

11

Microbial fact sheets

Fact sheets are provided on potential waterborne pathogens as well as on indicator and index microorganisms.

The potential waterborne pathogens include:

- bacteria, viruses, protozoa and helminths identified in Table 7.1 and Figure 7.1, with the exception of *Schistosoma*, which is primarily spread by contact with contaminated surface water during bathing and washing;
- potentially emerging pathogens, including *Helicobacter pylori*, *Tsukamurella*, *Isopora belli* and microsporidia, for which waterborne transmission is plausible but unconfirmed;
- *Bacillus*, which includes the foodborne pathogenic species *Bacillus cereus* but for which there is no evidence at this time of waterborne transmission; and
- hazardous cyanobacteria.

The human health effects caused by waterborne transmission vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis and typhoid fever. Contaminated water can be the source of large outbreaks of disease, including cholera, dysentery and cryptosporidiosis; for the majority of waterborne pathogens, however, there are other important sources of infection, such as person-to-person contact and food.

Most waterborne pathogens are introduced into drinking-water supplies in human or animal faeces, do not grow in water and initiate infection in the gastrointestinal tract following ingestion. However, *Legionella*, atypical mycobacteria, *Burkholderia pseudomallei* and *Naegleria fowleri* are environmental organisms that can grow in water and soil. Besides ingestion, other routes of transmission can include inhalation, leading to infections of the respiratory tract (e.g., *Legionella*, atypical mycobacteria), and contact, leading to infections at sites as diverse as the skin and brain (e.g., *Naegleria fowleri*, *Burkholderia pseudomallei*).

Of all the waterborne pathogens, the helminth *Dracunculus medinensis* is unique in that it is the only pathogen that is solely transmitted through drinking-water.

The fact sheets on potential pathogens include information on human health effects, sources and occurrence, routes of transmission and the significance of drinking-water as a source of infection. The fact sheets on microorganisms that can be used as indicators of the effectiveness of control measures or as indices for the potential presence of pathogenic microorganisms provide information on indicator value, source and occurrence, application and significance of detection.

11.1 Bacterial pathogens

Most bacterial pathogens potentially transmitted by water infect the gastrointestinal tract and are excreted in the faeces of infected humans and other animals. However, there are also some waterborne bacterial pathogens, such as *Legionella*, *Burkholderia pseudomallei* and atypical mycobacteria, that can grow in water and soil. The routes of transmission of these bacteria include inhalation and contact (bathing), with infections occurring in the respiratory tract, in skin lesions or in the brain.

11.1.1 *Acinetobacter*

General description

Acinetobacter spp. are Gram-negative, oxidase-negative, non-motile coccobacilli (short plump rods). Owing to difficulties in naming individual species and biovars, the term *Acinetobacter calcoaceticus baumannii* complex is used in some classification schemes to cover all subgroups of this species, such as *A. baumannii*, *A. iwoffii* and *A. junii*.

Human health effects

Acinetobacter spp. are usually commensal organisms, but they occasionally cause infections, predominantly in susceptible patients in hospitals. They are opportunistic pathogens that may cause urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections. These diseases are predisposed by factors such as malignancy, burns, major surgery and weakened immune systems, such as in neonates and elderly individuals. The emergence and rapid spread of multidrug-resistant *A. calcoaceticus baumannii* complex, causing nosocomial infections, are of concern in health care facilities.

Source and occurrence

Acinetobacter spp. are ubiquitous inhabitants of soil, water and sewage environments. *Acinetobacter* has been isolated from 97% of natural surface water samples in numbers of up to 100/ml. The organisms have been found to represent 1.0–5.5% of the HPC flora in drinking-water samples and have been isolated from 5–92% of distribution water samples. In a survey of untreated groundwater supplies in the USA, *Acinetobacter* spp. were detected in 38% of the groundwater supplies at an arithmetic mean density of 8/100 ml. The study also revealed that slime production, a virulence factor for *A. calcoaceticus*, was not significantly different between well water isolates and

clinical strains, suggesting some degree of pathogenic potential for strains isolated from groundwater. *Acinetobacter* spp. are part of the natural microbial flora of the skin and occasionally the respiratory tract of healthy individuals.

Routes of exposure

Environmental sources within hospitals and person-to-person transmission are the likely sources for most outbreaks of hospital infections. Infection is most commonly associated with contact with wounds and burns or inhalation by susceptible individuals. In patients with *Acinetobacter* bacteraemia, intravenous catheters have also been identified as a source of infection. Outbreaks of infection have been associated with water baths and room humidifiers. Ingestion is not a usual source of infection.

Significance in drinking-water

While *Acinetobacter* spp. are often detected in treated drinking-water supplies, an association between the presence of *Acinetobacter* spp. in drinking-water and clinical disease has not been confirmed. There is no evidence of gastrointestinal infection through ingestion of *Acinetobacter* spp. in drinking-water among the general population. However, transmission of non-gastrointestinal infections by drinking-water may be possible in susceptible individuals, particularly in settings such as health care facilities and hospitals. As discussed in chapter 6, specific WSPs should be developed for buildings, including hospitals and other health care facilities. These plans need to take account of particular sensitivities of occupants. *Acinetobacter* spp. are sensitive to disinfectants such as chlorine, and numbers will be low in the presence of a disinfectant residual. Control measures that can limit growth of the bacteria in distribution systems include treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals. *Acinetobacter* spp. are detected by HPC, which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms. However, *E. coli* (or, alternatively, thermo-tolerant coliforms) cannot be used as an index for the presence/absence of *Acinetobacter* spp.

Selected bibliography

- Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.
- Bergogne-Berezin E, Towner KJ (1996) *Acinetobacter* as nosocomial pathogens: microbiological, clinical and epidemiological features. *Clinical Microbiology Reviews*, 9:148–165.
- Bifulco JM, Shirey JJ, Bissonnette GK (1989) Detection of *Acinetobacter* spp. in rural drinking water supplies. *Applied and Environmental Microbiology*, 55:2214–2219.

Jellison TK, McKinnon PS, Rybak MJ (2001) Epidemiology, resistance and outcomes of *Acinetobacter baumannii* bacteremia treated with imipenem-cilastatin or ampicillin-sulbactam. *Pharmacotherapy*, 21:142–148.

Rusin PA et al. (1997) Risk assessment of opportunistic bacterial pathogens in drinking-water. *Reviews of Environmental Contamination and Toxicology*, 152:57–83.

11.1.2 *Aeromonas*

General description

Aeromonas spp. are Gram-negative, non-spore-forming, facultative anaerobic bacilli belonging to the family Vibrionaceae. They bear many similarities to the Enterobacteriaceae. The genus is divided into two groups. The group of psychrophilic non-motile aeromonads consists of only one species, *A. salmonicida*, an obligate fish pathogen that is not considered further here. The group of mesophilic motile (single polar flagellum) aeromonads is considered of potential human health significance and consists of the species *A. hydrophila*, *A. caviae*, *A. veronii* subsp. *sobria*, *A. jandaei*, *A. veronii* subsp. *veronii* and *A. schubertii*. The bacteria are normal inhabitants of fresh water and occur in water, soil and many foods, particularly meat and milk.

Human health effects

Aeromonas spp. can cause infections in humans, including septicaemia, particularly in immunocompromised patients, wound infections and respiratory tract infections. There have been some claims that *Aeromonas* spp. can cause gastrointestinal illness, but epidemiological evidence is not consistent. Despite marked toxin production by *Aeromonas* spp. *in vitro*, diarrhoea has not yet been introduced in test animals or human volunteers.

Source and occurrence

Aeromonas spp. occur in water, soil and food, particularly meat, fish and milk. *Aeromonas* spp. are generally readily found in most fresh waters, and they have been detected in many treated drinking-water supplies, mainly as a result of growth in distribution systems. The factors that affect the occurrence of *Aeromonas* spp. in water distribution systems are not fully understood, but organic content, temperature, the residence time of water in the distribution network and the presence of residual chlorine have been shown to influence population sizes.

Routes of exposure

Wound infections have been associated with contaminated soil and water-related activities, such as swimming, diving, boating and fishing. Septicaemia can follow from such wound infections. In immunocompromised individuals, septicaemia may arise from aeromonads present in their own gastrointestinal tract.

Significance in drinking-water

Despite frequent isolation of *Aeromonas* spp. from drinking-water, the body of evidence does not provide significant support for waterborne transmission. Aeromonads typically found in drinking-water do not belong to the same DNA homology groups as those associated with cases of gastroenteritis. The presence of *Aeromonas* spp. in drinking-water supplies is generally considered a nuisance. Entry of aeromonads into distribution systems can be minimized by adequate disinfection. Control measures that can limit growth of the bacteria in distribution systems include treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals. *Aeromonas* spp. are detected by HPC, which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms. However, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be used as an index for the presence/absence of *Aeromonas* spp.

Selected bibliography

- Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.
- Borchardt MA, Stemper ME, Standridge JH (2003) *Aeromonas* isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis. *Emerging Infectious Diseases*, 9:224–228.
- WHO (2002) *Aeromonas*. In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization.

11.1.3 *Bacillus*

General description

Bacillus spp. are large (4–10 µm), Gram-positive, strictly aerobic or facultatively anaerobic encapsulated bacilli. They have the important feature of producing spores that are exceptionally resistant to unfavourable conditions. *Bacillus* spp. are classified into the subgroups *B. polymyxa*, *B. subtilis* (which includes *B. cereus* and *B. licheniformis*), *B. brevis* and *B. anthracis*.

Human health effects

Although most *Bacillus* spp. are harmless, a few are pathogenic to humans and animals. *Bacillus cereus* causes food poisoning similar to staphylococcal food poisoning. Some strains produce heat-stable toxin in food that is associated with spore germination and gives rise to a syndrome of vomiting within 1–5 h of ingestion. Other strains produce a heat-labile enterotoxin after ingestion that causes diarrhoea within 10–15 h. *Bacillus cereus* is known to cause bacteraemia in immunocompromised patients as well as symptoms such as vomiting and diarrhoea. *Bacillus anthracis* causes anthrax in humans and animals.

Source and occurrence

Bacillus spp. commonly occur in a wide range of natural environments, such as soil and water. They form part of the HPC bacteria, which are readily detected in most drinking-water supplies.

Routes of exposure

Infection with *Bacillus* spp. is associated with the consumption of a variety of foods, especially rice, pastas and vegetables, as well as raw milk and meat products. Disease may result from the ingestion of the organisms or toxins produced by the organisms. Drinking-water has not been identified as a source of infection of pathogenic *Bacillus* spp., including *Bacillus cereus*. Waterborne transmission of *Bacillus* gastroenteritis has not been confirmed.

Significance in drinking-water

Bacillus spp. are often detected in drinking-water supplies, even supplies treated and disinfected by acceptable procedures. This is largely due to the resistance of spores to disinfection processes. Owing to a lack of evidence that waterborne *Bacillus* spp. are clinically significant, specific management strategies are not required.

Selected bibliography

Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.

11.1.4 *Burkholderia pseudomallei*

General description

Burkholderia pseudomallei is a Gram-negative bacillus commonly found in soil and muddy water, predominantly in tropical regions such as northern Australia and south-east Asia. The organism is acid tolerant and survives in water for prolonged periods in the absence of nutrients.

Human health effects

Burkholderia pseudomallei can cause the disease melioidosis, which is endemic in northern Australia and other tropical regions. The most common clinical manifestation is pneumonia, which may be fatal. In some of these areas, melioidosis is the most common cause of community-acquired pneumonia. Cases appear throughout the year but peak during the rainy season. Many patients present with milder forms of pneumonia, which respond well to appropriate antibiotics, but some may present with a severe septicaemic pneumonia. Other symptoms include skin abscesses or ulcers, abscesses in internal organs and unusual neurological illnesses, such as brainstem encephalitis and acute paraplegia. Although melioidosis can occur in healthy children and adults, it occurs mainly in people whose defence mechanisms against infection

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are impaired by underlying conditions or poor general health associated with poor nutrition or living conditions.

Source and occurrence

The organism occurs predominantly in tropical regions, typically in soil or surface-accumulated muddy water, from where it may reach raw water sources and also drinking-water supplies. The number of organisms in drinking-water that would constitute a significant risk of infection is not known.

Routes of exposure

Most infections appear to be through contact of skin cuts or abrasions with contaminated water. In south-east Asia, rice paddies represent a significant source of infection. Infection may also occur via other routes, particularly through inhalation or ingestion. The relative importance of these routes of infection is not known.

Significance in drinking-water

In two Australian outbreaks of melioidosis, indistinguishable isolates of *B. pseudomallei* were cultured from cases and the drinking-water supply. The detection of the organisms in one drinking-water supply followed replacement of water pipes and chlorination failure, while the second supply was unchlorinated. Within a WSP, control measures that should provide effective protection against this organism include application of established treatment and disinfection processes for drinking-water coupled with protection of the distribution system from contamination, including during repairs and maintenance. HPC and disinfectant residual as measures of water treatment effectiveness and application of appropriate mains repair procedures could be used to indicate protection against *B. pseudomallei*. Because of the environmental occurrence of *B. pseudomallei*, *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable index for the presence/absence of this organism.

Selected bibliography

- Ainsworth R, ed. (2004) *Safe, piped water: Managing microbial water quality in piped distribution systems*. IWA Publishing, London, for the World Health Organization, Geneva.
- Currie BJ (2000) The epidemiology of melioidosis in Australia and Papua New Guinea. *Acta Tropica*, 74:121–127.
- Currie BJ et al. (2001) A cluster of melioidosis cases from an endemic region is clonal and is linked to the water supply using molecular typing of *Burkholderia pseudomallei* isolates. *American Journal of Tropical Medicine and Hygiene*, 65:177–179.
- Inglis TJJ et al. (2000) Outbreak strain of *Burkholderia pseudomallei* traced to water treatment plant. *Emerging Infectious Diseases*, 6:56–59.

11.1.5 *Campylobacter*

General description

Campylobacter spp. are microaerophilic (require decreased oxygen) and capnophilic (require increased carbon dioxide), Gram-negative, curved spiral rods with a single unsheathed polar flagellum. *Campylobacter* spp. are one of the most important causes of acute gastroenteritis worldwide. *Campylobacter jejuni* is the most frequently isolated species from patients with acute diarrhoeal disease, whereas *C. coli*, *C. laridis* and *C. fetus* have also been isolated in a small proportion of cases. Two closely related genera, *Helicobacter* and *Archobacter*, include species previously classified as *Campylobacter* spp.

Human health effects

An important feature of *C. jejuni* is relatively high infectivity compared with other bacterial pathogens. As few as 1000 organisms can cause infection. Most symptomatic infections occur in infancy and early childhood. The incubation period is usually 2–4 days. Clinical symptoms of *C. jejuni* infection are characterized by abdominal pain, diarrhoea (with or without blood or faecal leukocytes), vomiting, chills and fever. The infection is self-limited and resolves in 3–7 days. Relapses may occur in 5–10% of untreated patients. Other clinical manifestations of *C. jejuni* infections in humans include reactive arthritis and meningitis. Several reports have associated *C. jejuni* infection with Guillain-Barré syndrome, an acute demyelinating disease of the peripheral nerves.

Source and occurrence

Campylobacter spp. occur in a variety of environments. Wild and domestic animals, especially poultry, wild birds and cattle, are important reservoirs. Pets and other animals may also be reservoirs. Food, including meat and unpasteurized milk, are important sources of *Campylobacter* infections. Water is also a significant source. The occurrence of the organisms in surface waters has proved to be strongly dependent on rainfall, water temperature and the presence of waterfowl.

Routes of exposure

Most *Campylobacter* infections are reported as sporadic in nature, with food considered a common source of infection. Transmission to humans typically occurs by the consumption of animal products. Meat, particularly poultry products, and unpasteurized milk are important sources of infection. Contaminated drinking-water supplies have been identified as a source of outbreaks. The number of cases in these outbreaks ranged from a few to several thousand, with sources including unchlorinated or inadequately chlorinated surface water supplies and faecal contamination of water storage reservoirs by wild birds.

Significance in drinking-water

Contaminated drinking-water supplies have been identified as a significant source of outbreaks of campylobacteriosis. The detection of waterborne outbreaks and cases appears to be increasing. Waterborne transmission has been confirmed by the isolation of the same strains from patients and drinking-water they had consumed. Within a WSP, control measures that can be applied to manage potential risk from *Campylobacter* spp. include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. Storages of treated and disinfected water should be protected from bird faeces. *Campylobacter* spp. are faecally borne pathogens and are not particularly resistant to disinfection. Hence, *E. coli* (or thermotolerant coliforms) is an appropriate indicator for the presence/absence of *Campylobacter* spp. in drinking-water supplies.

Selected bibliography

Frost JA (2001) Current epidemiological issues in human campylobacteriosis. *Journal of Applied Microbiology*, 90:85S–95S.

Koenraad PMFJ, Rombouts FM, Notermans SHW (1997) Epidemiological aspects of thermophilic *Campylobacter* in water-related environments: A review. *Water Environment Research*, 69:52–63.

Kuroki S et al. (1991) Guillain-Barré syndrome associated with *Campylobacter* infection. *Pediatric Infectious Diseases Journal*, 10:149–151.

11.1.6 *Escherichia coli* pathogenic strains

General description

Escherichia coli is present in large numbers in the normal intestinal flora of humans and animals, where it generally causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. A limited number of enteropathogenic strains can cause acute diarrhoea. Several classes of enteropathogenic *E. coli* have been identified on the basis of different virulence factors, including enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC). More is known about the first four classes named; the pathogenicity and prevalence of EAEC and DAEC strains are less well established.

Human health effects

EHEC serotypes, such as *E. coli* O157:H7 and *E. coli* O111, cause diarrhoea that ranges from mild and non-bloody to highly bloody, which is indistinguishable from haemorrhagic colitis. Between 2% and 7% of cases can develop the potentially fatal haemolytic uraemic syndrome (HUS), which is characterized by acute renal failure and haemolytic anaemia. Children under 5 years of age are at most risk of developing HUS. The infectivity of EHEC strains is substantially higher than that of the other

strains. As few as 100 EHEC organisms can cause infection. ETEC produces heat-labile or heat-stable *E. coli* enterotoxin, or both toxins simultaneously, and is an important cause of diarrhoea in developing countries, especially in young children. Symptoms of ETEC infection include mild watery diarrhoea, abdominal cramps, nausea and headache. Infection with EPEC has been associated with severe, chronic, non-bloody diarrhoea, vomiting and fever in infants. EPEC infections are rare in developed countries, but occur commonly in developing countries, with infants presenting with malnutrition, weight loss and growth retardation. EIEC causes watery and occasionally bloody diarrhoea where strains invade colon cells by a pathogenic mechanism similar to that of *Shigella*.

Source and occurrence

Enteropathogenic *E. coli* are enteric organisms, and humans are the major reservoir, particularly of EPEC, ETEC and EIEC strains. Livestock, such as cattle and sheep and, to a lesser extent, goats, pigs and chickens, are a major source of EHEC strains. The latter have also been associated with raw vegetables, such as bean sprouts. The pathogens have been detected in a variety of water environments.

Routes of exposure

Infection is associated with person-to-person transmission, contact with animals, food and consumption of contaminated water. Person-to-person transmissions are particularly prevalent in communities where there is close contact between individuals, such as nursing homes and day care centres.

Significance in drinking-water

Waterborne transmission of pathogenic *E. coli* has been well documented for recreational waters and contaminated drinking-water. A well publicized waterborne outbreak of illness caused by *E. coli* O157:H7 (and *Campylobacter jejuni*) occurred in the farming community of Walkerton in Ontario, Canada. The outbreak took place in May 2000 and led to 7 deaths and more than 2300 illnesses. The drinking-water supply was contaminated by rainwater runoff containing cattle excreta. Within a WSP, control measures that can be applied to manage potential risk from enteropathogenic *E. coli* include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. There is no indication that the response of enteropathogenic strains of *E. coli* to water treatment and disinfection procedures differs from that of other *E. coli*. Hence, conventional testing for *E. coli* (or, alternatively, thermotolerant coliform bacteria) provides an appropriate index for the enteropathogenic serotypes in drinking-water. This applies even though standard tests will generally not detect EHEC strains.

