GUIDANCE ON

WATERBORNE PATHOGENS IN DRINKING WATER

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Également disponible en français sous le titre :
Conseils sur les agents pathogènes d'origine hydrique dans l'eau potable

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Publication date: September 2022

Cat.: H144-107/2022E-PDF
ISBN: 978-0-660-44488-8
Pub.: 220319

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BACKGROUND ON GUIDANCE DOCUMENTS

Health Canada works with the provinces, territories and federal agencies to establish the Guidelines for Canadian Drinking Water Quality. Over the years, new methodologies and approaches have led Health Canada, in collaboration with the Federal-Provincial-Territorial Committee on Drinking Water, to develop a new type of document, guidance documents, to provide advice and guidance on issues related to drinking water quality for parameters that do not require a formal Guideline for Canadian Drinking Water Quality.

Guidance documents are developed to provide operational or management guidance related to specific drinking water–related issues (e.g., boil water advisories), in order to make health risk assessment information available when a guideline value is not deemed necessary.

Guidelines are established under the Guidelines for Canadian Drinking Water Quality specifically for contaminants that meet all of the following criteria:

1. exposure to the contaminant could lead to adverse health effects;
2. the contaminant is frequently detected or could be expected to be found in a large number of drinking water supplies throughout Canada; and
3. the contaminant is detected, or could be expected to be detected, at a level that is of possible health significance.

If a contaminant of interest does not meet all these criteria, Health Canada, in collaboration with the Federal-Provincial Territorial Committee on Drinking Water, may choose not to develop a Guideline Technical Document. In that case, a guidance document may be developed.

Guidance documents undergo a similar process as Guideline Technical Documents, including public consultations through the Health Canada Web site. They are offered as information for drinking water authorities and to help provide guidance in spill or other emergency situations.

Part A of this document provides guidance for managing risks associated with the pathogens discussed in this document; Part B provides scientific and technical information to support this guidance; Part C provides the references; and Part D provides the appendices.

EXECUTIVE **SUMMARY**

Many types of pathogenic microorganisms can spread through drinking which may lead to human illness. Some are present in human or animal feces and can cause gastrointestinal illness when fecally-contaminated water is consumed. Others are naturally found in aquatic environments and can cause opportunistic infections when the conditions in engineered water systems (e.g., drinking water distribution systems and building/premise plumbing) allow them to multiply and spread primarily to individuals who are susceptible to infection, such as infants, the elderly and immunocompromised individuals. The health effects caused by these opportunistic pathogens are diverse and range from respiratory illness to infections of the eye, skin, central nervous system or the gastrointestinal tract. In severe cases, illnesses caused by opportunistic pathogens can be fatal.

A basic understanding of the different types of waterborne pathogens, their sources, the measures that are important for their control and the people that are most at risk for becoming sick is necessary for effective drinking water management and for preventing waterborne disease. This guidance document was prepared in collaboration with the Federal-Provincial-Territorial Committee on Drinking Water and describes these microorganisms, their health effects, how they are transmitted and best practices to ensure safe drinking water.

Assessment

Setting maximum acceptable concentrations for the pathogens described in this document remains impractical and is not necessary in order for drinking water utilities to adequately manage risks. Implementing a source-to-tap risk management approach is a universally recommended strategy for minimizing the growth and transmission of waterborne pathogens in drinking water and controlling their potential risks. Important elements of this strategy include source water protection, treatment requirements based on health-based treatment goals for enteric protozoa (Giardia and Cryptosporidium) and enteric viruses, and minimizing microorganism survival and growth in drinking water distribution systems. Maintaining microbiological control in water systems in buildings and residences is also a critical component of providing safe drinking water at the consumer's tap.

The intent of this document is to provide stakeholders, such as provincial and territorial regulatory authorities, decision makers, water system owners and operators, building owners and managers and consultants with guidance on waterborne pathogens in drinking water that are not addressed in the Guidelines for Canadian Drinking Water Quality, with the objective of minimizing public health risks in Canadian water systems.

Part A

A.1 GOAL & SCOPE

The goal of this document is to provide provinces, territories, other government departments and stakeholders with guidance on waterborne pathogens of potential human health concern that are not addressed elsewhere in the *Guidelines for Canadian Drinking Water Quality*.

A wealth of important research has advanced understanding of the public health relevance of these waterborne pathogens in drinking water systems. Management strategy considerations focus on the treatment plant and the distribution system; however, some guidance is provided on source water protection and for plumbing systems in buildings and residences.

A.2 INTRODUCTION

The microorganisms covered in this document are listed in Table 1. This document addresses the waterborne bacterial pathogens that are of gastrointestinal origin and are known to cause illness when there is fecal contamination of inadequately treated drinking water. The document also describes naturally-occurring waterborne pathogens, as these microorganisms are often associated with infections, and in extreme cases, deaths in susceptible individuals (such as infants, the elderly and immunocompromised individuals), and are referred to as opportunistic pathogens. Engineered water systems (e.g., drinking water distribution systems and building/premise plumbing) are important habitats for naturally-occurring waterborne pathogens. Many possess features that present challenges for drinking water utilities and building owners/managers, such as increased resistance to disinfection, growth under low nutrient and oxygen conditions and growth in biofilms. Effective management of these microorganisms requires collaboration between water utilities that manage drinking water distribution systems and those responsible for building plumbing systems.

Overall strategies for management are summarized in Part A.3. In Part B, brief technical information is presented on the individual pathogens (See Table 1), their effects on human health, as well as sources and exposure. Analytical and treatment considerations are also summarized.

Table 1. Microorganisms addressed in this guidance document

Waterborne pathogens of gastrointestinal origin	Waterborne naturally-occurring pathogens
Campylobacter spp. Enteric pathogenic *Escherichia coli (E. coli)* and Shigella spp. *Helicobacter pylori* *Salmonella* spp. *Yersinia* spp.	**Bacteria:** *Aeromonas* spp. *Legionella* spp. *Mycobacterium* spp. *Pseudomonas* spp. **Protozoa:** *Acanthamoeba* spp. *Naegleria fowleri*

A.3 MANAGEMENT STRATEGIES

Setting maximum acceptable concentrations for these microorganisms remains impractical and is not required in order for drinking water utilities and building owners/managers to adequately manage risks. Instead, a priority focus on drinking water process management, for example, through the implementation of a source-to-tap or water safety plan approach, is the recommended strategy for water utilities to manage potential risks. Water utilities should also have education and outreach programs to make consumers aware of how water quality can deteriorate within residential and building plumbing systems. Building water management plans are the recommended best practice for building owners/managers.

It is important that those responsible for managing drinking water quality be aware that the absence of indicator bacteria (e.g., *Escherichia coli*, total coliforms) does not indicate the absence of opportunistic pathogens such as *Legionella* and *Mycobacterium*. The latter are more resistant to disinfection. As a result, water utilities and building owners/managers should have appropriate risk management strategies that utilize multiple parameters to assess the performance of drinking water treatment and/or distribution operations.

A.3.1 Source water protection

Source water protection is the first step in a source-to-tap, or water safety plan approach. Source water assessments should include: the identification of potential sources of microbiological contamination in the watershed/aquifer; potential pathways and/or events (low to high risk) by which microorganisms of concern can make their way into the source water and affect water quality; and conditions likely to lead to peak concentrations. Assessments for subsurface sources should include, at a minimum, a hydrogeological assessment, an evaluation of well integrity and a survey of activities and physical features in the area (Health Canada 2019b, 2019c). It is important that risks from both land-based and aerosol-generating activities (e.g., spray irrigation, pressure washers) be considered in the source water assessment.

Nutrients are critical for microorganisms to survive, grow and spread. Thus, surface and subsurface sources should be characterized with regard to organic and inorganic nutrient concentrations (Cantor, 2017).

Water temperature may also be an important consideration (Health Canada, 2021). Enteric bacteria (see Section B.1) survive longer at low temperatures whereas opportunistic pathogens (see Section B.2) thrive at warmer temperatures.

A.3.2 Water treatment plant

When properly designed and operated, physical removal and disinfection technologies commonly used in drinking water treatment are expected to control the risks associated with the microorganisms discussed in this guidance document. However, it is important to recognize that treated drinking water is not sterile and naturally-occurring bacterial pathogens (see Section B.2.1) have been detected in treated water. Adverse health effects from these opportunistic pathogens are not expected at the concentrations measured in treated water (Le Dantec et al., 2002) but these microorganisms can spread to and grow in downstream biofilms (Brooks et al., 2004). Water utilities should therefore aim to produce biologically stable water to minimize the potential for problems to occur in distribution and premise plumbing systems (see Sections A.3.3 and A.3.4) (Health Canada, 2022). Important elements related to drinking water treatment include:

- optimize treatment performance for turbidity and natural organic matter removal;
- properly apply primary disinfection technologies to meet appropriate CT (disinfectant concentration in mg/L × time in minutes) requirements when using chemical oxidants (i.e., free chlorine, ozone, chlorine dioxide) or IT (intensity measured in mW/cm^2 or W/m^2 × time measured in seconds resulting in a computed fluence in mJ/cm^2) requirements when using ultraviolet (UV) disinfection;

- minimize nutrient concentrations in treated water and have a good understanding of their concentrations in the distribution system;
- optimize treatment to minimize the amount of scaling and/or corrosion in the distribution system;
- properly apply secondary disinfection technologies (i.e., free chlorine or monochloramine) for residual maintenance in the distribution system;
- conduct performance testing using multiple parameters (e.g., disinfectant residual, microbiological indicators, pH, turbidity);
- provide operator training to assure the effectiveness of the water safety plan at all times.

It is important to note that monochloramine is recommended only for secondary disinfection and residual maintenance in the distribution system (Health Canada, 2019b).

A.3.3 Drinking water distribution system

Microorganisms can enter drinking water distribution systems as a result of inadequate treatment, passage through treatment barriers or through post-treatment contamination via intrusions, cross-connections or during construction or repairs. Biofilms and loose deposits in drinking water systems provide habitats that can support the survival, growth and dissemination of pathogenic microorganisms, particularly naturally-occurring pathogens (e.g., *Legionella*). Despite the detection of naturally-occurring bacterial pathogens in drinking water distribution systems, these systems are not expected to be a major source of exposure (LeChevallier, 2020). Maintaining an effective disinfectant residual is essential to manage risks. There is increasing recognition that a minimum disinfectant residual concentration greater than 0.2 mg/L is required to control microbiological (re)growth in the distribution system. Studies indicate that disinfectant residual concentrations in the order of 1.0 mg/L free chlorine (for systems that chlorinate) and 1.8 mg/L total chlorine (for systems that chloraminate) are required for controlling biofilm formation (Gagnon et al., 2008; Gillespie et al., 2014; Rand et al., 2014; LeChevallier et al., 2015a,b). Water utilities that regularly remove biofilms and loose deposits from their distribution systems may require lower concentrations. Cantor (2017) stressed the importance of removing accumulated material to meet water quality goals. Comprehensive, multi-parametric monitoring programs (e.g., disinfectant residual paired with temperature and biofilm formation rate) are recommended to confirm system-specific requirements (Health Canada, 2022).

The choice of secondary disinfectant for residual maintenance in the distribution system is a complex decision. Free chlorine and monochloramine possess different capabilities in terms of disinfectant power, reactivity with organic and inorganic material and biofilm penetration. Also, *Legionella* and mycobacteria have differing sensitivity to free chlorine and monochloramine. Thus, the choice of disinfectant residual should effectively balance concomitant water quality objectives related to: 1) microbial species (e.g., *Legionella*, mycobacteria); 2) water chemistry; 3) disinfection by-product concentrations; and 4) the complexity of the distribution system (Donohue et al., 2019a). Roth and Cornwell (2018) assessed the impact of increasing free chlorine residuals from 0.2 mg/L to 1.2 mg/L on disinfection by-product concentrations for 21 water utilities. The authors concluded that minimal impact was expected although system-specific assessments were recommended to avoid unintended consequences.

Additional information on managing microorganism survival and growth in drinking water distribution systems, and establishing multi-parametric monitoring programs, is found in these Health Canada publications: *Guidance on Monitoring the Biological Stability of Drinking Water in Distribution Systems* (Health Canada, 2022) and *Guidance on Natural Organic Matter in Drinking Water* (Health Canada, 2020a). Key distribution system operational and maintenance practices include:

- use proper construction materials;
- maintain an effective disinfectant residual and take preventive/corrective actions when low disinfectant residuals occur, particularly during warm water temperature conditions when biofilm growth accelerates;
- manage water age and the effects of temperature;
- minimize the potential for contaminant entry from external sources (e.g., maintain positive pressure, implement cross-connection/backflow control programs, practice strict hygiene during mains constructions and repairs);
- keep the distribution system clean by removing biofilm, loose deposits and sediment from watermains and storage facilities (e.g., use of appropriate flushing and cleaning techniques);
- conduct performance testing using multiple parameters (e.g., disinfectant residual, microbiological indicators, biological stability indicators, pH, pressure, temperature, turbidity); and
- provide operator training to assure the effectiveness of the water safety plan at all times.

A.3.4 Premise plumbing

Conditions in premise plumbing systems such as: 1) smaller pipe diameter; 2) increased temperature; 3) increased retention time; and 4) leaching of significant amounts of nutrients (e.g., carbon) from some pipe materials can lead to the growth of microorganisms, particularly opportunistic pathogens (e.g., *Legionella*) (Neu and Hammes, 2020). Thus, water utilities should educate their customers of the potential for water quality deterioration in premise plumbing.

Maintaining microbiological control in premise plumbing systems, is a critical component of providing safe drinking water at the consumer's tap. This is especially important in large buildings. Building owners/managers are responsible for managing water quality within their buildings and therefore should be aware of practices that reduce the risk of microorganism growth. Important elements of control strategies for plumbing systems include:

- limiting nutrient levels through an emphasis on system design and materials;
- minimizing areas of low flow/stagnation;
- keeping temperatures of cold and hot water systems outside of the ideal range for microorganism growth (e.g., cold water less than 20°C, hot water tank temperature greater than 60°C; hot water lines at distal points ideally greater than 55°C); and
- reducing the formation and transmission of contaminated aerosols from system components such as cooling towers, showers, faucets, hot tubs and humidifiers.

The increasing demand for conservation of energy, water and materials can have unplanned impacts on microbiological presence and growth. This is important for *Legionella* in particular but also has relevance for other opportunistic pathogens that can be problematic in premise plumbing. Changes to premise plumbing systems operations or features that involve the use of alternative water sources (e.g., reclaimed water, harvested rainwater), increased water age, reduced flows and changes to water temperatures in hot and cold building systems can have the unintended consequences of increasing the potential for growth of these pathogens (Bédard et al., 2016; Rhoads et al., 2016; NASEM, 2020).

Resources are available to assist building owners/managers develop a water management plan (WHO, 2007, 2011; HSE, 2013a, 2013b, 2014; PWGSC, 2016; CDC, 2017; ASHRAE, 2018). Other guidance documents provide standards and technical specifications for the design and installation of plumbing systems in buildings (NRCC, 2015a, 2015b; ASHRAE, 2018). In addition, building owners/managers should contact the responsible authority in the affected jurisdiction to confirm if specific requirements will apply to their system (e.g., cooling tower registry).

A.3.5 Roles and responsibilities

Water utilities and building owners/managers all have a role in effectively managing risks. Table 2 summarizes the key roles and responsibilities for stakeholders. Guidance is available to assist water utilities to develop education and outreach programs (Masters et al., 2019).

Table 2. Key roles and responsibilities to manage risks associated with waterborne pathogens

	Water utility			Building owner/manager
Section	Source	Treatment	Distribution System	Building plumbing system
Requirements	Source water protection plan	Appropriate treatment	Distribution system management plan	Building water management plan
Objective	Protect the source to minimize risks to public health and reduce water treatment costs	Produce water that is of acceptable microbiological quality and is biologically stable to minimize water quality degradation in distribution and building/premise plumbing systems	Establish policies and operational goals designed to protect water quality in the distribution system	Establish policies and operational goals designed to protect water quality in the building and premise plumbing system
Education and outreach	Establish education and outreach programs to inform water consumers about the risks posed by water quality degradation from the source to the tap			

A.3.6 Impacts of a Changing Climate

Weather events such as heavy or intense rainfall, severe storms, flooding, dry spells/drought and hotter temperatures can contribute to increased human exposure to waterborne pathogens through various mechanisms (Levy et al., 2016; Nichols et al., 2018; Walker, 2018; Semenza, 2020; Calero Preciado et al., 2021) including:

- transport and loading of pathogens and nutrients to drinking water sources;
- overwhelming or damaging drinking water infrastructure;
- influencing pathogen survival and growth in source waters and drinking water distribution and plumbing systems;

- extending transmission seasons or geographical ranges for pathogens;
- diminishing availability of water resources;
- influencing human activities or behaviours (e.g., increases in water consumption and use of heating, ventilation and cooling systems).

These events are forecasted to increase in frequency and severity with climate change (Levy et al., 2016; Nichols et al., 2018; Walker, 2018). The *Guidelines for Canadian Drinking Water Quality* do not discuss all the potential climate scenarios that could impact water utilities and building water systems. The responsible authority should be consulted to discuss relevant forecast scenarios. Climate change impacts will heighten the importance of water quality monitoring, proper treatment process selection, day-to-day process control, distribution system operation, building water system management, and education and outreach programs. Thus, water utilities and building owners/managers should integrate the risks associated with climate change into their management strategies to maximize the reliability, robustness and resilience of their water systems. Guidance is available to assist water utilities (AWWA, 2021).

Part B. Technical information

B.1 BACTERIA OF GASTROINTESTINAL ORIGIN

B.1.1 *Campylobacter* spp.

B.1.1.1 Description

Campylobacter (Class: Epsilonproteobacteria) is a bacterial genus that contains over 30 recognized species, but only some have relevance for human health (Wagenaar et al., 2015; Backert et al., 2017; LPSN, 2019). *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are the primary and secondary species of most relevance as causes of human gastrointestinal illness, accounting for greater than 90% of cases of human campylobacteriosis worldwide (Huang et al., 2015; Wagenaar et al., 2015). Other species are known to cause gastrointestinal illness, but their occurrence is rare or is associated with specific risk groups (e.g., immunocompromised individuals) or geographical areas (Wagenaar et al., 2015). Some *Campylobacter* species (spp.) have been associated with prenatal and neonatal infections and human periodontal disease (Backert et al., 2017; Huang et al., 2015).

Campylobacter spp. are Gram-negative, motile, curved or spiral rod-shaped cells (Percival and Williams, 2014b). They are fastidious and microaerophilic (require lower oxygen levels) bacteria that have a growth temperature of 30 to 45°C (optimum: 40–42°C) (Percival and Williams, 2014b; Wagenaar et al., 2015; Zautner and Masanta, 2016).

B.1.1.2 Health effects

Gastroenteritis caused by *Campylobacter* spp. includes a watery, profuse and sometimes bloody diarrhea occasionally accompanied by fever and abdominal pain (Backert et al., 2017; Percival and Williams, 2014b). Some severe infections may lead to hospitalization and can be life threatening, although fatalities are usually associated with the very young, very old, or patients with underlying disease or immune system deficiencies (Kvalsvig et al., 2014). Symptoms generally occur within one to five days of infection and the illness lasts less than seven to ten days (Backert et al., 2017). Shedding of the microorganisms in feces can continue for weeks following an episode of diarrhea; in most cases this

ceases within four weeks (Percival and Williams, 2014b; Lee et al., 2013). Asymptomatic infections with *Campylobacter* spp. are also possible (Percival and Williams, 2014b). While *Campylobacter* spp. can cause illness in healthy individuals across all age groups, in developed countries, infections are more prevalent in young children, young adults and the elderly (Kaakoush et al., 2015; PHAC, 2018c). The doses of *C. jejuni* required to cause infection and illness are not fully understood. Human challenge studies have shown that for some strains, ingestion of several hundred bacteria can be sufficient to cause infection and illness (Black et al., 1988). Meta-analyses of *C. jejuni* pathogenicity data from challenge studies and foodborne outbreaks show that the doses required to cause infection and illness may be orders of magnitude lower (Teunis et al., 2005; 2018).

Post-infection complications associated with *Campylobacter* spp. illness include Guillain–Barré Syndrome and reactive arthritis, though these are relatively rare (Backert et al., 2017; Percival and Williams, 2014b). *Campylobacter* spp. infection may be associated with the development of inflammatory bowel diseases such as Crohn's disease, ulcerative colitis and irritable bowel syndrome (Backert et al., 2017; Huang et al., 2015). A meta-analysis estimated that *Campylobacter* spp. cases develop long-term complications in the following proportions: Guillain–Barré Syndrome, 0.07% (95% confidence interval: 0.03–0.15%); reactive arthritis, 2.86% (95% CI: 1.40–5.61%); and, irritable bowel syndrome, 4.01% (95% CI: 1.41–10.88%) (Keithlin et al., 2014b). *Campylobacter* spp. is the leading cause of bacterial gastrointestinal illness in Canada and other developed countries worldwide (Backert et al., 2017; Huang et al., 2015). Cases of campylobacteriosis in Canada and internationally are predominantly sporadic, with most illness associated with consumption of contaminated food (Huang et al., 2015; Wagenaar et al., 2015). In Canada, reported annual incidence rates (all causes) over the period from 2013–2017 ranged from 25.4 to 29.2 (median: 28.4) cases per 100,000 population (PHAC, 2019b). Infections (all sources) are more common in the summer months (Fleury et al., 2006; Lal et al., 2012; Kaakoush et al., 2015).

llness caused by *Campylobacter* spp. is generally self-limiting, and antibiotics should only be prescribed in serious cases (Wagenaar et al., 2015). *Campylobacter* spp. are resistant to ciprofloxacin and azithromycin and have been classified as a "Serious Threat" by the Centers for Disease Control and Prevention (CDC, 2019a). The WHO and the Public Health Agency of Canada (PHAC) consider these microorganisms a medium to high priority for surveillance, research and public health attention (Garner et al., 2015; WHO, 2017b, PHAC, 2018a). No vaccines for *Campylobacter* spp. are available (Wagenaar et al., 2015).

