Potential Microbial Water Quality Impacts Resulting from Main Breaks in Edmonton, Alberta: A Quantitative Microbial Risk Assessment

By

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ABSTRACT

Few studies, and even fewer within Canada, have addressed the efficacy of water utility main break response and repair standard operating procedures (SOPs) for prevention of microbial contamination. To better understand such risks in the City of Edmonton, Alberta, ten main break events were evaluated to provide data for a quantitative microbial risk assessment. The motivation for this research comes from the current practice of not 'super-chlorinating' the repaired main and that the use of federal Guideline faecal indicators may not effectively index persistent pathogens that could have negative impacts on public health. Soil samples surrounding each break site as well as initial and final flush water samples were collected. Samples were analysed using relevant indicators of potential faecal contamination, including spores of *C. perfringens*, total coliforms and *E. coli* by culture and *Enterococcus* spp., human-targeted *Bacteroides* HF183 and *E. coli* by quantitative Polymerase Chain Reaction (qPCR). Ratios were then determined from concentrations of the faecal indicator bacteria (FIB) and reference pathogens, *Norovirus* GII, *Cryptosporidium*, *Giardia*, *Campylobacter* and *Mastadenovirus,* reported for raw sewage, the main contamination source of public health concern. These ratios were then applied to the results from each of the ten main break sites which had FIB above the method detection limits for both water and soil samples, assuming soil intrusion at three possible levels, 5 g, 50 g or 500 g. These scenarios were then applied to mains-break repairs for the whole population of Edmonton, AB and Quantitative Microbial Risk Assessment (QMRA) estimated risks were compared to the U.S. EPA infection benchmark of 1/10,000 people per year and the Health Canada drinking water gastrointestinal illness benchmark of one micro disability adjusted life-year (μDALY)/ person per year. Of the reference pathogens examined, *Mastadenovirus* (human adenovirus) was determined to have the highest risk to public health while *Norovirus* GII

had the lowest. Although many uncertainties were applied to this study, results showed scenarios ranging from having very minimal risk, up to 200 times higher risk than the recommended benchmarks. The ramifications of these results are discussed against current standard operating procedures.

ACKNOWLEDGEMENTS

My goal since starting this Master's Program was to finish before reaching the milestone of 30 years of age. With some curve balls that life threw at me, I barely achieved this, but the main thing is I achieved it and I learned a lot, not only about main breaks but, about myself along the way. It is now time for me to leave this project behind, allowing others to learn and build from, to continue making public health safer.

I would like to thank Dr. Nicholas Ashbolt for his patience, guidance and advice he provided throughout my time as his student. I would also like to thank his staff, in particular Ms. Candis Scott and Dr. Graham Banting for the training and recommendations they provided me in order to make this research possible.

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Finally, I would like to thank my dad for phoning me every week for four years to ask if I am done yet.

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LIST OF SYMBOLS

No. – number

> – greater than

% – percent

g – gram

L – Litre

mg/L – milligrams per Litre

°C – degrees Celsius

® – registered trademark

TM – trade-mark

LIST OF ABBREVIATIONS

AB – Alberta

AEP – Alberta Environment and Parks

AWWA – American Water Works Association

BAC – Biological Agents of Concern

cc – curb cock

CDC – Centers for Disease Control and Prevention

CFU – Colony-forming units

COP – Code of Practice

DALY– Disability Adjusted Life Year

DWA – Drinking Water Advisory

DWDS – Drinking Water Distribution System

DWWP – Drinking Water Works Permit

EPO – Environmental Project Officer

FIB – Faecal Indicator Bacteria

GCDWQ – Guidelines for Canadian Drinking Water Quality

GC – Genome or gene copy number

GI – Gastrointestinal

MOH – Medical Officer of Health

MSc - Master of Science

MST – Microbial Source Tracking

NTU – Nephelometric Turbidity Unit

OIP – Operator in Charge

PDWA - Precautionary Drinking Water Advisory

PHAC – Public Health Agency of Canada

SOP – Standard Operating Procedure

QMRA – Quantitative Microbial Risk Assessment

qPCR – quantitative Polymerase Chain Reaction

QSAP – Qualitative Surrogate Attribute Prioritization

UIS – Utility Information System

US – United States

US EPA – United States Environmental Protection Agency

WCWC – Walkerton Clean Water Center

WHO – World Health Organization

WRF – Water Research Foundation

WSA – Water Security Agency

WTP – Water Treatment Plant

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CHAPTER 1: INTRODUCTION

1.1: Problem Statement

Few studies, and even fewer within Canada, have addressed the efficacy of water utility main break response and repair standard operating procedures (SOPs) for prevention of microbial contamination. Of the studies conducted, the majority have been carried out using controlled laboratory conditions as opposed to actual main break repair sites. Further, even less data has been collected to compare water quality changes following a main break. Prior to the 2015 publication of "Microbial Risk Modeling for Main Breaks" (Yang *et al*., 2015), no quantitative microbial risk assessment (QMRA) study had been reported to evaluate microbial risks and control measures for main breaks.

There are many factors and events, both internal and external, which can affect the water quality within a drinking water distribution system (DWDS). Aging water distribution infrastructure experiences chemical and microbial induced corrosion as well as other age-related breakdown (Ingerson-Mahar and Reid, 2012). These factors can lead to possible violations of water quality standards as well as risks to public health (National Research Council, 2006).

Approximately 3,800 km of buried distribution system pipes deliver safe drinking water to Edmonton consumers. The Edmonton DWDS has approximately 400 breaks annually, based on the average over 11 years between 2005 and 2015 (EPCOR, 2005-2015). Due to Edmonton's severe weather and mobile soils these breaks are not evenly distributed throughout the year. Most breaks occur in the winter when ground shifts due to freezing and sudden extreme temperature changes occur. The majority of breaks that occur in the summer are usually from sudden changes in water pressure or a change in soil moisture content causing ground movement

(EPCOR, 2016). Other causes of water main breaks include, vibrations from heavy traffic above DWDS pipes, installation processes and unforeseen issues and pipe material, as some pipe material will corrode and break down faster than others (City of Calgary, 2017). Even with the number of main breaks in Edmonton decreasing with older cast iron and ductile iron pipe replacement with new pipe materials during mains renewal projects, water main failure will continue to occur due to aging infrastructure along with operational loads and environmental stressors like significant temperature and moisture changes (Francisque *et al*., 2013).

Edmonton's water utility (EPCOR Water Canada), follows industry standards to minimize the possibility of microbial contamination during main break repairs, but no studies have evaluated EPCOR's SOPs to determine if the SOPs provide sufficient protection to water quality and public health under local conditions.

As part of EPCOR's due diligence, and in response to studies undertaken in the U.S. and their recommendation for disinfection prior to reconnecting a new pipe section during a major repair (Kirmeyer *et al*., 2014), which is not current practice in Edmonton, this project focused on assessing soil/faecal contamination via assays of a range of indicator organisms by culture and molecular methods. The study organisms include human-targeted *Bacteroides*, spores of *Clostridium perfringens*, and traditional faecal indicators (*Enterococcus* spp., total coliforms and *Escherichia coli*) collected during initial and final flush samples and from soil within the vicinity of main repairs. This data was utilized to assess possible risks from various scenarios using QMRA modelling.

1.2: **Objectives**

The objective of this study was to examine possible microbial risks to public health resulting from DWDS main break repairs, by collecting field data to enable a QMRA of the current SOP as well as possible variations to the SOP for water main break repairs in the City of Edmonton.

Sub-objectives:

a) Identify variables that may be useful to predict microbial water quality deterioration during main breaks and/or repairs by combining historical data with new information collected during this study from 10 main breaks in the City of Edmonton;

b) Identify relevant indicators of potential faecal contamination using sites and analytes not currently tested for (e.g. spores of *C. perfringens* culture, and *Enterococcus* spp., human-targeted *Bacteroides* HF183 and *E. coli* by quantitative Polymerase Chain Reaction (qPCR), from local soil and first and final flush water samples during repair operations) to predict possible public health risks for those affected by main breaks in the City of Edmonton;

c) To aid in the interpretation of the microbial source tracking *Bacteroides* HF183 sewage marker, estimate its persistence (detectible qPCR signal) and decay rate in soils associated with the City of Edmonton's drinking water mains;

d) Perform a screening-level QMRA using local and literature data to evaluate possible risks associated with various contamination scenarios; and

e) To recommend future improvements to EPCOR's SOPs for main breaks and repairs.

CHAPTER 2: LITERATURE REVIEW

2.1: Main Breaks Within Drinking Water Distribution Systems

In the last century, construction of sanitary water delivery systems was one of the most important public health achievements in developed regions (CDC, 1999), which aided in ending large outbreaks of typhoid, cholera and other (traditional) waterborne diseases (Ercumen *et al*., 2014). While considerable water industry research has focused on monitoring the microbial quality and safety of finished water (treated drinking water produced at treatment plants), far less data is available describing changes in drinking water quality in the distribution system. Once water leaves the treatment facility and enters the distribution system it passes through many different pipes, reservoirs and valves before it reaches consumer taps. Like all other man-made environments, all these components have different microhabitats that house thousands of species of bacteria, archaea, algae, free-living protozoa, invertebrates, and their viruses, with the majority, in modern systems, being completely safe for humans (Ingerson-Mahar and Reid, 2012). The likelihood of pathogen contamination of water in a DWDS depends on numerous factors, yet verification of integrity is largely based on measuring coliform bacteria (Standridge, 2008). General factors impacting water quality include water source, treatment processes, characteristics and condition of the distribution system itself as well as at the user end-point (Payment *et al*., 1991; Craun, *et* al., 2010). These pipe, reservoir and valve components of DWDS undergo corrosion and deterioration over time and are subject to potential pathogen ingress via various mechanisms (Besner *et al*., 2011). Aging water distribution infrastructures also experience chemical and microbial induced corrosion as well as other age-related breakdown (Ingerson-Mahar and Reid, 2012; Cunliffe *et al*. 2014). As water distribution system infrastructure continues to age and deteriorate, it becomes more vulnerable to intrusions via

increased rates of main breaks (LeChevallier *et al*., 2014), yet the risk of intrusion is dependent in part on the type of break. The factors leading to the classification of the type of break include: type and size of pipe affected, pressure within the pipe, size of damage to pipe including both length of a fissure and/or size of an orifice and whether the repair can be done using a clamp or if it requires removing and replacing a section of pipe and/or valves.

Kirmeyer *et al*. (2014) developed guidelines to categorize types of breaks, ranging from Type I (lowest risk) to Type IV (highest risk), as well as recommended responses (Table 2-1).

Table 2-1 Categories and responses of main breaks

TYPE	PRESSURE	DISINFECTION	INTRUSION
I	Positive pressure maintained during the break and repair	Disinfect repairs parts	No signs of soil intrusion into pipe
II	Positive pressure maintained during the break until a shutdown is controlled	Disinfect repairs parts	No signs of soil intrusion into pipe
III	Possible local depressurization with a partial or fully uncontrolled shutdown	Disinfect repairs parts and super chlorinate	Possible soil intrusion into pipe
IV	Loss of pressure during break, widespread depressurization in system with a catastrophic failure	Disinfect repairs parts and super chlorinate	Possible/actual soil intrusion into pipe

Adapted from: http://www.waterrf.org/ExecutiveSummaryLibrary/4307a_ProjectSummary.pdf

When considering the responses given in main break guidelines (Table 2-1), approximately 5% of Edmonton's mains break repairs would fall within the Type I category, leaving 95% or roughly 380 breaks per year, in either the Type II, III or IV categories, with the majority being of Type III. Due to the nature of Edmonton's soil type and climate, allowing breaks to remain under positive pressure until excavation has exposed the pipe is not realistic, therefore most of the breaks fall into the Type III and IV categories. With safety being such a major focus at EPCOR, leaving breaks under pressure can lead to unnecessary hazardous conditions. Slips, trips and falls from water mixing with Edmonton's clay soils and/or freezing in the winter months causing very slippery surfaces, as well as exposing employees to flood zones as the trenches have to be dug at a depth, up to eight feet, more than the average height of a person, to access the pipes below the freezing region (frost line) of the ground. With the depths of the mains below the frost line to avoid freezing, being a major difference between Edmonton and warmer climates, the categories proposed by Kirmeyer *et al.* (2014) are more suitable to warmer climates where the mains are not buried at such depths. Under Edmonton conditions, doing a controlled shutdown is considered the best method by EPCOR Edmonton. However, as explained above, doing a controlled shutdown based on these categories would push the majority of breaks into the Type III and IV classification. Under Kirmeyer *et al.*'s scheme, Type III and IV require full disinfection of the water line post repair, which poses issues for EPCOR's customer service performance measure of water restored to customers within 24 hours 93.7% of the time.

In order to complete a full disinfection, two addition trenches would have to be dug to allow for new connections to enable the disinfection solution to be put into the system and flushed out. This would then lead to a number of other possible outcomes; such as closing every curb cock (cc) valve to isolate the area for the disinfection to avoid introducing the disinfection product

into house hold plumbing, which is not ideal given the age of the DWDS and the weather conditions in Edmonton. In addition, this isolation approach may be complicated by failure of cc valves not closing to begin with, or the ones that do close may not re-open, the cc valves could also break at any time during the process of trying to open or close them, which is common in Edmonton. In order to repair cc valves, more trenches would need to be excavated. With the City of Edmonton's current policy requiring 50 meters of full road restoration where utility cuts exist with less than 15 m of separation between them (City of Edmonton, 2015), a simple main break could turn into a much larger job resulting in longer periods of time that customers would be without water as well as hugely increasing the cost of repairs. EPCOR's performance standard is to restore water to its customers within 24 hours 93.7% of the time; if EPCOR were to follow Kirmeyer *et al*.'s protocol, this performance measure would become unattainable. When taking this into consideration, it can be proposed that a category between Type II and III would make most sense for Edmonton. Currently EPCOR Edmonton could classify most main breaks as Type 2.5, consisting of Type III response, excavate after local depressurization, and Type II disinfection, disinfect repair parts and conduct a flush of the area affected. Like many things, one method does not always work for every situation. Over an 11-year span from 2005 to the end of 2015, EPCOR has demonstrated success in repairing more than 4400 depressurizations, which includes any repair or maintenance to the DWDS where the system is depressurized, with less than 2% microbial contamination based on the samples collected post repair after system flushing and taken back to the lab for analysis. When microbial contamination does occur, re-samples are collected. Only 18% of re-samples come back also positive based on the current coliform testing protocol (Table 2-2; Figure 2-1). When a depressurization does produce a positive coliform result, a re-sample is taken from the original sample point, typically the first

hydrant downstream from the break, a different sample point within the same premise, as well as one sample upstream and one sample downstream from the original sample collection. Based on EPCOR's Approval to Operate, when you have a re-sample that is also positive, you are directed to the most current Edmonton Waterworks System Operations Program. Section 10.2.3 of the Operations Program states "If the resampling determines that the original bacteriological positive result was the result of poor sampling procedure, sample point contamination or contamination within the building plumbing, this will not be considered a violation of the Approval." With this being the outcome the majority of the time, a positive coliform test rarely results in a violation, which can be defined as a deviation from what the provincial regulators state as allowable parameters. .

Table 2-2 Depressurizations in the EPCOR Edmonton DWDS from 2005-2015

Year	Approx. No. of Depressurizations	No. of events total coliforms detected in original sample	No. of events *E. coli* detected in original sample	No. of events total coliforms detected in resamples**
2005	305	3	0	0
2006	430	6	1	0
2007	475	3	1	0
2008	583	10	0	3
2009	669	8	0	1
2010	333	7	0	1
2011	385	8	0	2
2012	365	2	0	0
2013	278	10	1	3
2014	389	7	0	2
2015	227	5	0	1
Total	**4439**	**69**	**3**	**13**

** *E. coli* was not detected in any resamples; resamples collected when the original sample fails.

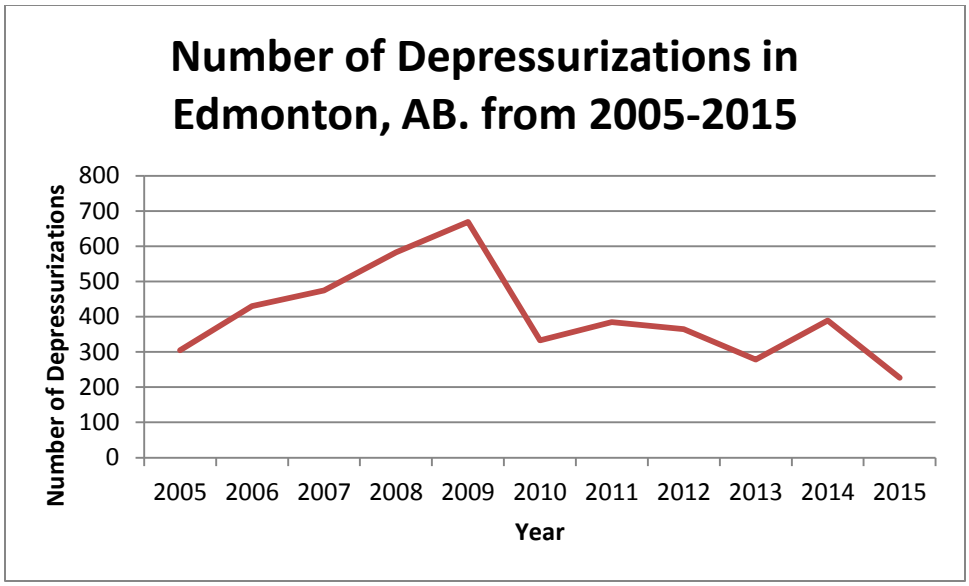

Figure 2-1 Depressurizations in the EPCOR Edmonton DWDS from 2005-2015

Although leaks and breaks, classified as main breaks are the focus of this study, there are other less frequently occurring events such as backflow from cross-connections and reservoir contamination events (Besner *et al.,* 2011), which may allow pathogen entry into the DWDS. Overall, any disruption of the DWDS increases risk of water contamination leading to gastrointestinal (GI) illness and other types of waterborne illness (Ercumen *et al.,* 2014).

2.2: Quantitative Microbial Risk Assessment Background

A quantitative microbial risk assessment (QMRA) is utilized to estimate and explore various scenarios of microbial risk to public health, typically as a part of preventative measures in microbial safety of drinking water (Petterson and Ashbolt, 2016). Health Canada created a QMRA model in 2007, revised in 2013, as a way to help users comprehend possible microbial risks associated with drinking water treatment systems and are promoting the use of QMRA for water utility companies to assess their specific system (WCWC, 2015). Regulatory agencies

internationally are using QMRA models or the outcome of these models for setting enteric viral, bacteria and parasitic protozoan treatment requirements (Petterson and Ashbolt, 2016). QMRA models may be developed that are too conservative or not conservative enough, both leading to a different set of problems. If a model is too conservative it can result in additional treatment being needed and a major increase in operational costs. If a model is not conservative enough, it can pose a risk to public health not being protected appropriately, which can lead to waterborne disease outbreaks (Payment, 2014).

For this Master of Science (MSc) study, QMRA was used as a tool to assess a range of scenarios that addressed possible uncertainties in microbial safety of drinking water post main break repairs within the City of Edmonton, AB. QMRA is a derivative of the chemical risk assessment model (NRC, 1983) and is composed of four basic stages modified to account for the properties of living organisms (Haas *et al*., 1999). Even more specifically, the framework for water-related QMRA includes (WHO, 2016):

1. Problem Formulation: Define the scope and purpose of the risk assessment in order to effectively manage the specific risk question that is being addressed.
2. Exposure Assessment: Determine how much and how often there is exposure to pathogens via the pathways and hazardous events defined during the problem formulation.
3. Health Effects Assessment: Compilation of data determining the public health impacts. This includes; health effects that may occur after initial infection, exposure dose to probability of infection or illness occurring (dose-response relationship), and probability

of morbidity and mortality. It could also be important to consider the portion and susceptibility of the public exposed.

4. Risk Characterization: Integrate the information collected from the exposure assessment and health effects assessment within a mathematical model to calculate risk of the exposed population. Analysis is then done to determine a range of possible risks including best and worst case scenarios as well as average risk scenarios.

2.3: Federal, Provincial and Municipal Compliance Monitor Testing

There is no direct correlation between federal, provincial and municipality potable water compliance monitoring testing and health outcome. For the most part no detectable presence of total coliforms, faecal coliforms or *Escherichia coli* indicators (faecal indicator bacteria, FIB) within a 100 mL sample is considered adequate for bacteriological testing after a main break repair (Cook *et al*., 2013). A major issue with this is a negative result from a 100 mL coliform-based test does not mean there are no waterborne pathogens present at an infectious dose (Ingerson-Mahar and Reid, 2012). The Federal-Provincial-Territorial Committee on Drinking Water develops the Guidelines for Canadian Drinking Water Quality (GCDWQ) (Government of Canada, 2016). With these being only "guidelines" there is no legal obligation to follow this document. Provinces and territories, however, may create their own framework from the GCDWQ as the federal government has no mandate over provincial drinking water quality. Alberta, Saskatchewan and Ontario have adopted the GCDWQ into standards to be used provincially, which then become a legal obligation when put into a Code of Practice (COP) or an Approval/Permit to Operate for a specific water treatment facility. This section reviews three Canadian provinces, assumed to have similar climate, looking at how their response/repair of

main breaks compares, as well as an intra-provincial comparison between two Alberta utility companies in Calgary and Edmonton.

2.3.1: ***Saskatchewan*** (Saskatchewan Water Security Agency, 2017)

Saskatchewan Water Security Agency (WSA) has developed a standard guidance document that they provide to waterworks owners and operators. Along with this document the WSA typically refers the waterworks owners to the most current version of the AWWA C651 Disinfecting Water Mains document when it comes to dealing with main breaks. However, each owner/operator is responsible for creating their own operating procedures for main break repairs. The waterworks permittee will develop operating procedures about the management of waterworks incidents then submit them to the appropriate Drinking Water Agency to be reviewed and approved as considered suitable.

All waterworks incidents that occur in Saskatchewan, are required to be reported so exact parameters necessary to isolate the incident from the rest of the distribution system can be determined by the reviewing Environmental Project Officer (EPO) and their manager. If at any point during the repair of the break, the water quality is expected to exceed what was in the accepted SOP, the WSA must be notified immediately. Saskatchewan adapted a main break management system from the Water Research Foundation (WRF) project #4307. The categories and responses for WRF project #4307 are the same as those discussed in Table 2-1. However, Saskatchewan has made the responses to each of the four categories more detailed in order to comply with their specific regulations (Table 2-3).

Table 2-3 Saskatchewan's response procedures to main breaks; adapted from WRF project #4307

Response Procedures			
Type I	**Type II**	**Type III**	**Type IV**
Reporting by email is sufficient	Reporting be email is sufficient	Immediate and Direct notification to WSA is required	Immediate and Direct notification to WSA is required
Excavate and repair under pressure >20 psi	Excavate until below break under pressure >20 psi, controlled shutdown while maintaining pit water level below break	Uncontrolled shutdown during excavation and repair. Possible contamination needs to be documented.	Widespread uncontrolled shutdown, shut off service lines in area affected. Possible contamination needs to be documented.
Disinfect repair parts	Disinfect repair parts	Disinfect repair parts	Disinfect repair parts
Confirm residual disinfectant level is acceptable	Low velocity flush of 3 pipe volumes.	Perform a scour flush and slug chlorination*	Perform a scour flush and slug chlorination
No DWA	Confirm residual disinfectant level is acceptable.	Confirm residual disinfectant level is acceptable.	Confirm residual disinfectant level is acceptable.
Microbiological samples required	Issue a DWA only if the area that was depressurized is larger than the area treated with flushing.	Notification for customers affected to flush premise plumbing when water back in service	Notification for customers affected to flush premise plumbing when water back in service
	Microbiological samples required	Self-managed DWA	WSA issued PDWA
		Microbiological samples required	Microbiological samples required

DWA -Drinking Water Advisory, PDWA- Precautionary Drinking Water Advisory
*slug chlorination – dosing chlorine to water entering the main for a sufficient period of time to build a solid column, or slug, of chlorinated water that will expose all interior surfaces to a high concentration allowing for disinfection (Melliger, 2006).

2.3.2: ***Ontario*** (Ontario Ministry of the Environment and Climate Change, 2016)

The province of Ontario has a procedure that is required to be followed in order to comply with the conditions implemented by the Director in a Drinking Water Works Permit (DWWP). All parts of water mains that come into contact with drinking water when replaced, repaired,

inspected or any activity that could cause contamination, must be disinfected. The AWWA C651- Standard for Disinfecting Water Mains or an equivalent procedure must be followed before they can be put back into service.

Ontario has come up with their own document, Watermain disinfection procedure, that the Ministry of the Environment and Climate Change has deemed equivalent to, and therefore, can replace the AWWA C651-Standard for Disinfecting Water Mains. Main breaks would fall under Section 2.0, Emergency/Unplanned repairs, of the document. Utility companies in Ontario must produce and document, operating procedures using the best management practices that go above the minimum requirements stated in the document issued by the province.

The procedure follows a set of minimum disinfection requirements with the goal to reduce any risk from drinking water to public health during these emergency/unplanned main breaks. The document also includes a set of standards for recording data/information and necessary notifications to the Ministry and Medical Officer of Health.

Steps during actual repair are as follows:

First the Operator in Charge (OIC) will determine if positive pressure can be maintained until excavation complete. If yes, positive pressure is maintained until an air gap is created. An air gap is an empty space or simple backflow prevention method that will help to avoid potable water and possible non-potable water from mixing (Balkan Sewer and Drain Cleaning Inc, 2018). The OIC will then do a visual check, post excavation, to classify the break into either category 1 or 2.

Category 1 is assigned when there is no evident or suspected contamination at any point during the break and repair. If during the process at any time contamination is suspected or the air gap is

not maintained, the repair is reclassified to Category 2, assigned when there is apparent or suspected contamination. Category 2 will also be assigned anytime there is more than six metres of pipe being replaced.

Shared practice between Category 1 and 2:

- all parts that will come into contact with drinking water must be disinfected with at least 1% solution of sodium hypochlorite;
- flushing until drinking water is free of discoloration and at least 0.2 mg/L Free Chlorine or 1.0 mg/L Total Chlorine is restored; and
- not required to report to the Spills Action Center or the local Medical Officer of Health (MOH) unless the MOH states they would like the option to be notified. Waterworks operators may contact the Medical Officer of Health at any time to request advice.

