *Cryptosporidium* (oo)cysts during water treatment of non-secure water sources are likely to also provide a high level of protection from these other emerging protozoan and fungal parasites. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that these emerging pathogenic organisms will be present in drinking-water. Authorities should remain aware of the pathogenic significance of these organisms and precautions taken to protect sourced water from human and animal faecal contamination and protection of distributed supplies.

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# *Isospora*

### Maximum Acceptable Value

No specific MAVs are proposed for *Isospora* but oocysts should not be present in New Zealand drinking-water. If *Isospora* are detected in drinking-water or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

Microbial agents (which include *Isospora*) are included in the [plan of work of the rolling revision](http://www.who.int/entity/water_sanitation_health/gdwqrevision/en/index.html) of the WHO *Guidelines for Drinking-water Quality*.

### Sources to drinking-water

Parasitic protozoa such as *Isospora* are recognised as emerging waterborne pathogens (Curry and Smith 1998). Unsporulated oocysts of *Isospora* (12 x 36 μm) are excreted in faeces but mature quickly to infectious oocysts within 1–2 days if temperatures are higher than 20°C. The oocysts of *Isospora* are likely to survive for many months in water environments.

There is limited information on the prevalence of *Isospora* in water environments. This is largely because sensitive and reliable techniques for the quantitative enumeration of oocysts in water environments are not available. However, waterborne transmission is considered possible since oocysts of *Isospora* have been detected in groundwater and surface waters.

### Health considerations

*Isospora spp* are coccidian, single-celled, obligate protozoan parasites related to *Cryptosporidium* and *Cyclospora*. Isosporiasis is not considered to be a zoonotic disease. There are many species of *Isospora* that infect animals, but only *I. belli* is known to infect humans, the only known host for this species. *Isospora belli* is one of the few coccidia that undergo sexual reproduction in the human intestine. Sporulated oocysts are ingested, and after complete asexual and sexual life cycles in the mucosal epithelium of the upper small intestine, unsporulated oocysts are released in faeces (Goodgame 1996).

*I. belli* is transmitted by the faecal–oral route with primary routes of exposure via contaminated food and water, although transmission by drinking-water has yet to be confirmed. Symptoms associated with infections by *Isospora* are similar to that of *Cryptosporidium* and *Giardia*. About one week after ingestion of viable cysts, a low-grade fever, lassitude and malaise may appear, followed soon by mild diarrhoea and vague abdominal pain. The infection is usually self-limited after 1–2 weeks, but occasionally diarrhoea, weight loss and fever may last for six weeks to six months.

Symptomatic isosporiasis is more common in children than in adults. Infection is often associated with immunocompromised patients, in whom symptoms are more severe and likely to be recurrent or chronic, leading to malabsorption and weight loss. Infections are usually sporadic and most common in the tropics and subtropics, although they also occur elsewhere, including industrialised countries. In immunocompetent individuals, infections with *I. belli* are frequently asymptomatic and self-limiting.

### New Zealand significance

*Isospora* *belli* occurs worldwide; infections have been reported from Central and South America, Africa and southeast Asia. Prevalence of *Isospora* is generally very low in developed countries with rates of <0.5 percent to 2 percent reported in the general population or immunocompromised hosts (Goodgame 1996).

### Treatment of drinking-water

There is very limited information regarding the effectiveness of water treatment processes on the removal of *Isospora*. It is likely that *I. belli* oocysts are relatively resistant to disinfection processes, particularly chloramination and chlorination. However, oocysts of *Isospora* are considerably larger than those of *Cryptosporidium* so may be more easily removed by flocculation and filtration treatment processes. Owing to the likely resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index of the presence/absence of *I. belli* in drinking-water supplies.

### Method of detection and identification

Oocysts of *Isospora* are shed typically in relatively low numbers. Acid-fast staining used for clinical diagnosis from faecal samples is not recommended for environmental samples due to variability in stain uptake. The development of new PCR-based molecular methods shows promise for the detection and identification of species and genotypes of *Isospora* in water so that sources of contamination can be distinguished. However, the sensitivity of these methods to reliably detect low levels of contamination for routine monitoring of water sources is as yet uncertain. Robust methods are also needed to assess virulence and to determine viability/infectivity of human infectious oocysts and spores.

