Health Impact Assessment of New Urban Water Concepts

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Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus prof. ir. K. C. A. M. Luyben;
voorzitter van het College voor Promoties,
in het openbaar te verdedigen op
woensdag 8 april 2015 om 10:00 uur

Door

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This research was performed at KWR Watercycle Research Institute and was funded by KWR Innovation Funds and by the Dutch National research program Knowledge for Climate.

Printed by Gildeprint Drukkerijen – The Netherlands
Cover design by Helena Sales Ortells
Original photo by Javier Simón Sánchez
ISBN: 978-94-6186-447-5

Summary

Water features in urban areas are increasingly perceived by citizens as a positive element because they provide aesthetic quality to the neighbourhood and offer recreation opportunities. They may also lead, however, to increased health risks due to the potential presence of waterborne pathogens. Microbial hazards may be present in water bodies due to input of faecal material such as sewage discharge containing human enteric pathogens (*Campylobacter*, *Cryptosporidium*, Norovirus, Rotavirus, etc.) or animal faecal input containing zoonotic pathogens, or growth of microorganisms in urban water bodies and features, such as toxic cyanobacteria in stagnant waters, or *Legionella pneumophila*, especially in warm water systems. Exposure of humans to pathogens in urban water occurs through recreational activities, household uses, occupational exposure, consumption of crops irrigated with contaminated water, or accidentally. Climate change affects these risks because 1. it results in urban water climate adaptations by urban planners (i.e., new water concepts and increase of water features in the city); 2. it modifies microbial populations and concentrations in water bodies (i.e. heavy rainfall leading to street run off and sewer overflows); 3. it results in different population exposure patterns (e.g., increased exposure to water because of temperature raise in the city). Therefore, research is needed on the new health risks derived from urban water exposure to inform urban water authorities and help them to implement risk control and mitigation measures.

Quantitative Microbial Risk Assessment (QMRA) is a useful tool to quantify the probability of developing a disease due to exposure to pathogenic microorganisms. It requires different knowledge steps: 1. the identification of the pathogenic microorganism(s) and its effects on human health; 2. the quantification of the microorganism in a single exposure (dose) which depends not only on the amount of the pathogen at the water source, but also on the population behaviour that determines the exposure pattern; 3. the translation of the dose to quantifiable health effects (for instance, by the use of dose-response models); 4. The integration of the previous steps to derive a risk estimate. To support risk management, the estimated risk is compared against health-based targets. Also, the knowledge collected in the QMRA process leads to understanding of the factors that are driving the risk and help to develop effective control measures.

In this thesis, the health risks of several urban water features have been assessed using QMRA tools. First of all, in **Chapter 2** several urban water features in Amsterdam were studied that were affected by climate change to a certain level, for instance because of increased pathogens concentration or increased magnitude of human exposure. At these locations, different activities take place that result in human-water contact with a certain degree of exposure. *Campylobacter spp.*, *Cryptosporidium*, norovirus, and *L. pneumophila* were the target pathogens, covering the main microorganism types and different diseases. Appropriate information about pathogen concentrations and exposure were selected from literature. Stochastic QMRA models were built for each water feature and exposure

combination with the aim of identifying the water features that are associated with the highest health risks. This is the first time that the risks of several kinds of water concepts and different pathogens are assessed together, helping water managers and authorities to set priorities for risk control measures. Higher risks were found for swimming and rowing at the river and lake, and for playing at a combined sewer overflow flooded street. Gathering site-specific pathogen data was proposed to reduce the uncertainty around the results and to help water managers in the decision making process.

Subsequently, a number of locations were selected and a summer monitoring campaign was conducted on a river, a lake, a pond, and a stormwater sedimentation pond, as well as a rain event study on the sedimentation pond and a bioswale (wadi) (**Chapter 3**). For this purpose, a methodology was developed to concentrate large volumes of water and molecular tools were used to determine the concentration of *Campylobacter spp.*, *Cryptosporidium*, adenovirus, and *L. pneumophila* in the water samples. Concentration of cyanobacteria (cyanochlorophyll-a) and microcystin were also determined. Pathogen concentrations were correlated with weather parameters to obtain information for risk assessment in future climate change scenarios. *Cryptosporidium* was not found at any location, adenovirus was found in the river and the lake occasionally in concentrations close to its limit of quantification, and *L. pneumophila* was found in the sedimentation pond (where formation of aerosols is not expected). *Campylobacter* was found at all locations in relatively high concentrations and these data were used to estimate the gastrointestinal risks derived from recreational exposure. The adenovirus data were used to determine the origin of the intestinal pathogens (human-faecal if present, animal-faecal if absent). High campylobacteriosis risks (above national incidence) were found at all locations, being highest for rowing in the river and playing at the wadi. Results of this study demonstrate the need of site-specific information for accurate risk assessment.

In **Chapter 4**, the study of the health risks from recreational exposure to a stormwater feature is described. Water plazas are new engineered water systems that deal with the excess of rain resulting from more frequent and strong storms due to climate change. They combine the stormwater storage function with a recreation facility for children. Water in a newly built water plaza was monitored during a rain simulation event. Molecular tools were used to determine the concentration of pathogens (*Campylobacter spp.*, *Cryptosporidium* and *L. pneumophila*). Furthermore, faecal source tracking tools, specifically human *Bacteroides*, avian *Helicobacter* and canine mitochondrial DNA, were used to determine the origin of these pathogens and, hence, their contribution to human disease. High concentrations of *Campylobacter spp.* were found, resulting in high risks (above the national incidence) and suggesting the need for further measures to reduce *Campylobacter* concentration in the water plaza or to limit recreational contact. The origin of *Campylobacter* was both animal and human, but the concentration was significantly higher in those samples where human *Bacteroides* was present, as compared to those were it was absent. The presence of human *Bacteroides* was not expected since the water plaza is

located in a separate sewer overflow system. Therefore, its presence indicates potential existence of cross-connections with the sanitary sewer that should be eliminated to ensure absence of human faecal contamination. Low concentrations of *L. pneumophila*, resulting in low risks, were found. Health risks could increase under future climate change scenarios.

The risks derived from consumption of lettuce that has been irrigated with reclaimed water containing human norovirus have been assessed in **Chapter 5**. Tertiary effluent is used in Catalonia for irrigation of lettuce with an overhead sprinkler system that allows close contact of the lettuce surface with the reclaimed water. The lettuce is subsequently sold at the local market. This study was the first to use norovirus site-specific data in a risk assessment of crops irrigated with reclaimed water, and the first one to assess the effects of virus internalization into lettuce crops. The concentration of norovirus was quantified in secondary and tertiary effluent with reverse-transcriptase quantitative PCR (RT-q-PCR). Norovirus concentration in tertiary effluent was not statistically different from the concentration in secondary effluent, indicating that the tertiary treatment is not efficient enough to reduce norovirus concentration, although the RT-q-PCR method is not able to discern between inactivated and infectious viruses. The risks were expressed in Disability Adjusted Life Years (DALYs) and were higher than the guideline threshold value, established by the WHO, of 10^{-6} DALYs/year. The additional norovirus reduction that was required to reach this guideline was computed. Further research is necessary to understand the internalization of viruses into crops and, hence, better quantify the health risks.

In **Chapter 6** a deterministic model was built using scientific literature to estimate the risk of developing Q fever (a disease caused by *Coxiella burnetii*) through exposure to drinking water produced from groundwater that is aerated with contaminated air during the Q fever outbreak in The Netherlands. *C. burnetii* emitted from a contaminated barnyard travels in the air and reaches the air inlet of a groundwater treatment plant for drinking water production. If the air is not filtered (or the filtration is not efficient), the intense contact between air and water in the aeration process results in transmission of *C. burnetii* cells to the water. Cells that survive the water treatment will reach the water faucets at the consumer's households, be aerosolized in the shower and inhaled by consumers. Cells that are deposited in the lower respiratory tract are able to produce Q fever disease. This study demonstrated that the risk through drinking water was negligible as compared to the airborne route of exposure, and stated that more research is needed in relation to *C. burnetii* dispersion, transfer and infection in order to reduce uncertainties.

Finally, general discussion, conclusions and recommendations are presented in **Chapter 7**. The water features assessed in this thesis showed high risks of gastrointestinal diseases (through *Campylobacter* or norovirus) but low risks of respiratory illness (legionellosis and Q fever). Uncertainties concerning each part of the QMRA and further research to improve the models (e.g. infectivity studies to determine concentrations of alive pathogens) are discussed. Measures to reduce the risks are proposed.

Samenvatting

Burgers waarderen water in de stad steeds meer vanwege de esthetische bijdrage aan de leefomgeving en de recreatiemogelijkheden. Water in de stad kan echter ook gezondheidsrisico's meebrengen, wanneer het water verontreinigd is met ziekteverwekkende micro-organismen. Ziekteverwekkers van het maagdarmkanaal (*Campylobacter*, *Cryptosporidium*, Norovirus, Rotavirus, etc.) kunnen in watersystemen aanwezig zijn via lozing van rioolwater, of door besmetting met faeces van dieren met daarin zoönotische ziekteverwekkers. Ook kunnen bepaalde ziekteverwekkende micro-organismen groeien in watersystemen, zoals toxische cyanobacteriën in stagnant water of *Legionella pneumophila* in warm water systemen. Blootstelling van mensen aan ziekteverwekkers in urbane watersystemen kan optreden bij waterrecreatie, aërosolen, gebruik in huis, werken aan watersystemen, eten van gewassen die zijn besproeid of gewassen met besmet water of bij ongelukken. Klimaatverandering kan deze risico's vergroten doordat 1) in de stedelijke planvorming wordt geanticipeerd op klimaatverandering, waarbij meer en nieuwe watersystemen in de stad worden aangelegd; 2) de microbiële populatie en concentratie in watersystemen kan veranderen door klimaatverandering; 3) er andere patronen voor de blootstelling van burgers aan watersystemen ontstaan (bijvoorbeeld toenemende waterrecreatie bij stijgende temperatuur in de stad). Daarom is onderzoek nodig naar (nieuwe) gezondheidsrisico's als gevolg van stedelijke watersystemen, om ontwikkelaars en beheerders te ondersteunen in ontwerp en beheer van veilige watersystemen.

Quantitative Microbial Risk Assessment (QMRA) is een geschikt instrument om te bepalen wat de gezondheidsconsequenties zijn van de blootstelling aan ziekteverwekkende micro-organismen. QMRA bestaat uit de volgende stappen: 1) de identificatie van de relevante ziekteverwekkende micro-organismen en hun gezondheidseffect; 2) de kwantificering van de hoeveelheid (dosis) micro-organismen waaraan burgers via watersystemen worden blootgesteld per blootstellingsgebeurtenis, welke wordt bepaald door de concentratie ziekteverwekkers in het watersysteem en door het gedrag van burgers in/rondom de watersystemen; 3) de gezondheidseffecten als gevolg van deze dosis (via het gebruik van dosis-respons modellen); 4) de integratie van de informatie uit de voorgaande stappen om een inschatting van het gezondheidsrisico te maken.

In dit proefschrift zijn de gezondheidsrisico's van diverse stedelijke watersystemen bepaald met QMRA. In **Hoofdstuk 2** zijn watersystemen in een wijk van Amsterdam onderzocht. Op de geselecteerde locaties vinden verschillende activiteiten in en om het watersysteem plaats die resulteren in verschillende mate van contact van burgers met water. *Campylobacter spp*, *Cryptosporidium*, norovirus, en *Legionella pneumophila* zijn gekozen als pathogenen, als referentie voor de belangrijkste typen micro-organismen en verschillende typen ziekte. Relevante informatie over concentratie ziekteverwekkers en blootstelling werden geselecteerd uit de literatuur. Stochastische QMRA modellen zijn

ontwikkeld voor elk van de watersystemen, elke pathogeen en elk type blootstelling, met als doel de watersystemen in te delen naar hun gezondheidsrisico en te bepalen welke watersystemen aanleiding geven tot verhoogde risico's en nader onderzoek. Dit is de eerste keer dat het risico van diverse watersystemen en diverse pathogenen in één analyse zijn onderzocht. Zwemmen en roeien in de rivier en het meer, en spelen in water op straat uit een overstromend gemengd riool leverde relatief hoge risico's. Hiermee waren de prioriteiten voor risicobeheersing voor de waterbeheerders bekend. Aanbevolen werd locatie-specifieke gegevens te verzamelen over pathogenen om de onzekerheid van de risicoanalyse te verkleinen, zodat de besluitvorming over risicobeheersing beter onderbouwd kan worden.

In de vervolgstudie zijn watersystemen uit Amsterdam geselecteerd en in de zomerperiode wekelijks doorgemeten op indicatororganismen en op de geselecteerde pathogenen. De locaties waren de rivier, het meer, een stadsvijver en een bezinkvijver voor opgevangen regenwater. Daarnaast is een wadi doorgemeten tijdens een regenbui (**Hoofdstuk 3**). Om dit te kunnen doen is een methode ontwikkeld om grote volumes water te concentreren en daar met moleculair microbiologische methoden (qPCR) de concentratie *Campylobacter spp.*, *Cryptosporidium*, adenovirus en *Legionella pneumophila* in het water te bepalen. Ook de concentratie cyanobacteriën (cyanochlorophyll-a) en microcystine werd bepaald. De correlatie tussen de concentratie pathogenen en weer parameters werd onderzocht om het effect van klimaatscenarios te kunnen inschatten. *Cryptosporidium* werd niet aangetroffen, adenovirus werd gevonden in de rivier en het meer (enkele malen) in concentraties dicht bij de detectielimiet. *L. pneumophila* is aangetroffen in de bezinkvijver (waar geen aerosolvorming van betekenis werd verwacht). *Campylobacter* is op alle locaties aangetroffen in relatief hoge concentraties. Deze gegevens zijn gebruikt om het risico op gastro-enteritis (GE) als gevolg van waterrecreatie te berekenen. De adenovirus data werden gebruikt om de herkomst van de fecale verontreiniging te bepalen (humaan-faecaal als aanwezig, animaal-faecaal als afwezig). Alle locaties gaven hoge campylobacteriose risico's te zien, boven de nationale GE incidentie. Het hoogst waren de risico's voor roeien op de rivier en spelen in de wadi. Deze studie onderbouwde het belang van locatie-specifieke metingen voor een accurate risicoanalyse.