Table 2-4 Difference in responses to Ontario's water utilities Category 1 and 2 main breaks

Category 1	Category 2
No addition decontamination necessary	Addition decontamination steps required Examples: Mechanical removal of contaminants, flushing into excavation, high velocity flushing
Microbiological samples are not required to be collected	Microbiological samples are mandatory to be collected (normal service can be restored prior to sample results)

2.3.3: ***Alberta*** (Alberta Environment and Parks, April 2012)

Standards and Guidelines for Municipal Waterworks, Wastewater and Storm Drainage Systems, is a document that Alberta Environment and Parks (AEP) supplies to large municipal waterworks, wastewater and storm drain facilities. Each utility owner is responsible for adhering

to AEP's regulatory requirements as well as to produce and deliver safe drinking water to its customers. Alberta requires new water mains to be disinfected and flushed before being put into service in accordance with the latest edition of AWWA Standard C651 for Disinfecting Water Mains. When it comes to main breaks AEP's standards and guidelines, it is recommended to flush the repaired pipe until chlorine residuals are greater than 0.1 mg/L and turbidity is less than 2.0 NTU. A bacteriological sample also needs to be collected at the same time as the chlorine and turbidity sample. The main can be put back into service before the bacteriological sample results are available so customers are not without water.

2.3.3.1 ***Calgary, AB*** (Huston, 2017)

Over the years the City of Calgary has been working to make improvements to their main break response and repair procedure in order to reduce risk and increase efficiencies. Since the late 1970's until the past couple years, the City of Calgary has reduced their number of main breaks from approximately 1800 per year, or 4-8 everyday, down to 200-300 per year which has been of major importance in reducing the risk of contamination into the DWDS. Another major difference since the 1970's is that the length of flushing time was determined by visual inspection of when the water appeared to be clear, there were no in-field or lab testing protocols. Other changes the City of Calgary has made in the attempt to reduce contamination of water mains includes a multi-barrier approach through maintaining positive pressure and only shutting down the mains when necessary, and capping any open ended pipe (either a pipe in need of repair or a piece of pipe that has been cut to replace a section of pipe) until it is being installed to prevent any debris from entering. This is especially helpful when a repair has to be left until the next day to complete.

Once the necessary repair to the main break is complete, the repair is tested to confirm that it is sufficient. A primary flush is then carried out to remove any debris that could have entered the pipe. The City of Calgary then follows a "return to service" procedure that is a two-step process to safeguard drinking water safety. The crews responsible for the repair are also responsible for the "primary flush". The primary flush consists of a one way flush using a hydrant, which ideally has a flow high enough in velocity to remove any debris that had entered the pipe but low enough that it does not scour the main causing a major increase in turbidity. Once the field tests reach more than 0.2 mg/L of chlorine residual and less than 1.0 NTU turbidity, customers are notified of safe water consumption. At this point the main is still controlled by keeping one valve closed to give water access to residents affected, but the section is still isolated and contained from the rest of the DWDS. Within 24 hours of the primary flush, a different crew will go back to the same location and carry out a secondary flush of all sections of pipe involved in the shutdown and repair of the main. Crews re-check turbidity and chlorine residual following the same limits as in the primary flush; a bacteriological sample to be analyzed in the lab is also collected at this time. If each analysis is acceptable, all valves are opened and normal operation of the system is restored.

2.3.3.2 ***Edmonton, AB*** (EPCOR, 2017)

As mentioned in Section 2.1, EPCOR targets to have 93.7% of main breaks repaired back in service within 24 hours. When water supply to customers is affected by a main break and is going to be off for a longer period of time, a temporary water source will be supplied. Once leak detection testing is complete, the main is depressurized. The break location is then excavated and crews will determine the best method for repair. EPCOR Edmonton has four repair methods that are used to repair main breaks:

1. Installation of Repair or Boss Clamp

   Installing a clamp is the most common method EPCOR uses to repair a main break. A boss clamp and a repair clamp are installed the same way placing the clamp around the breached area of the pipe and bolted together. The difference between the clamps is that a boss clamp is used when a pipe is broken at the site of a mainstop where the service line connects to the main. A boss clamp has a mainstop outlet allowing for reconnection of the service line.

2. Repair Lead Joint

   When there is a breach in the integrity of a lead joint that is joining pipe to fittings, tees or valve joints, it is repaired by caulking the leaded joint. If the joint is not repairable by this method it will be cut out and replace with a new joint.

3. Replace Pipe Section

   When a very large pipe area is broken or a complete sever of the pipe occurs, a clamp repair will not be sufficient. At this time the broken pipe is removed and replaced with a new section of pipe joined by couplings.

4. Repair Steel Transmission Main

   Main breaks that occur on steel transmission mains can sometimes be repaired using a clamp. If the pipe is too large for a clamp, a patch is welded onto the steel main then sprayed with a rubber coating to protect the steel patch from corrosion.

For clamp and pipe replacement methods, the existing pipe as well as the new cut piece of pipe, if necessary, are disinfected using a 1.0% chlorine solution. Once the repair has been completed the line is flushed until the total chlorine residual is greater than 1.0 mg/L and turbidity is less than 3.0 NTU determined using field kits. Water samples for total chlorine residual, turbidity and total coliform and *E. coli* are then collected and taken to the Rossdale Water Treatment Laboratory for analysis. Valves can then be turned back to the correct operating position prior to results from the lab.

2.4: **Summary of Federal, Provincial and Municipal Compliance Monitor Testing**

When comparing these three Canadian provinces and two intra-provincial cities, it is evident that there are clear differences in approaches to main break responses and repairs across Canada. Table 2-5 highlights some of the similarities and differences when it comes to basic operation during a routine main break.

Table 2-5 Similarities and differences in main break responses and repairs between Saskatchewan, Ontario, Alberta and between Edmonton and Calgary municipalities

Saskatchewan	Ontario	Alberta	
Use the GCDWQ to create framework for provincial regulations	Use the GCDWQ to create framework for provincial regulations	Use the GCDWQ to create framework for provincial regulations	
Recommend following AWWA C651 document for disinfection	Recommend following AWWA C651 document for disinfection	Recommend following AWWA C651 document for disinfection	
Main break assigned to 1 of 4 categories	Main break assigned to 1 of 2 categories	No categories	
Some repairs done under positive pressure	Some repairs done under positive pressure	No Repairs done under positive pressure	
Microbiological samples collected after every repair	Microbiological samples only collected for category 2 repairs	Microbiological samples collected after every repair	
		Calgary	**Edmonton**
		Main remains controlled until secondary flush and biological samples are analyzed and results pass	Main is put back into normal operation after test with field kits for residual chlorine and turbidity pass

2.5: Characteristics of Study Organisms

To explore a range of possible faecal contamination events, both molecular and culture-based methods were used in this MSc. study to quantify five different FIB surrounding and within ten main break sites in the City of Edmonton, Alberta, Canada: *Clostridium perfringens* spores,

Enterococcus spp., human-specific *Bacteroides* HF183, total coliforms and *Escherichia coli*. The Edmonton, AB region mainly has clay-soils, which are expected to preserve FIB and pathogens (Meschke and Sobsey, 1998), hence the rationale for also collecting nearby soil samples to explore possible sewage contamination in the region of the repair.

While most microbes are harmless and/or beneficial, there are some we classify as biological agents of concern (BAC). All BAC may cause illness, which can often be serious and even fatal, but they differ greatly in their physical characteristics, movement in the environment, and process of infection (WHO, 2016). Something to consider when dealing with drinking water is that pathogen concentrations may be below the detection limit of the method used to quantify the results, but can still pose a risk to public health (Petterson and Ashbolt, 2016). To aid in the constraints of pathogen detection and identification, FIB are commonly used as surrogates to substitute for the behavior of pathogens of interest (Harwood *et al*., 2005). There are hundreds of enteric bacteria, protozoa and viruses associated with waterborne disease, therefore FIB are typically used to represent the fate and transport of these pathogens in the environment. Unfortunately, there are problems with using indicators (Ashbolt *et al*., 2001). Traditional FIB come from animals, plants and soils and do not identify a specific source, and might not accurately imitate the behaviour of specific pathogens. When considering these complications, it is no surprise better indicators are always being sought after (US EPA, 2006). Figure 2-2 shows one strategy using a conceptual decision framework to determine the best surrogate for a particular study.

The main break sites tested in Edmonton, AB in this study would most likely get contaminated with FIB via leaking sewer pipes, animal faeces and storm water drains. In general, sewage is the

primary source of enteric pathogens (Bichai and Ashbolt, 2017) that could contaminate a main break repair site. Hence, the selected FIB are described within this context next.

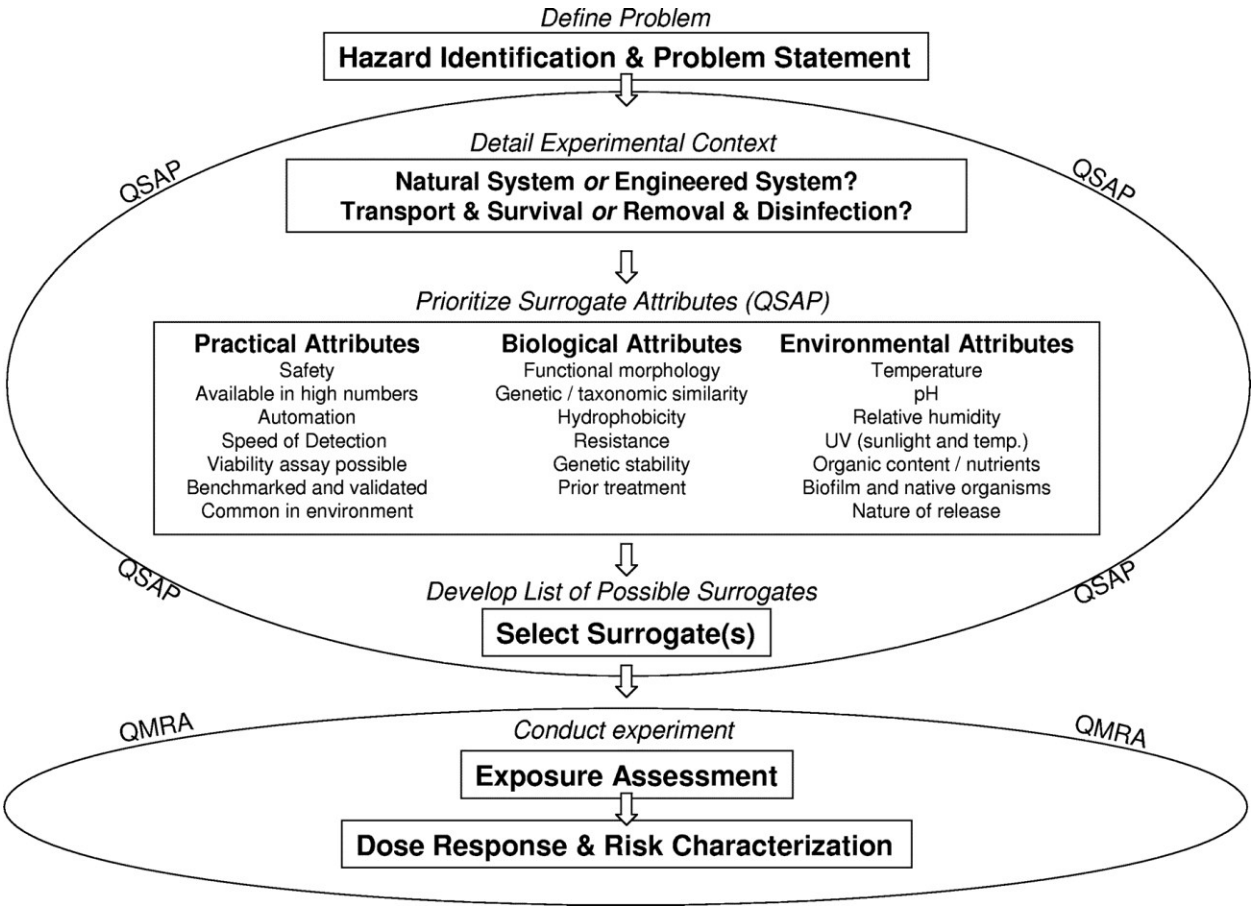

Figure 2-2 Conceptual decision framework for selecting a surrogate (Sinclair *et al*., 2012)
QSAP- qualitative surrogate attribute prioritization

2.5.1: Total Coliforms and *Escherichia coli*

2.5.1.1 *Characteristics and Sources*

Total coliforms are non-spore forming, Gram-negative rods that ferment lactose (using β-galactosidase) with gas and acid formation at 35°C in 48 h, but since the 1990's have simply

been defined by the enzyme β -galactosidase using specific substrate technology, such as Colilert^{TM}. They are present in the digestive tracts and excreted by both humans and other animals. However, they can also be common in plant and soil matrices as naturalized environmental strains, including strains of *E. coli* (Luo *et al.,* 2011).

Coliforms can be classified into three main groups, total coliforms, faecal coliforms, and *E. coli*, each indicating different drinking water quality and level of faecal pollution (Figure 2-3). Total coliforms are used as indicators of disinfection efficacy, as they include a very wide-range of bacteria, are common in soils and vegetation that have been influenced by surface water and for the most part are harmless to public health. The sub-group of total coliforms classified as faecal (thermotolerant) coliforms are more likely to indicate faeces from warm-blooded animals, including humans. The preferred faecal indicator, however, is *E. coli*, which usually indicates recent faecal contamination and is further indicated with the Colilert^{TM} medium by the activity of the enzyme β-glucuronidase.

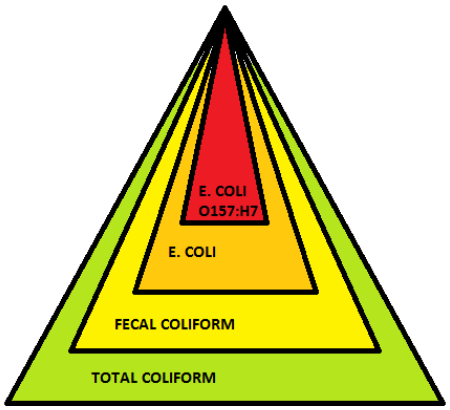

Figure 2-3 Coliform groupings and risk matrix.
Smaller groups are more specific faecal indicators and have higher likelihood to indicate pathogen presence

The majority of coliform bacteria do not cause disease. However, some uncommon strains of *E. coli*, especially 0157:H7, may cause a threat to public health, but due to their lack of the enzyme β -galactosidase are not detected by Colilert^{TM}. The *E. coli* O157:H7 strain has been identified in a range of domesticated animals (including cattle, chickens, pigs, and sheep). While human infections are more commonly associated with contamination with *E. coli* O157:H7 in poorly cooked hamburgers, contamination of drinking water supplies are rare but not unheard of (New York State, 2017). The most notorious *E. coli* O157:H7 drinking water contamination in Canada was the Walkerton, ON outbreak in 2000 (Hrudey and Hrudey, 2004).

2.5.1.2 *Applicability in Risk Assessment*

With pathogens being rarely found in potable water, and if present, are often well below detection limits yet of health concern, annual targets of 10^{-4} (1/10,000) infection risk regardless to whether symptoms occurred or not (U.S. EPA , 2006), and 10^{-6} Disability-Adjusted Life Year (DALY)/person or μDALY/person (Health Canada, 2012), direct detection is generally impractical. Therefore, using indicator organisms is more realistic when it comes to monitoring for faecal contamination.

The most basic test for bacterial contamination of a water supply is to test for total coliforms, which can give a general indication of the sanitary condition of a water supply, and more specifically the efficacy of disinfection (US EPA, 2006). Of the bacteria that make up the total coliform group, *E. coli* is generally the only one not found growing and reproducing in the environment. Therefore, *E. coli* is considered to be the species of coliform bacteria that is the best indicator of faecal pollution and the possible presence of pathogens (New York State, 2017). However, total coliforms are not considered to be a risk to public health and therefore, a health-based risk assessment is not usually relevant unless addressing disinfection efficacy. Risk

assessments, however, are more often undertaken for organisms that impact public health, such as the enteric viruses *Norovirus* and human adenovirus, and the parasitic protozoa *Cryptosporidium* and *Giardia* (Health Canada, 2011, 2012).

2.5.1.3 ***Fate and Transport***

Coliforms being easy to identify, found in large numbers, and behave the same as pathogenic bacteria in the environment, provide a reasonable indication of whether other pathogenic enteric bacteria may be present (New York State, 2017). People swimming/playing in water or working with soil, can be exposed to coliforms through cuts, abrasions and/or mucus membranes. A small sub-set of faecal coliforms may cause symptoms like upset stomach, diarrhea, ear infections, and rashes, all of which are considered minor. Pathogenic *E. coli* on the other hand can lead to severe health effects particularly in infants. Large numbers of faecal coliforms from sewage and manure may also imply contamination with excess nitrogen and phosphorus, leading to eutrophication. After excessive algal growth during eutrophication, bacteria deplete dissolved oxygen thereby affecting aquatic animals, pH shifts and unpleasant visual and odour aspects (Washington State Department of Ecology, 2005).

2.5.2: ***Enterococcus/*enterococci**

2.5.2.1 ***Characteristics and Sources***

Enterococcus spp. are facultative anaerobes that are catalase-negative, Gram-positive cocci (occurring in singles, pairs, or short chains) that grow with 6.5% sodium chloride at 37 °C (PHAC (b), 2017) but can survive temperatures of 60 °C for a short period. Enterococci are part of the normal microbiota of the intestinal tract of both humans and animals, but contain

pathogenic members often associated with urinary tract and female genital tract infections, and less commonly the oral cavity (Fraser, 2017).

2.5.2.2 *Applicability in Risk Assessment*

Enterococci are one of the four most commonly used FIB to describe water quality. They are usually found in high concentrations in human faeces (10^4 to 10^6 per gram weight) allowing for easier detection (Boehm and Sassoubre, 2014). *Enterococcus faecalis* and *Enterococcus faecium* are the most predominant species present in human faeces, and represent more than 90 % of clinical isolates. *E. faecium* is also responsible for the majority of vancomycin-resistant enterococci (VRE) infections (Fraser, 2017).

2.5.2.3 *Fate and Transport*

Enterococci are present in both human and animal faeces, but also in soils and insects associated with plants (Ashbolt *et al*., 2001). Types of sediment as well as light penetration, salinity and temperature have an effect on enterococci (and other bacterial) decay rates and play a major role in fate and transport (Gao *et al*., 2013).

2.5.3 Clostridium perfringens spores

2.5.2.4 *Characteristics and Sources*

C. perfringens is a strictly anaerobic, heat-resistant, spore-forming Gram-positive rod that is further identified by being catalase and superoxide dismutase negative. Some strains are human pathogens due to the production of toxins, types A-E (PHAC(a), 2017). Most notable as a FIB are its spores that may survive for many years in water/sediments/soils (Ashbolt *et al*., 2001). Although not reported to grow in receiving environments, within foods and the gastrointestinal

tract, spores germinate and vegetative cells rapidly grow (Miliotis *et al*., 2013).

2.5.2.5 ***Applicability in Risk Assessment***

C. perfringens is a suitable indicator of faecal contamination as it is the species of clostridia that is most often found in the faeces of humans, with lower concentrations from cattle, dogs and other warm-blooded animals (Ashbolt *et al*., 2001). Nonetheless, *C. perfringens* is only found in approximately 33% of adult and 36% of infant human faeces (Nagpal *et al*., 2015), making it a more useful faecal indicator from sewage networks rather than individual households. The main reason for utilizing *C. perfringens* is that the spores are very persistence in the environment. Therefore, older sewage material which could still be a risk to public health due to persistent human viruses and parasitic protozoa may be detected better using *C. perfringens* analysis over total coliform testing.

2.5.2.6 ***Environmental presence and consequence***

C. perfringens spores are broadly distributed in urbanized environments. While not of concern via water ingestion, food poisoning is associated with *C. perfringens* when a person ingests more than 10^8 vegetative cells which contain enterotoxin that causes the characteristic symptoms of diarrhea and abdominal cramping (University of Minnesota, 2003).

2.5.4 Bacteroides HF183

2.5.2.7 ***Characteristics and Sources***

Bacteroides HF183 is an aerotolerant, non-spore forming, Gram-positive rod that requires anaerobic conditions for growth and is resistant to kanamycin, vancomycin and colistin (Hecht, 2017). It is most useful as a microbial source tracking (MST) marker for human sewage (Shanks *et al.,* 2010), being a human gut microbiome member along with other species in the genus.

Bacteroides spp. are important in many metabolic activities in the human intestine and can even give some protection from invasive organisms.

2.5.2.8 ***Applicability in Risk Assessment***

Fiksdal *et al.* (1985) showed over 30 years ago that, *Bacteroides* spp. were considered a good indicator organism for detecting faecal contamination in water. Not only does the genera fill the criteria described for ideal FIB by Staley *et al*., (1987), but in addition to targeting its genome by qPCR, it has an antigen that can be detected for a lengthy amount of time after it has been exposed to aerobic waters and the organism has died. However, unlike traditional FIB, *Bacteroides* HF183 indicates the presence of human sewage, with a few minor exceptions (Harwood *et al.,* 2014), thereby providing the preferred FIB to sewage-related pathogens from its detection in the environment.

CHAPTER 3: METHODOLOGIES ADDRESSING MAIN BREAK SAMPLE ANALYSIS

3.1: Introduction

According to Besner *et al*. (2013), in order for contaminates to enter the drinking water distribution system (DWDS), three criteria must be met concurrently; sufficient pressure failure, a pathway for the contamination to enter, and contamination to be present outside the failed area of the distribution system. As described in Section 2.1, the majority of Edmonton's main breaks are repaired under local depressurization. With this being the case, it is very likely to meet this criteria allowing for contamination.

Throughout the current study all three criteria requirements were assessed. Local depressurization within the area of the break can occur multiple times during a single repair. After the initial depressurization and excavation, if the exact location of the break is hard to identify, the area is re-pressurized in order to determine where the water is exiting the distribution system. If the excavation was not done in the right location and further excavation was needed, re-pressurizing the system may again be necessary. Post repair, the system was pressurized to confirm that the break was repaired successfully. At this point the repair may not be adequate and water may still exit the system, leading to yet again another local depressurization. Another common occurrence at this point of the repair process when re-pressurization of the system occurs is to have another break happen. This is common because of the stress the pipe is put under during the repair. Vibrations from the excavation and repair process may lead to weakness in the pipe close to where the failure has already occurred. At this point local depressurization is required again and repair of the new break needed. Anytime there

is a breach in the integrity of the DWDS there is potential for contamination. Contamination present outside the failed area of the distribution system can come from many sources.

In this study, specific human faecal bacteria markers (*Bacteroides* HF183), most likely coming from nearby sewer pipe leakage was of highest interest. In Edmonton from January 2009 – August 2016 there were 113 drainage infrastructure damage events recorded. Of these 113 events, 84 were sanitary service or main sewer lines (Appendix 1). These events were assumed to be the main source of human faecal contamination in the vicinity of the distribution system mains.

3.2: **Study Area**

The City of Edmonton is the capital of the Province of Alberta. Edmonton's population started at 148 people in 1878 and following a Municipal census in 2016 was at 899,447 (City of Edmonton (a), 2017). EPCOR is one of the main utility companies in the Edmonton greater region offering power, and both water and wastewater services. EPCOR focuses on making Edmonton's drinking water safe, starting right from the source water which comes from the North Saskatchewan River that flows through the heart of the city (EPCOR, 2017). Source river water is withdrawn for two water treatment plants and distributed to customers, with EPCOR being responsible for the whole drinking water system (over 3,800 km) and associated monitoring programs (EPCOR, 2017). Edmonton's DWDS was the focus of this study and incorporates all of the sample locations including the positive and negative control sites. Given the sporadic occurrence of main breaks and repairs, the sample sites were unpredictable, with ten sample locations finally chosen based on when and where they occurred and the availability of sampling support during the duration of the Masters research. Sampling locations, including the negative

and positive controls, as well as EPCORs two Water Treatment Plants (WTP) and twelve Reservoirs are illustrated in Figure 3-1.

Sample locations were in a cluster relatively central in the city (Table 3-1/Figure 3-1). The City of Edmonton covers some 699.8 km^2 with the ten sample locations located within an approximate 84 km^2 section (City of Edmonton (b), 2017). The sampled locations reflect the higher frequency of DWDS breaks from older infrastructure as well nearby heavy loads of traffic, especially bus routes, and construction causing vibrations in the ground that can lead to main breaks.

The positive control site (sewage-impacted) was selected through unforeseen circumstances of being at a regular sample point location where the sewer line integrity was breached during the watermain break repair process. Due to the nature of the situation only molecular methods were utilized to examine soil samples. A cast iron water main renewal site that had no previous breaks in the pipe itself or surrounding utilities was used as a negative control; again only molecular methods were used. This negative control site was considered undisturbed since the original construction of the water main, approximately 60 years ago (UIS Production, 2017), and no treated water or wastewater had come into contact with the soil surrounding the main according to current EPCOR records.

Table 3-1 Locations of Sampling Sites in Edmonton, AB

Site	Date	Address	Number of Services/People Affected	Pipe Type	Length (m) of Pipe	Volume (L) of water in pipe
1	18-January-2016	163 Street & 110 Avenue (H5327)	17/44.2	6 inch Cast Iron	113.0	8.24
2	27-January-2016	104 Avenue & 148 Street (H1416)	4/10.4*	6 inch Cast iron	20.1	1.47
3	31-January-2016	106 Avenue & 82 Street (H1626)	21/54.6	6 inch Cast iron	102.4	7.47
4	15-February-2016	85 Street & 130 Avenue (H3547)	23/59.8	10 inch Cast iron	152.4	30.87
5	10-March-2016	86 Avenue & 91 Street (H886)	3 (large lots, not residential)	6 inch Cast iron	177.6	12.95
6	14-March-2016	90 Street & 124 Avenue (H587)	5/13.0	8 inch Cast iron	104.5	13.55
7	14-March-2016	121 Avenue & 49 Street (H4333)	20/52.0	6 inch Cast iron	180.9	13.19
8	23-March-2016	38 Street & 118 Avenue (H4312)	12/31.2	10 inch Cast iron	229.5	46.49
9	30-March-2016	89 Street & 118 Avenue (H2356)	2/5.2	Transmission main and 6 inch Cast iron (break at joint between the 2 pipes)	251.7	N/A
10	31-March-2016	67 Street & 107 Avenue (H2913)	No data available	6 inch Cast iron	No data available	N/A
+ Control	31-March-2016	67 Street & 107 Avenue	N/A	Sewer line broke during repair	N/A	N/A
- Control	2-September-2015	102 Avenue & 135 Street	N/A	Cast iron renewal (pipe had no mainbreaks)	N/A	N/A

*Assumption of four services because in a commercial non-residential area.
People affected are based on the 2011 Stats Canada census of 2.6 people per household on average.
H - Hydrant where initial and final flush samples were collected.