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Isospora belli* in the DWSNZ. However, it would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* compliance should ensure that the level of treatment selected to remove/inactivate *Giardia* and *Cryptosporidium* (oo)cysts during water treatment of non-secure water sources are likely to also provide a high level of protection from these other emerging protozoan and fungal parasites. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that these emerging pathogenic organisms will be present in drinking-water. Authorities should remain aware of the pathogenic significance of these organisms and precautions taken to protect sourced water from human and animal faecal contamination and protection of distributed supplies.

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# Microsporidia

### Maximum Acceptable Value

No specific MAVs are proposed for microsporidia but spores of these organisms should not be present in New Zealand drinking-water. If these organisms are detected in drinking-water or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

Microbial agents (which include microsporidia) are included in the [plan of work of the rolling revision](http://www.who.int/entity/water_sanitation_health/gdwqrevision/en/index.html) of the WHO Guidelines for Drinking-water Quality.

Microsporidia are spore-forming pathogens often referred to as obligate intracellular protozoa, but there is increasing argument to have them reclassified with the fungi.

### Sources to Drinking-water

Parasitic protozoa such as the microsporidia are recognised as emerging waterborne pathogens (Curry and Smith 1998). The term “microsporidia” is a non-taxonomic designation commonly used to describe a group of obligate intracellular protozoa belonging to the phylum Microspora. More than 100 microsporidial genera and almost 1,000 species have been identified. There may be more than one million species!

Microsporidia are among the smallest eukaryotes. Microsporidia produce unicellular spores (1–4.5 μm diameter) and a characteristic coiled polar filament for injecting the sporoplasm into a host cell to initiate infection. In an infected host, new spores are produced and released in faeces, urine, respiratory secretions or other body fluids depending on the species type and site of infection. The spores of microsporidia are likely to survive outside their host for many months, including in water environments. Certain animals, notably swine, may serve as a host for human infectious species.

There is limited information on the prevalence of microsporidia in water environments due to the lack of a quantification technique. However, waterborne transmission is considered possible since spores of microsporidia have been detected in groundwater and surface waters (Didier 2005). Indications are that their numbers in raw sewage may be similar to those of *Cryptosporidium* and *Giardia*.

Nine water catchment areas were studied in the north and east of Melbourne. A total of 610 faecal samples from wild deer, including sambar deer (516), red deer (77) and fallow deer (17) were collected from June 2009 to March 2017. *Enterocytozoon bieneusi* DNA was detected in 25 of the 610 (4.1 percent) faecal samples from wild deer by nested PCR-based sequencing of internal transcribed spacer (ITS) and exclusively in sambar deer in five of the nine water catchment areas (Zhang et al 2018).

A waterborne outbreak of microsporidiosis was suspected in Lyon, France although spores were not found in the water supply (Cotte 1999). To date, infection arising from contaminated drinking-water is considered plausible but unconfirmed.

### Health considerations

Their host range is extensive. Infections occur in every major animal group, including vertebrates and invertebrates. A number of genera of microsporidia have been associated with infections in humans (microsporidiosis) particularly in immunosuppressed hosts and travellers. These include *Enterocytozoon*, *Encephalitozoon* (including *Septata*)*, Pleistophera, Trachipleistophora*, *Vittaforma* and *Nosema,* as well as a collective group of unclassified microsporidia referred to as microsporidium(WHO 2004/2011; Didier 2005). The most prevalent microsporidia species found are *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. *Encephalitozoon* spp. are known to be zoonotic with spores found in the faeces of many mammals and birds.