Hoofdstuk 4 beschrijft een studie naar de gezondheidsrisico's van recreatie in een regenwateropvangsysteem. Water in een recent aangelegd waterplein (een engineered watersysteem dat lokale regenwateropvang combineert met waterrecreatie). Voor de studie werd een regenbui gesimuleerd. De concentratie pathogenen (*Campylobacter spp*, *Cryptosporidium* en *L. pneumophila*) werd gemeten met qPCR. Daarnaast is het water ook onderzocht op dierspecifieke merkers: humane *Bacteroides*, aviaire *Helicobacter* en canine mitochondriaal DNA om de herkomst van de verontreiniging met pathogenen te bepalen, en daarmee hun bijdrage aan het gezondheidsrisico. Ook hier werden hoge concentraties *Campylobacter* gevonden, wat resulteerde in hoge gezondheidsrisico's (boven de national GE incidentie). Op basis daarvan lijken maatregelen tot verdere reductie van de

Campylobacter concentratie in het water in het plein op zijn plaats en ook het beperken van de directe blootstelling. De *Campylobacter* bleek zowel van dieren als mensen afkomstig te kunnen zijn. De concentratie *Campylobacter* was significant hoger in de monsters waar ook humane *Bacteroides* aanwezig was dan in monsters waar geen humane *Bacteroides* aanwezig was. De aanwezigheid van humane *Bacteroides* op het plein was niet verwacht, omdat het plein een gescheiden rioolstelsel heeft. De aanwezigheid van een humane faecale merker is een aanwijzing dat er mogelijk een kruisverbinding bestaat tussen het gemengde riool in de nabijheid van het waterplein. Als dat zo blijkt te zijn, zou deze moeten worden verwijderd om geen faecale verontreiniging van humane herkomst op het plein toe te laten. *L. pneumophila* was aanwezig in lage concentraties en het berekende legionellose risico was eveneens laag. Dit zou toe kunnen nemen als door klimaatverandering verhoogde watertemperaturen voorkomen.

De gezondheidsrisico's van irrigatie van groente met gezuiverd rioolwater met daarin norovirus zijn bepaald in **Hoofdstuk** 5. In Catalonië wordt tertiair effluent gebruikt voor irrigatie van groente die rauw wordt gegeten (zoals sla). Door de sproei-irrigatie is er direct contact tussen water en groente. De groente wordt op de lokale markt verkocht. Deze studie gebruikt (voor het eerst) locatie-specifieke data over norovirus in rioolwater in een QMRA van irrigatie met rioolwater. Ook werd in deze studie voor het eerst het effect van internalisatie van virussen in de groente meegenomen. De concentratie norovirus werd gekwantificeerd in secundair en tertiair effluent met reverse-transcriptase quantitative PCR (RT-q-PCR). De norovirus concentratie in tertiair effluent was statistisch niet verschillend van de concentratie in secundair effluent. De tertiaire zuivering (hier chloor en UV) bleek niet effectief in het reduceren van de norovirus concentratie, hoewel de RT-q-PCR methode geen onderscheid maakt tussen levende en dode micro-organismen. De gezondheidsrisico's zijn in deze studie uitgedrukt in Disability Adjusted Life Years (DALYs) en de berekende risico's lagen boven de grenswaarde die wordt aanbevolen door de WHO: 10^{-6} DALYs/jaar. Dit betekent dat aanvullende norovirus reductie nodig is om de volksgezondheid afdoende te beschermen. Verder onderzoek is nodig naar de rol van internalisatie van virussen in groente gewassen, zodat de QMRA verder kan worden verbeterd.

In **Hoofdstuk 6** is een deterministisch model ontwikkeld, op basis van informatie uit de wetenschappelijke literatuur, om het gezondheidsrisico te bepalen van het ontwikkelen van Q-koorts (infectieziekte veroorzaakt door *Coxiella burnetii*) via blootstelling aan drinkwater uit (belucht) grondwater, ten tijde van de Q-koorts uitbraak in Nederland. *C. burnetii* die uit een stal in de lucht wordt geblazen reist via de lucht (ook) naar de luchtinlaat van de beluchting van een grondwaterzuivering voor de productie van drinkwater. Als de lucht niet wordt gefiltreerd bij de inname, of de filtratie is niet efficiënt, zouden door het intensieve lucht-watercontact *Coxiella* bacteriën kunnen worden overgedragen naar het grondwater. Cellen die de waterzuivering overleven zouden de tapkraan van woningen kunnen bereiken en daar via aerosolen op de mensen worden overgedragen. Cellen die terecht komen in de diepere luchtwegen kunnen Q koorts

veroorzaken. Deze studie demonstreerde dat het risico op overdracht van Q-koorts via drinkwater verwaarloosbaar laag is, vergeleken met directe blootstelling aan aerosolen in de omgeving van besmette stallen. Onderzoek naar de dispersie in de lucht, transport door de lucht en infectie/dosis-respons verbeterd inzicht in het risico op overdracht van *Coxiella* via de lucht en ook via drinkwatersystemen.

In het laatste hoofdstuk (**Hoofdstuk** 7) worden de algemene discussie, conclusies en aanbevelingen gepresenteerd. De watersystemen die in dit proefschrift zijn onderzocht vertoonden hoge risico's voor gastro-enterale infectieziektes (door *Campylobacter* of norovirus), maar lage risico's voor respiratoire infectieziekten (legionellose en Q koorts). Onzekerheden in de modellering worden besproken en aanbevelingen worden gedaan voor nader onderzoek om de QMRA modellen verder te verbeteren, zoals toepassing van methoden om de infectiviteit van pathogenen in de watersystemen te bepalen. Ook worden beheersmaatregelen om de risico's te reduceren voorgesteld.

Contents

Chapter 1: General Introduction

1. Introduction

The Netherlands has the second largest population density in Europe, with nearly 500 inhabitants/km^2 [1]. The amount of people living in urban areas has increased from 60% of the total population in 1960 to 89% in 2013 [2]. Urban developments to support growing communities impact the land and water [3], so sustainable measures are sought to reduce this impact. Furthermore, adaptation measures are needed to minimize unavoidable climate change effects [4]. Therefore, municipalities tend to implement new urban development projects that address current and future sustainability issues [3], including water sensitive urban development (WSUD), i.e. the sustainable use of water in the cities. Examples of WSUD are stormwater reservoirs (swales, wadis or water plazas), street water infiltration or water reservoirs behind the dikes. These WSUD features are added up to the already existing water bodies, such as rivers, lakes, canal or dams.

Citizens often perceive urban water and green spaces as positive elements: they provide aesthetic quality to the neighbourhood and offer recreational opportunities [5]. In The Netherlands, house pricing increases when houses have gardens facing water or are overlooking water or open green spaces [6]. Hence, WSUDs are often combined with ornamental fountains/ponds, water parks, spray parks or swimming pools/ponds. However, urban water features have also the disadvantage of potential health hazards as a result of human-water interaction [7-9].

A health hazard is anything that can cause harm (loss of life, injury, illness…). In water, these hazards are: drowning and near-drowning, unintentional injury, anxiety, infection [10] and intoxication through contact/inhalation of chemical hazards, e.g. chlorine disinfection-by products [11]. Microbial hazards can be classified as bacteria (e.g. enterohaemorrhagic *Escherichia coli*, *Campylobacter spp.*, *Legionella pneumophila*…), protozoa (e.g. *Cryptosporidium spp.*, *Giardia intestinalis*, *Toxoplasma gondii, Naegleria fowleri*…), viruses (norovirus, rotavirus, hepatitis A virus…), cyanobacteria (*Microcystis*, *Anabaena*…), nematodes, cestoda, and filamentous fungi (Figure 1−1) [12, 13]. Exposure to microbial hazards in water can result in gastrointestinal illnesses; fever; skin, ear and eye complaints; or more severe illnesses, such as hepatitis and meningitis [13].

Several sources of microbial hazards in urban water exist, depending on the kind of water system and the origin of the water. Sources are animals and humans, and ubiquitous microorganisms. Microbial hazards may be present in water bodies due to input of faecal material such as sewage discharge containing human enteric pathogens (*Campylobacter spp.*, *Cryptosporidium spp.*, norovirus, rotavirus, etc.) or animal faecal input (from waterfowl, dogs, and other domestic and wild animals) containing zoonotic pathogens [14,

15], or growth of microorganisms in urban water bodies and features, such as toxic cyanobacteria in stagnant waters [16], or *Legionella pneumophila*, especially in warm water systems [17]. Microbial risks are also influenced by climate change. A higher frequency and strength of storms and draughts affects the concentration of pathogens [14, 18][19], a temperature increase may promote formation of cyanobacterial blooms [20].

New water concepts introduce different ways of exposure of citizens to water and different ways of microbial contamination that may result in health risks. To protect public health adequately, these risks need to be understood: are pathogens present in these new water concepts? If so, what are the sources of contamination? How (often and intense) are people exposed to water (with pathogens) at these new concepts? What is the associated health risk? Is this risk significant compared to similar risks from other types of exposure (such as contaminated food)? Understanding the risks is the basis for determining if mitigating actions are needed, where they are needed most and what actions are most effective in reducing the risk. The research in this thesis aims to provide such understanding for several (new) water concepts and microbial hazards.

2. Urban Waters

Urban waters include different types and qualities, and different uses. Several of these uses might result in human exposure to hazards present in water. The kinds of urban waters, examples, contamination sources, uses, and exposure pathways are summarised in Table 1-1. Water uses that can result in human exposure to waterborne hazards are:

- Recreational exposure (through accidental ingestion or inhalation of aerosolized particles)
- Household exposure through domestic activities: gardening, showering, drinking, toilet flushing
- Consumption of crops irrigated with reclaimed water
- Occupational exposure: farmers using reclaimed water for crops or landscape irrigation (e.g. golf courses), water treatment plant workers, etc.
- Accidental/unintended exposure: inhalation of aerosols from cooling towers or fountains, falling in water, etc.

3. Health Impact Assessment and Quantitative Microbial Risk Assessment

Health Impact Assessment (HIA) is "A combination of procedures, methods and tools by which a policy, programme or project may be judged as to its potential effects on the health of a population, and the distribution of those effects within the population." [21]. In the context of this thesis, the effects on health of a population are those derived from infection with microbial pathogens present in urban water bodies. Different types of studies have been used to characterize health risks derived from human exposure to water, food, and the

environment, namely, microbial analyses, epidemiological studies and Quantitative Microbial risk Assessment (QMRA). Their advantages and limitations are summarized in Table 1−2.

Risk assessment is the process of quantifying the probability of a harmful effect to individuals or populations from certain human activities (here, infectious disease derived from human interaction with water in the city). Water quality studies do not provide information on health risks, and epidemiology studies are generally not specific and sensitive enough. This is important in gastrointestinal illnesses associated with water, since they are also associated with food and other exposures, and it might be difficult to differentiate the exposure source using epidemiological methods.

QMRA can estimate risk from a variety of different exposures and/or pathogens that would be too difficult to measure through epidemiological investigations due to the high cost and necessity of studying large populations [22]. QMRA is also useful to analyse rare events and to test "what if" scenarios, helping in targeting management interventions [23]. Therefore, in this thesis, QMRA is the method of choice to assess health risks, complemented with microbial analysis (when opportune). Furthermore, outputs of epidemiological studies are used in the QMRA studies, such as duration and severity of disease, mortality, and in dose-response functions where human challenge study data were complemented with data from outbreaks [24]. Also data from national disease surveillance studies were used as reference for the disease incidence/burden outcome of the QMRAs, and their outcomes are used for setting health based target levels of pathogens/indicators or risks in waters.

QMRA consists of four components: hazard identification, exposure assessment, hazard characterization and risk characterization [25]. In the hazard identification step, the system under evaluation is described and the hazards and hazardous events are identified. The exposure assessment aims to determine the amount of microorganisms that correspond to a single exposure (dose) or a set of exposures. In the hazard characterization step, the health outcomes associated with exposure to pathogens are determined and a dose-response relationship relates the dose of the agent with the quantitative health effects on the exposed population (disease, death…). The final step of the process, risk characterization, integrates the information from the exposure assessment and the hazard characterization into a risk estimate [26, 27]. In the following paragraphs, information on each of the QMRA steps, with relevant literature for conducting risk assessment of exposure to urban water, is provided.

Table 1-1: Urban related types of water, contamination sources, uses and human exposure.

Water Sources	Definition /examples	Contamination Source	Uses	Exposure
Natural surface waters	Naturally occurring water on the Earth surface (e.g. rivers, lakes, ponds, streams) and underground (aquifers, underground streams)	WW or effluent discharge, agricultural run-off, wild-life contamination (birds droppings, birds corpses, etc.), growth of waterborne microorganisms (algal blooms)	Recreational: high contact (swimming), low contact (rowing, playing, etc.); drinking water production	Accidental ingestion, inhalation of aerosolized particles, skin contact
Engi-neered surface waters	Man-made water channels and water retention structures (canals, dams)	WW or effluent discharge, agricultural run-off, wild-life contamination, growth of waterborne microorganisms	Recreational: high contact , low contact; drinking water production	Accidental ingestion, inhalation of aerosolized particles, skin contact
Coastal waters	Urban coastal waters	WW or effluent discharge, agricultural run-off, wild-life contamination	Recreational: high contact , low contact	Accidental ingestion, inhalation of aerosolized particles, skin contact
Grey water	Wastewater generated from wash hand basins, showers, baths, laundry, etc.	Human waste generated from the shower, laundry, etc.	Gardening, toilet flushing	Accidental ingestion, inhalation of aerosolized particles, consumption of crops irrigated with grey water

Table 1-1 Continued.

Water Sources	Definition/examples	Contamination Source	Uses	Exposure
Municipal WW	Water that has been adversely affected by human municipal use.	Human waste (faeces, toilet paper, urine, human fluids). If combined sewer systems also rainfall runoff (with wild-life droppings, etc.)	Treated waste water (reclaimed water) used for irrigation of landscape, crops, etc.	Consumption of crops irrigated with treated wastewater, accidental ingestion of water and inhalation of aerosolized particles by workers; exposure in flooded streets
Drinking water	Water safe enough to be ingested by humans without posing a health risk in short or long term	Not efficient treatment due to source excess contamination or treatment failure, recontamination during distribution (biofilms, leakage)	Drinking and domestic uses (showering, toilet flushing, etc.), municipal uses (decorative, etc.)	Ingestion, inhalation of aerosolized particles
Rain water	Roof collection systems, street collection, street runoff	Wild-life contamination (droppings, corpses), sewage from illicit cross-connections in separate sewers	Recreational, toilet flushing, discharge in engineered waters, decorative uses	Ingestion, inhalation of aerosolized particles

WW, waste water

Table 1-2: Comparison of types of studies used for the assessment of health risks[22].

