

Chapter 5: General microbiological quality

Contents

5.1	Introduction	2
5.2	Micro-organisms in drinking-water	5
5.2.1	Introduction	5
5.2.2	Controlling waterborne infection – historical overview	7
5.2.3	Maximum acceptable value (MAV)	9
5.3	Microbial indicators	10
5.3.1	Introduction	10
5.3.2	Bacterial indicators	12
5.3.3	Pathogenic protozoal indicators	14
5.3.4	Pathogenic viral indicators	16
5.3.5	Secondary bacterial indicators	17
5.3.6	Indicators of general quality	18
5.3.7	Indicators of effectiveness of treatment	20
5.4	Waterborne pathogens	21
5.4.1	Testing for specific pathogens	21
5.4.2	Bacterial pathogens from faecal contamination	24
5.4.3	Bacterial pathogens growing in the water supply	25
5.4.4	Viruses	25
5.4.5	Pathogenic protozoa	26
5.4.6	Helminths	31
5.4.7	Cyanobacteria (blue-green algae)	31
5.4.8	Disease from waterborne pathogens	32
5.5	Organisms causing problems other than disease	33
5.5.1	General	33
5.5.2	Organisms causing taste and odour	34
5.5.3	Micro-organisms causing colour	34
5.5.4	Iron and manganese deposits due to bacteria	34
5.5.5	Corrosion resulting from iron and sulphur bacteria activity	35

5.5.6	Large numbers of micro-organisms	36
5.5.7	Invertebrate inhabitants of water systems	36

References	37
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List of tables

Table 5.1:	Waterborne pathogens and their significance in water supplies	23
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5.1 Introduction

This chapter discusses the microbiological quality of drinking-water in general terms. Microbiological compliance issues are discussed as follows:

- Chapter 6: Bacterial Compliance
- Chapter 7: Virological Compliance
- Chapter 8: Protozoal Compliance
- Chapter 9: Cyanobacterial Compliance.

Infectious, water-related diseases are a major cause of morbidity and mortality worldwide. Newly-recognised pathogens and new strains of established pathogens are being discovered that present important additional challenges to both the water and public health sectors. Between 1972 and 1999, 35 new agents of disease were discovered and many more have re-emerged. Amongst these are pathogens that may be transmitted by water (WHO 2003b). There are suggestions that giardiasis was spread around the world by the mobility of armed forces during WWII. The first case of human cryptosporidiosis was reported in 1976, and by 1985 this 'new' pathogen was becoming more widely recognised.

The microbiological quality and the likelihood that a pathogen (disease causing organism) will be transmitted through drinking-water is dependent on numerous factors. Some of these reflect the characteristics of the pathogen itself, including resistance to environmental conditions such as ultraviolet light, desiccation, temperature, etc.

Many of these pathogens are zoonotic. Zoonoses are diseases caused by micro-organisms of animal origin that also infect humans. Zoonoses are of increasing concern for human health; next to pathogens with human-to-human transmission, they pose the greatest challenges to ensuring the safety of drinking-water and ambient water, now and in the future. See WHO (2004b) – a 528-page document.

The phenomena of 'emergence' and 're-emergence' of infectious diseases is well recognised. Up to 75 percent of emerging pathogens may be of zoonotic origin. WHO (2012) states in Chapter 2 that a pathogen or disease-causing agent is considered 'emerging' when it makes its appearance in a new host population or when there is a significant increase in its prevalence in a given population. A significant number of emerging and re-emerging waterborne pathogens have been recognised over recent decades; examples include *E. coli* O157:H7, *Campylobacter*, and *Cryptosporidium*. Public health scientists are increasingly discovering that the recent emergence or re-emergence of infectious diseases has an origin in environmental change. These environmental changes encompass social processes such as urbanisation and creation of transportation infrastructure, as well as ecologic processes such as land and water use, biodiversity loss, and climate change.

Between 1972 and 1999, 35 new agents of disease were discovered, and many more have re-emerged after long periods of inactivity or are expanding into areas where they have not previously been reported. From WHO (2017).

The frequency with which emerging communicable diseases are identified seems to be increasing. The rationale is well recognised as being the consequence of:

- increasing urbanisation with the movement of humans to major population centres being matched by the movement of vertebrate and invertebrate species into urban areas as well. The increased socialisation of individuals provides new opportunities for pathogen spread
- the phenomenal increase in international travel, in particular air travel, has provided opportunities for pathogens to travel along pathways between states with relative freedom and with increased speed and volume
- it is noted that since the end of the 1990s epidemiologists have been challenged by a succession of events which have featured either novel infections (SARs, H1N1, H5NI) or legacy infections that have been transferred to naïve populations (West Nile Virus in North America, Chikungunya in South Asia and Italy).

The overuse, careless use, inappropriate use and unregulated use of many antibiotics and other antimicrobial agents in both human and veterinary medicine are well documented, as is the extensive and largely unregulated use of these agents in animal agriculture and aquaculture, including for growth promotion. Uncontrolled release and disposal of these agents to sanitary sewers and landfills and in effluent discharges from pharmaceutical production facilities are also known to occur. Many enteric bacterial pathogens and associated faecal indicator bacteria, such as *Escherichia coli* and enterococci, are now multiple antimicrobial resistant, with some so resistant that infections cannot be effectively treated. A study has linked clinical isolates of multidrug-resistant enteric bacteria to the same bacteria found in environmental waters that were implicated in a possible waterborne community salmonellosis outbreak, and there are other cases of increased human mortality and morbidity caused by antimicrobial-resistant bacteria. New strains or variants of highly resistant enteric bacteria of human health concern continue to emerge, are detectable in environmental media such as water and soil and are spreading globally, thus posing increased human health risks (WHO 2014).

Along with trends in animal populations and husbandry, the presence of a given pathogen (eg, *Campylobacter*) may vary considerably from time to time, and the intensity of shedding may be influenced by factors with their own underlying trends, such as the seasonality and changes in farm control and management practices.

Securing the microbial safety of drinking-water supplies is based on the use of multiple barriers, from catchment to consumer, to prevent the contamination of drinking-water or to reduce contamination to levels not injurious to health.

Faecally derived pathogens from contamination by human, animal or bird faeces are the principal concerns in setting health-based targets for microbial safety. Microbial water quality often varies rapidly and over a wide range and short-term peaks in pathogen concentration may increase disease risks considerably, with greater reliance on treatment processes.

WHO (2012) stated:

- Although there are a large number of zoonotic pathogens that affect humans, five are known to cause illness around the world with high-frequency: *Cryptosporidium*, *Giardia*, *Campylobacter*, *Salmonella* and *E. coli* O157. Efforts to control these pathogens are likely to be effective in controlling other related zoonotic pathogens whether known, as-yet-unrecognised or emergent.
- Domestic animals such as poultry, cattle, sheep and pigs generate 85 percent of the world's animal faecal waste, proportionally a far greater amount than the contribution by the human population. The faecal production rate and contribution to the environment of these animals can be as high as 2.62×10^{13} kg/year.
- Limiting zoonotic pathogen-shedding in farm or production facilities for domestic animals should be accomplished by preventing illness in livestock, through minimising exposure to pathogens, by increasing immunity, by manipulation of the animal gastrointestinal tract microbial ecology and by managing (including treating) animal waste to reduce the release of zoonotic pathogens into the environment.

Being a significant exporter of animal sourced protein means that the relevance of zoonotic diseases will be potentially much greater in New Zealand than in many other countries. For a more detailed discussion relating to waterborne diseases reported in New Zealand, see Chapter 1, section 1.1.3.

The words cyst, oocyst and (oo)cyst appear frequently in this chapter and in Chapter 8: Protozoal Compliance. The definitions in the *Drinking-water Standards for New Zealand* (DWSNZ) are:

- an oocyst is a thick walled structure within which *Cryptosporidium* zygotes develop and which serves to transfer the organism to new hosts
- a cyst is the non-motile dormant form of *Giardia* which serves to transfer the organism to new hosts
- (oo)cyst is an abbreviation for cyst and oocyst.

FAO (2003) provided a practical framework and a structured approach for the characterisation of microbiological hazards, either in the context of a full microbiological risk assessment or as a stand-alone process. It is aimed at assisting governmental and research scientists to identify the points to be addressed, the methodology for incorporating data from different sources, and the methodology of dose-response modelling. The guidelines focus on the adverse impact of a hazard as a result of ingestion of contaminated food and water.

WHO (2004d) covers many water treatment processes suitable for pathogen control. WHO (2005a) published advice for travellers on how to make drinking-water safe. WHO (2009) is a 143-page publication devoted to just *Cryptosporidium*.

DWI (2015a) published a report on the wholesomeness of water used for toilet flushing. Waters used for purpose include rainwater, greywater, and reclaimed wastewater. It includes the statement "New Zealand has been looking at greywater/rainwater use in non-residential buildings but does not allow dual pipe systems in residential properties for potential cross-connection reasons. The use of greywater for toilet flushing is also banned for similar reasons".

5.2 Micro-organisms in drinking-water

5.2.1 Introduction

WHO (2004) stated:

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

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

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

New Zealand has many water supplies ranging from the fully treated large municipal supplies, to the small untreated supplies serving a community of say less than 100. Microbiological guidelines seek to ensure that water supplies are free from disease-causing micro-organisms. The provision of such a supply is of the utmost importance to the health of any community.

The most common and widespread health risk associated with drinking-water is contamination, either directly or indirectly through human, animal and occasionally bird faeces and with the micro-organisms contained in their faeces. If the contamination is recent and among the contributors there are carriers of communicable enteric diseases (diseases of the gut), some of the micro-organisms that cause these diseases may be present in the water. The degree of risk is related to the level of disease in the human or animal community at that time. Drinking this water or using it in food preparation may cause new cases of infection. Those at greatest risk of infection are infants and young children, people whose immune system is depressed, the sick and the elderly. Risebro et al (2012) found that "Contaminated small water supplies pose a substantial risk of infectious intestinal disease to young children who live in homes reliant on these supplies. By contrast older children and adults do not appear to be at increased risk".

The pathogenic organisms of concern in New Zealand include bacteria, viruses and protozoa. The diseases they cause vary in severity from mild gastroenteritis, to severe and sometimes fatal diarrhoea, dysentery, hepatitis, cholera, typhoid fever and campylobacteriosis.

A 15-month fortnightly survey of microbial health risk indicators and pathogens was carried out at 25 freshwater recreational and water supply sites distributed throughout New Zealand, for *E. coli*, *Clostridium perfringens* spores, F-RNA bacteriophage, somatic coliphage, human enteroviruses, human adenoviruses, *Cryptosporidium* oocysts, *Giardia* cysts, *Salmonella* and *Campylobacter* (MfE 2002 – the Bad Bugs Report). Viruses and *Campylobacter* were detected at all six water supply sites. There was very little difference between the drinking-water supply sites and the remaining site types with respect to the occurrence of pathogens and the concentrations of indicator organisms. The main issue for source waters is the high proportion of samples which contained *Campylobacter* (60 percent) and viruses (54 percent) and the ability of drinking-water treatment to inactivate or remove them. The widespread presence of *Campylobacter* indicates a need for considerable care with respect to small rural supplies, which have been implicated in campylobacteriosis previously (Eberhardt-Phillips et al 1997, Till et al 2008).