The sources of microsporidia infecting humans are uncertain. Spores are likely to be excreted in faeces and are also excreted in urine and respiratory secretions. Transmission of microsporidia is uncertain but it is likely to be via person-to-person contact and ingestion of spores in contaminated food or water. Zoonotic transmission of microsporidia is also likely given the wide range of animals infected with human species of microsporidia. Symptoms associated with infections by microsporidia are similar to that of *Cryptosporidium* and *Giardia* with diarrhoea, abdominal cramps, weight loss, anorexia and occasionally vomiting and/or fever. Microsporidia may also cause corneal, liver and bilary tract infections. Symptoms are more pronounced in immunocompromised hosts.

### New Zealand significance

Microsporidia occur worldwide, with prevalence rates ranging from 6 percent to 50 percent reported for immunocompromised patients (Goodgame 1996). There is very little information on the presence and distribution of these organisms in New Zealand. However, human infections of these organisms have been reported in immunocompromised groups and thus low-level intermittent infections within New Zealand seem possible (Everts et al 1997).

### Treatment of drinking-water

There is very limited information regarding the effectiveness of water treatment processes on the removal of microsporidia. Excretion of microsporidia spores in the faeces and urine of infected animals can contaminate water sources but little is known about the fate and behaviour of microsporidia spores during water treatment. The small size of the spores implies that in contrast to *Cyclospora* and *Isospora,* they are less likely to be removed by filtration processes. However, one study suggests that microsporidia spores may be susceptible to chemical disinfection, including chlorine (Wolk et al 2000). Owing to the lack of information on sensitivity of infectious species of microsporidia to disinfection, the reliability of *E. coli* (or, alternatively, thermotolerant coliforms) as an index for the presence/absence of these organisms from drinking-water supplies is unknown.

### Method of detection and identification

Methods for detection of microsporidia spores are very limited. The development of new PCR-based molecular methods shows promise for the detection and identification of species and genotypes of microsporidia in water so that sources of contamination can be distinguished. However, the sensitivity of these methods to reliably detect low levels of contamination for routine monitoring of water sources is as yet uncertain. Robust methods are also needed to assess virulence and to determine viability/infectivity of human infectious oocysts and spores (Sparfel et al 1997).

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for microsporidia in the DWSNZ. However, they would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* compliance should ensure that the level of treatment selected to remove/inactivate *Giardia* and *Cryptosporidium* (oo)cysts during water treatment of non-secure water sources are likely to also provide a high level of protection from these other emerging protozoan and fungal parasites. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that these emerging pathogenic organisms will be present in drinking-water. Authorities should remain aware of the pathogenic significance of these organisms and precautions taken to protect sourced water from human and animal faecal contamination and protection of distributed supplies.

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# *Naegleria*

### Maximum Acceptable Value

No specific MAV is proposed for *Naegleria fowleri* but cysts or trophozoites should not be present in New Zealand drinking-water. If *N. fowleri* is detected in drinking water, or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

More than 40 *Naegleria* species have been identified. *N. fowleri* is a damp soil/water-dwelling free-living single-celled thermophilic amoeboflagellate that feeds on bacteria. It has an amoebic trophozoite (10–20 μm), a non-feeding motile flagellate (7–11 μm), and a dormant cyst (7–15 μm) stage in its life cycle. The amoebic stage lives naturally in freshwater and its sediments and grows best at slightly elevated temperatures (36–42°C). It is therefore usually associated with thermally polluted waters. *N. fowleri* has been isolated from a variety of recreational warm water bodies, eg, hot springs, thermally polluted streams and rivers, geothermal water and heated swimming pools (Brown et al 1983; Marciano-Cabral et al 2003). The amoebae have also been detected in domestic water supplies and artificially heated industrial water source, eg, cooling waters particularly where water temperatures can exceed 25–30°C (Huizinga and McLaughlin 1990; Marciano-Cabral et al 2003). However, pathogenic *Naegleria* may also be present in sourced water at relatively low temperatures existing as dormant cysts and then increasing in numbers during the warmer summer months (Hoffman and Michel 2001).

Water supply systems should be considered at risk for occurrence of *N. fowleri* if water temperatures are consistently above 25°C for at least four months of the year, or if thermophilic *Naegleria* species have ever previously been detected in the system (Water Australia Factsheet 2015).