Type of study	Contributions	Limitations
Microbial analysis	- Determines concentrations of different pathogenic organisms in water - Provides data on pathogen die-off rates - Can help to identify sources of pathogens - Used to link pathogen to infection/disease	-Results are pathogen concentrations, not health risks -Expensive unless indicators are used, but there is no clear correlation between indicators and pathogens - Collection of samples and analysis may be time-consuming - Needs trained staff and laboratory facilities - Lack of standardized procedures for the detection of some pathogens or their recovery from food/water matrices. - Recovery percentages may show high variability - Some methods do not determine viability
Epidemiological studies	- Measure actual disease in an exposed population - Can be used to test different exposure hypotheses	- Expensive - Bias can affect results (e.g. underreported cases) - Large sample sizes needed to measure statistically significant health outcomes and discriminate waterborne exposure from other types of exposure - Ethical clearance needed - Need for balance between power of study and its sensitivity
QMRA	- Can estimate very low levels of risk of infection/disease - Low-cost method of predicting risk of infection/disease - Facilitates comparisons of different exposure routes -Provides understanding of the causes and pathways of the risk, so provides a basis for adequate risk management	- Exposure scenarios can vary significantly and are difficult to model - Validated data inputs are not available for every exposure scenario - Predicts risks from exposure to one type of pathogen at a time

3.1. Hazard Identification: Waterborne Pathogens and Diseases

Microbial hazards can be present in water through different pathways:

- Waterborne microorganisms: their natural habitat is water. Most of them are not pathogenic *per se* but can be in specific circumstances. For instance, exponential growth due to favourable environment factors can result in an increase in pathogen

concentration, enough to produce disease (*L. pneumophila*) or in production of toxins (cyanobacteria blooms). Also, opportunistic microorganisms which only cause a specific disease on immunocompromised hosts (e.g. *Pseudomonas aeruginosa* causes otitis in healthy people but can infect burns, wounds, lungs and urinary tract, and cause septicaemia in hospitalized patients) [16, 17, 28].

- Human faecal contamination: surface waters can be impacted with contamination from faecal origin because of WWTP effluent discharge, combined sewer overflows or discharge of untreated sewage. *Campylobacter spp.*, *Cryptosporidium spp.*, *G. intestinalis*, *E. coli*, norovirus, and hepatitis A virus are examples of human faecal pathogens [15].
- Animal faecal origin: dogs, birds, and other domestic and wild animals shed zoonotic pathogens in their depositions/droppings that can reach the water system through direct deposition, stormwater overflow, or subsurface runoff. Examples of zoonotic pathogens are *Campylobacter spp.* and *Cryptosporidium spp.* (in dogs and birds), *Leptospira* (in rodents), *T. gondii* (in cats), *Toxocara canis* (in dogs), etc. [14, 15].

Figure 1−1 shows waterborne pathogens, classified as human faecal, zoonotic faecal and non-faecal origin. Because of the large amount of pathogens that can be found in water, a selection of reference pathogens was made, based on the following criteria:

- Representation of the three major classes of microorganisms (bacteria, virus and protozoa).
- Inclusion of diseases of different nature (gastrointestinal, respiratory, skin)
- Their presence in water poses a hazard in the European setting, based on high incidence/prevalence in the population, infectivity and severity of disease, persistence in the environment, resistance to adverse environmental circumstances or water treatment, possibility of growth in the system (e.g., in biofilms or formation of blooms).

Campylobacter spp., *Cryptosporidium spp.*, adenovirus 40/41, norovirus, *L. pneumophila* and cyanobacteria were the selected pathogens. Furthermore, the zoonotic airborne pathogen *Coxiella burnetii* was also included in the study, to assess the possibility of Q fever transmission through water during the 2007-2012 outbreak spread via goat farms in The Netherlands [29]. The selected reference pathogens are discussed in more detail.

Gastrointestinal Pathogens

Campylobacter are non-spore forming, microaerophilic, Gram-negative zoonotic bacteria, 0.2 to 0.4 by 0.5 to 5 µm, presenting a curved or spiral shape [12]. The thermophilic species *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* are human pathogens [30]. Following an incubation period of one to eight days, acute diarrhoea appears. It can be preceded by flu-like illness, acute abdominal pain, or both. The diarrhoea can be profuse and watery in some cases, and it can contain blood or leukocytes [31]. Usually, *Campylobacter* is shed in

faeces for less than 3 weeks after infection, but asymptomatic carriers can shed it during 4 months. *C. jejuni* can also cause the Guillain-Barré syndrome, an acute flaccid paralysis, rheumatoid arthritis, irritable bowel syndrome, and inflammatory bowel disease [32].

Campylobacter can be found in water and sewage worldwide, including groundwater (probably due to infiltration of farm faecal material), streams, rivers, canals, ponds, ornamental lakes, reservoirs, drinking water, marine water, and sewage [33]. It can survive in water for many weeks, or months, at temperatures below 15°C, but only few hours in adverse conditions, being temperature the limiting factor for its survival [12]. Chlorine is an effective disinfectant, and *Campylobacter* shows susceptibility to chlorine similar to *E. coli*. At 0.1 mg/L of free chlorine, pH values of 6 and 25 ºC, 99% of *Campylobacter* where inactivated after 5 to 15 min [34, 35]. Among bacteria, *Campylobacter* was found the most common cause of gastroenteritis in a cohort study in the Netherlands [36]. In a laboratory surveillance study conducted between 1991 and 2001, *Campylobacter* was the main bacterial pathogen isolated from stools from the Dutch population [37].

Cryptosporidium is an obligate intracellular coccidian parasite with a monoxenus life cycle (it completes its cycle in a single host). It is transmitted via an environmentally resistant oocyst (of 4-6 µm in diameter) excreted in the faeces of the host (infected hosts can excrete 10^9 to 10^{10} oocysts) [38], including humans, dogs, cattle, horses and mice [39]. Transmission can be direct oral-faecal transmission or, due to the oocysts robustness, indirect through food, water or fomites contamination. Human disease is caused by the species *C. parvum, C. hominis, C. meleagridis, C. felis, C. canis, C. muris,* and *Cryptosporidium pig gentotype 1* [40]. Among them, *C. hominis* and *C. parvum* cause most infections in humans [41]. Presence of *Cryptosporidium* in water can be indicative of human or animal faecal contamination [38].

Cryptosporidiosis has a mean incubation period of 7 days and symptoms last for about one to two weeks. It consists of watery or mucoid diarrhoea with dehydration, weight loss, anorexia, abdominal pain, fever, nausea and vomiting. Oocysts are shed in the faeces 7 days after cessation of diarrhoea [39] and can remain infective in cool moist conditions for months, especially in northern countries where surface water temperatures remain cold but above freezing. Furthermore, they are resistant to chlorine, being frequently the cause of gastroenteritis outbreaks in swimming pools. *Cryptosporidium* occurs frequently in raw water world-wide. Water recreation has been associated with cryptosporidiosis outbreaks. Ground waters are also impacted [12].

In a cohort study, *Giardia lamblia* was identified as the main parasite cause of gastroenteritis (4%) followed by *Cryptosporidium* (2%) [36]. However, *Cryptosporidium* was responsible for 50.8% and *Giardia* for 40.6% of 325 water associated outbreaks of parasitic protozoan disease documented worldwide and 50.3% of outbreaks associated with recreational water were related to *Cryptosporidium*, while only 13.6% were related to *Giardia* [42]. On top of the epidemiological facts, *Cryptosporidium* is more relevant than *Giardia* for urban water concepts because it is a small parasite, so it is difficult to remove

by physical treatment, it is resistant to oxidizing disinfectants, and it has shown to survive longer in environmental waters [31].

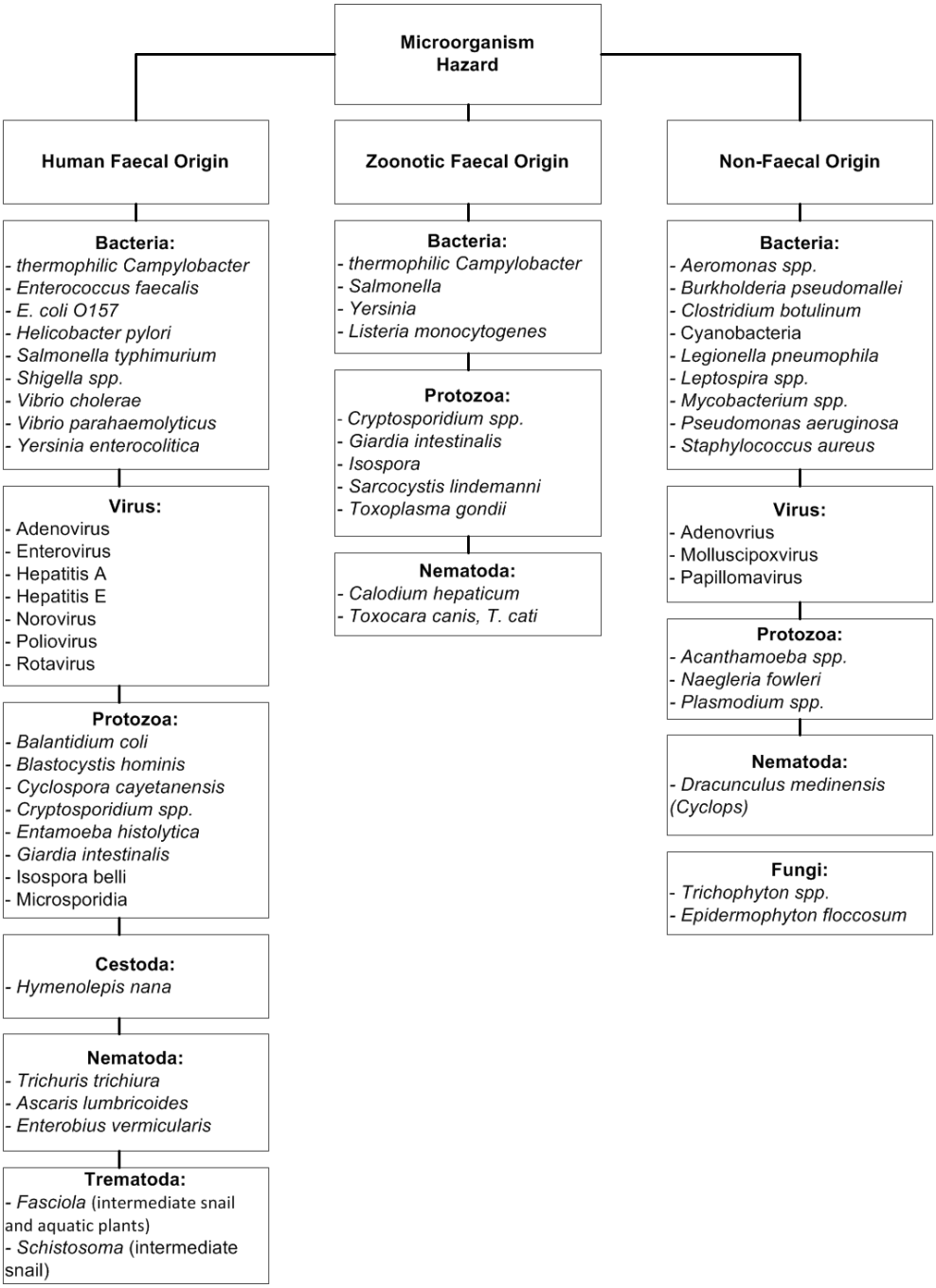

Figure 1−1: Pathogens in urban water [31, 43].

Human adenoviruses belong to the genus Mastadenovirus, from the Adenoviridae family. There are currently 51 identified serotypes (Ad1-Ad51), divided into six subgenera (A-F) and four hemagglutination groups (I-IV) [44]. Adenoviruses are non-envelope icosahedral virions containing linear double-stranded DNA of about 35 Kbases enclosed in a capsid of 90-100 nm [45]. Adenoviruses can survive in the environment for long periods, including in cold waters, and are resistant to heat, freezing, physical and chemical agents, and pH conditions [44].

Although some adenovirus infect animals, human adenoviruses are highly specific to humans [45]. Only one third of the known human adenovirus serotypes are pathogenic. They cause a wide variety of diseases, including upper and lower respiratory illness, conjunctivitis, cystitis, and gastroenteritis. Most illnesses are self-limited but the viruses (all of them) can remain in the gastrointestinal track and be shed for a long period of time. Therefore, contact with water of any kind (ingestion, inhalation, skin/mucosa contact) can be a source of infection. In children Ad1 and Ad2 are more prevalent, while in adults, infections are usually due to Ad3, Ad4 and Ad7, suggesting the existence of long-lasting immunity for Ad1 and Ad2 [44]. Enteric adenovirus (Ad40/41, group F) are responsible for most cases of adenovirus associated gastroenteritis, are resistant to conventional disinfection methods, are excreted in high rates by infected humans, and are highly present in the environment [46].

Contact with recreational water has been associated with adenovirus outbreaks, being the most common cause of outbreaks in swimming pools [44]. Enteric adenoviruses are important in urban waters because they are shed by many individuals (also asymptomatic ones), are environmentally robust, have been frequently detected all year round in (recreational) inland fresh waters, coastal waters, and wastewater [44, 46-49], and have been associated with recreational outbreaks in pools, lakes and ponds [50].

Noroviruses are RNA viruses belonging to the Caliciviridae family and consist of 5 different known genogroups. NoVGI and NoVGII are pathogenic for human [51], being NoVGII most frequently isolated in outbreaks [52]. Norovirus is the leading cause of diarrhoea worldwide among people of all ages. Outbreaks have a peak during cold months in temperate climates, although they happen all year-round. In children, peaks occur during spring and summer [53]. Noroviral gastroenteritis has an incubation period of 24-48 h and consists of acute onset of nausea, vomiting, abdominal cramps, myalgia and non-bloody diarrhoea. It is a self-limited disease, with symptoms resolving in 2-3 days. The disease is longer in hospitalized patients and it can cause death, and is associated with necrotizing enterocolitis. [53]

The faecal-oral spread, and through vomitus and environmental surfaces, are the most common ways of virus propagation. Several factors contribute to its high contagion rates: the high infectivity (the ID50 is 18 virus particles [54]), shedding of virus in faeces for a long time, even after the disease is resolved, its high resistance to chlorine, and lack of long-term immunity [53]. A prospective cohort study among the general Dutch population

conducted between 1998 and 1999 revealed norovirus as the main cause of gastroenteritis, causing 11% of the diseases [36]. In a more recent study, the disease burden of foodborne pathogens was evaluated for the year 2009, and norovirus was found, again, the pathogen with highest disease incidence [32]. Recreational surface water, including lakes, swimming pools, and recreational fountains, have been associated with norovirus outbreaks [55]. Hence, their presence and in urban waters may be a significant route of transmission.

