

7 MICROBIAL ASPECTS

As outlined in section 1.1.1, the greatest microbial risks are associated with contamination of drinking-water with human and animal excreta, although other hazards may also be significant. Drinking-water safety depends upon preventive approaches from catchment to tap.

7.1 Microbial hazards in drinking-water

7.1.1 Waterborne infections

Infectious diseases caused by pathogenic bacteria, viruses and protozoa or by parasites are the most common and widespread health risk associated with drinking-water. The pathogens that may be transmitted through contaminated drinking-water are diverse. While many of the pathogens are known it is unlikely that all water-borne pathogens have yet been recognised. For pathogens transmitted by the faecal-oral route, drinking-water is only one vehicle of transmission. Contamination of food, hands, utensils and clothing can also play a role, particularly when domestic sanitation and hygiene is poor.

Due to this multiplicity of transmission routes, improvements in the quality and availability of water, in excreta disposal and in general hygiene education are all important factors in achieving reductions in morbidity and mortality rates of faecal origin.

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Microbial water quality may vary rapidly and over a wide range. Short-term peaks in pathogen occurrence may increase disease risks considerably and may also trigger outbreaks of waterborne disease that may affect large numbers of persons. For these reasons reliance cannot be placed on water quality measurements alone, even when made frequently, to determine the safety of drinking-water.

In public health terms much attention has been paid to drinking-water as a cause of outbreaks of disease and the first priority in developing and applying controls on drinking-water should be the control of such outbreaks. Experience shows that systems for the detection of water borne disease outbreaks are typically inefficient in countries at all levels of socio-economic development and failure to detect outbreaks is not a guarantee that they do not occur, nor does it suggest that drinking-water should be considered safe.

Available evidence suggests that drinking-water also contributes to background rates of disease in non-outbreak situations and controls on drinking-water quality should therefore seek to control this also.

Some of the pathogens that are known to be transmitted through contaminated drinking-water lead to severe and sometimes life-threatening disease. Examples include typhoid, cholera, infectious hepatitis caused by hepatitis A virus (HAV) or hepatitis E virus (HEV), and disease

caused by *Shigella* spp and *E. coli* O157. Others are typically associated with less severe outcomes, such as self-limiting diarrhoeal disease (examples rotavirus, *Cryptosporidium*). Whilst the latter are of limited importance to healthy adults, diarrhoeal disease is associated with significant infant morbidity and mortality in some regions and amongst immuno-compromised. More widely it contributes to malnutrition and thereby to developmental problems. The impact of an episode of diarrhoea on a child in a developing country is typically greater than the impact on a child in a more developed country because of this. Other health outcomes may be more significant amongst other age groups.

The effects of exposure to pathogens are not the same for all individuals and as a consequence not for all populations. Repeated exposure to pathogens may be associated with a smaller probability or severity of illness because of the effects of acquired immunity. For some pathogens, immunity is lifelong (e.g. HAV) whereas for others the protective effects may be restricted to a few months to years (e.g. *Campylobacter*). On the other hand, sensitive subgroups (such groups as the young, the elderly, pregnant women and the immunocompromised) in the population may have a greater probability of illness or the illness may be more severe including mortality. Not all pathogens have greater effects in all sensitive subgroups.

The consequence of variable susceptibility to pathogens is that exposure to a particular quality drinking-water may lead to different health effects in different populations. For guideline derivation, it is necessary to define reference populations or, in some cases, to focus on specific sensitive subgroups. National or local authorities may wish to deviate from these and apply specific characteristics of their populations in deriving national standards.

Not all infected individuals will develop symptomatic disease. The ratio of asymptomatic carriers differs for pathogens, and also depends on population characteristics such as prevalence of immunity. Symptomatic and asymptomatic carriers may both contribute to secondary spread of pathogens, thereby boosting the effects of primary exposure to pathogens in water.

Drinking-water and drinking-water quality are not only related to faecal contamination. Some organisms grow in piped water distribution systems (e.g. *Legionella*) whilst other occur in source waters (Guinea worm) and may lead to outbreaks and individual cases. Some biological agents (e.g. toxic cyanobacteria) requires specific management approaches.

Pathogenic agents in water have several properties that distinguish them from chemical pollutants:

- pathogens are discrete and not in solution;
- pathogens are often clumped or adherent to suspended solids in water, so that the likelihood of acquiring an infective dose cannot be predicted from their average concentration in water;
- the likelihood of a successful challenge by a pathogen, resulting in infection, depends upon the invasiveness and virulence of the pathogen, as well as upon the immunity of the individual;
- if infection is established, pathogens multiply in their host, thereby perpetuating or even increasing the chances of infection; and
- unlike many chemical agents, the dose response of pathogens is not cumulative.

The human pathogens that are known to be transmitted by drinking-water consumption are listed in table 4.1, together with their health significance and main properties. Most of the pathogens described are distributed worldwide, however some such as outbreaks of cholera and infection by the Guinea worm *D. medinensis*, are regional. Eradication of *D. medinensis* is a recognized target of the World Health Assembly (WHA resolution WHA44.5, 1991).

Certain serious illnesses result from inhalation of water droplets (aerosols) in which the causative organisms have multiplied because of warm temperatures and the presence of nutrients. These include Legionnaires' disease (*Legionella* spp.) and those caused by the amoebae *Naegleria fowleri* (primary amoebic meningoencephalitis) and *Acanthamoeba* spp. (amoebic meningitis, pulmonary infections).

Schistosomiasis (bilharziasis) is a major parasitic disease of tropical and sub-tropical regions, and is primarily spread by contact with water during bathing or washing. The larval stage (cercariae) released by infected aquatic snails penetrates the skin. If safe drinking-water is readily available, it will be used for washing, and this will have the benefit of reducing the need to use contaminated surface water.

It is conceivable that unsafe drinking-water contaminated with soil or faeces could act as a carrier of other parasitic infections, such as balantidiasis (*Balantidium coli*), and certain helminths (species of *Fasciola*, *Fasciolopsis*, *Echinococcus*, *Spirometra*, *Ascaris*, *Trichuris*, *Toxocara*, *Necator*, *Ancylostoma*, *Strongyloides* and *Taenia solium*). However, in most of these, the normal mode of transmission is ingestion of the eggs in food contaminated with faeces or faecally contaminated soil (in the case of *Taenia solium*, ingestion of the larval cysticercus stage in uncooked pork) rather than ingestion of contaminated drinking-water.

Other pathogens transmitted by drinking-water cause disease opportunistically in subjects with low or impaired immunity, or have as their primary route of infection contact or inhalation, rather than by ingestion. Such pathogens may be naturally present in the environment. They are able to cause disease in people with impaired local or general defence mechanisms, such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy, or those with acquired immunodeficiency syndrome (AIDS). Water used by such patients for drinking or bathing, if it contains large numbers of these organisms, can produce various infections of the skin and the mucous membranes of the eye, ear, nose, and throat. Examples of such agents are *Pseudomonas aeruginosa* and species of *Flavobacterium*, *Acinetobacter*, *Klebsiella*, *Serratia*, *Aeromonas*, and certain "slow-growing" mycobacteria (see also section 6.1.6).

Table 7.1 - Orally transmitted waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Persistence in water supplies ^a	Resistance to chlorine ^b	Relative infective dose ^c	Important animal source
Bacteria					
<i>Campylobacter jejuni</i> , <i>C. coli</i>	High	Moderate	Low	Moderate	Yes
Pathogenic					
<i>Escherichia coli</i> - Pathogenic	High	Moderate	Low	High	Yes
<i>Escherichia coli</i> - Toxigenic					
<i>Salmonella typhi</i>	High	Moderate	Low	High ^d	No
Other <i>salmonellae</i>	High	Long	Low	High	Yes
<i>Shigella</i> spp.	High	Short	Low	Moderate	No
<i>Vibrio cholerae</i>	High	Short	Low	High	No
<i>Yersinia enterocolitica</i>	High	Long	Low	High(?)	Yes
<i>Pseudomonas aeruginosa</i> ^e	Moderate	May multiply	Moderate	High(?)	No
<i>Burkholderia pseudomallei</i>					
<i>Mycobacteria</i>					
<i>Legionella</i>					
Viruses					
Adenoviruses	High	?	Moderate	Low	No
Enteroviruses	High	Long	Moderate	Low	No
Hepatitis A	High	?	Moderate	Low	No
Hepatitis E	High	?	?	Low	No
Norwalk virus	High	?	?	Low	No
Rotavirus	High	?	?	Moderate	No(?)
Small round viruses	Moderate	?	?	Low(?)	No
Protozoa					
<i>Entamoeba histolytica</i>	High	Moderate	High	Low	No
<i>Giardia intestinalis</i>	High	Moderate	High	Low	Yes
<i>Cryptosporidium parvum</i>	High	Long	High	Low	Yes
<i>Acanthamoeba</i>					
<i>Toxoplasma</i>					
<i>Cyclospora</i>					
Helminths					
<i>Dracunculus medinensis</i>	High	Moderate	Moderate	Low	Yes

? not known or uncertain

^a Detection period for infective stage in water at 20°C: short, up to 1 week; moderate, 1 week to 1 month; long, over 1 month.^b When the infective stage is freely suspended in water treated at conventional doses and contact times. Resistance moderate, agent may not be completely destroyed.^c Dose required to cause infection in 50% of health adult volunteers; may be as little as one infective unit for some viruses.^d From experiments with human volunteers^e Main route of infections is by skin contact, but can infect immunosuppressed or cancer patients orally

7.1.2 Persistence and growth in water

While some organisms are able to persist in drinking-water, organisms generally do not grow or proliferate in water, with a few exceptions (e.g. *Legionella*, *V. cholera*, *Naegleria* and *Acanthamoeba* under some conditions). After leaving the body of their host, pathogens and parasites gradually lose viability and the ability to infect. The rate of decay is usually exponential, and a pathogen will become undetectable after a certain period. Pathogens with low persistence must rapidly find a new host and are more likely to be spread by person-to-person contact or faulty personal or food hygiene than by drinking-water. Because faecal contamination

is usually dispersed rapidly in surface waters, the most common waterborne pathogens and parasites are those that have high infectivity or possess high resistance to decay outside the body.

Persistence is affected by several factors, of which temperature is the most important. Decay is usually accelerated by increasing temperature of water and may be mediated by the lethal effects of ultraviolet radiation in sunlight acting near the water surface. Viruses and the resting stages of parasites (cysts, oocysts, ova) are unable to multiply in water. Conversely, relatively high amounts of biodegradable organic carbon, together with warm temperatures and low residual concentrations of chlorine, can permit growth of *Legionella*, *Naegleria fowleri* and *Acanthamoeba* and nuisance organisms during water distribution (see also WHO heterotrophic plate counts and public health).

7.1.3 Infective dose and infectivity

Waterborne transmission of the pathogens listed in Table 7.1 has been confirmed by epidemiological studies and case histories. Part of the demonstration of pathogenicity involves reproducing the disease in suitable hosts. Experimental studies of ‘infective dose’ provide relative information, as shown in Table 7.1, but it is doubtful whether the infective doses obtained are relevant to natural infections. For example, many epidemics of typhoid fever can be explained only by assuming that the infective dose was very low. Individuals vary widely in immunity, whether acquired by contact with a pathogen or influenced by such factors as age, sex, state of health, and living conditions. Pathogens are likely to be widely dispersed and diluted in drinking-water, and a large number of people will be exposed to relatively small numbers. Hence, the minimal infective doses and the attack rates are likely to be lower than in experimental studies. If food is contaminated by water containing pathogens that multiply subsequently, or if a susceptible person becomes infected by water, subsequently infecting others by person-to-person contact, the initial involvement of water may be unsuspected. Hence, improvements in water supply, sanitation, and hygiene are closely linked in control of disease in a community.

The multi-factorial natures of infection and immunity mean that experimental data from infectivity studies and epidemiology cannot be used to predict infectivity or risk precisely. However, probabilistic modelling has been used to predict the effects of water treatment in reducing attack rates from very low doses of viruses and *Giardia* and thereby to confirm water treatment criteria

7.2 Water-borne pathogens

Water may harbor a large variety of pathogens. To protect public health, attention should focus on those pathogens for which there is evidence of disease being caused by ingestion of drinking-water or by inhalation of droplets. Such evidence may originate from outbreak studies or from prospective studies in non-outbreak situations. The tables give general information on aspects of pathogens that are of relevance for water supply management. Source protection may be more difficult if there are multiple sources of contamination and is particularly important for environmentally resistant pathogens. Treatment effectiveness is illustrated by the effects of coagulation/sedimentation/filtration and chlorination, as these are commonly used throughout the

world. Pathogens that are able to regrow in the distribution system pose additional challenges to water treatment and management of distribution systems. The public health burden is related in particular to the severity of the illness(es) associated with the pathogens, and with their infective dose.

7.2.1 Bacterial pathogens of faecal origin

Acinetobacter

1. General description

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

2. Human health effects

Acinetobacter species may act as opportunistic pathogens causing nosocomial urinary tract infections, pneumonia, bacteremia, secondary meningitis and wound infections. These diseases are predisposed by factors such as malignancy, burns, major surgery as well as weakened immune systems such as in the very young or in elderly individuals. The emergence and rapid spread of multidrug-resistant *A. calcoaceticus baumannii* complex causing nosocomial infections are of great concern world-wide.

3. 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 0.1-100 cfu.ml⁻¹. It comprised 1.0% - 5.5% of the heterotrophic plate count (HPC) flora in drinking-water samples and has been isolated from 5% - 92% of distribution water samples.

4. Routes of exposure

Acinetobacter is naturally found in drinking-water. However, studies fail to confirm a clear link between the presence of *Acinetobacter* in drinking-water and clinical disease. *Acinetobacter* spp. are isolated as commensals from the skin and respiratory tract from healthy individuals. The intestine does not seem to be an important reservoir. The human skin is the likely source for most outbreaks of hospital infections.

5. Significance in drinking-water

Acinetobacter spp. form part of the HPC bacteria and have been suggested as being opportunistic pathogens when present in drinking-water. However, there is no credible evidence that consumption of drinking-water containing these organisms could be a source of infection.