### Health considerations

There are several species of *Naegleria;* *N. fowleri* causes disease in healthy humans. Infection by *N*. *fowleri* causes Primary Amoebic Meningoencephalitis (PAM), a rare but usually fatal disease of the central nervous system. The amoebic stage is infective. Water is the only known source of infection. The route of infection is via the nostrils. Amoebic trophozoites enter the nasal passages typically when diving or swimming in warm freshwater and then migrate via the olfactory nerve into the meninges (the membrane surrounding the brain) and often to the brain itself. There is a very short incubation period of generally 2–5 days. Infection is invariably fatal with death occurring rapidly within 5–10 days. *N. fowleri* does not require infection of a human host for the completion of its life cycle, and is thus not parasitic but an opportunistic pathogen. The infection cannot be transmitted from person to person. PAM is difficult to diagnose because it has non-specific symptoms which are similar to the more common viral and bacterial forms of meningitis.

Given the intranasal route of infection, PAM is strictly a waterborne disease exclusively associated with the use of warm water for swimming or domestic bathing (Cursons et al 1976). Water supplies can be potential sources of contamination in public and private swimming pools (Marciano-Cabral et al 2003). The infectious dose for *N. fowleri* is unknown. However, the frequency of infection is low even where large numbers of people have been exposed repeatedly. High levels of antibodies to the related non-pathogenic *N. gruberi* have been found in New Zealand human populations (Cursons et al 1980). Other research has suggested that high levels of sodium, potassium and calcium may be inhibitory as *N. fowleri* has not been isolated from thermal pools where these cations are found at high levels (Brown and Cursons 1987).

Details of deaths associated with municipal tap water in Louisiana (USA) have been published by researchers from the Centers for Disease Control and Prevention (Cope et al 2015). This is the first occasion on which a fatal case of primary amoebic meningoencephalitis has been confirmed in association with a treated (ie, disinfected) water supply in the US. Testing of 13 tap water samples revealed three which were positive for *N. fowleri* by direct PCR and culture, and one which was negative by direct PCR but positive by culture. Testing for total chlorine residual at distribution system sample sites showed all of the *N. fowleri* positive sample sites were below the detection limit, whereas seven of the eight negative sample sites tested had detectable chlorine levels (0.2–3.8 mg/L). Water temperatures in the system were warm (28–34°C among nine samples tested for this parameter), and the water delivered to the house was at 29°C. The tap water supply to this area is drawn from the Mississippi River and treated by filtration and primary disinfection with chlorine followed by addition of ammonia to form monochloramine.

NHMRC, NRMMC (2011) states: PAM cases have been recorded from South Australia, Western Australia, Queensland and New South Wales; *Naegleria fowleri* has been detected in water in each of these states and in the Northern Territory. Australia is the only country where *N. fowleri* has been detected in public water supplies (Dorsch et al 1983). Most of the available data on the density of *N. fowleri* in water relates to water supplies in South Australia (including the highest reported densities). In temperate Australia, significant seasonal cycles of density occur, from below one organism per litre to hundreds or thousands per litre in poorly disinfected water (Robinson and Christy 1984). *N. fowleri* detected at water temperatures below 18°C is likely to be present as cysts, which are not infectious, but which may seed a suitable environment.

### New Zealand significance

*Naegleria* spp have been responsible for nine recorded deaths in New Zealand since 1968; five cases were confirmed as *N*. *fowleri* (Cursons et al 2003). Victims were children and young adults with infections probably linked to prolonged exposure and submersion. All cases of death resulting from *Naegleria* infections in New Zealand have been associated with swimming in geothermal pools or rivers receiving geothermal waters in central North Island. Since 1976, considerable upgrading of thermal resorts in New Zealand has occurred and commercial geothermal pools must include stringent measures to exclude soil from the water sources and pools, filtration, adequate and sustained disinfection and/or high water turnover. One death in 2001 has occurred since PAM became a notifiable disease in New Zealand in 1976.