Respiratory Pathogens

Legionella are Gram negative coccobacilli of 0.3-0.9 µm width and 2-20 µm length. Currently, more than 50 species are known, and *L. pneumophila*, human pathogenic species, comprises 16 serogroups. *Legionella spp.* are ubiquitous bacteria, found in natural aquatic environments, moist soil and mud. Because they can survive chlorination, they are able to enter water supply systems and proliferate in thermal habitats, such as air-conditioning cooling towers, hot waters, shower heads, whirlpool spas, ornamental fountains, etc. [56]. Heavy rainfall has been associated with increased incidence of legionellosis [57, 58] and *L. pneumophila* has been found in rainwater on roads [59], and pluvial floods [60].

L. pneumophila is the causal agent of Legionnaire's disease (LD) a serious, sometimes fatal, pneumonia. *Legionella* is one of the three most common causes of severe pneumonia and is isolated in 1-40% of hospital acquired pneumonia. 90% of LD cases are originated by *L. pneumophila* serogroup 1 [56]. The LD incidence in The Netherlands was studied through three different methods and 1.15 (notified), 2.42 (ascertained) and 2.77 (estimated) cases in 100.000 habitants were found [61]. *L. pneumophila* is also responsible of a mild self-limited flu-like illness, Pontiac fever [56]. The incubation period of LD is between 2 and 10 days, and the disease can be preceded by headache, myalgia, asthenia and anorexia. Clinically, the disease cannot be distinguished from pneumococcal pneumonia, symptoms of which include fever, non-productive cough, myalgia, rigors, dyspnoea, and diarrhoea. Mortality rates range from less than 1% to 80%, depending on the underlying health status of the patient [56]. *L. pneumophila* is important in urban waters because of their ability to grow in engineered water systems and because they have been recently found in pluvial floods [59, 60].

Coxiella burnetii is an obligate intracellular member of the Gammaproteobacteria. Livestock (goats, sheep, cattle) and pets are major reservoirs of *C. burnetii* [62]. The environmental form of the bacteria is very resistant to drying, UV irradiation, acid or alkaline pH, disinfectants and other chemicals and at 4 °C, its viability is retained for 1 year in unchlorinated tap water [63].

Coxiella burnetii causes Q fever in humans. It does not usually cause clinical disease in its reservoirs, although high rates of abortion in goats and sheep have been linked to *C. burnetii* infection. Organisms are excreted in milk, urine, and feces of infected animals. Most importantly, during birthing the organisms are shed in high numbers within the

amniotic fluids and the placenta [64-67] and sheep placenta can contain up to 10^{10} infectious doses of *C.burnetii* per gram of tissue [68]. Infection of humans usually occurs by inhalation of these organisms from air that contains contaminated airborne barnyard dust. Humans are often very susceptible to the disease, and very few organisms may be required to cause infection [69].

Most acute cases of Q fever begin, after an incubation period of three to four weeks, with sudden onset of one or more of the following: high fevers (lasting from one to two weeks), severe headache, general malaise, myalgia, confusion, sore throat, chills, sweats, non-productive cough, nausea, vomiting, diarrhea, abdominal pain, and chest pain. Only about 40% of the people infected with *C. burnetii* show signs of clinical illness. Twenty percent of patients with a symptomatic infection will develop acute disease with pneumonia and/or hepatitis and 1%-3% of people with acute Q fever die of the disease [62, 70, 71]. Chronic Q fever, characterized by infection that persists for more than 6 months, is uncommon (developing in 1-5% of the acute Q fever cases), but is a much more serious disease that can result in endocarditis or hepatitis, and causes death on 65% of patients [72-74].

Q fever was rare in the Netherlands before 2007, with only around 15 cases reported annually. Since 2007, the number of cases increased, starting with an outbreak in Noord-Brabant in 2007 with 168 cases. In 2008, 1000 cases were reported in Noord-Brabant and the southern part of Gelderland and in 2009, 2354 cases were found in the Netherlands, with 6 fatalities. In 2010, 2011 and 2012, 504, 81 and 66 cases were reported [29]. Because of this outbreak was ongoing at the beginning of this thesis work, the potential risks of transmission of *C. burnetii* through water were assessed.

Other Pathogens

Cyanobacteria are a group of ubiquitous photosynthetic prokaryotes that occur specially in surface waters (lakes) but are also found in reservoirs and brackish waters worldwide [75]. In favourable conditions, they grow forming blooms and producing, as secondary metabolites, cyanotoxins that can pose a risk to human health. Massive growth often occurs during the summer months in surface waters [12]. A high variety of cyanotoxins exist that can be classified, according to the human health effects they produce, into hepatotoxins (e.g. microcystin), neurotoxins (e.g. anatoxin-a), cytotoxins (e.g. lipopolyyscaccharidic (LPS) cytotoxins), irritant and gastrointestinal toxins (e.g., aplysiatoxin), and other cyanotoxins (e.g. microviridin J) [76]. Each cyanotoxin can be produced by several cyanobacterial species and each species can produce several toxins. Moreover, within a single species, different genotypes occur with different ability for cyanotoxins production. Frequently, cyanobacterial blooms produce several cyanotoxins at the same time [75].

Humans can be exposed to cyanotoxins during water recreation activities through the oral route, dermal contact, or inhalation. Health outcomes that have been described after cyanotoxin exposure during water recreation include severe headache, pneumonia, fever,

myalgia, vertigo, and blistering in the mouth. Long inhalation exposure through canoeing or swimming in surface water led to respiratory symptoms and pneumonia cases. Allergic responses such as cutaneous effects, rhinitis, conjunctivitis, asthma, and urticaria are thought to be caused by cyanobacterial LPS endotoxins [75]. Microcystins, the most common human hazardous cyanotoxins, are very stable compounds and once airborne can potentially travel many kilometres without degrading. Therefore, airborne microcystins can pose a risk not only to surface water users but also to populations near contaminated lakes [77].

Occurrence of cyanobacteria (blooms) in surface waters during summer is increasingly reported and leading to beach closures and no-bathing advice. Between 1991 and 2007, cyanobacteria were identified as the causal agent for several outbreaks related to recreational water in The Netherlands. Concretely, 11.6% (8/69) cases of skin disease, 13.8% (8/58) of gastroenteritis, and 50% (4/8) of both gastroenteritis and skin complaints were positive for cyanobacteria [9]. Cyanobacteria are selected as reference pathogens in urban water studies because of their implication in illnesses and public awareness, their ubiquity and blooms in surface water, and because of the relation between the increase in cyanobacterial blooms and climate change [20].

3.2. Exposure Assessment

Exposure assessment is the quantitative estimation of the probability of exposure (through ingestion, inhalation or dermal contact) to pathogens in urban water (dose). This requires the assessment of the levels of pathogens in source water and the changes to these levels by water treatment, environmental conditions that affect die-off or multiplication, aerosolization, etc. Also, it needs information on the volume of water ingested, duration of exposure, etc. [27]

Pathogens concentration

Knowing the concentration of pathogens in exposure water is necessary in order to estimate a dose, endpoint of the dose-assessment step. It is not always possible to measure the pathogen concentration directly (e.g. drinking water), because of the low concentrations of pathogens in the water, or when the researcher does not have resources available to obtain specific information of the water source. In the first case, pathogens information is gathered on the contamination source or source water and, if the effects of the barriers in the water system (i.e. treatment processes, soil passage, inactivation by sunlight etc.) on the pathogen are known, its concentration in the exposure water can be estimated. In the second case, information can be obtained from published studies on similar water sources or, for instance, knowing the prevalence of a disease in a community and the microorganism excretion rate in faeces, as has been done for norovirus in grey water and wastewater [78, 79]. Moreover, natural processes can reduce or increase the concentration of pathogens in the water (inhibition due to atmospheric conditions or predation, multiplication due to

favourable nutrient conditions, street runoff after extreme rain events, etc.), and should be considered if site-specific sampling is not possible.

Ingestion

Gastrointestinal diseases are acquired through voluntary or accidental ingestion of water. Information needed to estimate the ingestion dose are the kind of activity conducted at the location, the volume of water ingested (which can vary from ingestion of small volumes through droplets generated by splashing or hand-to-mouth contact, to ingestion of mouthfuls), and the time spent.

Recently, studies have focused on describing the volumes of water ingested through recreational activities with the use of questionnaires [80-82], observation [7], or cyanuric acid measurements [82]. Water recreation activities investigated are swimming [80, 82], playing and splashing in urban flood water [81], and limited-contact recreational activities: canoeing, fishing, head immersion, kayaking, motor boating, rowing, wadeing/splashing, and walking [7, 82, 83].

Ingestion of waterborne microorganisms can also happen through consumption of raw crops irrigated with contaminated water, such as rainwater, reclaimed water, etc. Quantification of certain pathogens in crops can be a difficult task because of components of the crops that are released during sample processing and that can inhibit the response of the detection technique (e.g. PCR), leading to false negative results. If this is this case, information is needed on all the aspects that affect the concentration of pathogens from the initial concentration in the water, the water treatment (if any), the distribution, irrigation process, amount of pathogens that attach on the crops and/or are internalized into the edible parts, harvesting and storage of the crops, and the consumption patterns of the individual/population studied. These processes are reviewed in Chapter 5.

Aerosol generation and Inhalation

Respiratory illnesses are acquired through inhalation of aerosols containing the microbial pathogen. Information needed to assess the inhalation dose are the aerosolization produced at the locations (which depends on the water feature characteristics, e.g. fountain, and on the activities conducted, e.g. splashing), atmospheric conditions that will contribute to the characteristics of the aerosols (relative humidity influences on the size of the aerosols, which will determine their deposition pattern, and, therefore, the time that will be suspended in air and the portion of the respiratory tract that will reach), time spent at the location and respiratory minute volume (RMV). The USEPA has published tables with RMV for different activity degrees and ages [84].

Aerosol generation by decorative fountains and by domestic water uses has been studied. de Man, et al. [8] characterized the presence of endotoxins in the water and air downstream from several decorative fountains throughout The Netherlands. Anderson, et al. [85] studied the aerosolization of endotoxins from showers and humidifiers. The aerosolization of *L. pneumophila* from showering faucets has been studied by Dennis, et al.

[86], Bollin, et al. [87], Deloge-Abarkan, et al. [88], and Perkins, et al. [89]. O'Toole, et al. [90] characterized the size and concentration of water-based aerosols generated during domestic activities, specifically showering with water-efficient showerhead, car-washing with high pressure spray, and toilet flushing. Aerosolization from other facilities, such as cooling towers, whirlpool, spas, etc. has also been studied [91-93]. However, aerosolization from other sources of interest for urban water remains unknown, for instance, human splashing of water, car splashing, or rowing/sailing activities. Exposure assessments of these activities need to extrapolate the aerosolization rates from other activities/features.

The outdoor spread of aerosols has been widely studied and many models exist to predict the concentration of aerosols at different distances from the emission point [94, 95]. Aerosol dispersion downwind from the generation source depends on meteorological conditions (wind speed, insolation, temperature, humidity), height of the emission source, obstacles (high buildings, trees), etc. Microorganisms contained in aerosols can undergo inactivation during the dispersion, depending also on meteorological conditions. Types of aerosol dispersion models, with different complexity, include box models, Gaussian plume models, Langragian models, and computational fluid dynamic models [95]. For recreational exposure, concentration of aerosols at the exposure location is usually the aerosol source, or distances are so short that bioaerosol dispersion can be ignored for simplicity.

Aerosols with diameter lower than 7 µm are not filtered by the upper respiratory system and can be, therefore, inhaled (inhalable aerosols). However only particles between 1 and 3 µm are able to reach the lower respiratory tract (LRT) and deposit in the alveoli [90, 96]. Greater particles can deposit in the upper respiratory tract (URT) and be ingested [90]. The size distribution of aerosols formed in the shower and the inhaled dose during showering has been characterised. The particles sampled at the respiratory region were measured using a particle monitor. The inhaled dose was estimated using the model of a human respiratory tract [97].

Exposure duration and frequency

Exposure duration is depending on activity and has a high inherent variability associated. Exposure frequency is dependent on activity as well, but also on other factors, such as weather conditions. For instance, exposure to flood water in a certain area depends on the frequency of flooding, hence on the frequency of extreme rain events [81].

Research has been done to better characterize the exposure patterns of recreational and daily activities. Schets, et al. [80] characterized the duration of swimming in different water types using questionnaires. de Man, et al. [7], [81] studied the duration of exposure to urban floods also with questionnaire's, and the duration of exposure to urban splash parks by means of observational techniques. Sunger, et al. [98], on the other hand, used time-lapse cameras (validated with in-person surveys) to study the duration of recreational activities (namely jetski, kayaking, wading, swimming, boating, fishing, boat fishing, playing, and

playing with dog) in urban water. In the Netherlands, duration of water uses on different household activities, including shower duration, have been surveyed [99].

3.3. Hazard Characterization

The response of an organism to microbial pathogens exposures is highly variable and depends not only on the microbial dose but also on virulence characteristics of the pathogen, the general health and immune status of the host and the attributes of the matrix (food or water), which can alter the microbial or host status. The WHO included an outline of all information necessary for the hazard characterization step in their risk assessment guidelines [25]. These factors have to be considered to establish the uncertainty associated with dose-response models [100]. However, host responses to pathogens are difficult to assess and only information derived from young healthy subjects, with good immune status, is usually available.