Massey University's Gastrointestinal Protozoa, Research and Services have been testing source waters for the Ministry of Health. A total of 520 samples have been collected from 20 sites across New Zealand, ie, 26 quarterly samples per site over a 6.5-year period ending July 2016. Samples have been tested for *Cryptosporidium* oocysts, *Giardia* cysts, *E. coli* and *Campylobacter*. Based on the sites and probable land use, the 20 sites have been split into four groups:

1	Groundwater/springs	4 sites
2	Bush catchments	7 sites
3	Intermediate rivers	5 sites
4	Lowland rivers	4 sites

Observations: About 50 percent of lowland river samples contained protozoa. As expected, most *Cryptosporidium* oocysts in the 104 lowland river samples were found in autumn and spring. Only about 2 percent of samples from bush catchment and intermediate rivers contained protozoa. No samples from shallow groundwater/spring sites contained protozoa. Over 80 percent of samples from bush catchments and intermediate rivers contained *E. coli*, with arithmetic means around 4 to 175 per 100 mL. Both groups averaged about 10 percent of samples with *Campylobacter*. Results for the bush catchment and the intermediate river sites are not particularly different, the main difference being that the intermediate river samples contain about 2–3 times as many *E. coli* per 100 mL. Lowland rivers had *E. coli* in every sample, with an arithmetic mean around 275 per 100 mL. Lowland rivers also contained *Campylobacter* in more than 50 percent of samples. The sites with the most protozoa only need to meet 3-log removal.

While the classical waterborne diseases are caused by organisms originating in the gut of humans or animals, many organisms found in water are not, or at least not regularly, associated with the gut. Some of these may under certain circumstances cause disease in humans. They include the protozoan *Naegleria fowleri*, and a number of bacteria including *Aeromonas*, *Klebsiella*, *Legionella* spp, and some species of environmental mycobacteria. Refer to the individual datasheets for further information.

Infection is the main, but not the only problem associated with micro-organisms in drinking-water. Certain algae can produce toxins that affect humans and which may remain in the water even when the algae responsible have been removed, see Chapter 9: Cyanobacterial Compliance. Other 'nuisance organisms' can cause problems of taste, odour or colour, as well as deposits and corrosion, and while they may not cause disease, they are aesthetically unacceptable. The organisms concerned include iron, manganese, sulphur and nitrifying bacteria, nematodes, midges, crustacean, rotifers and mussels; these are discussed in AWWA (2004).

The supply of safe drinking-water involves the use of multiple barriers to prevent the entry and transmission of pathogens. The effectiveness of these multiple barriers should be monitored by a programme based on operational characteristics and testing for microbial indicators of faecal contamination and in some circumstances actual pathogens.

5.2.2 Controlling waterborne infection – historical overview

The value of a wholesome water supply has been recognised, at least in some quarters, for many centuries. Hippocrates described an association between water supplies and disease (Hippocrates, cited 1938) and Roman engineers went to great lengths to provide waters suitable in both quantity and quality for major cities.

Over recent centuries, urbanisation and industrialisation have increased the pressure upon water supplies and the systems of waste disposal. Thus it was that, by the middle of the 19th century, Britain was affected by major epidemics of cholera and endemic typhoid. John Snow and William Budd provided irrefutable evidence of the role of water in transmission of these two diseases.

Snow's case rested very simply on a comparison of cholera incidence among the customers of three London water companies (Snow 1855). One supplied filtered water; the second moved the source of its supply to a cleaner area of the River Thames, while the third persisted in supplying polluted River Thames water. Budd appreciated that the sewer was merely an extension of the diseased gut (Budd 1856) and applied what are now classical epidemiological concepts to the investigation of water as a vehicle for spreading typhoid.

As a result, filtration of river-derived water became legally required in London in 1859, and this practice gradually spread throughout Europe. By 1917, Sir Alexander Houston could draw attention to the effectiveness of London's systems of water treatment and delivery in stopping the waterborne transmission of typhoid. In America, he pointed out it was customary to consider as normal an annual mortality rate from typhoid of 20 or more per 100,000 of population (the rate in Minneapolis was 58.7). In London, however, the annual mortality from typhoid was 3.3 per 100,000 (Houston 1917).

Budd's relatively simple precautions against faecal-oral transmission of typhoid (use of strong disinfectants in the water-closet bucket) had been remarkably successful (Budd 1856). A century later, Hornick's experiments on volunteers helped to explain the success by showing the disease to be in some instances relatively difficult to catch (Hornick et al 1966). Around 10^7 *Salmonella* serovar Typhi bacteria caused disease in only fifty percent of his volunteer subjects. Kehr and Butterfield (1943), however, showed that a small minority of the population (about 1.5 percent) needed to ingest only a single typhoid organism to contract typhoid, and to protect these people clearly more elaborate precautions are needed.

When the need to protect drinking-water from faecal material was first recognised, the techniques available for the isolation of such organisms as *Salmonella* serovar Typhi and *Vibrio cholerae* were quite inadequate for practical purposes. Surrogates or indicators were needed, and the obvious candidates were common microflora from the gut, and so the use of indicator organisms became established. Testing water for ammonia was commonly used to indicate the presence of human wastes. An early consensus developed about the use of the coliform organisms, and in the early decades of the 20th century the work of Alexander Houston (1917) and Doris Bardsley (1934), among many others, helped to establish the validity of *Escherichia coli* (*E. coli*) as an indicator of faecal contamination.

Kehr and Butterfield (1943) showed the coliform test to be a useful indicator of *S. serovar* Typhi and they concluded that the presence of coliforms (as a bacterial group), even in moderate numbers, indicated a potential danger. They cited an outbreak in Detroit, Michigan, when on two successive days mean coliform counts in the water supply of only 3 and 10 per 100 mL were the indicator for an outbreak of waterborne typhoid. They also noted the very much higher risk of gastroenteritis associated with this low coliform count. For the eight cases of typhoid recorded in this outbreak, there were 45,000 cases of gastroenteritis.

Endemic and epidemic cholera and typhoid both still occur, transmitted through contaminated drinking-water, as demonstrated in Pristina (Yugoslav Typhoid Commission 1964), in South Africa (Kustner et al) and the cholera outbreak in Peru (Anderson 1991). The latest number of waterborne cholera cases from the World Health Organization is for the year 2000: 137,071 cases and almost 5,000 deaths. It should be noted that this figure does not include any cases from Bangladesh or Pakistan where cholera is endemic.

Fortunately, in New Zealand indigenous typhoid and cholera are now rare. Most cases are visitors from overseas or travellers returning to New Zealand. **Waterborne disease however remains a constant threat to public health.** Twenty cases of typhoid were notified in New Zealand in 2003, and one case of cholera. Typhoid carriers, and those people contracting the illness, have the potential to distribute large numbers of pathogens throughout the country where drinking-water protection and treatment systems are not operational. In addition there is the environmental risk from pathogens such as *Campylobacter*, *Salmonella*, *Cryptosporidium*, Hepatitis A and enterohaemorrhagic *E. coli* (EHEC). During 2003 nearly 15,000 cases of campylobacteriosis, 1,400 of salmonellosis, over 800 of cryptosporidiosis, 70 cases of Hepatitis A and 105 EHEC infections were reported in New Zealand (NZPHSR 2003).

5.2.3 Maximum acceptable value (MAV)

The use of a Maximum Acceptable Value (MAV) for *E. coli* for drinking-water requires an understanding of the use of microbiological indicator organisms as an indicator of the potential for the risk of pathogens being present. Whereas the MAV of a chemical determinand in a drinking-water represents its concentration that on the basis of present knowledge is not considered to cause any significant risk to the health of the consumer over a lifetime of consumption of the water, the use of MAVs for microbiological determinands is somewhat different.

The microbiological determinand *E. coli* is an indicator of recent faecal contamination. The quantification of *E. coli* is related to the absence or non-detectability of that micro-organism in a given volume of water. Such a value, when considered with the method of analysis and frequency of sampling for a given population, gives a probability that there is no significant risk of infection from micro-organisms of known health significance at the time of sampling. The presence of *E. coli* provides evidence of recent faecal contamination, and detection should lead to consideration of further action such as further sampling and investigation of inadequate treatment or breaches in distribution system integrity.

A MAV is given in the DWSNZ for *E. coli* as an indicator of the potential presence of pathogenic enteric bacteria, enteric viruses, and pathogenic protozoa. However, *E. coli* is not always a good indicator for viruses or protozoa.

A maximum indicator value (MIV) is a more appropriate parameter to use for bacteria than a MAV because *E. coli* is not monitored for health reasons, it is monitored as an indicator of faecal contamination, and therefore of the potential presence of pathogenic micro-organisms. However, for consistency with general (and historical) usage, the term MAV is used throughout the DWSNZ.

Historically and internationally, the guideline value, maximum contaminant level or MAV etc seems always to have been 'less than 1 per 100 mL', with the unit or test organism changing from *B. coli*, to total or presumptive coliforms, then faecal coliforms, to *E. coli*. Over the years, improved growth media and incubation conditions have enhanced selectivity, and quality assurance procedures have reduced the number of false positives and false negatives. But it's always been 'less than 1 per 100 mL'. The pattern was probably established with the original test methods over 100 years ago. It can't have had anything to do with infective doses, because only indicator organisms have been tested for, and the ratio of indicator organisms to pathogens would vary wildly. Retaining the 'less than 1 per 100 mL' for compliance testing has probably been more to do with pragmatism than science; water with 'less than 1 per 100 mL' seems not to have caused many illnesses over the years, ie, it seems to work! Water suppliers interested in more than just compliance testing are referred to Chapter 6, section 6.3.3.

5.3 Microbial indicators

5.3.1 Introduction

The detection of specific pathogens, including bacteria, viruses, protozoa and parasites is usually complex, expensive, time-consuming, and currently often not practically possible. It may take weeks to determine whether a sample actually contains a particular pathogen and whether it is infective. Furthermore, methods for parasitic cysts or oocysts (eg, *Giardia intestinalis*,¹ *Cryptosporidium hominis*² and *C. parvum*) have recovery efficiencies of typically less than 50 percent, and can be quite variable.

Therefore in monitoring microbiological quality, reliance is placed on relatively quick and simple tests for the presence of indicator organisms. At present this usually involves culturing the organisms on or in an appropriate growth medium. Selective media are usually chosen. These prevent or retard organisms other than the ones being targeted. There has been debate (Sinton 2006) whether culture techniques detect all the organisms. Are those that do not respond 'viable but non-culturable', or are they simply dead or injured beyond repair (ie, no longer pathogenic, ie, no longer infective)?