Selected bibliography

- Nataro JP, Kaper JB (1998) Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, 11:142–201.
- O'Connor DR (2002) *Report of the Walkerton Inquiry: The events of May 2000 and related issues. Part 1: A summary*. Toronto, Ontario, Ontario Ministry of the Attorney General, Queen's Printer for Ontario.

11.1.7 *Helicobacter pylori*

General description

Helicobacter pylori, originally classified as *Campylobacter pylori*, is a Gram-negative, microaerophilic, spiral-shaped, motile bacterium. There are at least 14 species of *Helicobacter*, but only *H. pylori* has been identified as a human pathogen.

Human health effects

Helicobacter pylori is found in the stomach; although most infections are asymptomatic, the organism is associated with chronic gastritis, which may lead to complications such as peptic and duodenal ulcer disease and gastric cancer. Whether the organism is truly the cause of these conditions remains unclear. The majority of *H. pylori* infections are initiated in childhood and without treatment are chronic. The infections are more prevalent in developing countries and are associated with overcrowded living conditions. Interfamilial clustering is common.

Source and occurrence

Humans appear to be the primary host of *H. pylori*. Other hosts may include domestic cats. There is evidence that *H. pylori* is sensitive to bile salts, which would reduce the likelihood of faecal excretion, although it has been isolated from faeces of young children. *Helicobacter pylori* has been detected in water. Although *H. pylori* is unlikely to grow in the environment, it has been found to survive for 3 weeks in biofilms and up to 20–30 days in surface waters. In a study conducted in the USA, *H. pylori* was found in the majority of surface water and shallow groundwater samples. The presence of *H. pylori* was not correlated with the presence of *E. coli*. Possible contamination of the environment can be through children with diarrhoea or through vomiting by children as well as adults.

Routes of exposure

Person-to-person contact within families has been identified as the most likely source of infection through oral–oral transmission. *Helicobacter pylori* can survive well in mucus or vomit. However, it is difficult to detect in mouth or faecal samples. Faecal–oral transmission is also considered possible.

Significance in drinking-water

Consumption of contaminated drinking-water has been suggested as a potential source of infection, but further investigation is required to establish any link with waterborne transmission. Humans are the principal source of *H. pylori*, and the organism is sensitive to oxidizing disinfectants. Hence, control measures that can be applied to protect drinking-water supplies from *H. pylori* include preventing contamination by human waste and adequate disinfection. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is not a reliable index for the presence/absence of this organism.

Selected bibliography

- Dunn BE, Cohen H, Blaser MJ (1997) *Helicobacter pylori*. *Clinical Microbiology Reviews*, 10:720–741.
- Hegarty JP, Dowd MT, Baker KH (1999) Occurrence of *Helicobacter pylori* in surface water in the United States. *Journal of Applied Microbiology*, 87:697–701.
- Hulten K et al. (1996) *Helicobacter pylori* in drinking-water in Peru. *Gastroenterology*, 110:1031–1035.
- Mazari-Hiriart M, López-Vidal Y, Calva JJ (2001) *Helicobacter pylori* in water systems for human use in Mexico City. *Water Science and Technology*, 43:93–98.

11.1.8 *Klebsiella*

General description

Klebsiella spp. are Gram-negative, non-motile bacilli that belong to the family Enterobacteriaceae. The genus *Klebsiella* consists of a number of species, including *K. pneumoniae*, *K. oxytoca*, *K. planticola* and *K. terrigena*. The outermost layer of *Klebsiella* spp. consists of a large polysaccharide capsule that distinguishes the organisms from other members of the family. Approximately 60–80% of all *Klebsiella* spp. isolated from faeces and clinical specimens are *K. pneumoniae* and are positive in the thermotolerant coliform test. *Klebsiella oxytoca* has also been identified as a pathogen.

Human health effects

Klebsiella spp. have been identified as colonizing hospital patients, where spread is associated with the frequent handling of patients (e.g., in intensive care units). Patients at highest risk are those with impaired immune systems, such as the elderly or very young, patients with burns or excessive wounds, those undergoing immunosuppressive therapy or those with HIV/AIDS infection. Colonization may lead to invasive infections. On rare occasions, *Klebsiella* spp., notably *K. pneumoniae* and *K. oxytoca*, may cause serious infections, such as destructive pneumonia.

Source and occurrence

Klebsiella spp. are natural inhabitants of many water environments, and they may multiply to high numbers in waters rich in nutrients, such as pulp mill wastes, textile finishing plants and sugar-cane processing operations. In drinking-water distribution

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systems, they are known to colonize washers in taps. The organisms can grow in water distribution systems. *Klebsiella* spp. are also excreted in the faeces of many healthy humans and animals, and they are readily detected in sewage-polluted water.

Routes of exposure

Klebsiella can cause nosocomial infections, and contaminated water and aerosols may be a potential source of the organisms in hospital environments and other health care facilities.

Significance in drinking-water

Klebsiella spp. are not considered to represent a source of gastrointestinal illness in the general population through ingestion of drinking-water. *Klebsiella* spp. detected in drinking-water are generally biofilm organisms and are unlikely to represent a health risk. The organisms are reasonably sensitive to disinfectants, and entry into distribution systems can be prevented by adequate treatment. Growth within distribution systems can be minimized by strategies that are designed to minimize biofilm growth, including treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals. *Klebsiella* is a coliform and can be detected by traditional tests for total coliforms.

Selected bibliography

- Ainsworth R, ed. (2004) *Safe, piped water: Managing microbial water quality in piped distribution systems*. IWA Publishing, London, for the World Health Organization, Geneva.
- Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.

11.1.9 Legionella

General description

The genus *Legionella*, a member of the family Legionellaceae, has at least 42 species. Legionellae are Gram-negative, rod-shaped, non-spore-forming bacteria that require L-cysteine for growth and primary isolation. *Legionella* spp. are heterotrophic bacteria found in a wide range of water environments and can proliferate at temperatures above 25°C.

Human health effects

Although all *Legionella* spp. are considered potentially pathogenic for humans, *L. pneumophila* is the major waterborne pathogen responsible for legionellosis, of which two clinical forms are known: Legionnaires' disease and Pontiac fever. The former is a pneumonic illness with an incubation period of 3–6 days. Host factors influence the likelihood of illness: males are more frequently affected than females, and most cases

occur in the 40- to 70-year age group. Risk factors include smoking, alcohol abuse, cancer, diabetes, chronic respiratory or kidney disease and immunosuppression, as in transplant recipients. Pontiac fever is a milder, self-limiting disease with a high attack rate and an onset (5 h to 3 days) and symptoms similar to those of influenza: fever, headache, nausea, vomiting, aching muscles and coughing. Studies of seroprevalence of antibodies indicate that many infections are asymptomatic.

Source and occurrence

Legionella spp. are members of the natural flora of many freshwater environments, such as rivers, streams and impoundments, where they occur in relatively low numbers. However, they thrive in certain human-made water environments, such as water cooling devices (cooling towers and evaporative condensers) associated with air conditioning systems, hot water distribution systems and spas, which provide suitable temperatures (25–50 °C) and conditions for their multiplication. Devices that support multiplication of *Legionella* have been associated with outbreaks of Legionnaires' disease. *Legionella* survive and grow in biofilms and sediments and are more easily detected from swab samples than from flowing water. Legionellae can be ingested by trophozoites of certain amoebae such as *Acanthamoeba*, *Hartmanella* and *Naegleria*, which may play a role in their persistence in water environments.

Routes of exposure

The most common route of infection is the inhalation of aerosols containing the bacteria. Such aerosols can be generated by contaminated cooling towers, warm water showers, humidifiers and spas. Aspiration has also been identified as a route of infection in some cases associated with contaminated water, food and ice. There is no evidence of person-to-person transmission.

Significance in drinking-water

Legionella spp. are common waterborne organisms, and devices such as cooling towers, hot water systems and spas that utilize mains water have been associated with outbreaks of infection. Owing to the prevalence of *Legionella*, the potential for ingress into drinking-water systems should be considered as a possibility, and control measures should be employed to reduce the likelihood of survival and multiplication. Disinfection strategies designed to minimize biofilm growth and temperature control can minimize the potential risk from *Legionella* spp. The organisms are sensitive to disinfection. Monochloramine has been shown to be particularly effective, probably due to its stability and greater effectiveness against biofilms. Water temperature is an important element of control strategies. Wherever possible, water temperatures should be kept outside the range of 25–50 °C. In hot water systems, storages should be maintained above 55 °C, and similar temperatures throughout associated pipework will prevent growth of the organism. However, maintaining temperatures of hot water above 50 °C may represent a scalding risk in young children, the elderly and other vul-

nerable groups. Where temperatures in hot or cold water distribution systems cannot be maintained outside the range of 25–50°C, greater attention to disinfection and strategies aimed at limiting development of biofilms are required. Accumulation of sludge, scale, rust, algae or slime deposits in water distribution systems supports the growth of *Legionella* spp., as does stagnant water. Systems that are kept clean and flowing are less likely to support excess growth of *Legionella* spp. Care should also be taken to select plumbing materials that do not support microbial growth and the development of biofilms.

Legionella spp. represent a particular concern in devices such as cooling towers and hot water systems in large buildings. As discussed in chapter 6, specific WSPs incorporating control measures for *Legionella* spp. should be developed for these buildings. *Legionella* are not detected by HPC techniques, and *E. coli* (or, alternatively, thermo-tolerant coliforms) is not a suitable index for the presence/absence of this organism.

Selected bibliography

- Codony F et al. (2002) Factors promoting colonization by legionellae in residential water distribution systems: an environmental case-control survey. *European Journal of Clinical Microbiology and Infectious Diseases*, 21:717–721.
- Emmerson AM (2001) Emerging waterborne infections in health-care settings. *Emerging Infectious Diseases*, 7:272–276.
- Rusin PA et al. (1997) Risk assessment of opportunistic bacterial pathogens in drinking-water. *Reviews of Environmental Contamination and Toxicology*, 152:57–83.
- WHO (in preparation) *Legionella and the prevention of legionellosis*. Geneva, World Health Organization.

11.1.10 Mycobacterium

General description

The tuberculous or “typical” species of *Mycobacterium*, such as *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. leprae*, have only human or animal reservoirs and are not transmitted by water. In contrast, the non-tuberculous or “atypical” species of *Mycobacterium* are natural inhabitants of a variety of water environments. These aerobic, rod-shaped and acid-fast bacteria grow slowly in suitable water environments and on culture media. Typical examples include the species *M. gordonae*, *M. kansasii*, *M. marinum*, *M. scrofulaceum*, *M. xenopi*, *M. intracellulare* and *M. avium* and the more rapid growers *M. chelonae* and *M. fortuitum*. The term *M. avium* complex has been used to describe a group of pathogenic species including *M. avium* and *M. intracellulare*. However, other atypical mycobacteria are also pathogenic. A distinct feature of all *Mycobacterium* spp. is a cell wall with high lipid content, which is used in identification of the organisms using acid-fast staining.

Human health effects

Atypical *Mycobacterium* spp. can cause a range of diseases involving the skeleton, lymph nodes, skin and soft tissues, as well as the respiratory, gastrointestinal and genitourinary tracts. Manifestations include pulmonary disease, Buruli ulcer, osteomyelitis and septic arthritis in people with no known predisposing factors. These bacteria are a major cause of disseminated infections in immunocompromised patients and are a common cause of death in HIV-positive persons.

Source and occurrence

Atypical *Mycobacterium* spp. multiply in a variety of suitable water environments, notably biofilms. One of the most commonly occurring species is *M. gordonae*. Other species have also been isolated from water, including *M. avium*, *M. intracellulare*, *M. kansasii*, *M. fortuitum* and *M. chelonae*. High numbers of atypical *Mycobacterium* spp. may occur in distribution systems after events that dislodge biofilms, such as flushing or flow reversals. They are relatively resistant to treatment and disinfection and have been detected in well operated and maintained drinking-water supplies with HPC less than 500/ml and total chlorine residuals of up to 2.8 mg/litre. The growth of these organisms in biofilms reduces the effectiveness of disinfection. In one survey, the organisms were detected in 54% of ice and 35% of public drinking-water samples.

Routes of exposure

Principal routes of infection appear to be inhalation, contact and ingestion of contaminated water. Infections by various species have been associated with their presence in drinking-water supplies. In 1968, an endemic of *M. kansasii* infections was associated with the presence of the organisms in the drinking-water supply, and the spread of the organisms was associated with aerosols from showerheads. In Rotterdam, Netherlands, an investigation into the frequent isolation of *M. kansasii* from clinical specimens revealed the presence of the same strains, confirmed by phage type and weak nitrate activity, in tap water. An increase in numbers of infections by the *M. avium* complex in Massachusetts, USA, has also been attributed to their incidence in drinking-water. In all these cases, there is only circumstantial evidence of a causal relationship between the occurrence of the bacteria in drinking-water and human disease. Infections have been linked to contaminated water in spas.

Significance in drinking-water

Detections of atypical mycobacteria in drinking-water and the identified routes of transmission suggest that drinking-water supplies are a plausible source of infection. There are limited data on the effectiveness of control measures that could be applied to reduce the potential risk from these organisms. One study showed that a water treatment plant could achieve a 99% reduction in numbers of mycobacteria from raw water. Atypical mycobacteria are relatively resistant to disinfection. Persistent residual disinfectant should reduce numbers of mycobacteria in the water column but is

unlikely to be effective against organisms present in biofilms. Control measures that are designed to minimize biofilm growth, including treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals, could result in less growth of these organisms. Mycobacteria are not detected by HPC techniques, and *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable index for the presence/absence of this organism.

Selected bibliography

- Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.
- Bartram J et al., eds. (2004) *Pathogenic mycobacteria in water: A guide to public health consequences, monitoring and management*. Geneva, World Health Organization.
- Covert TC et al. (1999) Occurrence of nontuberculous mycobacteria in environmental samples. *Applied and Environmental Microbiology*, 65:2492–2496.
- Falkinham JO, Norton CD, LeChevallier MW (2001) Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare* and other mycobacteria in drinking water distribution systems. *Applied and Environmental Microbiology*, 66:1225–1231.
- Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA*, 22:193–202.
- Rusin PA et al. (1997) Risk assessment of opportunistic bacterial pathogens in drinking-water. *Reviews of Environmental Contamination and Toxicology*, 152:57–83.
- Singh N, Yu VL (1994) Potable water and *Mycobacterium avium* complex in HIV patients: is prevention possible? *Lancet*, 343:1110–1111.
- Von Reyn CF et al. (1994) Persistent colonization of potable water as a source of *Mycobacterium avium* infection in AIDS. *Lancet*, 343:1137–1141.

11.1.11 *Pseudomonas aeruginosa*

General description

Pseudomonas aeruginosa is a member of the family Pseudomonadaceae and is a polarly flagellated, aerobic, Gram-negative rod. When grown in suitable media, it produces the non-fluorescent bluish pigment pyocyanin. Many strains also produce the fluorescent green pigment pyoverdine. *Pseudomonas aeruginosa*, like other fluorescent pseudomonads, produces catalase, oxidase and ammonia from arginine and can grow on citrate as the sole source of carbon.

Human health effects

Pseudomonas aeruginosa can cause a range of infections but rarely causes serious illness in healthy individuals without some predisposing factor. It predominantly colonizes damaged sites such as burn and surgical wounds, the respiratory tract of people

with underlying disease and physically damaged eyes. From these sites, it may invade the body, causing destructive lesions or septicaemia and meningitis. Cystic fibrosis and immunocompromised patients are prone to colonization with *P. aeruginosa*, which may lead to serious progressive pulmonary infections. Water-related folliculitis and ear infections are associated with warm, moist environments such as swimming pools and spas. Many strains are resistant to a range of antimicrobial agents, which can increase the significance of the organism in hospital settings.

Source and occurrence

Pseudomonas aeruginosa is a common environmental organism and can be found in faeces, soil, water and sewage. It can multiply in water environments and also on the surface of suitable organic materials in contact with water. *Pseudomonas aeruginosa* is a recognized cause of hospital-acquired infections with potentially serious complications. It has been isolated from a range of moist environments such as sinks, water baths, hot water systems, showers and spa pools.

Routes of exposure

The main route of infection is by exposure of susceptible tissue, notably wounds and mucous membranes, to contaminated water or contamination of surgical instruments. Cleaning of contact lenses with contaminated water can cause a form of keratitis. Ingestion of drinking-water is not an important source of infection.

Significance in drinking-water

Although *P. aeruginosa* can be significant in certain settings such as health care facilities, there is no evidence that normal uses of drinking-water supplies are a source of infection in the general population. However, the presence of high numbers of *P. aeruginosa* in potable water, notably in packaged water, can be associated with complaints about taste, odour and turbidity. *Pseudomonas aeruginosa* is sensitive to disinfection, and entry into distribution systems can be minimized by adequate disinfection. Control measures that are designed to minimize biofilm growth, including treatment to optimize organic carbon removal, restriction of the residence time of water in distribution systems and maintenance of disinfectant residuals, should reduce the growth of these organisms. *Pseudomonas aeruginosa* is detected by HPC, which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms. However, as *P. aeruginosa* is a common environmental organism, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be used for this purpose.

Selected bibliography

Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.

de Victorica J, Galván M (2001) *Pseudomonas aeruginosa* as an indicator of health risk in water for human consumption. *Water Science and Technology*, 43:49–52.

Hardalo C, Edberg SC (1997) *Pseudomonas aeruginosa*: Assessment of risk from drinking-water. *Critical Reviews in Microbiology*, 23:47–75.

11.1.12 *Salmonella*

General description

Salmonella spp. belong to the family Enterobacteriaceae. They are motile, Gram-negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. Originally, they were grouped into more than 2000 species (serotypes) according to their somatic (O) and flagellar (H) antigens (Kauffmann-White classification). It is now considered that this classification is below species level and that there are actually no more than 2–3 species (*Salmonella enterica* or *Salmonella choleraesuis*, *Salmonella bongori* and *Salmonella typhi*), with the serovars being subspecies. All of the enteric pathogens except *S. typhi* are members of the species *S. enterica*. Convention has dictated that subspecies are abbreviated, so that *S. enterica* serovar Paratyphi A becomes *S. Paratyphi A*.

Human health effects

Salmonella infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever / enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* spp. can be divided into two fairly distinct groups: the typhoidal species/serovars (*Salmonella typhi* and *S. Paratyphi*) and the remaining non-typhoidal species/serovars. Symptoms of non-typhoidal gastroenteritis appear from 6 to 72 h after ingestion of contaminated food or water. Diarrhoea lasts 3–5 days and is accompanied by fever and abdominal pain. Usually the disease is self-limiting. The incubation period for typhoid fever can be 1–14 days but is usually 3–5 days. Typhoid fever is a more severe illness and can be fatal. Although typhoid is uncommon in areas with good sanitary systems, it is still prevalent elsewhere, and there are many millions of cases each year.

Source and occurrence

Salmonella spp. are widely distributed in the environment, but some species or serovars show host specificity. Notably, *S. typhi* and generally *S. Paratyphi* are restricted to humans, although livestock can occasionally be a source of *S. Paratyphi*. A large number of serovars, including *S. Typhimurium* and *S. Enteritidis*, infect humans and also a wide range of animals, including poultry, cows, pigs, sheep, birds and even reptiles. The pathogens typically gain entry into water systems through faecal contamination from sewage discharges, livestock and wild animals. Contamination has been detected in a wide variety of foods and milk.