B.1.1.3 Sources and exposure

Campylobacter spp. are zoonotic pathogens (i.e., transmitted from animals to humans) that are naturally found in the intestinal tract of a wide range of wild and domestic birds and mammals (Wagenaar et al., 2015; Backert et al., 2017). Poultry are considered the

major reservoir (Wagenaar et al., 2015; Backert et al., 2017). Cattle, sheep and domestic pets are also important sources of the microorganisms (Wagenaar et al., 2015; Backert et al., 2017). Transmission of *Campylobacter* spp. occurs via the fecal-oral route, with the main pathways for exposure being contaminated food or water and direct contact with animals (Percival and Williams, 2014b; Wagenaar et al., 2015). Person-to-person transmission is uncommon (Percival and Williams, 2014b; Wagenaar et al., 2015). Important mechanisms for fecal contamination of drinking water sources (groundwater and surface water), are surface runoff containing livestock waste and sewage inputs (e.g., from wastewater discharges, leaking sanitary sewers) (Whiley et al., 2013). Intrusion of animal feces following heavy rainfall or snowmelt is a particularly important cause of contamination of vulnerable groundwater wells (Moreira and Bondelind, 2017).

Campylobacter spp. has been recognized as the enteric waterborne bacterial pathogen most frequently involved in drinking water outbreaks in developed countries (Moreira and Bondelind, 2017). According to United States (U.S.) data, *Campylobacter* spp. was identified as a causative or co-occurring agent in 11% of the drinking water outbreaks reported between 2001 and 2014 (the last year for which data is available). Outbreaks occurred in all months of the year, with the largest outbreaks occurring in the spring and summer months (CDC, 2004, 2006, 2008, 2011, 2013, 2015b, 2017d). Periods of higher risk for waterborne illness coincide with peak periods for precipitation-induced (e.g., rainfall, snowmelt) agricultural runoff (Sterk et al., 2013, Galanis et al., 2014).

The Walkerton outbreak of May 2000 is the most notable Canadian drinking water outbreak involving *Campylobacter* spp., with 105 confirmed cases; and was also caused by pathogenic *E. coli* (163 confirmed cases) (O'Connor 2002a, 2002b). The severity of this outbreak (greater than 2300 estimated cases, 7 deaths) led to substantial reform of drinking water regulatory policy in Canada (O'Connor, 2002b; Holme, 2003). Notable large drinking water outbreaks involving *Campylobacter* spp. to have occurred worldwide since Walkerton include New Zealand (2016: greater than 1000 cases), Denmark (2010: 409 cases), Ohio, U.S. (2004: 1450 cases) and Finland (2001: 1000 cases) (Hrudey and Hrudey, 2004, Government Inquiry into Havelock North Drinking Water, 2017; Moreira and Bondelind, 2017). Drinking water outbreaks have largely been associated with small drinking water supplies (i.e., private wells or small community supplies) with contamination from infiltration of animal feces or wastewater and inadequate disinfection reported as the most frequent causes (Moreira and Bondelind, 2017). Private and small community water systems are recognized as being more likely to contribute to cases of human enteric illness than municipally-operated systems (Hrudey and Hrudey, 2004; Murphy et al., 2016; Butler et al., 2016). Using a Quantitative Microbial Risk Assessment (QMRA) approach, Murphy et al. (2016) estimated that roughly 5% of the total number of Canadian cases of *Campylobacter* spp. acquired annually might be attributable to consumption of water from contaminated small drinking water

systems. In municipal drinking water systems, inadequate disinfection and post-treatment contamination via intrusions or cross-connections are the most common causes of *Campylobacter* spp. outbreaks (Moreira and Bondelind, 2017).

B.1.2 *Escherichia coli/Shigella* spp. (pathogenic strains)

B.1.2.1 Description

Escherichia coli (Class Gammaproteobacteria, Family: Enterobacteriaceae) are Gram-negative bacteria that are a member of the natural intestinal microbial community of humans and animals. They are facultatively anaerobic, motile or non-motile rod-shaped bacteria that can grow over a broad temperature range (7–45°C) with an optimal growth temperature of 37°C (Ishii and Sadowsky, 2008, Percival and Williams, 2014c). Whereas most strains (i.e., variants) of *E. coli* are harmless and function as indicators of fecal contamination, some have acquired virulence traits through gains and losses of genetic material (Croxen et al., 2013). These pathogenic *E. coli* strains can cause numerous human diseases including serious gastrointestinal infections, urinary tract and bloodstream infections and neonatal meningitis (Croxen et al., 2013; Percival and Williams, 2014c). Non-pathogenic *E. coli* strains and their role in drinking water risk management are addressed in Health Canada's Guideline Technical Document on *Escherichia coli* (Health Canada, 2019e).

Pathogenic *E. coli* are broadly categorized into functional groups based on the mechanisms with which they interact with their target cells and cause symptoms. Different types can bind to, invade, or cause structural alterations of cells and produce specific types of toxins. There are six major groups of pathogenic *E. coli* that cause gastrointestinal infections: enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and diffuse adherent *E. coli* (DAEC) (Croxen et al., 2013; Percival and Williams, 2014c). Categorization of pathogenic *E. coli* strains has previously been determined by biochemical testing and serotyping based on the classic Kauffmann-White classification scheme for surface O and H antigens (Croxen et al., 2013; Robins-Browne et al., 2016). Molecular methods have been developed that allow for more rapid detection and identification of the different pathogenic strains (Croxen et al., 2013; Robins-Browne et al., 2016). Serotyping information nevertheless remains helpful in outbreak investigations and disease surveillance (Robins-Browne et al., 2016). Additional pathogenic *E. coli* groups have been proposed, but these have not been completely defined. Comparative genomics studies have shown that these group designations are not always clear cut, and that there is considerable overlap in the virulence mechanisms possessed by different *E. coli* strains (Croxen et al., 2013).

Shigella spp. are closely related to *E. coli* but have historically been considered separate species. *Shigella* and EIEC strains are nearly indistinguishable based on their biochemical and serological properties (Croxen et al., 2013). Advanced molecular typing and sequencing analyses have demonstrated that *Shigella* spp. clearly belongs within the species *E. coli*, forming a single group with the EIEC (Croxen et al., 2013, Robins-Browne et al., 2016). A re-evaluation of the *Shigella* spp. classification may be required to take into account its genetic relationship to the *Escherichia* genus. The genus name *Shigella* spp. and disease name shigellosis (i.e., disease caused by *Shigella* spp.) are still used for historical purposes (Croxen et al., 2013). Conventionally, *Shigella* spp. has four major species (*S. dysenteriae, S. flexneri, S. boydii and S. sonnei*); with *Shigella sonnei* and *Shigella flexneri* being the most important in developed countries (Percival and Williams, 2014h).

Among the pathogenic *E. coli*, the EHEC (synonyms: shiga toxin-producing *Escherichia coli* and verotoxin-producing *Escherichia coli* (VTEC)) are of most concern to the drinking water industry (Percival and Williams, 2014c; Saxena et al., 2015). EHEC are the subset of *E. coli* that can produce one or more of the potent Shiga toxins and are considered to be highly pathogenic to humans. *E. coli* O157:H7 is the most prevalent EHEC serotype; however, other serotypes, i.e., O26, O45, O103, O111, O121, and O145 are also important causes of human illness (Croxen et al., 2013, Saxena et al., 2015; PHAC, 2018c).

B.1.2.2 Health effects

In developed countries, most *E. coli* illness occurs as sporadic cases or outbreaks associated with contaminated food and water or travel (Croxen et al., 2013; Saxena et al., 2015). In developing countries, enteric pathogenic *E. coli* are a significant cause of illness and mortality, particularly among children.

Enteric pathogenic *E. coli*/*Shigella* spp. cause diarrheal illness that can range in severity from mild and self-limiting to severe and life-threatening depending on the group and strain involved. The first symptom is watery diarrhea. This can be followed by bloody diarrhea in EHEC infections and occasionally during EIEC/*Shigella* spp. and EAEC infections. (Croxen et al., 2013, Percival and Williams 2014c; 2014h). Additional symptoms can include nausea, vomiting, abdominal pain, fever, headache and muscle pain. Symptom onset generally occurs within one to three days of infection. The duration of diarrhea is usually one to two weeks, but can persist longer with some strains (Croxen et al., 2013; Percival and Williams, 2014c, 2014h). Infected individuals can become asymptomatic carriers capable of shedding the microorganisms in their feces for weeks to months after infection (Croxen et al., 2013; Percival and Williams, 2014c, 2014h). Doses required to cause infection are estimated to range from less than 100 to 1000 organisms for EHEC and EIEC/*Shigella* spp. to greater than one million to ten billion organisms for the other groups (Kothary and Babu, 2001; Croxen et al., 2013, Percival and Williams, 2014c; 2014h).

EHEC illness is particularly concerning as it can progress to the serious and potentially life-threatening hemolytic uremic syndrome (HUS), which results in decreased blood cell and platelet counts and acute kidney failure. A meta-analysis showed that HUS was the predominant long-term complication following cases of *E. coli* O157 illness, with an estimated rate of occurrence between 4–17% (Keithlin et al., 2014a). HUS can also lead to further long-term effects in the pancreas, gastrointestinal system and central nervous system. (Spinale et al., 2013). Complications arising from non-EHEC infections are uncommon (Croxen et al., 2013). A link between infections with some pathogenic *E. coli* types (i.e., DAEC and some invasive *E. coli*) and chronic intestinal disorders such as irritable bowel syndrome and Crohn's disease has been suggested (Croxen et al., 2013). In developed countries, enteropathogenic *E. coli* can cause gastrointestinal infections in healthy individuals in all age groups. Young children and the elderly are at higher risk of experiencing illness and complications as a result of infection (Percival and Williams, 2014c, 2014h; Gargano et al., 2017).

EHEC and *Shigella* spp. are among the leading causes of bacterial gastrointestinal illness in Canada, the U.S. and Europe (Scallan et al., 2011; CDC, 2018; ECDC, 2018a; PHAC, 2019b). Case reports and outbreaks of *E. coli*-related diarrheal illness and shigellosis in North America have mostly been tied to food, and travel-related exposures, though waterborne exposure remains an important cause of infections (Croxen et al., 2013; PHAC, 2018c). Reported annual incidence rates in Canada for verocytotoxigenic *E. coli* infection and shigellosis (all causes) over the period from 2013 to 2017 were 1.78-2.24 (median: 1.82) cases per 100,000 persons and 1.94–2.53 (median: 2.28) cases per 100,000 persons, respectively (PHAC, 2019b). Seasonal trends in EHEC and *Shigella* spp. infections (all sources) have generally been observed worldwide, with more cases occurring in summer to early fall (Fleury et al., 2006; PHAC, 2010; Lal, 2012).

The CDC, WHO and PHAC have identified carbapenem-resistant *E. coli* and extended spectrum β-lactamase (ESBL)-producing *E. coli* as public health threats of serious to critical importance (CDC 2019a; WHO, 2017b, PHAC, 2018a). ESBL-producing *E. coli* are generally resistant to many antibacterial drugs; for persons with severe infections with these strains, carbapenems are one of the main treatment options. Resistance to carbapenems means resistance to one of the last available treatment options (CDC 2019a, WHO, 2017b). Pathogenic *E. coli* strains resistant to ESBL antibiotics and carbapenems have been recovered from humans and animals (Mir and Kudva, 2018). *Shigella* spp. resistant to ciprofloxacin and azithromycin also have been designated as a "Serious Threat" level by the CDC, and have been designated as a low to medium priority for research and surveillance by PHAC and the WHO (CDC, 2019a, Garner et al., 2015; WHO, 2017b). As *Shigella* spp. resistance to first-line drugs has increased, treatment has shifted

to reliance on these two drugs for treating resistant infections (CDC, 2013a; WHO, 2017b). A vaccine based on the cholera toxin (which is structurally similar to the ETEC heat-labile toxin) has been licensed for use to protect against ETEC-associated traveller's diarrhea (Croxen et al., 2013; O'Ryan et al. 2015). More data is required to determine the effectiveness of this and other candidate vaccines for ETEC (O'Ryan et al. 2015). No vaccines are available for other *E. coli* groups (Croxen et al., 2013).

B.1.2.3 Sources and exposure

Humans are the primary reservoir for the EPEC, ETEC and EAEC groups and the only known reservoir for EIEC/*Shigella* spp. (Croxen et al., 2013). EHEC are important zoonotic pathogens. Ruminants, particularly cattle, are the primary reservoir for EHEC; humans are a secondary reservoir (Croxen et al., 2013, Percival and Williams, 2014c). Animals (e.g., cattle, dogs, sheep, rabbits) are also a reservoir for certain EPEC strains (Croxen et al., 2013). Transmission of pathogenic *E. coli* occurs through the fecal-oral route and the main routes of infection are contaminated food or water, person-person spread and direct contact with animals. Surface runoff and sewage inputs are important mechanisms for fecal contamination of drinking water sources—much the same as those discussed for *Campylobacter* spp. (see Section B.1.1) (Hrudey and Hrudey, 2004; Moreira and Bondelind, 2017).

In the U.S., pathogenic *E. coli* (largely *E. coli* O157:H7) was identified as a causative or co-occurring agent in roughly 4% of the drinking water outbreaks reported over the period between 2001 and 2014 (the last year for which data is available) (CDC, 2004, 2006, 2008, 2011, 2013, 2015b, 2017d). Most *E. coli* drinking water outbreaks have been associated with small drinking water supplies (i.e., private wells or small community supplies) (Craun et al., 2010; CDC, 2011, 2013, 2015b, 2017d). QMRA estimates suggest that consumption of untreated or inadequately treated water from small drinking water supplies may be responsible for 4% of all cases of *E. coli* O157 illness in Canada (Murphy et al., 2016). The Walkerton outbreak of May 2000 is the most notable Canadian drinking water outbreak involving pathogenic *E. coli* [and *Campylobacter* (see Section B.1.1.3)], resulting in an estimated 2300 total cases, 163 confirmed cases of *E. coli* O157, 27 HUS cases and seven deaths (O'Connor 2002a, 2002b). Since the Walkerton outbreak, large drinking water outbreaks involving pathogenic *E. coli* have occurred in Missouri, U.S. (2010: 28 cases, 0 deaths) and Korea (2015: 188 cases, 0 deaths), (Hrudey and Hrudey, 2004; Missouri Department of Health and Senior Services, 2011; Park et al., 2018). *Shigella* spp. is rarely linked to drinking water outbreaks (Hrudey and Hrudey, 2004; Craun et al., 2010). Three reports of drinking water outbreaks involving *Shigella* spp. have been recorded in the U.S. between 2001 and 2014; all associated with irregular sources of drinking water (pond, lake water, commercially bottled water system) (CDC, 2006, 2011, 2015b).

Investigations of drinking water related illness in Canada have found that the majority of waterborne disease outbreaks occur in the spring and summer (Schuster et al. 2005, Thomas et al., 2006; Wilson et al., 2009). Weather variables (e.g., snowmelt, warmer temperatures, alternation between dry conditions and intense rain events) and increased human and animal activities in the watershed (e.g., grazing animals and land application of fecal wastes in agriculture) have been proposed as factors contributing to these trends (Tyrell and Quinton, 2003; Thomas et al., 2006; Wilson et al., 2009; Quilliam et al., 2011). Heavy rainfall causing flooding contributed to the Walkerton, Ontario *E. coli* O157:H7 and *Campylobacter* spp. outbreak of 2000 (O'Connor, 2002a).

B.1.3 *Helicobacter pylori*

B.1.3.1 Description

Helicobacter pylori (*H. pylori,* Class: Epsilonproteobacteria) is a pathogenic bacterium that can colonize the human stomach and is responsible for causing gastrointestinal diseases which can include gastritis, peptic ulcers and gastric cancer (Percival and Williams, 2014d; Posteraro et al., 2015). *Helicobacter* are closely related to the genus *Campylobacter* (Percival and Williams, 2014d). Over 20 different *Helicobacter* species have been determined by gene sequencing (Percival and Williams, 2014d; Posteraro et al., 2015). *H. pylori* is the predominant pathogenic species of the genus, accounting for the vast majority of human infections. Some other *Helicobacter* species have occasionally been associated with human gastrointestinal illness (Percival and Williams, 2014d).

H. pylori are Gram-negative, motile, fastidious and microaerophilic (require lower oxygen levels) bacteria that grow over the temperature range of 30–42°C (optimum: 37°C) (Mégraud and Lehours, 2007; Posteraro et al., 2015). They are not acidophilic (acid-loving) bacteria, but possess mechanisms that enable the bacteria to tolerate the acid conditions of the human stomach. *H. pylori* have two morphological forms: a spiral (S-shaped) rod form and a viable but non-culturable (VBNC) coccoid form that is adopted under conditions of environmental stress. The VBNC form is an important component of the organism's survival strategy; however, its role in pathogenesis is unknown (Percival and Williams, 2014d).

B.1.3.2 Health effects

The vast majority of *H. pylori* infections are asymptomatic (Percival and Williams, 2014d). *H. pylori* infection can cause a chronic and superficial gastritis, and some infections develop into peptic (i.e., duodenal or gastric) ulcers (Posteraro et al., 2015). Symptoms of gastritis and ulcers include nausea, abdominal pain, heartburn and bleeding (Percival and Williams, 2014d; Posteraro et al., 2015). In a very small fraction of the infected population, infections can develop into gastric cancer. *H. pylori* has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (IARC, 2014), and the organism is considered to be the single most common cause of gastric cancer worldwide (Percival and Williams, 2014d; Posteraro et al., 2015). The infectious dose of *H. pylori* is not known. Challenge studies suggest it is less than 10,000 cells (Solnick et al., 2001; Graham et al., 2004); however, evidence from case reports of illness suggests the dose could be orders of magnitude lower (Langenberg et al., 1990; Matysiak-Budnik et al., 1995).

The varying health outcomes of *H. pylori* infection seem to be related to human genetic variability, environment and dietary factors and differences in strain virulence (Brown, 2000; Posteraro et al., 2015). Since the majority of persons do not develop clinical disease, it can be difficult to determine when infection occurs (Brown, 2000). People living in low socioeconomic status, poor hygiene or sanitary conditions and crowded or high-density living conditions are associated with higher prevalence of *H. pylori* infections (Brown, 2000). Rates of infection are higher in developing countries and in at-risk populations, with most infections being acquired during childhood in these locations (Brown, 2000, Posteraro et al., 2015). Childhood rates in developed countries are lower and may be decreasing with improvements to sanitary practices (Brown, 2000). *H. pylori* have been cited as the most prevalent bacterial pathogen of humans (Posteraro at al., 2015). Roughly one half of the world's population is infected with *H. pylori* (Percival and Williams, 2014d). Rates of asymptomatic *H. pylori* infections vary widely by geographical area but are broadly estimated to fall in the range from 20 to 50% in developed regions and from 50 to >70% in developing countries (Brown, 2000; Hooi et al., 2017; Zamani et al. 2018). The rates of *H. pylori* infections in Canada are not well understood as they are not a reportable illnesses. Studies of *H. pylori* infections in Ontario among adults 50 to 80 years of age and Canadian children with upper gastrointestinal symptoms have reported rates of 23.1% and 7.1% respectively (Naja et al., 2007; Segal et al., 2008). Higher rates (>40%) have been reported among First Nations populations in Canada (Bernstein et al., 1999; Sethi et al., 2013; Fagan-Garcia et al., 2019).

Once colonized by *H. pylori*, infections can be lifelong unless intensive antimicrobial therapy is provided (Percival and Williams, 2014d). Eradication of *H. pylori* has been shown to be the definitive cure for duodenal and most gastric ulcers (Percival and Williams, 2014d). *Helicobacter* resistant to clarithromycin and multi-drug-resistant *Helicobacter* have been identified as a medium to high priority for research and the development of new antibiotic strategies by PHAC and the WHO (Garner et el., 2015; WHO, 2017b). No effective vaccines against *H. pylori* infection have been developed (Posteraro et al., 2015).

B.1.3.3 Sources and exposure

Hosts for *H. pylori* include humans, domestic cats and non-human primates (e.g., old world macaques) (Percival and Williams, 2014d). The human stomach is considered the major reservoir (Percival and Williams, 2014d). Domestic cats are also thought to be a source of the organism relevant for human infections (Percival and Williams, 2014d).