In addition to the indicator organisms specifically referred to in the DWSNZ, this section discusses heterotrophic plate counts (colony counts) that may be used to assess the general bacterial content of drinking water, and it considers phages. Chapter 7 discusses viruses.

Microbial indicators are micro-organisms that while not themselves pathogenic, indicate potential issues of microbiological water quality. The drinking-water industry commonly uses the following indicator organisms:

¹ Also known as *G. lamblia* and *G. duodenalis*.

² Previously called *Cryptosporidium parvum* (genotype 1).

- heterotrophic plate count (standard plate count, mesophilic plate count, aerobic plate count)
- total coliforms (presumptive coliforms)
- faecal coliforms (thermotolerant coliforms)
- *Escherichia coli* (*E. coli*).

An effective indicator organism for detecting faecal contamination of water should:

- always be present when faecal pathogens are present
- be present in faeces in large numbers so that the organisms can still be detected after considerable dilution
- be relatively easy and quick to detect
- survive in water at least as long as waterborne pathogens of faecal origin
- be as sensitive as pathogens to disinfection.

In assessing the results of their water study for the MoH, Massey University have found that *E. coli* and *Campylobacter* were important predictors for each other. A similar pattern was observed for *Giardia* and *Cryptosporidium*. However, the bacterial microbes were poor predictors of protozoan microbes and vice versa for the protozoan microbes.

Ideally, tests used to measure the numbers of indicator organisms in a sample must be specific to that organism, and they should encourage a high proportion of those present in the sample to grow. It has long been recognised that artificial culture media lead to only a very small fraction (0.01–1 percent) of the viable bacteria present being detected. Since MacConkey's development of selective media for *E. coli* and coliforms at the beginning of the 20th century, various workers have shown these selective agents inhibit environmentally or oxidatively stressed coliforms (WHO 2001, Chapter 13).

No single indicator fulfils all these considerations, nor is any suitable for all cases. All indicators have disadvantages that must be considered when interpreting test results, and expertise is thus mandatory in this area. Multiple indicator systems may be needed in certain circumstances. Nevertheless, if the indicators are satisfactory and monitoring is carried out appropriately, it should be possible to dispense with the use of complex tests for the specific pathogenic micro-organisms in all but a few cases. A vast amount of experience has accumulated from the use and interpretation of tests for indicator micro-organisms and considerable confidence can be placed on the results of these tests.

The most important point is that the presence of indicators of faecal contamination implies an increased risk of disease. For disease to occur, however, the indicators must be accompanied by pathogenic micro-organisms. The chances of this occurring are determined by the prevalence of the pathogens in the potential sources (people or animals) and in the catchment from which the water is drawn.

The occasional failure of indicators to predict disease underlines the prime importance of risk assessments and maintaining effective multiple barriers from catchment to tap to prevent faecal material from entering the water supply. Tests for the microbiological quality of water can only indicate breaches of the integrity of those barriers.

A history of the development of indicator organisms appears in Chapter 13 of WHO (2001).

5.3.2 Bacterial indicators

The coliform group of bacteria is a functionally-related group which belongs to a single taxonomic family (the Enterobacteriaceae) and comprises many genera and species. There are other genera in the Enterobacteriaceae family, such as *Salmonella* and *Shigella*, which are not considered coliforms. The Enterobacteriaceae includes the following genera which ferment lactose to acid and gas: *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*. When the definition was changed to producing only acid, the following genera were included: *Yersinia*, *Serratia*, *Hafnia*, *Pantoea* and *Kluyvera*. The introduction of the enzyme-based β -galactosidase test added the following genera to the coliforms: *Cedecea*, *Ewingella*, *Moellerella*, *Rahnella* and *Yokenella*. All coliforms other than *Escherichia coli* can be present in the environment as well as faeces; in fact, their colonisation in the gut of warm blooded animals is usually through interaction with the environment (Stevens et al 2003).

Total coliforms represent only about 1 percent of the total population of bacteria in human faeces, which can be found in concentrations of about 10^9 bacteria per gram. In 1948 a more specific test was developed that measured faecal (thermotolerant) coliforms; these grow can at 44.5°C. The vast majority of thermotolerant coliforms are *E. coli* and to a lesser extent *Klebsiella*, *Enterobacter* and *Citrobacter*.

The DWSNZ use *Escherichia coli* (*E. coli*) as the bacterial indicator, with a maximum acceptable value (MAV) of less than 1 per 100 mL. This is unchanged from earlier editions. Faecal coliforms (or thermotolerant coliforms) and total coliforms (or presumptive coliforms) can be monitored instead of *E. coli*, but with the proviso that a positive result for either should be treated as a positive *E. coli* result. Given that one can obtain either faecal coliforms or total coliforms in the absence of *E. coli*, this option is generally more demanding.

The total coliform test can be very useful for checking whether a bore water supply has been affected by surface influences, during commissioning or maintenance; see sections 3.2.4.1, 3.2.5.2, 3.2.5.3, 3.2.5.4 and 3.2.5.5 for further information. The USEPA's Revised Total Coliform Rule still uses total coliforms (along with *E. coli*) for monitoring drinking water. The USEPA considers total coliforms a useful indicator of other pathogens for drinking water. Total coliforms are used to determine the adequacy of water treatment and the integrity of the distribution system. Their presence requires water suppliers to perform assessments to identify sanitary defects and subsequently take action to correct them (USEPA 2013).

Frequently finding total coliforms in distribution system and service reservoir samples in the absence of *E. coli* suggests biofilm development, which tends to occur more often in the summer, or contamination from the environment; total plate counts usually increase at the same time. Maintaining a chlorine residue is an effective technique for controlling this problem, if not the cause. Biofilm development can also occur on the surface of the casing or fittings, or as a result of contamination at the bore head. WHO (2004e) discusses microbial contaminants and growth of microorganisms in distribution networks and the practices that contribute to ensuring drinking-water safety in piped distribution systems.

The bacterial compliance criteria in the DWSNZ have made use of the observation that *E. coli* is rarely found in drinking-water if the free available chlorine content is at least 0.2 mg/L.

A few strains of *E. coli* may be pathogenic in the gut. However, this is irrelevant to the use of *E. coli* as an indicator organism. Both pathogenic and non-pathogenic strains of *E. coli* are equally important as indicators of faecal contamination, as are animal and human sources.

The arguments for using *E. coli* for compliance testing are compelling:

- it is a strict indicator of faecal contamination, whereas the faecal coliforms and total coliforms are not
- it is an organism, whereas the other two are groups of bacteria
- it is most usually present when pathogens are present (eg, as found in the New Zealand Freshwater Microbiological Research Programme in fortnightly sampling at five drinking-water abstraction sites over a 15-month period, McBride et al 2002)
- *E. coli* can survive for considerable periods in water, which is generally similar to some of the waterborne faecal pathogens
- it is routinely associated with health risk effects in water ingestion studies (eg, Dufour 1984)
- it is now amenable to rapid and accurate enumeration, eg, using the Colilert™ MPN system (and acceptable equivalents). Colilert detects both total coliforms and *E. coli*.

The absence of *E. coli* does not necessarily guarantee the absence of faecal contamination (particularly where multiple barriers are absent as, for example, when reliance is placed on disinfection alone). Absence of evidence does not logically denote evidence of absence. Although their presence is a definite indication of pollution, their absence suggests that pathogenic bacteria and viruses are probably absent also. A study of outbreaks of waterborne disease in the USA found that one third of water supplies responsible for disease outbreaks did not have any total coliforms isolated from within the system (Craun et al 1997), concluded that the presence of coliforms is sometimes a useful indicator for viruses and bacteria, but not for protozoan parasites.

There are some indications that *E. coli* may grow in favourable environmental conditions, especially in warm climates (Fujioka et al 1999). *E. coli* growth has been reported in food (including *E. coli* O157:H7) (Doyle 1997), tropical water (Bermúdez and Hazen 1988), subtropical waters and soil (Hardina and Fujioka 1991), water in animal drinking troughs (Lejeune et al 2001) and in temperate waters and sediments in water reservoirs near Sydney (N Ashbolt, University of New South Wales, personal communication). However the New Zealand Freshwater Microbiological Programme did not find evidence of such growth occurring (McBride et al 2002). *E. coli* is unlikely to grow in New Zealand distribution systems, so if they are found there and not in the water leaving the treatment plant, it suggests post-treatment contamination with faecal matter has occurred.

To date, bacteria, including *E. coli*, have been defined by their biochemical reactions in the laboratory, rather than being identified by something more specific like DNA. Therefore there will always be debates about which test methods are the most appropriate, and which produce false positives or negatives. A recent study illustrates this (DWI 2010). Debate will continue about incubation temperatures, chlorine stress, and whether lactose fermentation or galactosidase and glucuronidase reaction is more appropriate.

Some enteric pathogens may occur even when few, if any, *E. coli* are present. For example, organisms such as *Giardia* cysts or oocysts of *Cryptosporidium*, and some viruses, are relatively resistant to chlorine disinfection in comparison with the indicators that are generally used. They may therefore survive a disinfection process that kills the indicator organisms. Likewise, UV disinfection is not particularly effective against some types of virus.

Clostridium perfringens spores are highly resistant in the environment, and vegetative cells appear not to reproduce in aquatic sediments, which can be a problem with traditional indicator bacteria. It is one of the most resistant micro-organisms in water, with a half-life (time for a 50 percent reduction in concentration) of 60 to >300 days (WHO 2003c). Like protozoa and some viruses, *Clostridium perfringens* is more resistant to some disinfection processes (WHO 2001, Chapter 13). Finding *Clostridia* in water leaving the treatment plant generally indicates that there is a fault in the chemical or physical treatment that requires investigation and appropriate remedial action. *Clostridium perfringens* can be used to detect faecal contamination of groundwater after the more traditional indicator organisms such as *E. coli* have died.

5.3.3 Pathogenic protozoal indicators

Giardia and *Cryptosporidium* are two protozoal pathogens that have been implicated in a number of outbreak and sporadic disease patterns in New Zealand (as elaborated in Chapter 1: Introduction, section 1. *Giardia* spp. and *Cryptosporidium* spp. are widespread in many New Zealand water sources; they are endemic in livestock, domestic and feral animals. Therefore surface waters, including shallow (particularly unconfined) groundwater, must be considered to be potentially contaminated.

For drinking waters, the MAV in the DWSNZ is for infectious pathogenic protozoa. Although new methods of assessing the infectiousness of protozoa by using human cell cultures have been developed, they are not yet suitable for routine monitoring of drinking-water. Therefore the MAV is effectively for total protozoa.

The analytical procedure to be used is based on method 1623 (USEPA 2003). This measures both *Giardia* cysts and *Cryptosporidium* oocysts, without identifying species. Until another method is developed, it is accepted that this method can be used to indicate total protozoal pathogens. There is very limited information about the removal and/or inactivation of emerging parasitic protozoa or opportunistically pathogenic protozoa during water treatment (see section 5.4.5); datasheets have been prepared for some. In the absence of information, the fate of these protozoal pathogens is considered similar to that of *Giardia* and *Cryptosporidium* during water treatment.