Routes of exposure

Salmonella is spread by the faecal–oral route. Infections with non-typhoidal serovars are primarily associated with person-to-person contact, the consumption of a variety of contaminated foods and exposure to animals. Infection by typhoid species is associated with the consumption of contaminated water or food, with direct person-to-person spread being uncommon.

Significance in drinking-water

Waterborne typhoid fever outbreaks have devastating public health implications. However, despite their widespread occurrence, non-typhoidal *Salmonella* spp. rarely cause drinking-water-borne outbreaks. Transmission, most commonly involving *S. Typhimurium*, has been associated with the consumption of contaminated ground-water and surface water supplies. In an outbreak of illness associated with a communal rainwater supply, bird faeces were implicated as a source of contamination. *Salmonella* spp. are relatively sensitive to disinfection. Within a WSP, control measures that can be applied to manage risk include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is a generally reliable index for *Salmonella* spp. in drinking-water supplies.

Selected bibliography

- Angulo FJ et al. (1997) A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. *American Journal of Public Health*, 87:580–584.
- Escartin EF et al. (2002) Potential *Salmonella* transmission from ornamental fountains. *Journal of Environmental Health*, 65:9–12.
- Koplan JP et al. (1978) Contaminated roof-collected rainwater as a possible cause of an outbreak of salmonellosis. *Journal of Hygiene*, 81:303–309.

11.1.13 Shigella**General description**

Shigella spp. are Gram-negative, non-spore-forming, non-motile, rod-like members of the family Enterobacteriaceae, which grow in the presence or absence of oxygen. Members of the genus have a complex antigenic pattern, and classification is based on their somatic O antigens, many of which are shared with other enteric bacilli, including *E. coli*. There are four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.

Human health effects

Shigella spp. can cause serious intestinal diseases, including bacillary dysentery. Over 2 million infections occur each year, resulting in about 600 000 deaths, predominantly in developing countries. Most cases of *Shigella* infection occur in children under 10 years of age. The incubation period for shigellosis is usually 24–72 h. Ingestion of as

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few as 10–100 organisms may lead to infection, which is substantially less than the infective dose of most other enteric bacteria. Abdominal cramps, fever and watery diarrhoea occur early in the disease. All species can produce severe disease, but illness due to *S. sonnei* is usually relatively mild and self-limiting. In the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process, with bloody diarrhoea and high concentrations of neutrophils in the stool. The production of Shiga toxin by the pathogen plays an important role in this outcome. *Shigella* spp. seem to be better adapted to cause human disease than most other enteric bacterial pathogens.

Source and occurrence

Humans and other higher primates appear to be the only natural hosts for the shigellae. The bacteria remain localized in the intestinal epithelial cells of their hosts. Epidemics of shigellosis occur in crowded communities and where hygiene is poor. Many cases of shigellosis are associated with day care centres, prisons and psychiatric institutions. Military field groups and travellers to areas with poor sanitation are also prone to infection.

Routes of exposure

Shigella spp. are enteric pathogens predominantly transmitted by the faecal–oral route through person-to-person contact, contaminated food and water. Flies have also been identified as a transmission vector from contaminated faecal waste.

Significance in drinking-water

A number of large waterborne outbreaks of shigellosis have been recorded. As the organisms are not particularly stable in water environments, their presence in drinking-water indicates recent human faecal pollution. Available data on prevalence in water supplies may be an underestimate, because detection techniques generally used can have a relatively low sensitivity and reliability. The control of *Shigella* spp. in drinking-water supplies is of special public health importance in view of the severity of the disease caused. *Shigella* spp. are relatively sensitive to disinfection. Within a WSP, control measures that can be applied to manage potential risk include protection of raw water supplies from human waste, adequate treatment and protection of water during distribution. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is a generally reliable index for *Shigella* spp. in drinking-water supplies.

Selected bibliography

- Alamanos Y et al. (2000) A community waterborne outbreak of gastro-enteritis attributed to *Shigella sonnei*. *Epidemiology and Infection*, 125:499–503.
- Pegram GC, Rollins N, Espay Q (1998) Estimating the cost of diarrhoea and epidemic dysentery in Kwa-Zulu-Natal and South Africa. *Water SA*, 24:11–20.

11.1.14 *Staphylococcus aureus***General description**

Staphylococcus aureus is an aerobic or anaerobic, non-motile, non-spore-forming, catalase- and coagulase-positive, Gram-positive coccus, usually arranged in grapelike irregular clusters. The genus *Staphylococcus* contains at least 15 different species. Apart from *S. aureus*, the species *S. epidermidis* and *S. saprophyticus* are also associated with disease in humans.

Human health effects

Although *Staphylococcus aureus* is a common member of the human microflora, it can produce disease through two different mechanisms. One is based on the ability of the organisms to multiply and spread widely in tissues, and the other is based on the ability of the organisms to produce extracellular enzymes and toxins. Infections based on the multiplication of the organisms are a significant problem in hospitals and other health care facilities. Multiplication in tissues can result in manifestations such as boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis and pneumonia. The onset of clinical symptoms for these infections is relatively long, usually several days. Gastrointestinal disease (enterocolitis or food poisoning) is caused by a heat-stable staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids. Onset of disease in this case has a characteristic short incubation period of 1–8 h. The same applies to the toxic shock syndrome caused by toxic shock syndrome toxin-1.

Source and occurrence

Staphylococcus aureus is relatively widespread in the environment but is found mainly on the skin and mucous membranes of animals. The organism is a member of the normal microbial flora of the human skin and is found in the nasopharynx of 20–30% of adults at any one time. Staphylococci are occasionally detected in the gastrointestinal tract and can be detected in sewage. *Staphylococcus aureus* can be released by human contact into water environments such as swimming pools, spa pools and other recreational waters. It has also been detected in drinking-water supplies.

Routes of exposure

Hand contact is by far the most common route of transmission. Inadequate hygiene can lead to contamination of food. Foods such as ham, poultry and potato and egg salads kept at room or higher temperature offer an ideal environment for the multiplication of *S. aureus* and the release of toxins. The consumption of foods containing *S. aureus* toxins can lead to enterotoxin food poisoning within a few hours.

Significance in drinking-water

Although *S. aureus* can occur in drinking-water supplies, there is no evidence of transmission through the consumption of such water. Although staphylococci are slightly more resistant to chlorine residuals than *E. coli*, their presence in water is readily controlled by conventional treatment and disinfection processes. Since faecal material is not their usual source, *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable index for *S. aureus* in drinking-water supplies.

Selected bibliography

- Antai SP (1987) Incidence of *Staphylococcus aureus*, coliforms and antibiotic-resistant strains of *Escherichia coli* in rural water supplies in Port Harcourt. *Journal of Applied Bacteriology*, 62:371–375.
- LeChevallier MW, Seidler RJ (1980) *Staphylococcus aureus* in rural drinking-water. *Applied and Environmental Microbiology*, 39:739–742.

11.1.15 Tsukamurella

General description

The genus *Tsukamurella* belongs to the family Nocardiaceae. *Tsukamurella* spp. are Gram-positive, weakly or variably acid-fast, non-motile, obligate aerobic, irregular rod-shaped bacteria. They are actinomycetes related to *Rhodococcus*, *Nocardia* and *Mycobacterium*. The genus was created in 1988 to accommodate a group of chemically unique organisms characterized by a series of very long chain (68–76 carbons), highly unsaturated mycolic acids, meso-diaminopimelic acid and arabinogalactan, common to the genus *Corynebacterium*. The type species is *T. paurometabola*, and the following additional species were proposed in the 1990s: *T. wratislaviensis*, *T. inchonensis*, *T. pulmonis*, *T. tyrosinosolvans* and *T. strandjordae*.

Human health effects

Tsukamurella spp. cause disease mainly in immunocompromised individuals. Infections with these microorganisms have been associated with chronic lung diseases, immune suppression (leukaemia, tumours, HIV/AIDS infection) and post-operative wound infections. *Tsukamurella* were reported in four cases of catheter-related bacteraemia and in individual cases including chronic lung infection, necrotizing tenosynovitis with subcutaneous abscesses, cutaneous and bone infections, meningitis and peritonitis.

Source and occurrence

Tsukamurella spp. exist primarily as environmental saprophytes in soil, water and foam (thick stable scum on aeration vessels and sedimentation tanks) of activated sludge. *Tsukamurella* are represented in HPC populations in drinking-water.

Routes of exposure

Tsukamurella spp. appear to be transmitted through devices such as catheters or lesions. The original source of the contaminating organisms is unknown.

Significance in drinking-water

Tsukamurella organisms have been detected in drinking-water supplies, but the significance is unclear. There is no evidence of a link between organisms in water and illness. As *Tsukamurella* is an environmental organism, *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable index for this organism.

Selected bibliography

- Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.
- Kattar MM et al. (2001) *Tsukamurella strandjordae* sp. nov., a proposed new species causing sepsis. *Journal of Clinical Microbiology*, 39:1467–1476.
- Larkin JA et al. (1999) Infection of a knee prosthesis with *Tsukamurella* species. *Southern Medical Journal*, 92:831–832.

11.1.16 *Vibrio*

General description

Vibrio spp. are small, curved (comma-shaped), Gram-negative bacteria with a single polar flagellum. Species are typed according to their O antigens. There are a number of pathogenic species, including *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. *Vibrio cholerae* is the only pathogenic species of significance from freshwater environments. While a number of serotypes can cause diarrhoea, only O1 and O139 currently cause the classical cholera symptoms in which a proportion of cases suffer fulminating and severe watery diarrhoea. The O1 serovar has been further divided into “classical” and “El Tor” biotypes. The latter is distinguished by features such as the ability to produce a dialysable heat-labile haemolysin, active against sheep and goat red blood cells. The classical biotype is considered responsible for the first six cholera pandemics, while the El Tor biotype is responsible for the seventh pandemic that commenced in 1961. Strains of *V. cholerae* O1 and O139 that cause cholera produce an enterotoxin (cholera toxin) that alters the ionic fluxes across the intestinal mucosa, resulting in substantial loss of water and electrolytes in liquid stools. Other factors associated with infection are an adhesion factor and an attachment pilus. Not all strains of serotypes O1 or O139 possess the virulence factors, and they are rarely possessed by non-O1/O139 strains.

Human health effects

Cholera outbreaks continue to occur in many areas of the developing world. Symptoms are caused by heat-labile cholera enterotoxin carried by toxigenic strains of *V.*

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cholerae O1/O139. A large percentage of infected persons do not develop illness; about 60% of the classical and 75% of the El Tor group infections are asymptomatic. Symptomatic illness ranges from mild or moderate to severe disease. The initial symptoms of cholera are an increase in peristalses followed by loose, watery and mucus-flecked “rice-water” stools that may cause a patient to lose as much as 10–15 litres of liquid per day. Decreasing gastric acidity by administration of sodium bicarbonate reduces the infective dose of *V. cholerae* O1 from more than 10^8 to about 10^4 organisms. Case fatality rates vary according to facilities and preparedness. As many as 60% of untreated patients may die as a result of severe dehydration and loss of electrolytes, but well established diarrhoeal disease control programmes can reduce fatalities to less than 1%. Non-toxicogenic strains of *V. cholerae* can cause self-limiting gastroenteritis, wound infections and bacteraemia.

Source and occurrence

Non-toxicogenic *V. cholerae* is widely distributed in water environments, but toxigenic strains are not distributed as widely. Humans are an established source of toxigenic *V. cholerae*; in the presence of disease, the organism can be detected in sewage. Although *V. cholerae* O1 can be isolated from water in areas without disease, the strains are not generally toxigenic. Toxigenic *V. cholerae* has also been found in association with live copepods as well as other aquatic organisms, including molluscs, crustaceans, plants, algae and cyanobacteria. Numbers associated with these aquatic organisms are often higher than in the water column. Non-toxicogenic *V. cholerae* has been isolated from birds and herbivores in areas far away from marine and coastal waters. The prevalence of *V. cholerae* decreases as water temperatures fall below 20°C.

Routes of exposure

Cholera is typically transmitted by the faecal–oral route, and the infection is predominantly contracted by the ingestion of faecally contaminated water and food. The high numbers required to cause infection make person-to-person contact an unlikely route of transmission.

Significance in drinking-water

Contamination of water due to poor sanitation is largely responsible for transmission, but this does not fully explain the seasonality of recurrence, and factors other than poor sanitation must play a role. The presence of the pathogenic *V. cholerae* O1 and O139 serotypes in drinking-water supplies is of major public health importance and can have serious health and economic implications in the affected communities. *Vibrio cholerae* is highly sensitive to disinfection processes. Within a WSP, control measures that can be applied to manage potential risk from toxigenic *V. cholerae* include protection of raw water supplies from human waste, adequate treatment and protection of water during distribution. *Vibrio cholerae* O1 and non-O1 have been

detected in the absence of *E. coli*, and this organism (or, alternatively, thermotolerant coliforms) is not a reliable index for *V. cholerae* in drinking-water.

Selected bibliography

- Kaper JB, Morris JG, Levine MM (1995) Cholera. *Clinical Microbiology Reviews*, 8:48–86.
- Ogg JE, Ryder RA, Smith HL (1989) Isolation of *Vibrio cholerae* from aquatic birds in Colorado and Utah. *Applied and Environmental Microbiology*, 55:95–99.
- Rhodes JB, Schweitzer D, Ogg JE (1985) Isolation of non-O1 *Vibrio cholerae* associated with enteric disease of herbivores in western Colorado. *Journal of Clinical Microbiology*, 22:572–575.
- WHO (2002) *Vibrio cholerae*. In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, pp. 119–142.

11.1.17 Yersinia

General description

The genus *Yersinia* is classified in the family Enterobacteriaceae and comprises seven species. The species *Y. pestis*, *Y. pseudotuberculosis* and certain serotypes of *Y. enterocolitica* are pathogens for humans. *Yersinia pestis* is the cause of bubonic plague through contact with rodents and their fleas. *Yersinia* spp. are Gram-negative rods that are motile at 25°C but not at 37°C.

Human health effects

Yersinia enterocolitica penetrates cells of the intestinal mucosa, causing ulcerations of the terminal ileum. Yersiniosis generally presents as an acute gastroenteritis with diarrhoea, fever and abdominal pain. Other clinical manifestations include greatly enlarged painful lymph nodes referred to as “buboes.” The disease seems to be more acute in children than in adults.

Source and occurrence

Domestic and wild animals are the principal reservoir for *Yersinia* spp.; pigs are the major reservoir of pathogenic *Y. enterocolitica*, whereas rodents and small animals are the major reservoir of *Y. pseudotuberculosis*. Pathogenic *Y. enterocolitica* has been detected in sewage and polluted surface waters. However, *Y. enterocolitica* strains detected in drinking-water are more commonly non-pathogenic strains of probable environmental origin. At least some species and strains of *Yersinia* seem to be able to replicate in water environments if at least trace amounts of organic nitrogen are present, even at temperatures as low as 4°C.

Routes of exposure

Yersinia spp. are transmitted by the faecal–oral route, with the major source of infection considered to be foods, particularly meat and meat products, milk and dairy products. Ingestion of contaminated water is also a potential source of infection. Direct transmission from person to person and from animals to humans is also known to occur.

Significance in drinking-water

Although most *Yersinia* spp. detected in water are probably non-pathogenic, circumstantial evidence has been presented to support transmission of *Y. enterocolitica* and *Y. pseudotuberculosis* to humans from untreated drinking-water. The most likely source of pathogenic *Yersinia* spp. is human or animal waste. The organisms are sensitive to disinfection processes. Within a WSP, control measures that can be used to minimize the presence of pathogenic *Yersinia* spp. in drinking-water supplies include protection of raw water supplies from human and animal waste, adequate disinfection and protection of water during distribution. Owing to the long survival and/or growth of some strains of *Yersinia* spp. in water, *E. coli* (or, alternatively, thermotolerant coliforms) is not a suitable index for the presence/absence of these organisms in drinking-water.

Selected bibliography

- Aleksic S, Bockemuhl J (1988) Serological and biochemical characteristics of 416 *Yersinia* strains from well water and drinking water plants in the Federal Republic of Germany: lack of evidence that these strains are of public health significance. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene B*, 185:527–533.
- Inoue M et al. (1988) Three outbreaks of *Yersinia pseudotuberculosis* infection. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene B*, 186:504–511.
- Ostroff SM et al. (1994) Sources of sporadic *Yersinia enterocolitica* infections in Norway: a prospective case control study. *Epidemiology and Infection*, 112:133–141.
- Waage AS et al. (1999) Detection of low numbers of pathogenic *Yersinia enterocolitica* in environmental water and sewage samples by nested polymerase chain reaction. *Journal of Applied Microbiology*, 87:814–821.

11.2 Viral pathogens

Viruses associated with waterborne transmission are predominantly those that can infect the gastrointestinal tract and are excreted in the faeces of infected humans (enteric viruses). With the exception of hepatitis E, humans are considered to be the only source of human infectious species. Enteric viruses typically cause acute disease with a short incubation period. Water may also play a role in the transmission of other viruses with different modes of action. As a group, viruses can cause a wide variety of infections and symptoms involving different routes of transmission, routes and sites

of infection and routes of excretion. The combination of these routes and sites of infection can vary and will not always follow expected patterns. For example, viruses that are considered to primarily cause respiratory infections and symptoms are usually transmitted by person-to-person spread of respiratory droplets. However, some of these respiratory viruses may be discharged in faeces, leading to potential contamination of water and subsequent transmission through aerosols and droplets. Another example is viruses excreted in urine, such as polyomaviruses, which could contaminate and then be potentially transmitted by water, with possible long-term health effects, such as cancer, that are not readily associated epidemiologically with water-borne transmission.

11.2.1 Adenoviruses

General description

The family Adenoviridae is classified into the two genera *Mastadenovirus* (mammal hosts) and *Aviadenovirus* (avian hosts). Adenoviruses are widespread in nature, infecting birds, mammals and amphibians. To date, 51 antigenic types of human adenoviruses (HAd) have been described. HAd have been classified into six groups (A–F) on the basis of their physical, chemical and biological properties. Adenoviruses consist of a double-stranded DNA genome in a non-enveloped icosahedral capsid with a diameter of about 80 nm and unique fibres. The subgroups A–E grow readily in cell culture, but serotypes 40 and 41 are fastidious and do not grow well. Identification of serotypes 40 and 41 in environmental samples is generally based on polymerase chain reaction (PCR) techniques with or without initial cell culture amplification.

Human health effects

HAd cause a wide range of infections with a spectrum of clinical manifestations. These include infections of the gastrointestinal tract (gastroenteritis), the respiratory tract (acute respiratory diseases, pneumonia, pharyngoconjunctival fever), the urinary tract (cervicitis, urethritis, haemorrhagic cystitis) and the eyes (epidemic keratoconjunctivitis, also known as “shipyard eye”; pharyngoconjunctival fever, also known as “swimming pool conjunctivitis”). Different serotypes are associated with specific illnesses; for example, types 40 and 41 are the main cause of enteric illness. Adenoviruses are an important source of childhood gastroenteritis. In general, infants and children are most susceptible to adenovirus infections, and many infections are asymptomatic. High attack rates in outbreaks imply that infecting doses are low.

Source and occurrence

Adenoviruses are excreted in large numbers in human faeces and are known to occur in sewage, raw water sources and treated drinking-water supplies worldwide. Although the subgroup of enteric adenoviruses (mainly types 40 and 41) is a major cause of gastroenteritis worldwide, notably in developing communities, little is known about the prevalence of these enteric adenoviruses in water sources. The limited availability

of information on enteric adenoviruses is largely due to the fact that they are not detectable by conventional cell culture isolation.