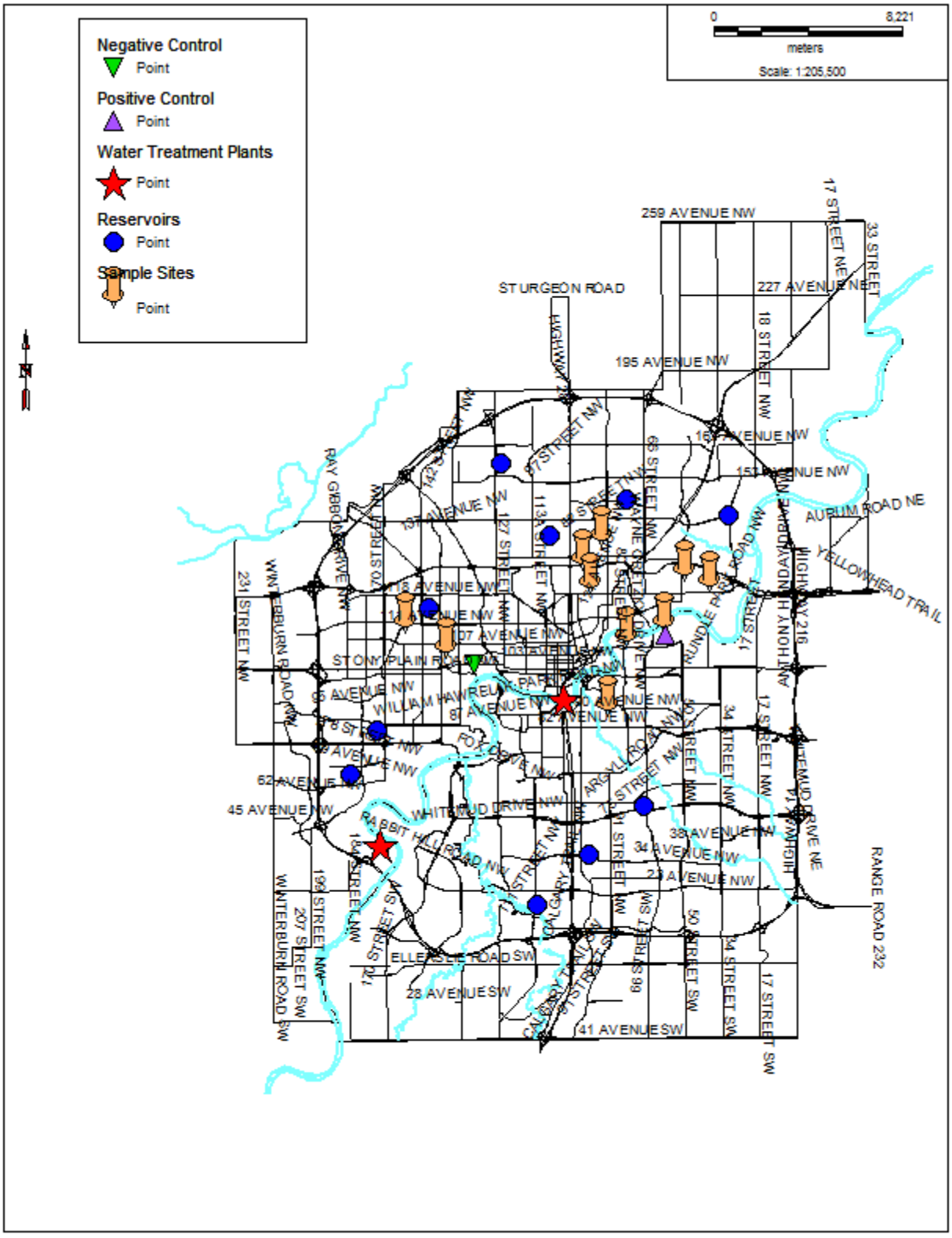

Figure 3-1 Map of Main Break Sampling Sites in Edmonton, AB

3.3: **Sampling**

Water and soil samples were collected from each of the ten sample sites identified in Figure 3-1 between January 18, 2016 and until March 31, 2016.

3.3.1: ***Soil Samples***

Five soil samples, approximately 100 g each, were aseptically collected into pristine zipper bags by folding inside out, roughly every 60 cm down from top layer of soil until beside the pipe undergoing repair (Figure 3-2). Positive and negative control sites were sampled as just described with the exception of soil samples only being available at the top layer of soil and beside the pipe (#1 and #5 of Figure 3-2, respectively).Also note that the positive control sample area was without drinking water supply and sewage flow for approximately five hours prior to excavation, leaving only a small amount of liquid leaking out of the sewer into the soil.

3.3.2: ***Water Samples***

Two water samples (10 L) were collected into sterile plastic carboys, one during the initial post repair flush and one during the final post repair flush. The initial post repair flush sample was collected based on the repair crew's experience of when stagnant water in the closest hydrant is flushed and initial water passing through the break/repair zone began to flow out of the hydrant. The final post repair sample was taken when chlorine and turbidity field kits passed acceptable criteria, total chlorine residual greater than 1.0 mg/L and turbidity less than 3.0 NTU, based on EPCOR Water quality standards.

Figure 3-2 Example soil sample collection Sites 1-5

3.4: Sample Analyses

All samples were stored in a refrigerator that was monitored daily and kept within 4-6 °C, and were analysed within 24 hours of collection.

3.4.1: *Soil Samples*

The 100 g soil samples were mixed thoroughly within the zipper bag until assumed homogenous then 0.250 g sub-samples were taken in triplicate and processed. For molecular analyses, DNA was extracted using PowerSoil® DNA Isolation Kit (Mo-Bio™) as per the manufacturer's protocol (Appendix B).

3.4.2: *Initial/Final Flush Water Samples*

The stored 10 L water samples were shaken vigorously for 30 seconds, then sub-samples filtered through sterile 0.45 µm filters (Millipore Corporation, Bedford, MA 01730). The actual volume filtered was unique to each sample depending on turbidity, ranging from 100 ml to 4500 mL. The 0.45 µm filters were then used for both molecular and culture based methods.

3.4.3: *DNA Extraction and (qPCR) Analysis of water samples*

The 0.45 µm filters used to concentrate target bacteria were rolled to fit into PowerBead tubes of the PowerSoil® DNA Isolation Kit (Mo-Bio™) using sterile forceps and processed the same as soil using the manufacturer's instructions (Appendix B). Extracted DNA was assayed by quantitative polymerase chain reaction (qPCR) using a Rotor-Gene Q thermal cycle (Q-series, Qiagen) using primers targeting *E. coli* (*uidA* gene primers), *Enterococcus* spp. (Entero1 gene primer), and *Bacteroides* (HF183 gene primers), TaqMan probes (supplied by Life Technologies), and cycler conditions described in Tables 3-2 and 3-3 respectively. PCR inhibition was evaluated using 5 µL of Internal Amplification Control (IAC) that was added to the sample DNA before running qPCR and multiplexed with HF183. All assays were directly quantitated using a standard curve consisting of serial dilutions (50,000 – 5 copies/reaction) of the plasmid (produced in house by Neumann Lab, UofA using TOPO® TA Cloning® Kit for Sequencing) containing the targeted sequences.

Table 3-2 DNA primers and TaqMan probes for faecal indicators

Primers	**Sequences**			**References**
*uid*A-F *uid*A-R	CGCAAGGTG CAC GGG AAT A CAGGCACAGCACATCAAAGAGA			Neumann Lab, UofA. 2012
Entero1-F Entero1-R	GAGAAATTCCAAACGAACTTG CAGTGCTCTACCTCCATCATT			US EPA, 2012
HF183-F HF184-R	ATCATGAGTTCACATGTCCG CGTAGGAGTTTGGACCGTGT			Haugland *et al*., 2010
IAC –F IAC-R	CTAACCTTCGTGATGAGCAATCG GATCAGCTACGTGAGGTCCTAC			Deer *et al*., 2012
Clos Perf 1– F Clos Perf 2 - R	AAA TGT AAC AGC AGG GGC A TGA AAT TGC AGC AAC TCT AGC			Karpowicz *et al*., 2009
Probes	**Sequences**	**3'quencher**	**5'dye**	
*uid*A-P	ACC CGA CGC GTC CGA TCA CCT	MGBNFQ	6FAM	Taskin *et a*l., 2011
Entero1-P	TGGTTCTCTCCGAAATAGCTTTAGGGCTA	TAMRA	6FAM	US EPA, 2012
HF183-P	CTGAGAGGAAGGTCCCCCACATTGGA	TAMRA	6FAM	Haugland *et al*., 2010
IAC-P	AGCTAGTCGATGCACTCCAGTCCTCCT	MGBNFQ	VIC	Deer *et al*., 2012
Cpn_60 - P	ATG TCT TCT TTT CCA TTT ACA GGC TTA GAA	BHQ®1	6FAM	Karpowicz *et al*., 2009

Table 3-3 qPCR Rotor-Gene Q thermal cycle profiles

Parameter	**UNG Incubation**	**Polymerase Activation**	**PCR (40 cycles)**	
	Hold	Hold	Denature	Anneal/extend
Temp. (°C)	50	95	95	60
Time (mm:ss)	02:00	00:30	00:03	00:30

Protocol adapted from: Applied Biosystems TaqMan® Fast Advanced Master Mix Product Insert. Polymerase Activation time was altered from 00:20 sec. to 00:30 sec.

3.4.3.1 *DNA Extraction Efficiency*

Membrane Filtration Samples

E. coli was used as the indicator bacteria to determine DNA extraction efficiency for membrane filtration samples identifying its *uid*A gene in duplicate. Overall filtered sample extraction efficiency was estimated at 25% as follows:

E. coli stock (boiled cells) = (292264 + 294303) / 2 = 293283.5 copies *uid*A/ 5µL

E. coli filter-collected stock = (73416 + 73339) / 2 = 73377.5 copies *uid*A / 5µL

(73377.5 / 293283.5) x 100 = 25.02% extraction efficiency

Soil Samples

Raw influent from the Gold Bar WWTP was used to determine DNA extraction efficiency for soil samples identifying the *Bacteroides* HF183 gene, at about 50 % as follows:

Gold Bar raw influent solution boiled = (8716 + 9774) / 2 = 9245 copies HF183 / 5 µL

Gold Bar raw influent solution DNA extracted = (4675 + 4484) / 2 = 4579.5 copies HF183 / 5µL

(4579.5 / 9245) x 100 = 49.53 % extraction efficiency

3.4.4: ***Culture of Faecal Indicators***

The filters used for culture based methods were analyzed using selective agars or following suspension of bacteria in liquid, in a most probable number (MPN) format. Enterococci were estimated as colony-forming units (CFU) by incubating membrane filters on mEI agar (Difco™) using standard amendments (nalidixic acid, µg/L, triphenyltetrazolium chloride, µg/L) and adjusted to pH 7.1 ± 0.2 using 0.1N NaOH at 35±0.5 °C for 24 hours.

Clostridium perfringens spores were estimated by counting CFU on *Clostridium* Agar (Oxoid™) (m-CP**)** plates incubated in anaerobic chambers at 44±1 °C for 24 h, which contained selective supplements (HIMEDIA antibiotic supplement D-Cycloserine),

phenolphthalein diphosphate, ferric chloride hexahydrate and indoxyl-β–glucoside additives as per the manufacturer's instructions.

Total coliforms and *E. coli* MPNs were estimated by using Colilert™ medium as per the manufacturer's instructions using the Quanti-Tray® system (IDEXX, Portland Maine). Membrane filters were first placed into 120 mL IDEXX vessels along with 100 mL of sterile DI water, vortexed for 15 seconds then poured into the Quanti-Trays/2000® (IDEXX).

CHAPTER 4: DECAY RATE OF HUMAN-SPECIFIC *BACTEROIDES* HF183

The experiment was performed in the Laboratory at Gold Bar Wastewater Treatment Plant Edmonton, AB over an eleven day period.

4.5: Soil and Sewage Samples

A 100 g soil sample was collected during a routine main break repair from a site in the Edmonton study area (Figure 3-1) and used for this FIB decay experiment. Four 10 g sub-samples of soil were dispensed into glass vials and amended with sewage or a sterile water control. A raw sewage sample (1 L), collected from the Gold Bar Wastewater Treatment Plant the morning the study commenced, was mixed and 15 mL aliquots added to three vials of soil, with the fourth being a non-sewage control amended with 15 mL of sterile water. All four samples were mixed until homogenous (illustrated in Figure 4-1).

Figure 4-1 Sequence to produce vials with soil-sewage or soil-water, then mixed for HF183 decay study

4.6: Sampling for MST Marker HF183

At the start of the experiment (day 1) and on days 3, 5, 7, 9 and 11 at room temperature, following vigorous vortexing, duplicate soil/sewage aliquots (250 µL) from the glass vials in

Figure 4-1 were pipetted into 2 mL Eppendorf tubes. The original sewage sample and soil/sterile water were also sampled (Figure 4-2). Negative controls consisted of 10 g soil and 15 mL sterile water and the original raw sewage sample not mixed with any soil was used to determine extraction efficacy (refer to section *3.3.3.1*). DNA was extracted using PowerSoil® DNA Isolation Kit (Mo-Bio™) (Appendix B).

Figure 4-2 Arrangement of tubes processed for DNA extraction

DNA was extracted using the PowerSoil® DNA Isolation Kit (Mo-Bio™) (Appendix B), qPCR undertaken as described before for all 48 samples in duplicate, but utilizing the *Bacteroides* HF183 gene primers and probes (Tables 3-2 and 3-3). All assays were directly quantitated using a standard curve consisting of serial dilutions (50,000 – 5 copies/reaction) of plasmid (produced in house by Neumann Lab, UofA using TOPO® TA Cloning® Kit for Sequencing) containing the targeted region.

The decay rate was estimated using a linear regression line from a plot of Log10 HF183 gene copies versus time (days). A logarithmic scale was used to account for skewness and to show multiplicative factors.

4.7: **Results of HF183 soil decay**

The 12 day experiment indicated the slope of the decay rate as -0.0871 and the y-intercept as 4.6535 (raw data can be found in Appendix E), as follows (Figure 4-3):

$y = mx + b$ where: m = slope = -0.087
b = y-intercept = 4.6535

$y = -0.0871\ X + 4.6535$

$0 - 4.6535 = -0.0871\ X$

$$\frac{-4.6535}{-0.0871} = \frac{-0.0871X}{-0.0871}$$

$X = 53.4$

This suggests that after approximately 53.4 days, *Bacteroides* HF183 was no longer detectable by qPCR using the above protocol with Edmonton clay-like soils.

Using raw sewage as the source of the HF183 marker rather than a pure culture in sterile water, the predicted linear decay had a reasonable R^2 value of 62%, but indicating the inherent variability in modeling its decay in soil.

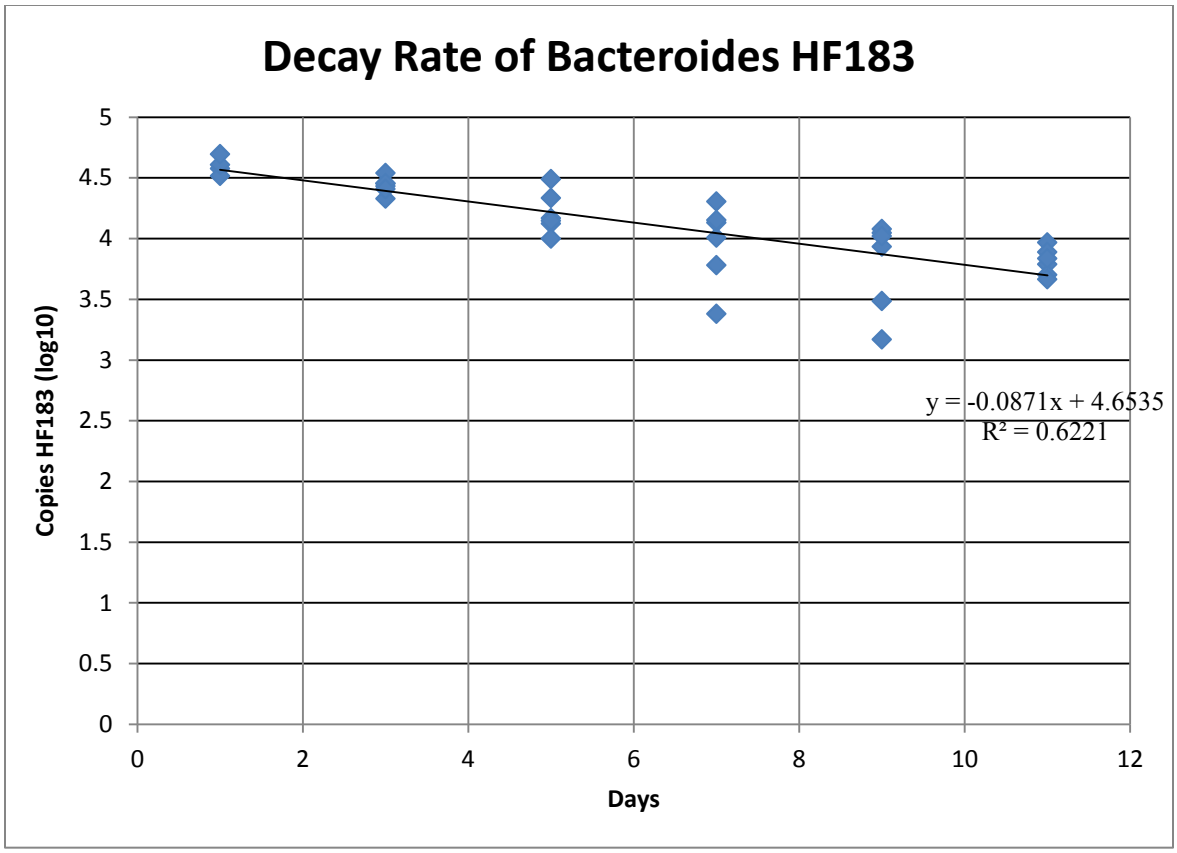

Figure 4-3 Decay rate of human specific Bacteroides HF183 based on a linear regression line from a plot of the log of HF183 gene copies versus time

CHAPTER 5: RESULTS AND DISCUSSION ADDRESSING MAIN BREAK SAMPLE ANALYSIS

5.8: Culture Analysis Results

Results for culture-based analysis, MPN (IDEXX Quanti-Trays/2000®), mEI agar (Difco™) and *Clostridium* Agar (OXOID™), for the ten main break sample sites within the City of Edmonton are presented below in Figures 5-1, 5-2, 5-3 and 5-4. Full data available in Appendix C.

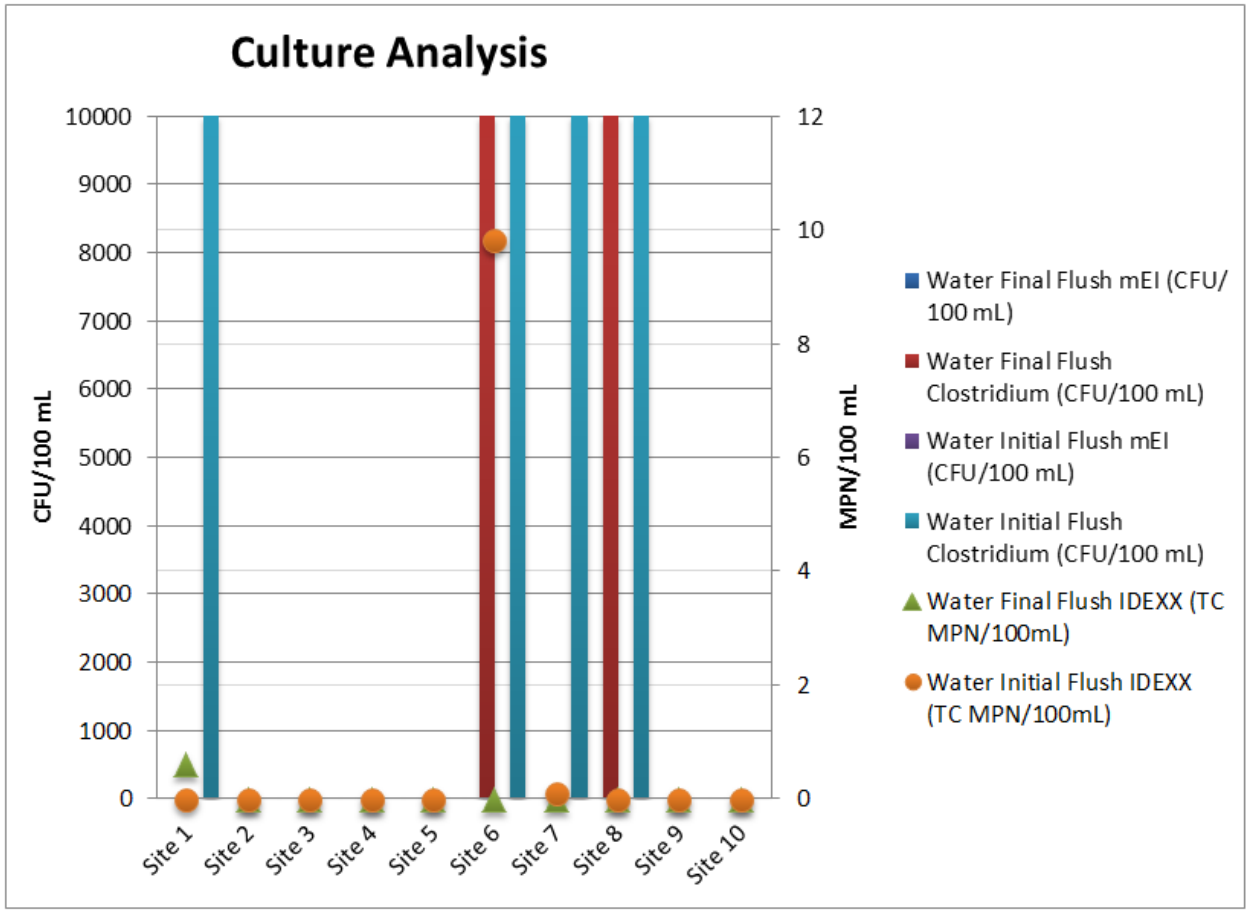

Figure 5-1 MPN (IDEXX Quanti-Trays/2000®), mEI agar (DifcoTM) and *Clostridium* Agar (OXOIDTM) initial and final water sample results from 10 main break sites around Edmonton, AB.

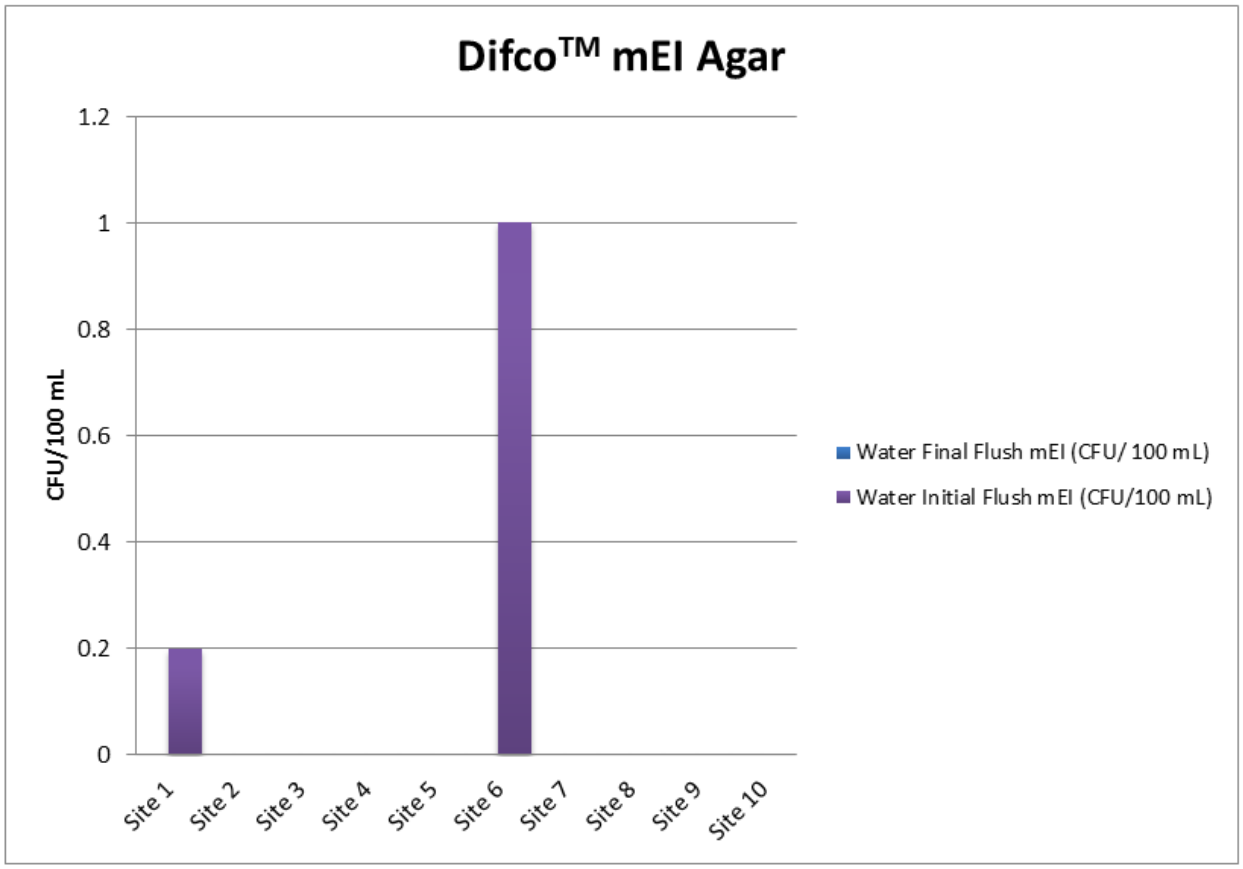

Figure 5-2 Enterococci by mEI agar (DifcoTM) CFU / 100 mL from ten main break sites around Edmonton, AB (detection limit was 1 CFU / 100 mL).