To control pathogenic protozoa, the DWSNZ require that water be treated to ensure their removal or inactivation, or that secure bore water is used. The level of treatment required for surface waters and non-secure bore water is determined from the concentration of *Cryptosporidium* in the source water, see section 5.2.1 of the DWSNZ, and Chapter 8: Protozoa Compliance, section 8.2 in the *Guidelines*. The premise is that *Cryptosporidium* is known to be very resistant to treatment processes, and is smaller than *Giardia*, so is used as an indicator for all pathogenic protozoa. Thus the level of treatment selected to remove *Cryptosporidium* should also provide a level of protection from other less resistant pathogenic protozoa, including *Giardia*.

When sewage is the source of these pathogens, the anaerobic spore-forming bacterium *Clostridium perfringens* appears to be a suitable index for enteric viruses and parasitic protozoa. Spores of *C. perfringens* are largely of faecal origin, and are always present in sewage (about 10^4 – 10^5 cfu per 100 mL). *Clostridium perfringens* is fairly resistant to lower doses of chlorine, so it has been suggested as an alternative indicator organism for protozoa; spores of *Clostridium perfringens* showed the strongest correlation ($r = 0.76$) with *Cryptosporidium* in a study on the River Meuse, a stronger correlation than thermotolerant coliforms or turbidity (WHO 2003c).

Methods for the detection of *Giardia* and *Cryptosporidium* in water have advanced considerably in the last few years. Detecting these protozoa involves the filtration of large volumes of water as the (oo)cysts are usually present in very low numbers. Methods have been developed using filtration and immuno-based techniques with monoclonal antibodies for separation (immunomagnetic separation, IMS) and detection (immunofluorescence assay, IFA) to determine concentrations of (oo)cysts with confirmation through vital dye staining (DAPI) and differential interference contrast (DIC) microscopy. However, the recovery success of this process can be variable, monoclonals may vary in their avidity and specificity to (oo)cysts or cross-react with other animal species, and the methods are costly. Routine monitoring for *Cryptosporidium* and *Giardia* in treated water is therefore not recommended in the DWSNZ as the methods do not reliably identify strains that are infective to humans, nor determine if those detected are infective (Quintero-Betancourt et al 2002).

Molecular-based methods and tissue cell culture assays show promise in detecting low level contamination in environmental waters, differentiating human pathogenic species from those that are not pathogenic and assessing infectivity but they are still being evaluated.

Over a period of seven years, four large supplies in Utah collected *Cryptosporidium*, *E. coli* and turbidity data from seven water treatment plants that treat both reservoir and stream sources. The data represented analyses completed by two USEPA-approved protozoan laboratories and local state-certified laboratories for *E. coli*. Results of the statistical analyses indicated poor correlation between *Cryptosporidium* and *E. coli* and between *Cryptosporidium* and turbidity in all monitored water sources throughout the entire monitoring period. The analyses indicate that elevated concentrations of *E. coli* are not indicative of the presence of *Cryptosporidium* in surface water. Both *E. coli* and turbidity are poor surrogates for occurrence of *Cryptosporidium* at treatment plant intakes (Nieminski et al 2010).

Instead of routine monitoring of *Giardia* and *Cryptosporidium* in treated drinking-waters, the DWSNZ require that water treatment performance is monitored using a variety of operational criteria as a substitute to protozoa testing in order to demonstrate compliance with the *Giardia* and *Cryptosporidium* standard for total pathogenic protozoa. See Chapter 8.

5.3.4 Pathogenic viral indicators

It has been suggested that a significant amount of viral disease in communities may be the result of low-level viral contamination of water. If this is the case, then viral indicators need to be sought. Epidemiological evidence is not clear on this point, though there is often a suggestion that recognised outbreaks of waterborne viral disease are generally associated with the presence of bacterial indicators in water.

Viral studies usually use polio viruses (mostly derived from live oral vaccines) as indicators because of their continual seeding into the aquatic environment and their relative resistance to accepted levels of disinfection. However, they may not be adequate indicators for all viral diseases likely to be associated with contaminated water.

The understanding of the fate and behaviour of viruses in drinking-water systems is not yet sufficiently advanced to enable an explicit standard to be made. Refer to Chapter 7 for further information about viruses. Sections 5.3.7 and 5.4.4 also mention viruses/coliphages.

The bacteriophages (viruses that infect bacteria) of *E. coli* have been proposed as indicators of the survival of viral pathogens. Phages are excreted by a certain percentage of humans and animals all the time whereas viruses are excreted only by infected individuals for a short period of time. The excretion of viruses depends heavily on variables such as the epidemiology of various viruses, outbreaks of viral infections and vaccination against viral infections. Consequently there is no direct correlation between numbers of phages and viruses excreted by humans. Enteric viruses have been detected in water environments in the absence of coliphages (WHO 2001, Chapter 13).

Human enteric viruses associated with waterborne diseases are excreted almost exclusively by humans. Phages used as models/surrogates in water quality assessment are excreted by humans and animals. In fact, the faeces of animals such as cows and pigs generally contain higher densities of coliphages than that of humans, and the percentage of many animals that excrete phages tends to be higher than for humans. Differences between phages and enteric viruses are also reflected by differences in the efficiency of adsorption-elution techniques for their recovery (from Chapter 13, WHO 2001).

International collaboration is now leading to meaningful, universally accepted guidelines for the recovery and detection of phages in water environments, such as those produced by the International Organisation for Standardisation (ISO).

5.3.5 Secondary bacterial indicators

Faecal streptococci are a species of gram-positive cocci belonging to two genera, *Enterococcus* and *Streptococcus*. The relevant species are linked by common biochemical antigenic properties and are found in the faeces of humans and other animals. Many will grow in 6.5 percent sodium chloride solutions and at 45°C. WHO (2001) defines these (and discusses them further) in Chapter 13 as:

Faecal streptococci (FS) are Gram-positive, catalase-negative cocci from selective media (eg, azide dextrose broth or m-Enterococcus agar) that grow on bile aesculin agar and at 45°C, belonging to the genera *Enterococcus* and *Streptococcus* possessing the Lancefield group D antigen.

Enterococci include all faecal streptococci that grow at pH 9.6, 10°C and 45°C and in 6.5 percent NaCl. Nearly all are members of the genus *Enterococcus*, and also fulfil the following criteria: resistance to 60°C for 30 minutes and ability to reduce 0.1 percent methylene blue. The enterococci are a subset of faecal streptococci that grow under the conditions outlined above. Alternatively, enterococci can be identified directly as micro-organisms capable of aerobic growth at 44±0.5°C and of hydrolysing 4-methylumbelliferyl-β-D-glucoside (MUD, detecting β-glucosidase activity by blue fluorescence at 366nm), in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyltetrazolium chloride (TTC, which is reduced to the red formazan) in the specified medium (ISO/FDIS 7899-1 1998).

The enterococci test is increasingly replacing faecal streptococci as an indicator, as enterococci are clearly of faecal origin from warm-blooded animals (OECD/WHO 2003). It is often used in place of *E. coli* when monitoring the quality of seawater, including in New Zealand. In Europe small water supplies are governed by the Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. In England that legislation was incorporated into The Private Water Supplies Regulations 2009, which requires both Enterococci and *Escherichia coli* to be absent in 100 mL.

Enterococcus and *Streptococcus* occur regularly in faeces but not in such numbers, or so invariably, as *E. coli*. Certain species of *Enterococcus* can be found free-living in soil and thus their presence in water may be from a non-faecal source (Leclerc et al 1996; Manero and Blanch 1999). Thus while the specificity of this indicator is acceptable, it is less sensitive than *E. coli*. Its persistence in water is less than that of *E. coli*, and it is generally a poorer indicator of the presence of certain pathogens that die off slowly (eg, viruses).

Until bifidobacteria were suggested as faecal indicators, *Clostridium perfringens* was the only obligately anaerobic, enteric micro-organism seriously considered as a possible indicator of the sanitary quality of water. *Clostridium perfringens* is a spore-former and may be highly persistent in the aquatic environment, and it can be found frequently in environmental material, eg, in soil. So as an indicator, its application is limited to specific circumstances, and the interpretation of its significance is often difficult.

Despite the first isolation of bifidobacteria in the late 1800s and very high numbers in human faeces (11 percent of culturable bacteria), their oxygen sensitivity (as with most other strict anaerobes) has limited their role as useful faecal indicators in waters (WHO 2001).

An alternative, the H₂S test, to measure *E. coli* has been suggested by the WHO (2002). This test uses less expensive equipment, and requires less operator skill, so will be particularly attractive in poorer countries. Some versions use ambient temperatures for incubation so it could be a useful test in remote areas or during emergencies, eg, when there is no electricity. While the H₂S producing organisms may not all be coliforms, they are organisms typically associated with the intestinal tracts of warm-blooded animals.

5.3.6 Indicators of general quality

The heterotrophic plate count (HPC) method uses a standard culture technique to grow a wide range of aerobic mesophilic bacteria on a non-selective agar medium. The bacteria that grow in these conditions are almost always present in drinking-water and are therefore an indicator of overall cleanliness of the water supply system.

The WHO (2003a) published *Heterotrophic Plate Counts and Drinking-water Safety: The significance of HPCs for water quality and the human health*. A quote from Chapter 1 follows:

HPC testing has a long history of use in water microbiology. At the end of the 19th century, HPC tests were employed as indicators of the proper functioning of processes (and of sand filtration in particular) and thereby as indirect indicators of water safety. Use as a safety indicator declined with the adoption of specific faecal indicator bacteria during the 20th century. HPC measurements nevertheless continue to figure in water regulations or guidelines in many countries. HPC 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
- 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 the water.

Piped water systems of large buildings may incur greater growth than encountered elsewhere (because of storage tanks, extensive internal distribution networks and temperature-related growth). The principal health concerns in these networks are cross-connections and growth of *Legionella* bacteria, which are not detected by the HPC test procedures.

Colony counts (heterotrophic plate counts) can be a useful indicator to monitor operational performance. They represent bacteria that have entered the water supply or that have survived the treatment processes and are able to grow and produce viable colonies on the growth medium used for the tests, under specified conditions (eg, incubation time, temperature, laboratory technique). Not all bacteria in water will, however, grow under these test conditions. It is usually not the absolute concentration of HPC but a change in HPC concentration that is useful to the water industry.

In groundwater the biologically available organic carbon and thus heterotrophic metabolism are often limited. Thus HPC levels in groundwater are generally low and stable over time. Groundwater affected by surface effects may show variable or higher than expected HPC counts; total or faecal coliform testing can also be helpful. These tests can also be useful for investigating parts of the distribution system that may experience biofilm growth. Health Canada (2012) contains some helpful information.