Routes of exposure

Owing to the diverse epidemiology of the wide spectrum of HAdS, exposure and infection are possible by a variety of routes. Person-to-person contact plays a major role in the transmission of illness; depending on the nature of illness, this can include faecal–oral, oral–oral and hand–eye contact transmission, as well as indirect transfer through contaminated surfaces or shared utensils. There have been numerous outbreaks associated with hospitals, military establishments, child care centres and schools. Symptoms recorded in most outbreaks were acute respiratory disease, keratoconjunctivitis and conjunctivitis. Outbreaks of gastroenteritis have also been reported. The consumption of contaminated food or water may be an important source of enteric illness, although there is no substantial evidence supporting this route of transmission. Eye infections may be contracted by the exposure of eyes to contaminated water, the sharing of towels at swimming pools or the sharing of goggles, as in the case of “shipyard eye.” Confirmed outbreaks of adenovirus infections associated with water have been limited to pharyngitis and/or conjunctivitis, with exposure arising from use of swimming pools.

Significance in drinking-water

HAdS have been shown to occur in substantial numbers in raw water sources and treated drinking-water supplies. In one study, the incidence of HAdS in such waters was exceeded only by the group of enteroviruses among viruses detectable by PCR-based techniques. In view of their prevalence as an enteric pathogen and detection in water, contaminated drinking-water represents a likely but unconfirmed source of HAd infections. HAdS are also considered important because they are exceptionally resistant to some water treatment and disinfection processes, notably UV light irradiation. HAdS have been detected in drinking-water supplies that met accepted specifications for treatment, disinfection and conventional indicator organisms. Within a WSP, control measures to reduce potential risk from HAdS should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove HAdS will require validation. Drinking-water supplies should also be protected from contamination during distribution. Because of the high resistance of the viruses to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of HAdS in drinking-water supplies.

Selected bibliography

Chapron CD et al. (2000) Detection of astroviruses, enteroviruses and adenoviruses types 40 and 41 in surface waters collected and evaluated by the information

collection rule and integrated cell culture-nested PCR procedure. *Applied and Environmental Microbiology*, 66:2520–2525.

D'Angelo LJ et al. (1979) Pharyngoconjunctival fever caused by adenovirus type 4: Report of a swimming pool-related outbreak with recovery of virus from pool water. *Journal of Infectious Diseases*, 140:42–47.

Grabow WOK, Taylor MB, de Villiers JC (2001) New methods for the detection of viruses: call for review of drinking water quality guidelines. *Water Science and Technology*, 43:1–8.

Puig M et al. (1994) Detection of adenoviruses and enteroviruses in polluted water by nested PCR amplification. *Applied and Environmental Microbiology*, 60:2963–2970.

11.2.2 Astroviruses

General description

Human and animal strains of astroviruses are single-stranded RNA viruses classified in the family Astroviridae. Astroviruses consist of a single-stranded RNA genome in a non-enveloped icosahedral capsid with a diameter of about 28 nm. In a proportion of the particles, a distinct surface star-shaped structure can be seen by electron microscopy. Eight different serotypes of human astroviruses (HAstVs) have been described. The most commonly identified is HAstV serotype 1. HAstVs can be detected in environmental samples using PCR techniques with or without initial cell culture amplification.

Human health effects

HAstVs cause gastroenteritis, predominantly diarrhoea, mainly in children under 5 years of age, although it has also been reported in adults. Seroprevalence studies showed that more than 80% of children between 5 and 10 years of age have antibodies against HAstVs. Occasional outbreaks in schools, nurseries and families have been reported. The illness is self-limiting, is of short duration and has a peak incidence in the winter. HAstVs are the cause of only a small proportion of reported gastroenteritis infections. However, the number of infections may be underestimated, since the illness is usually mild, and many cases will go unreported.

Source and occurrence

Infected individuals generally excrete large numbers of HAstVs in faeces; hence, the viruses will be present in sewage. HAstVs have been detected in water sources and in drinking-water supplies.

Routes of exposure

HAstVs are transmitted by the faecal–oral route. Person-to-person spread is considered the most common route of transmission, and clusters of cases are seen in child

care centres, paediatric wards, families, homes for the elderly and military establishments. Ingestion of contaminated food or water could also be important.

Significance in drinking-water

The presence of HAstVs in treated drinking-water supplies has been confirmed. Since the viruses are typically transmitted by the faecal–oral route, transmission by drinking-water seems likely, but has not been confirmed. HAstVs have been detected in drinking-water supplies that met accepted specifications for treatment, disinfection and conventional indicator organisms. Within a WSP, control measures to reduce potential risk from HAstVs should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove HAstVs will require validation. Drinking-water supplies should also be protected from contamination during distribution. Owing to the higher resistance of the viruses to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of HAstVs in drinking-water supplies.

Selected bibliography

- Grabow WOK, Taylor MB, de Villiers JC (2001) New methods for the detection of viruses: call for review of drinking water quality guidelines. *Water Science and Technology*, 43:1–8.
- Nadan S et al. (2003) Molecular characterization of astroviruses by reverse transcriptase PCR and sequence analysis: comparison of clinical and environmental isolates from South Africa. *Applied and Environmental Microbiology*, 69:747–753.
- Pintó RM et al. (2001) Astrovirus detection in wastewater. *Water Science and Technology*, 43:73–77.

11.2.3 Caliciviruses

General description

The family Caliciviridae consists of four genera of single-stranded RNA viruses with a non-enveloped capsid (diameter 35–40 nm), which generally displays a typical surface morphology resembling cup-like structures. Human caliciviruses (HuCVs) include the genera *Norovirus* (Norwalk-like viruses) and *Sapovirus* (Sapporo-like viruses). *Sapovirus* spp. demonstrate the typical calicivirus morphology and are called classical caliciviruses. Noroviruses generally fail to reveal the typical morphology and were in the past referred to as small round-structured viruses. The remaining two genera of the family contain viruses that infect animals other than humans. HuCVs cannot be propagated in available cell culture systems. The viruses were originally discovered by electron microscopy. Some *Norovirus* spp. can be detected by ELISA using antibodies raised against baculovirus-expressed *Norovirus* capsid proteins. Several reverse transcriptase PCR procedures have been described for the detection of HuCVs.

Human health effects

HuCVs are a major cause of acute viral gastroenteritis in all age groups. Symptoms include nausea, vomiting and abdominal cramps. Usually about 40% of infected individuals present with diarrhoea; some have fever, chills, headache and muscular pain. Since some cases present with vomiting only and no diarrhoea, the condition is also known as “winter vomiting disease.” Infections by HuCVs induce a short-lived immunity. The symptoms are usually relatively mild and rarely last for more than 3 days. High attack rates in outbreaks indicate that the infecting dose is low.

Source and occurrence

HuCVs are excreted in faeces of infected individuals and will therefore be present in domestic wastewaters as well as faecally contaminated food and water, including drinking-water supplies.

Routes of exposure

The epidemiology of the disease indicates that person-to-person contact and the inhalation of contaminated aerosols and dust particles, as well as airborne particles of vomitus, are the most common routes of transmission. Drinking-water and a wide variety of foods contaminated with human faeces have been confirmed as major sources of exposure. Numerous outbreaks have been associated with contaminated drinking-water, ice, water on cruise ships and recreational waters. Shellfish harvested from sewage-contaminated waters have also been identified as a source of outbreaks.

Significance in drinking-water

Many HuCV outbreaks have been epidemiologically linked to contaminated drinking-water supplies. Within a WSP, control measures to reduce potential risk from HuCV should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove HuCV will require validation. Drinking-water supplies should also be protected from contamination during distribution. Owing to the higher resistance of the viruses to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of HuCVs in drinking-water supplies.

Selected bibliography

- Berke T et al. (1997) Phylogenetic analysis of the Caliciviridae. *Journal of Medical Virology*, 52:419–424.
- Jiang X et al. (1999) Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *Journal of Virological Methods*, 83:145–154.

Mauer AM, Sturchler DA (2000) A waterborne outbreak of small round-structured virus, *Campylobacter* and *Shigella* co-infections in La Neuveville, Switzerland, 1998. *Epidemiology and Infection*, 125:325–332.

Monroe SS, Ando T, Glass R (2000) Introduction: Human enteric caliciviruses – An emerging pathogen whose time has come. *Journal of Infectious Diseases*, 181(Suppl. 2):S249–251.

11.2.4 Enteroviruses

General description

The genus *Enterovirus* is a member of the family Picornaviridae. This genus consists of 69 serotypes (species) that infect humans: poliovirus types 1–3, coxsackievirus types A1–A24, coxsackievirus types B1–B6, echovirus types 1–33 and the numbered enterovirus types EV68–EV73. Members of the genus are collectively referred to as enteroviruses. Other species of the genus infect animals other than humans – for instance, the bovine group of enteroviruses. Enteroviruses are among the smallest known viruses and consist of a single-stranded RNA genome in a non-enveloped icosahedral capsid with a diameter of 20–30 nm. Some members of the genus are readily isolated by cytopathogenic effect in cell cultures, notably poliovirus, coxsackievirus B, echovirus and enterovirus.

Human health effects

Enteroviruses are one of the most common causes of human infections. They have been estimated to cause about 30 million infections in the USA each year. The spectrum of diseases caused by enteroviruses is broad and ranges from a mild febrile illness to myocarditis, meningoencephalitis, poliomyelitis, herpangina, hand-foot-and-mouth disease and neonatal multi-organ failure. The persistence of the viruses in chronic conditions such as polymyositis, dilated cardiomyopathy and chronic fatigue syndrome has been described. Most infections, particularly in children, are asymptomatic, but still lead to the excretion of large numbers of the viruses, which may cause clinical disease in other individuals.

Source and occurrence

Enteroviruses are excreted in the faeces of infected individuals. Among the types of viruses detectable by conventional cell culture isolation, enteroviruses are generally the most numerous in sewage, water resources and treated drinking-water supplies. The viruses are also readily detected in many foods.

Routes of exposure

Person-to-person contact and inhalation of airborne viruses or viruses in respiratory droplets are considered to be the predominant routes of transmission of enteroviruses in communities. Transmission from drinking-water could also be important, but this has not yet been confirmed. Waterborne transmission of enteroviruses (coxsackievirus

A16 and B5) has been epidemiologically confirmed for only two outbreaks, and these were associated with children bathing in lake water in the 1970s.

Significance in drinking-water

Enteroviruses have been shown to occur in substantial numbers in raw water sources and treated drinking-water supplies. In view of their prevalence, drinking-water represents a likely, although unconfirmed, source of enterovirus infection. The limited knowledge on the role of waterborne transmission could be related to a number of factors, including the wide range of clinical symptoms, frequent asymptomatic infection, the diversity of serotypes and the dominance of person-to-person spread. Enteroviruses have been detected in drinking-water supplies that met accepted specifications for treatment, disinfection and conventional indicator organisms. Within a WSP, control measures to reduce potential risk from enteroviruses should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove enteroviruses will require validation. Drinking-water supplies should also be protected from contamination during distribution. Owing to the higher resistance of the viruses to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of enteroviruses in drinking-water supplies.

Selected bibliography

- Grabow WOK, Taylor MB, de Villiers JC (2001) New methods for the detection of viruses: call for review of drinking water quality guidelines. *Water Science and Technology*, 43:1–8.
- Hawley HB et al. (1973) Cocksackie B epidemic at a boys' summer camp. *Journal of the American Medical Association*, 226:33–36.

11.2.5 Hepatitis A virus

General description

HAV is the only species of the genus *Hepatovirus* in the family Picornaviridae. The virus shares basic structural and morphological features with other members of the family, as described for enteroviruses. Human and simian HAVs are genotypically distinguishable. HAV cannot be readily detected or cultivated in conventional cell culture systems, and identification in environmental samples is based on the use of PCR techniques.

Human health effects

HAV is highly infectious, and the infecting dose is considered to be low. The virus causes the disease hepatitis A, commonly known as “infectious hepatitis.” Like other members of the group enteric viruses, HAV enters the gastrointestinal tract by ingestion, where it infects epithelial cells. From here, the virus enters the bloodstream and reaches the liver, where it may cause severe damage to liver cells. In as many as 90%

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of cases, particularly in children, there is little, if any, liver damage, and the infection passes without clinical symptoms and elicits lifelong immunity. In general, the severity of illness increases with age. The damage to liver cells results in the release of liver-specific enzymes such as aspartate aminotransferase, which are detectable in the bloodstream and used as a diagnostic tool. The damage also results in the failure of the liver to remove bilirubin from the bloodstream; the accumulation of bilirubin causes the typical symptoms of jaundice and dark urine. After a relatively long incubation period of 28–30 days on average, there is a characteristic sudden onset of illness, including symptoms such as fever, malaise, nausea, anorexia, abdominal discomfort and eventually jaundice. Although mortality is generally less than 1%, repair of the liver damage is a slow process that may keep patients incapacitated for 6 weeks or longer. This has substantial burden of disease implications. Mortality is higher in those over 50 years of age.

Source and occurrence

HAV occurs worldwide, but the prevalence of clinical disease has typical geographically based characteristics. HAV is excreted in faecal material of infected people, and there is strong epidemiological evidence that faecally contaminated food and water are common sources of the virus. In areas with poor sanitation, children are often infected at a very early age and become immune for life without clinical symptoms of disease. In areas with good sanitation, infection tends to occur later in life.

Routes of exposure

Person-to-person spread is probably the most common route of transmission, but contaminated food and water are important sources of infection. There is stronger epidemiological evidence for waterborne transmission of HAV than for any other virus. Foodborne outbreaks are also relatively common, with sources of infection including infected food handlers, shellfish harvested from contaminated water and contaminated produce. Travel of people from areas with good sanitation to those with poor sanitation provides a high risk of infection. Infection can also be spread in association with injecting and non-injecting drug use.

Significance in drinking-water

The transmission of HAV by drinking-water supplies is well established, and the presence of HAV in drinking-water constitutes a substantial health risk. Within a WSP, control measures to reduce potential risk from HAV should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove HAV will require validation. Drinking-water supplies should also be protected from contamination during distribution. Owing to the higher resistance of the viruses to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of HAV in drinking-water supplies.

Selected bibliography

- Cuthbert JA (2001) Hepatitis A: Old and new. *Clinical Microbiology Reviews*, 14:38–58.
- WHO (2002) Enteric hepatitis viruses. In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, pp. 18–39.

11.2.6 Hepatitis E virus

General description

HEV consists of a single-stranded RNA genome in a non-enveloped icosahedral capsid with a diameter of 27–34 nm. HEV shares properties with a number of viruses, and classification is a challenge. At one stage, HEV was classified as a member of the family Caliciviridae, but most recently it has been placed in a separate family called hepatitis E-like viruses. There are indications of antigenic variation, and possibly even differences in serotypes of the virus, whereas human HAV consists of only one clearly defined serotype. HEV cannot be readily detected or cultivated in conventional cell culture systems, and identification in environmental samples is based on the use of PCR techniques.

Human health effects

HEV causes hepatitis that is in many respects similar to that caused by HAV. However, the incubation period tends to be longer (average 40 days), and infections typically have a mortality rate of up to 25% in pregnant women. In endemic regions, first infections are typically seen in young adults rather than young children. Despite evidence of antigenic variation, single infection appears to provide lifelong immunity to HEV. Global prevalence has a characteristic geographic distribution. HEV is endemic and causes clinical diseases in certain developing parts of the world, such as India, Nepal, central Asia, Mexico and parts of Africa. In many of these areas, HEV is the most important cause of viral hepatitis. Although seroprevalence can be high, clinical cases and outbreaks are rare in certain parts of the world, such as Japan, South Africa, the United Kingdom, North and South America, Australasia and central Europe. The reason for the lack of clinical cases in the presence of the virus is unknown.

Source and occurrence

HEV is excreted in faeces of infected people, and the virus has been detected in raw and treated sewage. Contaminated water has been associated with very large outbreaks. HEV is distinctive, in that it is the only enteric virus with a meaningful animal reservoir, including domestic animals, particularly pigs, as well as cattle, goats and even rodents.

Routes of exposure

Secondary transmission of HEV from cases to contacts and particularly nursing staff has been reported, but appears to be much less common than for HAV. The lower

level of person-to-person spread suggests that faecally polluted water could play a much more important role in the spread of HEV than of HAV. Waterborne outbreaks involving thousands of cases are on record. These include one outbreak in 1954 with approximately 40 000 cases in Delhi, India; one with more than 100 000 cases in 1986–1988 in the Xinjiang Uighar region of China; and one in 1991 with some 79 000 cases in Kanpur, India. Animal reservoirs may also serve as a route of exposure, but the extent to which humans contract HEV infection from animals remains to be elucidated.

Significance in drinking-water

The role of contaminated water as a source of HEV has been confirmed, and the presence of the virus in drinking-water constitutes a major health risk. There is no laboratory information on the resistance of the virus to disinfection processes, but data on waterborne outbreaks suggest that HEV may be as resistant as other enteric viruses. Within a WSP, control measures to reduce potential risk from HEV should focus on prevention of source water contamination by human and animal waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove HEV will require validation. Drinking-water supplies should also be protected from contamination during distribution. Due to the likelihood that the virus has a higher resistance to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of HEV in drinking-water supplies.

Selected bibliography

- Pina S et al. (1998) Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. *Applied and Environmental Microbiology*, 64:4485–4488.
- Van der Poel WHM et al. (2001) Hepatitis E virus sequence in swine related to sequences in humans, the Netherlands. *Emerging Infectious Diseases*, 7:970–976.
- WHO (2002) Enteric hepatitis viruses. In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, pp. 18–39.

11.2.7 Rotaviruses and orthoreoviruses

General description

Members of the genus *Rotavirus* consist of a segmented double-stranded RNA genome in a non-enveloped icosahedral capsid with a diameter of 50–65 nm. This capsid is surrounded by a double-layered shell, giving the virus the appearance of a wheel – hence the name rotavirus. The diameter of the entire virus is about 80 nm. *Rotavirus* and *Orthoreovirus* are the two genera of the family Reoviridae typically associated with human infection. Orthoreoviruses are readily isolated by cytopathogenic effect on cell cultures. The genus *Rotavirus* is serologically divided into seven groups, A–G, each of which consists of a number of subgroups; some of these subgroups specifically infect

humans, whereas others infect a wide spectrum of animals. Groups A–C are found in humans, with group A being the most important human pathogens. Wild-type strains of rotavirus group A are not readily grown in cell culture, but there are a number of PCR-based detection methods available for testing environmental samples.

Human health effects

Human rotaviruses (HRVs) are the most important single cause of infant death in the world. Typically, 50–60% of cases of acute gastroenteritis of hospitalized children throughout the world are caused by HRVs. The viruses infect cells in the villi of the small intestine, with disruption of sodium and glucose transport. Acute infection has an abrupt onset of severe watery diarrhoea with fever, abdominal pain and vomiting; dehydration and metabolic acidosis may develop, and the outcome may be fatal if the infection is not appropriately treated. The burden of disease of rotavirus infections is extremely high. Members of the genus *Orthoreovirus* infect many humans, but they are typical “orphan viruses” and not associated with any meaningful disease.

Source and occurrence

HRVs are excreted by patients in numbers up to 10^{11} per gram of faeces for periods of about 8 days. This implies that domestic sewage and any environments polluted with the human faeces are likely to contain large numbers of HRVs. The viruses have been detected in sewage, rivers, lakes and treated drinking-water. Orthoreoviruses generally occur in wastewater in substantial numbers.

Routes of exposure

HRVs are transmitted by the faecal–oral route. Person-to-person transmission and the inhalation of airborne HRVs or aerosols containing the viruses would appear to play a much more important role than ingestion of contaminated food or water. This is confirmed by the spread of infections in children’s wards in hospitals, which takes place much faster than can be accounted for by the ingestion of food or water contaminated by the faeces of infected patients. The role of contaminated water in transmission is lower than expected, given the prevalence of HRV infections and presence in contaminated water. However, occasional waterborne and foodborne outbreaks have been described. Two large outbreaks in China in 1982–1983 were linked to contaminated water supplies.