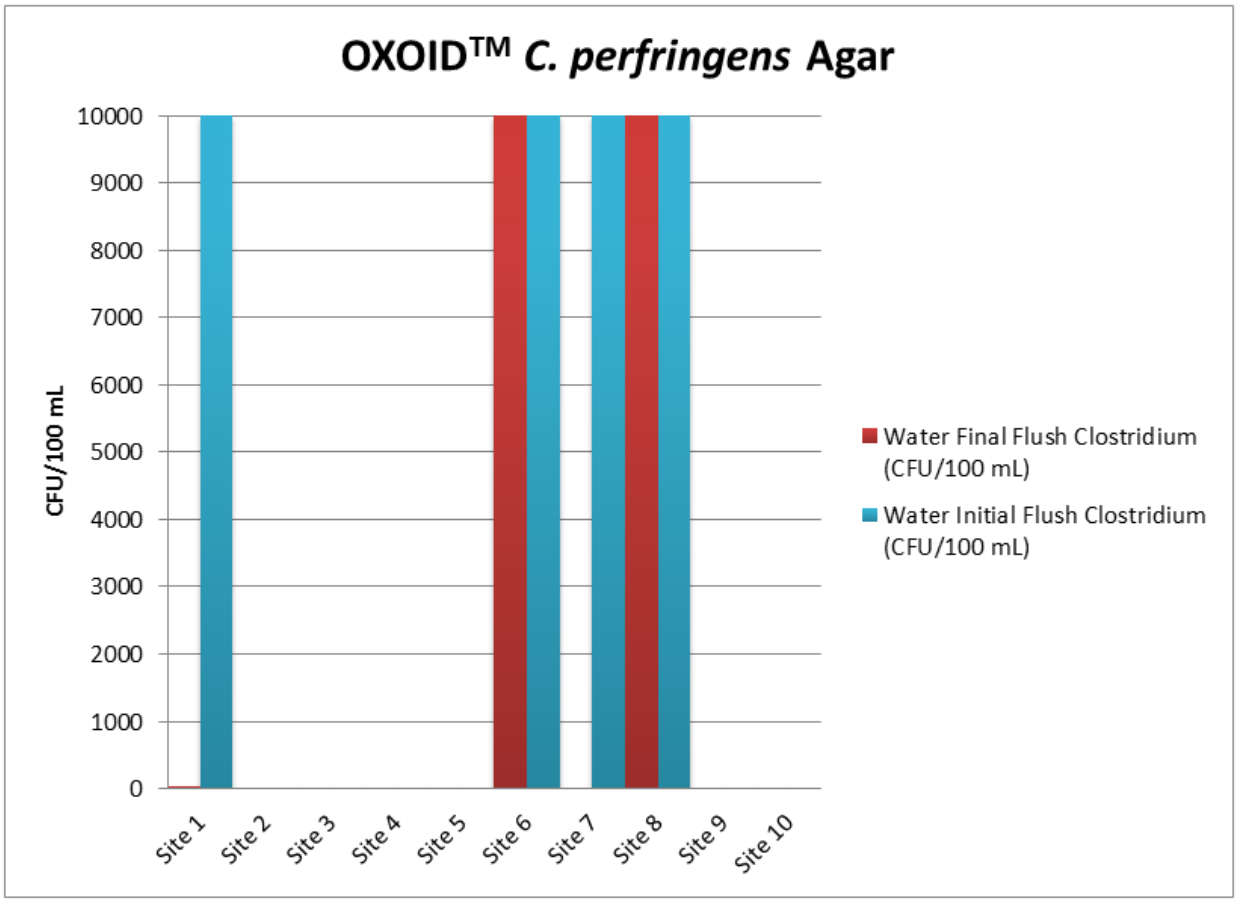

Figure 5-3 *C. perfringens* spore estimations by culture on *Clostridium* Agar (OXOID™) from ten main break sites around Edmonton, AB (detection limit was 1 CFU / 100 mL).

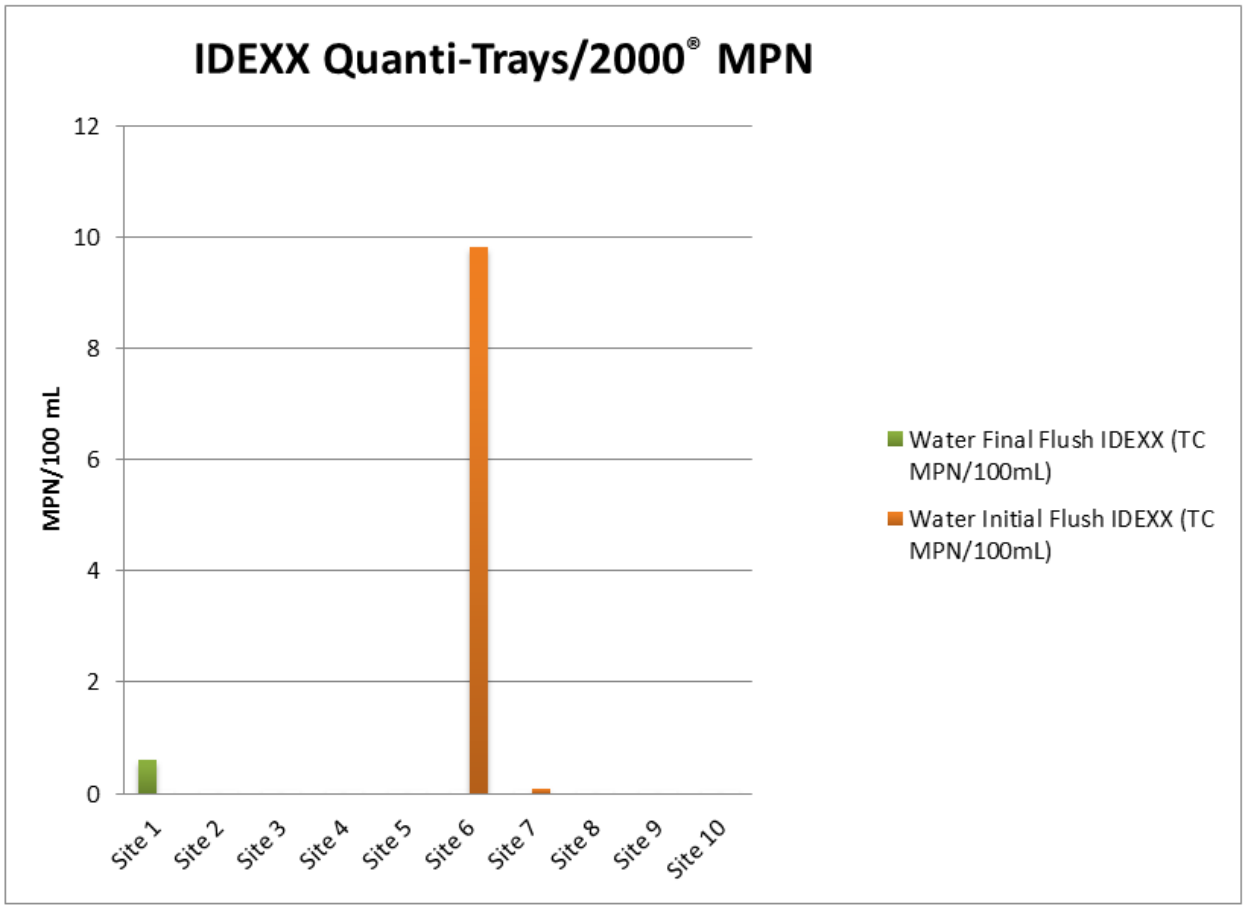

Figure 5-4 Coliform MPN estimates using Colilert (IDEXX Quanti-Trays/2000®) from ten main break sites around Edmonton, AB.

5.9: Summary of Culture Analysis Results

Culture-based methods were only used with the initial and final flush water samples collected at the end of the repair process. The results indicate initial flushes were positive at Site 1 with *Enterococcus* spp., and *C. perfringens* spores, Site 6 was positive with *Enterococcus* spp., *C. perfringens* spores and total coliforms. Sites 7 and 8 were positive for *C. perfringens* spores and all other sites were non-detects for culture-based methods. Of the ten sample sites the initial flushes were positive a total of 40 % of the time with one or more of the relevant indicators of potential faecal contamination. In contrast, final flushes were all negative for *Enterococcus* spp.,

but Site 1 was positive for total coliforms and Sites 6 and 8 were positive for *C. perfringens* spores. Of the ten sample sites the final flushes were positive a total of 30 % of the time with one or more of the relevant indicators of potential fecal contamination (Table 5-1). Positive *Enterococcus* spp., via mEI agar (Difco™) found in the initial and final flushes were deemed detectable but not quantifiable and with total coliforms being so common and as discussed in Section 2.1, rarely resulting in a violation to EPCOR's approval, therefore, neither of these FIB were used in further analysis.

Table 5-1 Positive detects of each indicator bacteria by culture analysis for each main break site sampled

Site	Initial Flush	Final Flush
1	+ *C. perfringens* + Enterococci	+ Total Coliforms
2	<1 CFU/ <1 MPN	<1 CFU/ <1 MPN
3	<1 CFU/ <1 MPN	<1 CFU/ <1 MPN
4	<1 CFU/ <1 MPN	<1 CFU/ <1 MPN
5	<1 CFU/ <1 MPN	<1 CFU/ <1 MPN
6	+ *C. perfringens* + Enterococci + Total Coliforms	+ *C. perfringens*
7	+ *C. perfringens*	<1 CFU/ <1 MPN
8	+ *C. perfringens*	+ *C. perfringens*
9	<1 CFU/ <1 MPN	<1 CFU/ <1 MPN
10	<1 CFU/ <1 MPN	<1 CFU/ <1 MPN

5.10: **Molecular Analysis Results**

Results for molecular-based analysis addressing the ten main break sample sites within the City of Edmonton are presented below for *Enterococcus* spp.*,* human-targeted *Bacteroides* and *E. coli* by qPCR. Figure 5-5 includes all targets and figure 5-6 includes all targets plus the positive and negative controls. Figures 5-7, 5-8 and 5-9 look at the individual targets *uid*A, HF183 and Entero1 respectively. Full data available in Appendix D.

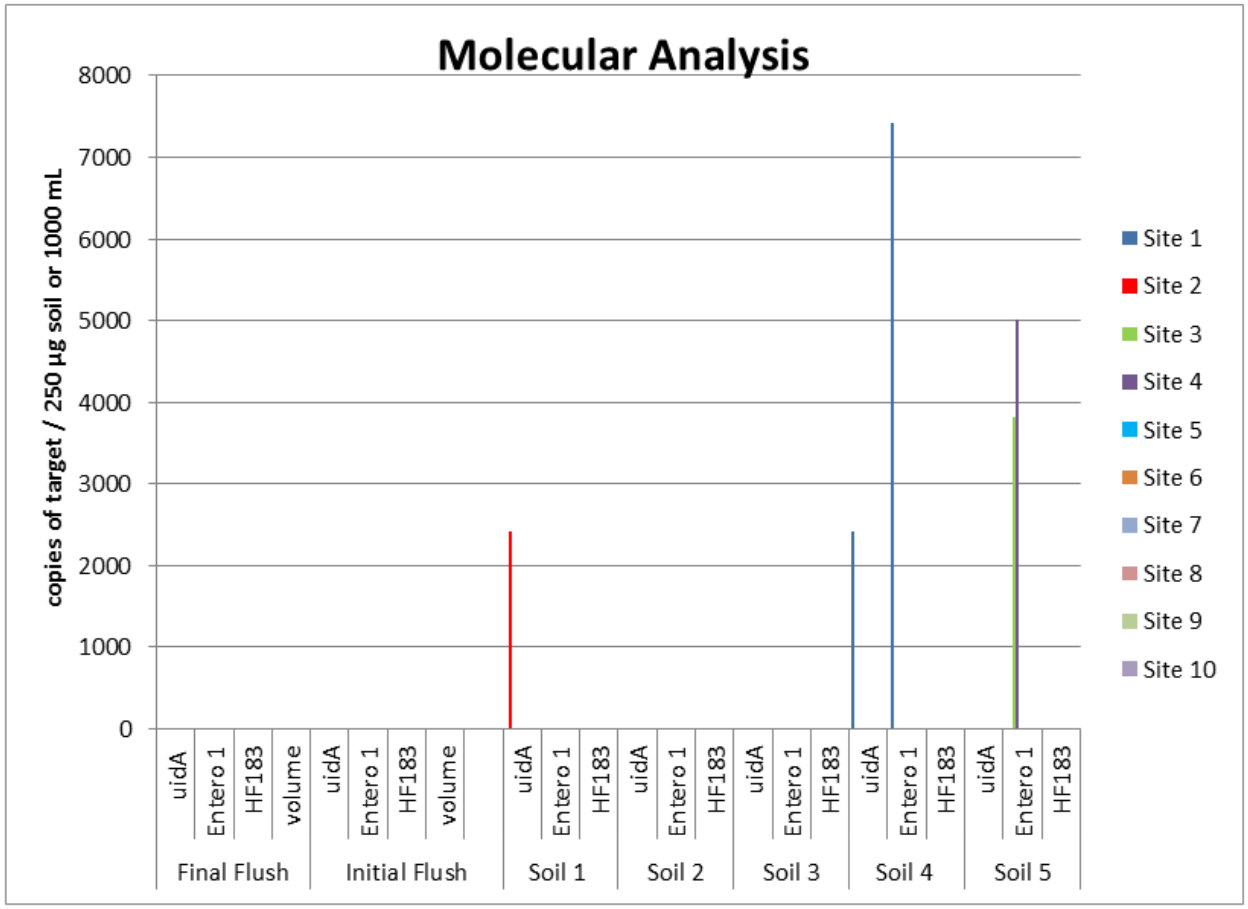

Figure 5-5 *Enterococcus* spp. (Entero1), human-targeted *Bacteroides* (HF183) and *E. coli* (*uid*A) in initial and final flush water and soil sample results from ten main break sites in Edmonton, AB.

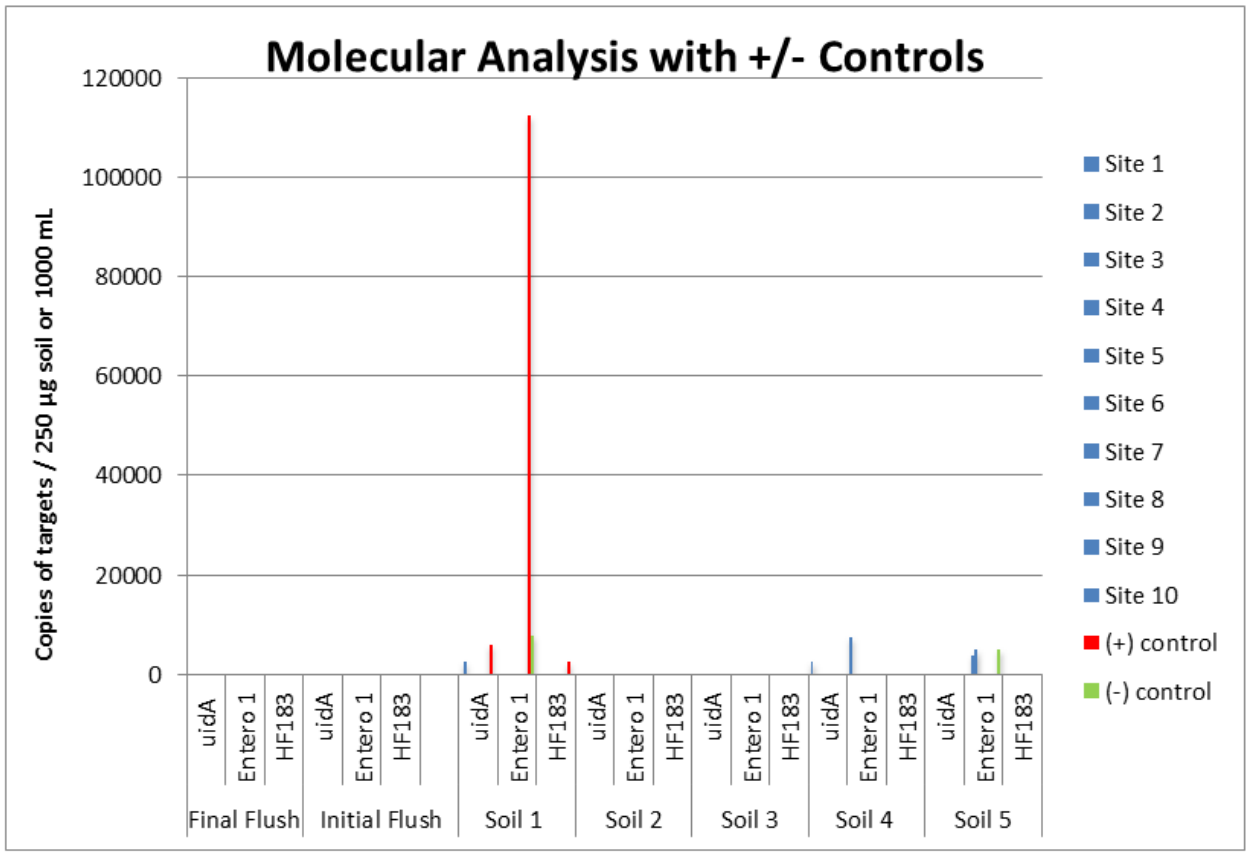

Figure 5-6 *Enterococcus* spp. (Entero1), human-targeted *Bacteroides* (HF183) and *E. coli* (*uid*A) initial and final flush water and soil sample results from ten main break sites in Edmonton, AB including the negative and positive control samples.

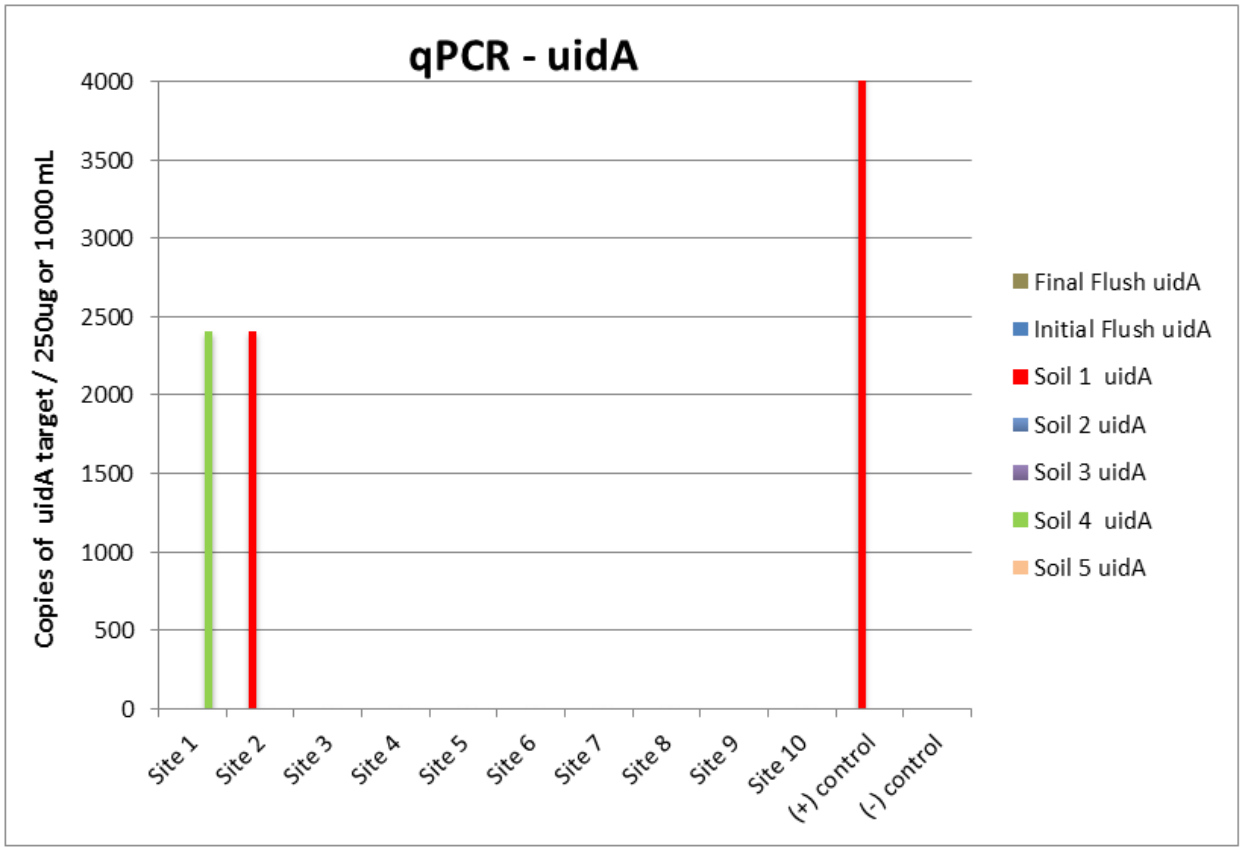

Figure 5-7 *E. coli* (*uid*A) initial and final flush water and soil sample results from ten main break sites in Edmonton, AB.

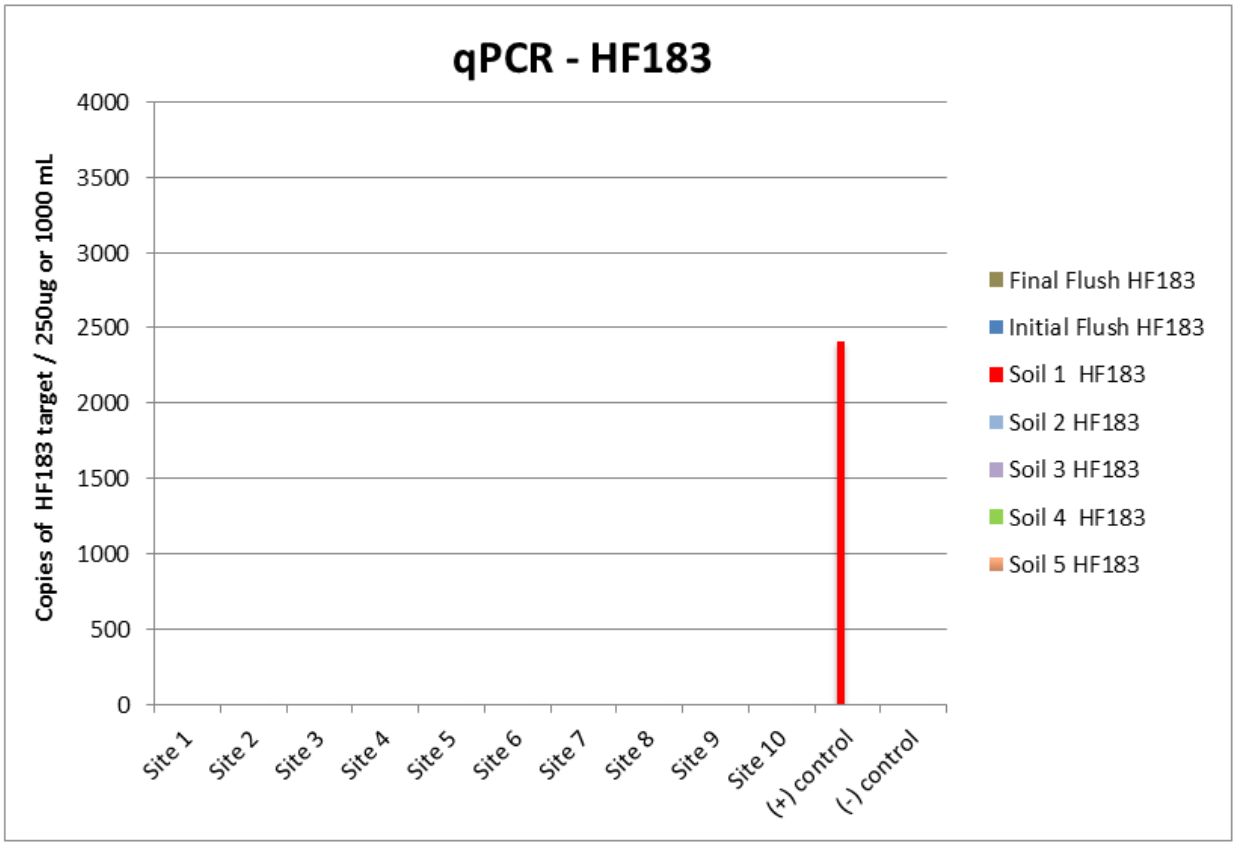

Figure 5-8 *Bacteroides* (HF183) initial and final flush water and soil sample results from ten main break sites in Edmonton, AB.

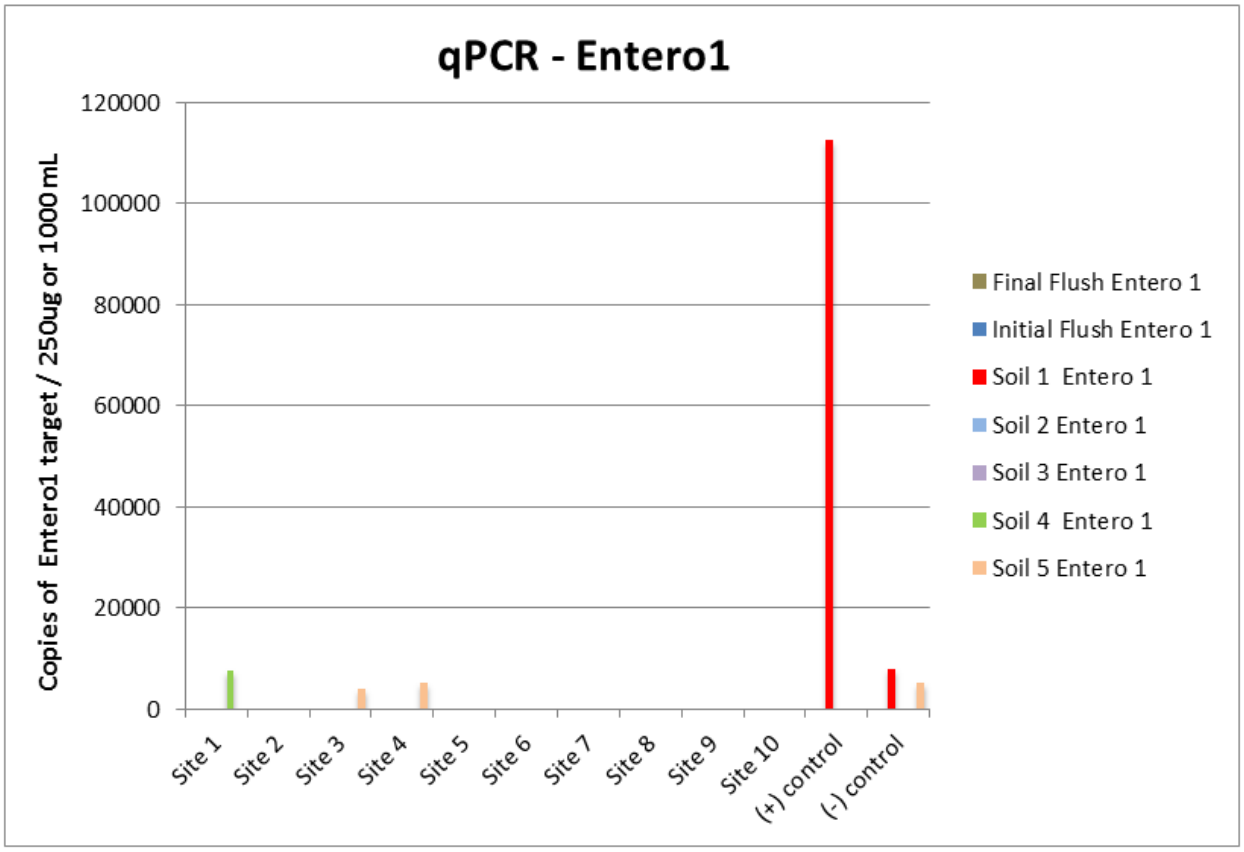

Figure 5-9 *Enterococcus* spp. (Entero1) initial and final flush water and soil sample results from ten main break sites in Edmonton, AB.