6. Key bibliography

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Aeromonas

1. General description

Aeromonas species are members of the family Vibrionaceae. They are Gram-negative, oxidase positive, rod-shaped, non-spore-forming, facultatively anaerobic bacteria and are able to move by means of a single polar flagellum. At present only six species of the mesophilic aeromonads are universally accepted as pathogens in humans, namely: *A. hydrophila*, *A. caviae*, *A. veronii subsp sobria*, *A. jandaei*, *A. veronii subsp veronii* and *A. schubertii*.

2. Human health effects

There is no dispute over the emergence of *Aeromonas* as an important human pathogen. These opportunistic pathogens have been involved in human infections, such as gastroenteritis, peritonitis, endocarditis, meningitis, septicemia, wound, respiratory as well as urinary tract infections. *Aeromonas hydrophila* causes mainly soft tissue infections, whereas *A. veronii subsp sobria* is associated with bacteraemia in immuno-compromised patients. *Aeromonas caviae* has mostly been implicated as diarrhoeal agent in humans.

3. Source and occurrence

Water is believed to be the main reservoir of *Aeromonas* spp. with strains found in most freshwater supplies and in some chlorinated water supplies. Evidence from surveys in the Netherlands indicates that regrowth of aeromonads can occur in drinking-waters derived from anaerobic groundwater sources. The presence of aeromonads in water systems can be enhanced by water temperature above 14°C, a raised organic carbon level and decreased chlorine levels.

4. Routes of exposure

Aeromonads have readily been isolated from sewage, all types of natural waters and even from chlorinated drinking-waters. According to a frequently cited study, a causal relationship exists between the presence of *Aeromonas* spp. in drinking-water and cases of human diarrhoea. This claim was based upon the detection of organisms with similar characteristics being found in drinking-water and in patients with diarrhoeal disease.

5. Significance in drinking-water

Despite frequent isolation of aeromonads (some with enterotoxigenic properties) from drinking-water there is a lack of epidemiological evidence demonstrating an association with illness in the community. A clear link remains to be established between the strains of *Aeromonas* found in drinking-water and those isolated from patients with diarrhoea. However, one strong association was found between untreated drinking-water and the occurrence of diarrhoea with the isolation of *Aeromonas* species. Therefore, it is advisable to adopt strategy that limits regrowth of these organisms in distribution systems, such as treatment to maximise organic carbon removal, shorter residence times in water distribution systems and better control of chlorine residuals.

6. Key bibliography

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Bacillus

1. General description

Bacillus spp. are Gram-positive, strictly aerobic or facultatively anaerobic rods and are important because they can form heat-resistant and desiccation-resistant endospores. *Bacillus* spp. have distinguishable physiologies that show strong correlation with spore morphologies and can be divided into four subgroups: 1) *B. polymyxa*; 2) *B. subtilis*, which includes *B. cereus* and *B. licheniformis*; 3) *B. brevis* and 4) *B. anthracis*.

2. Human health effects

Several *Bacillus* spp. are pathogenic for humans and animals. *Bacillus cereus* causes food poisoning (similar to staphylococcal food-poisoning), the commonest association being with reheated cooked rice and pulses. This organism is known to induce bacteremia in immuno-compromised hosts as well as symptoms such as vomiting and diarrhoea. *Bacillus anthracis* is the causative agent of anthrax in humans and animals.

3. Source and occurrence

Bacillus spp. are found in wide range of habitats including water distribution systems. They form part of the heterotrophic plate count (HPC) bacteria commonly found in drinking-water. *Bacillus* spp. produce resistant endospores which help these bacteria to survive chlorination, exposure to high temperature, low pH and other environmental conditions.

4. Routes of exposure

Bacillus spores are found in many foods, especially rice, pulses vegetables, tap water, bottled water and point-of-use filtration devices, and have been implicated in opportunistic infections as well as gastrointestinal diseases. There is a high association of *B. cereus* food poisoning with consumption of raw milk and meat products. Infection is acquired mainly by ingestion of organisms or toxins.

5. Significance in drinking-water

Research showed that 35% of gastrointestinal illness was attributed to the consumption of drinking-water meeting current water quality guidelines. In this study, a disproportionate percentage of the bacterial isolates belonged to the genus *Bacillus*. It was postulated that this genus could amplify in numbers in the water distribution system and result in low level gastroenteritis.

6. Key bibliography

Allen MJ, Edberg SC, Reasoner DJ (2002) Heterotrophic plate count (HPC) bacteria- what is their significance in drinking-water? Presented at the NSF International / WHO symposium on HPC bacteria in drinking-water, April 22-24, 2002, Geneva, Switzerland. pp.29-45.

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Campylobacter

1. General description

Campylobacter species are microaerophilic (require decreased O₂) and capnophilic (require increased CO₂), curved spiral rods with a single unsheathed polar flagellum. *Campylobacter* are one of the most important causes of acute gastroenteritis worldwide. *Campylobacter jejuni* is the most important human pathogen among the campylobacters and the organism most frequently isolated from patients with acute diarrhoeal disease.

2. Human health effects

The infectious dose of *C. jejuni* could be as few as 1 000 organisms. Most symptomatic infections occur in infancy and early childhood. The incubation period for the diarrhoeal disease is usually 2 to 4 days. Clinical symptoms of *C. jejuni* infection are characterised by crampy abdominal pain, diarrhoea (with or without blood or faecal leukocytes), chills and fever, which is self-limited and resolves in 3 to 7 days. Relapses may occur in 5% to 10% of untreated patients. Patients with severe disease will respond to treatment with oral erythromycin. Other clinical manifestations of *C. jejuni* infections in humans include septic arthritis, meningitis and proctocolitis secondary to *C. jejuni*. Several reports have associated *C. jejuni* infection with Guillain-Barré syndrome, an acute demyelinating disease of the peripheral nerves. *Campylobacter fetus* also causes human diarrhoeal disease, but this species is more likely to progress into a systemic infection resulting in vascular necrosis.

3. Source and occurrence

Campylobacter species occur in a variety of environments, but all appear to be inhabitants of the gastrointestinal tract of wild and domestic animals (household pets, chickens, turkeys and waterfowl). Meat and unpasteurized milk are important sources of campylobacter infections. The occurrence of campylobacters in surface waters has proved to be strongly dependant on rainfall, water temperature and the presence of waterfowl.

4. Routes of exposure

Transmission to humans occurs by the faecal-oral route, originating from animals and animal products by way of contaminated food and water.

5. Significance in drinking-water

In the past decade, numerous waterborne outbreaks of campylobacteriosis have been reported worldwide. *Campylobacter* species were introduced into the drinking-water supplies and storage systems of these communities by contamination with the faeces of wild birds and animals. Researchers described strong associations between gastrointestinal illness and consumption of water in these communities. Outbreaks of Campylobacteriosis were validated with the isolation of *Campylobacter* from both patients and water samples.

6. Key bibliography

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***Escherichia coli* pathogenic strains**

1. General description

Escherichia coli is the species most commonly isolated from human faecal samples and is part of the normal intestinal flora of healthy individuals. Generally, *E. coli* bacteria are not associated with adverse health effects. However, under certain circumstances *E. coli* may cause serious disease. Pathogenic strains of *E. coli* are responsible for urinary tract infections, bacteraemia, meningitis and diarrhoeal disease in humans. A number of virulence factors contribute to the pathogenicity of each group of *E. coli* bacteria. Four groups of diarrhoeagenic *E. coli* are recognised: enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC). Other putative diarrhoeagenic *E. coli* groups, such as enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) exist, but their clinical significance is unclear.

2. Human health effects

Escherichia coli O157:H7 and other EHEC serotypes cause illness that can present as mild non-bloody diarrhoea, haemorrhagic colitis (HC) and/or haemolytic uraemic syndrome (HUS). ETEC produces heat-labile- (LT) or heat stable (ST) *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 and young toddlers. EPEC are rare in developed countries, but occur commonly in developing countries in 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*.

3. Source and occurrence

Animals such as cattle are the main reservoir for pathogenic *E. coli*, but they also occur in other animal species such as chickens, goats and pigs.

4. Routes of exposure

Food- and waterborne transmission is the main route of *E. coli* infection. Pathogenic *E. coli* can be transmitted to humans via animal contact, contaminated food and water or between infected individuals. Person-to-person transmissions occur in communities where there is close contact between individuals such as nursing homes and day-care centres.

5. Significance in drinking-water

Waterborne transmission of pathogenic *E. coli* has been well documented and reported from both recreational waters and contaminated drinking-water. One of the most recent outbreaks of *E. coli* O157:H7 occurred in the water supply system of the farming community of Walkerton, Ontario in Canada in May 2000 where 7 people died and more than 2 300 people were left sickened. The outbreak serves as an example not only of the health risks constituted by pathogenic *E. coli* in water sources and supplies, but also of the legal and financial implications to all parties concerned.

6. Key bibliography

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Helicobacter

1. General description

Helicobacter pylori, originally classified as *Campylobacter pylori*, is a gram-negative microaerophilic spiral-shaped, motile bacterium. *Helicobacter pylori* is one of the most common organisms causing infections such as acute and chronic gastritis which may effect around half of the worlds population. There are at least 14 species of *Helicobacter* associated with different hosts.

2. Human health effects

Although most infections are silent, *H. pylori* is strongly associated with chronic gastritis, peptic and duodenal ulcer disease and gastric cancer. The majority of infections with *H. pylori* occur in children. The infections are more prevalent in developing countries and are associated with overcrowded living conditions.

3. Sources and occurrence

Humans appear to be the primary reservoir of *H. pylori*, but other possible sources include water, domestic cats and houseflies. In adults it seems that *H. pylori* is sensitive to the bile salts making it unlikely to be excreted in faeces. Possible contamination of the environment can be through children with diarrhoea or through vomiting by children as well as adults.

4. Routes of exposure

Although there is no direct evidence to implicate a single transmission route, epidemiological data postulate gastric-oral transmission as the most important route. *Helicobacter pylori* can survive well in mucus or vomit. However, it is difficult to detect in mouth or faecal samples. Other possible routes of infection include faecal-oral and oral-oral.

5. Significance in drinking-water

There has been a growing number of reports suggesting that water may be a route for spreading *H. pylori*. Although *H. pylori* is unlikely to grow in the environment, it can survive well in biofilms. *Helicobacter pylori* were found in the majority of surface and shallow groundwater samples tested in the USA and the survival capacity of these organisms in surface water was found to be up to 20-30 days. *Helicobacter pylori* is readily inactivated by chlorine, however, substandard municipal water supplies may be an important source of *H. pylori* infection.

6. Key bibliography

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Legionella

1. General description

The genus *Legionella*, a member of the family Legionellaceae, has 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 domestic water sources and cause a lung infections resembling pneumonia.

2. Human health effects

Legionella pneumophila is the major pathogen in the genus responsible for Pontiac fever and legionellosis (legionnaires' disease). Legionellosis is a form of pneumonia with an incubation period of 3-6 days. Pontiac fever is a milder disease, with a high attack rate, an onset (5 hours to 3 days) and symptoms similar to those of influenza: fever, headache, nausea, vomiting, aching muscles and coughing. However, all *Legionella* spp. are considered potentially pathogenic for humans.

3. Source and occurrence

Legionella spp. thrive in stagnant water at low temperatures and may survive chlorination by residing in sludge and scale or inside protozoa. After isolation of *Legionella* spp. from the air-conditioning system during the first outbreak in Philadelphia, the bacteria have been isolated from various sources in the environment especially from aquatic environments. The low nutrient content and total bacterial count in cooling tower systems may favour the occurrence of *Legionella* spp. but in waters with high nutrient content and low water temperature, *Legionella* spp. can not compete with other bacteria.

4. Routes of exposure

Infection is the result of inhalation of bacteria containing aerosols that are small enough to penetrate the lungs and be retained by the alveoli. The number of inhaled bacteria depends on the size of the aerosol generated (<5 µm pose the highest risk), the dispersal of the aerosol in the air and the duration of the exposure. Person to person transmission has not been proven.

5. Significance in drinking-water

In the environment, *Legionella* spp. can multiply extracellularly and may parasitise protozoa such as *Acanthamoeba* spp., *Hartmannella* spp. and *Tetrahymena* spp. Legionellosis outbreaks have been attributed to contaminated potable water, cooling towers or components of water distribution systems all over the world.

6. Key bibliography

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***Mycobacterium avium* complex**

1. General description

Mycobacterium avium complex (MAC) consists of the species *M. avium* and *M. intracellulare*. These non-tuberculous, aerobic, rod-shaped and acid fast bacteria grow relatively slow in suitable water environments and on culture media.

2. Human health effects

Mycobacterium avium complex causes human and animal infections of the lungs, lymph nodes, skin, bones, and the gastrointestinal and genitourinary tracts. Manifestations include pulmonary disease, osteomyelitis and septic arthritis in people with no known predisposing factors. These bacteria are a major cause of disseminated opportunistic infections in immuno-compromised patients and are the second most common cause of death in HIV-positive patients.

3. Source and occurrence

Mycobacterium avium complex are heterotrophic bacteria which multiply in suitable water environments, notably biofilms. High numbers may occur in distribution systems after events that dislodge biofilms, such as flushing or flow reversals. They are exceptionally resistant to treatment and disinfection and have been detected in well-operated and -maintained drinking-water supplies with heterotrophic plate counts less than 500 cfu.ml⁻¹ and total chlorine residuals of up to 2.8 mg.litre⁻¹. Reports indicate the presence of these organisms in 54% of ice and 35% of public drinking-water samples.

4. Routes of exposure

Their presence in drinking-water supplies confirms this as one route of exposure. This is supported by the absence of meaningful evidence of person-to-person transmission and the correlation of serotypes of MAC bacteria in human infections and water supplies.

5. Significance in drinking-water

One prospective epidemiological study did indeed implicate water as the source of infection, including two strains of MAC associated with nosocomial acquisition. Reasons for concern include their resistance to water disinfection processes, and the failure of conventional monitoring procedures, including heterotrophic plate counts, to detect these pathogens.

6. Key bibliography

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Pseudomonas

1. General description

Pseudomonas aeruginosa is a member of the family Pseudomonadaceae and is a mono-trichate, aerobic, Gram-negative rod. This organism produces a blue-green fluorescent pigment (pyocyanin).