The principal means through which *H. pylori* infections are acquired is unclear. Person-to-person transfer is presumed to be the most likely route of transmission via fecal-oral, gastric-oral or oral-oral routes (Percival and Williams, 2014d; Posteraro et al., 2015). Direct contact with domestic cats is also thought to be a pathway for infection; however, there is no confirmed data on transmission from animals to humans (Brown, 2000). Consumption of contaminated drinking water is suspected as a potential source of infection. Infection occurring through multiple transmission pathways is likely (Percival and Williams, 2014d). Attempts to culture *H. pylori* from environmental water samples have mostly been unsuccessful, and this absence of cultured data has limited epidemiological and risk assessments (Percival and Williams, 2014d). Evidence for a waterborne transmission comes largely from epidemiological studies conducted in developing countries (Percival and Williams, 2014d). Further support for water as vehicle of transmission has been provided by culture of *H. pylori* in feces of infected individuals; detection of *H. pylori* by molecular methods in drinking water supplies; and the finding of an association between *H. pylori* in untreated groundwater supplies and clinical infection in individuals drinking the water (Baker and Hagerty, 2001). In countries with adequate drinking water treatment, drinking water is unlikely to be a significant source of infection (Percival and Williams, 2014d). Nevertheless, further research on the role of water in the spread of *H. pylori* infections is needed. Studies on the detection of *H. pylori* in municipal drinking water supplies have been limited. Surveys of *H. pylori* presence in water and biofilm samples collected from the plumbing of private residences and public facilities receiving municipal water have reported detection of the microorganism by polymerase chain reaction (PCR) detection in 4–64% of the locations sampled (Watson et al., 2004; Santiago et al., 2015; Richards et al., 2018). *H. pylori* are not a recognized cause of waterborne outbreaks (Percival and Williams, 2014d).

B.1.4 *Salmonella* spp.

B.1.4.1 Description

Salmonella (Class Gammaproteobacteria, Family: Enterobacteriaceae) is a large and diverse group of bacteria that can cause gastrointestinal infections in animals and humans. Molecular methods have shown that the genus consists of only two species, *Salmonella enterica* and *Salmonella bongori* (Percival and Williams, 2014g; Graziani et al., 2017). *Salmonella enterica* is further divided into six subspecies and contains the majority of the over 2500 serotypes that have been identified (Grimont and Weill, 2007; Percival and Williams, 2014g). Early in *Salmonella* identification, serotypes were treated as species and given names that reflected the host or disease it was associated with or, later, the geographic location where it was found (Grimont and Weill, 2007). When the current taxonomic structure of *Salmonella* was established, these names had become so familiar that they have been maintained, substituting for the O and H group naming structure that is more commonly used with other bacterial species (Grimont and Weill, 2007).

Salmonella of human importance are broadly categorized into two main groups according to the type of disease they cause. The typhoidal *Salmonella* (*S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi) are the causative agents of enteric fever (also known as typhoid or parathyroid fever), a serious and life-threatening illness (Sanchez-Vargas et al., 2011). The non-typhoidal *Salmonella* are a large group containing the remainder of the *S. enterica* serotypes which cause gastrointestinal illness of varying severity (Sanchez-Vargas et al., 2011). In developed countries, non-typhoidal *Salmonella* are the most important as food and waterborne pathogens (Sanchez-Vargas et al., 2011; Percival and Williams, 2014g). *Salmonella* serotype Enteritidis (*S.* Enteritidis) and *S*almonella serotype Typhimurium (*S.* Typhimurium) are the serotypes most commonly encountered as causes of human infections (Sanchez-Vargas et al., 2011).

Salmonella are Gram-negative, facultative anaerobic, predominantly motile rod-shaped bacteria that can grow over the temperature range of 5–47°C, and optimally at 35–37°C (Graziani et al., 2017).

B.1.4.2 Health effects

Salmonella infections follow different courses of disease depending on whether the serotype is typhoidal or non-typhoidal (Sanchez-Vargas et al., 2011). Non-typhoidal *Salmonella* cause a gastroenteritis characterized by diarrhea, fever and abdominal pain (Percival and Williams, 2014g, Graziani et al., 2017). Symptoms occur within 12–72 hours of infection and the illness may last four to seven days. In severe cases, infection can spread to other parts of the body (e.g., blood, urine, joints, brain) and can be life-threatening (Percival and Williams, 2014g; Sanchez-Vargas et al., 2011). Children

have the highest incidence of *Salmonella* infections (Christenson, 2013; PHAC, 2018c). Severe infections and fatal cases are rare and are more commonly reported among the very young, the very old and those with compromised immune systems or underlying illness (Sanchez-Vargas et al., 2011; Dekker and Frank, 2015). A meta-analysis of non-typhoidal *Salmonella* cases developing long-term complications estimated the proportion developing reactive arthritis at 5.8% (95% CI: 3.2–10.3%) and irritable bowel syndrome at 3.3 % (95% CI: 1.6–6.6%) (Keithlin et al., 2015). Estimates for other outcomes (e.g., HUS, Guillain–Barré Syndrome) were impeded by a lack of data (Keithlin et al., 2015). Typhoidal *Salmonella* cause enteric fever, an invasive and systemic disease which involves high fever, vomiting, headaches and numerous potentially fatal complications (Sanchez-Vargas et al., 2011). Enteric fever predominantly occurs in developing countries. In developed countries the incidence of enteric fever is infrequent and mostly related to travel (Sanchez-Vargas et al., 2011). The dose required to cause infection varies depending on the serotype involved and the susceptibility of the host. Data suggests that the infective dose (non-typhoidal *Salmonella*) can range from a low of less than 100 organisms to a high of 100,000 to 10 billion organisms (Kothary and Babu, 2001).

Salmonella is the second-leading cause of bacterial gastrointestinal illness in Canada, the U.S. and Europe (Scallan et al., 2011; CDC, 2018; ECDC, 2019; PHAC, 2019b). In Canada, reported annual incidence rates of salmonellosis (all sources) in 2013–2017 ranged from 17.6 to 21.7 (median: 21.38) cases per 100,000 population (PHAC, 2019b). Cases of illness are predominantly sporadic, with most illness associated with consumption of contaminated food. Peak incidence of disease (all sources) occurs in the summer and fall (Fleury et al., 2006; Lal et al., 2012).

Infections with non-typhoidal *Salmonella* are generally self-limiting, and treatment involves fluid and electrolyte replacement (Percival and Williams, 2014g). Antibiotics can be prescribed in severe cases where there is increased risk of infection spread (Sanchez-Vargas et al., 2011; Percival and Williams, 2014g). No human vaccines are currently available for non-typhoidal *Salmonella* infections (Sanchez-Vargas et al., 2011). The CDC, WHO and PHAC have categorized non-typhoidal *Salmonella* resistant to ciprofloxacin, ceftriaxone or multiple classes (e.g., >3) of drugs as priority to "High-level Threats" (CDC 2019a; WHO, 2017b, PHAC, 2018a). In developed countries, antibiotic resistance in *Salmonella* has generally followed trends in the use of antimicrobials in food-producing animals, (McDermott et al., 2018). The most frequent types of resistances observed are for older antimicrobials (e.g., tetracycline, sulfonamides, streptomycin) (McDermott et al., 2018). Declines in resistance rates for critical drugs for animals and humans (third generation beta-lactam antibiotics, ciprofloxacin) have been reported in the U.S. and Canada and are consistent with policies limiting their use in agriculture (McDermott et al., 2018; PHAC, 2018a).

B.1.4.3 Sources and exposure

Non-typhoidal *Salmonella* are zoonotic pathogens. Chicken, pigs, turkey and cattle are considered the most important reservoirs (Graziani et al., 2017). Other animals (dogs, birds, rodents, reptiles) and humans (infected individuals and asymptomatic carriers) are also recognized as sources (Percival and Williams, 2014g; Graziani et al., 2017). Humans are the only known reservoir for the typhoidal *Salmonella* serotypes (Percival and Williams, 2014g).

Salmonella are spread by fecal-oral transmission. For the non-typhoidal serotypes, contaminated food is the most common pathway for infection; and person-to-person contact and direct contact with animals are significant exposure pathways (Percival and Williams, 2014g; Graziani et al., 2017). Ingestion of contaminated water is a recognized route for infection of non-typhoidal Salmonella (Graziani et al., 2017). For sources of contamination important to drinking water, see *Campylobacter* spp. (see Section B.1.1). Non-typhoidal *Salmonella* are very rarely linked to drinking water outbreaks (CDC, 2004, 2006, 2008, 2011, 2013, 2015b, 2017d; Hrudey and Hrudey, 2004).

B.1.5 *Yersinia* spp.

B.1.5.1 Description

The genus *Yersinia* (Class: Gammaproteobacteria, Family: Enterobacteriaceae) contains approximately 20 bacterial species, with only three recognized as human pathogens. Two species (*Yersinia enterocolitica*, *Yersinia paratuberculosis*) are recognized as food or waterborne enteropathogens that can cause acute gastroenteritis of mild to high severity (Percival and Williams, 2014i; Fredriksson-Ahomaa, 2015). *Yersinia enterocolitica* can be divided into six biotypes differentiated by physiochemical and biochemical tests, and into more than 30 serotypes based on variations in their surface O antigens (Sabina et al., 2011; Fredriksson-Ahomaa, 2015). Human infections have traditionally been associated with certain biotype and serotype combinations. Types 1b:O8, 2:O5,27, 2:O9, 3:O3, 4:O3 are most commonly associated with human disease worldwide (Todd, 2014; Fredriksson-Ahomaa, 2015, 2017). *Y. paratuberculosis* is more closely related to the plague bacteria (*Yersinia pestis*) than *Y. enterocolitica,* and is a less common cause of human infections (Todd, 2014). For *Y. paratuberculosis* there are over 20 serotypes based on O antigen variations, all of which are pathogenic (Percival and Williams, 2014i).

Members of the genus *Yersinia* are Gram-negative, motile, facultatively anaerobic, rod to coccobacilli-shaped cells that are able to grow at temperatures over the range of 4–43°C (optimum: 28–30°C) (Todd, 2014; Fredriksson-Ahomaa, 2015).

B.1.5.2 Health effects

Enteropathogenic *Yersinia* are enteroinvasive microorganisms which colonize and invade colon epithelial cells, causing diarrhea and inflammatory reactions (Percival and Williams, 2014i; Todd, 2014). Disease caused by *Y. enterocolitica* or *Y. paratuberculosis* is commonly referred to as yersiniosis (Fredriksson-Ahomaa, 2015). Symptoms of yersiniosis can differ depending on the age and immunity of the person infected, the strain involved and the infective dose (Todd, 2014). Diarrhea (often bloody), fever and abdominal pain are the most common symptoms in infants and young children under 5 years of age (Fredriksson-Ahomaa, 2015). In older children and adults, fever and right-sided abdominal pain may be the predominant symptoms and can be confused with appendicitis (Todd, 2014; Fredriksson-Ahomaa, 2015). Symptoms occur 1 to 11 days after exposure and can persist 1 to 3 days or longer (Todd, 2014; Fredriksson-Ahomaa, 2015). Asymptomatic infections with *Y. enterocolitica* and *Y. paratuberculosis* have been reported; and the pathogens can continue to be shed in feces for weeks after symptoms have ceased (Todd, 2014). Occasionally in severe cases, the bacteria can enter the lymph nodes and infection can be further spread by the bloodstream (Percival and Williams, 2014i; Fredriksson-Ahomaa, 2015). Complications from infections are uncommon, but can include joint pain (reactive arthritis) and skin rash (Percival and Williams, 2014i; Fredriksson-Ahomaa, 2015). Other symptoms less frequently associated with enteropathogenic *Yersinia* infection are various inflammatory reactions resulting from infection spread to other parts of the body (e.g., liver, spleen, lung, heart, brain, bones) (Percival and Williams, 2014i, Todd, 2014). Young children are more likely to become ill from infection with enteropathogenic *Yersinia* (Todd, 2014; PHAC, 2018c). Severe infections and fatal cases are rare and are typically observed in the elderly and immunosuppressed individuals (Todd, 2014). The infective dose is estimated to range between 10 thousand and 1 billion organisms for both *Y. enterocolitica* and Y. *paratuberculosis* (Todd, 2014); however, the dose is likely lower for immunosuppressed individuals (Fredriksson-Ahomaa, 2017).

Enteropathogenic *Yersinia* are a significant cause of bacterial gastrointestinal illness in Canada, the U.S. and Europe (PHAC 2019a; CDC 2018; ECDC, 2018b). National incidence data is not available as yersiniosis is a notifiable disease only in the province of Alberta (PHAC, 2019a). However, Canadian sentinel surveillance data from three locations (BC, AB and ON), reported endemic incidence rates of 10.16, 1.77 and 0.00 per 100,000 population respectively in 2018 (PHAC, 2019a). The majority of cases of *Yersinia*-associated illness are caused by *Y. enterocolitica* and are linked to the consumption of contaminated food (Todd, 2014; Fredriksson-Ahomaa, 2015; PHAC, 2018c). Generally, *Yersinia* infections are more frequently observed during the winter months (Todd, 2014; Fredriksson-Ahomaa, 2015).

Infections with *Y. enterocolitica* or *Y. paratuberculosis* are normally self-limiting, with treatment provided only in severe cases involving systemic infection or bacteremia (Todd, 2014; Fredriksson-Ahomaa, 2015). No human vaccines are available.

B.1.5.3 Sources and exposure

Pathogenic and non-pathogenic *Yersinia* spp. can be found in the gut of a variety of wild and domestic animals (Percival and Williams, 2014i; Fredriksson-Ahomaa, 2015). Pigs are the major reservoir of pathogenic strains of Y. *enterocolitica*; and ruminants (e.g., cattle, sheep, goats), dogs and cats are also important sources of the pathogen (Todd, 2014; Fredriksson-Ahomaa, 2015). Rodents and birds are considered the major reservoirs for *Y. paratuberculosis* (Todd, 2014; Fredriksson-Ahomaa, 2015). Pathogenic *Yersinia* spp. are zoonotic, thus can be transmitted from animals to humans via the fecal-oral route (Fredriksson-Ahomaa, 2015). Contaminated food sources are the most significant pathway for infections (Todd, 2014; Fredriksson-Ahomaa, 2015). Consumption of contaminated water and direct contact with animals are also important infection pathways (Todd, 2014; Fredriksson-Ahomaa, 2015). Person-to-person transmission is possible, but rare (Todd, 2014; Fredriksson-Ahomaa, 2015).

In most studies, it is the non-pathogenic species or strains that are more frequently isolated (Brennhovd et al., 1992; Cheyne et al., 2009; Schaffter and Parriaux, 2002). The low isolation frequency in environmental samples may be due to limitations in the sensitivity of culture-based methods (Fredriksson-Ahomaa and Korkeala, 2003). Cheyne et al. (2010) found that using PCR methods, *Yersinia* virulence genes were detected in 21–38% of samples taken from a heavily impacted river watershed that was used as a source for a drinking water treatment system.

Yersinia spp. have very rarely been linked to drinking water outbreaks. According to U.S. data for 2001–2014 (the last year for which data is available), *Yersinia enterocolitica* was associated with one drinking water outbreak as a co-occurring agent with *Campylobacter jejuni* (CDC, 2004, 2006, 2008, 2011, 2013, 2015b, 2017d). A contaminated untreated non-community groundwater supply was identified as the cause of the outbreak (CDC, 2004).

B.1.6 Analytical Methods

Standard methods are available for the detection of *Campylobacter* spp., pathogenic *E. coli /Shigella* spp., *Salmonella spp.* and *Yersinia* spp. in drinking water (APHA et al., 2017; ISO, 2019). The procedures for isolating and identifying these bacteria commonly involve steps such as enrichment and/or separation, plating, colony screening and identification using biochemical tests, serological techniques, molecular methods or commercial kits (e.g., for toxins) (APHA et al., 2017; ISO 2019).

No standard methods for the detection of viable *Helicobacter* spp. in water have been established (Percival and Williams, 2014d, APHA et al., 2017). Methods for the detection of *H. pylori* in water environments involve the use of culture-independent molecular techniques such as PCR or fluorescent in-situ hybridization (Watson et al., 2004; Percival and Williams, 2014d; Santiago et al., 2015; Richards et al., 2018).

For a review of methods used in research settings, please refer to: Ramírez-Castillo et al., 2015; Kennedy and Wilkinson, 2017; Gorski et al., 2019; Li et al., 2020.

B.1.7 Treatment considerations

When properly designed and operated, physical removal technologies—chemically-assisted, slow sand, diatomaceous earth and membrane filtration or an alternative proven technology—and primary disinfection methods—chlorine chlorine dioxide, ozone and ultraviolet (UV) light—commonly used in drinking water treatment are very effective in reducing or inactivating the enteric bacteria described in the preceding sections (LeChevallier and Au., 2004). Monochloramine should not be used for primary disinfection due to its low oxidation potential; monochloramine is recommended only for secondary disinfection (i.e., to maintain a disinfectant residual in the distribution system) (Health Canada, 2019b).

The CT requirements for chemical disinfectant inactivation of these bacteria are comparable to those for *E. coli* and less than those required for enteric protozoa and enteric viruses (Sobsey, 1989; Lund, 1996; Johnson et al., 1997; Rice et al., 1999; Baker et al., 2002; LeChevallier and Au, 2004; Rose et al., 2007; Wojcicka et al., 2007; Chauret et al., 2008; Rasheed et al., 2016; Jamil et al., 2017; Health Canada, 2019b, 2019c, 2020c). The UV dose (intensity × time) requirements for inactivation of these microorganisms are similarly comparable to those for *E. coli* and enteric protozoa, and less than those needed for many enteric viruses (Sommer et al., 2000; Zimmer and Slawson, 2002; Smeets et al., 2006; Hayes et al., 2006; Zimmer-Thomas et al., 2007; Hijnen et al., 2011; Health Canada, 2019b, 2019c, 2020c)

General operational and maintenance practises for the control of microbial growth and survival in drinking water distribution and plumbing systems are outlined in Part A (LeChevallier and Au, 2004; Friedman et al., 2017). These are necessary to manage biofilms which can provide a habitat for the survival of fecal pathogens that may have passed through drinking water treatment barriers or entered the distribution system directly via an integrity breach (Leclerc, 2003).

For residential-scale systems and private wells, regular physical inspection to identify deficiencies and testing of the water system (e.g., for *E. coli* and total coliforms) to confirm the microbiological quality of the water are important. General guidance on well construction, maintenance, protection and testing is typically available from provincial/territorial jurisdictions. Well owners can also consult the *Be Well Aware* series for information (Health Canada, 2019a). Where treatment is necessary, Health Canada recommends that consumers use devices certified by an accredited certification body as meeting the appropriate NSF International (NSF)/American National Standards Institute (ANSI) drinking water treatment unit standards (NSF/ANSI, 2018, 2019, 2020). Certification organizations provide assurance that a product conforms to applicable standards and must be accredited by the Standards Council of Canada (SCC). An up-to-date list of accredited certification organizations can be obtained from the SCC (2020).

B.1.8 International considerations

No drinking water guidelines for the enteric bacterial pathogens *Campylobacter* spp., enteric pathogenic *E. coli*/*Shigella* spp., *Helicobacter pylori*, *Salmonella* spp. and *Yersinia* spp. have been established by the WHO, the EU, the US EPA or the Australian National Health and Medical Research Council (NHMRC, NRMMC 2011; WHO, 2017a; European Commission, 2020; US EPA, 2021a). Similar to Health Canada's guidance document, the WHO and Australian drinking water guidelines contain fact sheets that provide information on waterborne pathogens of concern.

B.2 NATURALLY-OCCURRING PATHOGENS

B.2.1 Bacteria

B.2.1.1 *Aeromonas* spp.

B.2.1.1.1 Description

The bacterial genus *Aeromonas* (Class: Gammaproteobacteria) has a complex taxonomy. Around 30 species have been associated with the genus and potential new species continue to be described, although not all have been universally accepted (Janda and Abbott, 2010; Percival and Williams, 2014a; LPSN, 2019). The difficulties with *Aeromonas* identification

arises from the lack of clear-cut phenotypic characteristics and the absence of a consistent typing scheme for distinguishing species. As a result, the use of biochemical and molecular methods is required for an accurate classification. Clinically relevant *Aeromonas* spp. are opportunistic pathogens that have been linked to a variety of intestinal and extra-intestinal diseases and syndromes (Janda and Abbott, 2010, Liu, 2015). Fourteen species have been implicated in human illness, however most human infections (85%) are caused by strains of four species: *A. hydrophila*, *A. caviae*, *A. veronii* (biotype sobria) and *A. trota* (Percival and Williams, 2014a; Liu, 2015; Bhowmick and Battacharjee, 2018).

Aeromonads are Gram-negative, facultatively anaerobic, non-spore-forming rod-shaped bacteria (Janda and Abbott, 2010; Percival and Williams, 2014a). Strains associated with human infections grow optimally at temperatures of 35–37°C, although many strains can grow in 4–42°C (Janda and Abbott, 2010; Percival and Williams, 2014a; Liu, 2015).