Two of the most used dose-response models were introduced by Haas [101] and are the exponential and the beta-Poison model. Both are single-hit models, meaning that they assume that a single microorganism is able to initiate an infection if it is able to survive the host barriers, and the probability of (at least) one microorganisms surviving the host barriers and initiating and infection is dose-dependent. The exponential model does not include host and microorganism variability, and assumes that the pathogen distribution in the inoculum is random and characterized by a Poisson distribution (equation 1.1).

$$P_{inf} = 1 - e^{-rd} \tag{1.1}$$

where d is the exposure dose and r the probability of each microorganism of surviving host barriers and initiating infection. When d is very small, then $r \times d \ll 1$ and the exponential model can be simplified as equation 1.2:

$$P_{inf} = r \times d \tag{1.2}$$

The beta-Poisson model, on the other hand, assumes that the probability of initiating an infection differs for different hosts and microorganisms, and that the pathogen concentration in water follows a beta distribution. The beta-Poisson model can be approximated by solving the Kummer confluent Hypergeometric function (equation 1.3).

$$P_{inf} = 1 - {}_1F_1(\alpha, \alpha + \beta, -d) \tag{1.3}$$

where α and β are the shape parameters that define the host and microorganism variability. When $\alpha << \beta$ and $\beta >> 1$, this function can be simplified to the beta-Poisson formula (equation 1.1) [25].

$$P_{inf} = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha} \tag{1.4}$$

A summary of dose-response models of interest for waterborne diseases are shown in Table 1−3.

The adenovirus dose-response model is based on a data set of adenovirus type 4 administered by inhalation to a group of volunteers [102]. This has been widely used for gastrointestinal adenovirus because it is the only dose-response model available for this pathogen [47, 103]. The dose-response model indicates that adenovirus 4 is very infectious via the inhalation/intranasal route, and using it for enteric adenovirus assumes that adenovirus 40/41 has a similarly high infectivity through the ingestion route.

Table 1−3: Dose-response models for waterborne pathogens.

Microorganism	N50/TDI	Model	Parameters	Reference
Campylobacter	800	Approximate	α=0.145	[104]
		Beta- Poisson	β=7.59	
		Exact	α=0.024	[24]
		Beta- Poisson	β=0.011	
C. burnetii	1.54	Exponential	r=0.9	[69]
		Beta-Poisson	α=0.23	[105]
			β=0.18	
Cyanobacteria (microcystin)	0.04 (µg/kg/day)	-	-	[76]
L. pneumophila	11.7	Exponential	r=0.06	[106]
Cryptosporidium	9-1024	Exponential	r=0.0042	[26]
			r=0.0053	[107]
			r=0.0573	[107]
			r=0.009	[41]
		Exact	α=0.115	[107]
		Beta-Poisson	β=0.176	
Adenovirus (type 4, inhalation exposure)	1.66	Exponential	r=0.4172	[103]
Norovirus	18	Exact	α=0.04	[54]
		Beta-Poisson	β=0.055	

In most of the existing dose-response models, the studied response is infection. However, for QMRA it is more useful to express the output in terms of morbidity or mortality. Haas, et al. [108] proposed a simple assumption defining a conditional probability of developing an illness after acquired infection independent of the exposure level. A dose-response model has been derived for *Campylobacter* that relates dose to illness, using data from an outbreak originated from contaminated milk [24]. Teunis, et al.

[54] derived also a dose-dependent infection to illness model for norovirus. For *L. pneumophila*, an exponential dose-illness model is available [106].

3.4. Risk Characterization

In this step of the QMRA process, the information gathered in the other steps is combined to obtain a risk estimate. This can be the risk of infection or disease, either annual or per event. To estimate the annual risks, information on exposure frequency is needed. For drinking water, exposure frequency is 365 days/year, since people are believed to drink water daily. For recreational activities or food consumption, exposure frequency information can be obtained from surveys and questionnaires. In recreational activities, the exposure frequency depends on the activity and on the weather conditions that allow for the activity to take place or induce people to conduct or not certain activities (e.g. people swim more often in surface waters during warm weather conditions than during intense rainfall events).

QMRA can be conducted in a forward or reverse manner. Forward QMRA characterizes the risk of illness associated with a specific human exposure. Knowledge of abundance of pathogens in sources is used to predict risks of infection or illness associated with specific exposures. Reverse QMRA, on the other hand, is the assessment of required interventions to reduce the exposure given a level of tolerable risk (health target), i.e. the translation of a health target to a health-based treatment objective.

Furthermore, risk assessment can be qualitative, semi-quantitative, or quantitative, depending on the objective of the assessment and the type of data available. Quantitative QMRA can be deterministic or stochastic. Deterministic QMRA is performed when not much data are available, or as a preliminary step, i.e. screening-level QMRA, to determine if it is necessary to conduct more complex QMRA. Usually, a best estimate is calculated using average data, and variability/uncertainty is characterized by running again the model with a most conservative approach (using worst case scenario data at each step) and a less conservative approach (using the data leading to the lowest risk in each step) [109].

In the stochastic approach, each exposure assessment step is defined by a probability distribution that has been fit to experimental data (e.g. water samples) or has been obtained from the literature. Then, random samples from each probability distribution are collected in order to run different outputs of the model. This is done many times to obtain many possible outcomes through Monte Carlo simulation methods [109]. Usually, 10,000 iterations are considered enough [110].

Multiple exposure events

When exposure events are multiple, the researcher might want to estimate the annual risks. Ideally, when estimating annual risks, 10,000 iteration of each exposure day are conducted in order to ensure statistical independence of daily estimates and, hence, account for daily dose variability. This is done using the so-called Gold Standard estimation (equation 1.5).

$$P_y = 1 - \prod_{k=1}^{e}(1 - p_k) \quad (1.5)$$

where p_k is the kth daily infection probability and e the number of exposure events [111].

This is, however, not always possible or practical, and then a simpler annual infection probability equation (equation 1.6) is used that assumes a constant daily probability of infection and it is implemented many times (10,000) to account for variability.

$$P_y = 1 - (1 - P_d)^e \quad (1.6)$$

where P_d is the constant daily infection probability.

Risk end-point

As stated earlier, the QMRA output can be infection or disease. A disease output is preferred because it can be validated with epidemiological studies on disease incidence in a population. However, when the objective is to compare the risks of diseases that can be of very different nature, one step forward needs to be taken. The effect of a self-limited gastrointestinal disease is milder than that of a severe chronic disease. DALYs (Disability Adjusted Life Years) are summary measurements of health that allow for the comparison of effects across a wide range of health outcomes in terms of severity, including mortality and morbidity [10].

DALYs are the sum of the Years of Life lived with Disability (YLD) and the Years of Life Lost due to premature mortality (YLL). In QMRA, the YLD are calculated considering the estimated annual disease risk of a specific disease/pathogen among the population, the disease weight of each of the conditions derived from the disease (e.g. diarrhoea and chronic status), and the duration of each condition. Disability weights for several diseases and conditions are available online [112]. The YLL take into account the life expectancy at birth, and the years lost due to premature death respect to the life expectancy.

Multi-pathogen risk

Not only a specific pathogen can cause different diseases, but different pathogens can cause the same (or similar) disease. In urban water, several pathogens might be present that cause gastrointestinal disease, and it is more appropriate to assess the risk of developing the disease than the risk posed by specific pathogens. For this purpose, the risks derived from individual pathogens can be combined by adding up all individual probabilities, using equation 1.7.

$$P_y = 1 - [(1 - P_1)^{e_1} \times (1 - P_2)^{e_2} \times \ldots \times (1 - P_n)^{e_n}] \quad (1.7)$$

Variability and uncertainty

The output of a QMRA is not only a risk estimate, but also the variation inherent to that risk estimate. The variation derived from random sampling from probability distributions (Monte Carlo simulation) can be of two different natures: uncertainty and variability. Uncertainty represents the lack of knowledge of the parameter values and can be reduced by further sampling, while variability reflects the heterogeneity of the population and

cannot be reduced by further measurements [113]. For instance, the real concentration of pathogens in a water body is very uncertain if only one sample is taken, but this uncertainty can be reduced by gathering many samples and by considering the recovery of the method used. The volume ingested by children playing with water is very variable, since population is heterogenic, and this variability cannot be reduced by further population survey.

To assess the variability and uncertainty of a QMRA model, it is a good QMRA practice to include a sensitivity analysis. The sensitivity analysis is selected depending on the objective and on the type of QMRA analysis performed (e.g. stochastic or deterministic). Objectives of the sensitivity analysis can be rank ordering the importance of model inputs; identifying combination of input values that contribute to high exposure and/or risk scenarios, identifying and prioritizing key sources of variability and uncertainty; identifying critical limits; and evaluating the validity of the model [114]. In deterministic QMRA, nominal range sensitivity analysis or differential sensitivity analysis are commonly used. In stochastic QMRA, rank correlation analysis, scatter plots, regression tests, ANOVA are used, among others [115].

4. Risk Management

4.1. Health Targets and Safety Guidelines

Risk assessment outputs are used by risk managers to evaluate the safety of water features and plan/implement risk mitigation strategies, when necessary. For this purpose, the risk estimate is compared to a guideline level or to the disease level among the population (incidence derived from epidemiological studies), to determine if it is acceptable or not. No specific level of tolerable risk or water quality guidelines have been defined for urban waters, except for water for human consumption [116] and bathing water [117]. These provide quality levels based on levels of faecal indicators, and not on pathogens or disease risks.

The threshold level of *E. coli* for excellent and good bathing water quality in inland water bodies are 500 colony forming units (cfu)/100mL and 1,000 cfu/100mL, respectively. This was based on the tolerable daily level of gastrointestinal disease (3% for excellent and 5% for good bathing water quality) and of contracting acute feverish respiratory illness (1% for excellent and 2.5% for good standard) [117, 118]. The European Bathing Water directive aims at protecting bathers that are occasionally exposed to recreational waters. Therefore, it can also be applied to urban waters.

Direct application of the bathing water quality standards to urban waters does not recognize the large differences in exposure of the population to different water features. The standards in the bathing water directive were derived from epidemiological studies where healthy adult bathers were exposed for at least 10 min, with three head immersions. However, other types of water recreation may involve a much lower degree of water

contact (e.g., rowing, fishing, etc.) and hence a much lower risk than bathing in the same water.

Frequency and duration of exposure also differ between water uses and water features. For instance, in urban waters not designed for recreational purposes, the frequency of bathing is lower than in swimming pools, but the frequency of rowing activities can be higher. Therefore, the bathing water directive may not be protective enough for some urban water activities, and overprotective for others. On top of that, the water quality standards for recreational waters are based on studies with healthy adults, and so children, the elderly, and immunocompromised people may require further protection.

E. coli is also used by the Spanish regulation to protect health by different uses of reclaimed water. For instance, reclaimed water for irrigation of golf courses needs to contain less than 200 cfu/100 mL, while for irrigation of crops that are eaten raw through a method that allows direct contact of reclaimed water with edible parts of the crops (e.g. overhead sprinkler irrigation), the concentration of *E. coli* has to be lower than 100 cfu/100 mL [119].

In The Netherlands, a reference level of 10^{-4} infections/year for gastrointestinal diseases is defined in drinking water [120]. This is based on daily use of drinking water consumption (0.2-1L) by approximately 100% of the population. The World Health Organization recommends the use of DALYs to evaluate health risks derived from exposure to microbial hazards in waters and for the use of reclaimed water for agricultural purposes [22, 31]. The tolerable disease burden defined by the WHO guidelines is 10^{-6} DALY/person/year. This is approximately equivalent to a 10^{-5} excess lifetime risk of cancer (i.e. one case of cancer in 10,000 people ingesting drinking water at the quality-target daily over 70 years) [31]. The WHO Guidelines for the safe use of wastewater, excreta and greywater [22] are based on the revision of the previous guidelines by Blumenthal, et al. [121].

When no directives are available, an option is to compare with national incidences. In The Netherlands, national incidence of gastrointestinal disease and specific gastrointestinal pathogens are available, including DALYs figures [32], and so is the annual incidence of *L. pneumophila* disease [61]. National incidences, however, are derived from epidemiological studies and usually underestimate the incidence due to unreported cases, especially for gastrointestinal self-limited diseases [22].

For cyanobacteria, the EU Bathing Water Directive does not provide specific guidelines. The WHO has set guideline values for cyanobacteria in recreational water. A density of 20,000 cells/mL corresponds to low probability of adverse effects and 100,000 cells/mL corresponds to a moderate probability of adverse effects. The first figure (20,000 cells/L) corresponds to a production of 2 to 4 µg/L (or even 10 µg/L) of microcystin, if microcystin-producing cyanobacteria are dominant, while the second figure (100,000 cells/mL) corresponds to 20 µg of microcystin/L. If scum formation happens at densities of 100,000 cells/mL, very high cyanotoxin levels can be reached (1 to 10 mg/L or more). All

this is based in the Tolerable Daily Intake (TDI) for cyanotoxins, which is 2.4 µg/adult person (of 60kg of body weight) and 0.4 µg/child (of 10kg of body weight) [13, 75, 122].

Table 1−4: Health (based) targets and reference levels.

Health (based) Target	Reference level	Meaning	Source
E. coli	500 cfu/100mL	Excellent water quality for bathing (inland waters)	EU Bathing Water Directive [117]
	100 cfu/100mL	Appropriate quality for use of reclaimed water for irrigation of crops that are consumed raw with a method that allows direct contact of the reclaimed water with the edible part of the crop	Spanish regulation for use of reclaimed water [119]
Cyanochlorophill-a (fluoroprobe)	12.5 µg/L	Threshold for mild health risks	Stowa [123]
Microcystin	20 µg/L	Threshold for mild health risks	Stowa [123]
Gastrointestinal disease risk (event and annual)	3%	Excellent bathing water quality	EU Bathing Water Directive [117, 118]
Annual gastrointestinal infection risk	10^{-4} pppy	Tolerable risk of infection for gastrointestinal pathogens through drinking water	Dutch regulation on drinking water [120]
Annual DALYs (any disease)	10^{-6} pppy	Negligible disease burden	WHO Guidelines for drinking water quality [31]
National annual gastrointestinal disease incidence	29%	Negligible disease risk as compared to the baseline disease level in the population	Havelaar, et al. [32]
National annual legionellosis incidence	0.002%	Negligible disease risk as compared to the baseline disease level in the population	Beauté, et al. [61]

cfu, colony forming units; pppy, per person per year.

In The Netherlands, recreational waters are monitored fortnightly for cyanochlorophyll-a (through the Fluoroprobe method [124]) or biovolume, if no scum is present. Water is considered free of cyanobacterial hazards when cyanochlorophyll-a concentrations are below 12.5 µg/L or biovolume under 2.5 mm^3/L. If concentrations are higher or scum is observed in the water, the monitoring frequency is increased to weekly or daily. Small health-risks are present when cyanochlorophyll-a concentrations are in the range of 12.5 µg /L to 75 µg /L or biovolume between 2.5 and 15 mm^3/L. Under these conditions, a warning is given to bathers. If concentrations are higher, health risks are considered elevated and if more than 80% of genera are microcystin producing, microcystin has to be measured and bathing is dissuaded. Risks are considered high when microcystin is found at concentrations above 20 µg /L [123].