Pseudomonas aeruginosa, like other fluorescent pseudomonads that occur in natural waters, produce catalase, oxidase and ammonia from arginine and can grow with citrate as the sole source of carbon.

2. Human health effects

Pseudomonas aeruginosa cause mild and trivial diseases in healthy individuals. Several members of this group are involved in human and animal infections. *Pseudomonas aeruginosa* causes secondary infections of burn wounds, nosocomial pneumonia, nosocomial urinary tract infections and surgical wound infections. *Pseudomonas aeruginosa* is responsible for septicaemia, meningitis and infections of drainage sites resulting from trauma. Cystic fibrosis and immuno-compromised patients are prone to colonisation with mucoid morphotypes of *P. aeruginosa*, which have been associated with progressive pulmonary infections in these individuals. Waterborne infections are associated with warm, moist environments (i.e. indoor swimming pools and spas). Diseases result in skin rashes and pustules or outer ear canal infections.

3. Source and occurrence

Pseudomonas aeruginosa is found in faeces, soil, water and sewage. This organism may multiply in aquatic environment. *Pseudomonas aeruginosa* is predominantly an environmental organism and fresh water is an ideal reservoir. It proliferates in water piping systems and in hot water systems and spa pools.

4. Routes of exposure

Pseudomonas aeruginosa is an opportunistic pathogen. Water containing *P. aeruginosa* may contaminate food and pharmaceutical products, causing deterioration of these products, which may lead to secondary transmission by these products.

5. Significance in drinking-water

The presence of *P. aeruginosa* in potable water indicates a serious deterioration in bacteriological quality, is often associated with complaints about taste, odour and turbidity. This is linked to low rates of flow in the distribution system and a rise in water temperature. *Pseudomonas aeruginosa* may cause secondary infections due to contaminated water, but is not responsible for enteric infections following ingestion.

6. Key bibliography

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Salmonella

1. General description

The genus *Salmonella* is a member of the family Enterobacteriaceae. The salmonellae are actively motile, gram-negative bacilli which are capable of causing disease in both animals and humans. Members of the *Salmonella* genus are grouped according to their somatic (O) and flagellar (H) antigens. Salmonellae do not ferment lactose, but most form H₂S or gas from carbohydrate fermentation.

2. Human health effects

Four clinical types of *Salmonella* infection may be distinguished. Firstly, gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), secondly, bacteraemia or septicaemia (high spiking fever with positive blood cultures), thirdly, enteric fever (mild fever and diarrhoea) and lastly, a carrier state in persons with previous infections. Symptoms of gastroenteritis appear 4 to 5 days after ingestion of contaminated food or water. Diarrhoea lasts 3 to 5 days and is accompanied by fever and abdominal pain. *S. typhi*, *S. paratyphi* A and *S. paratyphi* B can cause enteric fever without any presentation of diarrhoea.

3. Source and occurrence

Salmonellae are hardy organisms that can survive in moist environments and in the frozen state for several months. Human infections with salmonellae are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Salmonellae are primarily found in lower animals (e.g. poultry, cows, pigs, sheep and birds) which are the principal source of non-typhoidal salmonellosis in humans. Salmonellosis has also been associated with direct or indirect contact with reptiles.

4. Routes of exposure

S. typhi and *S. paratyphi* A, unlike most other *Salmonella* species, are harbored by humans and not animals. Transmission of these species is from person-to-person via faecally contaminated food and water. *S. paratyphi* B can be transmitted through consumption of contaminated milk and dairy products. Most other salmonellae are primarily animal pathogens.

5. Significance in drinking-water

Most waterborne outbreaks have been associated with *S. typhi* and less frequently with other *Salmonella* serotypes. Waterborne outbreaks have been associated with the consumption of contaminated groundwater, surface water and insufficiently disinfected drinking-water supplies. Communities with deteriorating water systems risk widespread salmonellosis. Effective education to improve compliance during boil water orders is needed.

6. Key bibliography

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Shigella

1. General description

Members of the genus *Shigella* are pathogens that cause serious disease known as bacillary dysentery. *Shigella* species are gram-negative, non-spore-forming, non-motile bacilli which are serologically related to *E. coli*. Shigellae are serotyped according to their somatic O antigens. Both group and type antigens are distinguished, group antigenic determinants being common to a number of related types.

2. Human health effects

The incubation period for shigellosis is 36 to 72 hours. As few as 200 organisms could cause disease if ingested. Abdominal cramps, fever and watery diarrhoea occur early in the disease. Dysentery occurs during the ulceration process, with high concentrations of neutrophils in the stools. Symptoms could be mild or severe, depending on the species causing infection. The most severe illness are caused by *Shigella dysenteriae* type 1, which produces Shiga toxin.

3. Source and occurrence

The higher primates appear to be the only natural hosts for the shigellae, where they remain localised in the intestinal epithelial cells. Of the enteric bacterial pathogens, shigellae seem to be the best adapted to cause human disease. Epidemics of shigellosis occur in crowded communities where human carriers exist. *Shigella* can be spread by flies, fingers, food or faeces. Many cases of shigellosis are associated with day-care centres, prisons and institutions for the mentally retarded. Military field groups and travelers to countries with unsanitary conditions are also likely victims.

4. Routes of exposure

With few exceptions the shigellae are harbored by humans and transferred by the faecal-oral route. Shigellae are transferred from person-to-person by contaminated food, water and fomites.

5. Significance in drinking-water

Though shigella infection is not often spread by waterborne transmission, major outbreaks resulting from such transmission have been described. The presence of *Shigella* spp. in groundwater and other drinking-water supplies indicates recent human faecal pollution. Due to the severity of diseases caused by *Shigella* bacteria, it is of extreme public health importance to reduce the risk of waterborne outbreaks. Some cases of waterborne *E. coli* infections could be wrongfully diagnosed as *Shigella* infection without the necessary laboratory confirmation testing.

6. Key bibliography

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Staphylococcus

1. General description

Staphylococcus aureus is an aerobic, non-motile and non-sporeforming catalase- and coagulase-positive gram-positive coccus which causes a broad range of serious infections throughout the body. Staphylococcal disease is multifactorial and is usually due to the production of several pathogenic virulence factors.

2. Human health effects

Gastrointestinal disease caused by *S. aureus* (enterocolitis and food poisoning) occurs due to the production of staphylococcal enterotoxins which is characterised by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids.

3. Source and occurrence

Staphylococcus aureus is found in the external environment and in the nasopharynx of 20% to 40% of adults at any one time. Such carriers provide the reservoir for the spread of staphylococcal infections, most frequently by way of the hands. Although the organism is frequently part of the normal human microflora, it can cause significant opportunistic infections under the appropriate conditions.

4. Routes of exposure

Water and food products such as ham, poultry and potato and egg salads support the growth of *S. aureus*. Outbreaks of food poisoning occur when contaminated food or water is held at inappropriate temperatures long enough for the bacteria to grow and release enterotoxins. Food handlers or persons who have staphylococcal lesions on the skin, especially of the hands are the most likely to contaminate food and water destined for consumption.

5. Significance in drinking-water

Staphylococcus aureus is considered a useful indicator of recreational water pollution. The distribution of gram-positive bacteria is a crucial issue in groundwater systems. LeChevallier and co-workers isolated *S. aureus* from over 6% of 320 rural drinking-water samples originating from wells. Various researchers demonstrated the presence of gram-positive bacteria, such as *S. aureus* in natural water systems. Although the potential for transmission by drinking-water seems evident, this has not yet been confirmed.

6. Key bibliography

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Tsukamurella

1. 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. Moreover, they are actinomycetes related to *Rhodococcus*, *Nocardia* and *Mycobacteria*. 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 five additional species have been proposed in the 1990s such as *T. wratislaviensis*, *T. inchonensis*, *T. pulmonis*, *T. tyrosinosolvens* and *T. strandjordae*.

2. Human health effects

Tsukamurella spp. are opportunistic pathogens, causing mainly diseases in immuno-compromised individuals. Infections with these microorganisms have been associated with chronic lung diseases, immune suppression (leukemia, tumours, HIV-infection) and postoperative wound infections. *Tsukamurella* were reported in four cases of catheter-related bacteraemia and in individual cases including chronic lung infection, necrotising tenosynovitis with subcutaneous abscesses, cutaneous and bone infections, meningitis and peritonitis.

3. Source and occurrence

Tsukamurella spp. exist primarily as environmental saprophytes found in soil, water and foam (thick stable scum on aeration vessels and sedimentation tanks) of activated sludge. Since, these organisms are related to *Nocardia* and *Mycobacteria*, they form part of the Gram-positive heterotrophic plate count (HPC) bacteria found in drinking-water.

4. Routes of exposure

There is no doubt that *Tsukamurella* spp. can frequently be detected in a variety of waters. However, a clear link between the strains of *Tsukamurella* found in drinking-water and those isolated from patients with clinical diseases remains to be investigated. Some reports indicate that these organisms were isolated concomitantly with other bacteria and that they were considered environmental contaminants. These reports support the hypothesis of pseudoinfection with *Tsukamurella* spp.

5. Significance in drinking-water

Gram-positive bacteria occurring in natural and drinking-waters have been reported to belong to “arthrobacter-like” or “coryneform-like” bacteria and more rarely to *Tsukamurella*. The taxonomy of the genus *Tsukamurella* remains complex and unclear. The distribution of Gram-positive bacteria such as *Tsukamurella* is a critical issue in drinking-water distribution systems because of their high resistance to chlorine disinfection. However, up to date there are no published accounts of waterborne human infections due to *Tsukamurella* spp.

6. Key bibliography

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Yersinia

1. General description

The genus *Yersinia* consists of seven species within the family Enterobacteriaceae. *Y. enterocolitica* are Gram-negative rods and are motile at 25°C, but non-motile in cultures grown at 37°C. Strains involved in human disease belong to serotypes O:3; O:5; O:8; O:9; O:13a,13b; O:20; O:21, 27 with swine serving as a major reservoir for strains of serotypes O:3 and O:9.

2. Human health effects

Certain strains of *Y. enterocolitica* cause yersiniosis, an acute gastroenteritis with diarrhoea. *Yersinia enterocolitica* penetrates in the host cells. Children may have a more severe form of the disease than adults. Symptoms include: abdominal cramps, fever, headache, diarrhoea and light sensitivity. Vomiting, meningitis and eye infections may occur. The organism can be isolated from faeces, cerebrospinal fluid and infected eyes.

3. Source and occurrence

Many domestic and wild animals are considered to be possible reservoirs of *Y. enterocolitica*, because of the high isolation rate of the bacteria from these animals. Most isolates, except those isolated from swine, are not human pathogens. *Yersinia enterocolitica* species different from the serotypes associated with human disease, have been isolated from a variety of environmental samples, especially from water. The bacteria can grow at low temperatures (even 4°C) and survive for long periods in water environments.

4. Routes of exposure

Yersinia enterocolitica can be transmitted by ingestion of contaminated food and water. Direct transmission from person to person and from animals to humans occurs, but the health implications are not yet known.

5. Significance in drinking-water

Pathogenic strains of *Y. enterocolitica* can enter drinking-water via sewage contaminated water sources. Recent studies indicated the presence of human pathogenic serotypes of *Y. enterocolitica* in sewage and polluted surface water. Pathogenic types of *Y. enterocolitica* are not isolated from raw and treated drinking-water samples unless faecal pollution has occurred. Transmission of *Yersinia* can be avoided with standard chlorination methods if the treated water is of low turbidity. Free chlorine of 0.2 - 0.5 mg.l⁻¹ for 10 min at pH 7 completely eradicates the bacteria. Ozone (0.05 mg.l⁻¹) eradicates *Yersinia* after 1 min at any pH. *Yersinia enterocolitica* infections linked to consumption of water contaminated with serotypes O:3 have been reported and the consumption of untreated water was identified as a risk factor for sporadic infection with serotype O:3.

6. Key bibliography

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Vibrio

1. General description

Vibrio cholerae is a small, curved (comma-shaped) gram-negative organism possessing a single polar flagellum and can be grouped according to its O-antigen. *Vibrio cholerae* O1 has two biogroups, classical and El Tor associated with varying severity of disease. *Vibrio cholerae* O139 Bengal produces a capsule as well as cholera toxin and clinical disease caused by this serotype is the same as *V. cholerae* O1. *Vibrio cholerae* non-O1/O139 is responsible for causing self-limiting gastroenteritis, wound infections and bacteraemia. The O1 and O139 serotypes produce capsids which play a role in their ability to cause bacteraemia.

2. Human health effects

Symptoms of cholera result from chromosomally-mediated, heat-labile cholera enterotoxin. 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 (patient may lose as much as 10 to 15 litres of liquid per day). Gastric acidity reduces the infectious dose of *Vibrio cholerae* O1 to about 10^6 bacterial cells, whereas it is about 10^{11} bacteria at normal gastric activity. Death results in as many as 60% of untreated patients as a result of severe dehydration and loss of electrolytes.

3. Source and occurrence

Pathogenic *Vibrio* species are associated with molluscs and crustaceans found in freshwater lakes, rivers and marine environments in temperate and/or tropical regions throughout the world. *V. cholerae* has been isolated from birds and herbivores in areas removed from marine and coastal waters. The prevalence of *Vibrio* spp. decreases as water temperatures fall below 20°C.

4. Routes of exposure

Cholera, the disease caused by *V. cholerae*, is spread as a faecal-oral disease and people acquire the infection by the ingestion of faecally contaminated water and food.

5. Significance in drinking-water

Some serogroups of *V. cholerae* may be part of the normal bacterial populations of water. The presence of the pathogenic *V. cholerae* O1 and O139 in drinking-water supplies is of major public health importance and could have serious health and economic implications in the affected communities. *Vibrio cholerae* is extremely sensitive to disinfection processes and outbreaks of *V. cholerae* can be prevented by boiling practices and chlorine disinfection of drinking-water.

6. Key bibliography

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7.2.2 Viral pathogens of faecal origin

Adenoviruses

1. General description

Members of the family Adenoviridae comprise of two genera Mastadenovirus (mammal) and Aviadenovirus (avian). Adenoviruses are widespread in nature infecting birds, mammals and amphibians. Fifty-one antigenic types of human adenoviruses have been described to date. Human adenoviruses have been classified into six groups (A to F) based on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns, which have been confirmed by DNA homology studies.