Significance in drinking-water

Although ingestion of drinking-water is not the most common route of transmission, the presence of HRVs in drinking-water constitutes a public health risk. There is some evidence that the rotaviruses are more resistant to disinfection than other enteric viruses. Within a WSP, control measures to reduce potential risk from HRVs should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to

remove HRVs will require validation. Drinking-water supplies should also be protected from contamination during distribution. Due to a higher resistance of the viruses to disinfection, *E. coli* (or, alternatively, thermotolerant coliforms) is not a reliable index of the presence/absence of HRVs in drinking-water supplies.

Selected bibliography

- Baggi F, Peduzzi R (2000) Genotyping of rotaviruses in environmental water and stool samples in southern Switzerland by nucleotide sequence analysis of 189 base pairs at the 5' end of the VP7 gene. *Journal of Clinical Microbiology*, 38:3681–3685.
- Gerba CP et al. (1996) Waterborne rotavirus: a risk assessment. *Water Research*, 30:2929–2940.
- Hopkins RS et al. (1984) A community waterborne gastroenteritis outbreak: evidence for rotavirus as the agent. *American Journal of Public Health*, 74:263–265.
- Hung T et al. (1984) Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. *Lancet*, i:1139–1142.
- Sattar SA, Raphael RA, Springthorpe VS (1984) Rotavirus survival in conventionally treated drinking water. *Canadian Journal of Microbiology*, 30:653–656.

11.3 Protozoan pathogens

Protozoa and helminths are among the most common causes of infection and disease in humans and other animals. The diseases have a major public health and socio-economic impact. Water plays an important role in the transmission of some of these pathogens. The control of waterborne transmission presents real challenges, because most of the pathogens produce cysts, oocysts or eggs that are extremely resistant to processes generally used for the disinfection of water and in some cases can be difficult to remove by filtration processes. Some of these organisms cause “emerging diseases.” In the last 25 years, the most notable example of an emerging disease caused by a protozoan pathogen is cryptosporidiosis. Other examples are diseases caused by microsporidia and *Cyclospora*. As evidence for waterborne transmission of “emerging diseases” has been reported relatively recently, some questions about their epidemiology and behaviour in water treatment and disinfection processes remain to be elucidated. It would appear that the role of water in the transmission of this group of pathogens may increase substantially in importance and complexity as human and animal populations grow and the demands for potable drinking-water escalate.

Further information on emerging diseases is provided in *Emerging Issues in Water and Infectious Disease* (WHO, 2003) and associated texts.

11.3.1 *Acanthamoeba*

General description

Acanthamoeba spp. are free-living amoebae (10–50 µm in diameter) common in aquatic environments and one of the prominent protozoa in soil. 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 when evolving molecular biological knowledge is taken into consideration. *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.

Human health effects

Acanthamoeba culbertsoni causes granulomatous amoebic encephalitis (GAE), whereas *A. castellanii* and *A. polyphaga* are associated with acanthamoebic keratitis and acanthamoebic uveitis.

GAE is a multifocal, haemorrhagic and necrotizing encephalitis that is generally seen only in debilitated or immunodeficient persons. It is a rare but usually fatal disease. Early symptoms include drowsiness, personality changes, intense headaches, stiff neck, nausea, vomiting, sporadic low fevers, focal neurological changes, hemiparesis and seizures. This is followed by an altered mental status, diplopia, paresis, lethargy, cerebellar ataxia and coma. Death follows within a week to a year after the appearance of the first symptoms, usually as a result of bronchopneumonia. Associated disorders of GAE include skin ulcers, liver disease, pneumonitis, renal failure and pharyngitis.

Acanthamoebic keratitis is a painful infection of the cornea and can occur in healthy individuals, especially among contact lens wearers. It is a rare disease that may lead to impaired vision, permanent blindness and loss of the eye. 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.

Source and occurrence

The wide distribution of *Acanthamoeba* in the natural environment makes soil, air-borne dust and water all potential sources. *Acanthamoeba* can be found in many types of aquatic environments, including surface water, tap water, swimming pools and contact lens solutions. Depending on the species, *Acanthamoeba* can grow over a wide temperature range in water, with the optimum temperature for pathogenic species being 30°C. Trophozoites can exist and replicate in water while feeding on bacteria, yeasts and other organisms. Infections occur in most temperate and tropical regions of the world.

Routes of exposure

Acanthamoebic keratitis has been associated with soft contact lenses being washed with contaminated home-made saline solutions or contamination of the contact lens containers. Although the source of the contaminating organisms has not been established, tap water is one possibility. Warnings have been issued by a number of health agencies that only sterile water should be used to prepare wash solutions for contact

lenses. The mode of transmission of GAE has not been established, but water is not considered to be a source of infection. The more likely routes of transmission are via the blood from other sites of colonization, such as skin lesions or lungs.

Significance in drinking-water

Cases of acanthamoebic keratitis have been associated with drinking-water due to use of tap water in preparing solutions for washing contact lenses. Cleaning of contact lenses is not considered to be a normal use for tap water, and a higher-quality water may be required. Compared with *Cryptosporidium* and *Giardia*, *Acanthamoeba* is relatively large and is amenable to removal from raw water by filtration. Reducing the presence of biofilm organisms is likely to reduce food sources and growth of the organism in distribution systems, but the organism is highly resistant to disinfection. However, as normal uses of drinking-water lack significance as a source of infection, setting a health-based target for *Acanthamoeba* spp. is not warranted.

Selected bibliography

Marshall MM et al. (1997) Waterborne protozoan pathogens. *Clinical Microbiology Reviews*, 10:67–85.

Yagita K, Endo T, De Jonckheere JF (1999) Clustering of *Acanthamoeba* isolates from human eye infections by means of mitochondrial DNA digestion patterns. *Parasitology Research*, 85:284–289.

11.3.2 *Balantidium coli*

General description

Balantidium coli is a unicellular protozoan parasite with a length up to 200 µm, making it the largest of the human intestinal protozoa. The trophozoites are oval in shape and covered with cilia for motility. The cysts are 60–70 µm in length and resistant to unfavourable environmental conditions, such as pH and temperature extremes. *Balantidium coli* belongs to the largest protozoan group, the ciliates, with about 7200 species, of which only *B. coli* is known to infect humans.

Human health effects

Infections in humans are relatively rare, and most are asymptomatic. The trophozoites invade the mucosa and submucosa of the large intestine and destroy the host cells when multiplying. The multiplying parasites form nests and small abscesses that break down into oval, irregular ulcers. Clinical symptoms may include dysentery similar to amoebiasis, colitis, diarrhoea, nausea, vomiting, headache and anorexia. The infections are generally self-limiting, with complete recovery.

Source and occurrence

Humans seem to be the most important host of *B. coli*, and the organism can be detected in domestic sewage. Animal reservoirs, particularly swine, also contribute to

the prevalence of the cysts in the environment. The cysts have been detected in water sources, but the prevalence in tap water is unknown.

Routes of exposure

Transmission of *B. coli* is by the faecal–oral route, from person to person, from contact with infected swine or by consumption of contaminated water or food. One waterborne outbreak of balantidiasis has been reported. This outbreak occurred in 1971 when a drinking-water supply was contaminated with stormwater runoff containing swine faeces after a typhoon.

Significance in drinking-water

Although water does not appear to play an important role in the spread of this organism, one waterborne outbreak is on record. *Balantidium coli* is large and amenable to removal by filtration, but cysts are highly resistant to disinfection. Within a WSP, control measures to reduce potential risk from *B. coli* should focus on prevention of source water contamination by human and swine waste, followed by adequate treatment. 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.

Selected bibliography

Garcia LS (1999) Flagellates and ciliates. *Clinics in Laboratory Medicine*, 19:621–638.
Walzer PD et al. (1973) Balantidiasis outbreak in Truk. *American Journal of Tropical Medicine and Hygiene*, 22:33–41.

11.3.3 *Cryptosporidium*

General description

Cryptosporidium is an obligate, intracellular, coccidian parasite with a complex life cycle including sexual and asexual replication. Thick-walled oocysts with a diameter of 4–6 µm are shed in faeces. The genus *Cryptosporidium* has about eight species, of which *C. parvum* is responsible for most human infections, although other species can cause illness. *Cryptosporidium* is one of the best examples of an “emerging disease”-causing organism. It was discovered to infect humans only in 1976, and waterborne transmission was confirmed for the first time in 1984.

Human health effects

Cryptosporidium generally causes a self-limiting diarrhoea, sometimes including nausea, vomiting and fever, which usually resolves within a week in normally healthy people, but can last for a month or more. Severity of cryptosporidiosis varies according to age and immune status, and infections in severely immunocompromised people can be life-threatening. The impact of cryptosporidiosis outbreaks is relatively high due to the large numbers of people that may be involved and the associated socioe-

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conomic implications. The total cost of illness associated with the 1993 outbreak in Milwaukee, USA, has been estimated at US\$96.2 million.

Source and occurrence

A large range of animals are reservoirs of *C. parvum*, but humans and livestock, particularly young animals, are the most significant source of human infectious organisms. Calves can excrete 10^{10} oocysts per day. Concentrations of oocysts as high as 14000 per litre for raw sewage and 5800 per litre for surface water have been reported. Oocysts can survive for weeks to months in fresh water. *Cryptosporidium* oocysts have been detected in many drinking-water supplies. However, in most cases, there is little information about whether human infectious species were present. The currently available standard analytical techniques provide an indirect measure of viability and no indication of human infectivity. Oocysts also occur in recreational waters.

Routes of exposure

Cryptosporidium is transmitted by the faecal–oral route. The major route of infection is person-to-person contact. Other sources of infection include the consumption of contaminated food and water and direct contact with infected farm animals and possibly domestic pets. Contaminated drinking-water, recreational water and, to a lesser extent, food have been associated with outbreaks. In 1993, *Cryptosporidium* caused the largest waterborne outbreak of disease on record, when more than 400 000 people were infected by the drinking-water supply of Milwaukee, USA. The infectivity of *Cryptosporidium* oocysts is relatively high. Studies on healthy human volunteers revealed that ingestion of fewer than 10 oocysts can lead to infection.

Significance in drinking-water

The role of drinking-water in the transmission of *Cryptosporidium*, including in large outbreaks, is well established. Attention to these organisms is therefore important. The oocysts are extremely resistant to oxidizing disinfectants such as chlorine, but investigations based on assays for infectivity have shown that UV light irradiation inactivates oocysts. Within a WSP, control measures to reduce potential risk from *Cryptosporidium* should focus on prevention of source water contamination by human and livestock waste, adequate treatment and protection of water during distribution. Because of their relatively small size, the oocysts represent a challenge for removal by conventional granular media-based filtration processes. Acceptable removal requires well designed and operated systems. Membrane filtration processes that provide a direct physical barrier may represent a viable alternative for the effective removal of *Cryptosporidium* oocysts. Owing to the exceptional resistance of the oocysts to disinfectants, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index for the presence/absence of *Cryptosporidium* oocysts in drinking-water supplies.

Selected bibliography

- Corso PS et al. (2003) Cost of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerging Infectious Diseases*, 9:426–431.
- Haas CN et al. (1996) Risk assessment of *Cryptosporidium parvum* oocysts in drinking water. *Journal of the American Water Works Association*, 88:131–136.
- Leav BA, Mackay M, Ward HD (2003) *Cryptosporidium* species: new insight and old challenges. *Clinical Infectious Diseases*, 36:903–908.
- Linden KG, Shin G, Sobsey MD (2001) Comparative effectiveness of UV wavelengths for the inactivation of *Cryptosporidium parvum* oocysts in water. *Water Science and Technology*, 43:171–174.
- Okhuysen PC et al. (1999) Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *Journal of Infectious Diseases*, 180:1275–1281.
- WHO (2002) Protozoan parasites (*Cryptosporidium*, *Giardia*, *Cyclospora*). In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, pp. 70–118.

11.3.4 *Cyclospora cayetanensis*

General description

Cyclospora cayetanensis is a single-cell, obligate, intracellular, coccidian protozoan parasite, which belongs to the family Eimeriidae. It produces thick-walled oocysts of 8–10 µm in diameter that are excreted in the faeces of infected individuals. *Cyclospora cayetanensis* is considered an emerging waterborne pathogen.

Human health effects

Sporozoites are released from the oocysts when ingested and penetrate epithelial cells in the small intestine of susceptible individuals. Clinical symptoms of cyclosporiasis include watery diarrhoea, abdominal cramping, weight loss, anorexia, myalgia and occasionally vomiting and/or fever. Relapsing illness often occurs.

Source and occurrence

Humans are the only host identified for this parasite. 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. Due 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.

Routes of exposure

Cyclospora cayetanensis is transmitted by the faecal–oral route. Person-to-person transmission is virtually impossible, because the oocysts must sporulate outside the host to become infectious. 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 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.

Significance in drinking-water

Transmission of the pathogens by drinking-water has been confirmed. The oocysts are resistant to disinfection and are not inactivated by chlorination practices generally applied in the production of drinking-water. Within a WSP, control measures that can be applied to manage potential risk from *Cyclospora* include prevention of source water contamination by human waste, followed by adequate treatment and protection of water during distribution. 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.

Selected bibliography

- Curry A, Smith HV (1998) Emerging pathogens: *Isospora*, *Cyclospora* and microsporidia. *Parasitology*, 117:S143–159.
- Dowd SE et al. (2003) Confirmed detection of *Cyclospora cayetanensis*, *Encephalitozoon intestinalis* and *Cryptosporidium parvum* in water used for drinking. *Journal of Water and Health*, 1:117–123.
- Goodgame R (2003) Emerging causes of traveller's diarrhea: *Cryptosporidium*, *Cyclospora*, *Isospora* and microsporidia. *Current Infectious Disease Reports*, 5:66–73.
- Herwaldt BL (2000) *Cyclospora cayetanensis*: A review, focusing on the outbreaks of cyclosporiasis in the 1990s. *Clinical Infectious Diseases*, 31:1040–1057.
- Rabold JG et al. (1994) *Cyclospora* outbreak associated with chlorinated drinking-water [letter]. *Lancet*, 344:1360–1361.
- WHO (2002) Protozoan parasites (*Cryptosporidium*, *Giardia*, *Cyclospora*). In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, pp. 70–118.

11.3.5 *Entamoeba histolytica*

General description

Entamoeba histolytica is the most prevalent intestinal protozoan pathogen worldwide and belongs to the superclass Rhizopoda in the subphylum Sarcodina. *Entamoeba* has a feeding, replicative trophozoite (diameter 10–60 µm), which, under unfavourable conditions, will develop into a dormant cyst (diameter 10–20 µm). Infection is contracted by the ingestion of cysts. Recent studies with RNA and DNA probes demon-

strated genetic differences between pathogenic and non-pathogenic *E. histolytica*; the latter has been separated and reclassified as *E. dispar*.

Human health effects

About 85–95% of human infections with *E. histolytica* are asymptomatic. Acute intestinal amoebiasis has an incubation period of 1–14 weeks. Clinical disease results from the penetration of the epithelial cells in the gastrointestinal tract by the amoebic trophozoites. Approximately 10% of infected individuals present with dysentery or colitis. Symptoms of amoebic dysentery include diarrhoea with cramping, lower abdominal pain, low-grade fever and the presence of blood and mucus in the stool. The ulcers produced by the invasion of the trophozoites may deepen into the classic flask-shaped ulcers of amoebic colitis. *Entamoeba histolytica* may invade other parts of the body, such as the liver, lungs and brain, sometimes with fatal outcome.

Source and occurrence

Humans are the reservoir of infection, and there would not appear to be other meaningful animal reservoirs of *E. histolytica*. In the acute phase of infection, patients excrete only trophozoites that are not infectious. Chronic cases and asymptomatic carriers who excrete cysts are more important sources of infection and can discharge up to 1.5×10^7 cysts daily. *Entamoeba histolytica* can be present in sewage and contaminated water. Cysts may remain viable in suitable aquatic environments for several months at low temperature. The potential for waterborne transmission is greater in the tropics, where the carrier rate sometimes exceeds 50%, compared with more temperate regions, where the prevalence in the general population may be less than 10%.

Routes of exposure

Person-to-person contact and contamination of food by infected food handlers appear to be the most significant means of transmission, although contaminated water also plays a substantial role. Ingestion of faecally contaminated water and consumption of food crops irrigated with contaminated water can both lead to transmission of amoebiasis. Sexual transmission, particularly among male homosexuals, has also been documented.

Significance in drinking-water

The transmission of *E. histolytica* by contaminated drinking-water has been confirmed. The cysts are relatively resistant to disinfection and may not be inactivated by chlorination practices generally applied in the production of drinking-water. Within a WSP, control measures that can be applied to manage potential risk from *E. histolytica* include prevention of source water contamination by human waste, followed by adequate treatment and protection of water during distribution. 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.

Selected bibliography

Marshall MM et al. (1997) Waterborne protozoan pathogens. *Clinical Microbiology Reviews*, 10:67–85.

11.3.6 *Giardia intestinalis*

General description

Giardia spp. are flagellated protozoa that parasitize the gastrointestinal tract of humans and certain animals. The genus *Giardia* consists of a number of species, but human infection (giardiasis) is usually assigned to *G. intestinalis*, also known as *G. lamblia* or *G. duodenalis*. *Giardia* has a relatively simple life cycle consisting of a flagellate trophozoite that multiplies in the gastrointestinal tract and an infective thick-walled cyst that is shed intermittently but in large numbers in faeces. The trophozoites are bilaterally symmetrical and ellipsoidal in shape. The cysts are ovoid in shape and 8–12 µm in diameter.

Human health effects

Giardia has been known as a human parasite for 200 years. After ingestion and excystation of cysts, the trophozoites attach to surfaces of the gastrointestinal tract. Infections in both children and adults may be asymptomatic. In day care centres, as many as 20% of children may carry *Giardia* and excrete cysts without clinical symptoms. The symptoms of giardiasis may result from damage caused by the trophozoites, although the mechanisms by which *Giardia* causes diarrhoea and intestinal malabsorption remain controversial. Symptoms generally include diarrhoea and abdominal cramps; in severe cases, however, malabsorption deficiencies in the small intestine may be present, mostly among young children. Giardiasis is self-limiting in most cases, but it may be chronic in some patients, lasting more than 1 year, even in otherwise healthy people. Studies on human volunteers revealed that fewer than 10 cysts constitute a meaningful risk of infection.

Source and occurrence

Giardia can multiply in a wide range of animal species, including humans, which excrete cysts into the environment. Numbers of cysts as high as 88 000 per litre in raw sewage and 240 per litre in surface water resources have been reported. These cysts are robust and can survive for weeks to months in fresh water. The presence of cysts in raw water sources and drinking-water supplies has been confirmed. However, there is no information on whether human infectious species were present. The currently available standard analytical techniques provide an indirect measure of viability and no indication of human infectivity. Cysts also occur in recreational waters and contaminated food.

Routes of exposure

By far the most common route of transmission of *Giardia* is person-to-person contact, particularly between children. Contaminated drinking-water, recreational water and, to a lesser extent, food have been associated with outbreaks. Animals have been implicated as a source of human infectious *G. intestinalis*, but further investigations are required to determine their role.

Significance in drinking-water

Waterborne outbreaks of giardiasis have been associated with drinking-water supplies for over 30 years; at one stage, *Giardia* was the most commonly identified cause of waterborne outbreaks in the USA. *Giardia* cysts are more resistant than enteric bacteria to oxidative disinfectants such as chlorine, but they are not as resistant as *Cryptosporidium* oocysts. The time required for 90% inactivation at a free chlorine residual of 1 mg/litre is about 25–30 min. Within a WSP, control measures that can be applied to manage potential risk from *Giardia* include prevention of source water contamination by human and animal waste, followed by adequate treatment and disinfection and protection of water during distribution. 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.