### Treatment of drinking-water

Transmission of *N. fowleri* infection through ingestion of water is insignificant. However, the risk of PAM has been associated with the use of poorly chlorinated public water supplies in domestic swimming pools in South Australia and the use of unchlorinated private water supplies for bathtubs in the US (Marciano-Cabral et al 2003). Free chlorine or chloramines at 0.5 mg/L or higher will control *N. fowleri* but it is important that with chlorine the disinfectant level must be maintained constantly throughout the system (Robinson and Christy 1984) as *N. fowleri* can multiply to significant numbers in warm stagnant sections of water pipe (Miller et al 1982 cited Marciano-Cabral et al 2003). The cysts and trophozoites of *N. fowleri* are not generally as resistant to chemical disinfection compared with *Acanthamoeba* and therefore *N. fowleri* islikely to be removed more effectively by these processes. Physical treatment processes such as flocculation, sedimentation and filtration can be effective in their removal (Hoffmann and Michel 2001). *N. fowleri* is thermophilic and an elevated temperature is one of the most important factors accounting for increased levels of *N. fowleri* in water sources (Esterman et al 1984). Any water supply that seasonally exceeds 30°C or continually exceeds 25°C can support growth of *N. fowleri*. In New Zealand, the association of *N. fowleri* infection with warm water indicates that this organism may be present in water sources where the possibility of thermal pollution exists and *E coli* is present.

When *N. fowleri* are exposed to free chlorine at 30°C and pH 7.0, the two-log reduction Ct for trophozoites is <1.0 mg.min.L-1 and for cysts, 15 mg.min.L-1. Ct reduction values for *N. fowleri* exposed to monochloramine (NH2Cl) at pH 8.0 are approximately three-fold higher (CRC 2009). Adequate disinfection must be applied (to achieve a free chlorine residual concentration and contact time >30 mg/L-min) and the chlorine (or chloramine) residual should be maintained at 0.5 mg/L or above throughout the distribution system. Proper design, management and cleaning of physical assets (eg, pipes and storage tanks) is required to minimise sediment (which may harbour *Naegleria* cysts) and reduce water stagnation (which may lead to loss of disinfectant residual) (Water Australia Factsheet 2015).

### Method of detection and identification

Detection of *Naegleria* spp in thermal waters can be carried out by simple growth and enrichment techniques (Cursons et al 1979) followed by identification of *N. fowleri* using nested-PCR (Marciano-Cabral et al 2003). Any thermophilic amoeba species that is able to grow at 42°C or above is evidence that *N. fowleri* may be present (Griffin 1972). If samples include any *Naegleria*, **immediate** remedial action should be taken in the form of Amphotericin B and Rifampicin administration before final specific identification is carried out, as the onset of death may take as little as five days from exposure, and only hours after recognition of clinical symptoms. Owing to the environmental nature of this amoeba, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an indicator for the presence/absence of *N. fowleri* in drinking-water supplies.

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Naegleria fowleri* in the DWSNZ. However, it would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. In New Zealand the distribution of *Naegleria fowleri* is localised and although periodic prospective studies may be valuable, regular monitoring is not warranted unless *N. fowleri* is detected. The operational requirements in the DWSNZ for *Giardia* and *Cryptosporidium* compliance should ensure that the level of treatment selected to remove/inactivate these enteric parasitic protozoa during water treatment for non-secure sources are likely to also provide a high level of protection from pathogenic *Naegleria* organisms including trophozoites and cysts. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *Naegleria fowleri* will be present in drinking-water. Nevertheless, water authorities should remain aware of the pathogenic significance of this opportunistic pathogenic protozoa.

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# *Toxoplasma*

### Maximum Acceptable Value

No specific MAV is proposed for *Toxoplasma gondii* but oocysts should not be present in New Zealand drinking-water. If *T. gondii* is detected in drinking water or if drinking-water is suspected as a source of infection, advice should be sought from the relevant health authority. The Maximum Acceptable Value (MAV) for total pathogenic protozoa is less than 1 infectious (oo)cyst per 100 litres of drinking-water.