2. Human health effects

Human adenoviruses cause infections of the gastro-intestinal tract, eyes, respiratory tract and various sub-clinical infections. Infants and children are most susceptible to adenovirus infections i.e. gastro-enteritis, pharyngitis and pharyngeal-conjunctival fever.

3. Source and occurrence

The presence of adenoviruses in a variety of water environments has been confirmed. The only waterborne outbreaks of adenoviral diseases have been associated with swimming pools causing pharyngitis and conjunctivitis.

4. Routes of exposure

Initial infection may occur via the respiratory route, but faecal-oral transmission accounts for adenovirus infections in young children because of prolonged shedding of viruses in the faeces.

5. Significance in drinking-water

The importance of human adenoviruses and the role water play in the epidemiology of human adenoviruses as well as the potential health risks constituted by these viruses in water environments are widely recognised. This view is reflected by the “Candidate Contaminant List” (CCL) of the Environmental Protection Agency in the USA. The CCL contains a list of contaminants regarded of highest priority for research on the development of detection technology and inclusion in water quality specifications. Adenoviruses are one of only four viruses included in the list. The inclusion of adenoviruses in this list is based on their potential health implications and data which indicate that they occur in large numbers in water environments and that they are exceptionally resistant to purification and disinfection processes. The high resistance of enteric adenoviruses, compared with other enteric viruses, may be associated with the double-stranded nature of the DNA. Damaged DNA such as the formation of pyrimidine dimers may be repaired by the host cell DNA-repair mechanisms.

6. Key bibliography

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Astroviruses

1. General description

Animal and human strains of astroviruses are positive-sense, single-stranded RNA viruses classified in the family Astroviridae. The astroviruses are spherical particles about 28 nm in diameter with no envelope, but in a proportion of the particles a distinct surface star shaped structure can be seen by electron microscopy. Eight different serotypes of human astroviruses have been described. The most commonly identified is human astrovirus serotype 1.

2. Human health effects

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

3. Source and occurrence

Astroviruses are present in sewage and sewage-polluted water, since infected individuals may excrete large numbers of the viruses in faeces. Astroviruses have been detected in water that was shown to have passed all acceptable bacteriological standard tests.

4. Routes of exposure

The faecal-oral route is the predominant mode of transmission for astroviruses. Astrovirus-associated gastroenteritis have been associated with the consumption of contaminated food and water. Person-to-person spread is seen in nurseries, paediatric wards, families, homes for elderly and military camps.

5. Significance in drinking-water

Studies on marine bathing water have suggested that contact with contaminated water may be a risk factor, but there is no definite epidemiological evidence to support this. Astroviruses have the ability to survive in water environments, consequently, their presence in water sources used for domestic or recreational purposes could pose a potential health problem.

6. Key bibliography

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Caliciviruses

1. General description

Human caliciviruses (HuCVs) are positive-sense, single stranded RNA viruses and belong to the family Caliciviridae. Two genera of HuCVs, Norwalk-like viruses (NLVs) and Sapporo-like viruses (SLVs) have been described. SLVs reveal typical calicivirus morphology and are called classical caliciviruses. NLVs do not reveal the typical morphology and are referred to as small round-structured viruses. HuCVs can not be propagated in cell cultures and electron microscopy used to be the sole method of detection. Due to recent advances some NLVs can be detected with ELISA using antibodies raised to baculovirus expressed NLV capsid proteins. Several RT-PCR methods have been described for the detection of HuCVs.

2. Human health effects

HuCVs are a major cause of acute viral gastroenteritis, also known as winter vomiting disease, in all age groups. Symptoms include nausea, vomiting and diarrhea, lasting for one to three days. HuCVs induce a short-lived immunity. Although the symptoms are usually mild, the large numbers of people affected have economical implications in terms of medical costs and productivity.

3. Source and occurrence

HuCVs are enteric viruses and humans are the only known hosts. NLVs were first discovered during an outbreak of gastroenteritis at an school in Norwalk, Ohio in 1968. Food- and waterborne outbreaks have been described in densely populated environments such as institutions, resort camps, schools and on cruise ships. Foodborne gastroenteritis has been associated with uncooked salads, bakery products, minimally processed fruit, cold meats and raw or lightly cooked bivalve shellfish.

4. Routes of exposure

HuCVs are transmitted via the faecal-oral route. Transmission can occur by consumption of faecally contaminated food and water. Faecally contaminated irrigation or washing water, as well as associated minimally processed food can also be a route of infection. Person-to-person transmission can occur by aerosols of vomitus or by contact of contaminated surfaces and objects. HuCVs have an attack rate of 50% and only a few virus particles are required to cause disease.

5. Significance in drinking-water

HuCVs have been implicated as etiological agent in various outbreaks of waterborne gastroenteritis. In 1998 a thousand residents of a Swiss village were infected with NLVs during a waterborne outbreak. NLV sequences were recently detected in different brands of European bottled mineral water. Since HuCVs are shed in the faeces of infected individuals it may enter ground and surface water due to sewage leakage.

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Enteroviruses

1. General description

Enteroviruses comprise a large genus belonging to the family Picornaviridae. This genus consists of 68 different serotypes and is subdivided into poliovirus types 1 to 3, coxsackievirus types A1 to A24, coxsackievirus types B1 to B6, echovirus types 1 to 33 and the numbered enteroviruses EV68 to EV72.

2. Human health effects

Enteroviruses are among the most important viral pathogens of humans and may cause an estimated 30 million infections in the US each year. The spectrum of diseases ranges from a mild febrile illness to myocarditis, meningoencephalitis, poliomyelitis and neonatal multi-organ failure. Recent reports have described the persistence of enteroviruses in chronic diseases such as polymyositis, dilated cardiomyopathy and chronic fatigue syndrome.

3. Source and occurrence

Numerous studies have documented the presence of enteroviruses in raw and treated drinking-water. Enteroviruses are stable in the environment and are resistant to chlorine and UV disinfection treatment. In addition, enteroviruses may be recovered from houseflies, wastewater and sewage.

4. Routes of exposure

Enteroviruses have world-wide distribution. They are transmitted by the faecal-oral route, but person-to-person contact and respiratory spread are possible. Infection can be acquired through contaminated water, food or vomitus. Humans are the only known reservoirs of enteroviral infections. Risk factors for infection include poor sanitation, crowded living conditions and low socioeconomic class. Children younger than 5 years of age are the most susceptible to infection, due in part to a lack of prior immunity and to poor hygienic habits.

5. Significance in drinking-water

Recent epidemiological data associate substantial levels of enteric infections with drinking-water supplies which meet specifications for treatment, disinfection and counts of indicator organisms. A WHO Scientific Group has concluded that the presence of even a few enteric viruses in a large volume of drinking-water poses a threat to public health.

6. Key bibliography

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WHO Scientific Group (1979) Human viruses in water, wastewater and soil. *WHO Technical Report Series* 639. World Health Organization, Geneva.

Hepatitis A virus

1. General description

Hepatitis A virus (HAV) belongs to the family *Picornaviridae* and has been classified into its own genus, of which it is the only member, *Hepatovirus*. Until recently, it was believed that there was only one serotype worldwide, but recent reports classified the human and simian genotypes as two different serotypes. Hepatitis A virus is a non-enveloped, positive-sense single-stranded RNA virus with an icosahedral symmetry and a diameter of 27 nm.

2. Human health effects

The incubation period of hepatitis A (HA) ranges from 10 to 50 days with an average of 28-30 days, depending on the infection dose. Hepatitis A is usually a mild disease characterised by a sudden onset of fever, dark urine, malaise, nausea, anorexia and abdominal discomfort followed by jaundice. Mortality due to HA is <1.5% and mostly associated with old age, liver transplantations and underlying conditions such as immunodeficiencies, malnutrition and liver disease.

3. Source and occurrence

Hepatitis A virus is a major source of morbidity associated with faecally contaminated food and water worldwide. High risk groups include the staff and children in day-care centres, military personnel, patients and staff at care and rehabilitation centres, haemophiliacs and intravenous drug abusers.

4. Routes of exposure

Hepatitis A virus is transmitted via the faecal-oral route with person-to-person spread being the most prominent mode of transmission. Faecally polluted food and water as well as homosexual contact are also important modes of transmission.

5. Significance in drinking-water

Faecally contaminated water has been implicated in many HA outbreaks globally. Hepatitis A virus is readily inactivated by UV irradiation and a free-residual chlorine concentration of 2.0-2.5 mg.l⁻¹ is recommended for the inactivation of HAV.

6. Key bibliography

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Rotaviruses

1. General description

Rotaviruses are non-enveloped, wheel-shaped viruses and belong to the family *Reoviridae*. The viral particles are about 80 nm in diameter, with an inner capsid of icosahedral symmetry, 50-65 nm in size. The double-stranded RNA genome is organised in eleven segments. Rotaviruses can serologically be divided into seven groups (A-G), with group A being the most important human pathogen.

2. Human health aspects

Human rotaviruses (HRVs) are the most common cause of acute viral gastroenteritis in infants and young children in both developing and industrialized countries. The incubation period is estimated to be less than 48 hours and symptoms include fever, vomiting, chronic watery non-bloody diarrhoea and abdominal pain. Rotavirus infections have economic implications in terms of medical costs and the mortality rate. In developing countries 18 million infants are infected and 800 000 die due to rotavirus infection each year. Natural immunity results in a decreased incidence of disease with increasing age and protection increases with each subsequent infection.

3. Source and occurrence

Human rotaviruses are enteric viruses and contaminated water and food are potential sources. Since infected individuals can excrete up to 10^{11} viral particles per gram of faeces HRVs are present in sewage. Infants, immuno-compromised people, travelers to developing countries and the elderly are susceptible to HRV infection. Outbreaks in hospitals, day care centres and nursing homes have been reported.

4. Routes of exposure

Person-to-person transmission can occur via direct contact with infected persons, contaminated surfaces and diapers. Human rotaviruses are transmitted via the faecal-oral route and the infectious dose is ten to a hundred particles. Transmission can occur through droplets and aerosols via the respiratory route.

5. Significance in drinking-water

The first documented waterborne outbreak of gastroenteritis caused by HRV occurred in Colorado, USA in 1981. The presence of rotavirus in drinking-water or the occurrence of epidemics originating from contaminated drinking-water have been proven by several studies. The serotype predominantly detected during a HRV outbreak in Switzerland was also detected in river water where major sewage treatment plants were located.

6. Key bibliography

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Hepatitis E virus

1. General description

Hepatitis E virus (HEV) is a spherical, non-enveloped particle 27-34 nm in diameter, containing a polyadenylated, positive strand RNA genome of approximately 7.5 kb. Although a relationship with the caliciviruses was previously suggested, the new recommendations of the International Committee on the Taxonomy of Viruses now place HEV in a separate family called Hepatitis E-like viruses.

2. Human health effects

The clinical illness of HEV resembles other forms of acute viral hepatitis, especially acute viral hepatitis caused by hepatitis A virus (HAV). The onset of the disease follows an incubation period of 1 to 8 weeks (mean 40 days). Clinical symptoms of acute hepatitis E include abdominal pain, anorexia, dark urine, fever, hepatomegaly, jaundice, malaise, nausea and vomiting, with less common symptoms such as arthralgia, diarrhoea, pruritus and urticarial rash. The clinical disease occurs mainly in the young adult population. In areas where the disease is endemic, HEV is an important cause of death due to liver failure, especially in pregnant woman, with mortality rates of up to 25%.

3. Source and occurrence

The reservoir of HEV has not yet been established, although recent findings have suggested that at least some strains of HEV may be zoonotic. Humans, non-human primates, pigs and rats have been found to be susceptible to infection with HEV.

4. Routes of exposure

Hepatitis E outbreaks are usually associated with faecally contaminated drinking-water supply systems. Person-to-person transmission is minimal, as household contacts of HEV-infected patients do not appear to be at higher risk.

5. Significance in drinking-water

Notable epidemics, associated with contaminated water, have occurred in New Delhi, India (1955-56), Soviet Union (1955-56), Nepal (1973-74), Myanmar (1976-77), Kashmir, India (1978-82), China (1986-88) and Kanpur, India (1991).

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7.2.3 Protozoan pathogens of faecal origin

Acanthamoeba

1. General description

Acanthamoeba are free-living amoebae of the environment. Species include *A. zhysodes*, *A. hatchetti*, *A. astronyxis*, *A. palesinensis*, *A. castellanii*, *A. polyphaga*, and *A. culbertsoni*, but only the latter three are known to be human pathogens. *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 C to 56 C), disinfection and desiccation. There is no flagellate stage in *Acanthamoeba*'s life cycle.

2. Human health effects

A. culbertsoni causes granulomatous amoebic encephalitis (GAE), while *A. castellanii* and *A. polyphaga* are associated with acanthamoebic keratitis and acanthamoebic uveitis. GAE is a multifocal, haemorrhagic and necrotising encephalitis and usually afflicts 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 because of bronchopneumonia. Associated disorders of GAE include skin ulcers, liver disease, pneumonitis, renal failure and pharyngitis. *A. keratitis* is a painful infection of the cornea and can occur in healthy individuals. It is a rare disease that may lead to impaired vision, permanent blindness and loss of the eye.

3. Source and occurrence

Acanthamoeba can be found in soil, fresh and salt water. Infective cysts can be transmitted in dust and aerosols. *Acanthamoeba* keratitis outbreaks due to soft contact lenses being washed with contaminated tap water are well documented.

4. Routes of exposure

The primary infection site of GAE is the upper respiratory tract, lungs and skin. The amoeba spread from these lesions via the blood to the brain and central nervous system (CNS). When spreading to the CNS, the disease is known as GAE. Contact lens-related *Acanthamoeba* keratitis usually arises from contamination of the contact lens storage case, but the primary source is thought to be tap water where trophozoites can exist and replicate while feeding on bacteria, yeasts and other organisms.

5. Significance in drinking-water

Acanthamoeba is associated with any fresh or salty aquatic environment, including chlorinated swimming pools, drinking-water and wells, which could pose a possible health hazard to anyone using these sources for domestic or recreational purposes. *Acanthamoeba* cysts are fairly large and would be efficiently removed by filtration processes, but they are resistant to chlorine. The trophozoites, however, are sensitive to the levels of chlorine associated with adequately treated water supplies.