Table 1–4 shows a summary of the risk targets used in this thesis, based on the above discussed guidelines and epidemiological studies. Indicators or estimates of risk are compared to these figures. The risk estimates are considered negligible if they fall below the threshold values, and considerable if they fall above these values.

4.2. QMRA and Risk Management

QMRA of urban waters provides objective and scientific information for decision making (e.g. use of reclaimed water for irrigation of crops), for implementing risk management strategies (e.g. use of a specific water source for recreational purposes), and corrective or mitigation actions (e.g. eliminate wastewater discharge to a surface water body or add a water treatment step) or additional control measures when needed (e.g. increase the frequency of water quality monitoring in a water body) [27]. QMRA can help in decision making because it provides information on existing barriers that reduce these risks or on which barriers can be added to decrease them (e.g. chlorination/filtration of water, advice of non-swimming in a site after extreme rain events, etc.), and because it identifies the exposure pathways that may result in human infection and disease (e.g. accidental ingestion through swimming activities or inhalation of aerosols generated near fountains). Additional information that can help in the QMRA and risk management process is the identification of the source of origin of the risks, for instance, using faecal source tracking (FST) tools.

E. coli has been considered a good indicator of faecal contamination because it is shed in faeces from humans and animals, and does not multiply in environmental waters [125]. Although its quantification remains interesting for the sake of comparison with threshold values in water quality guidelines, it does not identify the source of faecal contamination and does not correlate with enteric bacteria, protozoa and viruses [126]. FST, on the other hand, uses characteristics that are specific of a faeces type or host source, and that can be identified in water if faeces have been in contact with it [126], identifying the origin of the faecal contamination. Some of these tools are identification of host-specific gut bacteria, host-specific viruses, detection of chemicals associated with human waste (sterols, caffeine, etc.), or mitochondrial DNA from gut cells that are shed through the faeces [127]. A good

FST marker is frequently found in faeces from a specific source in high numbers (sensitivity), and is not present in faecal material from other sources (specificity) [128]. Indicators of faecal contamination and host-specific markers of FST used in this Thesis (*E. coli*, human *Bacteroides*, avian *Helicobacter*, canine mitochondrial DNA, and adenovirus) are described below.

E. coli are Gram-negative, non-spore forming, usually motile, rod-shaped bacteria of 2-6 µm long and 1.1-1.5 µm wide. Pathogenic strains cause several types of gastroenteritis. Human and warm-blooded animals are reservoirs of *E. coli* in the intestines, and secrete them in the faeces. *E. coli* can survive in the environment but does not reproduce, except for tropical environments. Adequate chlorination effectively removes the bacteria [12].

Bacteroides spp. are gram-negative, non-spore forming, non-motile, anaerobic rod-shaped bacteria, present in the microflora of the gastrointestinal tract of humans and animals [129]. They do not survive for long periods outside the host, are host-specific, and are more abundant than *E. coli* or enterococcus in the gastrointestinal flora [130]. All these characteristics make them very suitable for FST. *Bacteroides* are difficult to cultivate, but different PCR methods to detect and quantify them exist. Specifically, the HF183 sequence, located in the 16S rRNA gene of *B. dorei* [128], was detected in 100% of 52 sewage samples and in none of the 155 animal samples by PCR [130].

Helicobacter are helicoidal Gram-negative bacteria, non-spore forming, motile due to multiple flagella, with optimal growth at 37°C [131]. Different species are found in stomachs of humans (*H. pylori*), and animals such as dogs (*H. canis*), cats (*H. felis*), rats (*H. nuridarum*), birds (*H. pullorum*), etc. Green, et al. [132] identified a *Helicobacter spp.* DNA sequence common to gulls, geese, ducks, and chickens. Heijnen, et al. [133] found this marker in high concentrations in avian faeces (average 2.4×10^7genomic copies (gc)/mg, 89.1% of samples were positive), while it was found only occasionally in other species (in humans, only 0.5% of faecal samples were positive, with average concentrations of 4.2×10^{-2} gc/mg). The marker was found in several fresh swimming water locations in The Netherlands in concentrations up to > 1,000 gc/mL.

Host epithelial cells are shed in the gut lumen of animals and secreted in faeces, and so are, therefore, host cell nucleic acids, including mitochondrial DNA (mtDNA). mtDNA contains species-specific sequences and is present in multiple copies in the mitochondria, several of which are contained in each cell. These characteristics make mtDNA an excellent target for FST. However, mtDNA can also be present in non-fecal sources such as fur, skin, and sputum. Still, this provides information on sources of pollution [127]. Dog mtDNA showed higher efficiency, sensitivity and specificity than dog-specific *Bacteroides* in a qPCR comparative study [134].

Human viruses are good indicators of human faecal contamination because viruses are host-specific. Recently, human adenovirus have been widely used in environmental waters as human faecal indicators because they are persistently shed by infected people (both symptomatic and asymptomatic) in faeces and urine [135].

5. Risk Assessment Studies in Urban Waters

The microbial health risks of several urban water features (urban freshwaters, urban flooding, splash parks, roof-harvested rainwater, ingestion of crops irrigated with reclaimed water, etc.) have been assessed. Table 1−5 shows a summary of several QMRA studies on urban water features (excluding drinking water), the pathogens studied, exposure routes, exposure assumptions made, and results of the assessment. The exposure assumptions are listed in the table without making any judgement on their validity/adequacy. For crop irrigation with reclaimed water, only virus studies have been included. Overall, these studies show that microbial health risks associated with exposure to urban waters are not negligible. Further research is needed on newly developed urban water concepts and using an holistic approach, i.e., including different water features and estimating the risks of several diseases, derived from multiple pathogens, for a better understanding of the extent of health risks associated with these features and how these risks can be managed.

6. Objectives

The objective of this research is to evaluate the microbial health impact of a series of water systems in the urban environment by using Quantitative Microbial Risk Assessment (QMRA) based tools. The research is focused on those urban water features that are newly designed to deal with global change (both climate and social) or that, as a result of it, require increasing attention. Different exposure routes are assessed (ingestion and inhalation) and different degrees of QMRA complexity are used depending on data availability and objective of each study.

First of all, a number of water locations in an urban area are studied to estimate the health risks derived from human-water interaction. The locations studied represent diversity in water source (and quality) and human uses (and exposure). A screening-risk assessment is conducted in 15 locations, to select a smaller number, based on health risks (**Chapter 2**). Next, the selected water locations are further studied during a water monitoring campaign and a rain event, gathering site-specific microbial data. This allows for a more specific estimation of the health risks at those sites (**Chapter 3**).

Then, the water quality and health risks derived from a newly built water plaza are studied. For this purpose, pathogens and FST markers were monitored during a rain simulation event (**Chapter 4**). **Chapter 5**, studies the risks derived from the consumption of lettuces irrigated with reclaimed water, using site-specific data. Urban wastewater is treated and the tertiary effluent is used to irrigate crop fields, which are subsequently sold in the local market. Finally, the increased urbanization in small countries like The Netherlands leads to close proximity of metropolitan areas with water treatment plants and farms, resulting in potential health risks. Therefore, in **Chapter 6**, a scenario is built on showering with water containing *C. burnetti* from a groundwater treatment plant that uses aeration with contaminated air from a nearby barnyard, during the Q fever outbreak that

occurred in The Netherlands from 2007 to 2012. General discussion, conclusions and recommendations are presented in **Chapter 7**.

Table 1−5: QMRA studies on urban (non-drinking) waters.

Water Feature	Pathogens	Exposure	Exposure Assessment Assumptions	Risk output	Ref
Surface waters					
Surface water (urban canals, rivers, ponds and lakes)	*Cryptospo-ridium, Giardia*	Ingestion via swimming (adults and children) and diving	Ingested volumes for swimmers derived from water ingestion in an indoor swimming pool. All the protozoa cysts detected by microscopy are assumed to be human pathogenic.	Mean risk of infection per event: *Cryptosporidium*: 6×10^{-5} %-0.006% in non-recreational sites;0-3×10^{-4} % at recreational sites. *Giardia*: 0.03-0.4% at non-recreational sites; 0-9×10^{-3} % at recreational sites.	[136]
Rainwater/Stormwater					
Roof harvested rainwater	*Salmonella spp., G. lamblia, L. pneumo-phila*.	Ingestion via drinking, showering and hosing; inhalation via showering and hosing	Equivalence of gc with cells/cysts. All PCR detected cells and cysts are viable and infective. Ingestion volumes, aerosol size distribution, alveolar deposition efficiency, breathing rate, shower duration, duration of hosing, inhalation during hosing only when person is downwind, worst case volume chosen for inhalation. Market survey data used to estimate proportion of household with rainwater tanks and uses. Assumption that all households use rainwater for garden irrigation and those that use it for drinking use it also for showering. Concentrations of pathogens measured in sampled tanks are representative of all households. Exposure events based on the proportion of the year that pathogens were present in the tanks.	Annual infection risk per 10,000 people *Salmonella*: 2.6×10^{-5} (hosing)-5.3×10^{1} (drinking) *G. lamblia*: 5.3×10^{-5} (hosing)– 1.3×10^{2} (drinking) *L. pneumophila*: 2.1×10^{-3} (hosing) – 7.3×10^{-3} (showering)	[137]

Table 1−5 Continued.

Water Feature	Pathogens	Exposure	Exposure Assessment Assumptions	Risk output	Ref
Rainwater/Stormwater Continued					
Flood water	*Campylo-bacter*, *C. parvum*, rotavirus	Accidental ingestion during withdrawal and clean-up process of flooded houses	Flood water quality based on combination of literature values and experimental data. Urban non-foul component assumed to have a dilution effect, containing negligible pathogen concentrations. Microbial concentrations assumed to decrease between the initial withdrawal and the clean-up. All residents were at home at the time of the flood, all chose to withdraw, estimation of the likelihood of immersion, ingestion volumes, only residents are present during clean-up, duration of clean-up, no additional die-off during clean-up, mean exposure per day, age percentage of people present during clean-up.	Number of Illness per event (out of 1,028 people): Overall: 29 illnesses, min (0.02) for Cryptosporidiosis during withdrawal, max (22.5) for viral gastroenteritis during clean-up	[138]
Stormwater (wet weather discharges into inland waters)	*Salmonella enterica*, *C. jejuni*, *Cryptospo-ridium*, *Giarida*, adenovirus, enterovirus, norovirus, rotavirus	Ingestion via swimming, inhalation, secondary contact	Combine hydrodynamic models and concentrations at the water source to estimate concentration of pathogens at the point-of –use. Ingestion rates and exposure duration based on literature data. Use of an empirical hockey-stick distributions of the pathogens concentration. All *Cryptosporidium* detected by microscopy are assumed to be human pathogenic.	Annual probability of illness for specific pathogens, sites and exposures.	[139]
Flood water (street runoff, and separate and combined sewer overflow)	*C. jejuni*, *Cryptospo-ridium*, *Giardia*, Norovirus, Enterovirus	Ingestion via playing, walking	Use of questionnaires to determine the volume of flood water ingested, the duration of exposure, and the frequency. Assumption of the volume corresponding to each self-reported ingestion (few drops, mouthful, etc.). All measured pathogens are assumed to be infectious; for enteroviruses, a ratio between infectious and defective particles is assumed. All *Cryptosporidium* detected by microscopy are assumed to be human pathogenic.	Mean risk of infection per event: Children: 33% CSO, 23% SSO, 3.5% surface runoff Adults: 3.9% CSO, 0.58% SSO, 0.039% surface runoff.	[81]

Table 1−5 Continued.

Water Feature	Pathogens	Exposure	Exposure Assessment Assumptions	Risk output	Authors
Rainwater/Stormwater Continued					
Splash parks that use rainwater	*Campylo-bacter*, *L. pneumo-phila*	Ingestion and inhalation via playing	Pathogens data are not site-specific. Volume of ingested water and inhaled particles, frequency of hand-to-mouth contact, volume of the inhalable water spray, inhalation rate. Frequency of different exposure types based on observation at two locations.	Infection risk: *L. pneumophila* 9.3×10^{-5} (children), 1.1×10^{-4} (adult); *Campylobacter* 3.6×10^{-2} (children)	[7]
Grey Water					
Treated greywater	*C. jejuni*, *Salmonella*, *C. parvum*, *G. lamblia*, rotavirus	Accidental ingestion, direct exposure after irrigation, drinking groundwater recharged with treated greywater	Pathogen concentrations based on concentration of indicators in greywater, population pathogen prevalence, excretion rate and faecal load. Reduction of pathogen concentrations due to greywater treatment based on indicators data. Ingestion volumes and exposure frequency are assumptions.	Median yearly risk of infection range: 10^{-11} (for *Salmonella* through exposure to groundwater after 3 months retention time in aquifer) to $10^{-0.2}$ (for rotavirus through groundwater after 1 month retention time and for irrigation).	[140]
Crop irrigation with grey water (from laundry or bathroom)	Norovirus	Ingestion of raw crops (washed and not washed)	Volume of water captured by lettuce, consumption of home-produced lettuce, loss of lettuce due to preparation, Norovirus concentration in greywater based on *E. coli* data, incidence, shedding rates, faeces on underwear, reduction from washing and laundry load volume. Crop internalization not considered. Decay rate based on *Bacilus fragilis* bacteriophage B40-8. Withholding period and virus reduction due to post-harvesting washing, proportion of population that washes vegetables and proportion of population that uses greywater to irrigate lettuce.	Median annual DALYs $>10^{-6}$ for consumers of crops irrigated with laundry water, lower for bathroom water.	[78]

Table 1−5 Continued.