Colony counts are usually determined after incubation at 20–22°C or at 35–37°C. Plate counts of bacteria able to grow at 20–22°C or at 35–37°C in a standard nutrient medium (heterotrophic counts) may be relevant to the nutrient status of the water but not the faecal pollution. In general, the practice in New Zealand is to use 22°C and 35°C. When reporting results it is important to include the test conditions.

The count at 22°C will favour many environmental organisms. It has little sanitary value but is useful in assessing the efficiency of water treatment, specifically the processes of coagulation, filtration and disinfection, each of which reduces bacterial numbers. It may be used to assess the cleanliness and integrity of the distribution system and the suitability of water for manufacturing food and drink where a high count may lead to spoilage.

The count at 35°C will include some environmental organisms and also some from faeces. A significant increase above normal in this count may be an early sign of contamination. For this reason, in many cases, the only heterotrophic plate count performed is that at 35°C.

Colony counts should only be used as an adjunct to routine monitoring for *E. coli*. When a large number of organisms is detected, some form of remedial action is recommended, such as cleaning of storage tanks or inspection and repair or disinfection of the reticulation system. It may be useful to identify the dominant organisms present, particularly where there is persistent bacterial growth in a reticulation system.

These counts are a useful measure of the general quality of a water supply and to some extent of the standard of treatment or the microbial condition of the distribution system. The numbers should fall substantially during treatment processes. Generally, well-maintained water supplies should have little difficulty in obtaining samples with colony counts as follows (using the pour-plate technique with standard plate count agar at 35°C for 48 hours):

- disinfected supply < 100 per mL colony-forming units
- undisinfected supply < 500 per mL colony-forming units.

New Zealand experience indicates that in a well-run large municipal supply the following counts can be readily attained:

- disinfected supply < 20 per mL colony-forming units
- undisinfected supply < 200 per mL colony-forming units.

It is not uncommon to find >1000 colony-forming units per mL in good quality drinking-water when incubating at 22°C for seven days.

5.3.7 Indicators of effectiveness of treatment

The effectiveness of treatment of raw water can be measured by following the progressive lowering of counts of coliforms, heterotrophic plate counts or *E. coli* following successive stages of treatment throughout the plant, leading, in the final stages, to their complete removal. Any viable bacterium detected after appropriate exposure to disinfectants provides a clear warning of a failure of treatment and therefore of a potential hazard to consumers.

When monitoring *E. coli*, most water suppliers test 100 mL samples, mainly because the MAV is expressed as <1 per 100 mL. It is tempting to think that a zero result means 'absence of' *E. coli*. Very large volumes of clean drinking-water can be tested using membrane filtration techniques. Results of say 1 per 10 L may be possible, ie, reporting 0.01 *E. coli* per 100 mL. Testing large volumes can be very useful when investigating treatment or distribution problems, particularly spasmodic problems, see section 6.3.3 in Chapter 6: Bacterial Compliance.

Other indicator systems such as faecal streptococci and, rarely, *Clostridium perfringens* spores, may also be used as they are particularly persistent to disinfection and so tend to indicate the efficacy of filtration processes.

While coliphages are common in sewage, somatic coliphage and F-specific RNA coliphages are found in low numbers in faeces so their presence in water is primarily as an index of sewage pollution rather than faecal contamination in drinking water (IAWPRC 1991). Nonetheless, a variety of coliphages (eg, F-RNA coliphage, MS2) have shown potential as model organisms for monitoring virus removal in drinking water treatment plants (Jofre et al 1995).

5.4 Waterborne pathogens

5.4.1 Testing for specific pathogens

A review of waterborne outbreaks in four Nordic countries during 1998–2012 showed caliciviruses (51 of 175 outbreaks) being the most common, followed by *Campylobacter* (36 outbreaks) and pathogenic *E. coli* (8 outbreaks). Among the calicivirus outbreaks, norovirus was identified in 86 percent (44 of 51 outbreaks), while in the remainder the specific type of calicivirus was not identified. Other identified pathogens included *Giardia* (5 outbreaks), *Cryptosporidium* (4), *Salmonella* (2), rotavirus (1) and *Shigella* (1 outbreak). There were also nine outbreaks where multiple pathogens were detected in patient specimens and/or water supplies (Guzman-Herrador et al 2015).

Tests for the presence of specific pathogenic organisms such as *Salmonella*, *Campylobacter* or *Cryptosporidium* are appropriate for special investigations and in the face of evidence of outbreaks of waterborne disease. These tests are not recommended for routine monitoring of water supplies, due to the cost, complexity of testing, and perhaps the interpretation of results. Under special circumstances they could become Priority 2 determinands.

Promising new techniques based on amplifying and identifying the specific gene or genetic fragments, for example, polymerase chain reaction (PCR), are revolutionising the monitoring and investigation of drinking-water supplies. They show particular potential for detection of pathogens. However, the technology has not yet been developed sufficiently for it to replace traditional methods and the costs in general are very high.

The use of monoclonal antibody techniques probably shows the most promising applications but this in addition still requires some development and application of cost factors to enable large-scale regular testing to be carried out as is required in the monitoring of water supplies.

Waterborne pathogens are discussed in AWWA (1999).

WHO (2011a) includes useful information (Annex 2: Potential biological and chemical hazards in building water supplies) about the incubation period, clinical symptoms and source of exposure for many bacteria and viruses. Covered are *Acinetobacter*, *Campylobacter*, *Escherichia coli* (enteroinvasive or enterotoxigenic), *E.coli* O157:H7 (enterohaemorrhagic), *Klebsiella* and other Gram-negative bacteria (*Serratia marcesans*, *Stentrophomonas maltophilia*, *Aeromonas*, *Burkholderia cepacia*, *Enterobacter*), *Legionella* spp, non-tuberculous or atypical *Mycobacterium* spp, (*M. gordonae*, *M. kansasii*, *M. marinum*, *M. xenopi*, *M. scrofulaceum*, *M. avium*, *M. chelonae*, *M. intracellulare* and *M. fortuitum*), *Pseudomonas aeruginosa*, *Salmonella*, *Salmonella* Typhi, *Shigella*, *Vibrio cholerae* 01 or 0139, adenoviruses, calicivirus: *Norovirus* and *Sapovirus*, enteroviruses, hepatitis A virus, rotavirus, *Cyclospora cayetanensis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia lamblia*, and some chemicals.

Health Canada (2013) issued their Guidance on Waterborne Bacterial Pathogens.

Section 2.5 in the chapter on management covers quantitative microbial risk assessment (QMRA). The QMRA method provides a framework for pathogenic micro-organisms that is analogous to the risk-based approach to setting guideline values for chemicals. It is based on WHO (2016).

Table 5.1 is a summary of the major waterborne pathogens and their significance in water supplies.

Table 5.1: Waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Persistence in water supplies ^a	Resistance to chlorine ^b	Relative infectivity ^c	Important animal source
Bacteria					
<i>Burkholderia pseudomallei</i>	High	May multiply	Low	Low	No
<i>Campylobacter jejuni</i> , <i>C. coli</i>	High	Moderate	Low	Moderate	Yes
<i>Escherichia coli</i> , Pathogenic ^d	High	Moderate	Low	Low	Yes
<i>E. coli</i> – Enterohaemorrhagic	High	Moderate	Low	High	Yes
<i>Francisella tularensis</i>	High	Long	Moderate	High	Yes
<i>Legionella</i> spp.	High	May multiply	Low	Moderate	No
<i>Leptospira</i>	High	Long	Low	High	Yes
Mycobacteria (non-tuberculous)	Low	May multiply	High	Low	No
<i>Salmonella</i> Typhi	High	Moderate	Low	Low	No
Other salmonellae	High	May multiply	Low	Low	Yes
<i>Shigella</i> spp.	High	Short	Low	High	No
<i>Vibrio cholerae</i>	High	Short to long	Low	Low	No
<i>Yersinia enterocolitica</i>	High	Long	Low	Low	Yes
Viruses					
Adenoviruses	Moderate	Long	Moderate	High	No
Astroviruses	Moderate	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A	High	Long	Moderate	High	No
Hepatitis E	High	Long	Moderate	High	Potentially
Noroviruses and Sapoviruses	High	Long	Moderate	High	Potentially
Rotavirus	High	Long	Moderate	High	No
Sapoviruses	High	Long	Moderate	High	Potentially
Protozoa					
<i>Acanthamoeba</i> spp.	High	May multiply	High	High	No
<i>Cryptosporidium parvum</i>	High	Long	High	High	Yes
<i>Cyclospora cayetanensis</i>	High	Long	High	High	No
<i>Entamoeba histolytica</i>	High	Moderate	High	High	No
<i>Giardia intestinalis</i>	High	Moderate	High	High	Yes
<i>Naegleria fowleri</i>	High	May multiply ^f	Low	Moderate	No
<i>Toxoplasma gondii</i>	High	Long	High	High	Yes
Helminths					
<i>Dracunculus medinensis</i>	High	Moderate	Moderate	High	No
<i>Schistosoma</i> spp.	High	Short	Moderate	High	Yes

Source: Ex WHO 2011 (Table 7.1).

- a This table contains pathogens for which there is some evidence of health significance related to their occurrence in drinking-water supplies. More information on these and other pathogens is presented in chapter 11 (WHO).
- b Health significance relates to the incidence and severity of disease, including association with outbreaks.
- c Detection period for infective stage in water at 20°C: short, up to one week; moderate, one week to one month; long, over one month.

- d When the infective stage is freely suspended in water treated at conventional doses and contact times and pH between 7 and 8. Low means 99% inactivation at 20°C generally in <1 minute, moderate 1–30 minutes and high >30 minutes. It should be noted that organisms that survive and grow in biofilms, such as *Legionella* and mycobacteria, will be protected from chlorination.
- e From experiments with human volunteers, from epidemiological evidence and from experimental animal studies. High means infective doses can be 1–10² organisms or particles, moderate 10²–10⁴ and low > 10⁴.
- f Includes enteropathogenic, enterotoxigenic, enteroinvasive, diffusely adherent and enteroaggregative.
- g *Vibrio cholerae* may persist for long periods in association with copepods and other aquatic organisms.
- h In warm water.

5.4.2 Bacterial pathogens from faecal contamination

The human bacterial pathogens that can be transmitted orally by drinking-water and which present a serious risk of disease include *Salmonella* spp, *Shigella* spp, enteropathogenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Campylobacter coli*.

While typical waterborne pathogens are able to persist in drinking-water, most do not grow or proliferate in water. Micro-organisms (eg, *E. coli* and *Campylobacter*) can accumulate in sediments and be mobilised with increased water flow or water flow fluctuations.

After being excreted in faeces from the body of their host, bacterial pathogens gradually lose viability and the ability to infect. The rate of decay varies with different bacteria. It is usually exponential and after a certain period a pathogen will become undetectable. The most common waterborne pathogens are those that are highly infectious or highly resistant to decay outside the body. Pathogens with a low persistence, ie, those that do not survive long outside the host, must rapidly find a new host and are more likely to be spread by person-to-person contact or by faulty personal or food hygiene than by drinking-water.