Selected bibliography

- LeChevallier MW, Norton WD, Lee RG (1991) Occurrence of *Giardia* and *Cryptosporidium* species in surface water supplies. *Applied and Environmental Microbiology*, 57:2610–2616.
- Ong C et al. (1996) Studies of *Giardia* spp. and *Cryptosporidium* spp. in two adjacent watersheds. *Applied and Environmental Microbiology*, 62:2798–2805.
- Rimhanen-Finne R et al. (2002) An IC-PCR method for detection of *Cryptosporidium* and *Giardia* in natural surface waters in Finland. *Journal of Microbiological Methods*, 50:299–303.
- Slifko TR, Smith HV, Rose JB (2000) Emerging parasite zoonoses associated with water and food. *International Journal for Parasitology*, 30:1379–1393.
- Stuart JM et al. (2003) Risk factors for sporadic giardiasis: a case–control study in southwestern England. *Emerging Infectious Diseases*, 9:229–233.
- WHO (2002) Protozoan parasites (*Cryptosporidium*, *Giardia*, *Cyclospora*). In: *Guidelines for drinking-water quality*, 2nd ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, pp. 70–118.

11.3.7 *Isospora belli*

General description

Isospora is a coccidian, single-celled, obligate parasite related to *Cryptosporidium* and *Cyclospora*. 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

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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.

Human health effects

Illness caused by *I. belli* is similar to that caused by *Cryptosporidium* and *Giardia*. About 1 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 6 weeks to 6 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 industrialized countries. They have been reported from Central and South America, Africa and south-east Asia.

Source and occurrence

Unsporulated oocysts are excreted in the faeces of infected individuals. The oocysts sporulate within 1–2 days in the environment to produce the potentially infectious form of the organism. Few data are available on numbers of oocysts in sewage and raw and treated water sources. This is largely because sensitive and reliable techniques for the quantitative enumeration of oocysts in water environments are not available. Little is known about the survival of oocysts in water and related environments.

Routes of exposure

Poor sanitation and faecally contaminated food and water are the most likely sources of infection, but waterborne transmission has not been confirmed. The oocysts are less likely than *Cryptosporidium* oocysts or *Giardia* cysts to be transmitted directly from person to person, because freshly shed *I. belli* oocysts require 1–2 days in the environment to sporulate before they are capable of infecting humans.

Significance in drinking-water

The characteristics of *I. belli* suggest that illness could be transmitted by contaminated drinking-water supplies, but this has not been confirmed. No information is available on the effectiveness of water treatment processes for removal of *I. belli*, but it is likely that the organism is relatively resistant to disinfectants. It is considerably larger than *Cryptosporidium* and should be easier to remove by filtration. Within a WSP, control measures that can be applied to manage potential risk from *I. belli* include prevention of source water contamination by human waste, followed by adequate treatment and disinfection and protection of water during distribution. 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.

Selected bibliography

- Ballal M et al. (1999) *Cryptosporidium* and *Isospora belli* diarrhoea in immunocompromised hosts. *Indian Journal of Cancer*, 36:38–42.
- Bialek R et al. (2002) Comparison of autofluorescence and iodine staining for detection of *Isospora belli* in feces. *American Journal of Tropical Medicine and Hygiene*, 67:304–305.
- Curry A, Smith HV (1998) Emerging pathogens: *Isospora*, *Cyclospora* and microsporidia. *Parasitology*, 117:S143–159.
- Goodgame R (2003) Emerging causes of traveller's diarrhea: *Cryptosporidium*, *Cyclospora*, *Isospora* and microsporidia. *Current Infectious Disease Reports*, 5:66–73.

11.3.8 Microsporidia

General description

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 1000 species have been identified. Infections occur in every major animal group, including vertebrates and invertebrates. A number of genera have been implicated in human infections, including *Enterocytozoon*, *Encephalitozoon* (including *Septata*), *Nosema*, *Pleistophora*, *Vittaforma* and *Trachipleistophora*, as well as a collective group of unclassified microsporidia referred to as microsporidium. Microsporidia are among the smallest eukaryotes. They produce unicellular spores with a diameter of 1.0–4.5 µm and a characteristic coiled polar filament for injecting the sporoplasm into a host cell to initiate infection. Within an infected cell, a complex process of multiplication takes place, and new spores are produced and released in faeces, urine, respiratory secretions or other body fluids, depending on the type of species and the site of infection.

Human health effects

Microsporidia are emerging human pathogens identified predominantly in persons with AIDS, but their ability to cause disease in immunologically normal hosts has been recognized. Reported human infections are globally dispersed and have been documented in persons from all continents. The most common clinical manifestation in AIDS patients is a severe enteritis involving chronic diarrhoea, dehydration and weight loss. Prolonged illness for up to 48 months has been reported. Infections in the general population are less pronounced. *Enterocytozoon* infection generally appears to be limited to intestinal enterocytes and biliary epithelium. *Encephalitozoon* spp. infect a variety of cells, including epithelial and endothelial cells, fibroblasts, kidney tubule cells, macrophages and possibly other cell types. Unusual complications include keratoconjunctivitis, myositis and hepatitis.

Source and occurrence

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. Due to the lack of a quantification technique, there is limited information on the prevalence of microsporidia spores in water environments. However, microsporidia have been detected in sewage and water sources. Indications are that their numbers in raw sewage may be similar to those of *Cryptosporidium* and *Giardia*, and they may survive in certain water environments for many months. Certain animals, notably swine, may serve as a host for human infectious species.

Routes of exposure

Little is known about transmission of microsporidia. Person-to-person contact and ingestion of spores in water or food contaminated with human faeces or urine are probably important routes of exposure. A waterborne outbreak of microsporidiosis has been reported involving about 200 cases in Lyon, France, during the summer of 1995. However, the source of the organism and faecal contamination of the drinking-water supply were not demonstrated. Transmission by the inhalation of airborne spores or aerosols containing spores seems possible. The role of animals in transmission to humans remains unclear. Epidemiological and experimental studies in mammals suggest that *Encephalitozoon* spp. can be transmitted transplacentally from mother to offspring. No information is available on the infectivity of the spores. However, in view of the infectivity of spores of closely related species, the infectivity of microsporidia may be high.

Significance in drinking-water

Waterborne transmission has been reported, and infection arising from contaminated drinking-water is plausible but unconfirmed. Little is known about the response of microsporidia to water treatment processes. One study has suggested that the spores may be susceptible to chlorine. The small size of the organism is likely to make them difficult to remove by filtration processes. Within a WSP, control measures that can be applied to manage potential risk from microsporidia include prevention of source water contamination by human and animal waste, followed by adequate treatment and disinfection and protection of water during distribution. 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.

Selected bibliography

Coote L et al. (2000) Waterborne outbreak of intestinal microsporidiosis in persons with and without human immunodeficiency virus infection. *Journal of Infectious Diseases*, 180:2003–2008.

- Dowd SE et al. (2003) Confirmed detection of *Cyclospora cayetanensis*, *Encephalitozoon intestinalis* and *Cryptosporidium parvum* in water used for drinking. *Journal of Water and Health*, 1:117–123.
- Goodgame R (2003) Emerging causes of traveller's diarrhea: *Cryptosporidium*, *Cyclospora*, *Isospora* and microsporidia. *Current Infectious Disease Reports*, 5:66–73.
- Joyson DHM (1999) Emerging parasitic infections in man. *The Infectious Disease Review*, 1:131–134.
- Slifko TR, Smith HV, Rose JB (2000) Emerging parasite zoonoses associated with water and food. *International Journal for Parasitology*, 30:1379–1393.

11.3.9 *Naegleria fowleri*

General description

Naegleria are free-living amoeboflagellates distributed widely in the environment. There are several species of *Naegleria*, of which *N. fowleri* is the primary infectious species. *Naegleria* spp. exist as a trophozoite, a flagellate and a cyst stage. The trophozoite (10–20 µm) moves by eruptive pseudopod formation feeding on bacteria and reproduces by binary fission. The trophozoite can transform into a flagellate stage with two anterior flagella. The flagellate does not divide but reverts to the trophozoite stage. Under adverse conditions, the trophozoite transforms into a circular cyst (7–15 µm), which is resistant to unfavourable conditions.

Human health effects

Naegleria fowleri causes primary amoebic meningoencephalitis (PAM) in healthy individuals. The amoeba enters the brain by penetrating the olfactory mucosa and cribriform plate. The disease is acute, and patients often die within 5–10 days and before the infectious agent can be diagnosed. Treatment is difficult. Although the infection is rare, new cases are reported every year.

Source and occurrence

Naegleria fowleri is thermophilic and grows well at temperatures up to 45 °C. It occurs naturally in fresh water of suitable temperature, and prevalence is only indirectly related to human activity, inasmuch as such activity may modify temperature or promote bacterial (food source) production. The pathogen has been reported from many countries, usually associated with thermally polluted water environments such as geothermal water or heated swimming pools. However, the organism has been detected in drinking-water supplies, particularly where water temperature can exceed 25–30 °C. Water is the only known source of infection. The first cases of amoebic meningitis were diagnosed in 1965 in Australia and Florida. Since that time, about 100 cases of PAM have been reported throughout the world.

Routes of exposure

Infection with *N. fowleri* is almost exclusively contracted by exposure of the nasal passages to contaminated water. Infection is predominantly associated with recreational use of water, including swimming pools and spas, as well as surface waters naturally heated by the sun, industrial cooling waters and geothermal springs. In a limited number of cases, a link to recreational water exposure is lacking. The occurrence of PAM is highest during hot summer months, when many people engage in water recreation and when the temperature of water is conducive to growth of the organism. Consumption of contaminated water or food and person-to-person spread have not been reported as routes of transmission.

Significance in drinking-water

Naegleria fowleri has been detected in drinking-water supplies. Although unproven, a direct or indirect role of drinking-water-derived organisms – for example, through use of drinking-water in swimming pools – is possible. Any water supply that seasonally exceeds 30°C or that continually exceeds 25°C can potentially support the growth of *N. fowleri*. In such cases, a periodic prospective study would be valuable. Free chlorine or monochloramine residuals in excess of 0.5 mg/litre have been shown to control *N. fowleri*, providing the disinfectant persists through the water distribution system. In addition to maintaining persistent disinfectant residuals, other control measures aimed at limiting the presence of biofilm organisms will reduce food sources and hence growth of the organism in distribution systems. Owing to the environmental nature of this amoeba, *E. coli* (or, alternatively, thermotolerant coliforms) cannot be relied upon as an index for the presence/absence of *N. fowleri* in drinking-water supplies.

Selected bibliography

- Behets J et al. (2003) Detection of *Naegleria* spp. and *Naegleria fowleri*: a comparison of flagellation tests, ELISA and PCR. *Water Science and Technology*, 47:117–122.
- Cabanes P-A et al. (2001) Assessing the risk of primary amoebic meningoencephalitis from swimming in the presence of environmental *Naegleria fowleri*. *Applied and Environmental Microbiology*, 67:2927–2931.
- Dorsch MM, Cameron AS, Robinson BS (1983) The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 77:372–377.
- Martinez AJ, Visvesvara GS (1997) Free-living amphizoic and opportunistic amebas. *Brain Pathology*, 7:583–598.
- Parija SC, Jayakeerthee SR (1999) *Naegleria fowleri*: a free living amoeba of emerging medical importance. *Communicable Diseases*, 31:153–159.

11.3.10 *Toxoplasma gondii*

General description

Many species of *Toxoplasma* and *Toxoplasma*-like organisms have been described, but it would appear that *T. gondii* is the only human infectious species. *Toxoplasma gondii* is a coccidian parasite, and the cat is the definitive host. Only cats harbour the parasite in the intestinal tract, where sexual reproduction takes place. The actively multiplying asexual form in the human host is an obligate, intracellular parasite (diameter 3–6 µm) called a tachyzoite. A chronic phase of the disease develops as the tachyzoites transform into slowly replicating bradyzoites, which eventually become cysts in the host tissue. In the natural cycle, mice and rats containing infective cysts are eaten by cats, which host the sexual stage of the parasite. The cyst wall is digested, and bradyzoites penetrate epithelial cells of the small intestine. Several generations of intracellular multiplication lead to the development of micro- and macrogametes. Fertilization of the latter leads to the development of oocysts that are excreted in faeces as early as 5 days after a cat has ingested the cysts. Oocysts require 1–5 days to sporulate in the environment. Sporulated oocysts and tissue-borne cysts can both cause infections in susceptible hosts.

Human health effects

Toxoplasmosis is usually asymptomatic in humans. In a small percentage of cases, flu-like symptoms, lymphadenopathy and hepatosplenomegaly present 5–23 days after the ingestion of cysts or oocysts. Dormant cysts, formed in organ tissue after primary infection, can be reactivated when the immune system becomes suppressed, producing disseminated disease involving the central nervous system and lungs and leading to severe neurological disorders or pneumonia. When these infection sites are involved, the disease can be fatal in immunocompromised patients. Congenital toxoplasmosis is mostly asymptomatic, but can produce chorioretinitis, cerebral calcifications, hydrocephalus, severe thrombocytopenia and convulsions. Primary infection during early pregnancy can lead to spontaneous abortion, stillbirth or fetal abnormality.

Source and occurrence

Toxoplasmosis is found worldwide. Estimates indicate that in many parts of the world, 15–30% of lamb and pork meat is infected with cysts. The prevalence of oocyst-shedding cats may be 1%. By the third decade of life, about 50% of the European population is infected, and in France this proportion is close to 80%. *Toxoplasma gondii* oocysts may occur in water sources and supplies contaminated with the faeces of infected cats. Due to a lack of practical methods for the detection of *T. gondii* oocysts, there is little information on the prevalence of the oocysts in raw and treated water supplies. Details on the survival and behaviour of the oocysts in water environments are also not available. However, qualitative evidence of the presence of oocysts in faecally polluted water has been reported, and results suggest that *T. gondii*

oocysts may be as resistant to unfavourable conditions in water environments as the oocysts of related parasites.

Routes of exposure

Both *T. gondii* oocysts that sporulate after excretion by cats and tissue-borne cysts are potentially infectious. Humans can become infected by ingestion of oocysts excreted by cats by direct contact or through contact with contaminated soil or water. Two outbreaks of toxoplasmosis have been associated with consumption of contaminated water. In Panama, creek water contaminated by oocysts from jungle cats was identified as the most likely source of infection, while in 1995, an outbreak in Canada was associated with a drinking-water reservoir being contaminated by excreta from domestic or wild cats. A study in Brazil during 1997–1999 identified the consumption of unfiltered drinking-water as a risk factor for *T. gondii* seropositivity. More commonly, humans contract toxoplasmosis through the consumption of undercooked or raw meat and meat products containing *T. gondii* cysts. Transplacental infection also occurs.

Significance in drinking-water

Contaminated drinking-water has been identified as a source of toxoplasmosis outbreaks. Little is known about the response of *T. gondii* to water treatment processes. The oocysts are larger than *Cryptosporidium* oocysts and should be amenable to removal by filtration. Within a WSP, control measures to manage potential risk from *T. gondii* should be focused on prevention of source water contamination by wild and domesticated cats. If necessary, the organisms can be removed by filtration. 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.

Selected bibliography

- Aramini JJ et al. (1999) Potential contamination of drinking water with *Toxoplasma gondii* oocysts. *Epidemiology and Infection*, 122:305–315.
- Bahia-Oliveira LMG et al. (2003) Highly endemic, waterborne toxoplasmosis in North Rio de Janeiro State, Brazil. *Emerging Infectious Diseases*, 9:55–62.
- Bowie WR et al. (1997) Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma Investigation Team. *Lancet*, 350:173–177.
- Kourenti C et al. (2003) Development and application of different methods for the detection of *Toxoplasma gondii* in water. *Applied and Environmental Microbiology*, 69:102–106.

11.4 Helminth pathogens

The word “helminth” comes from the Greek word meaning “worm” and refers to all types of worms, both free-living and parasitic. The major parasitic worms are classi-

fied primarily in the phylum Nematoda (roundworms) and the phylum Platyhelminthes (flatworms including trematodes). Helminth parasites infect a large number of people and animals worldwide. For most helminths, drinking-water is not a significant route of transmission. There are two exceptions: *Dracunculus medinensis* (guinea worm) and *Fasciola* spp. (*F. hepatica* and *F. gigantica*) (liver flukes). Dracunculiasis and fascioliasis both require intermediate hosts to complete their life cycles but are transmitted through drinking-water by different mechanisms. Other helminthiasis can be transmitted through water contact (schistosomiasis) or are associated with the use of untreated wastewater in agriculture (ascariasis, trichuriasis, hookworm infections and strongyloidiasis) but are not usually transmitted through drinking-water.

11.4.1 *Dracunculus medinensis*

Dracunculus medinensis, commonly known as “guinea worm,” belongs to the phylum Nematoda and is the only nematode associated with significant transmission by drinking-water.

The eradication of guinea worm infection from the world by 1995 was a target of the International Drinking Water Supply and Sanitation Decade (1981–1990), and the World Health Assembly formally committed itself to this goal in 1991. The Dracunculus Eradication Programme has achieved a massive reduction in the number of cases. There were an estimated 3.3 million cases in 1986, 625 000 cases in 1990 and fewer than 60 000 cases in 2002, with the majority occurring in Sudan. Dracunculiasis is restricted to a central belt of countries in sub-Saharan Africa.

General description

The *D. medinensis* worms inhabit the cutaneous and subcutaneous tissues of infected individuals, the female reaching a length of up to 700 mm, and the male 25 mm. When the female is ready to discharge larvae (embryos), its anterior end emerges from a blister or ulcer, usually on the foot or lower limb, and releases large numbers of rhabditiform larvae when the affected part of the body is immersed in water. The larvae can move about in water for approximately 3 days and during that time can be ingested by many species of *Cyclops* (cyclopoid Copepoda, Crustacea). The larvae penetrate into the haemocoelom, moult twice and are infective to a new host in about 2 weeks. If the *Cyclops* (0.5–2.0 mm) are swallowed in drinking-water, the larvae are released in the stomach, penetrate the intestinal and peritoneal walls and inhabit the subcutaneous tissues.

Human health effects

The onset of symptoms occurs just prior to the local eruption of the worm. The early manifestations of urticaria, erythema, dyspnoea, vomiting, pruritus and giddiness are of an allergic nature. In about 50% of cases, the whole worm is extruded in a few weeks; the lesion then heals rapidly, and disability is of limited duration. In the

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remaining cases, however, complications ensue, and the track of the worm becomes secondarily infected, leading to a severe inflammatory reaction that may result in abscess formation with disabling pain that lasts for months. Mortality is extremely rare, but permanent disability can result from contractures of tendons and chronic arthritis. The economic impact can be substantial. One study reported an 11% annual reduction in rice production from an area of eastern Nigeria, at a cost of US\$20 million.

Source and occurrence

Infection with guinea worm is geographically limited to a central belt of countries in sub-Saharan Africa. Drinking-water containing infected *Cyclops* is the only source of infection with *Dracunculus*. The disease typically occurs in rural areas where piped water supplies are not available. Transmission tends to be highly seasonal, depending on changes in water sources. For instance, transmission is highest in the early rainy season in a dry savannah zone of Mali with under 800 mm annual rainfall but in the dry season in the humid savannah area of southern Nigeria with over 1300 mm annual rainfall. The eradication strategy combines a variety of interventions, including integrated surveillance systems, intensified case containment measures, provision of safe water and health education.

Routes of exposure

The only route of exposure is the consumption of drinking-water containing *Cyclops* spp. carrying infectious *Dracunculus* larvae.