6. Key bibliography

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Balantidium

1. General description

Balantidium coli is a unicellular protozoan parasite, which can be up to 200 µm in length, making it the largest of the human intestinal parasites. This parasite produces trophozoites, which are oval in shape and are covered with cilia which enable them to move. The cysts of *B. coli* are 60 µm to 70 µm in length and are resistant to environmental conditions such as, pH and temperature. *Balantidium coli* belongs to the largest protozoan group, the ciliates (7 200 species).

2. Human health effects

Infection with *B. coli* is most often asymptomatic. Clinical manifestations may include dysentery similar to amoebiasis, colitis, diarrhea, nausea, vomiting, headache and anorexia. Ulcerative abscesses and haemorrhagic lesions can occur due to the invasion of the submucosa of the large bowel.

3. Source and occurrence

Balantidium coli exists in animal reservoirs such as swine and chimpanzees. This is the only pathogenic ciliate known to infect humans. The cysts, which are shed in the environment by infected animals and humans, are resistant to chlorine at concentrations used for drinking-water treatment.

4. Routes of exposure

Balantidium coli is common in swine and direct contact with pigs is the most important route of transmission of this parasite. Infection with *B. coli* is also associated with the ingestion of faecally contaminated water or food. Poor sanitary conditions can lead to a higher incidence of infection of *B. coli*.

5. Significance in drinking-water

The only reported waterborne outbreak of balantidiasis occurred in 1971 as a result of contamination of drinking-water supplies by pig faeces after the event of a typhoon. The incidence of balantidiasis in humans is low.

6. Key bibliography

Volk WA, Benjamin DC, Kadner RJ and Parsons JT (1991) Protozoa. In Essentials of Medical Microbiology. JB Lippencott Company, Philadelphia. pp. 733-758.

Cryptosporidium

1. General description

Cryptosporidium is an obligate, intracellular, coccidian protozoan parasite, which produces environmentally resistant oocysts (4 µm to 6 µm in diameter). These oocysts are excreted in the faeces of infected individuals. There are 8 known species of *Cryptosporidium*, of which *C. parvum* is responsible for most human infections.

2. Human health effects

Cryptosporidium parvum infects the intestinal epithelia of humans and animals causing severe, life-threatening diarrhea in immuno-compromised individuals or mild, self-limiting disease in immuno-competent individuals. Clinical manifestations may also include nausea, vomiting and fever. The infective dose of this protozoan parasite was found to be 132 oocysts in healthy volunteers and can be as low as 30 oocysts.

3. Source and occurrence

Cryptosporidiosis has been associated with either drinking or recreational water. *Cryptosporidium* oocysts are resistant to chlorine and most other disinfectants, like iodine and bromine, at concentrations used for drinking-water treatment.

4. Routes of exposure

Contamination of drinking-water supplies with human or animal faeces may lead to outbreaks of cryptosporidiosis if the levels of oocysts are high enough. More than half (56%) of the waterborne cryptosporidiosis outbreaks between 1984 and 1999 were associated with drinking-water, while 44% of the outbreaks were associated with the use of recreational water facilities.

5. Significance in drinking-water

The waterborne transmission of *C. parvum* has been well documented. In 1993, an outbreak of cryptosporidiosis, associated with the public water supply in Milwaukee, resulted in diarrheal illness in an estimated 403 000 persons. Rapid and effective monitoring procedures are needed at drinking-water facilities, which would improve the decisions concerning treatment, contamination and public health risks.

6. Key bibliography

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Cyclospora

1. General description

Cyclospora cayetanensis is a single cell, obligate, intracellular, coccidian protozoan parasite, which belongs to the family Eimeriidae. This parasite produces resistant oocysts (8 µm to 10 µm in diameter), which are excreted in the faeces of infected individuals

2. Human health effects

Cyclospora cayetanensis penetrates the small intestine of susceptible humans after ingestion of sporulated oocysts. Infected individuals may experience watery diarrhoea, abdominal cramping, weight loss, anorexia, myalgia and occasionally vomiting and/or fever. Relapsing of cyclosporiasis often occurs.

3. Source and occurrence

Humans seem to be the only true host for this parasite. The *C. cayetanensis* oocysts are exceptionally resistant to disinfection and have been detected in chlorinated drinking-water.

4. Routes of exposure

Although *C. cayetanensis* is transmitted more often via contaminated produce, contaminated drinking-water, irrigation water and irrigated crops are potential sources for cyclosporiasis. It has been suggested that person-to-person transmission is unlikely.

5. Significance in drinking-water

To date, only two waterborne *Cyclospora* outbreaks have been associated with the consumption of contaminated drinking-water. The first reported outbreak in 1990, occurred in the staff of a Chicago hospital. The infections were associated with drinking tap water possibly contaminated with stagnant water from a rooftop storage reservoir. The second waterborne outbreak, associated with drinking-water consisting of a mixture of river and municipal water, occurred in 12 of 14 British soldiers. *Cyclospora* oocysts have been isolated from wastewater, which introduces the potential for contamination of drinking-water sources. Membrane filtration and light microscopy have successfully been used to detect *Cyclospora* oocysts in drinking-water and wastewater. The identity of the *Cyclospora* species is determined by nested PCR and RFLP (restriction fragment length polymorphisms). No monoclonal antibodies are available to detect *Cyclospora* oocysts in environmental samples.

6. Key bibliography

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Entamoeba

1. General description

Entamoeba histolytica is an amebic parasite, which causes amebiasis in humans. This parasite produces trophozoites, 10 µm to 60 µm in diameter, as well as environmentally resistant, non-motile cysts, 10 µm to 20 µm in diameter.

2. Human health effects

The majority of infections with *E. histolytica* are asymptomatic (85%-95%). Intestinal disease results from the penetration of the intestinal tissues by the amoebic trophozoites. Approximately 10% of infected individuals present with dysentery, colitis or amoeboma. Symptoms of amebic dysentery include diarrhea 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, deepen into the classic flask-shaped ulcers of amebic colitis. *Entamoeba histolytica* may invade other parts of the body, such as the lungs, liver and brain.

3. Source and occurrence

It is estimated that more than 10% of the world's population is infected with *E. histolytica*. Humans are the primary reservoir of this parasite.

4. Routes of exposure

Entamoeba histolytica cysts are excreted in the faeces of infected individuals. Infection with *E. histolytica* occurs by the ingestion of these cysts from faecally contaminated food and water. Sexual transmission has been documented.

5. Significance in drinking-water

Drinking-water can play a role in the transmission of *E. histolytica* as a result of contamination of water supplies with domestic sewage. Cysts may remain viable for as long as three months in an aquatic environment. In the United States most epidemics are as a result of faecal contamination due to a faulty water supply. Cysts present in water can be destroyed by hyperchlorination or iodination.

6. Key bibliography

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Giardia

1. General description

There are 3 known species of *Giardia*, of which *Giardia intestinales* is responsible for the most human and mammal infections. These organisms are flagelated protozoa which parasitise the intestines of humans and animals. *Giardia* produces trophozoites (reproductive stage), as well as environmentally resistant cysts. The trophozoites are bilaterally symmetrical and ellipsoidal in shape. The cysts are ovoid in shape and 8 µm to 12 µm in diameter.

2. Human health effects

Followed by ingestion and excystation of infective cysts, the trophozoites attach to intestinal surfaces. Infections in adults may be asymptomatic. Symptoms of giardiasis may include diarrhoea and abdominal cramps, but in severe cases malabsorption deficiencies in the small intestine may be present.

3. Source and occurrence

Giardia can multiply in several host animal species, including humans, which excrete cysts into the environment. *Giardia* cysts survive standard concentrations of chlorine used for water purification systems.

4. Routes of exposure

The *Giardia* cysts can survive in aquatic environments and if viable, can infect susceptible individuals after oral ingestion of faecally contaminated food or water. Drinking-water, recreational water, food and person-to-person contact have been reported to play a role in the transmission of this parasite.

5. Significance in drinking-water

The waterborne transmission of *Giardia* has been well documented and outbreaks have been reported worldwide. *Giardia* has been detected in a large percentage of surface waters studied in the North America. Outbreaks have been associated with unfiltered surface water systems in which chlorination was the only treatment. The destruction of the cysts requires longer contact times and higher concentrations of chlorine. Filtration through cartridge membrane and density gradient centrifugation have been used to recover *Giardia* cysts from water samples. Molecular methods based on PCR have been shown to increase detection sensitivity in comparison to immunofluorescence microscopy. An immunocapture polymerase chain reaction (IC-PCR) assay has been developed for the simultaneous detection of *Cryptosporidium* and *Giardia* in surface water.

6. Key bibliography

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Isospora

1. General description

Isospora are coccidian, single celled obligate parasites related to *Cryptosporidium* and *Cyclospora*. Although there is many species, only *I. belli* is known to infect humans. *Isospora hominis* is commonly found in homosexual men. *Isospora canis* and *I. felis* infect dogs and cats respectively and is usually harmless to healthy animals, but can cause serious illness in debilitated or immune compromised animals, puppies and kittens. Reptiles and birds can also contract *Isospora* infections. The life cycle is completed within a single host, and includes (a) mature oocysts, the survival stage, (b) motile sporozoites which infect the small bowel after ingestion of oocysts, and (c) motile merozoites which spread infection further through the gut tissues.

2. Human health effects

Infection with *I. belli* may cause colicky pain, loose stools or watery diarrhoea, malabsorption, weight loss and fever, but these symptoms are usually mild and self-limiting in patients with normal immune status. Recurrent and even chronic symptoms have been documented though. Usually, infection is associated with immunocompromised patients and symptoms are more severe and likely to be recurrent or chronic.

3. Source and occurrence

Infection follows ingestion of mature sporocyst-containing oocysts via the faecal-oral route. Poor hygiene can result in faecally contaminated food and water. *Isospora* infections are associated with the tropical and sub-tropical areas globally.

4. Routes of exposure

The ingestion of mature oocysts via faecally contaminated food and water could result in *I. belli* infections. The sporocysts excyst in the small intestine and the released sporozoites then invade the epithelial cells and cause infection. The cysts are also likely to be transmitted directly from person-to-person through poor hygiene, hand-to-mouth transmission and homosexual contact.

5. Significance in drinking-water

There is no conclusive evidence that water is a mode of transmission, but faecally contaminated food and water has been suspected. Coagulation-sedimentation and filtration processes are probably important in drinking-water treatment because they may effectively remove the large oocysts which are relatively resistant to disinfection processes.

6. Key bibliography

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Microsporidium

1. General description

The members of the class Microsporea in the phylum Microspora are commonly called *Microsporidia*. *Microsporidia* are among the smallest eukaryotes and are obligate intercellular protozoan parasites. These organisms produce unicellular spores, 1 to 4.5 µm in diameter, containing a uninucleate or binucleate sporoplasm with a polar filament and polar cap. *Microsporidia* cause infections in both vertebrates and invertebrates. Human *Microsporidia* pathogens appear to have animal reservoirs such as pigs. These protozoa are too small to be observed by a light microscope but may be visualized by electron microscopy. *Microsporidia* can also be grown in cell cultures but require a minimum of 28 days of incubation.

2. Human health effects

Microsporidia have only recently been recognized to cause disease in humans, increasing its public health significance. Microsporidiosis is more common in immuno-compromised individuals, usually in AIDS patients, reflecting the opportunistic nature of these organisms. Only a few cases of infection occur among immuno-competent individuals where the organisms can cause ocular or encephalitic infections, but rarely diarrhoea. *Microsporidia* enter individuals via ingestion or inhalation. Spores are excreted in faeces and urine and possibly through mucous secretions. *Microsporidia* that most frequently cause infections in human are *Enterocytozoon bieneusi*. Others include *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, *Encephalitozoon intestinalis* and *Nosema corneum*.

3. Source and occurrence

Lack of practical methods for the detection of these organisms has delayed surveys of their waterborne. The levels of *Microsporidia* found in raw sewage are comparable to that of *Cryptosporidium* and *Giardia*. *Microsporidia* spores have been shown to survive prolonged period of up to 4 months of time in water and have been detected in surface water.

4. Routes of exposure

Microsporidia enter individuals via ingestion or inhalation. Resistant spores are formed within the host and are excreted in faeces and urine. These pathogens may therefore, find their way into drinking-water sources causing waterborne outbreaks.

5. Significance in drinking-water

During the summer of 1995, *Microsporidia* were identified as the causative agent in a waterborne outbreak in Lyon, France. These organisms have been listed in the top 10 on the United States Environmental Protection Agency's (USEPA) pathogens priority list, as well as, important emerging pathogens by the Centers for Disease Control and Prevention (CDC) and the National Institute of Health. During this incident 200 persons were infected, but no source or faecal contamination was found. *Microsporidia* have been found in treated wastewater, surface water and groundwater. The spores of *Microsporidia* have been shown to be resistant to chlorination, which is the primary water treatment method in many drinking-water processes.

6. Key bibliography

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Toxoplasma

1. General description

Toxoplasma is an obligate intracellular parasite. The life cycle of *Toxoplasma* consists of two phases: (a) the intestinal/enteroepithelial phase in cats where resistant oocysts are produced, and (b) the extraintestinal phase in infected mammals that results in tachyzoite (trophozoite), bradyzoite and zoitocyst production. *Toxoplasma gondii* is the etiological agent of toxoplasmosis.

2. Human health effects

Most human *T. gondii* infections are asymptomatic, but flu-like symptoms, lymphadenopathy and hepatosplenomegaly can present in a small number of cases 5-23 days post exposure. Dormant tissue cysts, formed in organ tissue after primary infection, can be reactivated when the infected individual becomes immunosuppressed to produce disseminate disease involving the central nervous system and lungs leading to severe neurological disorders or pneumonia. When both of these infection sites are involved, the disease can be fatal in an immune compromised person. Congenital toxoplasmosis is mostly asymptomatic, but can produce chorioretinitis, cerebral calcifications, hydrocephalus, severe thrombocytopenia and convulsions. These illnesses lead to mental retardation and visual handicaps. Depending on the age of the fetus, as well as the virulence of the parasite, foetal infections can lead to spontaneous abortion, a still born or retarded child.