Water Feature	Pathogens	Exposure	Exposure Assessment Assumptions	Risk output	Authors
Reclaimed Wastewater *					
Salad crop irrigation with wastewater (secondary effluent)	Enterovirus	Ingestion of raw crops	Pathogens data based on literature. Virus retention on crop surface assumes all irrigation water is retained and all virus attach to crop. Decay rate based on Bacilus fragilis bacteriophage B40-8. Crop internalization not considered. Consumption assumed to occur 14 days following the last irrigation. Decay after harvesting not considered. Amount of lettuce consumed per event.	Mean likelihood of infection: 0.11 people/10,000 exposed	[141]
Wastewater and sewage sludge treatment and reuse	EHEC, *Salmonella*, *Giardia*, *Cryptospo-ridium*, rotavirus, adenovirus	Ingestion by WWTP workers, unintentional immersion at wetland inlet, child playing at wetland inlet, swimming, child playing at sludge storage, entrepreneur spreading sludge, consumption of raw vegetables	Volume ingested (through immersion and hand to mouth), frequency of exposure and number of people affected. Pathogen concentrations based on literature data. Reduction of the concentrations at the wastewater treatment plant based on indicators. Concentration of EHEC in sewage based on epidemiological prevalence data and expected excretion diluted in the volume of water produced. Recreational water assumed to be fully mixed and under steady-state conditions. Use of first-order pathogen inactivation equations. Concentration of pathogens in raw sludge based on measured concentrations in sewage and calculated ratios between raw sewage and sludge. Inactivation during anaerobic digestion based on literature. 50% of pathogens assumed to be attached to sludge particles in the dewatering process. No inactivation in protozoan and particles before exposure to sludge. EHEC and *Salmonella* concentrations back to initial levels, after sludge die-off, because of regrowth. Sewage sludge homogeneously mixed into the top 25cm of soil. Crop internalization not considered.	Median number of annual infections (different number of exposed people per scenario): <0.0001(for *Cryptosporidium* for child playing at wetland inlet) to 2 (for adenovirus for entrepreneur spreading sludge)	[142]

Table 1−5 Continued.

Water Feature	Pathogens	Exposure	Exposure Assessment Assumptions	Risk output	Authors
Reclaimed Wastewater Continued					
Crop irrigation with wastewater	Enteric viruses	Ingestion of raw crops	No site-specific data. Crop internalization not considered. Virus retention on crop surface assumes all irrigation water is retained and all virus attach to crop. For cucumber and lettuce, volume of water on surface based on literature data derived from immersion of vegetables in water. Virus kinetic decay constant based on *Bacilus fragilis* bacteriophage B40-8. Viral decay post-harvest is assumed negligible. Daily consumption crop data from a different country.	Annual infection risk: 5.4×10^{-8} (broccoli, 14 days environmental exposure) to 2×10^{-1} (lettuce, 1 day environmental exposure)	[143]
Crop irrigation with wastewater	Norovirus	Ingestion of raw crops	Based on assumptions on *E. coli* concentrations. Assumption that all irrigation water is retained and all virus attach to crop. Crop internalization not considered. Lettuce consumption pattern, pathogen die-off per day on crop surface, pathogen reduction due to produce washing, disinfection and peeling.	Annual infection risk: 3.6×10^{-5} to 1pppy. 4-6 log reductions are needed	[144]
Crop irrigation with wastewater	*Campylo-bacter*, *Cryptospo-ridium*, Rotavirus	Accidental aerosol ingestion by irrigators/ growers and consumption of crops	Pathogens data are not site-specific, assumptions for water treatment efficiencies of the multi-barrier model for pathogens reduction; pathogen decay rate is assumed to be constant with time; faecal indicators decay rates are used at some sites. Ingestion volume, amount of lettuce ingested, water retained on lettuce, washing removal and exposures per year. Method of irrigation is a conservative assumption.	Median values $<10^{-6}$ DALYs/year except for one location	[145]
Crop irrigation with wastewater (different water treatments)	Norovirus	Ingestion of raw crops	Norovirus concentration based on population disease incidence, population of the study area, shedding rate, faecal weight and daily wastewater volume in the study area. Removal of virus through conventional and advanced treatment. Virus retention on crop surface assumes all irrigation water is retained and all virus attach to crop. Crop internalization not considered. Decay rate based on *B. fragilis* bacteriophage B40-8. Proportion of population that wash vegetables and reduction of virus concentration due to washing. Vegetables consumptions rates are based on foreign surveys (for some vegetables).	Annual DALYs: All treatments and crops result in $>10^{-6}$ DALYs (except for cucumbers) but combination of treatments would reduce risks below this level	[79]

Table 1−5 Continued.

Water Feature	Pathogens	Exposure	Exposure Assessment Assumptions	Risk output	Authors
Miscellaneous waters					
Surface water open drainage channels, grey water in tertiary drains, storage containers, unprotected springs, contaminated soil, and tap water in slums	*E. coli O157:H7*, *Salmonella spp.*, rotavirus, human adenovirus F and G	Ingestion (voluntary, accidental), dermal contact and inhalation	Drinking water: ingestion volume, fraction of the population storing drinking water in household, days that drinking water is available in the pipe system. Grey water, surface water, and soil: ingestion volume, frequency of exposure. The microbial risks associated with inhalation of bacteria and viruses are minor compared to the ingestion route.	680 DALYs/1,000 persons/year, highest through surface water open drainage channels (39%), lowest tap water (0.02%), Highest number of infections caused by *E. coli O157:H7* (41%), lowest by *Salmonella* (7%)	[146]
Car wash with reclaimed water (urban wastewater, car/bus/truck wastewater, rainwater)	*E. coli*	Ingestion for users and operators	Use of *E. coli* quantified with culture (so, no distinction between pathogenic and non-pathogenic). Frequency of car wash, volume of water used, ingestion doses by car wash based on irrigation studies.	Annual infection risk: Users: 1.7×10^{-5} Operator: $10^{-3} – 10^{-1}$	[147]

**Crops irrigation with wastewater studies: only those that target viruses are included. EHEC, Enterohaemorrhagic E. coli*

Chapter 2: Screening-Level Microbial Risk Assessment of Urban Water Locations: A Tool for Prioritization

Abstract

People in urban areas are exposed to microbial hazards in urban waters. In this study, various hazards, diseases, and water systems, where different recreation activities take place, are compared in an integrated quantitative microbial risk assessment (QMRA). The event and annual probability of gastrointestinal illness (GI) and Legionnaires'disease (LD) were analysed in QMRA models using selected literature data. Highest mean event probabilities of GI were found for playing in pluvial flood from a combined sewer overflow (34%), swimming (18%), and rowing (13%) in the river, swimming (8.7%) and rowing (4.5%) in the lake, and playing in a water playground (3.7%) and in the pluvial flood from stormwater sewers (4.7%). At these locations, the GI probability was above the EU Bathing Water Directive threshold for excellent water quality (3%). All the annual risk medians were below the national incidence of legionellosis of 0.002%. The illness probability was most sensitive to the pathogens concentration (particularly *Campylobacter*, norovirus, and *Legionella*) and exposure frequency. Therefore, site-specific pathogen data collection is the best next step to strengthen the certainty of the risk estimates. This study created an evidence-base that was used by water authorities to understand the health risks and set priorities for risk management.

This chapter is based on:

Sales-Ortells, H. and Medema, G. 2014. Screening-Level Microbial Risk Assessment of Urban Water Locations: a Tool for Prioritization. *Environmental Science and Technology* 48(16), 9780-9789.

1. Introduction

Nearly half of the world population lives in urban areas [148]. The Netherlands is a highly urbanized country. As a result, water bodies and features in metropolitan areas are abundant and human contact with water in urban public spaces happens often during recreation in and around ponds in parks and other blue-green areas, urban rivers and lakes, water playgrounds, public swimming pools, street drinking water taps, or urban canals.

Climate change is expected to lead to an increase in frequency and intensity of storm events. Consequently, urban sewage systems will be overwhelmed more often, and street flooding with stormwater will occur more frequently [149]. New urban water concepts such as water plazas and bioswales (wadis) are emerging in cities. These features serve as stormwater temporary storage, reducing pluvial flooding during intense rainstorms, and making stormwater available for other purposes, such as landscape irrigation or recreation.

Exposure of humans to urban water concepts may lead to health risks when pathogenic microorganisms are present [150]. Microbial hazards may be present in water bodies due to input of faecal material, such as sewage discharge containing human enteric pathogens (*Campylobacter*, *Cryptosporidium*, norovirus, rotavirus, etc.) [14, 15] or animal faecal input (from waterfowl, dogs, and other domestic and wild animals) containing zoonotic pathogens, or growth of microorganisms in urban water bodies and features, such as toxic cyanobacteria in stagnant waters [16] or *Legionella pneumophila*, especially in warm water systems [17]. Microbial risks are also influenced by climate change. A higher frequency and strength of storms and draughts affects the concentration of pathogens [14, 18, 19], a temperature increase may promote formation of cyanobacterial blooms [20].

Several studies have assessed microbial quality and risks of urban waters in the past [7, 81, 136, 137, 151-153]. Those studies usually focused on single urban water systems and/or single pathogens. In reality, exposure to urban water bodies may result in several microbial risks. Moreover, urban planners and water managers are faced with an array of water bodies and water contact and desire an evidence-base to set priorities for risk control measures. The objective of the present study is to conduct an integrated, scientific evidence-based analysis of the health risk associated with all water systems and relevant microbial hazards in an urban area. This informs planners and utilities about the sources and level of risk associated with the different water systems, guides priorities for risk management, and select sites for pathogen monitoring. This screening-level quantitative microbial risk assessment (QMRA) study combined scientific data and assumptions into a site-specific assessment. The use of assumptions is common in QMRA, although not always explicit. Assumptions are the best option available in the absence of (site-specific) data [154, 155], and the assumptions in this study are justified in Appendix A.4.

2. Study Site Description

The Watergraafsmeer is a polder located in the south eastern part of the city of Amsterdam. The polder is an urban settlement protected from flooding by man-made dikes. It is surrounded by the river Amstel, a ring of canals that are fed by the river, and the Nieuwe Diep lake. The water that flows through the polder is stormwater overflow from combined sewers (CSO) in some areas and separated sewers (SSO) in others. Stormwater is stored in reservoirs that act as sedimentation ponds. When there is not enough rainfall, weirs are opened to allow the flow of surface water from the ring of canals.

Several municipalities are located in the river Amstel basin, with secondary treated sewage discharges and some stormwater overflows. Several recreation activities take place in the river, especially rowing and other low-contact activities, but also swimming during hot summer days.

Other water bodies and features are present in the Watergraafsmeer where contact between humans and water occurs, which differ in the water source (and quality) and the type of human exposure. Other water features are wadis (bioswales, climate change adaptation measures for temporary stormwater storage in the area), an ornamental fountain, public taps fed by drinking water, and a chlorinated swimming pool fed by drinking water inside the river Amstel. The river, canals, the lake, and a water playground are used for full contact water recreation, although they are not designated bathing water locations under the EU Bathing Water Directive [117].

A variety of activities may bring humans in contact with the water of these features. Minimal exposure activities are, for instance, walking near features that are aerosol sources (cars splashing water when crossing flooded streets, dogs splashing water after swimming in a pond, or the fountain where aerosols are continuously generated), and fishing; intermediate exposure activities are rowing and other sailing activities in the river or lake, and playing in stormwater systems (flooded streets, wadis), water playgrounds, and ornamental fountains; high contact activities are swimming and head immersion in the water. Specific water sources and activities can be found in Appendix A.1.

Twenty water bodies/features were identified with potential exposure of humans to contaminated water by the water utility of the city of Amsterdam. An expert judgment was made of the expected water quality and human exposure for each location. The locations were ranked according to water quality and exposure and 15 sites were selected for further analysis. More information on this study, the members of the expert team, the different locations evaluated, and a map of the area with the 15 selected sites can be found in Appendix A.1.

3. Screening-Level Risk Assessment

3.1. Hazard Identification

Cryptosporidium, *Campylobacter*, norovirus, and *L. pneumophila* were the microorganisms selected for the study, as the most relevant human pathogens causing gastrointestinal and respiratory illness in The Netherlands [9, 36, 37]. The rationale for selection of these pathogens has been described in the introduction of this thesis.

3.2. Exposure Assessment

An exposure assessment model was built for each location, pathogen, and type of human – water interaction.

Concentration of Pathogens

Literature on the occurrence of pathogens in various water bodies was reviewed. Selection of the data was made based on the location of the studies (selecting those with similar climate and socio-economic characteristics). When arrays of data were available, statistical distributions were fitted using the maximum likelihood estimation or the matching moments estimation methods, and the goodness of fit was evaluated with cumulative density function graphs, and the Kolmogorov – Smirnov test. When only statistical data were available (mean, quantiles, etc.), log-normal or gamma distributions were fitted to those data. These distributions were chosen because they have been shown to provide a good fit to concentrations of microorganisms in water [156, 157]. Occasionally, fitted distributions to concentrations of pathogens in water were found in the literature, and these were used as data input [7, 156]. Distributions and parameters used for each water body and literature sources are shown in Table 2−1.

For the river and lake, site-specific data on *Cryptosporidium* and *Escherichia coli* were available [136]. For *Campylobacter*, data from The Netherlands in a sewage impacted river and in waterfowl impacted lakes were available [158]. Norovirus data were also available for a sewage impacted river in The Netherlands [156]. However, no quantitative information on norovirus in lakes was found. Average concentrations of *E. coli* were 1.3 logs lower in the lake Nieuwe Diep than in the river Amstel [136]. We used this data to extrapolate the concentration of norovirus in the river to the lake. Gamma distributions were fitted to *L. penumophila* data on different rivers, stream, ponds and lakes in The Netherlands [159]. Because the data array was not large, all the data were pooled together. However, two scenarios were assessed: one including data from a lake that was impacted by a wastewater discharge rich in *L. pneumophila*, and one excluding those data.

Table 2−1: Concentration of pathogens.

Location	*Pathogen*	Cistribution[a]	Parameters[d]	Units[e]	Literature
River	*Cryptosporidium*	Lognormal	μ=-1.92 σ = 0.4	Log10 (oocysts/100 mL)	[136]
	Campylobacter	Lorgnormal	μ=1.8 σ = 0.7	Log10 (MPN/100 mL)	[158]
	Norovirus	Gamma	r = 8.55×10^{-2} $\lambda = 3.7\times10^{2}$	Pdu/L	[156]
	L. pneumophila	Gamma	r =8.8×10^{-2} $\lambda =1.4\times10^{3}$	Lpn/L	[159]
	L. pneumophila (Impacted)	Gamma	r =1.4×10^{-2} $\lambda =9.8\times10^{5}$	Lpn/L	[159]
Lake and surface water playground	*Cryptosporidium*	Lognormal	μ=-3 σ = 1	Log10 (oocysts/100 mL)	[136]
	Campylobacter	Lognormal	μ=1.1 σ = 0.7	Log10 (MPN/100 mL)	[158]
	Norovirus	Gamma	r = 8.55×10^{-2} $\lambda = 3.7\times10^{2}$ 1.3 log reduction	Pdu/L	[156], [136]
	L. pneumophila	Gamma	r =8.8×10^{-2} $\lambda =1.4\times10^{3}$	Lpn/L	[159]
	L. pneumophila (Impacted)	Gamma	r =1.4×10^{-2} $\lambda =9.8\times10^{5}$	Lpn/L	[159]

Table 2−1 Continued.