Significance in drinking-water

Dracunculus medinensis is the only human parasite that may be eradicated in the near future by the provision of safe drinking-water. Infection can be prevented by a number of relatively simple control measures. These include intervention strategies to prevent the release of *D. medinensis* larvae from female worms in infected patients into water and control of *Cyclops* spp. in water resources by means of fish. Prevention can also be achieved through the provision of boreholes and safe wells. Wells and springs should be surrounded by cement curbing, and bathing and washing in these waters should be avoided. Other control measures include filtration of water carrying infectious *Dracunculus* larvae through a fine mesh cloth to remove *Cyclops* spp. or inactivation of *Cyclops* spp. in drinking-water by treatment with chlorine.

Selected bibliography

- Cairncross S, Muller R, Zagaria N (2002) Dracunculiasis (guinea worm disease) and the eradication initiative. *Clinical Microbiology Reviews*, 15:223–246.
- Hopkins DR, Ruiz-Tiben E (1991) Strategies for dracunculiasis eradication. *Bulletin of the World Health Organization*, 69:533–540.

11.4.2 *Fasciola* spp.

Fascioliasis is caused by two trematode species of the genus *Fasciola*: *F. hepatica*, present in Europe, Africa, Asia, the Americas and Oceania, and *F. gigantica*, mainly distributed in Africa and Asia. Human fascioliasis was considered a secondary zoonotic disease until the mid-1990s. In most regions, fascioliasis is a foodborne disease. However, the discovery of floating metacercariae in hyperendemic regions (including the Andean Altiplano region in South America) indicates that drinking-water may be a significant transmission route for fascioliasis in certain locations.

General description

The life cycle of *F. hepatica* and *F. gigantica* takes about 14–23 weeks and requires two hosts. The life cycle comprises four phases. In the first phase, the definitive host ingests metacercariae. The metacercariae excyst in the intestinal tract and then migrate to the liver and bile ducts. After 3–4 months, the flukes attain sexual maturity and produce eggs, which are excreted into the bile and intestine. Adult flukes can live for 9–14 years in the host. In the second phase, the eggs are excreted by the human or animal. Once in fresh water, a miracidium develops inside. In the third phase, miracidia penetrate a snail host and develop into cercaria, which are released into the water. In the fourth and final phase, cercaria swim for a short period of time until they reach a suitable attachment site (aquatic plants), where they encyst to form metacercariae, which become infective within 24 h. Some metacercariae do not attach to plants but remain floating in the water.

Human health effects

The parasites inhabit the large biliary passages and the gall-bladder. Disease symptoms are different for the acute and chronic phases of the infection. The invasive or acute phase may last from 2 to 4 months and is characterized by symptoms such as dyspepsia, nausea and vomiting, abdominal pain and a high fever (up to 40°C). Anaemia and allergic responses (e.g., pruritis, urticaria) may also occur. In children, the acute infection can be accompanied by severe symptoms and sometimes causes death. The obstructive or chronic phase (after months to years of infection) may be characterized by painful liver enlargement and in some cases obstructive jaundice, chest pains, loss of weight and cholelithiasis. The most important pathogenic sequelae are hepatic lesions and fibrosis and chronic inflammation of the bile ducts. Immature flukes may deviate during migration, enter other organs and cause ectopic fascioliasis in a range of subcutaneous tissues. Fascioliasis can be treated with triclabendazole.

Source and occurrence

Human cases have been increasing in 51 countries on five continents. Estimates of the numbers of humans with fascioliasis range from 2.4 to 17 million people or even higher, depending on unquantified prevalence in many African and Asian countries.

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Analysis of the geographical distribution of human cases shows that the correlation between animal and human fascioliasis occurs only at a basic level. High prevalences in humans are not necessarily related to areas where fascioliasis is a great veterinary problem. Major health problems associated with fascioliasis occur in Andean countries (Bolivia, Peru, Chile, Ecuador), the Caribbean (Cuba), northern Africa (Egypt), Near East (Iran and neighbouring countries) and western Europe (Portugal, France and Spain).

Routes of exposure

Humans can contract fascioliasis when they ingest infective metacercariae by eating raw aquatic plants (and, in some cases, terrestrial plants, such as lettuce, irrigated with contaminated water), drinking contaminated water, using utensils washed in contaminated water or eating raw liver infected with immature flukes.

Significance in drinking-water

Water is often cited as a human infection source. In the Bolivian Altiplano, 13% of metacercariae isolates are floating. Untreated drinking-water in hyperendemic regions often contains floating metacercariae; for example, a small stream crossing in the Altiplano region of Bolivia contained up to 7 metacercariae per 500 ml. The importance of fascioliasis transmission through water is supported by indirect evidence. There are significant positive associations between liver fluke infection and infection by other waterborne protozoans and helminths in Andean countries and in Egypt. In many human hyperendemic areas of the Americas, people do not have a history of eating watercress or other water plants. In the Nile Delta region, people living in houses with piped water had a higher infection risk. Metacercariae are likely to be resistant to chlorine disinfection but should be removed by various filtration processes. For example, in Tiba, Egypt, human prevalence was markedly decreased after filtered water was supplied to specially constructed washing units.

Selected bibliography

- Mas-Coma S (2004) Human fascioliasis. In: *Waterborne zoonoses: Identification, causes, and controls*. IWA Publishing, London, on behalf of the World Health Organization, Geneva.
- Mas-Coma S, Esteban JG, Bargues MD (1999) Epidemiology of human fascioliasis: a review and proposed new classification. *Bulletin of the World Health Organization*, 77(4):340–346.
- WHO (1995) *Control of foodborne trematode infections*. Geneva, World Health Organization (WHO Technical Report Series 849).

11.5 Toxic cyanobacteria

More detailed information on toxic cyanobacteria is available in the supporting document *Toxic Cyanobacteria in Water* (see section 1.3).

General description

Cyanobacteria are photosynthetic bacteria that share some properties with algae. Notably, they possess chlorophyll-a and liberate oxygen during photosynthesis. The first species to be recognized were blue-green in colour; hence, a common term for these organisms is blue-green algae. However, owing to the production of different pigments, there are a large number that are not blue-green, and they can range in colour from blue-green to yellow-brown and red. Most cyanobacteria are aerobic phototrophs, but some exhibit heterotrophic growth. They may grow as separate cells or in multicellular filaments or colonies. They can be identified by their morphology to genus level under a microscope. Some species form surface blooms or scums, while others stay mixed in the water column or are bottom dwelling (benthic). Some cyanobacteria possess the ability to regulate their buoyancy via intracellular gas vacuoles, and some species can fix elemental nitrogen dissolved in water. The most notable feature of cyanobacteria in terms of public health impact is that a range of species can produce toxins.

Human health effects

Many cyanobacteria produce potent toxins, as shown in Table 11.1. Cyanobacterial toxins are also discussed in section 8.5.6. Each toxin has specific properties, with distinct concerns including liver damage, neurotoxicity and tumour promotion. Acute symptoms reported after exposure include gastrointestinal disorders, fever and irritations of the skin, ears, eyes, throat and respiratory tract. Cyanobacteria do not multiply in the human body and hence are not infectious.

Source and occurrence

Cyanobacteria are widespread and found in a diverse range of environments, including soils, seawater and, most notably, freshwater environments. Some environmental conditions, including sunlight, warm weather, low turbulence and high nutrient levels, can promote growth. Depending on the species, this may result in greenish discol-

Table 11.1 Cyanotoxins produced by cyanobacteria

Toxic species	Cyanotoxin
Potentially <i>Anabaena</i> spp.	Anatoxin-a(S), anatoxin-a, microcystins, saxitoxins
<i>Anabaenopsis millenii</i>	Microcystins
<i>Aphanizomenon</i> spp.	Anatoxin-a, saxitoxins, cylindrospermopsin
<i>Cylindrospermum</i> spp.	Cylindrospermopsin, saxitoxins, anatoxin-a
<i>Lyngbya</i> spp.	Saxitoxins, lyngbyatoxins
<i>Microcystis</i> spp.	Microcystins, anatoxin-a (minor amounts)
<i>Nodularia</i> spp.	Nodularins
<i>Nostoc</i> spp.	Microcystins
<i>Oscillatoria</i> spp.	Anatoxin-a, microcystins
<i>Planktothrix</i> spp.	Anatoxin-a, homoanatoxin-a, microcystins
<i>Raphidiopsis curvata</i>	Cylindrospermopsin
<i>Umezakia natans</i>	Cylindrospermopsin

oration of water due to a high density of suspended cells and, in some cases, the formation of surface scums. Such cell accumulations may lead to high toxin concentrations.

Routes of exposure

Potential health concerns arise from exposure to the toxins through ingestion of drinking-water, during recreation, through showering and potentially through consumption of algal food supplement tablets. Repeated or chronic exposure is the primary concern for many of the cyanotoxins; in some cases, however, acute toxicity is more important (e.g., lyngbyatoxins and the neurotoxins saxitoxin and anatoxin). Human fatalities have occurred through use of inadequately treated water containing high cyanotoxin levels for renal dialysis. Dermal exposure may lead to irritation of the skin and mucous membranes and to allergic reactions.

Significance in drinking-water

Cyanobacteria occur in low cell density in most surface waters. However, in suitable environmental conditions, high-density “blooms” can occur. Eutrophication (increased biological growth associated with increased nutrients) can support the development of cyanobacterial blooms (see also section 8.5.6).

Selected bibliography

- Backer LC (2002) Cyanobacterial harmful algal blooms (CyanoHABs): Developing a public health response. *Lake and Reservoir Management*, 18:20–31.
- Chorus I, Bartram J, eds. (1999) *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*. Published by E & FN Spon, London, on behalf of the World Health Organization, Geneva.
- Lahti K et al. (2001) Occurrence of microcystins in raw water sources and treated drinking water of Finnish waterworks. *Water Science and Technology*, 43:225–228.

11.6 Indicator and index organisms

Owing to issues relating to complexity, cost and timeliness of obtaining results, testing for specific pathogens is generally limited to validation, where monitoring is used to determine whether a treatment or other process is effective in removing target organisms. Very occasionally, pathogen testing may be performed to verify that a specific treatment or process has been effective. However, microbial testing included as part of operational and verification (including surveillance) monitoring is usually limited to that for indicator organisms, either to measure the effectiveness of control measures or as an index of faecal pollution.

The concept of using indicator organisms as signals of faecal pollution is a well established practice in the assessment of drinking-water quality. The criteria determined for such indicators were that they should not be pathogens themselves and should:

- be universally present in faeces of humans and animals in large numbers;
- not multiply in natural waters;
- persist in water in a similar manner to faecal pathogens;
- be present in higher numbers than faecal pathogens;
- respond to treatment processes in a similar fashion to faecal pathogens; and
- be readily detected by simple, inexpensive methods.

These criteria reflect an assumption that the same indicator organism could be used as both an index of faecal pollution and an indicator of treatment/process efficacy. However, it has become clear that one indicator cannot fulfil these two roles. Increased attention has focused on shortcomings of traditional indicators, such as *E. coli*, as surrogates for enteric viruses and protozoa, and alternative indicators of these pathogens, such as bacteriophages and bacterial spores, have been suggested. In addition, greater reliance is being placed on parameters that can be used as indicators for the effectiveness of treatments and processes designed to remove faecal pathogens, including bacteria, viruses, protozoa and helminths.

It is important to distinguish between microbial testing undertaken to signal the presence of faecal pathogens or alternatively to measure the effectiveness of treatments/processes. As a first step, the separate terms *index* and *indicator* have been proposed, whereby:

- an *index organism* is one that points to the presence of pathogenic organisms – for example, as an index of faecal pathogens; and
- an *indicator organism* is one that is used to measure the effectiveness of a process – for example, a process indicator or disinfection indicator.

These terms can also be applied to non-microbial parameters; hence, turbidity can be used a filtration indicator.

Further discussion on index and indicator organisms is contained in the supporting document *Assessing Microbial Safety of Drinking Water* (see section 1.3).

11.6.1 Total coliform bacteria

General description

Total coliform bacteria include a wide range of aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 h at 35–37°C. *Escherichia coli* and thermotolerant coliforms are a subset of the total coliform group that can ferment lactose at higher temperatures (see section 11.6.2). As part of lactose fermentation, total coliforms produce the enzyme β -galactosidase. Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter*, but the group is more heterogeneous and includes a wider range of genera, such as *Serratia* and *Hafnia*. The total coliform group includes both faecal and environmental species.

Indicator value

Total coliforms include organisms that can survive and grow in water. Hence, they are not useful as an index of faecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms. However, there are better indicators for these purposes. As a disinfection indicator, the test for total coliforms is far slower and less reliable than direct measurement of disinfectant residual. In addition, total coliforms are far more sensitive to disinfection than are enteric viruses and protozoa. HPC measurements detect a wider range of microorganisms and are generally considered a better indicator of distribution system integrity and cleanliness.

Source and occurrence

Total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters. Some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environments. Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms.

Application in practice

Total coliforms are generally measured in 100-ml samples of water. A variety of relatively simple procedures are available based on the production of acid from lactose or the production of the enzyme β -galactosidase. The procedures include membrane filtration followed by incubation of the membranes on selective media at 35–37°C and counting of colonies after 24h. Alternative methods include most probable number procedures using tubes or micro-titre plates and P/A tests. Field test kits are available.

Significance in drinking-water

Total coliforms should be absent immediately after disinfection, and the presence of these organisms indicates inadequate treatment. The presence of total coliforms in distribution systems and stored water supplies can reveal regrowth and possible biofilm formation or contamination through ingress of foreign material, including soil or plants.

Selected bibliography

- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA*, 22:193–202.

Sueiro RA et al. (2001) Evaluation of Coli-ID and MUG Plus media for recovering *Escherichia coli* and other coliform bacteria from groundwater samples. *Water Science and Technology*, 43:213–216.

11.6.2 *Escherichia coli* and thermotolerant coliform bacteria

General description

Total coliform bacteria that are able to ferment lactose at 44–45°C are known as thermotolerant coliforms. In most waters, the predominant genus is *Escherichia*, but some types of *Citrobacter*, *Klebsiella* and *Enterobacter* are also thermotolerant. *Escherichia coli* can be differentiated from the other thermotolerant coliforms by the ability to produce indole from tryptophan or by the production of the enzyme β -glucuronidase. *Escherichia coli* is present in very high numbers in human and animal faeces and is rarely found in the absence of faecal pollution, although there is some evidence for growth in tropical soils. Thermotolerant coliform species other than *E. coli* can include environmental organisms.

Indicator value

Escherichia coli is considered the most suitable index of faecal contamination. In most circumstances, populations of thermotolerant coliforms are composed predominantly of *E. coli*; as a result, this group is regarded as a less reliable but acceptable index of faecal pollution. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is the first organism of choice in monitoring programmes for verification, including surveillance of drinking-water quality. These organisms are also used as disinfection indicators, but testing is far slower and less reliable than direct measurement of disinfectant residual. In addition, *E. coli* is far more sensitive to disinfection than are enteric viruses and protozoa.

Source and occurrence

Escherichia coli occurs in high numbers in human and animal faeces, sewage and water subject to recent faecal pollution. Water temperatures and nutrient conditions present in drinking-water distribution systems are highly unlikely to support the growth of these organisms.

Application in practice

Escherichia coli (or, alternatively, thermotolerant coliforms) are generally measured in 100-ml samples of water. A variety of relatively simple procedures are available based on the production of acid and gas from lactose or the production of the enzyme β -glucuronidase. The procedures include membrane filtration followed by incubation of the membranes on selective media at 44–45°C and counting of colonies after 24 h. Alternative methods include most probable number procedures using tubes or microtitre plates and P/A tests, some for volumes of water larger than 100 ml. Field test kits are available.

Significance in drinking-water

The presence of *E. coli* (or, alternatively, thermotolerant coliforms) provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity.

Selected bibliography

- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- George I et al. (2001) Use of rapid enzymatic assays to study the distribution of faecal coliforms in the Seine river (France). *Water Science and Technology*, 43:77–80.
- Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA*, 22:193–202.
- Sueiro RA et al. (2001) Evaluation of Coli-ID and MUG Plus media for recovering *Escherichia coli* and other coliform bacteria from groundwater samples. *Water Science and Technology*, 43:213–216.

11.6.3 Heterotrophic plate counts

A substantial review of the use of HPC is available (Bartram et al., 2003).

General description

HPC measurement detects a wide spectrum of heterotrophic microorganisms, including bacteria and fungi, based on the ability of the organisms to grow on rich growth media, without inhibitory or selective agents, over a specified incubation period and at a defined temperature. The spectrum of organisms detected by HPC testing includes organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers; and organisms that rapidly proliferate in treated water in the absence of residual disinfectants. The tests detect only a small proportion of the microorganisms that are present in water. The population recovered will differ according to the method and conditions applied. Although standard methods have been developed, there is no single universal HPC measurement. A range of media is available, incubation temperatures used vary from 20°C to 37°C and incubation periods range from a few hours to 7 days or more.

Indicator value

The test has little value as an index of pathogen presence but can be useful in operational monitoring as a treatment and disinfectant indicator, where the objective is to keep numbers as low as possible. In addition, HPC measurement can be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms.

Source and occurrence

Heterotrophic microorganisms include both members of the natural (typically non-hazardous) microbial flora of water environments and organisms present in a range of pollution sources. They occur in large numbers in raw water sources. The actual organisms detected by HPC tests vary widely between locations and between consecutive samples. Some drinking-water treatment processes, such as coagulation and sedimentation, reduce the number of HPC organisms in water. However, the organisms proliferate in other treatment processes, such as biologically active carbon and sand filtration. Numbers of HPC organisms are reduced significantly by disinfection practices, such as chlorination, ozonation and UV light irradiation. However, in practice, none of the disinfection processes sterilizes water; under suitable conditions, such as the absence of disinfectant residuals, HPC organisms can grow rapidly. HPC organisms can grow in water and on surfaces in contact with water as biofilms. The principal determinants of growth or “regrowth” are temperature, availability of nutrients, including assimilable organic carbon, lack of disinfectant residual and stagnation.

Application in practice

No sophisticated laboratory facilities or highly trained staff are required. Results on simple aerobically incubated agar plates are available within hours to days, depending on the characteristics of the procedure used.

Significance in drinking-water

After disinfection, numbers would be expected to be low; for most uses of HPC test results, however, actual numbers are of less value than changes in numbers at particular locations. In distribution systems, increasing numbers can indicate a deterioration in cleanliness, possibly stagnation and the potential development of biofilms. HPC can include potentially “opportunistic” pathogens such as *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Klebsiella*, *Moraxella*, *Serratia*, *Pseudomonas* and *Xanthomonas*. However, there is no evidence of an association of any of these organisms with gastrointestinal infection through ingestion of drinking-water in the general population.

Selected bibliography

- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- Bartram J et al., eds. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing.

11.6.4 Intestinal enterococci

General description

Intestinal enterococci are a subgroup of the larger group of organisms defined as faecal streptococci, comprising species of the genus *Streptococcus*. These bacteria are Gram-positive and relatively tolerant of sodium chloride and alkaline pH levels. They are facultatively anaerobic and occur singly, in pairs or as short chains. Faecal streptococci including intestinal enterococci all give a positive reaction with Lancefield's Group D antisera and have been isolated from the faeces of warm-blooded animals. The subgroup intestinal enterococci consists of the species *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae*. This group was separated from the rest of the faecal streptococci because they are relatively specific for faecal pollution. However, some intestinal enterococci isolated from water may occasionally also originate from other habitats, including soil, in the absence of faecal pollution.