5.11: Summary of Molecular Analysis Results

Molecular based methods were run on initial and final flush water samples as well as soil samples collected throughout excavation. The results show that there was no molecular targets of interest detected in any final flush sample across the ten sites. Site 9 was positive for *uid*A in the initial flush sample but was right at the method detection limit and was not used in further analysis. Of the five soil samples collected at each site, Site 1 was positive for *uid*A and Entero1, Site 2 was positive for only *uid*A and Sites 3 and 4 were only positive for Entero1, all other sites

were non-detects for the qPCR method (Table 5-2). Of the four positive sites, three were positive in the lower two layers of soil and one positive in the top layer (Table 5-3). HF183 was non-detectable at all sites in both water and soil samples except for the positive control sewer main break.

The results collected from Chapters 4 & 5 formed the input data to estimate pathogen risks as described in Chapter 6.

Table 5-2 Positive detects of each indicator bacteria by qPCR for each site sampled

Site	Soil (layer)	Initial Flush/ 100 mL	Final Flush/ 100 mL
1	+*uid*A (4) + Entero1 (4)	<35.0 copies	<8.75 copies
2	+*uid*A (1)	<11.7 copies	<8.75 copies
3	+Entero1 (5)	<5.00 copies	<6.36 copies
4	+Entero1 (5)	<8.00 copies	<5.38 copies
5	<200 copies/ 250µg soil	<11.7 copies	<5.83 copies
6	<200 copies/ 250µg soil	<175 copies	<5.00 copies
7	<200 copies/ 250µg soil	<117 copies	<5.00 copies
8	<200 copies/ 250µg soil	<11.7 copies	<5.00 copies
9	<200 copies/ 250µg soil	+ *uid*A	<8.75 copies
10	<200 copies/ 250µg soil	<17.5 copies	<13.46 copies

Table 5-3 Percentage positive results in soil profile (1-5) for *E. coli* (*uid*A), enterococci (Entero1) and sewage (HF183) detected by qPCR

	uidA	Entero1	HF183
Soil 1	10%	0%	0%
Soil 2	0%	0%	0%
Soil 3	0%	0%	0%
Soil 4	10%	10%	0%
Soil 5	0%	20%	0%
Total	**20%**	**30%**	**0%**

CHAPTER 6: QUANTITATIVE MICROBIAL RISK ASSESSMENT

6.1: Problem Formation

Main breaks, as elsewhere, occur on a regular basis in the City of Edmonton, Alberta, Canada. While general faecal indicators are addressed in the federal Guidelines for Canadian Drinking Water Quality, numerous, more persistent pathogens that are not routinely monitored could have negative impacts on public health. Therefore, in this chapter a QMRA model was developed to aid the interpretation of possible health effects and management options using data collected from the ten main breaks investigated. In particular, this chapter addresses: 1) possible gastrointestinal public health risks arising from degradation of potable water quality within the Edmonton distribution system based on the evaluation of ten main breaks; 2) how effective Edmonton's water utility (EPCOR) main break repair standard operating procedures (SOPs) could be in preventing water quality degradation; and 3) identify whether post main break testing results for *Clostridium perfringens*, *Enterococcus* and human-specific *Bacteroides* are informative for reducing public health risk. This risk analysis focused on possible gastrointestinal illness from enteric pathogens associated with ingestion of contaminated tap water post main break repairs within the Edmonton DWDS using ratios determined from concentrations of FIB and reference pathogens in sewage, the main source of public health concern pathogens.

6.2: Exposure Assessment

As discussed above, the risk assessment is focused on exposure scenarios and resulting possible gastrointestinal disease risk via ingesting one liter of tap water assuming no flushing of premise plumbing affected by a main break. The obtained results were used to suggest management measures. The concentration of reference pathogens was based on ranges reported for raw sewage for each FIB and reference pathogen (Table 6-1), so that possible pathogen

concentrations in the soil or flush water could be estimated from the assayed FIB in soil/water, assuming no further die-off around each main break.

Table 6-1 Raw Sewage microbial concentrations used to determine reference pathogens to faecal indicator bacteria ratios

Pathogen/indicator Amount in Raw sewage	Unit /100mL	Concentration (distribution or mean/100 mL)	Value used to determine ratios with FIB	Source
Norovirus GII	Gene copies	N(3.9, 1.4)	7.94×10^2	Schoen *et al*., 2017
Cryptosporidium	Oocysts	U(-0.5, 3.72)	5.25×10^2	Schoen *et al*., 2017
Giardia	Cysts	U(0.5, 4.0)	1.00×10^3	Schoen *et al*., 2017
Campylobacter	MPN	U(2.95, 4.60)	3.98×10^3	Schoen *et al*., 2017
Mastadenovirus	Infectious units	U(1.75, 3.84)	6.92×10^2	Schoen *et al*., 2017
HF183 (a)	Gene copies	5.40×10^8	5.40×10^8	Ahmed *et al*., 2014
HF183 (b)	Gene copies	3.60×10^7	3.60×10^7	Appendix G
***E. coli* (a)**	CFU	1.40×10^6	1.40×10^6	Ahmed *et al*., 2014
***E. coli* (*uid*A) (b)**	Gene copies	1.30×10^6	1.30×10^6	Appendix G
***Enterococcus* (a)**	Gene copies	3.10×10^6	3.10×10^6	Ahmed *et al*., 2014
***Enterococcus* (Entero1) (b)**	Gene copies	1.35×10^7	1.35×10^7	Appendix G
***C. perfringens* (a)**	Spores	6.00×10^4 – 8.00×10^4	7.00×10^4	WHO, 2003(a)
***C. perfringens* (Cpn_60) (b)**	Gene copies	9.68×10^4	9.68×10^4	Appendix G

N – Normal Distribution, U -Uniform distribution.

Soil samples (100 g) were sub-sampled in aliquots of 250 µg, to assay for FIB at each of the ten main break sites (Chapter 5). FIB concentration estimates were then extrapolated to determine the number of FIB associated with 5 g, 50 g and 500 g of the same soil to simulate that level of soil intrusion during a main break/repair (Table 6-2).

Table 6-2 FIB in 250 µg soil samples collected at ten main break sites in Edmonton, AB extrapolated on the assumption of 5, 50 or 500 g of soil intruding during a repair

Site	Indicator qPCR target	Copies/250 µg	Copies/5 g	Copies/50 g	Copies/500 g
1	**uidA**	2.41×10^3	4.82×10^4	4.82×10^5	4.82×10^6
	Entero1	7.42×10^3	1.48×10^5	1.48×10^6	1.48×10^7
2	**uidA**	2.41×10^3	4.82×10^4	4.82×10^5	4.82×10^6
3	**Entero1**	3.81×10^3	7.62×10^4	7.62×10^5	7.62×10^6
4	**Entero1**	5.02×10^3	1.00×10^5	1.00×10^6	1.00×10^7
5	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND
9	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND

ND – non detect

The pipe volume at each of the ten break sites was determined by the length of pipe between the two valves that were shut off to isolate the break and the diameter of the pipe. Tables 6-3 and 6-4 demonstrate how the pipe volumes were used to estimate the actual concentration of FIB inside each isolated pipe section if 5 g, 50 g, or 500 g were to enter the DWDS at each of the ten main break sites.

Table 6-3 Concentrations of *uid*A copies per 1 litre if 5 g, 50 g or 500 g of soil were to enter the DWDS at each main break site in Edmonton, AB

Site	Pipe Radius (m)	Pipe Length (m)	Volume (L)	Concentration of *uid*A (copies/L) if 5 g of soil entered DWDS	Concentration of *uid*A (copies/L) if 50 g of soil entered DWDS	Concentration of *uid*A (copies/L) if 500 g of soil entered DWDS
1	0.15	113	2060	23.4	2.34×10^2	2.34×10^3
2	0.15	20.1	370	130	1.30×10^3	1.30×10^4
3	0.15	102	1870	ND	ND	ND
4	0.25	152	7720	ND	ND	ND
5	0.15	178	3240	ND	ND	ND
6	0.20	105	3390	ND	ND	ND
7	0.15	181	3300	ND	ND	ND
8	0.25	230	1.16×10^4	ND	ND	ND
9	n/a	n/a	n/a	ND	ND	ND
10	n/a	n/a	n/a	ND	ND	ND

ND – non-detect; n/a – data is not available

Table 6-4 Concentrations of Entero1 copies per 1 litre if 5 g, 50 g and 500 g of soil were to enter the DWDS at each main break site in Edmonton, AB

Site	Pipe Diameter (m)	Pipe Length (m)	Volume (L)	Concentration of Entero1 copies/L if 5.0g of soil entered DWDS	Concentration of Entero1 copies/L if 50.0g of soil entered DWDS	Concentration of Entero1 copies/L if 500.0g of soil entered DWDS
1	0.15	113	2060	72.1	721	7.21×10^3
2	0.15	20.1	370	ND	ND	ND
3	0.15	102	1870	40.8	408	4.08×10^3
4	0.25	152	7720	13.0	130	1.30×10^3
5	0.15	178	3240	ND	ND	ND
6	0.20	105	3390	ND	ND	ND
7	0.15	181	3300	ND	ND	ND
8	0.25	230	1.16×10^4	ND	ND	ND
9	n/a	n/a	n/a	ND	ND	ND
10	n/a	n/a	n/a	ND	ND	ND

ND – non-detect; n/a – data is not available

The ratios between the FIB and reference pathogens were determined based on the assumption that the reference pathogens occur in the same ratio to FIB in the water and soil samples as they are reported to occur in raw sewage (Table 6-1). The reference pathogens used include

Norovirus GII, *Cryptosporidium*, *Giardia*, *Campylobacter* and *Mastadenovirus* (i.e. human adenoviruses). The soil and water samples collected post main break repair were quantified using FIB to reference pathogen ratios (Table 6-5).

Table 6-5 Estimated FIB:pathogen ratios assuming raw sewage contamination (Table 6-1)

Indicator	**Pathogen**				
per 100 mL of raw sewage	*Norovirus* GII 7.94×10^2	*Cryptosporidium* 5.25×10^2	*Giardia* 1.00×10^3	*Campylobacter* 3.98×10^3	*Mastadenovirus* 6.92×10^2
C. perfringens (a) 7.00×10^4	88.2:1	133:1	70.0:1	17.6:1	101:1
C. perfringens (Cpn_60) (b) 9.68×10^4	122:1	184:1	96.8:1	24.3:1	140:1
E. coli (a) 1.40×10^6	1760:1	2670:1	1400:1	352:1	2020:1
E. coli (uidA) (b) 1.30×10^6	1640:1	2480:1	1300:1	327:1	1880:1
Enterococcus (a) 3.10×10^6	3900:1	5900:1	3100:1	779:1	4480:1
Enterococcus (Entero1) (b) 1.35×10^7	17000:1	25700:1	13500:1	3390:1	19500:1
HF183 (a) 5.40×10^8	680000:1	1030000:1	540000:1	136000:1	780000:1
HF183 (b) 3.60×10^7	45300:1	68600:1	36000:1	9050:1	52000:1

Norovirus GII units = gene copies; *Giardia* units = cysts; *Cryptosporidium* units = oocysts; *Mastadenovirus* units = infectious units; *Campylobacter* units = MPN; *C. perfringens* (a) units = spores; *C. perfringens* (b) units = gene copies

The reference pathogens reported were the most important of eight pathogens described to account for over 95% of all non-foodborne gastrointestinal illnesses associated with water in the US (Soller *et al.,* 2010). The behaviour of the enteric bacterial pathogens not assayed in the current study (pathogenic *E. coli* and *Salmonella enterica*) and virus (rotavirus) from those used by Soller *et al.* (2010) were assumed to be addressed by chosen reference pathogens.

6.3: **Health Affects Assessment**

Messner *et al.* (2006) estimated that DWDSs could be responsible for millions of cases of acute GI illness in the United States every year, yet there is no clear relationship described by FIB. Although GI illness does not often result in a physician visit, it can cause overall societal costs due to lost work time. In the City of Edmonton, it was assumed that FIB detected within the distribution system would most likely enter via a main break occurring in a sewage impacted site. Sewage impacted sites are an outcome of sewer mains running in close proximity to water mains that have a breach in their integrity, resulting in sewage within the surrounding soil. Some of the FIB of interest, particularly *Clostridium perfringens*, are very stable as they form spores that are environmentally resistant (Ashbolt *et al.*, 2001). Hence, they represent the most conservative estimate of persistent enteric pathogen risks in the scenarios investigated.

As described above, ratios (Table 6-5) were used to estimate possible concentrations of the reference pathogens. These ratios were used to estimate possible doses of reference pathogens based on the estimated FIB detected at the ten main break locations and assuming one litre of water ingestion. Table 6-6 shows the dose-response models used for each reference pathogen in the risk assessment. The models assume no co-infection and that all infections were independent of any other pathogen that could be present. These dose-response models describe the probability of GI infection and infection to DALY factor due to ingestion of each reference pathogen in drinking water associated with a water main break daily across the population of Edmonton. Three different scenarios were considered for each main break site where FIB were detected; being that either 5, 50 or 500 g of soil entered the distribution system during the main break and repair process. Other assumptions made were even mixing of the soil within the affected distribution system, contaminated ‘first flush’ water reached customers service lines and these

were not flushed by households prior to ingestion.

Table 6-6 Dose-Response Models used to calculate probability of infection, conditional probability of illness and Disability Adjusted Life Year (DALY) factor

Reference Pathogen	Dose-Response Parameters	Model	Units	Probability of Infection[1]	Conditional probability of illness[3]	Illness to DALY factor[4]
a:Norovirus GII b:Norovirus GII	P = 0.72 μ = 1106 Messner *et al.,* 2014	Fractional Poisson	Genome copies	$P_{inf}(Dose, P, \mu) = P \times [1 - e^{(-Dose/\mu)}]$	60%	5.0
a:*Cryptosporidium* b:*Cryptosporidium*	r = 0.09 US EPA, 2005.	Exponential	oocysts	$P_{inf}(Dose, r) = 1 - e^{(-Dose \times r)}$	50%	17.0
a:*Giardia* b:*Giardia*	r = 0.0199 Rose *et al*., 1991.	Exponential	cysts	$P_{inf}(Dose, r) = 1 - e^{(-Dose \times r)}$	40%	16.0
a:*Campylobacter* b:*Campylobacter*	α = 0.145 β = 7.589 Madema *et al*., 1996	Beta Poisson	CFU	$P_{inf}(Dose, \alpha, \beta) = 1 - [1 + Dose/\beta]^{(-\alpha)}$	30%	23.5
a:*Mastadenovirus* b:*Mastadenovirus*	r = 0.4172 Rose *et al*., 1996	Exponential	TCID50[2]	$P_{inf}(Dose, r) = 1 - e^{(-Dose \times r)}$	60%	3.3

[1]Probability of infection formulas from Teunis and Havelaar, 2000.
[2] Assumption that $TCID_{50}$ = Infectious units
[3]Conditional probability of illness given infection from Soller et al., 2010
[4] Infection to DALY factor = DALY/10,000 cases from Gibney *et al.,* 2014, except for *Cryptosporidium* (a) from Gaunt *et al*., 2011

6.4: **Risk Characterization**

Each main break location was treated as a separate scenario, using the number of services affected by the break and an average number of people per household to be 2.4 based on Statistics Canada 2016 Census. These results were then generalized across the population of Edmonton, AB, 899,447 (Edmonton Municipal Census, 2016) and assumed to occur once a day somewhere in the DWDS for a year, to estimate the annual risk. One break per day was estimated based on the average number of depressurizations that occurred in Edmonton from 2005-2015 (Table 2-2). The results are all based on worst case scenarios and the assumption that FIB are detectable at every break that occurs once a day for a year. Table 6-7 indicates all

assumptions used during this analysis. Actual results showed FIB to be only detected at 40% of initial flush samples, 30% of final flush samples and 40% of soil samples. The acceptable criteria for each situation was set at 10^{-4} (1/10,000) infection risk regardless to whether symptoms occurred or not, and one micro disability adjusted life-year (μDALY)/ person based on waterborne exposure as the benchmarks used by the U.S. EPA (2006) and Health Canada (2012) respectively.

Tables 6-8 and 6-9 show the probability of infection from the five reference pathogens if someone were to ingest one litre of water from the initial flush and final flush samples post main break repair that were detected to have FIB. The health impacts are shown by infections/10,000 people per year in Edmonton and μDALY/ person per year in Edmonton based on each of these scenarios occurring once per day for 365 days.

Table 6-7 Assumptions used in QMRA Analysis

Assumption	Justification
1 L water ingestion	Consistent with what WHO uses for drinking water scenarios
No flushing of premise plumbing and FIB are present at every main break site	Goal was to determine worst case scenario
5 g, 50 g, and 500 g soil intrusion	Depending on the type of break, a small fissure, orifice or a replacement of a section of pipe, will allow or not allow different amounts of soil intrusion
1 main break a day for 1 year	Edmonton, AB has an average of approximately 400 main breaks a year, for simplicity one main break per day was assigned
Even mixing in the distribution system after soil intrusion	For this study there was no method to determine how much mixing occurred in the DWDS once soil intrusion occurred

Table 6-8 Probability of infection and health impact of ingestion of 1 L of water containing reference pathogens based on ratios determined from indicator bacteria *C. perfringens* in water collected during the initial flush post main break repair

Site	Indicator	Initial Flush (CFU/ L)	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of people infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
1	*C. perfringens*	100000	a:*Norovirus* GII	1.13×10^3	0.46	44	20	0.60	82.8	0.50	0.02	2.48
			b:*Norovirus* GII	2.62×10^3	0.65		29		117		0.04	3.51
			a:*Cryptosporidium*	750	1.00		44	0.50	179	1.70	0.15	15.3
			b:*Cryptosporidium*	1.73×10^3	1.00		44		179		0.15	15.3
			a:*Giardia*	1.43×10^3	1.00		44	0.45	179	1.60	0.13	12.9
			b:*Giardia*	3.30×10^3	1.00		44		179		0.13	12.9
			a:*Campylobacter*	5.69×10^3	0.62		27	0.30	111	23.5	0.78	78.0
			b:*Campylobacter*	1.31×10^4	0.66		29		119		0.84	83.6
			a:*Mastadenovirus*	989	1.00		44	0.60	179	3.30	0.36	35.5
			b:*Mastadenovirus*	2.28×10^3	1.00		44		179		0.36	35.5
2	ND											
3	ND											
4	ND											
5	ND											
6	*C. perfringens*	100000	a:*Norovirus* GII	1.13×10^3	0.46	13	6.0	0.60	24.4	0.50	0.01	0.73
			b:*Norovirus* GII	2.62×10^3	0.65		8.5		34.4		0.01	1.03
			a:*Cryptosporidium*	750	1.00		13	0.50	52.8	1.70	0.04	4.48
			b:*Cryptosporidium*	1.73×10^3	1.00		13		52.8		0.04	4.48
			a:*Giardia*	1.43×10^3	1.00		13	0.45	52.8	1.60	0.04	3.80
			b:*Giardia*	3.30×10^3	1.00		13		52.8		0.04	3.80
			a:*Campylobacter*	5.69×10^3	0.62		8.0	0.30	32.6	23.5	0.23	23.0
			b:*Campylobacter*	1.31×10^4	0.66		8.6		34.9		0.25	24.6
			a:*Mastadenovirus*	989	1.00		13	0.60	52.8	3.30	0.10	10.5

Site	Indicator	Initial Flush (CFU/ L)	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of people infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			b:*Mastadenovirus*	2.28×10^3	1.00		13		52.8		0.10	10.5
7	*C. perfringens*	100000	a:*Norovirus* GII	1.13×10^3	0.46	52	24	0.60	97.5	0.50	0.03	2.92
			b:*Norovirus* GII	2.62×10^3	0.65		34		137		0.04	4.13
			a:*Cryptosporidium*	750	1.00		52	0.50	211	1.70	0.18	17.9
			b:*Cryptosporidium*	1.73×10^3	1.00		52		211		0.18	17.9
			a:*Giardia*	1.43×10^3	1.00		52	0.45	211	1.60	0.15	15.2
			b:*Giardia*	3.30×10^3	1.00		52		211		0.15	15.2
			a:*Campylobacter*	5.69×10^3	0.62		32	0.30	130	23.5	0.92	91.8
			b:*Campylobacter*	1.31×10^4	0.66		34		139		0.98	98.3
			a:*Mastadenovirus*	989	1.00		52	0.60	211	3.30	0.42	41.8
			b:*Mastadenovirus*	2.28×10^3	1.00		52		211		0.42	41.8
8	*C. perfringens*	100000	a:*Norovirus* GII	1.13×10^3	0.46	31	14	0.60	58.5	0.50	0.02	1.75
			b:*Norovirus* GII	2.62×10^3	0.65		20		82.6		0.02	2.48
			a:*Cryptosporidium*	750	1.00		31	0.50	127	1.70	0.11	10.8
			b:*Cryptosporidium*	1.73×10^3	1.00		31		127		0.11	10.8
			a:*Giardia*	1.43×10^3	1.00		31	0.45	127	1.60	0.09	9.12
			b:*Giardia*	3.30×10^3	1.00		31		127		0.09	9.12
			a:*Campylobacter*	5.69×10^3	0.62		19	0.30	78.1	23.5	0.55	55.1
			b:*Campylobacter*	1.31×10^4	0.66		21		83.7		0.59	59.0
			a:*Mastadenovirus*	989	1.00		31	0.60	127	3.30	0.25	25.1
			b:*Mastadenovirus*	2.28×10^3	1.00		31		127		0.25	25.1
9	ND											
10	ND											

ND – non-detect; Benchmarks

Norovirus GII units = gene copies; *Giardia* units = cysts; *Cryptosporidium* units = oocysts; *Mastadenovirus* units = infectious units; *Campylobacter* units = MPN; *C. perfringens* (a) units = spores; *C. perfringens* (b) units = gene copies

Table 6-9 Probability of infection and health impact of ingestion of 1 L of water containing reference pathogens based on ratios determined from faecal indicator bacteria *C. perfringens* in water collected during the final flush post main break repair

Site	Indicator	Final Flush (CFU/ L)	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of people infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
1	ND											
2	ND											
3	ND											
4	ND											
5	ND											
6	*C. perfringens*	100000	a:*Norovirus* GII	1.13×10^3	0.46	13	6.0	0.60	24.4	0.50	0.01	0.73
			b:*Norovirus* GII	2.62×10^3	0.65		8.5	0.60	34.4		0.01	1.03
			a:*Cryptosporidium*	750	1.00		13	0.50	52.8	1.70	0.04	4.48
			b:*Cryptosporidium*	1.73×10^3	1.00		13	0.50	52.8		0.04	4.48
			a:*Giardia*	1.43×10^3	1.00		13	0.45	52.8	1.60	0.04	3.80
			b:*Giardia*	3.30×10^3	1.00		13	0.45	52.8		0.04	3.80
			a:*Campylobacter*	5.69×10^3	0.62		8.0	0.30	32.6	23.5	0.23	23.0
			b:*Campylobacter*	1.31×10^4	0.66		8.6	0.30	34.9		0.25	24.6
			a:*Mastadenovirus*	989	1.00		13	0.60	52.8	3.30	0.10	10.5
			b:*Mastadenovirus*	2.28×10^3	1.00		13	0.60	52.8		0.10	10.5
7	ND											
8	ND	100000	a:*Norovirus* GII	1.13×10^3	0.46	31	14	0.60	58.5	0.50	0.02	1.75
			b:*Norovirus* GII	2.62×10^3	0.65		20	0.60	82.6		0.02	2.48
			a:*Cryptosporidium*	750	1.00		31	0.50	127	1.70	0.11	10.8
			b:*Cryptosporidium*	1.73×10^3	1.00		31	0.50	127		0.11	10.8
			a:*Giardia*	1.43×10^3	1.00		31	0.45	127	1.60	0.09	9.12
			b:*Giardia*	3.30×10^3	1.00		31	0.45	127		0.09	9.12
			a:*Campylobacter*	5.69×10^3	0.62		19	0.30	78.1	23.5	0.55	55.1

Site	Indicator	Final Flush (CFU/ L)	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of people infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			b:*Campylobacter*	1.31×10^4	0.66		20.62	0.30	83.7		0.59	59.0
			a:*Mastadenovirus*	989	1.00		31.20	0.60	127	3.30	0.25	25.1
			b:*Mastadenovirus*	2.28×10^3	1.00		31.20	0.60	127		0.25	25.1
9	ND											
10	ND											
ND – non-detect;		Benchmarks										

Norovirus GII units = gene copies; *Giardia* units = cysts; *Cryptosporidium* units = oocysts; *Mastadenovirus* units = infectious units; *Campylobacter* units = MPN; *C. perfringens* (a) units = spores; *C. perfringens* (b) units = gene copies

Tables 6-10, 6-11 and 6-12 show the probability of infection from the five reference pathogens if someone were to ingest one liter of water from three different scenarios where 5-500 g of contaminated soil entered the DWDS during the four main breaks that were detected to have FIB. The health impacts are shown by infections/10,000 people per year in Edmonton and µDALY/ person per year in Edmonton based on each of these scenarios occurring once per day for 365 days.