If present in drinking-water, faecal contamination and hence the related waterborne bacterial pathogens are likely to be dispersed widely and rapidly. Outbreaks of waterborne disease are therefore frequently characterised by an infection across a whole community.

Although bacterial contamination normally can be thought of as a short-term event, there are examples of long-term after effects. One example was at Queenstown (see Chapter 1). Another occurred at Walkerton in 2000 (also mentioned in Chapter 1). A seven-year follow-up study of Walkerton residents showed that many continued to experience long-term adverse health effects. One of the most severe complications of *E. coli* O157 infection is HUS (haemolytic uremic syndrome) and survivors of HUS may have permanent kidney damage, potentially requiring a kidney transplant later in life. Therefore there has been a particular focus on children who suffered HUS during the outbreak. The Year 3 follow-up of children who had HUS showed that 32 percent had microalbuminuria (trace amounts of albumin in the urine) compared to 5 percent of children who had not had HUS. However by Year 5 these rates had dropped to 20 percent and 3 percent respectively, and the Year 7 follow-up showed no worsening of the condition or any overt kidney disease in the children. These findings were more

favourable than had been predicted from previous literature on HUS, but continued monitoring of kidney function is still deemed desirable in HUS survivors. Also, it was found that among those who had experienced severe gastroenteritis during the outbreak, 36 percent had developed Irritable Bowel Syndrome, compared to 28 percent of those who had moderate gastroenteritis and 10 percent of those who had not been ill (WQRA 2010).

5.4.3 Bacterial pathogens growing in the water supply

Various bacteria that occur naturally in the environment may cause disease opportunistically in humans. Those most at risk are the elderly, the very young, people with burns or excessive wounds, those undergoing immunosuppressive therapy, or those with acquired immunodeficiency syndrome (AIDS). Water used by such people for drinking or bathing, if it contains large numbers of these opportunistic pathogens, can occasionally produce infections of the skin and of the mucous membranes of the eye, ear, nose and throat. Examples of such agents are *Pseudomonas aeruginosa*, species of *Klebsiella* and *Aeromonas*, and certain slow-growing mycobacteria such as *Mycobacterium avium*.

The World Health Organization (WHO 2004c) has published an excellent book bringing together a great deal of what is currently known about the mycobacteria. Refer also to the Datasheet in Volume 3 of these Guidelines.

Legionellosis, caused by the bacterium *Legionella pneumophila*, can be a serious illness. It results from inhalation of aerosols in which the causative organisms have been able to multiply because of warm conditions and the presence of nutrients. The WHO produced Fact Sheet #285 (February 2005) on legionellosis, available on www.who.int/entity/mediacentre/factsheets/fs285/en/. This was updated in book form (WHO 2007). Refer also to the Datasheet in Volume 3 of these Guidelines.

CRC (2009) covers the opportunistic growth of *Aeromonas hydrophilla* and *Burkholderia pseudomallei* in the distribution system.

5.4.4 Viruses

Viruses are among the smallest and more resilient of infectious agents. In essence they are nucleic acid molecules that can enter cells and replicate in them. The virus particle consists of a genome, either RNA or DNA, surrounded by a protective protein shell, the capsid. Frequently this shell is enclosed in an envelope that contains both protein and lipid. Viruses replicate only inside specific host cells and they are absolutely dependent on the host cells' synthetic and energy-yielding apparatus for producing new virus particles. Thus viruses are not known to multiply in the environment.

The viruses of most significance in relation to drinking-water are those that multiply in human gut tissues and are excreted in large numbers in the faeces and urine of infected individuals. Although they cannot multiply outside the tissues of infected hosts, some enteric viruses can survive in the environment and remain infective for long periods. Human enteric viruses occur in water largely as a result of contamination by sewage and human excreta. The numbers of viruses present and their species distribution will reflect the extent that the population is carrying them.

The different analytical methods currently available can also lead to wide variations in numbers of viruses found in sewage.

Sewage treatment may reduce numbers by ten to ten thousand-fold, depending on the nature and degree of treatment. However, even tertiary treatment of sewage will not eliminate all viruses. As sewage mixes with the receiving water, viruses are carried downstream and the length of time they remain detectable depends on temperature, their degree of absorption into sediments, penetration of sunlight into the water, pH and other factors. Consequently, enteric viruses can be found in sewage-polluted water at the intakes to water treatment plants.

Proper treatment and disinfection, however, should produce drinking-water that is essentially virus-free. The occurrence of human viruses in source waters and the effectiveness of various drinking water treatment approaches are discussed in Chapter 7: Virological Compliance.

5.4.5 Pathogenic protozoa

The majority of free-living protozoa (FLP) are aquatic organisms of no significance to public health. Protozoa can be differentiated into three general types: ciliates, flagellates and amoebae. They generally feed on other micro-organisms such as bacteria, algae, cyanobacteria, or other protozoa.

FLP are ubiquitous where bacteria, their main food source, are found. That they are present in distributed water is not a surprise, and it would be logical that the types and numbers of active FLP in distributed water relate directly to the quality and quantity of their food source present in infrastructure biofilms, and perhaps to a lesser extent in the bulk water. Over many years, water has been implicated as a source of opportunistic pathogens in healthcare and community disease outbreaks, particularly for the opportunistic respiratory pathogens *Legionella* spp. and *Mycobacterium* spp. Epidemiological data, along with laboratory reports of pathogens resisting digestion by amoebae, replicating inside amoebae, and dispersed by amoebae, have raised the spectre of FLP as Trojan horses delivering pathogens throughout distribution networks, and hence being real villains in the battle to provide safe drinking water. On the other hand, FLP almost certainly provide significant benefits to drinking water by removing/digesting bacteria in biofilms and bulk water. The issue of bacterial-FLP interactions is especially important for large volume water users and in recycled and warm water systems that are most implicated in the epidemiology of legionellae and mycobacterial infections (DWI 2015).

Protozoa likely to be found in drinking-waters and of public health significance can be grouped into those of enteric or environmental origin:

- enteric protozoa occur widely as parasites in the intestine of humans and other mammals and involve at least two stages (trophozoite and (oo)cyst) in their life cycle (see section 5.4.5.1)
- some free-living protozoa are opportunistic pathogens in humans and are responsible for some serious diseases of the nervous system and the eye (see section 5.4.5.2)
- an occurrence survey reported in DWI (2015) confirmed the ubiquity of FLP in all types of water tested. It identified a wide variety of FLP from all systems of distributed water. These included acanthamoebae and hartmannellae, but the most commonly observed and isolated FLP in all systems were cercomonads. These small FLP are flagellates with an amoeboid stage that are avid consumers of bacteria in bulk water as well as in biofilms and belong to the most common group of predators in soils and waters. They are not implicated in the epidemiology of either legionellae or mycobacteria and might be considered as beneficial consumers of bacteria including biofilms.

5.4.5.1 Enteric parasitic protozoa

The most prevalent enteric protozoal parasites associated with waterborne disease include *Giardia intestinalis*, *Cryptosporidium hominis* and *C. parvum*. *Toxoplasma gondii*, *Entamoeba histolytica*, and *Balantidium coli* have also been associated with waterborne outbreaks. *Cryptosporidium*, a coccidian protozoal parasite, was only identified as a human pathogen in 1976. It can cause diarrhoeal illness in the immunocompetent but with dire consequences in immunocompromised individuals. The disease is endemic throughout the world. The incidence of infection is also high, illustrated by the finding that in the USA 20 percent of young adults have evidence of infection by *Cryptosporidium*. This rate was over 90 percent amongst children under one year old in a Brazilian shanty town (quoted in WHO 2003(b): *Emerging issues in water and infectious diseases*). Analysis of a Swedish outbreak of *Cryptosporidium hominis* showed that nearly half the inhabitants were infected (Widerström et al 2014).

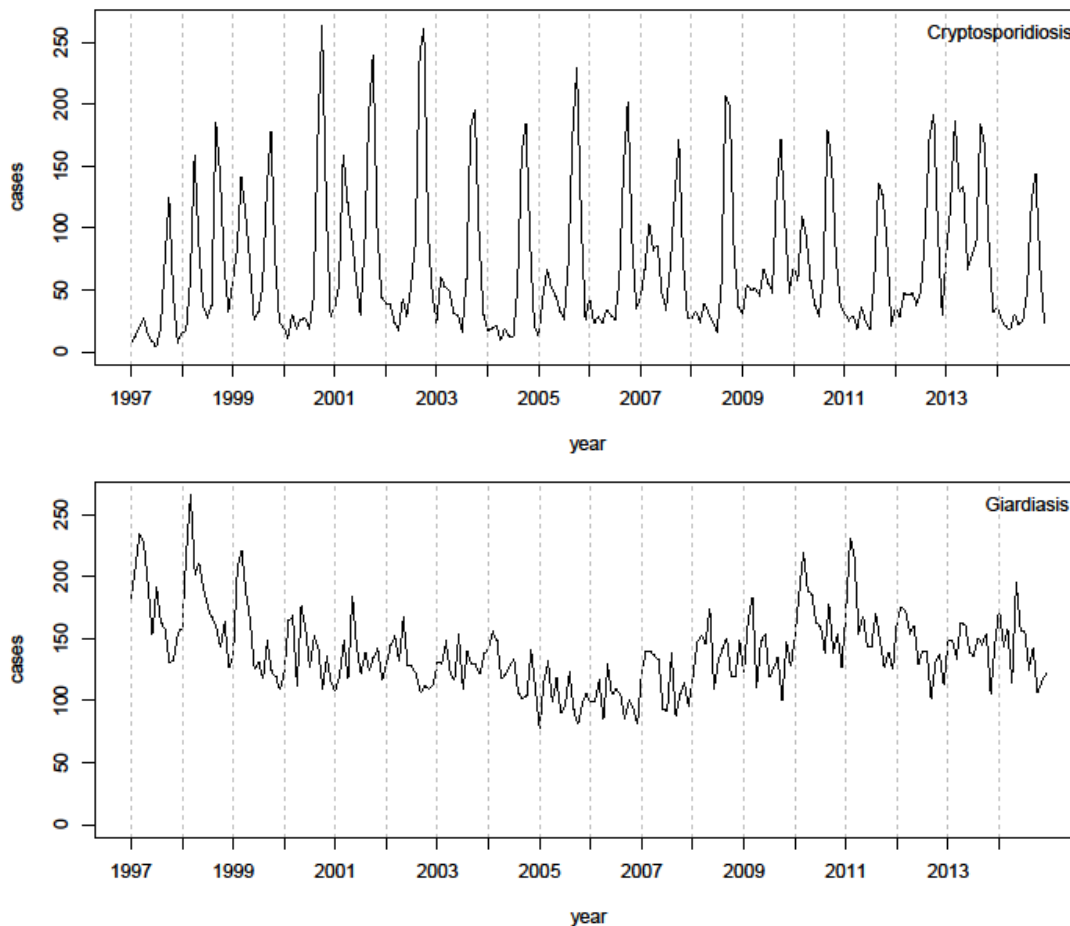
Epidemiological studies often report cases as incidence per (say 1000) population. Sometimes prevalence is used, being a better indicator of disease burden due to the longer duration of cryptosporidiosis. Incidence is defined as the number of incidents and prevalence as the number of days with diarrhoea in a given time period.