Indicator value

The intestinal enterococci group can be used as an index of faecal pollution. Most species do not multiply in water environments. The numbers of intestinal enterococci in human faeces are generally about an order of magnitude lower than those of *E. coli*. Important advantages of this group are that they tend to survive longer in water environments than *E. coli* (or thermotolerant coliforms), are more resistant to drying and are more resistant to chlorination. Intestinal enterococci have been used in testing of raw water as an index of faecal pathogens that survive longer than *E. coli* and in drinking-water to augment testing for *E. coli*. In addition, they have been used to test water quality after repairs to distribution systems or after new mains have been laid.

Source and occurrence

Intestinal enterococci are typically excreted in the faeces of humans and other warm-blooded animals. Some members of the group have also been detected in soil in the absence of faecal contamination. Intestinal enterococci are present in large numbers in sewage and water environments polluted by sewage or wastes from humans and animals.

Application in practice

Enterococci are detectable by simple, inexpensive cultural methods that require basic bacteriology laboratory facilities. Commonly used methods include membrane filtration with incubation of membranes on selective media and counting of colonies after incubation at 35–37°C for 48 h. Other methods include a most probable number technique using micro-titre plates where detection is based on the ability of intestinal enterococci to hydrolyse 4-methyl-umbelliferyl- β -D-glucoside in the presence of thallium acetate and nalidixic acid within 36 h at 41 °C.

Significance in drinking-water

The presence of intestinal enterococci provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity.

Selected bibliography

- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- Grabow WOK (1996) Waterborne diseases: Update on water quality assessment and control. *Water SA*, 22:193–202.
- Junco TT et al. (2001) Identification and antibiotic resistance of faecal enterococci isolated from water samples. *International Journal of Hygiene and Environmental Health*, 203:363–368.
- Pinto B et al. (1999) Characterization of “faecal streptococci” as indicators of faecal pollution and distribution in the environment. *Letters in Applied Microbiology*, 29:258–263.

11.6.5 *Clostridium perfringens*

General description

Clostridium spp. are Gram-positive, anaerobic, sulfite-reducing bacilli. They produce spores that are exceptionally resistant to unfavourable conditions in water environments, including UV irradiation, temperature and pH extremes, and disinfection processes, such as chlorination. The characteristic species of the genus, *C. perfringens*, is a member of the normal intestinal flora of 13–35% of humans and other warm-blooded animals. Other species are not exclusively of faecal origin. Like *E. coli*, *C. perfringens* does not multiply in most water environments and is a highly specific indicator of faecal pollution.

Indicator value

In view of the exceptional resistance of *C. perfringens* spores to disinfection processes and other unfavourable environmental conditions, *C. perfringens* has been proposed as an index of enteric viruses and protozoa in treated drinking-water supplies. In addition, *C. perfringens* can serve as an index of faecal pollution that took place previously and hence indicate sources liable to intermittent contamination. *Clostridium perfringens* is not recommended for routine monitoring, as the exceptionally long survival times of its spores are likely to far exceed those of enteric pathogens, including viruses and protozoa. *Clostridium perfringens* spores are smaller than protozoan (oo)cysts and may be useful indicators of the effectiveness of filtration processes. Low numbers in

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some source waters suggest that use of *C. perfringens* spores for this purpose may be limited to validation of processes rather than routine monitoring.

Source and occurrence

Clostridium perfringens and its spores are virtually always present in sewage. The organism does not multiply in water environments. *Clostridium perfringens* is present more often and in higher numbers in the faeces of some animals, such as dogs, than in the faeces of humans and less often in the faeces of many other warm-blooded animals. The numbers excreted in faeces are normally substantially lower than those of *E. coli*.

Application in practice

Vegetative cells and spores of *C. perfringens* are usually detected by membrane filtration techniques in which membranes are incubated on selective media under strict anaerobic conditions. These detection techniques are not as simple and inexpensive as those for other indicators, such as *E. coli* and intestinal enterococci.

Significance in drinking-water

The presence of *C. perfringens* in drinking-water can be an index of intermittent faecal contamination. Potential sources of contamination should be investigated. Filtration processes designed to remove enteric viruses or protozoa should also remove *C. perfringens*. Detection in water immediately after treatment should lead to investigation of filtration plant performance.

Selected bibliography

- Araujo M et al. (2001) Evaluation of fluorogenic TSC agar for recovering *Clostridium perfringens* in groundwater samples. *Water Science and Technology*, 43:201–204.
- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- Nieminski EC, Bellamy WD, Moss LR (2000) Using surrogates to improve plant performance. *Journal of the American Water Works Association*, 92(3):67–78.
- Payment P, Franco E (1993) *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking-water treatment for viruses and protozoan cysts. *Applied and Environmental Microbiology*, 59:2418–2424.

11.6.6 Coliphages

General description

Bacteriophages (phages) are viruses that use only bacteria as hosts for replication. Coliphages use *E. coli* and closely related species as hosts and hence can be released

by these bacterial hosts into the faeces of humans and other warm-blooded animals. Coliphages used in water quality assessment are divided into the major groups of somatic coliphages and F-RNA coliphages. Differences between the two groups include the route of infection.

Somatic coliphages initiate infection by attaching to receptors permanently located on the cell wall of hosts. They replicate more frequently in the gastrointestinal tract of warm-blooded animals but can also replicate in water environments. Somatic coliphages consist of a wide range of phages (members of the phage families Myoviridae, Siphoviridae, Podoviridae and Microviridae) with a spectrum of morphological types.

F-RNA coliphages initiate infection by attaching to fertility (F-, sex) fimbriae on *E. coli* hosts. These F-fimbriae are produced only by bacteria carrying the fertility (F-) plasmid. Since F-fimbriae are produced only in the logarithmic growth phase at temperatures above 30 °C, F-RNA phages are not likely to replicate in environments other than the gastrointestinal tract of warm-blooded animals. F-RNA coliphages comprise a restricted group of closely related phages, which belong to the family Leviviridae, and consist of a single-stranded RNA genome and an icosahedral capsid that is morphologically similar to that of picornaviruses. F-RNA coliphages have been divided into serological types I–IV, which can be identified as genotypes by molecular techniques such as gene probe hybridization. Members of groups I and IV have to date been found exclusively in animal faeces, and group III in human faeces. Group II phages have been detected in human faeces and no animal faeces other than about 28% of porcine faeces. This specificity, which is not fully understood, offers a potential tool to distinguish between faecal pollution of human and animal origin under certain conditions and limitations.

Indicator value

Phages share many properties with human viruses, notably composition, morphology, structure and mode of replication. As a result, coliphages are useful models or surrogates to assess the behaviour of enteric viruses in water environments and the sensitivity to treatment and disinfection processes. In this regard, they are superior to faecal bacteria. However, there is no direct correlation between numbers of coliphages and numbers of enteric viruses. In addition, coliphages cannot be absolutely relied upon as an index for enteric viruses. This has been confirmed by the isolation of enteric viruses from treated and disinfected drinking-water supplies that yielded negative results in conventional tests for coliphages.

F-RNA coliphages provide a more specific index of faecal pollution than somatic phages. In addition, F-RNA coliphages are better indicators of the behaviour of enteric viruses in water environments and their response to treatment and disinfection processes than are somatic coliphages. This has been confirmed by studies in which the behaviour and survival of F-RNA coliphages, somatic phages, faecal bacteria and enteric viruses have been compared. Available data indicate that the specificity of F-

RNA serogroups (genotypes) for human and animal excreta may prove useful in the distinction between faecal pollution of human and animal origin. However, there are shortcomings and conflicting data that need to be resolved, and the extent to which this tool can be applied in practice remains to be elucidated. Due to the limitations of coliphages, they are best used in laboratory investigations, pilot trials and possibly validation testing. They are not suitable for operational or verification (including surveillance) monitoring.

Source and occurrence

Coliphages are excreted by humans and animals in relatively low numbers. As a result of their respective modes of replication and host specificity, somatic coliphages are generally excreted by most humans and animals, whereas F-RNA coliphages are excreted by a variable and generally lower percentage of humans and animals. Available data indicate that in some communities, F-RNA phages are detectable in 10% of human, 45% of bovine, 60% of porcine and 70% of poultry faecal specimens. Somatic coliphages have been found to generally outnumber F-RNA phages in water environments by a factor of about 5 and cytopathogenic human viruses by a factor of about 500, although these ratios vary considerably. Sewage contains somatic coliphages in numbers of the order of 10^6 – 10^8 per litre; in one study, slaughterhouse wastewater was found to contain somatic coliphages in numbers up to 10^{10} per litre. There are indications that they may multiply in sewage, and somatic coliphages may multiply in natural water environments using saprophytic hosts. Somatic phages and F-RNA phages have been detected in numbers up to 10^5 per litre in lake and river water.

Application in practice

Somatic coliphages are detectable by relatively simple and inexpensive plaque assays, which yield results within 24h. Plaque assays for F-RNA coliphages are not quite as simple, because the culture of host bacteria has to be in the logarithmic growth phase at a temperature above 30 °C to ensure that F-fimbriae are present. Plaque assays using large petri dishes have been designed for the quantitative enumeration of plaques in 100-ml samples, and P/A tests have been developed for volumes of water of 500 ml or more.

Significance in drinking-water

Since coliphages typically replicate in the gastrointestinal tract of humans and warm-blooded animals, their presence in drinking-water provides an index of faecal pollution and hence the potential presence of enteric viruses and possibly also other pathogens. The presence of coliphages in drinking-water also indicates shortcomings in treatment and disinfection processes designed to remove enteric viruses. F-RNA coliphages provide a more specific index for faecal pollution. The absence of coliphages from treated drinking-water supplies does not confirm the absence of pathogens such as enteric viruses and protozoan parasites.

Selected bibliography

- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- Grabow WOK (2001) Bacteriophages: Update on application as models for viruses in water. *Water SA*, 27:251–268.
- Mooijman KA et al. (2001) Optimisation of the ISO-method on enumeration of somatic coliphages (draft ISO 10705–2). *Water Science and Technology*, 43:205–208.
- Schaper M et al. (2002) Distribution of genotypes of F-specific RNA bacteriophages in human and non-human sources of faecal pollution in South Africa and Spain. *Journal of Applied Microbiology*, 92:657–667.
- Storey MV, Ashbolt NJ (2001) Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. *Water Science and Technology*, 43:133–138.

11.6.7 *Bacteroides fragilis* phages

General description

The bacterial genus *Bacteroides* inhabits the human gastrointestinal tract in greater numbers than *E. coli*. Faeces can contain 10^9 – 10^{10} *Bacteroides* per gram compared with 10^6 – 10^8 *E. coli* per gram. *Bacteroides* are rapidly inactivated by environmental oxygen levels, but *Bacteroides* bacteriophages are resistant to unfavourable conditions. Two groups of *B. fragilis* phages are used as indicators in water quality assessment. One is a restricted group of phages that specifically uses *B. fragilis* strain HSP40 as host. This group of phages appears unique, because it is found only in human faeces and not in faeces of other animals. The numbers of these phages in sewage appear to be relatively low, and they are almost absent in some geographical areas. The *B. fragilis* HSP40 phages belong to the family Siphoviridae, with flexible non-contractile tails, double-stranded DNA and capsids with a diameter of up to 60 nm. The second group of *Bacteroides* phages used as indicators is those that use *B. fragilis* strain RYC2056 as a host. This group includes a substantially wider spectrum of phages, occurring in the faeces of humans and many other animals. The numbers of these phages in sewage are generally substantially higher than those of *B. fragilis* HSP40 phages.

Indicator value

Bacteroides bacteriophages have been proposed as a possible index of faecal pollution due to their specific association with faecal material and exceptional resistance to environmental conditions. In particular, *B. fragilis* HSP40 phages are found only in human faeces. *Bacteroides fragilis* phage B40-8, a typical member of the group of *B. fragilis* HSP40 phages, has been found to be more resistant to inactivation by chlorine than poliovirus type 1, simian rotavirus SA11, coliphage f2, *E. coli* and *Streptococcus faecalis*. *Bacteroides fragilis* strain RYC2056 phages seem to be likewise relatively resistant

to disinfection. Indicator shortcomings of *B. fragilis* phages include relatively low numbers in sewage and polluted water environments. This applies in particular to *B. fragilis* HSP40 phages. Human enteric viruses have been detected in drinking-water supplies that yielded negative results in conventional tests for *B. fragilis* HSP40 phages. Owing to the limitations of *Bacteroides* bacteriophages, they are best used in laboratory investigations, pilot trials and possibly validation testing. They are not suitable for operational or verification (including surveillance) monitoring.

Source and occurrence

Bacteroides fragilis HSP40 phages are excreted by about 10–20% of humans in certain parts of the world; consequently, their numbers in sewage are substantially lower than those of somatic and even F-RNA coliphages. A mean count of 67 *B. fragilis* HSP40 phages per litre in a sewage-polluted river has been reported. In some parts of the world, *B. fragilis* HSP40 phages would appear not to be detectable in sewage at all. Phages using *B. fragilis* RYC2056 as host are excreted in larger numbers and seem to occur more universally. On average, these phages are excreted by more than 25% of humans. In a survey of water environments, *B. fragilis* HSP40 phages have been found to outnumber cytopathogenic enteric viruses on average by only about 5-fold. Theoretically, wastewaters could be expected to contain higher levels of *B. fragilis* phages than those detected. The reason for the discrepancy may be due to failure in maintaining sufficiently anaerobic conditions during the performance of plaque assays. Improvement of detection methods may result in the recording of higher numbers of *B. fragilis* phages in sewage and polluted water environments.

Application in practice

Disadvantages of *B. fragilis* phages are that the detection methods are more complex and expensive than those for coliphages. Costs are increased by the need to use antibiotics for purposes of selection and to incubate cultures and plaque assays under absolute anaerobic conditions. Results of plaque assays are usually available after about 24 h compared with about 8 h for coliphages.

Significance in drinking-water

The presence of *B. fragilis* phages in drinking-water is sound evidence of faecal pollution as well as shortcomings in water treatment and disinfection processes. In addition, the presence of *B. fragilis* HSP40 phages strongly indicates faecal pollution of human origin. However, *B. fragilis* phages occur in relatively low numbers in sewage, polluted water environments and drinking-water supplies. This implies that the absence of *B. fragilis* phages from treated drinking-water supplies does not confirm the absence of pathogens such as enteric viruses and protozoan parasites.

Selected bibliography

- Bradley G et al. (1999) Distribution of the human faecal bacterium *Bacteroides fragilis* and their relationship to current sewage pollution indicators in bathing waters. *Journal of Applied Microbiology*, 85(Suppl.):90S–100S.
- Grabow WOK (2001) Bacteriophages: Update on application as models for viruses in water. *Water SA*, 27:251–268.
- Puig A et al. (1999) Diversity of *Bacteroides fragilis* strains in their capacity to recover phages from human and animal wastes and from fecally polluted wastewater. *Applied and Environmental Microbiology*, 65:1772–1776.
- Storey MV, Ashbolt NJ (2001) Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. *Water Science and Technology*, 43:133–138.
- Tartera C, Lucena F, Jofre J (1989) Human origin of *Bacteroides fragilis* bacteriophages present in the environment. *Applied and Environmental Microbiology*, 10:2696–2701.

11.6.8 Enteric viruses

General description

The viruses referred to here are a combined group of those that infect the human gastrointestinal tract and are predominantly transmitted by the faecal–oral route. Well known members of this group include the enteroviruses, astroviruses, enteric adenoviruses, orthoreoviruses, rotaviruses, caliciviruses and hepatitis A and E viruses. The enteric viruses cover a wide spectrum of viruses, members of which are a major cause of morbidity and mortality worldwide. Members of the group of enteric viruses differ with regard to structure, composition, nucleic acid and morphology. There are also differences in the numbers and frequency of excretion, survival in the environment and resistance to water treatment processes. Enteric viruses have robust capsids that enable them to survive unfavourable conditions in the environment as well as allowing passage through the acidic and proteolytic conditions in the stomach on their way to the duodenum, where they infect susceptible epithelial cells.

Indicator value

The use of enteric viruses as indicator or index organisms is based on the shortcomings of the existing choices. The survival of faecal bacteria in water environments and the sensitivity to treatment and disinfection processes differ substantially from those of enteric viruses. Monitoring based on one or more representatives of the large group of enteric viruses themselves would, therefore, be more valuable for assessment of the presence of any of the enteric viruses in water and the response to control measures.

Source and occurrence

Enteric viruses are excreted by individuals worldwide at a frequency and in numbers that result in many of these viruses being universally present in substantial numbers

in wastewater. However, the prevalence of individual members may vary to a large extent due to variations in rates of infection and excretion. Much higher numbers would be present during outbreaks.

Application in practice

Practical methods are not yet available for the routine monitoring of water supplies for a broad spectrum of enteric viruses. Viruses that are more readily detectable include members of the enterovirus, adenovirus and orthoreovirus groups. These viruses occur in polluted environments in relatively high numbers and can be detected by reasonably practical and moderate-cost techniques based on cytopathogenic effect in cell culture that yield results within 3–12 days (depending on the type of virus). In addition, progress in technology and expertise is decreasing costs. The cost for the recovery of enteric viruses from large volumes of drinking-water has been reduced extensively. Some techniques – for instance, those based on glass wool adsorption–elution – are inexpensive. The cost of cell culture procedures has also been reduced. Consequently, the cost of testing drinking-water supplies for cytopathogenic viruses has become acceptable for certain purposes. Testing could be used to validate effectiveness of treatment processes and, in certain circumstances, as part of specific investigations to verify performance of processes. The incubation times, cost and relative complexity of testing mean that enteric virus testing is not suitable for operational or verification (including surveillance) monitoring. Orthoreoviruses, and at least the vaccine strains of polioviruses detected in many water environments, also have the advantage of not constituting a health risk to laboratory workers.

Significance in drinking-water

The presence of any enteric viruses in drinking-water should be regarded as an index for the potential presence of other enteric viruses, is conclusive evidence of faecal pollution and also provides evidence of shortcomings in water treatment and disinfection processes.

Selected bibliography

- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J, eds. *Water quality: Guidelines, standards and health – Assessment of risk and risk management for water-related infectious disease*. WHO Water Series. London, IWA Publishing, pp. 289–315.
- Grabow WOK, Taylor MB, de Villiers JC (2001) New methods for the detection of viruses: call for review of drinking-water quality guidelines. *Water Science and Technology*, 43:1–8.

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Chemical fact sheets

The background documents referred to in this chapter may be found on the Water Sanitation and Health website at http://www.who.int/water_sanitation_health/dwq/guidelines/en/.

12.1 Acrylamide

Residual acrylamide monomer occurs in polyacrylamide coagulants used in the treatment of drinking-water. In general, the maximum authorized dose of polymer is 1 mg/litre. At a monomer content of 0.05%, this corresponds to a maximum theoretical concentration of 0.5 µg/litre of the monomer in water. Practical concentrations may be lower by a factor of 2–3. This applies to the anionic and non-ionic polyacrylamides, but residual levels from cationic polyacrylamides may be higher. Polyacrylamides are also used as grouting agents in the construction of drinking-water reservoirs and wells. Additional human exposure might result from food, owing to the use of polyacrylamide in food processing and the potential formation of acrylamide in foods cooked at high temperatures.

Guideline value	0.0005 mg/litre (0.5 µg/litre)
Occurrence	Concentrations of a few micrograms per litre have been detected in tap water.
Basis of guideline derivation	Combined mammary, thyroid and uterine tumours observed in female rats in a drinking-water study, and using the linearized multistage model
Limit of detection	0.032 µg/litre by GC; 0.2 µg/litre by HPLC; 10 µg/litre by HPLC with UV detection
Treatment achievability	Conventional treatment processes do not remove acrylamide. Acrylamide concentrations in drinking-water are controlled by limiting either the acrylamide content of polyacrylamide flocculants or the dose used, or both.
Additional comments	Although the practical quantification level for acrylamide in most laboratories is above the guideline value (generally in the order of 1 µg/litre), concentrations in drinking-water can be controlled by product and dose specification.