B.2.1.1.2 Health effects

Gastroenteritis is the most commonly encountered disease associated with *Aeromonas* infection (Janda and Abbott, 2010). Forms of the disease range from a watery enteritis accompanied by low-grade fever, vomiting and abdominal pain (most common) to a dysenteric form involving bloody stools (rare), to a cholera-like illness (very rare) (Janda and Abbott, 2010, Liu, 2015). *Aeromonas* spp. are an infrequent cause of traveller's diarrhea and they can also be associated with a subacute or chronic intestinal infection (Janda and Abbott, 2010, Liu, 2015).

The time between infection and symptom onset is one to two days for *Aeromonas*-associated traveller's diarrhea (Janda and Abbott, 2010). Subacute cases of diarrhea are defined as those lasting from two weeks to two months, whereas chronic cases persist for longer periods (Janda and Abbot, 2010). Complications that have been associated with more severe cases of *Aeromonas* gastroenteritis include ulcerative colitis, haemolytic uremic syndrome and inflammatory bowel disease (Janda and Abbott, 2010, Liu, 2015). The dose of *Aeromonas* spp. necessary to cause gastrointestinal infection is not clear. The only published challenge study showed that only 2/5 strains produced infection (14/57 individuals) and diarrhea (2/57 individuals) at high concentrations (ten thousand to ten billion colony forming units (CFU)) (Morgan et al., 1985). Data provided from foodborne outbreaks that have been observed suggests that the concentration required to cause infection could be orders of magnitude lower for some *Aeromonas* strains (Teunis and Figueras, 2016).

Skin and soft tissue infections are the second most common forms of *Aeromonas*-related disease. *Aeromonas* spp. can be associated with a variety of infections ranging from mild irritations (e.g., pus-filled lesions) to serious or life-threatening infections such as cellulitis or flesh-eating disease (Janda and Abbott, 2010; Bhowmick and Battacharjee, 2018).

Aeromonads have also been implicated in blood-borne infections, which largely arise through the transfer of bacteria from gastrointestinal tract or wound infections. Common features associated with these infections are fever, jaundice, abdominal pain and septic shock (Janda and Abbott, 2010). Other less frequent diseases linked to *Aeromonas* infection include respiratory tract, urogenital tract and ocular infections (Janda and Abbott, 2010). High mortality rates have been observed with *Aeromonas* septicemia and severe wound infections in high at-risk individuals (Janda and Abbot, 2010; Liu, 2015).

Aeromonas-associated diarrhea has been encountered worldwide in healthy persons across all age groups (Janda and Abbot, 2010; Percival and Williams, 2014a; Teunis and Figueras, 2016). Still, given that *Aeromonas* spp. are widely encountered in food and water, illness is observed in relatively few individuals who are exposed to the bacteria (Janda and Abbott, 2010). Gastrointestinal infections are more prevalent in developing countries (Ghenghesh et al., 2008). Susceptible groups include infants, young children, the elderly and persons with lowered immune status or underlying disease such as liver disease and malignant illnesses (Ghenghesh et al., 2008; Liu, 2015). Skin and soft tissue infections are often the result of trauma or penetrating injury and occur more frequently in adults than children (Janda and Abbot, 2010). For *Aeromonas*-associated bacteremia, the vast majority of cases are in immunocompromised individuals (Janda and Abbot, 2010). Antibiotics can be prescribed in severe cases where there is increased risk of infection spread (Percival and Williams, 2014a; Liu et al., 2015). PHAC has categorized drug-resistant *Aeromonas* spp. as a low priority for research and surveillance compared to other antimicrobial resistant pathogens (Garner et al., 2015). No human vaccines are currently available for *Aeromonas* infections (Liu et al., 2015).

Aeromonas–associated infections are not reportable illnesses in North America or in most countries worldwide. Case reports and outbreaks of illness have mostly been tied to food, hospital exposures, travel, non-water environments or unknown causes (Teunis and Figueras, 2016). Infections are more frequently observed during the warmer months (Janda and Abbot, 2010; Bhowmick and Battacharjee, 2018).

B.2.1.1.3 Sources and exposure

Aeromonas spp. can exist in virtually every ecosystem niche, including aquatic habitats, soils, vertebrate and invertebrate animal species, insects and foods (Janda and Abbot, 2010; Percival and Williams, 2014a). They are found in water and aquatic environments (e.g., lakes, rivers, groundwater, seawater, potable water supplies, wastewater and sewage) in all but the most extreme conditions of pH, temperature and salinity (Janda and Abbot, 2010). Members of the genus are found in the gastrointestinal tract of cold-blooded and warm-blooded animals including fish, birds, reptiles and domestic livestock. *Aeromonas* spp. can be isolated from the feces of healthy humans as a result of consumption of food and

water containing the microorganisms (Percival and Williams, 2014a). They can be found in high concentrations in wastewater (Janda and Abbott, 2010; Percival and Williams, 2014a). Aeromonads grow optimally at elevated temperatures, thus concentrations in water are at their highest during the warmer months (LeChevallier et al., 1982; Gavriel et al., 2008; Chauret et al., 2001; Egorov et al., 2011).

Ingestion of contaminated food and water are considered the main routes of transmission for *Aeromonas*-associated gastroenteritis. Direct body contact with contaminated water is the primary method of transmission for *Aeromonas* spp. in water-related skin and soft tissue infections. Contaminated floodwaters in natural disaster settings have been identified as an important vehicle for these types of illnesses (Tempark et al., 2013). Person-to-person transfer is not considered a risk with *Aeromonas* infections.

Aeromonads are not commonly detected in the bulk water in municipal distribution systems with a disinfectant residual (Chauret et al., 2001; Egorov et al., 2011). In a survey of 293 public water systems in the U.S., *Aeromonas* spp. were detected by culture methods at 42 systems (14.3%), with concentrations ranging from 0.2 to 880 (median 1.6) CFU per 100 mL (Egorov et al., 2011). Groundwater is expected to have lower numbers of Aeromonads than surface waters, but drinking water wells can become colonized by the bacteria (Borchardt et al., 2003; Percival and Williams, 2014a; Katz et al. 2015). Aeromonads are capable of growth and persistence in distribution system biofilms and this can contribute to an increased recovery of the microorganisms from drinking water supplies (Gavriel et al., 1998; Chauret et al., 2001).

The importance of drinking water as a route of transmission for *Aeromonas*-related gastrointestinal illness is not clearly understood. Species of *Aeromonas* possessing multiple virulence genes have been detected in drinking water supplies in North America and in other countries (Handfield et al., 1996; Kühn et al., 1997; Sen and Rogers, 2004; Robertson et al., 2014b). Some investigations attempting to link strains recovered from drinking water supplies to patient isolates have been unsuccessful (Havelaar et al., 1992; Borchardt et al., 2003). Other studies have presented evidence of an epidemiological link between *Aeromonas* in clinical samples and drinking water as a source of infection (Khajanchi et al., 2010; Katz et al., 2015). It is generally accepted that only a subset of *Aeromonas* strains can cause gastrointestinal illness in humans (Teunis and Figueras, 2016). Furthermore, it is believed that infection is a complex process involving the virulence of the *Aeromonas* strain, its interaction with other microbes present in the gut (as co-infecting pathogens or as part of the natural microbiota) and the health status of the host (Teunis and Figueras, 2016). As a result, the presence of *Aeromonas* spp. in drinking water on its own is not sufficient to signify that a health risk exists (Edberg et al., 2007). More work is needed to determine the specific combination of host, environment

and pathogen factors that lead to the occurrence of gastrointestinal illness associated with *Aeromonas* infections (Teunis and Figueras, 2016). No known drinking water outbreaks associated with *Aeromonas* have been recorded (Janda and Abbot, 2010; Teunis and Figueras, 2016).

B.2.1.1.4 Analytical methods

Standard methods for the detection of *Aeromonas* in drinking water are available (US EPA, 2001; APHA et al., 2017). However, there is no established universally-accepted culture-based method capable of detecting all Aeromonads in water samples (APHA et al., 2017). *Aeromonas* spp. are heterotrophic bacteria and are detected by heterotrophic plate count (HPC) tests; however, no direct correlation between HPC counts and *Aeromonas* concentrations exists.

B.2.1.1.5 Treatment considerations

When properly designed and operated, physical removal—chemically-assisted, slow sand, diatomaceous earth and membrane filtration or an alternative proven technology—and primary disinfection methods—chlorine, chlorine dioxide, ozone and UV—commonly used in drinking water treatment are very effective in reducing or inactivating *Aeromonas* spp. (Chauret et al., 2001; WHO, 2002; US EPA, 2006a; Yu et al., 2008). Monochloramine should not be used for primary disinfection due to its low oxidation potential; monochloramine is recommended only for secondary disinfection (i.e., to maintain a disinfectant residual in the distribution system) (Health Canada, 2019b). The use of granulated activated carbon (GAC) in water treatment may provide nutrient sources for aeromonads which can contribute to their presence and survival in drinking water distribution systems (WHO, 2002; US EPA, 2006a).

Aeromonas are as sensitive to chemical disinfectants as *E. coli* and other waterborne bacteria (Knøchel, 1991; Medema et al., 1991; Sisti et al., 1998; WHO, 2002; US EPA, 2006a). The CT requirements for inactivation of *Aeromonas* spp. by chemical disinfectants are less than those required for numerous enteric viruses. The UV dose requirements are comparable to other waterborne enteric bacterial pathogens and the enteric protozoa *Giardia* and *Cryptosporidium* and are less than those needed for many enteric viruses (Massa et al., 1999; Gerba et al., 2003; US EPA, 2006a; Health Canada, 2019b, 2019c).

General operational and maintenance practises for managing microbial survival and growth in drinking water distribution and plumbing systems as outlined in Part A are important for the control of *Aeromonas* spp. (Chauret et al., 2001; WHO, 2002; Percival and Williams, 2014a).

For residential-scale systems and private wells, regular physical inspection to identify deficiencies and testing of the water system (e.g., for *E. coli* and total coliforms) to confirm the microbiological quality of the water, are important. Where problems with the microbiological quality of the drinking water are suspected, it may be useful to include additional parameters (e.g., HPC) in the analysis (WHO and OECD, 2003, Health Canada, 2020c). Specific guidance on construction, operation, maintenance and testing should be obtained from the responsible drinking water authority in the affected jurisdiction.

B.2.1.1.6 International considerations

No guideline for *Aeromonas* spp. in drinking water has been established by the WHO, the EU, the US EPA or the Australian National Health and Medical Research Council (NHMRC, NRMMC 2011; WHO, 2017a; European Commission, 2020; US EPA, 2021a). In the Netherlands, Dutch drinking water legislation specifies a monitoring requirement for *Aeromonas* as an operational parameter with a target limit of < 1000 CFU/100 mL (Smeets et al., 2009). This target is based on treatment achievability and not on public health significance (WHO, 2002).

B.2.1.2 *Legionella* spp.

B.2.1.2.1 Description

The bacterial genus *Legionella* (Class: Gammaproteobacteria) comprises 61 species and 3 subspecies (LPSN, 2019). At least 30 species have been known to cause human infection (Cuhna et al., 2016; Burillo et al., 2017). Pathogenic *Legionella* spp. are opportunistic pathogens that cause respiratory illness in two main forms: Legionnaires' disease and Pontiac fever (Percival and Williams, 2014e, NASEM, 2020). *Legionella* have also been associated with extrapulmonary infections, although these are much rarer (NASEM, 2020, CDC, 2021a). Illnesses caused by *Legionella* spp. are collectively known as legionellosis. *Legionella pneumophila* (mainly serogroup 1) is the most common and virulent pathogen of the genus, responsible for 65–90% of all cases of Legionnaires' disease (Fields et al., 2002; Edelstein and Roy, 2015; Percival and Williams, 2014e, Prussin II et al., 2017). Diagnostic clinical tests are optimized for the detection of this species and serogroup, thus the proportion of disease caused by non-pneumophila, non-serogroup 1 *Legionella* is likely underestimated (NASEM, 2020). Other *Legionella* species can lead to disease, including: *L. micdadei*, *L. bozmanae*, *L. dumoffii* and *L. longbeachae* (Edelstein and Roy, 2015; Percival and Williams, 2014e; Cuhna et al., 2016).

The bacteria are Gram-negative, obligately aerobic, predominantly motile, short rod-shaped cells that require specific nutrients (L-cystine and iron) for growth (Percival and Williams, 2014e). During its life cycle, *Legionella* can adapt to fluctuating conditions by differentiating into cell types that vary in their infectivity and resistance to disinfection (Robertson et al., 2014a; NASEM, 2020).

B.2.1.2.2 Health effects

Legionnaires' disease is a severe respiratory illness involving pneumonia, with symptoms that include fever, cough, chills, neurological aspects (confusion), muscle pain, headache and gastrointestinal problems (diarrhea, nausea, vomiting) (Castillo et al., 2016, Cunha et al., 2016; Edelstein and Roy, 2015). Symptom onset generally occurs 2 to 14 days after becoming infected (NASEM, 2020), and the disease can persist for weeks to several months (Palusińska-Szysz and Cendrowska-Pinkosz, 2009). Although many individuals are exposed to *Legionella* bacteria, few develop illness (Castillo et al., 2016). Legionnaires' disease has a low attack rate, affecting less than 1–5% of the general population and less than 1–14% of hospital patients who are exposed to the bacteria during outbreaks (Hornei et al., 2007; Edelstein and Roy, 2015; Leoni et al., 2018). Legionnaires' disease is more likely to occur in older adults or immunocompromised individuals, however healthy individuals can acquire Legionnaires' disease if they are exposed to a high enough concentration of the bacteria (Springston and Yocavitch, 2017). Reports of Legionnaires' disease in healthy children are extremely rare (McDonough et al., 2007, Greenberg et al., 2006). Factors associated with an increased susceptibility to Legionnaires' disease following exposure are male gender, age beginning at 40–50 years, smoking, chronic heart or lung disease, diabetes, chronic renal failure, immunosuppression, organ transplantation and some forms of cancer (Fields et al., 2002; Edelstein and Roy, 2015, Castillo et al., 2016; Cuhna et al., 2016; NASEM, 2020). The case fatality rate associated with Legionnaires' disease depends on the underlying health of the patients, how quickly therapy is delivered and whether the cases are sporadic, hospital-acquired or outbreak-related (Edelstein and Roy, 2015). Mortality is estimated at less than 10–15% for community acquired cases, but can be higher than 25% for hospital acquired cases (Benin et al., 2002; Howden et al., 2003; Dominguez et al., 2009; Soda et al., 2017; Leoni et al., 2018).

Pontiac fever is a milder, flu-like, self-limiting and non-pneumonic disease associated with exposure to *Legionella*. The disease has mainly been diagnosed in outbreaks where individuals have flu-like symptoms and share exposure to aerosols from a common source (Lüttichau et al., 1998). How Pontiac fever develops is poorly understood and why some persons develop this disease while others develop Legionnaires' disease is not known (Fields, et al., 2001; Edelstein, 2007). It has been proposed that Pontiac fever may be due to exposure to some combination of live and dead microorganisms (either *Legionella* species

or coexisting microorganisms) and their products (including endotoxins) (Edelstein, 2007). Pontiac fever has a high attack rate, affecting as high as 80–90% of exposed individuals during outbreaks (Leoni et al., 2018). Symptoms appear from five hours to three days after infection and last for two to seven days. Long-term complications are not observed and the disease is not fatal (Tossa et al., 2006; Edelstein and Roy, 2015). There appear to be no predisposing host factors for Pontiac fever (Edelstein and Roy, 2015). Cases of Pontiac fever in children have been reported during outbreaks of the disease (Lüttichau et al., 1998; Goldberg et al., 1989, Jones et al., 2003; Burnsed et al., 2007).

Legionnaire's disease and Pontiac fever are the most common manifestations of legionellosis; extrapulmonary disease caused by *Legionella* is extremely rare (NASEM, 2020, CDC, 2021a). Reported sites of extrapulmonary infections include the skin, joints and soft tissues which line the heart (Padrnos et al., 2014; Ibranosyan et al., 2019; CDC, 2021a). These infections have been most frequently observed in individuals who are immunosuppressed or who have concurrent *Legionella* pulmonary infections (Padrnos et al., 2014; Ibranosyan et al., 2019). Extrapulmonary infections are predominantly caused by *Legionella* species and strains other than *L. pneumophila* and *L. pneumophila* serogroup 1 (Padrnos et al., 2014; Ibranosyan et al., 2019).

Dose-response models have been developed for a few specific *Legionella* strains, derived from animal experiments (NASEM, 2020). No expert consensus exists on whether there is a threshold for detectable *Legionella* below which there is no risk of infection (NASEM, 2020).

Legionella is the major cause of waterborne illness outbreaks in the U.S. (Neil and Berkleman, 2008; CDC, 2017d; Friedman et al., 2017). Large *Legionella* outbreaks receive the most attention given their substantial health impact. However, it is estimated that less than 20% of all reported legionellosis cases are outbreak-related (Fields et al., 2002; Neil and Berkleman, 2008; Burillo et al., 2017). In Canada, reported rates of legionellosis in 2006–2016 (the latest year for which data have been published) were 0.37–1.39 (median: 0.71) per 100,000 population (PHAC, 2019b). Reported rates from the U.S. were 1.0–1.89 (median 1.18) per 100,000 population over the same period (Adams et al., 2016, 2017). As legionellosis is underdiagnosed and underreported, the actual number of cases is expected to be much higher (Castillo et al., 2016; PHAC, 2018d). A 22 month, multicentre Canada-wide study reported that 3.2% of patients diagnosed with community-acquired pneumonia (28/850) had Legionnaires' disease (Marrie et al., 2003). Another Canadian study examining the frequency of Legionnaires' disease in the summer months (May to October) found that 28% (9/33) of patients diagnosed with pneumonia tested positive for *Legionella* (Spiegelman et al., 2020). Legionellosis follows a distinct seasonal pattern, with

the peak number of cases occurring during summer and fall (Prussin II et al., 2017, Cuhna et al., 2016). The yearly incidences of legionellosis in Canada and the U.S. are on the rise (Adams et al., 2016, 2017; PHAC, 2019b). Factors contributing to the yearly incidence rate include a true increase in the number of cases, greater use of diagnostic testing, and increased reporting (Burillo et al., 2017).

As *Legionella* are intracellular pathogens, treating Legionnaires' disease requires the use of antibiotic agents capable of reaching therapeutic concentrations within human cells (Fields et al., 2002; Edelstein and Roy, 2015, Castillo et al., 2016; Wilson et al., 2018). No human vaccine for the disease exists (Edelstein and Roy, 2015). Most individuals with Pontiac fever do not become ill enough to seek medical attention, and antibiotic treatment is generally not required (Edelstein and Roy, 2015, Castillo et al., 2016). Trends in antibiotic resistance in *Legionella* spp. are not well understood (Wilson et al., 2018). Data on resistance of clinical isolates to antibiotics is not well documented due to the absence of easily performed tests (Wilson et al., 2018).

B.2.1.2.3 Sources and exposure

Legionella has two habitats—a primary reservoir in the natural environment and a secondary habitat in engineered water systems (NASEM, 2020). Its growth in these habitats is predominantly within free-living protozoa that reside within biofilms (Devos et al., 2005; NASEM, 2020). *Legionella* has been detected in freshwater and soil environments including lakes, rivers, sediments and groundwater worldwide (Fields et al., 2002; Percival and Williams, 2014e, Burillo et al., 2017; NASEM, 2020). Human and animal feces are not considered a source of *Legionella*, although it can be detected in the feces of infected individuals experiencing diarrhea symptoms. Animals can be infected by *Legionella*, but zoonotic transmission of the organism has not been documented (Surman-Lee et al., 2007; Edelstein and Roy, 2015).

As noted above, *Legionella* multiply inside protozoa (e.g., amoebae and ciliates) that are found in biofilms in natural waters and engineered water systems. Among them are: *Acanthamoeba* (see Section B.3.2.1), *Hartmanella*, *Naegleria* (see Section B.3.2.2), *Valkampfia*, *Vermamoeba* (formerly *Hartmanella*), *Echinamoeba* and *Tetrahymena* (Fields et al., 2002; Lau and Ashbolt, 2009; Buse et al., 2012, Percival and Williams, 2014e; NASEM, 2020). Survival within these protozoa provides a source of nutrients, a protective environment against disinfectants and other adverse conditions (such as elevated temperatures) and a means of transport (Percival and Williams, 2014e, Buse et al., 2012; NASEM, 2020). *Legionella* are also capable of persisting in biofilms in the absence of host protozoa (NASEM, 2020).

Legionella can be found in engineered water systems and equipment that support biofilm growth, including drinking water distribution systems, building and residential plumbing systems and cooling towers (NASEM, 2020). Low levels of *Legionella* can pass through treatment barriers, and grow in distribution system biofilms, where conditions are favourable. While *Legionella* is rarely detected in treated drinking water as it leaves the treatment plant (King et al., 2016; Hull et al., 2017), it is occasionally detected in drinking water distribution systems (Brooks et al., 2004; Pryor et al., 2004; Wang et al., 2012a; Lu et al., 2016; Hull et al., 2017; Waak et al., 2018; Dias et al., 2019; LeChevallier, 2019a,b). However, municipal drinking water distribution systems are not thought to be a major reservoir of *Legionella* (NASEM, 2020). It is important to note that storage facility sediments are known to harbour opportunistic pathogens, including *Legionella* (Lu et al., 2015; Qin et al., 2017). One legionellosis outbreak was attributed to a storage facility that had a low (<0.2 mg/L) free chlorine residual (Cohn et al., 2015).