Table 6-10 Probability of infection and health impact of ingestion of 1 L of water containing reference pathogens based on ratios determined from faecal indicator bacteria assuming 5 g of soil entered the DWDS during each main break

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
1	*E. coli* (*uid*A)	5 g	a:*Norovirus* GII	0.13	0.00	44	0.00	0.60	0.02	0.50	0.00	0.00
			b:*Norovirus* GII	0.14	0.00		0.00		0.02		0.00	0.00
			a:*Cryptosporidium*	0.09	0.01		0.35	0.50	1.41	1.70	0.00	0.12
			b:*Cryptosporidium*	0.09	0.01		0.37		1.52		0.00	0.13
			a:*Giardia*	0.17	0.00		0.15	0.45	0.60	1.60	0.00	0.04
			b:*Giardia*	0.18	0.00		0.16		0.64		0.00	0.05
			a:*Campylobacter*	0.66	0.01		0.53	0.30	2.17	23.5	0.02	1.53
			b:*Campylobacter*	0.72	0.01		0.57		2.33		0.02	1.64
			a:*Mastadenovirus*	0.12	0.05		2.1	0.60	8.44	3.30	0.02	1.67
			b:*Mastadenovirus*	0.12	0.05		2.2		9.08		0.02	1.80
	Enterococcus (Entero 1)		a:*Norovirus* GII	0.18	0.00		0.01	0.60	0.02	0.50	0.00	0.00
			b:*Norovirus* GII	0.04	0.00		0.00		0.00		0.00	0.00
			a:*Cryptosporidium*	0.12	0.01		0.47	0.50	1.93	1.70	0.00	0.16
			b:*Cryptosporidium*	0.03	0.00		0.12		0.48		0.00	0.04
			a:*Giardia*	0.23	0.00		0.20	0.45	0.82	1.60	0.00	0.06
			b:*Giardia*	0.05	0.00		0.04		0.18		0.00	0.01
			a:*Campylobacter*	0.93	0.02		0.73	0.30	2.98	23.5	0.02	2.10
			b:*Campylobacter*	0.21	0.00		0.17		0.71		0.00	0.50
			a:*Mastadenovirus*	0.16	0.06		2.9	0.60	11.6	3.30	0.02	2.29
			b:*Mastadenovirus*	0.04	0.02		0.73		2.97		0.01	0.59
2	*E. coli* (*uid*A)	5 g	a:*Norovirus* GII	0.74	0.00	10	0.00	0.60	0.02	0.50	0.00	0.00
			b:*Norovirus* GII	0.79	0.00		0.01		0.02		0.00	0.00

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			a:*Cryptosporidium*	0.49	0.04		0.45	0.50	1.81	1.70	0.00	0.15
			b:*Cryptosporidium*	0.53	0.05		0.48		1.95		0.00	0.17
			a:*Giardia*	0.93	0.02		0.19	0.45	0.77	1.60	0.00	0.06
			b:*Giardia*	1.00	0.02		0.21		0.83		0.00	0.06
			a:*Campylobacter*	3.70	0.06		0.58	0.30	2.36	23.5	0.02	1.66
			b:*Campylobacter*	3.98	0.06		0.62		2.51		0.02	1.77
			a:*Mastadenovirus*	0.64	0.24		2.5	0.60	9.93	3.30	0.02	1.97
			b:*Mastadenovirus*	0.69	0.25		2.6		10.59		0.02	2.10
3	Enterococcus (Entero 1)	5 g	a:*Norovirus* GII	0.10	0.00	55	0.00	0.60	0.01	0.50	0.00	0.00
			b:*Norovirus* GII	0.02	0.00		0.00		0.00		0.00	0.00
			a:*Cryptosporidium*	0.07	0.01		0.34	0.50	1.39	1.70	0.00	0.12
			b:*Cryptosporidium*	0.02	0.00		0.10		0.40		0.00	0.03
			a:*Giardia*	0.13	0.00		0.14	0.45	0.57	1.60	0.00	0.04
			b:*Giardia*	0.03	0.00		0.03		0.13		0.00	0.01
			a:*Campylobacter*	0.52	0.01		0.52	0.30	2.12	23.5	0.01	1.49
			b:*Campylobacter*	0.12	0.00		0.12		0.50		0.00	0.35
			a:*Mastadenovirus*	0.09	0.04		2.0	0.60	8.17	3.30	0.02	1.62
			b:*Mastadenovirus*	0.02	0.01		0.45		1.84		0.00	0.36
4	Enterococcus (Entero 1)	5 g	a:*Norovirus* GII	0.03	0.00	59	0.00	0.60	0.00	0.50	0.00	0.00
			b:*Norovirus* GII	0.01	0.00		0.00		0.00		0.00	0.00
			a:*Cryptosporidium*	0.02	0.00		0.11	0.50	0.43	1.70	0.00	0.04
			b:*Cryptosporidium*	0.01	0.00		0.05		0.22		0.00	0.02
			a:*Giardia*	0.04	0.00		0.05	0.45	0.19	1.60	0.00	0.01
			b:*Giardia*	0.01	0.00		0.01		0.05		0.00	0.00
			a:*Campylobacter*	0.17	0.00		0.19	0.30	0.77	23.5	0.01	0.54

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/ 1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			b:*Campylobacter*	0.04	0.00		0.04		0.18		0.00	0.13
			a:*Mastadenovirus*	0.03	0.01		0.73	0.60	2.97	3.30	0.01	0.59
			b:*Mastadenovirus*	0.01	0.00		0.25		1.00		0.00	0.20
5	ND											
6	ND											
7	ND											
8	ND											
9	ND											
10	ND											

ND – non-detect; Benchmarks

Norovirus GII units = gene copies; *Giardia* units = cysts; *Cryptosporidium* units = oocysts; *Mastadenovirus* units = infectious units; *Campylobacter* units = MPN; *C. perfringens* (a) units = spores; *C. perfringens* (b) units = gene copies

Table 6-11 Probability of infection and health impact of ingestion of 1 L of water containing reference pathogens based on ratios determined from faecal indicator bacteria assuming 50 g of soil entered the DWDS during each main break

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
1	*E. coli* (*uid*A)	50 g	a:*Norovirus* GII	1.33	0.00	44	0.04	0.60	0.15	0.50	0.00	0.00
			b:*Norovirus* GII	1.43	0.00		0.04		0.17		0.00	0.00
			a:*Cryptosporidium*	0.88	0.08		3.4	0.50	13.6	1.70	0.01	1.16
			b:*Cryptosporidium*	0.94	0.08		3.6		14.6		0.01	1.24
			a:*Giardia*	1.67	0.03		1.4	0.45	5.86	1.60	0.00	0.42
			b:*Giardia*	1.80	0.04		1.6		6.31		0.00	0.45
			a:*Campylobacter*	6.65	0.09		3.9	0.30	15.6	23.5	0.11	11.0
			b:*Campylobacter*	7.16	0.09		4.1		16.5		0.12	11.6
			a:*Mastadenovirus*	1.16	0.38		17	0.60	68.6	3.30	0.14	13.6
			b:*Mastadenovirus*	1.24	0.41		18		72.7		0.14	14.4
	Enterococcus (Entero 1)		a:*Norovirus* GII	1.85	0.00		0.05	0.60	0.22	0.50	0.00	0.01
			b:*Norovirus* GII	0.42	0.00		0.01		0.05		0.00	0.00
			a:*Cryptosporidium*	1.22	0.10		4.6	0.50	18.7	1.70	0.02	1.59
			b:*Cryptosporidium*	0.28	0.02		1.1		4.46		0.00	0.38
			a:*Giardia*	2.32	0.05		2.0	0.45	8.09	1.60	0.01	0.58
			b:*Giardia*	0.53	0.01		0.46		1.88		0.00	0.14
			a:*Campylobacter*	9.25	0.11		4.8	0.30	19.6	23.5	0.14	13.8
			b:*Campylobacter*	2.12	0.04		1.6		6.29		0.04	4.44
			a:*Mastadenovirus*	1.61	0.49		22	0.60	87.7	3.30	0.17	17.4
			b:*Mastadenovirus*	0.37	0.14		6.3		25.7		0.05	5.08
2	*E. coli* (*uid*A)	50 g	a:*Norovirus* GII	7.38	0.00	10	0.05	0.60	0.20	0.50	0.00	0.01
			b:*Norovirus* GII	7.95	0.00		0.05		0.22		0.00	0.01

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			a:*Cryptosporidium*	4.88	0.36		3.7	0.50	15.0	1.70	0.01	1.28
			b:*Cryptosporidium*	5.26	0.38		3.9		15.9		0.01	1.35
			a:*Giardia*	9.30	0.17		1.8	0.45	7.13	1.60	0.01	0.51
			b:*Giardia*	10.01	0.18		1.9		7.62		0.01	0.55
			a:*Campylobacter*	37.00	0.23		2.4	0.30	9.56	23.5	0.07	6.74
			b:*Campylobacter*	39.84	0.23		2.4		9.85		0.07	6.94
			a:*Mastadenovirus*	6.43	0.93		9.7	0.60	39.3	3.30	0.08	7.79
			b:*Mastadenovirus*	6.93	0.94		9.8		39.9		0.08	7.89
3	Enterococcus (Entero 1)	50 g	a:*Norovirus* GII	1.04	0.00	55	0.04	0.60	0.15	0.50	0.00	0.00
			b:*Norovirus* GII	0.24	0.00		0.01		0.03		0.00	0.00
			a:*Cryptosporidium*	0.69	0.06		3.3	0.50	13.3	1.70	0.01	1.13
			b:*Cryptosporidium*	0.16	0.01		0.78		3.17		0.00	0.27
			a:*Giardia*	1.32	0.03		1.4	0.45	5.74	1.60	0.00	0.41
			b:*Giardia*	0.30	0.01		0.32		1.32		0.00	0.09
			a:*Campylobacter*	5.23	0.07		4.00	0.30	16.2	23.5	0.11	11.4
			b:*Campylobacter*	1.20	0.02		1.2		4.67		0.03	3.29
			a:*Mastadenovirus*	0.91	0.32		17	0.60	70.0	3.30	0.14	13.9
			b:*Mastadenovirus*	0.21	0.08		4.6		18.6		0.04	3.68
4	Enterococcus (Entero 1)	50 g	a:*Norovirus* GII	0.33	0.00	59	0.01	0.60	0.05	0.50	0.00	0.00
			b:*Norovirus* GII	0.08	0.00		0.00		0.01		0.00	0.00
			a:*Cryptosporidium*	0.22	0.02		1.2	0.50	4.69	1.70	0.00	0.40
			b:*Cryptosporidium*	0.05	0.00		0.26		1.07		0.00	0.09
			a:*Giardia*	0.42	0.01		0.49	0.45	1.99	1.60	0.00	0.14
			b:*Giardia*	0.10	0.00		0.12		0.48		0.00	0.03
			a:*Campylobacter*	1.67	0.03		1.7	0.30	6.79	23.5	0.05	4.79

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			b:*Campylobacter*	0.38	0.01		0.42		1.69		0.01	1.19
			a:*Mastadenovirus*	0.29	0.11		6.7	0.60	27.2	3.30	0.05	5.39
			b:*Mastadenovirus*	0.07	0.03		1.7		6.88		0.01	1.36
5	ND											
6	ND											
7	ND											
8	ND											
9	ND											
10	ND											

Benchmarks

ND – non-detect;
Norovirus GII units = gene copies; *Giardia* units = cysts; *Cryptosporidium* units = oocysts; *Mastadenovirus* units = infectious units; *Campylobacter* units = MPN; *C. perfringens* (a) units = spores; *C. perfringens* (b) units = gene copies

Table 6-12 Probability of infection and health impact of ingestion of 1 L of water containing reference pathogens based on ratios determined from faecal indicator bacteria assuming 500 g of soil entered the DWDS during each main break

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
1	*E. coli* (*uid*A)	500 g	a:*Norovirus* GII	13.26	0.01	44	0.38	0.60	1.54	0.50	0.00	0.05
			b:*Norovirus* GII	14.28	0.01		0.41		1.66		0.00	0.05
			a:*Cryptosporidium*	8.77	0.55		24	0.50	97.9	1.70	0.08	8.32
			b:*Cryptosporidium*	9.44	0.57		25		103		0.09	8.73
			a:*Giardia*	16.70	0.28		13	0.45	50.7	1.60	0.04	3.65
			b:*Giardia*	17.98	0.30		13		54.0		0.04	3.89
			a:*Campylobacter*	66.47	0.28		12	0.30	50.5	23.5	0.36	35.57
			b:*Campylobacter*	71.58	0.29		13		51.7		0.36	36.45
			a:*Mastadenovirus*	11.56	0.99		44	0.60	178	3.30	0.35	35.23
			b:*Mastadenovirus*	12.45	0.99		44		178		0.35	35.32
	Enterococcus (Entero 1)		a:*Norovirus* GII	18.46	0.01		0.53	0.60	2.14	0.50	0.00	0.06
			b:*Norovirus* GII	4.24	0.00		0.12		0.49		0.00	0.01
			a:*Cryptosporidium*	12.21	0.67		29	0.50	120	1.70	0.10	10.17
			b:*Cryptosporidium*	2.80	0.22		9.9		40.0		0.03	3.40
			a:*Giardia*	23.25	0.37		16	0.45	66.4	1.60	0.05	4.78
			b:*Giardia*	5.34	0.10		4.5		18.1		0.01	1.30
			a:*Campylobacter*	92.53	0.31		14	0.30	56.0	23.5	0.39	39.46
			b:*Campylobacter*	21.25	0.18		7.8		31.6		0.22	22.26
			a:*Mastadenovirus*	16.09	1.00		44	0.60	179	3.30	0.35	35.47
			b:*Mastadenovirus*	3.69	0.79		35		141		0.28	27.90
2	*E. coli* (*uid*A)	500 g	a:*Norovirus* GII	73.81	0.05	10	0.48	0.60	1.96	0.50	0.00	0.06
			b:*Norovirus* GII	79.49	0.05		0.52		2.11		0.00	0.06

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			a:*Cryptosporidium*	48.80	0.99		10	0.50	41.7	1.70	0.04	3.54
			b:*Cryptosporidium*	52.56	0.99		10		41.8		0.04	3.56
			a:*Giardia*	92.96	0.84		8.8	0.45	35.6	1.60	0.03	2.56
			b:*Giardia*	100.11	0.86		9.0		36.5		0.03	2.62
			a:*Campylobacter*	369.97	0.43		4.5	0.30	18.3	23.5	0.13	12.9
			b:*Campylobacter*	398.43	0.44		4.6		18.5		0.13	13.1
			a:*Mastadenovirus*	64.33	1.00		10	0.60	42.2	3.30	0.08	8.36
			b:*Mastadenovirus*	69.27	1.00		10		42.2		0.08	8.36
3	Enterococcus (Entero 1)	500 g	a:*Norovirus* GII	10.44	0.01	55	0.37	0.60	1.50	0.50	0.00	0.04
			b:*Norovirus* GII	2.40	0.00		0.09		0.35		0.00	0.01
			a:*Cryptosporidium*	6.90	0.46		25	0.50	103	1.70	0.09	8.71
			b:*Cryptosporidium*	1.59	0.13		7.3		29.5		0.03	2.51
			a:*Giardia*	13.15	0.23		12	0.45	51.0	1.60	0.04	3.67
			b:*Giardia*	3.02	0.06		3.2		12.9		0.01	0.93
			a:*Campylobacter*	52.34	0.26		14	0.30	57.4	23.5	0.40	40.4
			b:*Campylobacter*	12.02	0.13		7.0		28.5		0.20	20.1
			a:*Mastadenovirus*	9.10	0.98		53	0.60	217	3.30	0.43	42.9
			b:*Mastadenovirus*	2.09	0.58		32		129		0.26	25.5
4	Enterococcus (Entero 1)	500 g	a:*Norovirus* GII	3.33	0.00	59	0.13	0.60	0.52	0.50	0.00	0.02
			b:*Norovirus* GII	0.76	0.00		0.03		0.12		0.00	0.00
			a:*Cryptosporidium*	2.20	0.18		11	0.50	42.9	1.70	0.04	3.65
			b:*Cryptosporidium*	0.51	0.04		2.6		10.7		0.01	0.91
			a:*Giardia*	4.19	0.08		4.7	0.45	19.1	1.60	0.01	1.38
			b:*Giardia*	0.96	0.02		1.1		4.50		0.00	0.33
			a:*Campylobacter*	16.68	0.16		9.1	0.30	37.1	23.5	0.26	26.1

Site	Indicator	Soil intrusion into DWDS	Pathogen	Concentration of Pathogen/1L	Probability of Infection	People affected by main break	Actual number of People infected	Conditional probability of illness given infection	Infections per 10,000 people per year in Edmonton	Illness to DALY factor (DALY/1000 cases)	DALY/ 10,000 person per year	µDALY/ person per year in Edmonton
			b:*Campylobacter*	3.83	0.06		3.4		13.8		0.10	9.69
			a:*Mastadenovirus*	2.90	0.70		41	0.60	168	3.30	0.33	33.2
			b:*Mastadenovirus*	0.67	0.24		14		58.3		0.12	11.5
5	ND											
6	ND											
7	ND											
8	ND											
9	ND											
10	ND											

ND – non-detect; Benchmarks

Norovirus GII units = gene copies; *Giardia* units = cysts; *Cryptosporidium* units = oocysts; *Mastadenovirus* units = infectious units; *Campylobacter* units = MPN; *C. perfringens* (a) units = spores; *C. perfringens* (b) units = gene copies

6.5: **Summary of QMRA**

All results in this QMRA are based on worst case scenarios using assumptions discussed in Section 6.3. Best case scenario and average risk scenarios are discussed in the Chapter 7.

Initial flush samples were interpreted as only positive for one FIB, *C. perfringens,* and only found at four of the ten sample sites. Based on the ratios determined using *C. perfringens* and reference pathogens, the highest probability of infection at all four positive sites was equal between *Mastadenovirus*, *Giardia* and *Cryptosporidium* at 100 %. *Campylobacter* and *Norovirus* GII probability of infection was calculated to be the same at all four sites of approximately 65 % and 55 % respectively. When generalized across the population of Edmonton the approximate highest health impact was equal among *Mastadenovirus*, *Giardia* and *Cryptosporidium* at 50-210 times the EPA infection benchmark, and *Campylobacter* at 20-100 times and *Mastadenovirus* at 10-40 times the Health Canada benchmark. The lowest pathogen impact was *Norovirus* GII at 30-140 times the EPA infection benchmark, and <1-4 times the Health Canada benchmark.

Final flush samples were only positive for one FIB, *C. perfringens,* and only found at two of the ten sample sites. Based on the ratios determined using *C. perfringens* and reference pathogens, the highest probability of infection at both sites was equal between *Mastadenovirus*, *Giardia* and *Cryptosporidium* at 100 %. *Campylobacter* and *Norovirus* GII probability of infection calculated to be the same at both sites of approximately 65 % and 55 % respectively. When generalized across the population of Edmonton the highest health impact (some 100 x benchmark) was equal among *Mastadenovirus*, *Giardia* and *Cryptosporidium* based on infections/10,000 people per year, and *Campylobacter* based on μDALY/ person per year (some 10-fold above the

benchmark). The lowest pathogen impact was *Norovirus* GII at 20-80 times the EPA infection benchmark, and <1-3 times the Health Canada benchmark.

Soil samples were positive for two FIB, *E. coli* and *Enterococcus* at Site 1, only *E. coli* at Site 2 and only *Enterococcus* at Sites 3 and 4. Sites 5 through 10 identified no FIB above the method detection limit of qPCR. Hence, ratios using the two detected FIB were used to determine the highest probability of infection and health impacts at the four positive sites.

For the 5 g soil intrusion scenarios, the highest probability of infection at the four positive sites was *Mastadenovirus*, ranging from <1.0 % to 25 %. The probability of infection of the remaining four pathogens, *Giardia*, *Cryptosporidium, Campylobacter* and *Norovirus* GII, were all very similar ranging from <1.0 % to 6.0 %. When generalized across the population of Edmonton the highest health impact was from *Mastadenovirus* followed by *Campylobacter*, *Cryptosporidium, Giardia*, and *Norovirus* GII respectively, based on both the EPA and Health Canada benchmarks. The EPA benchmark was exceeded by up to a factor of 10 by *Mastadenovirus* with the rest of the pathogens coming in around 2 or less times. All pathogens in all scenarios came in around 2 or less times the Health Canada benchmark and *Norovirus* GII specifically, came in below both benchmarks.

For the 50 g soil intrusion scenarios, the highest probability of infection at the four positive sites was *Mastadenovirus*, ranging from 3.0 % to 94 %. The probability of infection of the *Giardia*, *Cryptosporidium, Campylobacter*, were all very similar ranging from <1.0 % to 38 % and *Norovirus* GII having the lowest probability of infection at <1.0 %. When generalized across the

population of Edmonton the highest health impact was from *Mastadenovirus* followed by *Campylobacter*, *Cryptosporidium, Giardia*, and *Norovirus* GII respectively, based on both the EPA and Health Canada benchmarks. The EPA benchmark was exceeded up to approximately a factor of 90 by *Mastadenovirus* with the rest of the pathogens coming in around 20 or less times. *Mastadenovirus* and *Campylobacter* in all scenarios came in around 5-10 times the Health Canada benchmark and *Norovirus* GII specifically, came in below both benchmarks.

For the 500 g soil intrusion scenarios, the highest probability of infection at the four positive sites was *Mastadenovirus*, ranging from 24 % to 100 %. The probability of infection of *Cryptosporidium* was the second highest ranging from 4 % to 99 %. *Campylobacter* and *Giardia* were similar ranging from 2.0 % to 86 % and *Norovirus* GII having the lowest probability of infection at <1.0 % to 5.0 %. When generalized across the population of Edmonton the highest health impact was from *Mastadenovirus* followed by *Cryptosporidium, Campylobacter*, *Giardia*, and *Norovirus* GII respectively, based on the EPA benchmark, and *Mastadenovirus* followed by *Campylobacter*, *Cryptosporidium, Giardia*, and *Norovirus* GII based on the Health Canada benchmark. Every scenario excluding *Norovirus* GII at one of the main break sites, exceeded the EPA benchmark by >1-220 times. The Health Canada benchmark was exceeded by all pathogens by >1-40 times except for *Norovirus* GII that came in below the benchmark at every main break site.

CHAPTER 7: CONCLUSION & RECOMMENDATIONS

The objective of this study was to investigate microbial risks to public health during DWDS main breaks by using collected field and literature data to enable a QMRA to explore risks that may occur during the current EPCOR main break response and repair procedure in Edmonton, AB.

Due to the difficulty in proving the source of contamination for GI illness, models are often needed to estimate exposure events (WHO, 2003). While there are many studies assessing water quality factor for GI illness, few studies examine water quality post main break repairs. Samples from ten main break sites were collected and analyzed over a period of two years to determine a risk assessment of the efficacy of EPCOR's standard operating procedure for main break responses and repairs. The results from the QMRA show that there is definitely risk exceeding the benchmarks used by the EPA (up to 220-fold) and Health Canada (up to 100-fold) when considering worst-case scenarios.

The results from the initial flush samples are most relevant to the exposure customers would come into contact with during the worst-case scenarios that were used in this study. When considering both, infection and μDALY, every scenario with every reference pathogen except for *Norovirus* GII in only one of the four main breaks, exceeded both benchmarks. The EPA benchmark of 10^{-4} infections/ y in Edmonton had the highest risk equally from *Mastadenovirus, Cryptosporidium* and *Giardia* exceeding the benchmark of one, by 50 to 180 times respectively. However, when considering μDALY/ person per year in Edmonton the highest risk comes from *Campylobacter* exceeding the benchmark of one, by 20 to 90 times. With these being the results for the worst-case scenarios of one main break occurring every day for 365 days and having *C. perfringens* intrusion into the DWDS while customers do not flush their premise plumbing

post main break repair, it is important to keep in mind that only 40 % of the sites were positive which would cut the risk down drastically.

Final flush samples are more realistic to the exposure customers will actually come into contact with. Following the same trend as the initial flush samples, the EPA benchmark of 10^{-4} infections/ year in Edmonton had the highest risk equally from *Mastadenovirus, Cryptosporidium* and *Giardia* exceeding the benchmark by 50 to 130 times and the Health Canada benchmark of 1 μDALY/ person per year in Edmonton has the highest risk coming from *Campylobacter* exceeding the benchmark by 20 to 60 times respectively. Taking into consideration only 20 % of sites were positive for FIB, cuts the risk down by a very large margin which would be more relevant as an average risk scenario.

Due to *C. perfringens* only being analyzed in the water samples, it is undetermined whether this contamination came from soil intrusion due to the main break, or if it was already in the DWDS in a biofilm. The disruption of the pipe due to the main break could easily cause a piece of the biofilm to break free. With the results being either at the very high end or none quantifiable, it seems the latter is more probable. In reality, *Campylobacter* spp. and other enteric bacterial pathogens are not considered to be very persistent, so spores of *C. perfringens* are probably far too conservative in estimating their presence unless other bacterial indicators are detected. On the other hand, human enteric viruses may last up to a year in soils (Lipson *et al.*, 1984; Yeager and O'Brien, 1979), indicating that human adenoviruses appear to present the highest risks under the above scenarios.