3. Source and occurrence

Toxoplasmosis is the result of ingestion of oocysts, from cat faeces, unwashed foods, or hands after handling pet cats, contaminated soil etc., or bradyzoites in raw or undercooked meat. Toxoplasmosis can be transmitted transplacentally via tachyzoites and causes severe infection in the foetal tissue.

4. Routes of exposure

Oocysts are excreted in cat faeces and cause toxoplasmosis when ingested by an intermediate host such as humans or rodents. Oocysts sporulate and the infective sporozoite stage develops. Tachyzoites travel to various organs via the blood stream and will encyst when threatened by an immune response.

5. Significance in drinking-water

There is no information on the occurrence of *Toxoplasma* in water sources, but the highly resistant oocysts could be found in faecally contaminated water. Few outbreaks of toxoplasmosis have been associated with drinking-water. One outbreak occurred in Canada in 1995 and was linked to a drinking-water reservoir and/or its feeder streams being contaminated by faeces from wild and/or domestic cats. One seroprevalence study undertaken in Rio de Janeiro State, Brazil, 1997-1999, identified drinking unfiltered drinking-water as a risk factor for *T. gondii* seropositivity in an endemic area. Filtration and coagulation-sedimentation processes during drinking-water treatment are important to remove the chlorine-resistant oocysts.

6. Key bibliography

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<http://www.extension.iastate.edu/foodsafety/toxo.htm>

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7.2.4 Indicator organisms

Heterotrophic plate count bacteria

1. General description

Heterotrophic Plate Count (HPC) bacteria require simple organic carbon rather than carbon dioxide for growth. HPCs represent those microbes isolated by a particular method, whose variables include media composition, time of incubation, temperature of incubation and means of medium inoculation. Other terms that have been used to describe this group of bacteria in water include “standard plate count” or “total plate count”.

2. Indicator value

The heterotrophic plate count method determines the general microbiological quality of treated drinking-water. The recommended test method includes pour plates using a rich growth medium such as Yeast Extract Agar and incubation for 48 h at 37°C. Drinking-water quality specifications used worldwide allow HPCs of 100 cfu.ml⁻¹ and in some cases as high as 500 cfu.ml⁻¹.

3. Source and occurrence

Heterotrophic bacteria are found in tap, bottled as well as other sources of potable water. In drinking-water distribution systems they show bacterial regrowth, aftergrowth and biofilm formation.

4. Application in practice

Regular heterotrophic plate count analyses determine water quality after storage. This test is simple and inexpensive, yields results in a relatively short time and proved one of the most reliable and sensitive indicators of disinfection failure. One reason is that the heterotrophic plate count method detects bacteria and bacterial spores, organisms of faecal origin, as well as natural inhabitants of water environments, which may result in aesthetic problems or opportunistic infections.

5. Significance in drinking-water

Heterotrophic plate counts are sensitive and practical indicators of disinfection efficiency as well as aftergrowth and biofilm formation. Some of the heterotrophic plate count bacteria identified as opportunistic pathogens include: *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Klebsiella*, *Legionella*, *Moraxella*, *Mycobacterium*, *Serratia*, *Pseudomonas* and *Xanthomonas*. These microorganisms can be found in source waters and in treated drinking-water and can be enumerated on HPC media. Opportunistic pathogens are naturally present in the environment and the consumption or exposure to water containing large numbers of HPC organisms can lead to diseases such as gastroenteritis, skin and mucous membrane infections particularly in persons whose immune systems are compromised by AIDS, organ transplantation or chemotherapy.

6. Key bibliography

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WHO (2001) *Guidelines for Drinking-Water Quality* (2nd edn.), vol 1. *Microbiological Methods*. World Health Organization, Geneva.

Coliform bacteria including thermotolerant coliforms and *E.coli*

1. General description

Total coliform bacteria include a wide range of aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli that ferment lactose and produce gas within 24 h at 35°C. Many of these bacteria are of faecal origin, but some are heterotrophic and able to multiply in various water environments. Members of this group which are able to ferment lactose and produce gas at 45.5°C, and are indole-positive, are known as faecal (thermotolerant) coliform bacteria consisting predominantly of *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter* species. Faecal coliforms are much more closely associated with faecal pollution than total coliforms. However, some of them, notably *Klebsiella*, may multiply in suitable water environments. The only coliform organism that rarely if ever multiplies in water environments is *Escherichia coli*. All coliform bacteria are excreted by humans and other warm-blooded animals.

2. Indicator value

Total coliforms are widely used as indicators of the general sanitary quality of treated drinking-water. The tests have the benefit that isolates can be typed to determine the presence of *E. coli*. Faecal coliforms give a much closer indication of faecal pollution, and are generally used to assess faecal pollution of waste water discharges and raw water sources intended for the production of drinking-water. *Escherichia coli* is commonly used as specific indicator of faecal pollution. The introduction of coliform bacteria as indicators of faecal pollution has revolutionised water quality assessment. These indicators are likely to play a fundamental role in water quality control for a long time to come. However, despite many benefits, coliforms do have shortcomings. Among these are that under circumstances they fail to indicate the presence of resistant pathogens such as viruses and protozoan parasites in treated drinking-water supplies.

3. Source and occurrence

Coliform bacteria occur in high numbers in sewage and polluted water sources. They generally outnumber waterborne pathogens by a substantial margin. The ratio of coliform bacteria to pathogens such as viruses and protozoan parasites decreases as conditions for survival deteriorate.

4. Application in practice

Coliform bacteria are detectable by relatively simple and inexpensive tests based on cultivation using selective growth media. Most techniques for total and faecal coliforms yield results within 24 h. Confirmation of the presence of *E. coli* may take another 24 h. Tests for coliform bacteria tend to be the most common tool used for water quality assessment in drinking-water laboratories all over the world.

5. Significance in drinking-water

The presence of total coliforms in drinking-water indicates potential faecal pollution and shortcomings in treatment and disinfection procedures. Faecal coliforms yield much stronger evidence of faecal pollution and the presence of these bacteria in drinking-water is generally considered not acceptable. The presence of *E. coli* confirms faecal pollution of drinking-water which is not acceptable.

6. Key Bibliography

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Enterococci

1. General description

Enterococci are Gram-positive bacteria which are tolerant to sodium chloride and alkaline pH conditions. These bacteria are facultatively anaerobic and occur singly, in pairs or as short chains. They appear in human and animal faeces, but in lower numbers than the total or faecal coliforms and are more resistant than coliform bacteria. The enterococci were once part of the faecal streptococci group, but are now classified separately. There are 19 species included in this group with the predominant species of the intestinal enterococci being *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae*.

2. Indicator value

Enterococci seem to have gained the most acceptance as indicator of faecal contamination of source water. These bacteria have a number of advantages as indicators over the total coliforms and even *E. coli*, including that they do not grow in the environment and they can survive longer under these conditions. Although they can be detected in an order of magnitude less numerous than faecal coliforms and *E. coli* in human faeces, their numbers are still abundant enough to be detected.

3. Source and occurrence

Members of enterococci are present in faeces of humans and animals. Enterococci have also been detected in soil. Although their presence in water is not necessarily indicative of faecal pollution, recent research indicated that the majority of enterococci (84%) isolated from various polluted water sources were true faecal species.

4. Application in practice

Enterococci are detectable by simple, inexpensive cultural methods that require basic routine bacteriology laboratory facilities. The low level or infrequent occurrence of enterococci in source water limits their use as indicators of drinking-water treatment processes..

5. Significance in drinking-water

The main value of enterococci in assessing water quality is as an additional indicator of treatment efficiency or as a secondary indicator for re-sampling after the detection of coliforms or *E. coli* in distribution systems. Enterococci are highly resistant to drying and may be valuable for routine monitoring after new water mains are laid, or after repairs to distribution systems. These bacteria provide valuable supplementary data on the bacteriological quality of natural water systems, because they rarely multiply in water.

6. Key bibliography

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Clostridium perfringens

1. General description

Clostridia are Gram-positive, rod-shaped, anaerobic spore-forming bacteria. *Clostridium* spp. produce surviving structures or spores that are resistant to environmental conditions such as temperature, pH, UV as well as water treatment processes and disinfection. The most common member of the group, *Clostridium perfringens*, is normally present in human faeces, though in much smaller numbers than *E. coli*. *Clostridium perfringens* are sulphite-reducing clostridia.

2. Indicator value

There is evidence to show that *C. perfringens* may be a suitable indicator for viruses and parasitic protozoa when sewage is the suspected cause of contamination. However they are not recommended for the routine monitoring of distribution systems as they tend to survive and accumulate and may be detected long after pollution had occurred.

3. Source and occurrence

Spores of *C. perfringens* are largely of faecal origin and are always present in sewage, while vegetative cells appear not to reproduce in aquatic sediments, unlike many traditional indicator bacteria. *Clostridium perfringens* is present in higher numbers in the faeces of animals such as dogs than in humans and is generally lower or absent in other warm-blooded animals.

4. Application in practice

Detection methods for *C. perfringens* are easy to perform, even though a pasteurisation step is required for the enumeration of spores and strict anaerobic conditions are needed.

5. Significance in drinking-water

Clostridium perfringens is a reliable indicator for the survival of viruses and protozoan cysts or oocysts in treated drinking-water. Their presence in treated water suggests deficiencies in removal by treatment, or failure of disinfection processes, or recontamination of the treated water.

6. Key bibliography

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WHO (2001) *Guidelines for drinking-water quality*. 2nd Edition Vol 1: *Microbiological Methods*. World Health Organization, Geneva.

Coliphages

1. General description

Bacteriophages (phages) are viruses which infect bacteria. Coliphages infect *Escherichia coli* and closely related species. Phages share many properties with human viruses, notably composition, morphology, structure and mode of replication. For instance, F-RNA coliphages and picornaviruses are morphologically hardly distinguishable, and both consist of a single-strand RNA molecule in a spherical protein capsid.

2. Indicator value

Due to the many similarities, phages resemble the behaviour of enteric viruses in water environments much closer than commonly used indicators such as faecal bacteria. Like viruses, phages used as indicators also fail to replicate in water environments, with rare exceptions such as somatic coliphages which may be replicated by host bacteria in suitable water environments.

3. Source and occurrence

Since the habitat of host bacteria is typically the gastrointestinal tract of humans and other warm-blooded animals, coliphages are excreted in faeces. Somatic coliphages infect any host bacteria and are produced in large numbers. F-RNA coliphages are produced only by host bacteria which carry fertility fimbriae. Wastewater does, therefore, typically contain somatic coliphages in large numbers and F-RNA coliphages in much smaller numbers.

4. Application in practice

Coliphages are generally used as indicators for the behaviour and survival of enteric viruses in water treatment and disinfection processes. The phages are detectable by relatively simple and inexpensive techniques which yield results within 24 hours. F-RNA coliphages have the additional benefit of occurring in four genotypes which are selectively excreted by either humans or animals. Typing of F-RNA coliphages is, therefore, a valuable tool to distinguish between faecal pollution of human and animal origin.

5. Significance in drinking-water

Since coliphages are typically excreted in faeces, generally outnumber enteric viruses in raw water sources, and are at least as resistant to most water treatment and disinfection processes, they are valuable tools for assessment of the potential incidence of enteric viruses in drinking-water supplies. In addition, they are detectable by practical and sensitive techniques, and typing of F-RNA coliphages even casts valuable light on the human or animal origin of faecal pollution which has benefits for the assessment of virological health risks.

6. Key bibliography

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***Bacteroides fragilis* phages**

1. General description

Two groups of *B. fragilis* phages are used as indicators in water quality assessment. One is a wide spectrum of phages which infect any *B. fragilis* bacteria, and the other is phages which specifically infect *B. fragilis* strain HSP40. *Bacteroides fragilis* bacteria are obligate anaerobic members of the intestinal flora of humans and other warm-blooded animals. They are, therefore, valuable indicators of faecal pollution, particularly since they fail to multiply in water environments. Consequently phages which infect these bacteria are likewise valuable indicators of faecal pollution. *Bacteroides fragilis* HSP40 has the additional feature of being highly specific for the human gastrointestinal tract, which implies that the phages which infect this strain are excreted only by humans. The *B. fragilis* HSP40 phages belong to the family *Siphoviridae* with flexible non-contractile tails, double-strand DNA, and capsids with a diameter of up to 60 nm. *Bacteroides fragilis* strain RYC2056 is generally used to detect the wider spectrum of *B. fragilis* phages.

2. Indicator value

In addition to being valuable indicators of faecal pollution and useful tools to distinguish between faecal pollution of human and animal origin, *B. fragilis* HSP40 phages proved exceptionally resistant to unfavourable environmental conditions. Evidence has been presented that they survive longer in a variety of water environments than typical human enteric viruses. This implies that the absence of *B. fragilis* HSP40 phages in water sources is a meaningful indication of the absence of human enteric viruses.

3. Source and occurrence

Bacteroides fragilis HSP40 phages are excreted by about 10-20% of humans in certain parts of the world. This implies that they are not excreted by as many people as coliphages, and consequently their numbers in sewage are substantially lower than those of somatic and even F-RNA coliphages. Unfortunately it would appear that *B. fragilis* HSP40 phages occur in extremely low numbers or not at all in some parts of the world. Phages infecting any strain of *B. fragilis* bacteria are excreted in larger numbers and seem to occur more universally.

4. Application in Practice

The detection of *B. fragilis* phages is not as simple and economic as the detection of coliphages because plaque assays have to be carried out under strict anaerobic conditions using complex growth media and plaques are visible only after two days. For this reason, and the relatively low number of the phages in many water environments, the phages are not used as widely as coliphages in water quality assessment. However, the merits of these phages for particular purposes, including assessment of the efficiency of treatment and disinfection processes, is widely recognised.

5. 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.

6. Key Bibliography

Grabow WOK, Neubrech TE, Holtzhausen CS, and Jofre J (1995) *Bacteroides fragilis* and *Escherichia coli* bacteriophages: Excretion by human and animals. *Water Science and Technology* 31: 223-230.
 Tartera C, Lucena F, Jofre J (1989) Human Origin of *Bacteroides fragilis* bacteriophages Present in the Environment. *Applied Environmental Microbiology* 10: 2696-2701.