### Sources to drinking-water

*Toxoplasma gondii* is a zoonotic coccidian protozoan parasite that infects primarily cats, but can also cause infections in humans including unborn babies. *T. gondii* has several stages of development in the human host including the asexual forms tachyzoites
(3–6 μm) and cysts (up to 60 μm diameter). In cats, the parasite can also develop thick-walled immature oocysts (11–13 μm), which are excreted into the environment. Cats are the only animal in which the parasite replicates sexually. After release, the oocysts take around five days to mature to sporulated oocysts before they become infective. Oocysts are environmentally robust and can remain viable for many months in moist environments (Dubey 2004).

Drinking-water may be an important route of transmission for *T. gondii*. Several outbreaks of toxoplasmosis have been associated with the consumption of water from contaminated water sources and municipal water supplies (Bowie et al 1997, WHO 2004). Although *T. gondii* oocysts were not detected in the British Columbia municipal reservoir (Isaac-Renton et al 1998), runoff from soil contaminated with oocysts from infected wild or domestic cats was considered the probable source (Aramini et al 1999).

WHO (2012, Chapter 2 in particular) summarises recent experience on the occurrence and effects of *Toxoplasma*.

### Health considerations

There are many species of the protozoan parasite *Toxoplasma* but *T. gondii* is the only species infecting humans (as an intermediate host). The prevalence of oocyst-shedding cats may be 1 percent. By the third decade of life, about 50 percent of the human population in Europe is infected, and in France this proportion is close to 80 percent. *T. gondii* can also infect a wide range of other intermediate hosts including most livestock but the cat is the definitive host. Estimates indicate that in many parts of the world,
15–30 percent of lamb and pork meat is infected with cysts. *T. gondii* can be transmitted to humans via the ingestion of infected meat containing cysts of the parasite, by the faecal-oral route, from contaminated soil or water containing oocysts excreted in the faeces of cats, or congenitally (Petersen and Dubey 2001). A total of 158 laboratory-confirmed cases of toxoplasmosis were reported in the UK during 2009 (DEFRA 2011).

When oocysts are ingested they migrate through the body and form permanent cysts in various organs and tissues, frequently in the brain. There have been reports that *T. gondii* can be behaviour-altering. Very few oocysts (10 or less) may be required to initiate an infection (Dubey et al1996). Most human infections are asymptomatic. However, for a few cases (about 10 percent), the ingestion of tissue-borne cysts or mature sporulated oocysts causes flu-like symptoms, headache, malaise and enlargement of liver and spleen. After an initial infection, people develop immunity. However, the parasite commonly establishes a long-lived “dormant” infection in the form of tissue cysts and, if the immune system subsequently becomes compromised, the infection may re-activate. Disease is more severe in immuno-compromised individuals and pregnant women where intense generalised infection occurs involving the central nervous system and lungs with serious and sometimes fatal consequences for the host or unborn child.

### New Zealand significance

Toxoplasmosis is found worldwide. In developed countries, seroprevalence estimates of human *T. gondii* infection can reach 50–80 percent (Tenter et al 2000; Dubey 2004). Serological evidence suggests that *T. gondii* also has a high prevalence in New Zealand although cases are usually asymptomatic or present with mild illness. Over 50 percent of people showed antibodies against *Toxoplasma* in several New Zealand blood donor surveys and higher prevalence’s of positive titres were found in sheep farmers (Charleston, 1994). A recent study found serological evidence of historic and recent *T. gondii* infection in 33 percent of pregnant women in Auckland (Morris and Croxson 2004). High serological prevalence of *Toxoplasma* infection has also been found in New Zealand sheep (up to 93 percent serologically positive) and goats (up to 37 percent) indicating that the ingestion of tissue-borne cysts could be an important route of infection (Charleston 1994). However, domestic cats are a major source of environmental contamination as they are common and can produce large numbers of *T. gondii* oocysts. Antibodies are detected commonly in around 50 percent of domestic cats although the prevalence of cats shedding oocysts may be 1 percent in New Zealand (Charleston 1994).