Premise plumbing systems along with equipment supplied by these systems (e.g., cooling towers, hot tubs) are the priority area of concern for *Legionella* growth. Large complex premise plumbing systems, such as those found in hospitals, hotels, apartment buildings, community centres, industrial buildings and cruise ships, are significant sources of *Legionella*. Plumbing systems possess unique characteristics, which can promote the growth of *Legionella* to high concentrations. These characteristics include increased water temperatures, higher surface area to volume ratios, longer stagnation times, losses of disinfectant residual and the presence of nutrients. *Legionella* has been detected in both residential and non-residential buildings, in cold and hot water, at varying frequencies (Alary and Joly, 1991; Stout et al., 1992; Bates et al., 2000; Mathys et al., 2008; Donohue et al., 2014, 2019b; Bédard et al., 2015; Dilger et al., 2016; Collins et al., 2017; Hull et al., 2017; Dias et al., 2019; Gora et al., 2020). In general, the hot water supply system, because of its typically lower disinfectant residual concentrations and higher temperatures, tends to favour *Legionella* growth. Cold water supplies held at temperatures above 25°C can also have an increased risk of *Legionella* colonization (Donohue et al., 2014; Schwake et al. 2016).

Cooling towers or evaporative condensers in buildings and industry also represent a significant area of concern for *Legionella* growth. High rates of detection of *Legionella,* including *L. pneumophila,* have been reported for cooling towers (Llewellyn et al., 2017). Other potential sources of *Legionella* are car washes, decorative fountains and supermarket produce misters (NASEM, 2020).

GROWTH

Legionella typically grows at temperatures between 25 and 45°C, with an optimum temperature range between 25 and 35°C. Thus, water temperatures in this range support the highest levels of growth of this microorganism (NASEM, 2020). *Legionella* is also thermotolerant, meaning that it can survive at high temperatures, between 55 and 70°C (Allegra et al., 2008; Cervero-Aragó, 2015; 2019). Survival of *Legionella* in protozoan cysts following exposure to 80°C has been demonstrated (NASEM, 2020). Climate change and its associated temperature increases may facilitate *Legionella* growth (Cuhna and Cuhna, 2017; MacIntyre et al., 2018).

TRANSMISSION

Legionella has a water-to-air transmission, meaning inhalation of aerosols (size 2–10 µm) containing the bacteria is the main route of transmission (Percival and Williams, 2014e; Castillo et al., 2016). Generally, consumption of drinking water is not a recognized route of *Legionella* transmission (Percival and Williams, 2014e; Prussin II et al., 2017). It is hypothesized that microaspiration that occurs during drinking, or is associated with certain clinical conditions or procedures, is a potential source of exposure (NASEM, 2020). Inoculation of surgical wounds is another less common route of infection (Cuhna et al., 2016; Burillo et al., 2017). Person-to-person transmission generally does not occur (Percival and Williams, 2014e; Edelstein and Roy, 2015), but one probable case has been reported (Correia et al., 2016).

Given its route of transmission, fittings and equipment (e.g., showerheads, faucets, cooling towers, hot tubs, humidifiers and nebulizers, indoor fountains) capable of developing biofilms and of generating aerosols, represent potential sources of exposure to *Legionella* (NASEM, 2020).

WATERBORNE ILLNESS

Data on *Legionella* concentrations from epidemiological investigations and occurrence studies is limited, making it difficult to assess the extent of *Legionella* risk from various sources (NASEM, 2020). Epidemiological investigations of Legionnaires' disease show that outbreaks are most commonly linked to building water systems, cooling towers and recreational facilities such as hot tubs (NASEM, 2020). The building categories most frequently involved are hotels and resorts, hospitals, long-term care facilities and industrial buildings (Walser et al., 2013; Garrison et al., 2016; Beauté, 2017). Notable outbreaks of Legionnaires' disease in North America include Brooklyn, New York (2015: 138 cases, 16 deaths), Quincy, Illinois (2015: 58 cases, 12 deaths), Genessee County, Michigan (2014–2015: 87 cases, 12 deaths), Quebec City, Quebec (2012: 182 cases, 13 deaths) and Scarborough, Ontario (2005: 112 cases, 23 deaths) (Gilmour et al., 2007, Levesque et al., 2014, CDC, 2015a;

MDHHS, 2016, Weiss et al., 2017). The collective understanding of the origins of sporadic cases of legionellosis is limited. Residential and non-residential building water systems appear to contribute to a substantial proportion of sporadic disease; and cooling towers are also a potentially significant source (Orkis et al., 2018). However, definitive linkage of cases to specific sources is difficult (Orkis et al., 2018). The importance of domestic water systems as a source of *Legionella* infection is not clear (Bates et al., 2000; Prussin II et al., 2017). Immunocompromised individuals are at greater risk for acquiring legionellosis from contaminated residential plumbing systems (NASEM, 2020).

Although legionellosis case rates are highest in the summer and fall, the report of a community-associated outbreak of Legionnaires' disease in Calgary, Alberta in November and December of 2012 suggests that *Legionella* transmission can occur during the late fall and winter months in Canada (Knox et al., 2017). Rainfall and humidity have been associated with an increased risk of disease (Fisman et al., 2005; Beauté et al., 2016). Further changes in seasonality may be observed as result of climate change.

A comprehensive review and meta-analysis of *Legionella* occurrence data from outbreaks, sporadic cases and routine sampling programs at various water systems (cooling towers, wastewater treatment plants recreational facilities, buildings and residences) concluded that a *Legionella* concentration of 50,000 CFU/L warrants concern and should be considered an action level to trigger remedial activities). A lower action level may be necessary to protect individuals at higher risk for legionellosis, such as hospital patients (NASEM, 2020). Guidance material produced by numerous agencies vary in their recommendations for actions levels for *Legionella* or *L. pneumophila* in water, including for cooling towers (range: >1000 to >1,000,000 CFU/L) and potable water systems (range: >1000 to >10,000 CFU/L) (NASEM, 2020). More information is needed about environmental exposures that result in disease in order to inform health risk-based numerical values for *Legionella* in water.

B.2.1.2.4 Analytical methods

Standard methods for the detection of *Legionella* in drinking water are available (APHA et al., 2017; ISO 2019; AFNOR, 2021). Other methods may be approved for use in other jurisdictions. The literature can also be consulted for details on specific methods (Mercante and Winchell, 2015; Wang et al., 2017, Petricek and Hall, 2018). Building water systems vary substantially in their design, complexity and propensity for *Legionella* transmission. Thus environmental monitoring at individual facilities should be informed by a site-specific risk assessment as part of a Water Management Plan (HSE, 2013b; CDC, 2017a; ASHRAE, 2018). In general, monitoring programs consist of routine monitoring of general microbiological quality, as an indication of system control, in conjunction with

testing for *Legionella* at regular time intervals (HSE 2013a; 2014; 2019; PWGSC, 2016). Historically, culture-based methods have been applied for monitoring (NASEM, 2020). Culture-based methods provide an acceptable measure of viability, but are time consuming and do not detect viable but non-culturable (VBNC) cells (Wang et al., 2017; NASEM, 2020). Quantitative PCR methods provide greater specificity and sensitivity and a shorter turnaround time when compared to culture-based methods; and they can detect VBNC cells (Wang et al., 2017; NASEM, 2020). A drawback of qPCR methods is that they capture all DNA, even from dead cells (NASEM, 2020). Both culture-based and qPCR methods can be applied for establishing baseline numbers of *Legionella*, flagging concerns and providing information indicative of growth, death or changes in the system (Wang et al., 2017, NASEM, 2020). Pulsed-field gel electrophoresis (PFGE) and sequence-based typing (SBT) are two common approaches to the molecular subtyping of *Legionella* (Raphael et al., 2016; Mercante and Winchell, 2015). For epidemiological investigations, sequence-based typing methods are the current gold standard for comparing environmental and patient isolates of *Legionella* (Gaia et al., 2005; Mercante and Winchell, 2015; APHA et al., 2017).

B.2.1.2.5 Treatment considerations

The general advice outlined in Part A is important for the control of *Legionella* spp. from source to tap (Falkinham et al., 2015b). The advice in Part A should be consulted along with the information provided below.

Treatment: When properly designed and operated, physical removal technologies—chemically-assisted, slow sand, diatomaceous earth and membrane filtration or an alternative proven technology—will reduce the number of *Legionella* present in drinking water (US EPA 1989, 2006b; Hijnen and Medema, 2010). Data from a 2019 study by the US EPA suggest that planktonic (i.e., freely floating) *Legionella* should be easily inactivated by free chlorine at CT values commonly applied during water treatment (see Appendix D). One study reported detection of *Legionella* by qPCR at a facility that chloraminated the water (King et al., 2016). This detection serves as an important reminder that monochloramine should not be used for primary disinfection due to its low oxidation potential (Health Canada, 2019b). Biofilm-associated *Legionella* is much more resistant to disinfection (see distribution system section below). Research data indicates that UV dose requirements are greater than those needed for *Giardia* and *Cryptosporidium*, but are less than those needed for many enteric viruses (Hijnen et al., 2011; Health Canada, 2019b, 2019c). Providing effective control of free-living protozoa in drinking water (e.g., *Acanthamoeba*, *Naegleria*—see Section B.2.2) is also necessary for reducing *Legionella* populations (Loret and Greub, 2010; Thomas and Ashbolt, 2011; NASEM, 2020).

Drinking water distribution system: Buse et al. (2019) evaluated the protective effect that biofilms can have on the resistance of *Legionella pneumophila* to secondary disinfectants. CT values were determined for *Legionella pneumophila* strain Philadelphia-1 serogroup 1 when associated with biofilm on PVC material (see Table 3). As Table 3 demonstrates, free chlorine is a more powerful oxidant than monochloramine. However, water utilities typically maintain lower residuals with free chlorine. This has a significant impact on the amount of time required to inactivate *Legionella* when it is associated with biofilm as shown in Table 4. Thus, maintaining an effective disinfectant residual in the distribution system is essential to control the growth of *Legionella*.

Table 3. CT values for inactivation of biofilm-associated *Legionella pneumophila* strain Philadelphia-1 serogroup 1 using free chlorine and monochloramine (pH = 8, temperature = 21°C)

Log inactivation	Free chlorine (mg·min/L)	Monochloramine (mg·min/L)
2 log	8.86	17.16
3 log	36.11	62.80
4 log	63.67	108.44

Table 4. Required time in a distribution system for 2 log inactivation of biofilm-associated *Legionella pneumophila* strain Philadelphia-1 serogroup 1 using free chlorine and monochloramine (assuming CT values presented in Table 3)

Residual type	Residual concentration (mg/L)	Time required to achieve 2 log inactivation (minutes)
Free chlorine	0.2	44.3
Free chlorine	0.5	17.7
Free chlorine	1.0[a]	8.9
Monochloramine	1.0	17.2
Monochloramine	1.5	11.4
Monochloramine	1.8[a]	9.5

[a] Suggested concentrations to control biofilm growth (Gagnon et al., 2008; Gillespie et al., 2014; Rand et al., 2014; LeChevallier et al., 2015a,b).

There is conflicting evidence with regards to whether free chlorine or monochloramine provides better control of *Legionella* (Donohue et al., 2019a; LeChevallier, 2019a,b). Monochloramine may trigger free-living amoeba trophozoites to form cysts which cannot

host and support the growth of *Legionella* (Bukhari et al., 2018). However, the use of monochloramine may increase *Mycobacterium* spp. detections (Donohue et al., 2019a). Additional research is needed to determine optimized strategies for minimizing risks from opportunistic pathogens in drinking water distribution and premise plumbing systems (NASEM, 2020). Ideally, selected controls for *Legionella* should have benefits for the control of other pathogens in water systems (NASEM, 2020). The requirements for disinfectant residuals necessary to control *Legionella* in drinking water systems are under review by the US EPA (US EPA, 2021b).

The 2014–15 outbreaks of Legionnaires' disease in Genesee County, Michigan which coincided with the Flint water crisis, provide an example of the unintentional consequences of changes in drinking water systems operations on distribution system water quality. A switch in the source of drinking water to Flint's municipal system resulted in changes in water composition and distribution system conditions that ultimately resulted in a reduced free chlorine residual (Zahran et al., 2018). It is hypothesized that this disruption in water quality stimulated the growth of *L. pneumophila* in Flint's distribution and plumbing systems and was responsible for the outbreaks (Zahran et al., 2018; Garner et al., 2019).

Premise plumbing: Many resources are available which address measures for reducing the risk of exposure to *Legionella* in building water systems. The National Building Code of Canada (NRCC, 2015a) and the National Plumbing Code of Canada (NPC) (NRCC 2015b) set out standards and technical provisions for the design and installation of HVAC systems and plumbing systems in buildings, respectively. Both contain provisions dealing with *Legionella* in building systems. The American National Standards Institute/American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ANSI/ASHRAE) Standard 188 (ASHRAE, 2018) establishes minimum *Legionella* risk management requirements for building water systems intended for use by those involved in design, construction, installation, commissioning, operation, maintenance and service of centralized building water systems and components. Guidance documents recommend the use of water management/water safety plans for the management of *Legionella* in building water systems. Healthcare and long-term care facilities and buildings with cooling towers are identified as buildings with a particular need for water management programs to reduce the risk of *Legionella* growth and spread (WHO, 2007, HSE 2013a, CDC, 2017a). Publications to assist building managers in developing water management/water safety plans are available (WHO, 2007, 2011; HSE, 2013a, 2014; PWGSC, 2016; CDC, 2017a; ASHRAE, 2018). In general, the NASEM Committee on Management of *Legionella* in Water Systems recommends requirements for water management plans in all public buildings and establishing cooling tower registries as two policy initiatives that can improve public health protection from exposure to *Legionella* (NASEM, 2020).

The Province of Quebec enacted building safety legislation in 2013 which included regulations for the maintenance and operation of cooling towers (Government of Quebec, 2020). The regulations outline the requirements for owners that include registering their system with the regulator, implementing a water management plan and conducting regular testing for *Legionella pneumophila.* The city of Vancouver has also updated its building bylaws, requiring operational permits, maintainance logs and *Legionella* testing for cooling towers, evaporative condensers, decorative water features and rainwater harvesting and alternative water systems (City of Vancouver, 2021). Mandatory requirements for cooling tower registration have been in place in the City of Hamilton since 2011 (City of Hamilton, 2019).

For plumbing systems, temperature management, i.e., the use of control measures to keep the hot and cold water systems outside the microorganism's growth range of 25–43°C, is a fundamental aspect of a *Legionella* control strategy (Bédard et al., 2016a; Boppe et al., 2016; NASEM, 2020). Maintaining a minimum hot water tank temperature of 60°C is a key threshold for reducing positive detection of *Legionella* in buildings (WHO, 2011; HSE, 2014; NRCC 2015b, NASEM, 2020). The NPC specifies that storing hot water at temperatures below 60°C in hot water tanks and delivery systems may lead to growth of *Legionella* bacteria. The NPC further specifies that electric storage-type water heaters should be pre-set to a temperature of 60°C as a result of the temperature stratification that can occur with this type of heater. Temperature stratification is not a concern for other types of water heaters with different designs that use different fuels (NRCC, 2015b). Adjusting temperature regimes to achieve temperature greater than 55°C at distal points in the system has also been recommended as an effective measure for reducing *Legionella* colonization (WHO, 2011; HSE, 2014, NASEM, 2020). The hot water temperatures required to prevent *Legionella* growth are associated with a higher scalding risk (NRCC 2015b, NASEM, 2020). Applications of temperature management strategies should operate in accordance with regulations in place regarding maximum allowable temperatures at the tap. The NPC specifies that water valves supplying showerheads and bathtubs should be capable of maintaining a water outlet temperature that does not exceed 49°C in order to reduce the risk of scalding (NRCC, 2015b). To meet temperature requirements, plumbing codes dictate the use of devices such as thermostatic mixing valves to ensure appropriate water temperatures (NASEM, 2020). These devices allow elevated water heater temperatures while protecting against scalding risk at the tap, yet can also provide surfaces for *Legionella* attachment and create favourable growth temperatures if located too far from taps and outlets (NASEM, 2020; Singh et al., 2020). The overall impact of these devices on control strategies is not clear (NASEM, 2020; Singh et al., 2020). Mixing valves should be positioned as close as possible to the point of use and provide access for maintenance and cleaning (WHO, 2007, NASEM, 2020). Temporarily elevating the water temperature, or heat

shock (e.g., a stringent thermal shock of 70°C for 30 minutes) has been utilized as a control measure in building systems. However, the efficacy of this procedure is controversial, and it is considered an extreme remediation measure (NASEM, 2020).

Avoiding stagnation through proper system design and the use of flushing regimes is also essential for effective *Legionella* control (WHO, 2007, 2011; PWGSC, 2016; ASHRAE, 2018; NASEM, 2020). There is no consensus on the optimal flushing frequency required to mitigate *Legionella* risks (NASEM, 2020; Singh et al., 2020). Site assessments as part of a water management plan are recommended for informing and developing specific control strategies. Risks of stagnation in peripheral parts of plumbing systems can be minimized by regular use of outlets (HSE, 2014). Guidance documents for building systems recommend minimum weekly flushing of low flow pipe runs, dead ends/dead legs and infrequently used fittings or outlets (ECDC, 2017; HSE 2014, CDC, 2021b). For buildings with larger numbers of at-risk individuals, more frequent flushing may be needed, as determined by the risk assessment (WHO, 2007; HSE, 2014). Care should be taken during plumbing flushing procedures. Flushing can disturb biofilms and may generate contaminated aerosols containing *Legionella* (WHO, 2007; Singh et al., 2020). Resources for developing building flushing programs are available (Purdue University, 2020).

The use of on-site disinfection technologies can also be an important part of a *Legionella* control strategy in large building water systems (Bartram et al., 2007; US EPA, 2016; NASEM, 2020). Various disinfection technologies (free chlorine, monochloramine, chlorine dioxide, copper-silver ionization, UV light, ozone, point-of-use (POU)/point-of-entry (POE) filtration technologies) have demonstrated some level of effectiveness against *Legionella* (Bentham et al., 2007, Exner et al., 2007; US EPA, 2016; Springston and Yocavitch, 2017, NASEM, 2020). A water treatment professional should be consulted before applying any supplemental disinfection. Guidance materials on *Legionella* control in plumbing systems for health-care facilities have recommended minimum disinfectant residual targets of 0.3 mg/L (Moore and Shelton, 2004; WHO, 2007) to 0.5 mg/L (Australian Government, 2015) for free chlorine and 1.5 mg/L for monochloramine (Moore and Shelton, 2004). There is evidence that a monochloramine residual provides better control of *Legionella* in building water systems compared to free chlorine, although the reasons for the improved performance are not fully clear (NASEM, 2020).

The selection of any control strategy requires a detailed understanding of the complexity of the system and the composition of the water and system materials (Bartram et al., 2007; US EPA, 2016; NASEM, 2020). Advances in monitoring equipment (e.g., low cost sensors for temperature, oxidation-reduction potential) and data acquisition/analytics facilitates the implementation of real-time status and control of complex plumbing systems to identify and manage risks (Bédard et al., 2015; Saetta et al., 2021). "Smart" water meters can also provide useful data (e.g., water use, temperature, flow direction, pressure).

For homeowners, recommendations for the control of *Legionella* in household plumbing systems involve maintaining a minimum hot water tank temperature of 60°C, consistent with NPC specifications (NRCC 2015b; WHO, 2007; NASEM, 2020). Educating immunocompromised individuals on the potential risks from in-home equipment that create aerosols and may support *Legionella* growth (e.g., humidifiers, nebulizers) is a useful component of *Legionella* risk management in the home (NASEM, 2020).

B.2.1.2.6 International Considerations

The WHO, and the Australian National Health and Medical Research Council have not established a limit for *Legionella* in drinking water (WHO, 2017a; NHMRC, NRMMC 2011). The US EPA established a maximum contaminant level goal (MCLG, a non-enforceable guideline) of zero *Legionella* in drinking water in its 1989 Surface Water Treatment Rule (US EPA, 1989). The 2020 European Union Drinking Water Directive includes an action level of 1000 CFU/L for *Legionella* in premise plumbing systems (European Commission, 2020). Other guidelines or standards established for *Legionella* spp. in Canada, the U.S. and other countries worldwide relate to the operation and maintenance of premise plumbing in buildings.

B.2.1.3 *Mycobacterium* spp.

B.2.1.3.1 Description

The genus *Mycobacterium* (Class: Actinobacteria) contains over 200 recognized species. Bacteria belonging to this genus are diverse in their ability to cause disease in humans. Some are strict pathogens, whereas others cause opportunistic infections or are non-pathogenic. Tuberculosis and leprosy are two illnesses caused by *Mycobacterium* species; however, these particular species are not relevant to drinking water. The mycobacteria of concern for drinking water providers are the species collectively referred to as the non-tuberculous mycobacteria (NTM).