Other emerging protozoal parasites of concern include *Cyclospora cayetanensis* and *Isospora belli*. Microsporidia are also emerging pathogens of public health importance and, although recently classified as fungi, their fate and behaviour in water can be similar to that of the parasitic protozoa.

The transmissive/infective stages of these parasites are cysts (*Giardia*, *Balantidium*, *Entamoeba*), oocysts (*Cryptosporidium*, *Cyclospora*, *Isospora*, *Toxoplasma*) or spores (Microsporidia). These forms are excreted in faeces of infected hosts as fully infectious agents (*Giardia*, *Cryptosporidium*, Microsporidia, *Balantidium*) or as immature stages (*Cyclospora*, *Isospora*, *Toxoplasma*) requiring a short period of development in the environment to reach the mature stage. They can get into drinking-water supplies by

contamination with human or animal faeces. All are widely dispersed and have been associated with outbreaks of infection resulting from drinking contaminated water, see datasheets (Volume 3).

Giardia and *Cryptosporidium* are the most widely reported causes of waterborne parasitic disease in developed countries. In New Zealand giardiasis and cryptosporidiosis are the third and fourth most commonly notified diseases, respectively. The following plots show the temporal trends in cryptosporidiosis and giardiasis in New Zealand; data are from ESR, and were included in a report from Massey University to the MoH in 2016.



These organisms cause varying degrees of enteric condition that can be manifested from violent diarrhoea symptoms to being asymptomatic. Immunocompetent people typically recover without intervention. Dehydration is the most frequent symptom requiring attention in severely affected individuals.

The (oo)cysts of *Giardia* and *Cryptosporidium* are widespread in environmental waters of New Zealand especially in water from areas of intensive stock farming and they can occur in high concentrations. A recent study of measures that can be taken to reduce the numbers of (oo)cysts in water appears in Victorian Department of Health (2011). Coliforms, faecal coliforms, and *E. coli* have been shown to be poor indicators of the presence of pathogenic protozoa in drinking-water, so *Giardia* and *Cryptosporidium* are considered as Priority 1 determinands in the DWSNZ.

The organisms can survive for a long time in cold water. Medema et al (1997) conducted bench scale studies of the influence of temperature on the die-off rate of *Cryptosporidium* oocysts. Die-off rates were determined at 5°C and 15°C. Both excystation and vital dye staining were used to determine oocyst viability. At 5°C, the die-off rate was 0.010 log₁₀/day, assuming first order kinetics. This translates to 0.5 log reduction at 50 days. At 15°C, the die-off rate in natural river water approximately doubled to 0.024 log₁₀/day (excystation) and 0.018 log₁₀/day (dye staining).

Sattar et al (1999) evaluated factors impacting *Cryptosporidium* and *Giardia* survival. Microtubes containing untreated river water were inoculated with purified oocysts and cysts. Samples were incubated at temperatures ranging from 4 to 30°C; viability of oocysts and cysts was measured by excystation. At 20°C and 30°C, reductions in viable *Cryptosporidium* oocysts ranged from approximately 0.6 to 2.0 log after 30 days. Relatively little inactivation took place when oocysts were incubated at 4°C.

The significance of waterborne transmission in New Zealand is still not clear. The prevalence of *Giardia* and *Cryptosporidium* infection in livestock, domestic, and feral animals suggests a significant reservoir for zoonotic transmission. However, information is needed on the presence of human and animal specific genotypes in water in order to clarify the relative importance of human or animal derived waterborne infections. The datasheets provide further detailed descriptions of the enteric protozoa. Chapter 8: Protozoa Compliance also provides further information relating to *Cryptosporidium*.

A thorough discussion on the impact of waterborne *Giardia* and *Cryptosporidium* internationally appears in WHO (2012, see Chapter 2 in particular).

5.4.5.2 Opportunistically pathogenic free-living protozoa

Free-living protozoa (FLP) are numerous in open surface waters including water supply sources but greatest numbers can be found in nutrient enriched environments where their bacterivorous feeding activities are of great benefit, eg, in biological wastewater treatment systems. FLP are ubiquitous in aquatic environments with a wide tolerance to environmental conditions ranging from geothermal waters, thermally polluted waters, to water distribution pipes.

The most well-known free-living, opportunistically pathogenic protozoa are the free-living amoebae, *Naegleria*, *Acanthamoeba* and more recently *Balamuthia*, which cause cerebral or corneal diseases. Infection is opportunistic and usually associated with recreational bathing-water contact or domestic use of water other than drinking. The occurrence of *Naegleria* and *Acanthamoeba* in water is not necessarily associated with faecal contamination.

Naegleria spp have been responsible for nine recorded deaths in New Zealand since 1968: five cases were confirmed as *N. fowleri* (Cursons et al 2003). Infection by *N. fowleri* is strictly waterborne and can cause a cerebral infection known as primary amoebic meningoencephalitis (PAM), a rare but usually fatal condition. All cases of death resulting from *Naegleria* infections in New Zealand have been associated with swimming in geothermal pools or rivers receiving geothermal waters. These deaths led to more control of geothermal tourist areas with specific advice on pool care including

exclusion of soil from the water sources and pools, filtration, disinfection, and rate of water turnover. CRC (2009) covers the opportunistic growth of *Naegleria fowleri* in the distribution system.

Acanthamoeba species are commonly found in soil and water and cause diseases of the central nervous system (granulomatous amoebic encephalitis GAE) and a disease of the eye called keratitis. GAE is invariably fatal but no cases of GAE have been reported in New Zealand to date. However, although GAE is not associated with swimming, a species known to cause the disease in humans, *Acanthamoeba culbertsoni*, has been isolated from New Zealand thermal waters. In contrast, amoebic keratitis does occur in New Zealand and there have been eight reported cases since 1995 (Ellis-Pegler 2003). The disease has been associated with people who wear soft contact lenses. *Acanthamoeba* spp has been isolated from contact lens washing fluid on several occasions.

Balamuthia mandrillalis causes GAE in humans and other animals. Little is known about the ecology of *Balamuthia*. They are present in soil and possibly water but there is no obvious association of waterborne transmission with those cases reported of *Balamuthia* infection (Schuster and Visvesvara 2004).

Both *Acanthamoeba* and *Naegleria* as well as other free-living amoebae are known to ingest bacterial pathogens such as *Legionella* (Brown and Barker 1999). *Legionella* spp. have adapted to replicate inside amoebae and thus the amoebae containing *Legionella* within their vacuoles can act as vectors for packets of *Legionella* infection.

Free-living amoebae can be found in source water and isolated from water distribution pipes. Their presence is usually associated with thermally polluted waters (eg, *Naegleria*) or inadequate disinfection of treated supplies.

Information is increasing on emerging enteric protozoa such as *Blastocystis* spp, *Dientamoeba fragilis* and *Endolimax nana*. Researchers have recently agreed that *Blastocystis* spp are pathogenic, causing intestinal disorders. Datasheets have been prepared for:

- *Acanthamoeba* sp.
- *Balantidium coli*
- *Blastocystis*
- *Cryptosporidium*
- *Cyclospora*
- *Entamoeba histolytica*
- *Giardia intestinalis* (*lamblia*)
- *Hartmanella vermiformis*
- *Isospora*
- *Microsporidia*
- *Naegleria fowleri*
- *Toxoplasma*.

5.4.6 Helminths

A variety of human and zoonotic helminth (worm parasite) diseases have been found in New Zealand, including *Fasciola*, an economically important zoonotic helminth parasite in cattle.

However, reports of helminth infections in the New Zealand human population occur rarely; infection is most often associated with recent immigrants or travellers returning from areas where disease is endemic.

Whilst infective helminth parasites should not be present in drinking-water, the low prevalence of helminth infection in New Zealand indicates that a Maximum Acceptable Value (MAV) in the DWSNZ is impractical for these disease organisms. Physical treatment processes used for the removal of protozoal parasites during drinking-water treatment should also remove helminths if these are present in the source water, as they are generally excluded by their size. Helminth infective stages are typically larger and heavier than protozoal (oo)cysts (>20 µm). Care is needed with microscopic identification of organisms from water supplies as adult worms and larvae are more likely to belong to free-living nematode groups such as *Turbatrix* or *Rhabditis*.

The majority of helminths are not typically transmitted through drinking-water. Exceptions are *Dracunculus* (Guinea worm) and in some endemic situations, *Fasciola* spp (liver fluke). Most helminth infections are acquired through direct faecal-oral contact (eg, *Enterobius*), ingestion of faecally contaminated food (eg, *Ascaris*, *Trichuris*), or through contact with contaminated soil or surface water (eg, hookworm, *Schistosoma* spp). However, helminth parasites can produce large numbers of transmissive stages (infective egg or larvae) that can sometimes be found in water, and there have been reports of incidental disease transmission due to consumption of contaminated water.

Although no MAV is prescribed for helminths in the DWSNZ, precautions should be taken to protect source water supplies from zoonotic helminth contamination, particularly in rural communities where livestock may be considered a viable reservoir and to ensure security of water during and post treatment.

Further information is provided in the helminth and nematode datasheet.

5.4.7 Cyanobacteria (blue-green algae)

Cyanobacterial cells or colonies do not usually cause a health problem in drinking-water. They will have been removed from properly treated water. They can interfere with water treatment processes if in large numbers in the raw water, which may lead to other problems.

Their main health problem is the toxins that they can produce. This is discussed in Chapter 9: Cyanobacterial Compliance. See also the datasheets for cyanobacteria and for cyanotoxins.

5.4.8 Disease from waterborne pathogens

Drinking-water is an important source of infectious agents, particularly ones that cause enteric infections. Many of the great epidemics of history have been caused by faecal contamination of drinking-water. While person-to-person contact is equally important it is common for the population to indicate water as a source of disease.

The use of chemical disinfectants in water treatment usually results in the formation of chemical by-products. However, the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection efficacy not be compromised in attempting to control such by-products (WHO 2017, section 1.1.3, and Ch 3 of these Guidelines).

The significance of any particular organism varies with the disease caused under local water supply conditions. Not all individual members of any population will be susceptible to a pathogenic organism in the water. Waterborne infections will depend on the following:

- the concentration of any pathogenic organism in drinking-water
- the virulence of the strain
- the amount of water taken in by individuals which has not been adequately disinfected
- the minimum infectious dose (MID) of the pathogen in question
- the immune capability or susceptibility of individuals
- the incidence of the infection in a community, thus determining the number of enteric pathogens that would be shed into a potential receiving water source.