Enteric Viruses

1. General description

The viruses referred to here typically infect the human gastrointestinal tract and are typically transmitted by the faecal-oral route. Well-known members of this group include the enteroviruses (polio, coxsackie A and B, echo and entero), and astro, enteric adeno, reo, calici and hepatitis A and E viruses. These viruses are relatively host specific, and related viruses which infect animals are not included in the term "enteric viruses" used for water quality assessment. The enteric viruses cover a wide spectrum of viruses, members of which are a major cause of morbidity and mortality world-wide. Clinical manifestations include gastroenteritis, respiratory disease, meningitis, paralysis, hepatitis and conjunctivitis. Members of the group of enteric viruses differ substantially with regard to structure, composition, nucleic acid and morphology. Consequently there are far-reaching differences in the numbers and frequency of excretion and in their survival in the environment and resistance to water treatment processes.

2. Indicator value

Commonly used indicators such as faecal bacteria have meaningful shortcomings for indicating the presence or behaviour of enteric viruses in water environments. Among the reasons are that faecal bacteria are excreted at a relatively consistent rate by all people at all times, while enteric viruses are excreted only by infected individuals usually for a restricted period of time. In addition, the survival of faecal bacteria differs substantially from that of enteric viruses. Data on enteric viruses themselves would, therefore, be more valuable for assessment of the incidence and behaviour of enteric viruses in water than data on faecal bacteria. Even though it is not yet possible to analyse water for the entire spectrum of enteric viruses, information on any member of the group is considered more relevant for other members of the group than data on faecal bacteria.

3. Source and occurrence

Enteric viruses are excreted by individuals world-wide at a frequency and in numbers which result in these viruses being universally present in substantial numbers in waste waters. However, the incidence of individual members may vary to large extent because it is subject to the epidemiology of the viruses, with much higher numbers during outbreaks.

4. Application in practice

Practical methods are not yet available to monitor water supplies for a meaningful spectrum of enteric viruses. Water quality assessment is therefore generally limited to those viruses which are more readily detectable. These include members of the group enteroviruses, the group of adenoviruses and reoviruses. It so happens that these viruses have distinct benefits as indicators for other enteric viruses because they occur in polluted water environments in relatively large numbers and they are relatively resistant to unfavourable conditions including water treatment and disinfection processes.

5. Significance in drinking-water

The presence of any enteric viruses in drinking-water implies the potential presence of other enteric viruses and represents sound evidence of shortcomings in water treatment and disinfection processes.

6. Key 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.

7.2.5 Bacterial pathogens from regrowth

Microorganisms will normally grow in water, and on surfaces in contact with water as biofilms. Growth following drinking-water treatment is normally referred to as 'regrowth'. Growth is typically reflected in higher Heterotrophic plate counts (HPC) values measured in water samples. HPC are broadly defined as microorganisms that require organic carbon for growth. They include bacteria, yeasts and moulds measurements are used:

- to indicate the effectiveness of water treatment processes, thus as an indirect indication of pathogen removal;
- as a measure of numbers of regrowth organisms that may or may not have sanitary significance; and
- as a measure of possible interference with coliform measurements in lactose-based culture methods. This application is of declining value as lactose-based culture media are being replaced by alternative methods that are lactose-free.

Elevated HPC levels occur especially in stagnant parts of piped distribution systems, in domestic plumbing, in bottled water and in plumbed-in devices such as softeners, carbon filters, and vending machines. The principal determinants of regrowth are temperature, availability of nutrients, and lack of residual disinfectant. Nutrients may derive from the water body and/or materials in contact with water.[cross-reference HPC publication]

7.2.6 Other microorganisms

Dracunculus medinensis

1. General description

Dracunculus medinensis, or guinea worm is a tissue roundworm or nematode found in Africa and large areas of Asia (India, Pakistan and Yemen). These parasites have been observed in the West Indian Islands and Brazil but they are no longer believed to cause human disease in the Western Hemisphere. These parasites are grouped with the filariae.

2. Human health effects

Diagnosis depends on the appearance of the female worm on the surface of the skin. Dracunculiasis is a debilitating disease that causes little direct mortality but provokes a broad spectrum of clinical symptoms. Ulceration of the skin with a burning sensation develops at the site of the skin penetration. Secondary bacterial infections of the ulcerating lesion can disable the host and complicate recovery. Systemic symptoms of vomiting, diarrhoea, hives and shortness of breath may result from an allergic reaction rather than from direct toxicity of the parasite. Symptoms last for several weeks while the female discharges larvae. Local healing occurs when the female parasite dies after being expelled from the body.

3. Source and occurrence

Unlike other nematode infections of blood and tissue, *D. medinensis* infections are acquired by drinking-water that contains the intermediate host for this parasite, infected copepods (minute freshwater and marine crustaceans). Although much progress have been made with the eradication of dracunculiasis in the world, there are still a number of countries in Africa and Asia where this disease is rampant. More than three quarters of the remaining cases of Dracunculiasis in the world are found in Sudan.

4. Routes of exposure

After ingestion of these copepods the larvae penetrate the intestinal wall and migrate to the deep subcutaneous tissues where they develop into adult worms. The female worm moves to a position just beneath the skin where an ulcer develops. Numerous larvae (microfilariae) are discharged when the infected person comes in contact with water. This initiates a new cycle for human infection.

5. Significance in drinking-water

Drinking-water is the only source of infection with *D. medinensis*. This is the only human parasite that can be eradicated by the provision of safe drinking-water. Guinea worm infections can be controlled by the provision of piped water or covered wells for drinking-water. Measures to prevent contamination of water sources include chemical treatment as well as filtration of drinking-water through a fine mesh cloth.

6. Key bibliography

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- Koneman EW, Allen SD, Janda WM, Schreckenberger PC and Winn WC (1997) Color Atlas and Textbook of Diagnostic Microbiology. Fifth Edition. Lippincott Williams & Wilkins. Lippincott-Raven Publishers, Philadelphia, PA, USA.
- Tison DL (1999) *Vibrio*. p. 497-506. In: PR Murray, EJ Baron, MA Tenover and RH Tenover (ed.). *Manual of Clinical Microbiology*. 7th edition. American Society for Microbiology. ASM Press, Washington DC.
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Cyanobacteria

[Text Pending]

7.3 Principles for controlling microbial hazards in drinking-water

The provision of a safe supply of drinking-water depends upon use of either protected high-quality water sources, or properly selected and operated series of interventions (barriers) capable of reducing pathogens and other contaminants to levels not injurious to health and prevention of re-contamination in distribution. Multiple barriers should provide protection against transmission of infection and include catchment management practices, use of appropriate treatment technologies including disinfection and protection of water quality in distribution. The key actions required to achieve this goal are:

- adopting water quality targets within the context of public health, and available resources (see Chapter 3);
- planning the design, implementation and upgrading of water supply systems to meet adopted water quality targets (see Chapter 4);
- implementing management systems to ensure that water quality targets are continuously met, including;
 - assessment and monitoring to demonstrate that both design and implementation are adequate to meet water quality targets (see Chapter 4);
 - establishing monitoring and testing plans for routine and emergency management (see Chapter 4);
 - carry out active surveillance to ensure that the public health objectives are achieved. (see Chapter 5)

In common with other sectors where microbial hazards are of significant concern for human health (such as food safety), emphasis is placed upon ensuring the adequacy of safeguards that are put in place to protect public health, and the direct verification of the adequate functioning of these safeguards. The tools used include planning, direct inspection and water quality analysis (for both microbial and non-microbial parameters).

The processes preceding terminal disinfection should be capable of producing safe water so that terminal disinfection becomes a final safeguard rather than the primary method of preventing microbial contamination in drinking-water. Disinfection is also most efficient when the water has already been treated to remove turbidity and when substances exerting a disinfectant demand, or capable of protecting pathogens from disinfection, have been removed as far as possible.

It is neither possible nor necessary to consider all pathogens in order to design and operate safe drinking-water supplies. Waterborne pathogens vary in size, in their ability to survive in the environment, through different water treatment processes and in the distribution system; they also vary in their infectivity and in the severity of the diseases they cause. In identifying specific pathogens that by their characteristics can represent a group of similar pathogens, it is possible to limit the necessary information and considerations. Such pathogens can provide a reference for developing design and implementation guidelines to meet water quality goals for an entire group of pathogens. In order to protect public health such pathogens should be those within the group that are most difficult to remove or control and that have the largest associated health burden, both on a population and on individual basis. Ideally, there should also be ample high quality

data on each aspect of relevance to assessing and managing risks. In the absence of such data (which is often the case), data on other pathogens or even non-pathogenic organisms may be useful as proxies.

7.4 Health-based Target setting

The general approaches to health-based target setting are described in section 2.1.1.

7.4.1 Epidemiological approach

Health based targets provide the basis for the application of the Guidelines for Drinking-Water Quality to community and household supplies. In many parts of the world faecally contaminated drinking water is a major contributor to the community burden of enteric disease because available water sources are faecally contaminated and untreated, inadequately treated or become contaminated during collection, handling, storage and use. Under such conditions, effective drinking water treatment at the community or household has the potential to appreciably reduce the overall risks of enteric disease transmission. Therefore, as a first step in the application of health based targets to achieve safe drinking water supply, a community can set as their health target a quantifiable reduction in the overall level of diarrhoeal disease. Such a reduction could be reached by the implementation of water treatment at the household or community level (by disinfection and related processes) capable of achieving a significant reduction of pathogen loads in the water. Water quality can be quantified on the basis of standard tests of microbial quality (e.g., presence or absence of *E. coli* in 100-ml samples) and/or easily measured physical or chemical surrogate parameters of microbial water quality (e.g., chlorine residual or turbidity).

The effectiveness of the treatment technology or intervention to achieve both a water quality target and the health based target (reduction in enteric disease attributable to water) can be determined by community based, intervention type epidemiological studies. In these studies, the drinking-water treatment intervention is introduced into a representative number of households but not into a representative number of control households, or it is introduced into one of two otherwise similar communities. Then, the quality of the water and the occurrence of enteric disease are monitored regularly in both intervention and control households and communities. Analysis of the water quality and enteric illness data from such studies typically documents appreciable reductions in both fecal contamination of water (e.g., lower proportions of 100-ml drinking water samples positive for *E. coli* or other waterborne enteric microbes) and lower enteric illness rates in the water treatment intervention households or communities than in the control households or communities. For example, a study of non-piped household water in a peri-urban section of Dhaka, Bangladesh, the use chlorine and a safe water storage container was reported to reduce the percentage of 100-ml samples positive for *E. coli* from 45 to 13% and reduced the household rate of diarrhoeal disease from 2.2 to 1.25 episodes per month.

The use of an epidemiological approach to directly measure the achievement of a health risk target expressed as a measurable reduction in community enteric disease in relation to achieving a water quality target based on absence of fecal indicator microbes in samples of drinking water subjected to treatment is a powerful tool to demonstrate the achievement of safer drinking water. In many situations this can be an effective first incremental step in the eventual goal of achieving

even safer drinking water based on even greater levels of health protection with lower health risk targets and higher water quality targets by higher levels of source water quality, treatment and protection.

7.4.2 Risk assessment approach

In the typical health situation of Established Market Economies, the overall burden of enteric disease is considerably lower than in lesser-developed countries, and the possible effects of water quality interventions are less easily measured by epidemiological studies. In order to relate the effects of improved drinking water quality to health risks in the population, risk assessment models can be constructed as an alternative. Table 7.2 represents the type of data that would normally be used to construct such a risk assessment model for one or more waterborne pathogens. This table presents data for representatives of the three major groups of pathogens (bacteria, viruses and protozoa) from a range of sources. These choices of pathogens were mainly based on availability of data. Where possible, other aspects such as resistance to water treatment, infectivity and disease burden were also taken into account. The pathogens illustrated may not be priority pathogens in all regions of the world, and countries may wish to base their WSP on risk assessments for other pathogens.

Table 7.2. Linking tolerable disease burden and source water quality for reference pathogens*

Pathogen group	Disease basis for DALYs estimate	DALYs / case	Tolerable cases/yr	Dose response (cases/particle)	Particles/L	Particles/L adjusted for fraction of lifestage
Protozoa	Watery diarrhea over lifetime	0.001 Cryptosporidium)	10^{-3} Cryptosporidium	10^{-3} Cryptosporidium	10^{-2} Cryptosporidium	10^{-2} Cryptosporidium
Viruses	Infectious hepatitis in adults	0.1 HAV	10^{-5} HAV	10^{-1} HAV	10^{-6} HAV	10^{-6} HAV
Bacteria	HUS in children	5 E. coli O157	10^{-7} E. coli O157	10^{-2} E. coli O157	10^{-7} E. coli O157	10^{-6} E. coli O157

* based on a tolerable disease burden of 10^{-6} DALYs per person per year, see section xx

Table 7.2b Linking tolerable disease burden

River water (human and animal pollution)		Crypto-sporidium	Campylo-bacter	Rotavirus	
		low income regions		high income regions	
Raw water quality	Organisms per L	10	100	10	10
Treatment effect	Percent reduction	99.994%	99.99987%	99.999995%	99.99998%
(performance target)	Log-reduction	4.2	5.9	8.3	6.8
Drinking water quality	Organisms per L	6.3E-04	1.3E-04	5.0E-08	1.6E-06
(water quality target)	Log organisms per L	-3.2	-3.9	-7.3	-5.8
Consumption of unheated drinking	L per day	1	1	1	1

water					
Exposure by drinking water	Organisms per day	6.3E-04	1.3E-04	5.0E-08	1.6E-06
Dose-response	Infections per organism	4.0E-03	1.8E-02	2.7E-01	2.7E-01
Risk of infection	Per day	2.5E-06	2.3E-06	1.4E-08	4.3E-07
	Per year	9.2E-04	8.3E-04	4.9E-06	1.6E-04
Risk of (diarrhoeal) illness given infection		0.7	0.3	0.5	0.5
Risk of (diarrhoeal) illness	Per year	6.4E-04	2.5E-04	2.5E-06	7.8E-05
Disease burden	DALYs per case	1.5E-03	4.6E-03	4.8E-01	1.4E-02
Susceptible fraction	Percent of population	100%	100%	6%	6%
Disease burden	DALYs per year	1E-06	1E-06	1E-06	1E-06

Table 7.3 Pathogen levels in contaminated river water

Pathogen	Concentration (per L)
Campylobacter	100
Salmonella	1000
Cryptosporidium	10
Giardia	10
Viruses	10

The data in the table represents reasonable default values, based on current data availability. It is recognized that there is considerable variability in each of the parameters entered in the tables. Wherever possible, country- or site-specific information should be used to replace the default values.