NTM are a group of over 150 distinct species that are considered to be opportunistic human pathogens (Falkinham, 2016a, 2016b). Members of the *M. avium* complex—which includes *M. avium* and its subspecies *M. intracellulare* and *M. chimaera*—are the microorganisms most frequently associated with human illness. Other medically-relevant species include *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. malmoense* and *M. xenopi* (Nichols et al., 2004; Hoefsloot et al., 2013; Falkinham, 2016a).

Mycobacteria are Gram-negative, aerobic to microaerophilic, non-motile, non-spore-forming rod-shaped bacteria. Species are categorized as either rapid growers or slow growers based on the time required to produce colonies on growth media (Cangelosi et al., 2004; Falkinham, 2015b). In general, mycobacteria grow at temperatures between 15–45°C (George et al., 1980; Cangelosi et al., 2004). Optimal growth temperatures for individual species vary within the range of 30–45°C (De Groote, 2004a; Stinear et al., 2004). The bacteria are relatively heat-resistant, capable of surviving at temperatures greater than 50°C (Schulze-Robbecke and Buchholtz, 1992; Falkinham, 2016a). Mycobacteria can utilize many substances as nutrient sources and are able survive on very simple substrates (Kaur, 2014). All mycobacteria possess a thick and lipid-rich cell wall that makes the microorganisms relatively impermeable to hydrophilic compounds. This provides the bacteria with increased resistance to acid/alkaline conditions, disinfectants and antibiotics.

B.2.1.3.2 Health effects

NTM species cause a wide spectrum of diseases in humans (Whiley et al., 2012), even though disease in healthy humans is rarely reported. NTM infections occur largely in individuals who have weakened or suppressed immune status or in persons with underlying respiratory conditions. Risk factors for NTM diseases vary according to the type of disease.

Pulmonary disease is the most common form of NTM-associated illness (Griffith et al., 2007; Sharma and Upadhyay, 2020). There are two forms of NTM pulmonary disease. The first is the more traditional form, presenting with cavitary lesions and is seen in adults with underlying lung disease (De Groote, 2004b). Disease tends to occur more frequently in older males with a history of smoking, alcoholism or lung damage from occupational exposure to dusts and persons with chronic disease conditions that affect the lungs (e.g., cystic fibrosis, lung cancer, chronic obstructive pulmonary disease) (De Groote, 2004b; Falkinham, 2015c). The second form of disease presents as inflamed bronchi accompanied by nodules and is seen in individuals that lack classic risk factors or underlying disease (De Groote, 2004b). Disease tends to occur more frequently in older non-smoking females (De Groote, 2004b; Falkinham, 2015c). Features common to both diseases include persistent cough, weakness and night sweats (De Groote, 2004b; Falkinham, 2015c). The attack rates and time to onset of symptoms for pulmonary disease are not known. Infections can be difficult to diagnose from general respiratory illness and patients may have a long history of symptoms (e.g., months to years) before a diagnosis of mycobacterial disease is made (Falkinham et al., 2015b). Hypersensitivity pneumonitis, a form of pulmonary illness where inflammation within the lung is attributable to the body's immune response to mycobacterial antigens, has also been associated with NTM-exposure (Whiley et al., 2012; Adjemian et al., 2018).

NTM may also cause disease involving extrapulmonary sites such as lymph nodes, skin and soft tissues, the bloodstream and other body sites (Whiley et al., 2012; Sharma and Upadhyay, 2020). Cervical lymphadenitis caused by NTM is a disease of childhood, marked by swollen lymph nodes in the head or neck (Bayazit et al., 2004; von Reyn et al., 2004). The majority of cases are seen in otherwise healthy children ranging in age from 18 months to 5 years (von Reyn et al., 2004, Falkinham, 2015c). It is suggested that erupting teeth may have a role in how this age group acquires the disease (Bayazit et al., 2004; Falkinham, 2015c). Specific risk factors for the disease in children remain unclear (von Reyn et al., 2004). Skin and soft tissue infections caused by NTM range from localized skin lesions or nodules to widespread ulcerative or necrotizing disease (Percival and Williams, 2014f). Gastrointestinal tract infections are common in individuals with acquired immunodeficiency syndrome (AIDS) (von Reyn et al., 2004). In immunodeficient individuals, NTM infections can spread to various parts of the body including joints, skin, blood, the liver and the brain (Percival and Williams, 2014f). NTM-associated bacteremia is a common and life-threatening infection in individuals with human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS) (Falkinham, 2015c). The NTM member *M. avium* subspecies *paratuberculosis*, is hypothesized to be a cause of Crohn's disease, though evidence of an association is inconclusive (Waddell et al., 2015; 2016). Risk factors for non-pulmonary NTM diseases include other comorbidities that result in a compromised immunity, such as underlying immunological disorders and HIV infection; as well as injuries or procedures that can introduce infection through trauma (Adjemian et al., 2018). The infective doses for NTM species are not known (Stout et al., 2016; Hamilton et al., 2017; Adjemian et al., 2018). Mortality rates associated with cases of NTM illness are not well understood (Adjemian et al., 2018). In the U.S., the overall mortality burden associated with NTM disease has been estimated at 2.3 deaths per 1,000,000 person years (Vinnard et al., 2016).

The incidence of illness associated with NTM in Canada is not known, as cases of illness are not reportable. In Ontario, the annual rates of pulmonary NTM disease were estimated at 9.7–10.7 cases per 100,000 population for the years 2006–2010 (Marras et al., 2013). In the U.S., NTM-associated infections are a reportable condition in only a small number of states (Donohue and Wymer, 2016; Adjemian et al., 2018). Donohue and Wymer (2016) reported that across five states (Maryland, Mississippi, Missouri, Ohio, and Wisconsin) the average annual rate of NTM cases of any type (pulmonary and extra-pulmonary), ranged from 8.7–13.9 cases per 100,000 population between 2008 and 2013. Population-based studies have shown that pulmonary cases can account for roughly 77–93% of the reported NTM cases (Cassidy et al., 2009; Donahue and Wymer, 2016). Data suggests the prevalence of pulmonary NTM disease is continually increasing in North America and worldwide (Marras et al., 2013; Donohue and Wymer, 2016; Stout et al., 2016, Adjemian et al., 2018).

Few data are available on extra-pulmonary NTM disease prevalence in North America. In U.S. studies, estimates of the incidence of extra-pulmonary disease range from 1.5–1.9 cases per 100,000 population (Cassidy et al., 2009; Henkle et al., 2017, Adjemian et al., 2018). Little information is available on the impacts of seasonal- or climate-related factors on infections (Falkinham, 2004; Adjemian et al., 2018).

NTM are resistant to many commonly used antibiotics. Treatment typically requires a combination of antimicrobial antibiotics including clarithromycin, arithromycin, rifampin and others (Percival and Williams, 2014f; Falkinham, 2015c, Halstrom et al., 2015).

B.2.1.3.3 Sources and exposure

NTM are found in the environment in soil and in water habitats such as marine waters, lakes, rivers, streams, groundwater and swamps. Soils, in particular, acidic, peat-rich soils, are the primary reservoir (Falkinham, 2016b). Groundwater generally contains lower numbers of NTM than surface waters (Falkinham, 2015c). Wastewater and sewage sludge can contain high numbers of these microorganisms (Radomski et al., 2011; Percival and Williams, 2014f). Engineered water systems (e.g., drinking water distribution systems and building/premise plumbing) along with equipment supplied by plumbing systems (e.g., humidifiers, hot tubs, swimming pools) are important sources of NTM. These systems and equipment can provide nutrient, temperature and disinfection protection conditions that allow the bacteria to increase to high numbers. Mycobacteria are ideally suited to life in these environments because they are able to grow under low nutrient and oxygen conditions, are resistant to disinfectants, possess thermal tolerance, and can survive and grow in biofilms and free-living protozoa (Falkinham, 2015a).

Mycobacterium spp. have been frequently detected, by culture or qPCR, in treated drinking water; and at varying frequencies, in drinking water distribution systems (Le Dantec et al., 2002; Hilborn et al., 2006; Wang et al., 2012a; Thomson et al., 2013; Holinger et al., 2014; Whiley et al., 2014; Lu et al., 2016; King et al., 2016; Hull et al., 2017; Dias et al., 2019; Gora et al., 2020). The occurrence and concentrations of mycobacteria in municipal distribution systems using groundwater or surface water can be similar, despite typically different concentrations at the source (see above) (Covert et al., 1999; Lu et al., 2015). Sediments in drinking water storage facilities are also known to harbour *Mycobacterium* spp. (Lu et al., 2015; Qin et al., 2017).

Premise plumbing systems are a significant environment of concern for the growth of NTM. The microorganisms have been detected in both residential and non-residential buildings, in cold and hot tap water, at varying frequencies (Hilborn et al., 2006; Feazel et al., 2009; Wang et al., 2012a; Holinger et al., 2014; Dias et al., 2019; Donohue et al., 2019b; Lande et al., 2019; Gora et al., 2020). Higher rates of NTM contamination are found in

buildings with recirculating hot water systems (e.g., hospitals, condominiums, apartment buildings) compared to private residences (Falkinham, 2015b; Li et al., 2017). NTM have been isolated from premise plumbing fittings and equipment supplied by plumbing systems including faucets, showerheads, hot tubs/spas, ice machines, swimming pools, footbaths and medical nebulizers (Percival and Williams, 2014f; Nichols et al., 2004). Gebert et al. (2018) reported that mycobacteria were far less abundant in showerheads from US homes receiving water from private wells as compared to those receiving municipal water.

TRANSMISSION

Inhalation of aerosols is the primary route of transmission for NTM pulmonary disease (De Groote, 2004b; Halstrom et al., 2015). Premise plumbing systems are the most credible sources for aerosol exposure (De Groote et al., 2004b; Halstrom et al., 2015). Soils and dust have also been identified as potential sources of aerosols (Adjemian et al., 2018; Halstrom et al., 2015). Ingestion of contaminated water is considered as a possible route of transmission that is relevant for gastrointestinal infections in HIV/AIDS patients (von Reyn et al., 2004; Corti and Palmero, 2008) and cervical lymphadenitis infections in children (Bayazit et al., 2004; Falkinham, 2015c). For NTM infections involving skin and soft-tissues and other body sites (e.g., blood, joints, bones, organs) transmission occurs through injuries or procedures (medical, cosmetic) that expose the site to contaminated water, soil or medical devices; or are a result of disseminated infections among immuno-compromised individuals (De Groote and Johnson, 2004; von Reyn et al., 2004; Piersimoni and Scarparo, 2009; Falkinham, 2015b; Sharma and Upadhyay, 2020). Indirect person-to-person transfer (i.e., via contaminated objects) may be a relevant route of transmission for persons with cystic fibrosis (Bryant et al., 2013; Bryant et al., 2016, Sood and Parrish, 2017). *M. avium ssp. paratuberculosis* has a fecal-oral route of transmission in cattle; however, the pathogenic role of this organism in human disease and potential sources of infection are topics of considerable debate (Harris and Barrletta, 2001; Waddell et al., 2016). In general, animal-human transmission is not thought to be a significant route of exposure (Whiley et al., 2012; Falkinham 2015c).

WATERBORNE ILLNESS

It is difficult to identify the specific transmission routes and infection sources for NTM microorganisms. NTM are ubiquitous in water environments and the associated diseases have long incubation times (Nishiuchi et al., 2017; Adjemian et al., 2018). Verification of infection sources requires the identification of an identical genotype between clinical and environmental isolates (Halstrom et al., 2015; Nishiuchi et al., 2017; Ratnatunga et al., 2020). Infections with NTM are generally sporadic. Most outbreaks associated with NTM have been linked to treated recreational water facilities (swimming pools, hot tubs) or

medical or cosmetic procedures (De Groote et al., 2004; De Groote and Johnson, 2004). No outbreaks of NTM disease have been linked to the consumption of drinking water in Canada or the U.S. (CDC, 2013, 2015b; 2017d).

NTM are particularly problematic in healthcare facility water systems, causing a variety of diseases. Li et al. (2017) conducted a systematic review of NTM waterborne infections in health care facilities. Outbreaks of mycobacterial infection were found to occur in a range of settings including inpatient hospital units, outpatient procedure clinics, hemodialysis centers and operating rooms. Types of mycobacterial disease varied based on the route of exposure. Reported sites of infection included the bloodstream, respiratory tract, soft-tissues and other specific sites related to surgical procedures (Li et al., 2017). The most frequent routes of transmission were through exposure of catheters or surgical wounds to contaminated water supplies and exposures to non-sterile water during medical procedures (Li et al., 2017).

Residential plumbing systems have also been proposed as an important source of NTM exposure (Whiley et al., 2012; Halstrom et al., 2015). NTM pulmonary infections have been linked to exposure to aerosols from household water supplies and residential hot tubs (Halstrom et al., 2015). Skin infections from NTM associated with residential hot tub use have also been reported (Whiley et al., 2012). Other settings for case reports of extrapulmonary NTM infections tied to water uses have included: footbaths at a nail salon, home aquarium cleaning and tattoo parlours (Halstrom et al., 2015; Griffin et al., 2019). Monitoring results from premise plumbing systems in residential and non-residential buildings show that there are opportunities for human exposure to NTM but other factors (e.g., aerosols and host) are necessary for disease transmission (Donohue et al 2019b).

B.2.1.3.4 Analytical methods

Methods for isolation and culture-based detection of *Mycobacterium* spp. in drinking water have been described; however, there is presently no standardized approach to testing (APHA et al., 2017). Isolates can be identified to the genus and species level using PCR or DNA-sequencing methods (Stinear et al., 2004; Falkinham, 2015c). Identification to the subspecies or strain level requires more advanced molecular techniques (Stinear et al., 2004; Falkinham, 2015c). The literature can be consulted for details on specific methods (Stinear et al., 2004; Thomson et al., 2008; Wang et al., 2017).

B.2.1.3.5 Treatment considerations

The general advice outlined in Part A is important for the control of *Mycobacterium* spp. from source to tap (Falkinham et al., 2015a, 2015b). The advice in Part A should be consulted along with the information provided below.

Treatment: When properly designed and operated, physical removal technologies—chemically-assisted, slow sand, diatomaceous earth and membrane filtration or an alternative proven technology—are capable of reducing the number of mycobacteria in drinking water (LeChevallier et al., 2001; Le Dantec et al., 2002; LeChevallier, 2004). However, specific features of mycobacteria such as hydrophobicity and surface charge affect treatment processes in different ways (LeChevallier, 2004; Wong and Shin, 2015). Due to their highly hydrophobic cell wall, mycobacteria have an increased tendency to attach to particles (LeChevallier, 2004). Correlations between turbidity removal and removal of mycobacteria have been demonstrated (Falkinham et al., 2001; Wong and Shin, 2015). The use of GAC filters can provide conditions (accumulated nutrients, neutralized disinfectant residuals) which support the growth of mycobacteria (Le Dantec et al., 2002; LeChevallier, 2004).

Mycobacteria are very resistant to commonly used chemical disinfectants. Individual species and strains show significant variations in disinfectant sensitivity (Taylor et al., 2000; WHO, 2004); and reported CT values differ among investigators (WHO, 2004). The CT values reported by Taylor et al. (2000) for free chlorine (51–1552 mg·min/L, see Appendix D) would be technically challenging to achieve at a treatment facility. Jacangelo et al. (2002) observed that the inactivation of *Mycobacterium fortuitum* required CT values for ozone equal to or greater than those required for *Cryptosporidium*.

The UV dose required to inactivate *Mycobacterium avium* complex microorganisms can be greater than those needed for *Giardia* and *Cryptosporidium* and comparable to that required for some enteric viruses. It has been reported that the inactivation of some strains of *M. avium and M. fortuitum* require UV doses comparable to those required for adenovirus (Gerba et. al., 2003; Schiavano et al., 2018).

Even with effective treatment and disinfection in place, NTM have a strong tolerance for disinfection and can pass into distribution and plumbing systems in low numbers.

Drinking water distribution system: Full-scale studies suggest that free chlorine is more successful than monochloramine as a secondary disinfectant for controlling mycobacteria (Pryor et al., 2004; Wang et al., 2012a; Rhoads et al., 2017; Donohue et al., 2019b). Monochloramine may provide better control in biofilms on certain pipe materials such as corroded iron surfaces (Norton et al., 2004).

Premise plumbing: Guidance documents recommend the use of water management/water safety plans for the management of mycobacteria in building water systems (WHO, 2007). Resources are available to provide information for building managers (WHO, 2007; 2011). In health care facilities, control of mycobacteria will be achieved in part through management plans designed to reduce risk from *Legionella* (Ford et al., 2004). However, it should be recognized that mycobacteria and *Legionella* have differing sensitivity to drinking water disinfectants (Jacangelo et al., 2002, Pryor et al., 2004; Moore et al., 2006b).

Supplemental strategies described for control in hospital and health care facilities have included superheat and flush disinfection with hot water to temperatures above 50–70°C, the use of various disinfection strategies (free chlorine hyperchlorination, chlorine dioxide) and the use of POU membrane filtration technologies (LeChevallier, 2004; Sebakova et al., 2008; Williams et al., 2011; Hsu et al., 2016). Additional actions recommended as part of a water safety plan include regular cleaning and maintenance of plumbing fittings and equipment that can support biofilm growth and aerosol formation (faucets, showerheads, hot tubs/spas, cooling towers) (Ford et al., 2004).

For homeowners, maintaining hot water tank temperature consistent with NPC specifications for the control of *Legionella* (i.e., minimum of 60°C) (NRCC 2015b) has been recommended as part of a general strategy for minimizing the risks of exposure to opportunistic premise plumbing pathogens in the home (WHO, 2011; Falkinham et al., 2015a, 2015b; NASEM, 2020).

B.2.1.3.6 International considerations

No drinking water guideline for *Mycobacterium* spp. has been established by the WHO, the EU, the US EPA or the Australian National Health and Medical Research Council (NHMRC, NRMMC 2011; WHO, 2017a; European Commission, 2020; US EPA, 2021a).

B.2.1.4 *Pseudomonas* spp.

B.2.1.4.1 Description

The bacterial genus *Pseudomonas* (Class: Gammaproteobacteria) includes over 30 species (Chakravarty and Anderson, 2015). *Pseudomonas aeruginosa* is the most clinically relevant species and is an opportunistic pathogen capable of causing a variety of infections in humans (Chakravarty and Anderson, 2015; Daniels and Gregory, 2015). Other species (*P. fluorescens*, *P. putida*, *P. stutzeri*) have been infrequently reported in human infections (Chakravarty and Anderson, 2015).

Pseudomonas spp. are Gram-negative, strictly aerobic, motile, straight or slightly curved rod-shaped bacteria that grow over the range of 4–42°C (optimum: 28–37°C) (Moore et al., 2006a; Chakravarty and Anderson, 2015). They are metabolically versatile, capable of utilizing numerous substances as nutrient sources and surviving under low nutrient conditions (Chakravarty and Anderson, 2015; Falkinham et al., 2015a). *Pseudomonas* spp. are also significant due to their capacity to join or form biofilms in water environments (Bédard et al., 2016b).

B.2.1.4.2 Health effects

P. aeruginosa causes disease following colonization in patients where some predisposing factor (e.g., reduced immunity, underlying disease, traumatic injury or medical procedure) has made them more vulnerable to infection (Chakravarty and Anderson, 2015). The respiratory tract is the most common site of human infections. Symptoms typically include fever, chills, cough and laboured breathing; the onset can be sudden and severe (Daniels and Gregory, 2015). Cystic fibrosis patients are particularly prone to respiratory infection with *P. aeruginosa*, and the organism is a leading cause of morbidity and mortality in these individuals (Chakravarty and Anderson, 2015). *P. aeruginosa* is an important cause of infections involving the skin, eyes, ears and urinary tract (Chakravarty and Anderson, 2015; Daniels and Gregory, 2015). Bloodstream infections resulting from lung, skin or urinary tract infections can result in spread of the organism to other parts of the body. High mortality rates have been observed with *P. aeruginosa* septicaemia in high risk individuals (Chakravarty and Anderson, 2015; Daniels and Gregory, 2015). Individuals at higher risk for infections include those that have lowered immune status (e.g., patients with low neutrophil counts or HIV/AIDS); have underlying diseases (cystic fibrosis, diabetes, chronic pulmonary disease); are undergoing procedures with invasive medical devices (vascular and urinary catheters, ventilator, endotracheal tubes); or have breaches in host defenses as a result of burns or penetrating trauma (surgical incisions, wounds) (Daniels and Gregory, 2015). The doses of *P. aeruginosa* required to cause infection via the various transmission pathways are not well understood (Roser et al., 2014). *P. aeruginosa* infections in healthy individuals are rare.

Infections caused by *Pseudomonas* are not reportable illnesses in North America or in most countries worldwide. A review of outbreaks associated with water systems in healthcare settings identified the types of infection most commonly attributed to *Pseudomonas* as bloodstream, lung and urinary tract infections (Kanamori et al., 2016). Skin infections are the cause of the vast majority of *Pseudomonas*-associated outbreaks linked to recreational water venues that use treated water in the United States, with hotels the leading setting (Hlavsa et al., 2018).