Soil sample scenarios are completely based on assumption that there is contaminated soil intrusion during every main break that occurs. For all three situations of 5 g, 50 g or 500 g of contaminated soil intrusion, all results show the highest health impact being due to *Mastadenovirus*. The worst case scenarios based on both 5 g and 50 g of soil intrusion have very similar results where the highest to lowest health impacts for both infections and µDALY are *Mastadenovirus, Campylobacter*, *Cryptosporidium, Giardia* then *Norovirus* GII. The health impacts for both benchmarks appear to increase by nine times when increasing the soil intrusion amount by ten-fold (5 g to 50 g). However, when moving up to 500 g of soil intrusion the highest to lowest health impacts remain the same for the Health Canada benchmark but the EPA benchmark changes. *Mastadenovirus* still has the highest infection impact but is followed by *Cryptosporidium, Giardia, Campylobacter* and *Norovirus* GII. At 5 g of soil intrusion there is very minimal risk using Health Canada's µDALY benchmark. There are only a few cases above one µDALY with the highest being 2.29 µDALY/ person per year in Edmonton due to *Mastadenovirus*. Infections due to *Mastadenovirus* come in a little higher with the highest being 12 infections/ 10,000 people per year in Edmonton and the remainder of the reference pathogens being less than three with the majority coming in below the bench mark at less than one. When soil intrusion is increased by ten-fold to 50 g, *Mastadenovirus* and *Campylobacter* come in consistently above the benchmark ranging from 6 to 18 µDALY and infections/ 10,000 people have all pathogens, except *Norovirus* GII, above the benchmark. Perceivably 500 g of soil could intrude, resulting in infections/ 10,000 well above the benchmarks for *Mastadenovirus* with one even being over 200. C*ampylobacter*, *Cryptosporidium,* and *Giardia* are all above the benchmarks for both infections and µDALY. *Norovirus* GII being the lowest threat is below the

bench mark for μDALY and gets just above the bench mark for infections four out of five times keeping it at a relatively low risk in all scenarios.

Of the ten sites sampled, three sites were determined to have no quantifiable FIB in any of the water or soil samples. With only 70 % of all breaks showing any quantifiable FIB, and needing more than 5 g of soil to enter the DWDS, risk is decreased significantly. It is also fair to say that not every person will drink one liter of water directly from the tap as soon as their water is turned back on without any form of flushing. Risk will decrease drastically as the amount of exposure to contaminated water decreases. Nonetheless, results from this study are consistent with QMRA results reported by Yang *et al*. (2015), which showed *Cryptosporidium* and pathogenic *E. coli* exceeding the 10^{-4} infection risk benchmark when no additional flushing or disinfection occurs post main break repairs, but did not align with the results for *Norovirus* GII. Yang *et al*. (2015) showed *Norovirus* GII to exceed the benchmarks whereas the current study showed very minimal risk from *Norovirus* GII. The discrepancy in the results for *Norovirus* GII could be due to Yang *et al*. (2015) using Monte Carlo simulation where variables like external pathogens, reduction of pathogen levels, amount of water ingested and pathogen infectivity were randomly generated. The present study had a set of variables based on results from actual break scenarios. Another difference was that Yang *et al*. (2015) only looked at infection of the first customer downstream of the break and did not take into consideration all service lines affected by the break. A very similar study by Nygård *et al*. (2007) showed that breaks and other work on the DWDSs lead to an increase in GI illness to those affected. Nygård *et al*. (2007) considered different utility companies that chlorinated and/or flushed. Through interviewing the affected households they showed the chlorination and/or flushing both caused an increase of acute GI

illness compared to the non-affected households. In fact, the risk was as high as two times in some cases. While the present study does not show actual evidence that anyone affected by a main break got acute GI illness, the QMRA scenarios showing the exceedance of two benchmarks aligns with a possible outcome of increased GI illness in those who have their service lines exposed to a main break.

Although results from the current study are based on numerous assumptions and risk is most likely over estimated, it does support Cook *et al's.* (2013) paper regarding microbial testing of drinking water. Current testing post main repairs for total coliforms and *E. coli* or faecal coliforms, may not be sufficient to be protective of risks from *Giardia, Cryptosporidium* and enteric viruses, which require their own surrogate testing from DWDS samples. Adding more microbial testing is a recommendation for future improvements to EPCOR's SOPs for main breaks and repairs. If you consider the QMRA in reverse and calculate the dose of pathogens needed to meet the EPA and Health Canada benchmarks, it is apparent why FIB or surrogate testing is necessary. To relate this specifically to this study, Site 1 with the assumption that 500 g of soil intrusion occurred, considering *Mastadenovirus,* which had the highest health impact, resulted in 178 infections/ 10,000 people per year in the City of Edmonton. When back calculating to estimate what the critical concentration of pathogens in drinking water would need to be at or below in order to come in under the benchmark risk levels (one infection for 10,000/y or uDALY/y), the concentration of *Mastadenovirus* in this situation would need to be 0.015 pathogens/ L. This value is nearly impossible to detect in any reasonable quantity of sample, especially on a routine basis. Therefore, sampling directly for pathogens will still not guarantee safety. Analysis of *E. coli* (*uid*A) which has a ratio in raw sewage of 1880 *uid*A gene copies for every 1 *Mastadenovirus* infectious unit, is a much more realistic scenario. With *E. coli* are

generally not found growing and reproducing in the environment it could be considered to be one of the best indicators of faecal pollution and the possible presence of pathogens (New York State, 2017), but is easily inactivated by a chlorine residual whereas chlorine-resistant enteric viruses and protozoa could remain infectious.

This project has only grazed the surface of microbial water quality as a result of main breaks when assessing impacts of faecal matter/soil intrusion events. In order to progress this work and establish more accurate risks to public health I recommend five follow-up areas to consider:

a) A pipe study to estimate enteric pathogen risks when super-chlorination is undertaken as part of the main break repair procedure, particularly its impact on contaminated water reaching service lines to premises. It would also be beneficial to know which situation causes the larger risk, microbial or disinfection by-product/chlorine? The results of this would allow for more information regarding possible future improvements of EPCOR's SOPs for main breaks and repairs.

b) Determining how much soil is actually entering the DWDS during main breaks is a key component to this study. Due to all the soil intrusion scenarios in this study being assumed, determining whether soil is always entering the DWDS or not dependent on the type of break, would eliminate a lot of uncertainty from the current risk assessment.

c) Looking at the same microbial targets and possibly more, determining which are least and most impactful in different situations so the risks can be generalized outside the City of Edmonton. One situation to look at could be whether different soil types make a difference as to how much soil/sewage intrusion occurs (sand verses clay soils).

d) With every scenario being based on no flushing, doing a survey/study to determine how many customers affected by main breaks are actually flushing their premise plumbing post main break repairs, would be a very simple and cost efficient way to eliminate major uncertainty in the current estimates.

e) With the negative control/ background FIB levels in soil coming in higher than most main break sites, it would be beneficial to know if chlorinated water exiting the DWDS through the main break has a positive impact on the safety of public health through dilution and disinfection or if the release of chlorinated water into the environment has a worse overall impact.

Overall, main breaks are a very common event in all urban areas. Even though most utility companies are actively working on pipe replacement programs, the amount of pipe associated with DWDSs, regardless of material and age, will not allow main breaks to be eradicated anytime soon. Therefore, it is somewhat perplexing as to what incentives are required to work on the above in Canada and generally.

LITERATURE CITED

Chapter 1 Introduction

1.1 Problem Statement

City of Calgary, 2017. http://www.calgary.ca/UEP/Water/Pages/construction-projects/Water-main-repairs-and-maintenance/Water-Main-Breaks-FAQ.aspx#cause (accessed 24-April-2017).

EPCOR. 2016. EWDT-PRO-WM-007 – Test and Repair Main breaks. Standard Operating Procedure.

EPCOR. 2005-2015. Annual Performance Reports.

Francisque, A., Shahriar, A., Islam, N., Betrie, G., Siddiqui, R.B., Tesfamariam, S. and R. Sadiq. 2013. A decision support tool for water mains renewal for small to medium sized utilities: a risk index approach. *Journal of Water Supply: Research and Technology – Aqua*. **63:** 281-302

Ingerson-Mahar, M. and A. Reid. The microbiology of water distribution systems. A report on an American academy of microbiology colloquium. A report from the American academy of Microbiology. April 2012

Kirmeyer, J.G., Thomure, T.M., Rahman, R., Marie, J.L., LeChevallier, M.W.,Yang, J., Hughes, D.M., and Schneider, O. 2014. Effective Microbial Control Strategies for Main Breaks and Depressurization. Water Research Foundation.

National Research Council. 2006. *Drinking Water Distribution Systems: Assessing and Reducing Risks*. Washington, DC: The National Academies Press, p. 39. DOI:https://doi.org/10.17226/11728.

Yang, J., Schneider, O.D., JJemba, P.K., LeChevallier, M.W. 2015. Microbial Risk Modeling for Main Breaks. *Journal AWWA* **107**: 84-85

Chapter 2 Literature Review

2.1 Main Breaks Within Water Distribution Systems

Balkan Sewer and Drain Cleaning Inc. 2018. http://www.balkandraincleaning.com/air-gap-plumbing-pipe-systems/ (Accessed 14-Sept-2018).

Besner, M. C.; Prévost, M.; Regli, S. 2011. Assessing the public health risk of microbial intrusion events in distribution systems: conceptual model, available data, and challenges. *Water Research***, 45:** 961-79.

CDC. 1999. A Century of U.S. Water Chlorination and Treatment: One of the Ten Greatest Public Health Achievements of the 20th Century. *Morbidity and Mortality Weekly Report.* **48**:621-9

City of Edmonton. 2015. Design and Construction Standards Volume 2: Roadways Section 02965 Utility Cut Restoration Clause 3.2.4.

Craun, G.F., Brunkard, J.M., Yoder, J.S., Roberts, V.A., Carpenter, J., Wade, T. 2010. Cause of outbreaks associated with drinking water in the United States from 1971-2006. *Clinical Microbiology Reviews*. **23:**507-528

Cunliffe, D.; Ashbolt, N.; D'Anglada, L.; Greiner, P.; Gupta, R.; Hearn, J.; Jayaratne, A.; Cheong, K.; O'Connor, N.; Purkiss, D.; Toh, I.; Viola, D.; Wong, K. W., *Water Safety in Distribution Systems. WHO/FWC/WSH/14.03*. World Health Organization: Geneva, 2014.

EPCOR. 2014. Performance Report/ Annual Report.

Ercumen, A., Gruber, J.S. and J.M. Jr. Colford. 2014. Water Distribution System Deficiencies and Gastrointestinal Illness: A Systematic Review and Meta-Analysis. *Environmental Health Perspectives*. **122**: 651-660.

Ingerson-Mahar, M. and A. Reid. The microbiology of water distribution systems. A report on an American academy of microbiology colloquium. A report from the American academy of Microbiology. April 2012

Kirmeyer, J.G., Thomure, T.M., Rahman, R., Marie, J.L., LeChevallier, M.W.,Yang, J., Hughes, D.M., and Schneider, O. 2014. Effective Microbial Control Strategies for Main Breaks and Depressurization. Water Research Foundation.

LeChevallier, M. W., Yang, Y., Xu, M., Hughes, D. and Kunkel, G. *Pressure Management: Industry Practices and Monitoring Procedures*; Water Research Foundation: Denver, CO, 2014; p 177.

Melliger, J. 2006. Water Main Flushing and Disinfection. Lincoln Water System, University of Nebraska, Department of Civil Engineering.

Payment, P., Richardson, L., Siemiatycki, J., Dewar, R., Edwards, M.G., Franco, E. 1991. A randomized trial to evaluate the risk of gastrointestinal disease due to the consumption of drinking water meeting current microbiological standards. *American Journal of Public Health.* **8:** 703-708.

Standridge, J. 2008. *E. coli* as a public health indicator of drinking water quality. *Journal-American Water Works Association.* **100:** 65-75.

2.2 Quantitative Microbial Risk Assessment Background

Haas, C. N., Rose, J. B. Gerba, C. P. 1999. *Quantitative Microbial Risk Assessment*. John Wiley & Sons, Inc: New York; p 449.

Payment, P. 2014. Assessing Waterborne Health Risks Through Quantitative Risk Assessment Models. Centre Inrs-Institut Armand-Frappier.

Petterson, S. R., Ashbolt, N. J. 2016. QMRA and water safety management: review of application in drinking water systems. *J Water Health* **14,** (4), 571-589.

National Research Council. 1983. *Risk Assessment in the Federal Government: Managing the Process*. National Academy Press: Washington, DC.

Walkerton Clean Water Center (WCWC), 2015. Fact Sheet: Quantitative Microbial Risk Assessment: What, Why, How? Issue: 3, Volume: 1, Year: 2015

World Health Organization (WHO), 2016. Quantitative microbial risk assessment Application for water safety management. World Health Organization, Geneva.

2.3 Federal, Provincial and Municipal Compliance Monitor Testing

Alberta Environment and Parks. 2012. Standards and Guidelines for Municipal Waterworks. Wastewater and Storm Drainage Systems, April 2012.

Cook, C., Prystajecky, N., Feze, I.N., Joly, Y., Dunn, G., Kirby, E., Ozdemir, V. and J. Isaac-Renton. 2013. A comparison of the regulatory frameworks governing microbial testing of drinking water in three Canadian provinces. *Canadian Water Resources Journal*. **38**:185-195.

EPCOR, 2017. EWDR-PRO-V-Test and Repair Main Breaks.

Government of Canada. 2016. Canadian Drinking Water Guidelines. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/water-quality/drinking-water/canadian-drinking-water-guidelines.html (Accessed 19-Sept-2017).

Huston, C. Manager, Drinking Water Distribution, City of Calgary. Personal Communication. 22-June-2017.

Ingerson-Mahar, M. and A. Reid. The microbiology of water distribution systems. A report on an American academy of microbiology colloquium. A report from the American academy of Microbiology. April 2012

Ontario Ministry of the Environment and Climate Change, 2016. Watermain disinfection procedure. https://www.ontario.ca/page/water-main-disinfection-procedure (Accessed 21-Sept-2017).

Saskatchewan Water Security Agency. 2017. Permittee Guideline for Self-Managed Waterworks Upsets, Drinking Water Advisories and Consumer Notifications. April 2017.

2.5 Characteristics of Study Organisms

Ashbolt, N. J.; Grabow, W. O. K.; Snozzi, M., Indicators of microbial water quality. In *Water Quality: Guidelines, Standards and Health. Risk Assessment and Risk Management for Water-Related Infectious Disease*, Fewtrell, L.; Bartram, J., Eds. IWA Publishing: London, 2001; pp 289-315.

Bichai, F. and Ashbolt, N. 2017. Public health and water quality management in low-exposure stormwater schemes: A critical review of regulatory frameworks and path forward. *Sustainable Cities and Society***. 28**: 453-465.

Boehm, A.B. and Sassoubre, L.M. 2014. Enterococci as Indicators of Environmental Fecal Contamination. In Enterococci: From Commensals to Leading Causes of Drug Resistant Infections, M.S. Gilmore, D. B. Clewell, Y. Ike et al., Eds., Massachusetts Eye and Ear Infirmary, Boston, Mass, USA, 2014.

Hrudey, S. E. and Hrudey, E. J. *Safe Drinking Water. Lessons from Recent Outbreaks in Affluent Nations*. IWA Publishing: London, 2004; p 514.

Fiksdal, L., Maki, J.S., LaCroix, S.J. and Staley, J.T. 1985. Survival and detection of *Bacteroides spp*., prospective indicator bacteria. *Applied Environmental Microbiology.* **49**:148–150.

Fraser, S.L. MD Infectious Diseases Physician, Infections Limited, Northwest Medical Specialties http://emedicine.medscape.com/article/216993-overview (Accessed 14-March-2017)

Gao, G., Falconer, R.A., Lin, B. 2013. Modeling importance of sediment effects on fate and transport of enterococci in the Severn Estuary, UK. *Marine Pollution Bulletin*. **67**:45-54.

Harwood, V.J., Levine, A.D., Scott, M.S., Chivukula, V., Lukasik, J., Farrah, S.R. and Rose, J.B. 2005. Validity of Indicator Organism Paradigm for Pathogen Reduction in Reclaimed Water and Public Health Protection. *Applied and Environmental Microbiology.* **71**: 3163-3170.

Harwood, J. J. 2014. Molecular markers for identifying municipal, domestic and agricultural sources of organic matter in natural waters. *Chemosphere***. 95:** 3-8.

Health Canada. 2011. Guidelines for Canadian drinking water quality: guideline technical document - enteric viruses. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H129-6/2011E).

Health Canada. 2012. Guidelines for Canadian drinking water quality: guideline technical document -- enteric protozoa: *Giardia* and *Cryptosporidium*. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H129-23/2013E-PDF).

Health Canada. 2013. *Guidance on the Use of the Microbiological Drinking Water Quality Guidelines*; Ottawa, Ontario.

Hecht, D.W. 2017. Anti-microbe, Infectious Disease & Antimicrobial Agents: Bacteroides species. http://www.antimicrobe.org/b85.asp (Accessed 20-March-2017).

Luo, C., Walk, S.T., Gordon, D. M., Feldgarden, M., Tiedje, J. M. and Konstantinidis, K. T. 2011. Genome sequencing of environmental *Escherichia coli* expands understanding of the ecology and speciation of the model bacterial species. *Proc. Natl. Acad. Sci. U.S.A.* **108,** (17), 7200-5.

Meschke, J. S.; Sobsey, M. D. 1998. Comparative adsorption of Norwalk virus, poliovirus 1 and F+ RNA coliphage MS2 to soils suspended in treated wastewater. *Water Science and Technology.* **38**, (12), 187-189.

Miliotis, M. D., and Bier, J. W. 2003. International handbook of foodborne pathogens. New York: Marcel Dekker.

McQuaig, S., Griffith, J. and Harwood V.J. 2012. Association of Fecal Indicator Bacteria with Human Viruses and Microbial Source Tracking Markers at Coastal Beaches Impacted by Nonpoint Source Pollution. *Applied and Environmental Microbiology*. **78:** 6423-6432.

Nagpal R., Ogata K., Tsuji H., Matsuda, K., Takahashi, T., Nomoto, K., Suzuki, Y., Kawashima, K., Nagata, S. and Yamashiro, Y. 2015. Sensitive quantification of Clostridium perfringens in human feces by quantitative real-time PCR targeting alpha-toxin and enterotoxin genes. *BMC Microbiology*. **15**: 219

New York State. 2017. Department of Health: Coliform Bacteria in Drinking Water Supplies. https://www.health.ny.gov/environmental/water/drinking/coliform_bacteria.htm (Accessed 9-November-2017).

Petterson S, Ashbolt N. 2016. Exposure Assessment, p 3.5.2-1-3.5.2-18. *In* Yates M, Nakatsu C, Miller R, Pillai S (ed), *Manual of Environmental Microbiology, Fourth Edition*. ASM Press, Washington, DC. doi: 10.1128/9781555818821.ch3.5.2

Public Health Agency of Canada (PHAC (a)). *Clostridium perfringens* Pathogen Safety Data Sheet - Infectious Substances. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/clostridium-perfringens-eng.php (Accessed 2-March-2017).

Public Health Agency of Canada (PHAC (b)). *Enterococcus faecalis* Pathogen Safety Data Sheet - Infectious Substanceshttp://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/enterococcus-eng.php (Accessed 14-March-2017).

Shanks, O. C., White, K., Kelty, C. A., Sivaganesan, M., Blannon, J., Meckes, M., Varma, M., Haugland, R. A. 2010. Performance of PCR-based assays targeting *Bacteroidales* genetic markers of human fecal pollution in sewage and fecal samples. *Environ Sci Technol.* **44,** (16), 6281-8.

Sinclair, R.G., Rose, J.B., Hashsham, S.A., Gerba, C.P., and Haas, C.N. 2012. Criteria for Selection of Surrogates Used to Study the Fate and Control of Pathogens in the Environment. *Applied and Environmental Microbiology.* **78**: 1969-1977.

Staley, J.T., Moench, T.T., Ward, N.R. and Johnstone, D.L. 1987. Evaluation of Bacteroides as Indicator Bacteria in Drinking Water. USEPA. Water Engineering Research Laboratory.

University of Minnesota. 2003. *Clostridium perfringens* Enterotoxin. http://enhs.umn.edu/current/5103_spring2003/perfringens/perintro.html (Accessed: 3-Nov-2017).

US EPA. 2006. Chapter 17: Bacteria Indicators of Potential Pathogens. This document is Chapter 17 of the Volunteer Estuary Monitoring Manual, A Methods Manual, Second Edition, EPA-842-B-06-003.

Washington State Department of Ecology. 2005. Focus on Fecal Coliform Bacteria. Ecology's Water Quality Program.

Washington State Department of Health. 2016. Coliform Bacteria and Drinking Water. Division of Environmental Health Office of Drinking Water.

World Health Organization (WHO), 2016. Quantitative microbial risk assessment Application for water safety management. World Health Organization, Geneva.

Chapter 3 Methodologies Addressing Main Break Sample Analysis

Besner, M.C., Ebacher, G., and Provost, M. 2013. Public Health Risk of microbial intrusion events in distribution systems. In U. Borchers, J. Gray and K.C. Thompson (Eds.) *Water Contamination Emergencies* (pp.145-155).

3.1 Study Area

City of Edmonton (a). 2017. https://www.edmonton.ca/city_government/facts_figures/population-history.aspx (Accessed 20-Sept-2017).

City Of Edmonton (b). 2017. https://www.edmonton.ca/business_economy/land-location-and-buildings.aspx (Accessed 2-October-2017).

Deer DM, Lampel KA, González-Escalona N. 2010. A versatile internal control for use as DNA in real-time PCR and as RNA in real-time reverse transcription PCR assays. *Letters in Applied Microbiology*, **50**: 366–372.

EPCOR, 2017. https://www.epcor.com/learn/efficiency-conservation/envirovista-champion-report/Pages/protecting-drinking-water-supply.aspx (Accessed 20-Sept-2017).

Haugland R.A., Varma, M., Sivaganesan, M., Kelty, C., Peed, L., and Shanks, O.C. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. *Systematic and Applied Microbiology*. **33**: 348–357.

Karpowicz, E., Novinscak, A., Bärlocher, F. and Filion, M. 2009. qPCR Quantification and Genetic Characterization of *Clostridium perfringens* Populations in Biosolids Composted for 2 Years. *Journal of Applied Microbiology.* **108**: 571-581.

Neumann, N Laboratory (Technician: Candis Scott) 2012. University of Alberta, School of Public Health, Environmental Health Sciences.

Stats Canada. 2011. https://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/famil53c-eng.htm (Accessed 21-May-2018).

Taskin B, Gozen AG, Duran M. 2011. Selective quantification of viable Escherichia coli bacteria in biosolids by quantitative PCR with propidium monoazide modification. *Applied and Environmental Microbiology*. **77:** 4329–35.

UIS Production Program. EPCOR Internal Database. (Accessed 23-Oct-2017).

US EPA. Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay: October 2012.

Chapter 6 Quantitative Microbial Risk Assessment

Ahmed, W., Gyawali, P., Sidhu, J. and Toze, S. 2014. Relative inactivation of faecal indicator bacteria and sewage markers in freshwater and seawater microcosms. *Lett. Appl. Microbiol*., **59** (3), 348-354.

Ashbolt, N. J.; Grabow, W. O. K.; Snozzi, M., Indicators of microbial water quality. In *Water Quality: Guidelines, Standards and Health. Risk Assessment and Risk Management for Water-Related Infectious Disease*, Fewtrell, L.; Bartram, J., Eds. IWA Publishing: London, 2001; pp 289-315.

Bitton, G. *Microbiology of Drinking Water Production and Distribution*, 1st ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2014; p. 312.

Edmonton Municipal Census. 2016. https://www.edmonton.ca/city_government/municipal-census.aspx (Accessed 7-Jun-2018).

Gaunt E.R., Harvala H., McIntyre C., Templeton K.E., Simmonds P. 2011. Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model. *J Clin Virol.* **52**(3):215-21.

Gibney, K.B., O'Toole, J., Sinclair, M., Leder, K., 2014. Disease burden of selected gastrointestinal pathogens in Australia, 2010. *Int. J. Infect. Dis*. **28**:176–185.

Health Canada *Guidelines for Canadian Drinking Water Quality - Summary Table*; Ottawa, Ontario, 2012.

Lisle, J.T., Smith, J.J., Edwards, D.D. and McFeters, G.A. 2004. Occurrence of Microbial Indicators and *Clostridium perfringens* in Wastewater, Water Column Samples, Sediments, Drinking Water, and Weddell Seal Feces Collected at McMurdo Station, Antarctica. *Appl Environ Microbiol.* **70**(12): 7269–7276.

Medema, G., Teunis, P., Havelaar, A. , Haas, C. , 1996. Assessment of the dose-response relationship of *Campylobacter jejuni* . Int. J. Food Microbiol. **30**(1): 101–111.

Messner, M.J., Berger, P., and Nappier, S.P. 2014. Fractional Poisson—a simple dose-response model for human Norovirus. *Risk Anal.* **34**(10): 1820–1829.

Rose, J.B., Haas, C.N., and Gerba, C.P. 1996. Risk Assessment for Microbial Contaminants in Water. Report for the AWWA Research Foundation. AWWA Research Foundation and AWWA.

Rose, J.B., Haas, C.N., and Regli, S. 1991. Risk assessment and control of waterborne giardiasis. *Am. J. Public Health***. 81**(6): 709–713.

Schoen, M., N. Ashbolt, M. Jahne, and J. Garland. 2017. Risk-based enteric pathogen reduction targets for non-potable and direct potable use of roof runoff, stormwater, and greywater. *Microbial Risk Analysis.* **5**: 32-43.

Soller, J.A., Bartrand, T., Ashbolt, N.J., Ravenscroft, J. and Wade, T.J. 2010. Estimating the Primary etiologic agents in recreational freshwaters impacted by human courses of faecal contamination. *Water Research*. **44**: 4736-4747.

Teunis, P.F.M. and Havelaar, A.H. 2000. The Beta Poisson Dose-Response Model is Not a Single-Hit Model. *Risk Analysis*. **20**(4): 513-520

U.S. EPA, 2005. Occurrence and Exposure Assessment For the Final Long Term 2 Enhanced Surface Water Treatment Rule. EPA 815-R-06-002. United States Environmental Protection Agency, Washington, DC.

U.S. EPA, 2006. National primary drinking water regulations: Long Term 2 Enhanced Surface Water Treatment Rule. *Federal Register*, **71**, (3), 654-702.

WHO. 2003(a). Guidelines for safe recreational water environments. Volume 1: coastal and fresh waters.

Chapter 7 Conclusions and Recommendations

Cook, C., Prystajecky, N., Feze, I.N., Joly, Y., Dunn, G., Kirby, E., Ozdemir, V. and J. Isaac-Renton. 2013. A comparison of the regulatory frameworks governing microbial testing of drinking water in three Canadian provinces. *Canadian Water Resources Journal.* **38**:185-195.

Lipson, S. M.; Stotzky, G. 1984. Effect of proteins on reovirus adsorption to clay minerals. *Appl Environ Microbiol.* **48,** (3), 525-530.

New York State. 2017. Department of Health: Coliform Bacteria in Drinking Water Supplies. https://www.health.ny.gov/environmental/water/drinking/coliform_bacteria.htm (Accessed 9-November-2017).