The concentration in drinking water is directly related to the concentration in raw water, as defined by the treatment effect. The treatment effects should be adjusted so that the resulting disease burden is equal to the tolerable level of risk of 10^{-6} DALYs per year. These treatment effects can therefore be used as Performance Targets in order to meet the proposed tolerable risk level. Likewise, the concentrations in drinking water as presented in the sixth column represent the Water Quality Targets that meet the proposed tolerable risk level. These values are considerably below levels that may be reasonably monitored in drinking-water. This and the facts that actual contaminants vary widely and rapidly; and that the pathogens used are acting as indicators for a wide group of pathogens with similar basic characteristics indicate that they are unsuitable for use as targets in direct testing.

To link drinking water quality to disease burden, the following default assumptions were made. Drinking water consumption was assumed to be 1 liter per day. This value is lower than used for deriving chemical WQT, and refers to consumption of unheated water (through food and beverages) only as heating will rapidly inactivate pathogens. By multiplication of the concentration in water and the daily consumption, a daily dose can be calculated. For example,

exposure to *Cryptosporidium* oocysts is 1.0×10^{-3} per day. This indicates that, on average, each day 1 out of every 1000 consumers would be exposed to a single oocyst. The probability of an adverse health effect follows from a dose response model. Available dose-response data have mainly been obtained in studies using healthy, adult volunteers. Several subgroups in the population, such as children, the elderly and immunocompromised persons are more sensitive to infectious disease but currently, data are lacking to account for this. At low exposures, such as would typically occur in drinking water, the dose-response model is approximately linear. Hence, the probability of infection can easily be calculated as the product of the exposure to one organism and the probability that exposure to one organism would result in infection. The probability of infection per day is simply multiplied by 365 to calculate the probability of infection per year. Again, this is a simplification that is justified for low risks only. Not all infected individuals will develop clinical illness; asymptomatic infection is common for most pathogens. The percentage of infected persons that will develop clinical illness depends on the pathogen, but also on other factors such as the immune status of the host. Risk of illness per year is obtained by multiplying the probability of infection by the probability of illness given infection.

The disease burden per case not only reflects the effects of diarrhoeal illness but also the effects of more serious endpoints (e.g. Guillain-Barré syndrome by *Campylobacter*). Hence, these values reflect situations that vary widely by region of the world. For example, the disease burden per 1000 cases of rotavirus diarrhea is 10 DALYs in low-income regions, where child mortality frequently occurs. However, it is only 0.1 DALY per 1000 cases in high-income regions, where hospital facilities are accessible to the great majority of the population. As a consequence, countries are encouraged to modify the default disease burden estimates for their specific situations. No accounting is made for efforts on immunocompromised persons (e.g. cryptosporidiosis in AIDS patients).

The table provides substantiation for a simplified version of a full risk assessment model for pathogens in drinking water. Most importantly, it only presents point estimates and does not account for variability and uncertainty. Full risk assessment models would incorporate such factors by representing the input variables by statistical distributions rather than by point estimates. However, such models are currently beyond the means of many countries and data to define such distributions are scarce.

When applying the simplified risk assessment model, it is important to choose the most appropriate point estimate for each of the variables. Theoretical considerations show that risks are directly proportional to the arithmetic mean of the ingested dose. Hence, arithmetic means of variables such as concentration in raw water, removal by treatment and consumption of drinking water are recommended. This recommendation is different from the usual practice among microbiologists and engineers to convert concentrations and treatment effects to log-values and make calculations or specifications on the log-scale. Such calculations result in estimates of the geometric mean rather than the arithmetic mean, and these may significantly underestimate risks. Analyzing site-specific data may therefore require going back to the raw data rather than relying upon reported log-transformed values.

7.5 Occurrence of pathogens in water

Tables 7.3-7.7 present estimates of pathogen occurrence derived from a systematic review of published data, supplemented with data provided from external agencies. The detailed information is published as WHO *Occurrence of Pathogens in Surface water and their relationship with Indicator Parameter*.

The occurrence data provides useful guide to the incidence of enteric pathogens and *E.coli* as an indicator organism in a variety of sources. However, there are a number of limitations and sources of uncertainty, including:

- questions concerning the sensitivity of analytical techniques, particularly for viruses and protozoa;
- questions concerning the viability and human infectivity of *Cryptosporidium* oocysts and *Giardia* cysts identified by the various methods used in the different studies; and
- lack of knowledge about the human infectivity of the bacterial pathogens.

The concentrations are shown as orders of magnitude as a reflection of this uncertainty. While the concentrations provide an indication of concentrations that might be present in water sources, the most accurate way of determining pathogen loads in specific catchments and water sources is by analysing water quality over a period of time taking care to include consideration of seasonal variation and peak events.

Table 7.3 Summary of sources of pathogens in drinking-water catchments

Source of Pathogen	Lakes and Reservoirs	Rivers and Streams	Groundwater	
			Confined	Unconfined
Human				
Animal				
Both				
None				

Table 7.4 Pathogen numbers from various sources in lakes and reservoirs

Source of Pathogen	Bacteria/L	Protozoa/L	Virus/L	<i>E. coli</i> /100 mL
Human		<i>Cryptosporidium</i> <i>Giardia</i> 1		
Animal		<i>Cryptosporidium</i> 100 <i>Giardia</i> 10		
Both		<i>Cryptosporidium</i> 100 <i>Giardia</i> 10		
None		<i>Cryptosporidium</i> 1 <i>Giardia</i> 0.1		

Table 7.5 Pathogen numbers from various sources in rivers and streams

Source of Pathogen	Bacteria/L	Protozoa/L	Virus/L	<i>E. coli</i> /100 mL
Human	<i>Campylobacter</i> 10 <i>Salmonella</i> -	<i>Cryptosporidium</i> 10 <i>Giardia</i> 100	100	10000
Animal	<i>Campylobacter</i> 1000 <i>Salmonella</i> 100	<i>Cryptosporidium</i> 1000 <i>Giardia</i> 10		100000
Both	<i>Campylobacter</i> 10 <i>Salmonella</i> –1000	<i>Cryptosporidium</i> 100 <i>Giardia</i> 100	100	10000
None	<i>Campylobacter</i> 0 <i>Salmonella</i> -	<i>Cryptosporidium</i> 100 <i>Giardia</i> 10	0.1	100

Table 7.6 Pathogen numbers from various sources in confined groundwater

Source of Pathogen	Bacteria	Protozoa	Virus	<i>E. coli</i>
Human				
Animal				
Both				
None				0

Table 7.7 Pathogen numbers from various sources in unconfined groundwater

Source of Pathogen	Bacteria/L	Protozoa/L	Virus/L	<i>E. coli</i> /100 mL
Human		<i>Cryptosporidium</i> <i>Giardia</i>		1000
Animal		<i>Cryptosporidium</i> <i>Giardia</i>		1000
Both		<i>Cryptosporidium</i> 10 <i>Giardia</i> 1	10	
None	<i>Campylobacter</i> <i>Salmonella</i>	<i>Cryptosporidium</i> 10 <i>Giardia</i> 0.1		100

7.6 Verification of microbial safety and quality

The control of faecal contamination in drinking-water systems and sources is of primary importance. Faecal- specific indicator bacteria such as *E. coli* are the parameters of first importance in verification of microbial quality.

Faecal indicator bacteria should fulfil certain criteria to give meaningful results. They should be universally present in high numbers in the faeces of humans and warm-blooded animals, readily detectable by simple methods and they should not grow in natural water. Furthermore, their persistence in water and their degree of removal in treatment of water should be similar to those of waterborne pathogens. The primary indicator organism of faecal pollution is *Escherichia coli*. Thermotolerant coliforms can be used as an alternative to the test for *E. coli* in many circumstances.

The absence of faecal indicator organisms does not mean that drinking-water is free from microbial contamination. Protozoa and some enteroviruses are more resistant to disinfection by chlorine and may be present in drinking-water in the absence of *E. coli*, for example, following disinfection. Therefore verification may require analysis of a range of organisms, such as faecal streptococci, *Clostridia perfringens* and bacteriophages.

Table 7.8 Guideline values for verification of microbial quality^a

Organisms	Guideline value
All water directly intended for drinking	
<i>E. coli</i> or thermotolerant coliform bacteria ^{b,c}	Must not be detectable in any 100-ml sample
Treated water entering the distribution system	
<i>E. coli</i> or thermotolerant coliform bacteria ^b	Must not be detectable in any 100-ml sample
Treated water in the distribution system	
<i>E. coli</i> or thermotolerant coliform bacteria ^b	Must not be detectable in any 100-ml sample

^a Immediate investigative action must be taken if either *E. coli* are detected.

^b Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

^c It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies.

Pathogens more resistant to conventional treatment technologies may be present in treated drinking-water in the absence of *E. coli*. Retrospective studies of waterborne disease outbreaks and advances in the understanding of the behaviour of pathogens in water, has shown that continued reliance on these bacterial indicators alone, and assumptions surrounding the absence or presence of *E. coli* does not ensure that informed decisions are made regarding water quality.

7.7 Sampling

7.7.1 Sample collection and preservation

Care must be taken to ensure that samples are representative of the water to be examined and that no accidental contamination occurs during sampling. Sample collectors should, therefore, be trained and made aware of the responsible nature of their work. Samples should be clearly labeled with the site, date, time, nature of the work, and other relevant information and sent to the laboratory for analysis without delay.

If the water to be examined is likely to contain chlorine, chloramine, chlorine dioxide, or ozone, then sodium thiosulfate solution should be added to neutralize any residual disinfectant. A properly controlled concentration of thiosulfate has no significant effect on coliform organisms, including *E. coli*, either in chlorinated or in unchlorinated water samples during storage.

When samples of disinfected water are taken, the concentration of residual disinfectant at the sampling point and the pH should be determined at the time of collection.

When a number of samples are to be taken for various purposes from the same location, the sample for bacteriological examination should be collected first to avoid the danger of contamination of the sampling point.

The changes that may occur in the bacterial content of water on storage can be reduced to a minimum by ensuring that samples are not exposed to light and are kept cool, preferably between 4 °C and 10 °C, but not frozen. Examination should begin as soon as possible after sampling and certainly within 24 hours. If samples cannot be cooled, they must be examined within 2 hours of sampling. If neither condition can be met, the sample should not be analysed. The box used to carry samples should be cleaned and disinfected after each use to avoid contaminating the surfaces of bottles and the sampler's hands.

7.7.2 Sample locations

Samples must be taken from different parts of the distribution system to ensure that all parts of the system are tested. When streams, lakes, or cisterns are being sampled, the water must be taken from below the surface, away from banks, sides of tanks, and stagnant zones, and without stirring up sediments. Taps, sampling ports, and the orifices of pumps should, if possible, be disinfected and a quantity of water run to waste to flush out the standing water in the pipe, before the sample is taken. Sampling ports in treatment processes and on water mains must be carefully sited, to ensure that samples are representative. The length of pipework to the tap should be as short as possible.

7.7.3 Sampling frequency

The resources available will determine the frequency of sampling for verification of water quality. Verification of drinking-water quality is more representative if samples are taken at different times of day and on different days of the week. Frequent examination by a simple method is more valuable than less frequent examination by a complex test or series of tests.

Sampling frequencies for raw water sources will depend upon their overall quality, their size, the likelihood of contamination, and the season of the year. They should be established by local control agencies and are often specified in national regulations and guidelines. The results and information from sanitary inspection of the gathering grounds will often indicate whether increased vigilance is needed.

The frequency of sampling must be greater where the number of people supplied is large, because of the higher number of people at risk. Advice on the design of sampling programmes and on the frequency of sampling is given in ISO standards (Table). The minimum frequencies shown in Table 5.1 are recommended for water in distribution.

Samples should be spaced randomly within each month and from month to month, and should be taken both from fixed points, such as pumping stations and tanks, and from random locations throughout the distribution system, including points near its extremities and taps connected directly to the mains in houses and large multi-occupancy buildings, where there is a greater risk of contamination through cross-connections and back-siphonage. Frequency of sampling should be increased at times of epidemics, flooding, emergency operations, or following interruptions of supply or repair work. Especially with systems serving small communities, periodic sanitary surveys are likely to yield more information than infrequent sampling.

7.8 Methods of detection

Analysis for faecal indicator bacteria provides the most sensitive, although not the most rapid, indication of pollution of drinking-water supplies. Unlike chemical or physical analysis, however, it is a search for very small numbers of viable organisms and not for a defined chemical entity or physical property. Because the growth medium and the conditions of incubation, as well as the nature and age of the water sample, can influence the species isolated and the count, microbiological examinations may have variable accuracy. This means that the standardization of methods and of laboratory procedures is of great importance if criteria for microbiological quality of water are to be uniform in different laboratories and internationally. International standard methods should be evaluated under local circumstances before being adopted in national surveillance programmes. Established standard methods are available, such as those of the International Organization for Standardization (ISO) (Table 2) and of the American Public Health Association (APHA). It is desirable that established standard methods be used for routine examinations. Whatever method is chosen for detection of *E. coli* or thermotolerant coliforms, some step for “resuscitating” or recovering environmentally-or disinfectant-damaged strains must be used, such as pre-incubation for a short period at a lower temperature.

Table 7.9. International Organization for Standardization (ISO) standards for detection and enumeration of faecal indicator bacteria in water

ISO standard	Title (water quality)
6461-1:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 1: Method by enrichment in a liquid medium
6461-2:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 2: Method by membrane filtration
7704:1985	Evaluation of membrane filters used for microbiological analyses
7899-1:1984	Detection and enumeration of faecal streptococci - Part 1: Method by enrichment in a liquid medium
7899-2:1984	Detection and enumeration of faecal streptococci - Part 2: Method by membrane filtration
9308-1:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive <i>Escherichia coli</i> - Part 1: Membrane filtration method
9308-2:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive <i>Escherichia coli</i> - Part 2: Multiple tube (most probable number) method.