Treatment of *P. aeruginosa* infections is difficult as a result of increasing antibiotic resistance (Falkinham et al., 2015a). Some strains have been found to be resistant to nearly all or all antibiotics including later generation beta-lactam antibiotics, fluoroquinolones and carbapenems (CDC, 2019a). Multidrug-resistant *P. aeruginosa* has been categorized as a "Serious Threat" by the CDC, and a priority for risk management attention by PHAC (CDC, 2019a; Garner et al., 2015). Carbapenem-resistant *P. aeruginosa* in particular have been identified by the WHO as a critical priority for developing new antibiotic strategies (WHO, 2017b).

B.2.1.4.3 Sources and exposure

Pseudomonas spp. are ubiquitous bacteria, found in a wide variety of habitats including soil, aquatic environments (fresh and marine surface waters, groundwater, potable water supplies) and vegetation (Falkinham et al., 2015a; Degnan, 2006). Human and animal feces are not a significant source; however, the microorganisms can be found in large numbers in sewage and wastewater (Degnan, 2006). Premise plumbing and equipment supplied by these systems that can provide the proper conditions for growth (e.g., nutrients, temperature, protection from disinfectants) are habitats for *P. aeruginosa* (Bédard et al., 2016b). Water supply systems in hospitals and other health-care settings are important sources of *P. aeruginosa* (Bédard et al., 2016b). Confirmed reservoirs in these settings include potable water faucets, sink and shower drains, humidifiers, water baths, hydrotherapy pools and bathing basins (Falkinham, 2015a; Bédard et al., 2016b). In community settings, hot tubs/spas and swimming pools can also be important sources of infections (Bédard et al., 2016b).

P. aeruginosa can be transmitted through person-to-person contact or through direct contact with contaminated objects or water (Falkinham, 2015a; Bédard et al., 2016b). Consumption of drinking water is not a recognized route of infection (Bédard et al., 2016b).

Studies involving both culture-based and molecular detection methods have found that *P. aeruginosa* is sporadically detected in treated water as it leaves the treatment plant and water and sediment samples from drinking water distribution systems (Wingender and Flemming, 2004; Van der Wielen and van der Kooij, 2013; Lu et al., 2015, 2016; Dias et al., 2019) and can be more frequently detected in samples collected from premise plumbing systems (Reuter et al., 2002; Rogues et al., 2007; Lavenir et al., 2008; Van der Wielen and van der Kooij, 2013; Charron et al., 2015). The amplification of *P. aeruginosa* populations within biofilms in premise plumbing or plumbing fittings is proposed as the reason for the increased detection in these samples (Bédard et al., 2016b). Within biofilms, *Pseudomonas* can survive and grow following ingestion by free-living amoebae such as *Acanthamoeba* spp. (see Section B.3.2.1) (Thomas and Ashbolt, 2011; Bédard et al., 2016b).

The protective environment and nutrients provided by this interaction can contribute to the enhanced survival and dispersal of *P. aeruginosa* in drinking water distribution and premise-plumbing systems (Thomas and Ashbolt, 2011). No known drinking water outbreaks associated with *P. aeruginosa* have been recorded (CDC, 2004, 2006, 2008, 2011, 2013, 2015b, 2017d).

B.2.1.4.4 Analytical methods

Standard methods for the detection of *Pseudomonas spp.* in drinking water are available (APHA et al., 2017; ISO, 2019). The literature can also be consulted for details on specific methods (Wang et al., 2017). *Pseudomonas* spp. are heterotrophic bacteria and are detected by HPC tests; however, no direct correlation between HPC counts and *P. aeruginosa* concentrations exists.

B.2.1.4.5 Treatment considerations

The general advice outlined in Part A is important for the control of *Pseudomonas* spp. from source to tap (Falkinham et al., 2015a; Bédard et al., 2016b). The advice in Part A should be consulted along with the information provided below.

Treatment: When properly designed and operated, physical removal technologies—chemically-assisted, slow sand, diatomaceous earth and membrane filtration or an alternative proven technology—and primary disinfection methods—chlorine, chlorine dioxide, ozone and UV—commonly used in drinking water treatment are effective at removing or inactivating *P. aeruginosa* (LeChevallier and Au., 2004; Clauβ, 2006; Xue et al., 2013; Behnke and Camper, 2012, Zuma et al., 2009; Garvey et al., 2014; Zhang et al., 2015). For the inactivation of *P. aeruginosa*, the CT requirements for chlorine are less than those required for the inactivation of many enteric viruses and the UV dose requirements are less than those required for the enteric protozoa *Giardia* and *Cryptosporidium* (Clauβ, 2006; Xue et al., 2013; Health Canada, 2019b, 2019c). Monochloramine should not be used for primary disinfection due to its low oxidation potential; monochloramine is recommended only for secondary disinfection (i.e., to maintain a residual disinfectant in the distribution system) (Health Canada, 2019b).

Drinking water distribution system: Resistance to chlorination will vary depending on the strain and the protective effects provided by biofilms (Bédard et al., 2016; Mao et al., 2018). Laboratory-scale and pilot-scale studies suggest that maintaining free chlorine residuals above 0.3 mg/L is useful for control of *Pseudomonas* spp. in bulk water (Wang et al., 2012b; Mao et al., 2018). Mao et al., (2018) highlighted that long-term, continuous exposure to an effective free chlorine residual is important in order to prevent regrowth of *Pseudomonas* and the selection of resistant strains. Further research on the effects of chlorine-based disinfectants on *P. aeruginosa* in premise plumbing water and biofilms is needed (Bédard et al., 2016).

Premise plumbing: Water management/water safety plans are recommended for the management of *Pseudomonas aeruginosa* in building water systems (WHO, 2011). Supplemental strategies described as control measures in hospital and health care facilities have included superheat and flush disinfection with hot water to temperatures above 50–70°C, and the use of POU membrane filtration technologies (Falkinham et al., 2015a; Bédard et al., 2016b).

For homeowners, no specific actions have been identified as necessary to reduce their risk of *P. aeruginosa* infections. However, homeowners can minimize their risk of exposure to opportunistic waterborne pathogens by maintaining the temperature of their hot water tank at a minimum of 60°C (WHO, 2011; Falkinham et al., 2015a, 2015b).

B.2.1.4.6 International considerations

No drinking water guideline for *P. aeruginosa* has been established by the WHO, the EU, the US EPA or the Australian National Health and Medical Research Council (NHMRC, NRMMC 2011; WHO, 2017a; European Commission, 2020; US EPA, 2021a). Guidelines or standards developed for *Pseudomonas* spp. in Canada and the U.S. and other countries worldwide relate to control of the organism in building water systems outside of municipal distribution system networks.

B.2.2 Protozoa

B.2.2.1 *Acanthamoeba* spp.

B.2.2.1.1 Description

Acanthamoeba spp. are free-living amoebae commonly found in soil and aquatic environments. They are opportunistic pathogens that can cause rare but severe human diseases affecting the eye, skin, lungs, brain and central nervous system (Visvesvara et al., 2007; Chalmers, 2014a). Species of *Acanthamoeba* were originally classified based on differences in life stage (e.g., cyst—see below) morphology; however, genotyping is currently used to classify members of the genus (Visvesvara et al., 2007; Juárez et al., 2018). Approximately 20 different genotypes of *Acanthamoeba* have been identified based on gene sequence differences (Juárez et al., 2018). *Acanthamoeba* genotype T4 is the predominant type encountered in cases of illness and in the environment; however, other genotypes have also been associated with disease (Chalmers, 2014a; Juárez et al., 2018). *Acanthamoeba* spp. are also significant due to their ability to act as hosts for certain pathogenic microorganisms within drinking water systems.

Acanthamoeba spp. have low nutrient requirements and grow over the range of 12–45°C (optimum 30°C) (Chalmers, 2014a). Their lifecycle consists of two stages: a feeding trophozoite (25–40 µm) and resistant cyst (10–30 µm) that can withstand temperatures of -20°C–56°C and provide resistance to desiccation and disinfection (Chalmers, 2014a; Juárez et al., 2018).

B.2.2.1.2 Health effects

Acanthamoeba infections are rare in the general population (Visvesvara et al., 2007; Juárez et al., 2018). Acanthamoeba keratitis (AK) is the most common form of illness (Juárez et al., 2018). Early symptoms of AK include blurred vision, intense pain and photosensitivity, usually in one eye (Chalmers, 2014a; Juárez et al., 2018). In advanced and severe cases, symptoms include ulceration, swelling, glaucoma, cataract and blindness (Juárez et al., 2018). AK has a slow onset, taking days to several weeks to develop after infection, and the disease has a slow but severe progression (Köhsler et al., 2016, Juárez et al., 2018). In developed countries, AK primarily occurs among individuals who wear contact lenses (Chalmers, 2014a). Persons at increased risk of exposure include those who use unsterile tap water to store, wash or disinfect contact lenses; and persons who swim, use hot tubs or showers while wearing contact lenses (Chalmers, 2014a; Juárez et al., 2018). In the minority of AK cases that are not associated with contact lenses, the infections are generally associated with ocular trauma or environmental contamination (Chalmers, 2014a).

Other expressions of *Acanthamoeba*-associated disease are disseminated infections originating in the skin or lungs that can spread to areas such as the kidneys and adrenal glands; and granulomatous amoebic encephalitis (GAE), a fatal disease which occurs when infection spreads to the brain and central nervous system (Visvesvara et al., 2007; Chalmers, 2014a). These are very rare forms of illness and primarily affect individuals who have weakened or suppressed immune status or underlying disease (e.g., persons with HIV/AIDS, cancer, diabetes, liver disease or who are undergoing chemotherapy or organ transplantation) (Visvesvara et al., 2007; Chalmers, 2014a; Guimaraes et al., 2016). The numbers of *Acanthamoeba* spp. necessary to cause infections are not known.

Despite the widespread occurrence of the organism in environmental waters, the number of cases of illness caused by *Acanthamoeba* spp. is low. The estimated incidence of AK in developed countries is one to 33 cases per million contact lens wearers (CDC, 2017b). Treatment of AK is difficult, as the cysts are resistant to most antimicrobials at concentrations tolerated by the human eye (Juárez et al., 2018). Prolonged treatment with a combination of drugs is needed (Visvesvara et al., 2007).

B.2.2.1.3 Sources and exposure

Acanthamoeba spp. are ubiquitous in soil and water worldwide; and are one of the most common free-living amoebae occurring in the environment (Visvesvara et al., 2007; Juárez et al., 2018). The amoebae have been isolated from an abundance of natural and man-made environments including soil, mud, fresh and brackish waters, swimming pools, hot tubs/spas, cooling towers, humidifiers, heating, ventilation and air conditioning equipment, drinking water and airborne dust (Visvesvara et al., 2007; Chalmers, 2014a).

The relative importance of water as a pathway for infection is unclear. The ubiquitous presence of *Acanthamoeba* in the environment makes it difficult to determine sources of infection. Drinking and inhalation of contaminated water are not considered routes of infection (Chalmers, 2014a). No outbreaks of AK as a result of exposure to drinking water have been reported in North America (Kilvington et al., 2004; Craun et al., 2010; Yoder et al., 2012b). Cases of AK have been associated with the use of nonsterile tap water in the preparation of contact-lens solutions (Visvesvara et al., 2007). Disseminated infections and GAE caused by *Acanthamoeba* spp. are not thought to be waterborne (Chalmers, 2014a).

Acanthamoeba spp. can be commonly detected in drinking water distribution systems in North America and internationally (Magnet et al., 2012; Lu et al., 2016; Qin et al., 2017). Multiplication of *Acanthamoeba* spp. can occur in biofilms and loose deposits in drinking water distribution and premise plumbing systems (Thomas and Ashbolt, 2011; Wang et al., 2012a; Qin et al., 2017). In the U.S., *Acanthamoeba* spp. have been detected by PCR in 40–63% of municipal storage tank sediments (Lu et al., 2015; Qin et al., 2017).

Acanthamoeba spp. may serve as hosts for pathogenic amoebae-resisting microorganisms, providing conditions (nutrients, protection from environmental stresses) critical for the survival, amplification and transport of these organisms (Thomas and Ashbolt, 2011). It has been proposed that passage in free-living protozoa increases the virulence of amoebae-resisting microorganisms (Visvesvara et al., 2007; Thomas and Ashbolt, 2011; Chalmers, 2014a). Pathogenic bacteria isolated from *Acanthamoeba* spp. include *Legionella pneumophila*, *Mycobacterium avium*, *Helicobacter pylori*, *Escherichia coli* serotype O157, *Listeria monocytogenes, Pseudomonas spp.* and *Vibrio cholerae* (Visvesvara et al., 2007; Juárez et al., 2018). *Acanthamoeba* spp. are also able to harbour protozoa, fungi and viruses (Köhsler et al., 2016; Juárez et al., 2018). More research is needed to determine the implications of the interactions between free-living amoeba species and pathogenic amoeba-resisting microorganisms in drinking water (Thomas and Ashbolt, 2011).

B.2.2.1.4 Analytical methods

No standardized methods have been established for the detection and identification of *Acanthamoeba spp.* in drinking water. Procedures for the isolation of *Acanthamoeba* in water samples involve concentration by membrane filtration or centrifugation; plaque screening and identification using molecular methods (Chalmers, 2014a). The literature can be consulted for details on specific methods (Wang et al., 2017).

B.2.2.1.5 Treatment considerations

Acanthamoeba cysts are larger than *Giardia* cysts and *Cryptosporidium* oocysts (Chalmers, 2014a; Health Canada, 2019b), thus physical removal mechanisms used during drinking water treatment are expected to remove these cysts. The cysts are very resistant to commonly used chemical disinfectants and UV (Loret et al., 2008; Hijnen et al., 2011). For the inactivation of cysts of *Acanthamoeba spp.*, the CT value reported by Loret et al. (2008) for free chlorine (1300 mg·min/L, see Appendix D) would be technically challenging to achieve at a treatment facility. The CT values for chlorine dioxide and ozone are greater than those reported for *Giardia* and *Cryptosporidium* (Loret et al., 2008). The UV dose requirements for the inactivation of cysts of *Acanthamoeba* spp. are similar to those required for adenovirus (Hijnen et al., 2011; Health Canada, 2019b).

The general operational and maintenance practises important for the control of waterborne pathogens, including *Acanthamoeba* spp., in drinking water distribution and plumbing systems are outlined in Part A (Chalmers, 2014a; Ashbolt, 2015). As part of a general facility water management plan, building system managers may implement regular cleaning and maintenance of plumbing fittings and equipment that can support biofilm growth and aerosol formation (e.g., faucets, showerheads, hot tubs/spas, cooling towers). Control of *Acanthamoeba* spp. may be particularly important in some specialised uses of water such as emergency eye-wash stations (Chalmers, 2014a)

No specific homeowner actions are necessary. However, homeowners can minimize risks of exposure to opportunistic waterborne microorganisms in the home by maintaining the temperature of their hot water tank at a minimum of 60°C (WHO, 2011). Individuals in the home who wear contact lenses should also follow guidance from their eye care providers on proper lens handling, cleaning and wear (CDC, 2017b).

B.2.2.1.6 International considerations

No drinking water guideline for *Acanthamoeba* spp. has been established by the WHO, the EU, the US EPA or the Australian National Health and Medical Research Council (NHMRC, NRMMC 2011; WHO, 2017a; European Commission, 2020; US EPA, 2021a).

B.2.2.2 *Naegleria fowleri*

B.2.2.2.1 Description

Naegleria fowleri is a pathogenic free-living amoeba that causes primary amebic meningoencephalitis (PAM) in humans, a rare but almost always fatal disease. Over 40 species of *Naegleria* spp. have been identified. However, to date, only *N. fowleri* has proven to be pathogenic to humans (Marciano-Cabral & Cabral et al. 2007; Yoder et al. 2010). Eight known *N. fowleri* genotypes have been found worldwide, and all are suspected to be pathogenic to humans (Bartrand et al., 2014; Chalmers, 2014b). *N. fowleri* are thermophilic, grow well at 25–40°C (optimum: 37°C) and can tolerate temperatures exceeding 50–60°C (Hallenbeck & Brenniman, 1989; Visvesvara et al., 2007; Zaongo et al., 2018). There are three distinct phases in the *N. fowleri* life cycle: a feeding and infectious trophozoite stage, an intermediate flagellate stage and a resistant cyst stage (Bartrand et al., 2014, Chalmers, 2014b). Cysts can survive for long periods at temperatures well below those optimal for growth and are considerably resistant to disinfectants (Bartrand et al., 2014).

B.2.2.2.2 Health effects

Symptoms of PAM are clinically similar to bacterial or viral meningitis, beginning with headache, fever, nausea and vomiting and then moving to stiff neck, altered mental status, occasional hallucinations, seizures and coma (Visvesvara et al., 2007; Chalmers, 2014b). Onset of symptoms occurs within one to seven days of exposure and the disease progresses rapidly, with death generally occurring within five days (Visvesvara et al., 2007; Chalmers, 2014b). PAM has an extremely high fatality rate (greater than 97%) (De Jonckheere, 2011; Capewell et al., 2015). Among documented cases in the U.S., there have been only five known survivors (Capewell et al., 2015). Infection occurs when water containing *N. fowleri* enters the nasal passages. The amoebae invade the mucous membranes and travel along the olfactory nerve to the brain where they consume nerve and blood cells, causing inflammation and cell damage leading to death (Chalmers, 2014b; Siddiqui et al., 2016). The dose of *N. fowleri* necessary to cause infection is not well understood (Bartrand et al., 2014).

PAM occurs in otherwise healthy individuals with exposure to warm, untreated or poorly disinfected water (Yoder et al., 2010). The disease primarily affects children and young adults with a history of exposure to warm, recreational freshwaters. Reasons for the age distribution are unclear, but it is proposed that these age groups more frequently engage in water activities (e.g., diving, head submersion) that increase the risk of water moving forcefully up the nose (Visvesvara et al., 2007; Yoder et al., 2010). Despite the widespread occurrence of *N. fowleri* in environmental waters, PAM occurs infrequently. As of 2011, only 235 cases of PAM had been reported globally, with the majority occurring in the U.S. (De Jonckheere, 2011). Cases most often occur during the hot summer months.

PAM is difficult to detect, with most cases progressing so rapidly that diagnosis is made following death (Chalmers, 2014b). Several drugs have demonstrated effectiveness against *N. fowleri* in the laboratory, but their effectiveness in clinical treatment remains unclear (Capewell et al., 2015; Siddiqui et al., 2016). Survival of infection has been demonstrated in two cases using a combination of antimicrobial agents and aggressive management of brain-swelling (CDC, 2019b) Continued testing of therapies is necessary (Capewell et al., 2015; Siddiqui et al., 2016). No human vaccines for PAM exists (Siddiqui et al., 2016).

B.2.2.2.3 Sources and exposure

N. fowleri are naturally found in warm freshwater environments and soils worldwide (Chalmers, 2014b). The microorganisms have been isolated from a wide variety of natural and human-made warm water sources, including lakes, rivers, ponds, hot springs, geothermal groundwater, water receiving thermal discharges from power plants or industrial facilities and poorly maintained swimming pools (Chalmers, 2014b, Bartrand et al., 2014). In the U.S., *N. fowleri* has been most commonly encountered in natural waters in southern-tier states. However, PAM cases have also been reported from more northern states in the U.S. including Kansas, Indiana and Minnesota (Yoder et al., 2010; 2012a; Cope and Ali, 2016). *N. fowleri* has been isolated from drinking water and premise plumbing supplies in Australia and in Arizona, Louisiana and Texas in the U.S. (Bartrand et al., 2014, Villegas, 2020). Increasing ambient temperatures as a result of climate change may increase the geographical range of *N. fowleri* (Bartrand et al., 2014; Chalmers, 2014b).

Transmission of *N. fowleri* occurs through the intranasal route. While PAM cases have been reported from several countries, the most complete data comes from the U.S. (Cope and Ali, 2016. In the United States, most infections have been associated with recreational activities (e.g., swimming, diving, jumping, underwater play) in warm, fresh recreational waters (Yoder et al., 2010). The majority of exposures have occurred at lakes and ponds; exposures at rivers or streams have been less frequently reported (Yoder et al., 2010). Cases of illness have also been documented where improperly maintained swimming pools were the probable sources of exposure (Yoder et al., 2010; Cope and Ali, 2016). In very rare cases, infections have been linked to contaminated drinking water supplies, through activities such as nasal cleansing, bathing or recreational water uses (Yoder et al., 2010, 2012a, Bartrand et al., 2014). Drinking contaminated water is not a route of infection.

There is very little data on the occurrence of *N. fowleri* in North American drinking water distribution and premise plumbing systems (Bartrand et al., 2014). *N. fowleri* is widespread in environmental reservoirs but at low numbers, unless the environment provides conditions for amplification such as optimal growth temperatures, availability of nutrients and the absence of a disinfectant residual (Bartrand et al., 2014). Drinking water systems vulnerable to *N. fowleri* contamination are those where the temperature of the water

supply continually exceeds 25°C and where effective disinfectant residuals are not maintained (Bartrand et al., 2014). Long-term survival of the cysts at temperatures below optimal growth temperatures is possible, and N. *fowleri* has the ability to survive over winter in lakes in subtropical and temperate regions (Bartrand et al., 2014). Laboratory-scale and full-scale studies have demonstrated that *N. fowleri* can persist and grow in distribution system and premise plumbing biofilms (Bartrand et al., 2014).