The significance of water transmission of *T. gondii* infections in New Zealand is not known. There is no available information on the prevalence of oocysts in water sources. Small rural sourced supplies could be at risk from contamination by domestic or feral cats. Most cats in New Zealand have antibodies to *T. gondii* that indicates exposure to the parasite at some stage. Toxoplasmosis was a notifiable disease in New Zealand between 1987 and 1996 (MPI 2008).

*T. gondii* has been monitored as part of the Massey University quarterly project since September 2016. As at June 2018, 160 samples water samples have been tested; *T. gondii* has been absent in all samples.

### Treatment of drinking-water

*T. gondii* oocysts are likely to be able to survive unfavourable conditions in water environments, as are the oocysts of related parasites such as *Cryptosporidium*. Oocysts are extremely resistant to many detergent and disinfectant solutions, and can remain viable in 2 percent sulphuric acid, 2.5 percent potassium dichromate and sodium hypochlorite; MPI (2010). However, as the oocysts are larger than those of *Cryptosporidium*, *T. gondii* oocysts may be more likely to be removed by physical treatment processes. Measures to prevent contamination of source waters by domestic and feral cats should be considered.

An outbreak of 100 cases in Canada was reported. Water was drawn from an open surface water reservoir, with no water filtration and the chloramination treatment was insufficient to destroy oocysts. Domestic cats and wild cougars were probably shedding oocysts in the catchment area; taken from MPI (2010).

### Method of detection and identification

Detection of *T. gondii* in water is difficult as there are no standardised methods available to detect the relatively few oocysts that are likely to be present. Methods involving concentration by centrifugation, density gradient purification, and PCR detection have been applied to water. *Toxoplasma* DNA was detected in 8 percent of raw water, groundwater and drinking-water samples (Villena et al 2004). Immuno-based techniques (eg, IMS, IFA) are not currently available due to the lack of suitable monoclonal antibodies for *T. gondii* oocysts. Owing to the lack of information on sensitivity of *T. gondii* to disinfection, the reliability of *E. coli* (or, alternatively, thermotolerant coliforms) as an indicator for the presence/absence of these organisms in drinking-water supplies is unknown.

### Derivation of Maximum Acceptable Value

No specific MAV is proposed for *Toxoplasma gondii* in the DWSNZ 2005. However, it would be covered by the total pathogenic protozoa MAV of less than 1 infectious (oo)cyst per 100 litres of drinking-water sample. The level of *T. gondii* in environmental waters is unknown and there are several routes of transmission. The MAV for *Giardia* and *Cryptosporidium* should ensure that the level of treatment selected to remove/inactivate these enteric parasitic protozoa during water treatment for non-secure sources are likely to also provide a high level of protection from *T. gondii* oocysts. If water leaving the treatment plant satisfies the appropriate operational criteria, and if the bacterial compliance criteria for water are satisfied, then it is considered unlikely that *T. gondii* will be present in drinking-water. Nevertheless, water authorities should remain aware of the public health significance of this protozoan parasite and take precautions to protect water sources from contamination.

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1. Formerly named *Cryptosporidium parvum* genotype I or the human genotype of *C. parvum*. [↑](#footnote-ref-1)
2. *C. parvum* now used for *Cryptosporidium* previously known as *C. parvum* genotype II or the bovine/cattle strain. [↑](#footnote-ref-2)
3. The role of contact with farm animals as a source of *Cryptosporidium* infections was highlighted in the UK during the foot and mouth outbreaks when restricted farm access coincided with a dramatic decline of human cases of cryptosporidiosis and infections with *C. parvum* (bovine) (Hunter et al 2003). [↑](#footnote-ref-3)
4. Product of disinfectant concentration and contact time. [↑](#footnote-ref-4)
5. Refer to Chapter 8 in the Guidelines and Chapter 5 in the *Drinking-water Standards for New Zealand*. [↑](#footnote-ref-5)
6. The five other assemblages appear to be confined to specific animal hosts and include dogs (assemblages C and D), livestock (assemblage E), cats (assemblage F) , rats (assemblage G) and voles/muskrats (Thompson 2004). [↑](#footnote-ref-6)
7. Product of disinfectant concentration and contact time. [↑](#footnote-ref-7)