Paradoxically, if a particular infection has been received repeatedly from a contaminated water source the community may have become immune to some of the pathogens. This situation develops in countries where the number of pathogens in water is high and the standard of drinking-water is low. Conversely, visitors who drink from such water frequently become ill while the locals have far fewer ill effects. This is a population immunity but it is acquired at the cost of illness and death among children and is not considered acceptable in developed countries.

Where indicators of faecal pollution are found in water the population using that water may not be showing enteric disease. However, the presence of indicators of faecal pollution means that the likelihood of faecal pathogens occurring in that water is high. Continual vigilance is required to determine the need for treatment. If an infection occurs in a community, follow-up epidemiological studies should be carried out such that the source and route of infection can be determined and treated.

The diseases most frequently associated with water are enteric infections such as infectious diarrhoea. In many cases the disease is mild and self-limiting. However, a proportion of the population will suffer more severe outcomes. Several waterborne pathogens such as *Vibrio cholerae*, hepatitis E virus and *E. coli* O157:H7 have high mortality rates.

Since the 1990s evidence that microbial infections are associated with chronic disease started to accumulate. Several waterborne pathogens have been associated with serious sequelae (ie, severe illness or chronic or recurrent disease that appears long after the initial exposure to contaminated water). Examples of sequelae that could potentially be associated with acute waterborne include (WHO 2003c):

- diabetes which has been linked to Coxsackie B4 virus
- myocarditis which has been linked to echovirus
- Guillian-Barré syndrome associated with *Campylobacter* spp
- gastric cancer which has been linked to *Helicobacter* sp
- reactive arthritis which has been linked to *Klebsiella* sp.

5.5 Organisms causing problems other than disease

5.5.1 General

People in the western world demand water that is free from pathogenic organisms, has a pleasant taste and odour, is colourless and free from toxic chemical substances and corrosive properties. In addition to the pathogenic micro-organisms discussed, waters may also contain (AWWA 2004):

- cyanobacteria (other than those producing cyanotoxins)
- iron, manganese, sulphur and nitrifying bacteria
- actinomycetes and fungi
- large eukaryote organisms such as algae, crustacea, nematodes and protozoa
- insect larvae, eg, the midge and mosquito, usually in storage tanks.

Intermittent problems can occur when some or all of these organisms get into distribution systems where their maintenance or growth is encouraged. Excessive quantities of organic matter will usually support bacteria and fungi that in turn can support protozoa and crustacea. Many eukaryotes (cellular organisms with a nucleus, ie, not including viruses) and invertebrates can then feed on bacteria, fungi and protozoa.

Normally, treated water does not contain sufficient nutrient to support the growth of these organisms. However, the use of any form of filter bed inevitably retains large amounts of organic matter providing substrate and shelter. The filter is therefore an excellent growth medium for bacteria and other organisms higher up the food chain, which feed directly or indirectly on the bacteria. Filter backwashing is used to control this build-up.

Quantitative limits for this heterogeneous group of micro-organisms are not recommended.

5.5.2 Organisms causing taste and odour

Unpleasant tastes and smells can result from compounds that are produced by a range of eukaryote micro-organisms. These include protozoa and cyanobacteria. Protozoa from the amoebae and the ciliates are likely to produce odorous compounds. The amoeba of the genera *Vanella*, *Saccamoeba*, *Ripidomyxa*, all of which have bacterial symbionts in their cytoplasm, can produce geosmin or methylisoborneol (MIB). Other sources of the same compounds are cyanobacteria and the actinomycetes. Thus it seems likely that the symbionts of the protozoa are the source of these compounds. Free-swimming ciliates that contain algal symbionts (zoochlorella), including the genera *Stentor* and *Paramecium*, also contribute to odours in water if they reach high numbers.

See AWWA (2004) for discussion on biology, ecology, identification and control strategies. See EA (1998) and EA (2004) for some microbiological and chemical methods for assessing taste and odour problems caused by micro-organisms.

Refer to Chapter 18: Aesthetic Determinands for additional information.

5.5.3 Micro-organisms causing colour

Explosive growths of algae, cyanobacteria and other bacteria, can produce unwanted colour in water. *Botryococcus* is one such alga; it can develop high levels of hydrocarbons, which being lighter than water, can result in floating orange blooms. Algal blooms can be controlled by careful application of copper sulphate to the water. If pigmented organisms such as cyanobacteria and algae are crushed on filters to the extent that the cells are disrupted to release pigment, they can create colour. Micro-algae that pass through filters can cause additional turbidity problems.

5.5.4 Iron and manganese deposits due to bacteria

A wide range of micro-organisms (bacteria, fungi and sometimes protozoa) can be categorised as chemolithotrophic or photolithotrophic, that is, they are able to oxidise metal salts as part of their metabolism and in doing so cause problems by encrusting pipes, bores or filters. The elements involved in this are mostly iron, manganese and sulphur. The problems are usually identifiable by coloured deposits on equipment. In water containing ferrous or manganese salts, bacteria able to oxidise the compounds can form rust-coloured or black deposits in tanks and on the walls of pipes where the water flow is slow. If the water flow increases, the deposits may be detached to cause colour problems in domestic supplies. The slurry may also contain organic compounds that can break down and produce odour problems. AWWA (2004) discusses the biology, ecology, identification and control strategies.

Manganese-oxidising organisms are responsible for deposits in wells and water pipes and they can reduce yield, clog bore pipes, and reduce flow capacity in water pipes. They may also damage equipment for measuring water flows and produce black-coloured water that can stain in the domestic environment. Bacteria may attach to the deposits and if disturbed will increase a colony count of that water. Prevention is based on the elimination of manganese and iron from raw water if the concentration exceeds 0.1 mg/L iron and 0.04 mg/L manganese.

These chemolithotrophic organisms can impair water quality, but they are usually an intermittent problem and it is therefore not practical to monitor them routinely because of their diverse nature and unpredictable occurrence. Consumer concern or operational problems should be the stimulus for action.

Minimising the problems due to iron and manganese bacteria in groundwater is discussed in Chapter 3: Source Waters, section 3.2.3.4. Methods for removing iron and manganese from water are covered in Chapter 18: Aesthetic Considerations, section 18.3.

5.5.5 Corrosion resulting from iron and sulphur bacteria activity

Iron and steel pipes have always been at the mercy of activity by iron and sulphur bacteria. The iron and steel are nowadays often protected with cement or other coatings, or replaced by other materials such as PVC. Microbial corrosion of pipe materials results from:

- depletion of oxygen
- liberation of corrosive metabolites
- production of sulphuric acid
- inclusion of sulphate reducing bacteria in cathodic processes under anaerobic conditions.

Some micro-organisms in water indicate the corrosion of cast iron. Still other micro-organisms can be responsible for the biodeterioration of non-metallic materials such as plastic, rubber and pipelining materials which provide organic nutrients and encourage micro-organism growth, eg, *Pseudomonas aeruginosa* and some coliform organisms (not *E. coli*). Unchlorinated water, or water in which the chlorine demand is high and therefore the chlorine residual has disappeared, supports higher rates of attack than water in which chlorine is still detectable. See AWWA (2004) for further information.

5.5.6 Large numbers of micro-organisms

It must always be remembered that water prior to treatment contains a heterogeneous population of micro-organisms. These are mostly aerobic heterotrophic bacteria but their presence may mask the interpretation of a test based on coliform counts, or even restrict their growth, and thus yield false results. A case in point has been demonstrated with strains of *Aeromonas* that produce acid and gas with coliform media at 44.5°C. Control of such micro-organisms is by reduction of the organic carbon source. However, this may not be possible since most catchments have some organic runoff. The less nutrient-rich a water supply is, the better in terms of the reduction of possible micro-organism growth.

Shallow groundwaters often have large numbers of bacteria that grow on general bacteriological media at 37°C, often in the absence of faecal coliforms; total or presumptive coliforms can reach high numbers in these waters too. These are likely to include naturally-occurring soil bacteria. They may also be bacteria from septic tank overflow that has passed through an extensive drainage field where the faecal indicator bacteria have died out or been grazed by larger organisms.

See section 5.3.6 for a discussion on heterotrophic bacteria in water supplies, and Chapter 8: Protozoa Compliance, section 8.5: Challenge Testing, for how measuring the population density of these bacteria can be helpful.

Ainsworth (2004) and Bartrum (2003) discuss the occurrence and control of these bacteria.

5.5.7 Invertebrate inhabitants of water systems

Problems caused by the presence of invertebrate animals in large water supplies are uncommon in New Zealand. Poorly operated slow sand filters can give rise to large populations in the distribution system. Invertebrate animals may be present in shallow wells. Such animals derive their food from bacteria, algae and protozoa present in the water or on slime growths or deposits. They include freshwater sponges (the porifera), coelenterates, bryozoans, crustacea, molluscan bivalves, snails and nematodes. Freshwater mussels have caused major problems overseas by blocking pipes. The problems caused by many of these, and control strategies, are discussed in AWWA (2004).

For convenience, the types of organisms can be divided into two sections:

- free-swimming organisms such as the crustacean *Paracalliope* spp (freshwater hopper), *Paranephrops* (freshwater crayfish) and copepods
- animals that move along surfaces or are anchored to them such isopods (water lice), snails, and other molluscs, bryozoans, nematodes and the larvae of chironomids.

In warm weather, slow sand filters can discharge larvae of midges and mosquitoes (eg, *Chironomus* and *Culex* spp) into the water. Such filters may also be heavily infested with adults and larvae of the genus *Psychodidae*. If the top layer of a filter collapses, insect larvae and adults may be drawn down into the unfiltered water. Penetration of invertebrate animals into water supplies through a water filtration plant is much more likely to occur when low quality raw waters are used and where high rate filtration processes are used, especially if the filter depth is less than a metre, or where large sand grains (say more than three mm) have been chosen for the filters. Pre-chlorination destroys the invertebrates and thereby assists their removal by filtration, but promotes increased formation of chlorinated organic compounds. Maintaining chlorine residuals in the distribution system and regularly cleaning mains by flushing can usually prevent infestation.

The removal of isopods and other crustacea from the distribution system has been effected by permethrin treatment of water (in parts of the system that have been isolated) at an average dose not exceeding 0.01 mg/L for 24 to 48 hours. Water treated this way must not be discharged into watercourses as it will be toxic to fish and other aquatic life. Before using permethrin, the proposed procedures should be discussed with the Medical Officer of Health.

Note, however, that adding permethrin directly to drinking-water for public health purposes is not recommended by the WHO, as part of its policy to exclude the use of any pyrethroids for larviciding of mosquito vectors of human disease. This policy is based on concern over the possible accelerated development of vector resistance to synthetic pyrethroids, which, in their application to insecticide-treated mosquito nets, are crucial in the current global malaria strategy.

Renal dialysis units must not be supplied with permethrin-treated water, and those rearing fish should be warned not to replenish their fish tanks with mains water while it is being treated. The treated water can be discharged safely to sewers for treatment at sewage works. In circumstances where such concerns exist, relevant specialist expertise must be sought.

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