N. fowleri can act as a reservoir for amoeba-resisting microorganisms (Thomas and Ashbolt, 2011; Bartrand et al., 2014). *Naegleria* species are regarded as a host for *L. pneumophila* and can provide conditions which permit the replication, protection and distribution of this pathogen in the environment (Thomas and Ashbolt, 2011; Bartrand et al., 2014; Siddiqui et al., 2016). Significant research is needed in order to understand the interactions between free-living amoeba species and pathogenic amoeba-resisting microorganisms in order to quantify any risk to human health (Thomas and Ashbolt, 2011). Only six of 132 U.S. cases of *N. fowleri* reported between 1962 and 2013 have resulted from exposures related to drinking water (Yoder *et al.* 2010; CDC, 2017c). Three of the cases were linked to nasal cleansing (Louisiana (2): 2011, U.S. Virgin Islands (1), 2012), two were related to bathing (Arizona, 2002), and one was related to tap water exposure on an outdoor play slide (Louisiana, 2013) (Yoder *et al.* 2010, 2012a; Bartrand et al., 2014). The two Louisiana cases represent the first time disinfected tap water has been implicated in *N. fowleri* infection in the U.S. (Yoder et al., 2012a). To date, there have been no known cases of PAM documented in Canada.

B.2.2.2.4 Analytical methods

The detection and identification of *N. fowleri* in drinking water requires highly specialized laboratories (Bartrand et al., 2014, Chalmers, 2014b). No standardized methods have been established. Procedures for the isolation of *N. fowleri* involve concentration (membrane filtration or centrifugation) or separation; plaque screening and identification using immunofluorescence or molecular assays (Bartrand et al., 2014; Chalmers, 2014b, Wang et al., 2017). The literature can be consulted for details on specific methods (Bartrand et al., 2017; Wang et al., 2017).

B.2.2.2.5 Treatment considerations

When properly designed and operated, physical removal technologies—chemically-assisted, slow sand, diatomaceous earth and membrane filtration or an alternative proven technology—commonly used in drinking water treatment are expected to remove *N. fowleri*. The cysts are very resistant to commonly used primary disinfectants—chlorine and UV. *N. fowleri* cysts are very similar in size to *Giardia* cysts (Chalmers, 2014b, Health Canada, 2019b). For the inactivation of cysts of *Naegleria spp.* the CT requirements for

free chlorine are similar to those needed for *Giardia*, whereas the UV dose requirements are greater than those needed for enteric protozoa but less than those needed for adenovirus (Sarkar and Gerba, 2012; Goudot et al., 2014; Health Canada, 2019b, 2019c). Monochloramine should not be used for primary disinfection due to its low oxidation potential; monochloramine is recommended only for secondary disinfection (i.e., to maintain a disinfectant residual in the distribution system) (Health Canada, 2019b).

The general operational and maintenance practises important for the control of waterborne pathogens, including *Naegleria* spp., in drinking water distribution and plumbing systems are outlined in Part A (Bartrand et al., 2014). Maintaining a minimum free chlorine residual of 0.5 mg/L throughout the distribution system is recommended for the control of *N. fowleri* in vulnerable drinking water systems (NHMRC, NRMMC, 2011; Louisiana Department of Health and Hospitals, 2013). Bartrand et al., 2014). The suggested best practice for a chloramine residual of greater than 1.5 mg/L throughout the distribution system (Health Canada, 2020b) is sufficient for *N. fowleri* control (NHMRC, NRMMC, 2011).

N. fowleri is not an immediate risk for drinking water systems in Canada. However, individuals should ensure that they conduct nasal rinses using water that has been boiled and cooled, or distilled water.

B.2.2.2.6 International considerations

No drinking water guideline for *N. fowleri* has been established by the WHO, the EU, the US EPA or the Australian National Health and Medical Research Council (NHMRC, NRMMC 2011; WHO, 2017a; European Commission, 2020; US EPA, 2021a).

Part C. References

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Part D. Appendices

APPENDIX A
LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
AK	Acanthamoeba keratitis
ANSI	American National Standards Institute
ASHRAE	American Society of Heating, Refrigerating, and Air-Conditioning Engineers
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
CT	concentration (C) × time (T)
DAEC	diffuse adherent *Escherichia coli*
DNA	deoxyribonucleic acid
EAEC	enteroaggregative *Escherichia coli*
E. coli	*Escherichia coli*
EHEC	enterohaemorrhagic *Escherichia coli*
EIEC	enteroinvasive *Escherichia coli*
EPEC	enteropathogenic *Escherichia coli*
ESBL	extended spectrum β-lactamase
ETEC	enterotoxigenic *Escherichia coli*
EU	European Union
GAC	granulated activated carbon
GAE	granulomatous amoebic encephalitis
HIV	human immunodeficiency virus
HPC	heterotrophic plate count

HUS	hemolytic uremic syndrome
HVAC	heating, ventilation and air conditioning
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
NASEM	National Academies of Sciences, Engineering and Medicine
NPC	National Plumbing Code (Canada)
NSF	NSF International
NTM	non-tuberculous mycobacteria
PAM	primary amebic meningoencephalitis
PCR	polymerase chain reaction
PHAC	Public Health Agency of Canada
POE	point-of-entry
POU	point-of-use
QMRA	quantitative microbial risk assessment
SCC	Standards Council of Canada
spp.	species
US EPA	United States Environmental Protection Agency
U.S.	United States
UV	ultraviolet
VBNC	viable but non-culturable
VTEC	verotoxin-producing *Escherichia coli*
WHO	World Health Organization

APPENDIX B

Table B1. Summary of waterborne pathogens of gastrointestinal origin

Pathogen	Members most frequently associated with human illness	Health Effects	Groups at higher risk for illness	Main reservoirs	Major route for waterborne transmission	Significance as a drinking water pathogen
Campylobacter spp.	*C. jejuni, C. coli*	Gastroenteritis (symptoms: watery, profuse and sometimes bloody diarrhea; fever and abdominal pain).	Young children, young adults, the elderly.	Poultry, cattle, sheep, domestic pets.	Ingestion of fecally-contaminated water.	Well-documented as a cause of outbreaks associated with contaminated drinking water.
Pathogenic *Escherichia coli/ Shigella* spp.	Enterohaemorrhagic *E. coli* (EHEC) group. *E. coli* serotype O157:H7 is the most prevalent.	Gastroenteritis (symptoms: watery diarrhea that can be accompanied by blood, nausea, vomiting, abdominal pain, fever). EHEC illness can progress to the hemolytic uremic syndrome (HUS), a potentially life-threatening condition involving decreased blood cell and platelet counts and acute kidney failure.	Young children, the elderly.	Cattle and other ruminants, humans.	Ingestion of fecally-contaminated drinking water.	Well-documented as a cause of outbreaks associated with contaminated drinking water.
	Shigella spp.: *S. sonnei* and *S. flexneri*	Gastroenteritis (symptoms: watery diarrhea that can be accompanied by blood, abdominal pain, fever).	Young children.	Humans.	Ingestion of fecally-contaminated water.	Rarely linked to drinking water outbreaks.

Pathogen	Members most frequently associated with human illness	Health Effects	Groups at higher risk for illness	Main reservoirs	Major route for waterborne transmission	Significance as a drinking water pathogen
Helicobacter pylori	*H. pylori*	Asymptomatic superficial gastritis. Some infections develop into peptic (e.g., duodenal or gastric) ulcers.	Individuals living in areas with crowded or high density living conditions and/or poor sanitation.	Humans.	Ingestion of fecally-contaminated water suspected as a possible route.	Further research is needed on the importance of drinking water as a source of infection.
Salmonella spp.	Non-typhoidal Salmonella group[a], particularly: *S. serotype* Enteritidis and *S. serotype* Typhimurium.	Gastroenteritis (diarrhea, fever and abdominal pain). Severe cases with immunocompromised individuals: can spread to other parts of the body (e.g., blood, urine, joints, brain)	Young children, the elderly, persons with weakened immune systems.	Chicken, pigs, turkey and cattle.	Ingestion of fecally-contaminated water.	Rarely linked to drinking water outbreaks.
Yersinia spp.	*Y. enterocolitica, Y. paratuberculosis*	Gastroenteritis ranging in severity depending on the strain (symptoms: diarrhea that can be accompanied by blood, fever and abdominal pain in children; appendicitis-like symptoms in adults).	Young children, the elderly, persons with weakened immune systems.	*Y. enterocolitica*: Pigs, ruminants, dogs, cats. *Y. paratuberculosis*: rodents, birds	Ingestion of fecally-contaminated water.	Rarely linked to drinking water outbreaks.

[a] The non-typhoidal group of Salmonella contains the serotypes of most importance for developed countries (see Section B.1.4).

APPENDIX C

Table C1. Summary of waterborne naturally-occurring pathogens

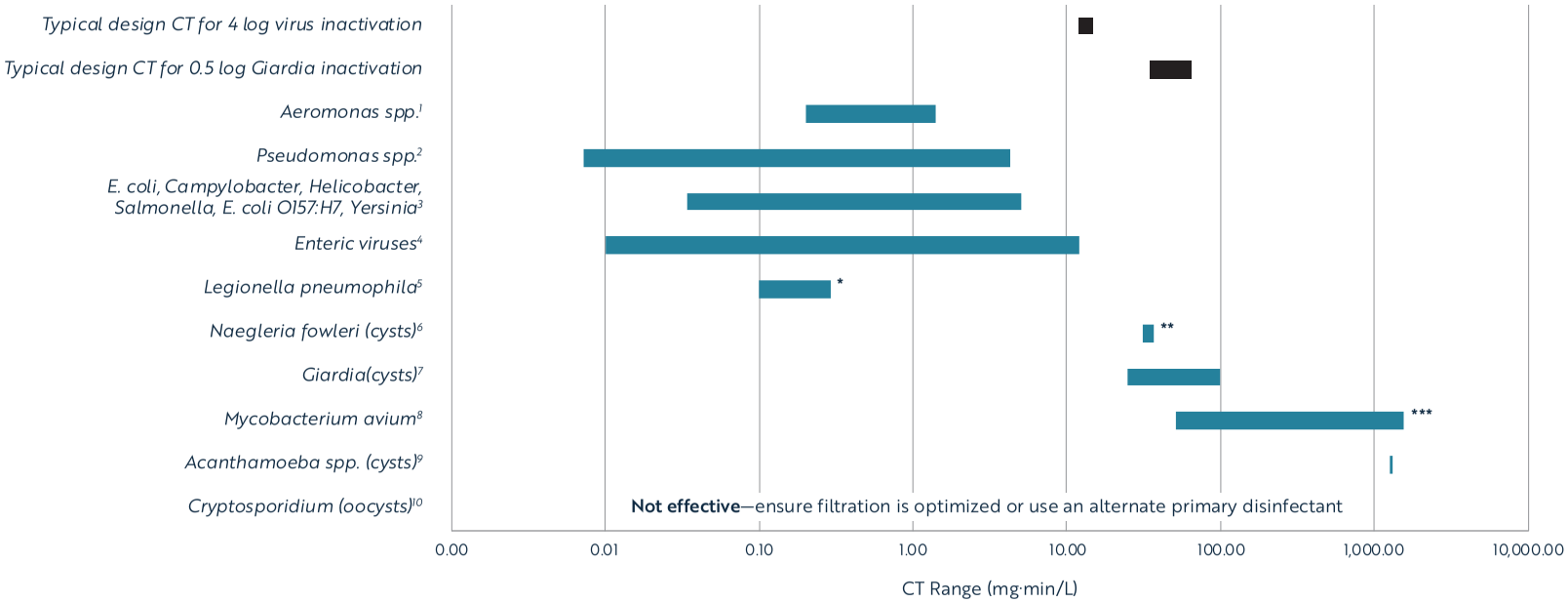
Pathogen	Members most frequently associated with human illness	Health Effects	Groups at higher risk for illness	Main reservoirs	Major route for waterborne transmission	Significance as a drinking water pathogen
Aeromonas spp.	*A. hydrophila, A. caviae, A. veronii* (biotype sobria), *A. trota*	Linked to a variety of intestinal and extra-intestinal diseases and syndromes. Gastroenteritis is the most common disease (symptoms: watery diarrhea along with low grade fever, vomiting and abdominal pain).	Young children, the elderly, persons with weakened immune systems.	Ubiquitous bacteria, found in a wide variety of habitats, including aquatic habitats, soils, vertebrate and invertebrate animal species, insects and foods.	Ingestion of contaminated water.	Further research is needed on the importance of drinking water as a source of infection.
Legionella spp.	*L. pneumophila* (mainly serogroup 1)	Legionnaires' disease: severe respiratory illness involving pneumonia. Pontiac Fever: milder, flu-like, self-limiting and non-pneumonic disease. Other disease (extremely rare): extrapulmonary infections involving the skin or soft tissues.	Older adults beginning at age 40–50, smokers, persons with weakened immune systems or underlying disease.	Free-living protozoa that can be found within biofilms in the natural environment and in engineered water systems and equipment (large plumbing systems, cooling towers, drinking water distribution systems).	Inhalation of contaminated aerosols generated by fittings and equipment associated with plumbing systems, HVAC systems, and humidification equipment.	Well-documented as a cause of outbreaks associated with water systems (cooling towers, plumbing systems) of large buildings (most commonly hospitals, long-term care facilities, hotels and resorts).

Pathogen	Members most frequently associated with human illness	Health Effects	Groups at higher risk for illness	Main reservoirs	Major route for waterborne transmission	Significance as a drinking water pathogen
Mycobacterium spp.	Non-tuberculous mycobacteria (NTM), group, particularly: *M. avium* complex (MAC): *M. avium* and its subspecies, *M. intracellulare*, and *M. chimaera*	Pulmonary disease. Symptoms: persistent cough, weakness and night sweats. Severe cases with immunocompromised individuals, infection can spread to other parts of the body (e.g., joints, liver, brain). Other diseases: cervical lymphadenitis, skin and soft tissue infections.	Individuals with weakened immune systems or underlying respiratory conditions.	Soils, water habitats, biofilms in engineered water systems (plumbing systems, drinking water distribution systems).	Inhalation of contaminated aerosols generated by fittings and equipment associated with plumbing systems and humidification equipment.	No reported outbreaks associated with drinking water. Contaminated water can be an important source for infections in specific settings (e.g., health care facilities) for at risk groups.
Pseudomonas spp.	*P. aeruginosa*	Respiratory infections (symptoms: fever, chills, cough and laboured breathing); infections involving the skin, eyes, ears and urinary tract.	Individuals with weakened immune systems or underlying diseases (particularly cystic fibrosis), patients undergoing procedures with invasive medical devices or have burns or penetrating trauma (surgical incisions, wounds).	Ubiquitous bacteria found in a wide variety of habitats, including soil, aquatic environments, vegetation and biofilms in engineered water systems (plumbing systems).	Direct body contact with contaminated water or medical devices in contact with contaminated water from engineered water systems.	No reported outbreaks associated with drinking water. Contaminated water can be an important source for infections in specific settings (e.g., health care facilities) for at risk groups.

Pathogen	Members most frequently associated with human illness	Health Effects	Groups at higher risk for illness	Main reservoirs	Major route for waterborne transmission	Significance as a drinking water pathogen
Acanthamoeba spp.	*Acanthamoeba* genotype T4	Acanthamoeba keratitis (AK), a vision-threatening disease (symptoms: blurred vision, intense pain and photosensitivity; later, severe cases, ulceration, swelling, glaucoma, cataracts and blindness).	Contact lens wearers.	Ubiquitous in soil and water; also present in biofilms in engineered water systems and equipment (plumbing systems, drinking water distribution systems cooling towers) and in airborne dust.	Eye contact with lenses exposed to water containing the organisms during lens washing, storage or wear.	Incidence of disease is rare. Can act as hosts for pathogenic bacteria including *Legionella* and non-tuberculous mycobacteria.
Naegleria spp.	*N. fowleri*	Primary Amebic Meningoencephalitis (symptoms similar to viral or bacterial meningitis: headache, fever, nausea and vomiting; later: stiff neck, altered mental status, occasional hallucinations seizures, coma). Infections almost always fatal.	Children and young adults engaging in recreational water activities where organism is prevalent; individuals performing nasal cleansing with non-sterile water.	Warm freshwater environments (lakes, rivers, hot springs) and soils. Can adapt to growth in biofilms in distribution and plumbing systems if conditions are favourable (optimal growth temperature, absence of disinfectant).	Intranasal contact with contaminated water through diving, swimming, bathing, splashing or nasal cleansing.	Incidence of disease is rare. Infections and isolations from piped water systems have primarily occurred in areas with a subtropical climate. Can act as hosts for pathogenic bacteria including Legionella and non-tuberculous mycobacteria.

APPENDIX D

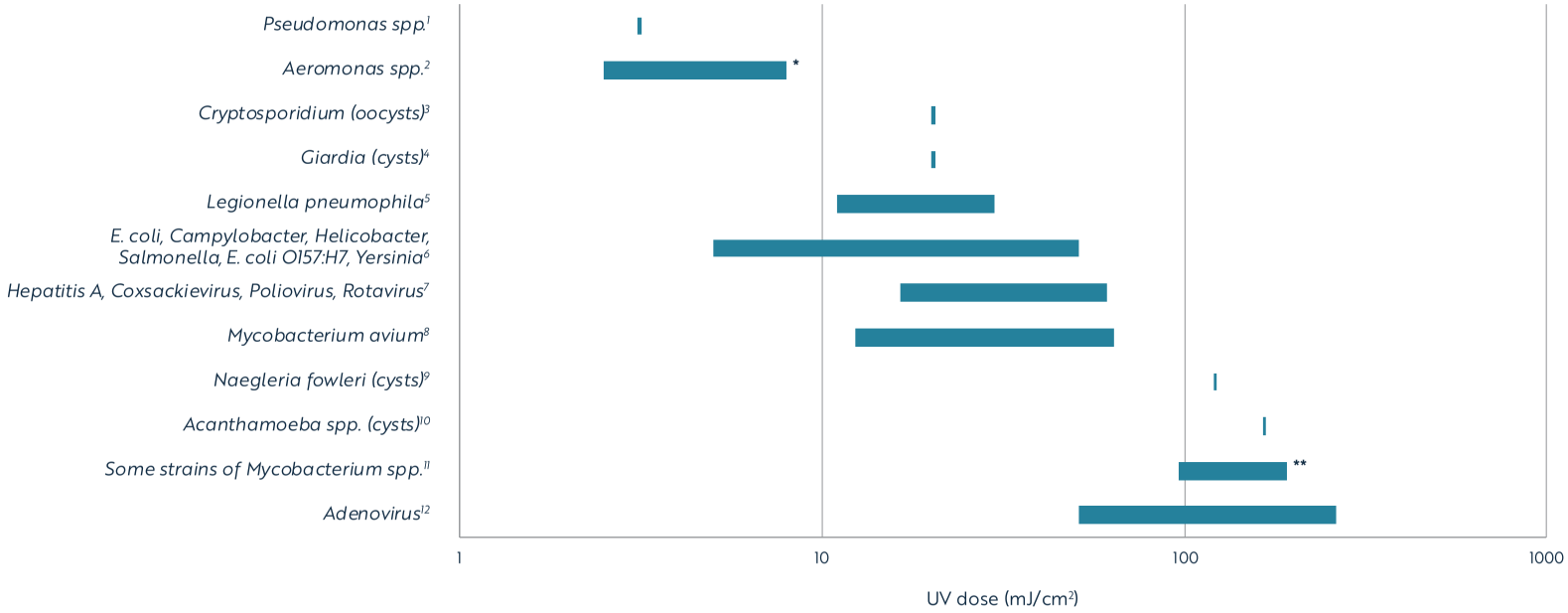
Figure D1. Relative CT values for various waterborne pathogens, Free chlorine (2 log inactivation, 5-25°C, pH 6-9)



1 Massa et al., 1999, Gerba et al., 2003
2 Perez-Recuerda et al., 1998; Xue et al., 2013
3 Hoff, 1986; Lund et al., 1996; Johnson et al., 1997; Perez-Recuerda et al., 1998; Rice et al., 1999; Baker et al., 2002; Wojcicka et al., 2007; Rasheed et al., 2016
4 Health Canada, 2019c
5 Buse et al., 2019
6 Sakar and Gerba, 2012
7 Health Canada, 2019b
8 Taylor et al., 2000
9 Loret et al., 2008
10 Health Canada, 2019b
* 3-4 log removal
** 4-log removal
*** 3-log removal

APPENDIX E

Figure E1. Relative UV dose requirements for various waterborne pathogens (4 log inactivation)



1 Clauß et al., 2006
2 Gerba et al., 2003
3 Health Canada, 2019b
4 Health Canada, 2019b
5 Hijnen et al., 2011
6 Zimmer and Slawson, 2002; Hayes et al., 2006; Zimmer-Thomas et al., 2007; Hijnen et al., 2011
7 Health Canada, 2019c
8 Hayes et al., 2008; Schiavano et al., 2018
9 Sakar and Gerba, 2012
10 Hijnen et al., 2011
11 Gerba et al., 2003, Schiavano et al., 2018
12 Health Canada, 2019c
* 2 log removal
** 2-5 log removal