Nygård, K., Wahl, E., Krogh, T., Tveit, O.A., Bøhleng, E., Tverdal, A. and Aavitsland, P. 2007. Breaks and maintenance work in the water distribution systems and gastrointestinal illness: a cohort study. *International Journal of Epidemiology*. **36:**873–880.

WHO. 2003. Hazard Characterization for Pathogens in Food and Water. Microbiological Risk Assessment Series, No. 3.

Yang, J., Schneider, O.D., JJemba, P.K., LeChevallier, M.W. 2015. Microbial Risk Modeling for Main Breaks. *Journal AWWA*. **107**: 84-85

Yeager, J. G.; O'Brien, R. T. 1979. Enterovirus inactivation in soil. *Appl Environ Microbiol* **38,** (4), 694-701.

APPENDICES

Appendix A: Drainage infrastructure damage events from January 2009-August 2016 highlighting sanitary service and main sewer lines

Year Reported	Location	Date of Loss	Cause of Damage	Drainage Infrastructure Damaged
2009	11225 53 ST	12-Jan-09	Water damage	Sanitary service
2009	9516 180 AVE	14-Mar-09	Water damage	Sanitary service
2009	10145 113 ST	23-Apr-09	Water damage	Storm sewer
2009	12311 104 ST	18-Mar-09	Water damage	Sanitary service
2009	135 ST and Dovercourt AVE	30-Mar-09	Water damage	Catch basin/lead
2009	92 ST and 115 AVE	22-Dec-08	Excavation damage	Main sewer line
2009	10775 164 ST	27-Mar-09	Water damage	Catch basin/lead
2009	76 ST and 101 AVE	6-Apr-09	Water damage	Storm sewer
2009	5611 101A AVE	17-Oct-09	Excavation damage	Main sewer line
2009	12051 69 ST	16-Oct-09	Excavation damage	Sanitary service
2010	8605 159 ST	26-Apr-10	Excavation damage	Sanitary service
2010	9729 73 AVE	8-Feb-10	Excavation damage	Sanitary service
2010	13427 124 ST	6-Mar-10	Water damage	Sanitary service
2010	9311 75 AVE	28-Jan-10	Water damage	Sanitary service
2010	15101 87 AVE	5-May-10	Excavation damage	Storm sewer
2011	11123 75 AVE	14-Apr-11	Water damage	Sanitary service
2011	12028 136 AVE	20-Mar-11	Water damage	Sanitary service
2011	100A ST and 95 AVE	17-Feb-11	Excavation damage	Catch basin/lead
2011	16155 110B AVE	15-Mar-11	Water damage	Sanitary service
2011	12011 122 ST	6-Apr-11	Water damage	Sanitary service
2011	96 ST and 101 AVE	19-Apr-11	Water damage	Sanitary service
2011	76 ST and Rowland Road	25-Mar-11	Water damage	Catch basin/lead
2011	10502 70 AVE	23-Jan-11	Water damage	Sanitary service
2011	15213 106 AVE	23-Jul-11	Water damage	Manhole
2011	9670 84 AVE	30-May-11	Excavation damage	Sanitary service
2011	10536 44 ST	13-Jul-11	Water damage	Main sewer line
2011	11218 95 ST	24-May-11	Excavation damage	Sanitary service
2012	10511 153 ST	2-May-11	Excavation damage	Sanitary service
2012	11335 Tower Road	22-Aug-12	Water damage	Sanitary service
2012	11525 90 ST	19-May-11	Excavation damage	Sanitary service
2012	11745 78 ST	27-Jan-12	Excavation damage	Sanitary service
2012	11938 36 ST	13-May-12	Unknown	Sanitary service
2012	12241 54 ST	6-Feb-12	Water damage	Sanitary service
2012	13029 66 ST	8-Jun-11	Excavation damage	Sanitary service

2012	9831 90 AVE	30-Jun-11	Excavation damage	Sanitary service
2012	Saskatchewan Drive and 111 ST	8-May-12	Water damage	Main sewer line
2013	10706 135 ST	13-Oct-11	Excavation damage	Sanitary service
2013	111 ST & 107 AVE	12-Sep-11	Main Renewal	Storm sewer
2013	108 ST & 104 AVE	29-Sep-11	Main Renewal	Main sewer line
2013	10535 137 ST	21-Jul-12	Unknown	Sanitary service
2013	5176 Mullen Road	24-May-12	Excavation damage	Main sewer line and services
2013	11335 Tower Road	30-Jul-12	Water damage	Sanitary service
2013	934 Knottwood Road South	3-Jan-12	Water damage	Catch basin/lead
2013	6805 Yellowhead Trail	18-Apr-12	Excavation damage	Storm sewer
2013	50 ST & 94B AVE	18-Jul-12	Excavation damage	Catch basin/lead
2013	7406 119 ST	5-Jan-12	Water damage	Sanitary service
2013	8440 105 AVE	8-May-12	Excavation damage	Catch basin/lead
2013	8007 Argyll Road	29-Aug-12	Excavation damage	Storm sewer
2013	67 ST & 124 AVE	2-Jan-13	Excavation damage	Catch basin/lead
2013	12605 107 AVE	22-Oct-12	Excavation damage	Sanitary service
2013	9673 86 AVE	22-Jan-13	Water damage	Sanitary service
2013	15628 80 AVE	23-Apr-13	Water damage	Sanitary service
2013	11841 97 ST	28-Jan-13	Water damage	Sanitary service
2013	104A ST & 137 AVE	22-Jan-13	Excavation damage	Catch basin/lead
2013	16423 99A AVE	9-Oct-12	Excavation damage	Storm sewer
2013	108 ST & 109 AVE	15-Feb-11	Water damage	Main sewer line
2013	9672 86 AVE	3-Jun-13	Excavation damage	Sanitary service
2013	96 ST & 112 AVE	1-May-13	Water damage	Catch basin/lead
2013	11110 127 ST	5-Oct-10	Main Renewal	Sanitary service
2013	11733 96 ST	24-Jun-13	Backfill Process	Sanitary service
2013	120 ST and 114 AVE	9-Sep-13	Water damage	Catch basin/lead
2013	127 ST and 111 AVE	13-Apr-13	Main Renewal	Storm sewer
2013	82 ST and 116 AVE	9-Sep-13	Excavation damage	Main sewer line
2013	Argyll Road and 77 ST	16-Jul-13	Unknown	Main sewer line
2014	10335 84 AVE	24-Jul-13	Excavation damage	Sanitary service
2014	8927 Strathearn Drive	30-Jul-14	Excavation damage	Catch basin/lead
2014	10639 50 ST & 10640 48 ST	9-Jul-14	Water damage	Sanitary service
2014	7140 136 AVE	21-Aug-14	Water damage	Sanitary service
2014	10673 161 AVE	26-Feb-14	Excavation damage	Sanitary service
2014	10710 68 AVE	1-Dec-14	Unknown	Sanitary service
2014	11239 95A ST	18-Mar-14	Water damage	Sanitary service
2014	11302 89 ST	18-Mar-14	Water damage	Sanitary service
2014	115 ST and 43 AVE	7-Mar-14	Water damage	Main sewer line

2014	12307 102 ST	13-Nov-13	Unknown	Sanitary service
2014	12550 72 ST	12-Sep-14	Excavation damage	Sanitary service
2014	128 ST and 116 AVE	29-May-14	Directional Drill	Main sewer line
2014	12941 63 ST	1-Jul-13	Water damage	Sanitary service
2014	13435 136 ST	21-Oct-14	Unknown	Sanitary service
2014	149 ST and 100 AVE	9-Apr-14	Water damage	Catch basin/lead
2014	8133 150 ST	18-Mar-14	Excavation damage	Sanitary service
2014	9745 106 ST	24-Nov-14	Excavation damage	Sanitary service
2014	9842 81 AVE	Unknown	Main Renewal	Main sewer line
2015	82 ST and Jasper AVE	Unknown	Unknown	Catch basin/lead
2015	10239 146 ST	13-Oct-15	Directional Drill	Sanitary service
2015	103 ST and 98 AVE	26-Feb-15	Water damage	Sanitary service
2015	10350 121 ST	25-Oct-15	Excavation damage	Sanitary service
2015	97 ST and 86 AVE	2-Jan-15	Water damage	Catch basin/lead
2015	10509 71 AVE	10-Sep-15	Directional Drill	Sanitary service
2015	106 ST and 51 AVE	9-Apr-15	Water damage	Catch basin/lead
2015	10618 163 ST	27-Apr-15	Water damage	Sanitary service
2015	10937 86 AVE	25-May-15	Directional Drill	Sanitary service
2015	11008 129 ST	6-Feb-15	Directional Drill	Sanitary service
2015	11149 66 ST	30-Mar-15	Water damage	Sanitary service
2015	11153 66 ST	30-Mar-15	Water damage	Sanitary service
2015	11207 University Ave	28-Jul-15	Excavation damage	Sanitary service
2015	11835 67 ST	23-Apr-15	Excavation damage	Sanitary service
2015	12831 97 ST	30-Dec-15	Water damage	Sanitary service
2015	12841 87 ST	6-Feb-15	Water damage	Sanitary service
2015	4319 115 ST	20-Jul-15	Water damage	Sanitary service
2015	9507 133 AVE	13-Jul-15	Water damage	Sanitary service
2015	9739 94 ST	13-May-15	Excavation damage	Sanitary service
2015	9813 74 AVE	10-Jun-15	Excavation damage	Sanitary service
2015	9820 79 ST	1-May-15	Water damage	Sanitary service
2015	Connors RD and 90 ST	11-Dec-15	Main Renewal	Catch basin/lead
2015	Connors RD and 92 AVE	16-Sep-15	Main Renewal	Main sewer line
2016	5806 110 ST	1-Jan-87	Directional Drill	Catch basin/lead
2016	107 ST and 79 AVE	5-Jul-14	Main Renewal	Main sewer line
2016	11126 81 AVE	15-Mar-16	Excavation damage	Sanitary service
2016	12109 35 ST	29-Feb-16	Water damage	Sanitary service
2016	12849 87 ST	8-Oct-15	Excavation damage	Sanitary service
2016	6625 106 ST	27-Sep-16	Main Renewal	Sanitary service
2016	80 ST and 120 AVE	16-Jun-16	Water damage	Catch basin/lead
2016	9267 Ottewell RD	20-Aug-11	Main Renewal	Sanitary service

Appendix B: Power Soil® DNA Isolation Kit Method

1. To the **PowerBead Tubes** provided, add 0.250g soil, 250µl soil liquid mix, or 10 mm 44 micron filter.

2. Gently vortex to mix.

3. Check **Solution C1**. If **Solution C1** is precipitated, heat solution to 60°C until dissolved before use.

4. Add 60 µl of **Solution C1** and invert several times or vortex briefly.

5. Secure **PowerBead Tubes** horizontally using the MO BIO 24 place Vortex Adapter tube holder for the vortex. For 12 preps or less Vortex at maximum speed for 10 minutes, for more than 12 preps, increase the vortex time by 5-10 minutes.

6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature.

7. Transfer 500µl of supernatant to a clean **2 mL Collection Tube**.

8. Add 250µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

9. Centrifuge tubes at 10,000 x g for 1 minute at room temperature.

10. Avoiding the pellet, transfer 600 µl of supernatant to a clean **2 mL Collection Tube**.

11. Add 200µl of **Solution C3** and vortex briefly. Incubate at 4°C for 5 minutes.

12. Centrifuge tubes at 10,000 x g for 1 minute at room temperature.

13. Avoiding the pellet, transfer 700µl of supernatant to a clean **2 mL Collection Tube**.

14. Shake to mix **Solution C4** before use. Add 1200µl of **Solution C4** to the supernatant and vortex for 5 seconds.

15. Load approximately 675µl onto a **Spin Filter** and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add another 675µl to the **Spin Filter** and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the **Spin Filter** and centrifuge at 10,000 x g for 1 minute at room temperature.

16. Add 500µl of **Solution C5** and centrifuge at 10,000 x g for 30 seconds at room temperature.

17. Discard the flow through.

18. Centrifuge again at 10,000 x g for 1 minute at room temperature.

19. Carefully place spin filter in a clean **2 mL Collection Tube**. Avoid splashing any **Solution C5** onto the **Spin Filter**.

20. Add 100 µl of **Solution C6** to the center of the white filter membrane.

21. Centrifuge at 10,000 x g for 30 seconds at room temperature.

22. Discard the Spin Filter. The DNA in the tube is now ready for downstream application. Freeze DNA at -20°C to -80°C.

Appendix C: Culture analysis results from initial and final flush samples collected from ten main break sites around Edmonton, AB

Culture Analysis Water – Initial Flush							
Site	Difco™ mEI Agar (CFU/100mL)	Difco™ mEI Agar (Raw data)	*Clostridium* Agar (Oxoid™) (m-CP**)** (CFU/100 mL)	*Clostridium* Agar (Oxoid™) (m-CP**)** (raw data)	IDEXX Quanti-Trays/2000® (MPN/100mL)	IDEXX Quanti-Trays/2000® MPN (TC raw data)	**Comments**
1	<1.0	4 CFU/1500mL	10000	TNTC/1500mL	<15	<1.0 MPN/ 1500mL	No EC found in any IDEXX all results are TC. Assumption TNTC = 10000 CFU/100mL
2	<1.0	<22.5 CFU/2250mL	<1.0	<10.5 CFU/1050mL	<15	<1.0 MPN/ 1500mL	
3	<1.0	<25 CFU/2500mL	<1.0	<25 CFU/2500mL	<30	<1.0 MPN/ 3000mL	
4	<1.0	<30 CFU/3000mL	<1.0	<25 CFU/2500mL	<30	<1.0 MPN/ 3000mL	
5	<1.0	<25 CFU/2500mL	<1.0	<2.8 CFU /2750mL	<30	<1.0 MPN/ 3000mL	
6	1.0	3 CFU/300mL	10000	TNTC/300mL	9.8	39.3 MPN/ 400mL	
7	<1.0	<4.5 CFU/450mL	10000	TNTC/450mL	<12.5	<1.0 MPN/ 1250mL	
8	<1.0	<15 CFU/1500mL	10000	TNTC/2000mL	<30	<1.0 MPN/ 3000mL	
9	<1.0	<17.5 CFU/1750mL	<1.0	<17.5 CFU/1750mL	<25	<1.0 MPN/ 2500mL	
10	<1.0	<17.5 CFU/1750mL	<1.0	<17.5 CFU/1750mL	<25	<1.0 MPN/ 2500mL	

TC- total coliform*;* EC – *E. coli*; TNTC- too numerous to count; CFU – colony forming units

Culture Analysis Water – Final Flush							
Site	Difco™ mEI Agar (CFU/100mL)	Difco™ mEI Agar (Raw data)	*Clostridium* Agar (Oxoid™) (m-CP**)** (CFU/100 mL)	*Clostridium* Agar (Oxoid™) (m-CP**)** (raw data)	IDEXX Quanti-Trays/2000® (MPN/100mL)	IDEXX Quanti-Trays/2000® MPN (TC raw data)	**Comments**
1	<1.0	<20 CFU/2000mL	<1.0	2 CFU/2000mL	<1.0	20.3 MPN/ 3000 mL	No EC found in any IDEXX all results are TC. Assumption TNTC = 10000 CFU/100mL
2	<1.0	<45 CFU/4500mL	<1.0	<10 CFU/1000mL	<30	<1.0 MPN/ 3000mL	
3	<1.0	<30 CFU/3000mL	<1.0	<25 CFU/2500mL	<30	<1.0 MPN/ 3000mL	
4	<1.0	<30 CFU/3000mL	<1.0	<30 CFU/3000mL	<30	<1.0 MPN/ 3000mL	
5	<1.0	<30 CFU/3000mL	<1.0	<30 CFU/3000mL	<30	<1.0 MPN/ 3000mL	
6	<1.0	<30 CFU/3000mL	10000	TNTC/2000mL	<30	<1.0 MPN/ 3000mL	
7	<1.0	<25 CFU/2500mL	<1.0	<25 CFU/2500mL	<30	<1.0 MPN/ 3000mL	
8	<1.0	<25 CFU/2500mL	10000	TNTC/2500mL	<35	<1.0 MPN/ 3500mL	
9	<1.0	<30 CFU/3000mL	<1.0	<30 CFU/3000mL	<35	<1.0 MPN/ 3500mL	
10	<1.0	<30 CFU/3000mL	<1.0	<30 CFU/3000mL	<35	<1.0 MPN/ 3500mL	

TC- total coliform*;* EC – *E. coli*; TNTC- too numerous to count; CFU – colony forming units

Appendix D: Molecular qPCR results from initial and final flush samples as well as soil samples from the ten main break sites sampled around Edmonton, AB

Molecular (qPCR) Analysis Water - Initial Flush						
Site	*uid*A copies/100 mL	*uid*A - raw data copies/ volume	Entero 1 copies/100 mL	Entero1 - raw data copies/ volume	HF183 copies/100 mL	HF183 - raw data copies/volume
1	<35.00	<175/500 mL	<35.00	<175/500 mL	<35.00	<175/500 mL
2	<11.67	<175/1500 mL	<11.67	<175/1500 mL	<11.67	<175/1500 mL
3	<5.00	<175/3500 mL	<5.00	<175/3500 mL	<5.00	<175/3500 mL
4	<8.00	<175/2500 mL	<8.00	<175/2500 mL	<8.00	<175/2500 mL
5	<11.67	<175/1500 mL	<11.67	<175/1500 mL	<11.67	<175/1500 mL
6	<175.00	<175/100 mL	<175.00	<175/100 mL	<175.00	<175/100 mL
7	<116.67	<175/150 mL	<116.67	<175/150 mL	<116.67	<175/150 mL
8	<11.67	<175/1500 mL	<11.67	<175/1500 mL	<11.67	<175/1500 mL
9	11.67	175/1500 mL	<11.67	<175/1500 mL	<11.67	<175/1500 mL
10	<17.50	<175/1000 mL	<17.50	<175/1000 mL	<17.50	<175/1000 mL

Molecular (qPCR) Analysis Water - Final Flush						
Site	*uid*A copies/100 mL	*uid*A - raw data copies/ volume	Entero 1 copies/100 mL	Entero1 - raw data copies/ volume	HF183 copies/100 mL	HF183 - raw data copies/volume
1	<8.75	<175/2000 mL	<8.75	<175/2000 mL	<8.75	<175/2000 mL
2	<8.75	<175/2000 mL	<8.75	<175/2000 mL	<8.75	<175/2000 mL
3	<6.36	<175/2750 mL	<6.36	<175/2750 mL	<6.36	<175/2750 mL
4	<5.38	<175/3250 mL	<5.38	<175/3250 mL	<5.38	<175/3250 mL
5	<5.83	<175/3000 mL	<5.83	<175/3000 mL	<5.83	<175/3000 mL
6	<5.00	<175/3500 mL	<5.00	<175/3500 mL	<5.00	<175/3500 mL
7	<5.00	<175/3500 mL	<5.00	<175/3500 mL	<5.00	<175/3500 mL
8	<5.00	<175/3500 mL	<5.00	<175/3500 mL	<5.00	<175/3500 mL
9	<8.75	<175/2000 mL	<8.75	<175/2000 mL	<8.75	<175/2000 mL
10	<13.46	<175/1300 mL	<13.46	<175/1300 mL	<13.46	<175/1300 mL

Molecular (qPCR) Analysis Soil (copies/250µg of soil)															
Site	**Soil 1**			**Soil 2**			**Soil 3**			**Soil 4**			**Soil 5**		
	*uid*A	Entero1	HF183	*uid*A	Entero 1	HF183	*uid*A	Entero1	HF183	*uid*A	Entero 1	HF183	*uid*A	Entero1	HF183
1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	2407.52	7423.29	<200	<200	<200	<200
2	2407.52	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
3	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	3812.01	<200
4	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	5015.77	<200
5	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
6	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
7	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
8	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
9	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
10	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
(+) control	6018.80	112350.83	2407.52	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	<200	<200	<200
(-) control	<200	7824.44	<200	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	<200	5015.77	<200

n/a – sample not available

Appendix E: HF183 decay experiment results (Chapter 4)

HF183 Time (Days)	Sample Extraction Duplicates	Ct value	Sub-Sample 1 copies/ 5 µL (Raw Data)	Sub-Sample 1 copies/ 250 µg soil	Ct value	Sub-Sample 1 Duplicate copies/ 5 µL (Raw Data)	Sub-Sample 1 Duplicate copies/ 250 µg soil	Sub-Sample 1 Average copies/ 250 µg soil	Sub-Sample 1 Log10 Average
1	A	27.98	1282	38580.51	28.05	1224	36835.06	37707.78	4.58
	B		<5			<5			
3	A	28.31	1032	31057.01	28.59	856	25760.46	28408.74	4.45
	B	28.17	1132	34066.41	28.12	1168	35149.79	34608.10	4.54
5	A	28.91	692	20825.05	28.81	737	22179.28	21502.16	4.33
	B	29.64	424	12759.86	29.53	456	13722.86	13241.36	4.12
7	A	32.26	68	2046.39	31.83	91	2738.55	2392.47	3.38
	B	30.15	290	8727.26	29.74	385	11586.19	10156.73	4.01
9	A	30.33	256	7704.06	30.04	312	9389.33	8546.70	3.93
	B	29.80	368	11074.59	29.78	373	11225.06	11149.83	4.05
11	A	30.50	227	6831.34	30.83	181	5447.01	6139.18	3.79
	B	31.28	133	4002.50	30.68	201	6048.89	5025.70	3.70

Not used during analysis

HF183 Time (Days)	Sample Extraction Duplicates	Ct value	Sub-Sample 2 copies/ 5 µL (Raw Data)	Sub-Sample 2 copies/ 250 µg soil	Ct value	Sub-Sample 2 Duplicate copies/ 5 µL (Raw Data)	Sub-Sample 2 Duplicate copies/ 250 µg soil	Sub-Sample 2 Average copies/ 250 µg soil	Sub-Sample 2 Log10 Average
1	A	28.32	1021	30725.97	28.14	1152	34668.29	32697.13	4.51
	B	27.89	1363	41018.12	27.93	1322	39784.27	40401.20	4.61
3	A	28.49	915	27536.01	28.55	875	26332.25	26934.13	4.43
	B	28.92	684	20584.30	28.82	733	22058.90	21321.60	4.33
5	A	30.5	239	7192.47	29.65	422	12699.67	9946.07	4.00
	B	29.49	468	14083.99	29.37	509	15317.85	14700.92	4.17
7	A	29.59	427	12850.14	29.45	469	14114.09	13482.11	4.13
	B	28.98	650	19561.10	28.89	691	20794.95	20178.03	4.30
9	A	33.88	22	662.07	30.83	181	5447.01	3054.54	3.48
	B	32.93	43	1294.04	32.56	55	1655.17	1474.61	3.17
11	A	29.93	338	10171.77	30.21	278	8366.13	9268.95	3.97
	B	30.54	221	6650.77	30.13	294	8847.64	7749.21	3.89

HF183 Time (Days)	Sample Extraction Duplicates	Ct value	Sub-Sample 3 copies/ 5 µL (Raw Data)	Sub-Sample 3 copies/ 250 µg soil	Ct value	Sub-Sample 3 Duplicate copies/ 5 µL (Raw Data)	Sub-Sample 3 Duplicate copies/ 250 µg soil	Sub-Sample 3 Average copies/ 250 µg soil	Sub-Sample 3 Log10 Average
1	A	27.73	1509	45411.85	27.48	1784	53687.70	49549.77	4.70
	B		184	5537.30		242	7282.75	6410.02	
3	A	28.29	1041	31327.85	28.58	858	25820.65	28574.25	4.46
	B	28.47	923	27776.76	28.73	776	23352.94	25564.85	4.41
5	A	28.33	1014	30515.32	28.3	1037	31207.48	30861.40	4.49
	B	29.47	474	14264.56	29.53	458	13783.05	14023.80	4.15
7	A	29.73	387	11646.38	29.21	553	16641.98	14144.18	4.15
	B	30.35	252	7583.69	31.13	148	4453.91	6018.80	3.78
9	A	29.69	398	11977.41	29.7	395	11887.13	11932.27	4.08
	B	29.69	397	11947.32	30.1	301	9058.29	10502.81	4.02
11	A	31.07	154	4634.48	31.09	152	4574.29	4604.38	3.66
	B	30.66	203	6109.08	30.35	252	7583.69	6846.39	3.84

Not used during analysis

Appendix F: Faecal indicator bacteria analyzed by qPCR found in raw sewage collected from GoldBar Wastewater Treatment Plant influent in copies/ 5µL (raw data)

HF183 Time (Days)	Raw Sewage	Raw Sewage Duplicate	Raw Sewage Average	Blank Soil	Blank Soil Duplicate	Blank Soil Average
1	4488.00	4600.00	4544.00	<5.00	<5.00	<5.00
3	1779.00	1883.00	1831.00	<5.00	<5.00	<5.00
5	13.00	33.00	23.00	<5.00	<5.00	<5.00
7	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
9	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
11	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00

*uid*A Time (Days)	Raw Sewage	Raw Sewage Duplicate	Raw Sewage Average	Blank Soil	Blank Soil Duplicate	Blank Soil Average
1	161.00	208.00	184.50	<5.00	<5.00	<5.00
3	257.00	320.00	288.50	<5.00	<5.00	<5.00
5	12.00	32.00	22.00	<5.00	<5.00	<5.00
7	16.00	12.00	14.00	<5.00	<5.00	<5.00
9	24.00	26.00	25.00	<5.00	<5.00	<5.00
11	18.00	16.00	17.00	<5.00	<5.00	<5.00

Entero1 Time (Days)	Raw Sewage	Raw Sewage Duplicate	Raw Sewage Average	Blank Soil	Blank Soil Duplicate	Blank Soil Average
1	1685.00	1646.00	1665.50	<5.00	<5.00	<5.00
3	1253.00	1633.00	1443.00	<5.00	<5.00	<5.00
5	203.00	61.00	132.00	<5.00	<5.00	<5.00
7	129.00	176.00	152.50	<5.00	<5.00	<5.00
9	294.00	321.00	307.50	<5.00	<5.00	<5.00
11	110.00	124.00	117.00	<5.00	<5.00	<5.00

Cpn_60 Time (Days)	Raw Sewage	Raw Sewage Duplicate	Raw Sewage Average	Blank Soil	Blank Soil Duplicate	Blank Soil Average
1	8.06	<5.00	6.02	<5.00	<5.00	<5.00