Location	*Pathogen*	Cistribution[a]	Parameters[d]	Units[e]	Literature
Ornamental fountain, drinking water playground, and public water taps	*L. pneumophila*	Gamma	$r = 8.48\times10^{-2}$ $\lambda = 3.06\times10^{4}$	Lpn/L	[160]
Swimming pool	*Cryptosporidium*	Gamma	$r = 6.5\times10^{-2}$ $\lambda = 1.88$	Oocysts/L	[151]
Sedimentation pond[b], pond in green area[c], pond in a park[3], SSO in traffic road, SSO in residential area, Wadi	*Cryptosporidium*	Lognormal	$\mu = -2.7$ $\sigma = 1$	Log (oocysts/L)	[81] [161]
	Campylobacter	Lognormal	$\mu = 1.4$ $\sigma = 0.25$	Log (MPN/L)	[81]
	Norovirus	Lognormal	$\mu = 1.8$ $\sigma = 0.4$	Log (pdu/L)	[81]
CSO	*L. pneumophila*	Gamma	$r = 4.5\times10^{-2}$ $\lambda = 2.6\times10^{4}$	Lpn/L	[7]
	Cryptosporidium	Lognormal	$\mu = 7.6\times10^{-2}$ $\sigma = 0.35$	Log (oocysts/L)	[81]
	Cryptosporidium	Lognormal	$\mu = 2.4$ $\sigma = 0.2$	MPN/L	[81]
	Campylobacter	Lognormal	$\mu = 3.75$ $\sigma = 0.45$	Pdu/L	[81]
	Norovirus	Gamma	$r = 4.6\times10^{-2}$ $\lambda = 3.14\times10^{10}$	Lpn/L	[162]

[a]Lognormal is normal distribution with logdata[143]; [b]Concentration in JB is 1 log lower because of sedimentation; [c]Concentration in JV and FD is lower because of sedimentation (1 log) and dilution effects (triangular (0.1, 1, 2) logs); [d]parameters of the gamma distribution are r=shape and λ=scale; [e]MPN is Most Probable Number, lpn is Legionella pneumophila cells, pdu is PCR detection units.

Julianabak is a reservoir receiving water runoff from a stormwater sewer system. It is also a sedimentation pond, reducing the concentration of particles suspended in water. Sedimentation ponds reduce the contamination of thermotolerant coliforms by 1.5 logs and 0.5 logs approximately in the dry and wet season, respectively [163]. For simplicity, we assumed a 1 log reduction for each pathogen in the sedimentation pond.

The sedimentation pond drains into the net of canals in the polder. During dry periods water from the outer ring is used to supplement the water in the canals. A triangular distribution (0.1, 1, 2) based on *E. coli* data (not shown) was used to describe the log reduction in pathogen concentrations due to dilution and natural processes (sedimentation, sunlight radiation inactivation, and predation) in the green area canals and the park.

L. pneumophila has been reported in drinking water systems [160, 164]. Fourteen out of 357 tap water samples from 250 different buildings throughout The Netherlands showed the presence of *L. pneumophila* (with culture or PCR) [164] and in five out of eight drinking water distribution systems[160].

For the chlorinated pool, only *Cryptosporidium* was considered in the model, because, unlike the other pathogens, it is able to resist the level of chlorine in the pool water [39].

During CSOs, domestic wastewater is diluted by stormwater. A 10-fold dilution was assumed to extrapolate the concentrations of pathogens in domestic wastewater [152].

Water Ingestion

Water ingestion routes considered were accidental ingestion after head immersion in the water (children swimming), hand-to-mouth contact (fishing), or ingestion of droplets and aerosols (rowing, splashing, etc.). The volumes of water ingested for each activity and at each location are shown in Table 2-2. Distributions fitted to ingestions volumes of water by children, derived from Dutch population, during swimming activities in swimming pools and surface water were used [80]. For children playing in stromwater systems (wadis and flooded streets) data from a study on exposure to floodwater were used, also derived from Dutch population [81]. For other activities, like playing in surface water, rowing, fishing, or walking, Dutch data were not found in the literature. Hence, data from an exposure study in the U.S.[82], or data extrapolated from other activities, were used (Table 2−2).

Exposure Duration

Table 2−3 shows the duration of exposure used in every activity and location [80, 81, 98]. The dose of pathogens (d) per person per day (pppd) derived from ingestion is calculated with equation 2.1:

$$d = \mu_w \times V \times t \tag{2.1}$$

where μ_w is the concentration of pathogens in the water (per mL), V the volume ingested (in mL/h) and t the time spent (in h).

Table 2−2: Ingestion Volume per activity and location.

Activity	Distribution	Parameters	Units	Literature
Rowing/ Sailing/ surfing in surface water	Triangle	Min = 0.1 Mode = 3.1 Max = 7	mL/h	[82], [165]
Swimming in surface water	Gamma	r = 0.64 λ = 58	mL/ event	[80]
Swimming in swimming pool water	Gamma	r = 0.81 λ = 63	mL/ event	[80]
Playing in wadi/ street-runoff/overflow	Triangle	Min = 0 Mode= 0,051 Max = 5	mL/event	Mean and 95% CI from[81].
Playing in surface water	Triangle	Min = 0.1 Mode = 2.5 Max = 11.2	mL/h	[82], min is an assumption
Walking on flooded street (getting splashed by cars)	Triangle	Min = 0.1 Mode = 3.5 Max = 10.6	mL/h	[82]*, min is an assumption
Walking the dog in the park	Triangle	Min = 0.1 Mode = 3.5 Max = 10.6	mL/h	[82]*, min is an assumption
Fishing	Triangle	Min = 0.1 Mode = 3.5 Max = 7	mL/ event	[82, 165], min is an assumption

**Data from people walking around a swimming pool where limited contact recreation is taking place.*

Inhalation

All activities except for fishing were assumed to lead to formation of aerosols. An aerosolization ratio (a) was used to translate concentrations of *L. pneumophila* in water into concentrations in the air. No literature was found on aerosol generation due to recreational activities such as swimming, rowing, wadding, splashing, etc. Generation of aerosols has only been studied from aerosolizers [166], showers [88], and decorative fountains [8]. A normal distribution was fitted to the log10-transformed aerosolization ratios from the decorative fountains study ($\mu = -8.07$, $\sigma = 0.3$) to calculate the concentration of *Legionella* in the air.

Respiratory minute volumes (RMV) are different for every activity and age group [84]. Intensity levels were assigned to the different activities. Rowing and swimming were considered high, playing moderate, and walking light intensity activities. A log-normal

distribution was used fitting mean and 95th percentile of the RMV for different activities and age groups [84].

A deposition in the lower respiratory tract (I) of 12.7% of inhaled aerosols was assumed [167]. The dose (pppd) of *L. pneumophila* received through inhalation was calculated as indicated with equation 2.2:

$$d = \mu_w \times a \times RMV \times I \times t \quad (2.2)$$

Table 2−3: Exposure duration per activity and location.

Activity	Distribution	Parameters	Units	Literature
Rowing/ sailing/ surfing in freshwater	Triangle	Min = 1 Mode = 2 Max =4	h	[98] (max is based on information provided by rowers)
Playing in rainwater reservoirs/ street runoff	Normal	μ = 21 σ = 5	min	Mean [81], distribution shape and σ are assumptions
Playing in surface water playground*	Lognormal	μ = 4.1 σ = 0.80	min	[80] (surface water)
Playing in drinking water playground*	Lognormal	μ = 4.2 σ = 0.55	min	[80] (swimming pool)
Walking on flooded street (getting splashed by cars)	Triangle	Min = 1 Mode = 5 Max = 10	sec	Assumption
Walking the dog	Triangle	Min = 15 Mode = 30 Max =60	min	Assumption
Public water taps	Point estimate	1	min	Assumption

**Due to the lack of data available for exposure duration of playing in fresh and drinking water, data from swimming in surface water and swimming pool are used here.*

3.3. Dose–Response Assessment

Dose–response models were combined with the dose derived from the exposure assessment to calculate expected individual probability of infection per event for the enteric pathogens and for *L. pneumophila*. Probability of disease for enteric pathogens was estimated with published probabilities of developing illness given infection [24, 30, 41, 54, 168-175] (Table 2−4).

Table 2−4: Dose–response models for the waterborne pathogens.

Pathogen	Infection Model and Paramters	Disease given infection	Rationale	Literature
Campylobacter	Hypergeometric; $\alpha = 0.024$; $\beta = 0.011$	0.33	Most conservative model, included outbreak data.	[24, 30]
Cryptosporidium	Exponential; r = 0.09	0.5	Most conservative model, combines data from different strains	[41, 170]
Norovirus	Hypergeometric; $\alpha = 0.04$, $\beta = 0.055$	0.67	Most conservative	[54, 174]
L. pneumophila	Exponential; r = 0.06	Exponential; $r = 1.07\times10^{-4}$	Only model available	[175]

3.4. Risk Characterization

The annual probability was estimated considering the assumed exposure events for each activity and location and using equation 2.3 [26]. This annual probability corresponds to the population exposed to the hazard, and not to the total population.

$$P_y = 1 - (1 - P_M)^f \tag{2.3}$$

where P_M is the daily probability, and f the exposure frequency (in days per year) at each location. The frequency of swimming follows a negative binomial distribution [80]. For playing in flooded streets and wadis, a negative binomial distribution was fitted to literature data [81]. For other activities, assumptions were made and step uniform, binomial, or negative binomial distributions were used (see Table 2−5).

Frequency of rowing in the river was considered to have a high variability: from those who do it once per year, to regular rowers, who row three times per week during the 9 months rowing season, and all intermediate possibilities. In the lake, however, exposure frequency was based on the courses offered by the sailing school located at the lake.

The infection and illness probability derived from *Cryptosporidium*, *Campylobacter*, and norovirus exposure was computed as total Gastrointestinal Illness (GI) probability, according to equation 2.4.

$$P_y = 1 - [(1 - P_C)^f \times (1 - P_P)^f \times (1 - P_N)^f] \tag{2.4}$$

where P_C, P_P, and P_N are daily probabilities for *Campylobacter*, *Cryptosporidium*, and norovirus, respectively.

Table 2−5: Exposure frequency per activity and location (days per year).

Activity	Distribution	Parameters	Literature
Rowing in the river	Step Uniform	Min = 1 Max = 108	Assumption
Rowing in the lake	Negative binomial	μ = 5.1 k = 12	Assumption based on courses offered by Zeilschool Nieuwe Diep
Swimming in surface water	Negative binomial	μ = 8 k = 1.3	[80]
Swimming in swimming pool water	Negative binomial	μ = 24 k = 1	[80]
Fishing	Binomial	N = 12 P = 0.2	Assumption [165]
Wading / splashing / walking in flooded streets and wadi	Negative binomial	μ = 8 k = 2	[81, 165]
Wading / splashing in water playgrounds and ornamental fountain	Binomial	N = 12 P = 0.2	Assumption [165]
Walking the dog, walking close to public water taps	Binomial	N = 12 P = 0.2	Assumption [165]

Risks were calculated using Monte Carlo simulations with random sampling of 10,000 values from each distribution input. Distributions represent the variability within the data (or assumption) of each model parameter. Parameter or assumption uncertainty is not incorporated.

Sensitivity analysis was conducted to determine how sensitive the model outputs are to each of the input parameters to determine whether the model is sensitive to input parameters that are more or less certain. Spearman rank correlation coefficients (SR) and p-values were calculated between the model output (probability of illness) and each input parameter. SR correlates the variability of the output with the variability of each input parameter. When a high correlation is found, the variability in the parameter is largely responsible for the variability of the output [176]. Fitting distributions, Monte Carlo simulations, and sensitivity analysis were performed with R version 3.0.1.[177].

4. Results

Results of the risk assessment are presented as the distribution parameters (in Box Whisker plots) of the calculated probability of illness per person per exposure event (pppd) and per (exposed) person per year (pppy) (Figure 2−1 and Figure 2−2). Results from those locations and activities that had the same values in each step of the model are presented together. These are wading in pluvial floods from SSO and in the wadi, for both GI and Legionnaire's disease (LD) probability of illness, and swimming in the river and the lake and playing at the freshwater playground, for LD probability of illness.

4.1. Probability of Gastrointestinal Illness

The estimated probability of GI for single exposure events at the different water bodies are presented in Figure 2−1. Mean and 95 percentiles of the probability of infection and illness can be found in Appendix A.2. Highest mean event probabilities were found for playing in pluvial flood from the CSO (34%), swimming and rowing in the river (18% and 13%, respectively), and swimming in the lake (8.7%). Slightly lower probabilities were found for rowing on the lake (4.5%), and playing at the surface water playground (3.7%) and in the pluvial flood from the stormwater sewer and in the wadi (SSO) (4.7%). At each of these locations, the probability of GI was above the 3% tolerable GI level for excellent bathing water quality [117].

The annual probabilities of GI of 84% for rowing and 52% for swimming in the river, 18% and 33% for rowing and swimming in the lake are clearly above the 3% level. Playing in the surface water playground (8%) and in pluvial flood from SSO or the wadi (9.4%), fishing in the sedimentation pond (5.3%), and swimming in the green area pond (8.6%) were closer to the 3% level. Finally, fishing in the green area pond (0.8%), walking the dog at the park (0.3%), crossing the traffic road (0.05%), and swimming in the pool (0.5%) are below the 3% level.

4.2. Legionellosis Risks

The calculated probabilities of LD (Figure 2−2) were more variable than the probabilities of GI. This was due to the large variability in the data on *L. pneumophila* concentrations in different waters (see Table 2−1). The highest probability of LD per event was obtained for playing in pluvial floodwater from the CSO (1%), followed by rowing on the river (1.4×10^{-3}%) and the lake (1.4×10^{-3}%), and playing at the surface water playground (3.4×10^{-4}%). The probabilities derived from activities in freshwater locations were considerably lower when the *L. pneumophila* concentration data from the lake impacted by the *L. pneumophila* rich wastewater treatment plant effluent were not included in the models. The lowest risks were obtained for the park, the traffic road, and the public water taps.

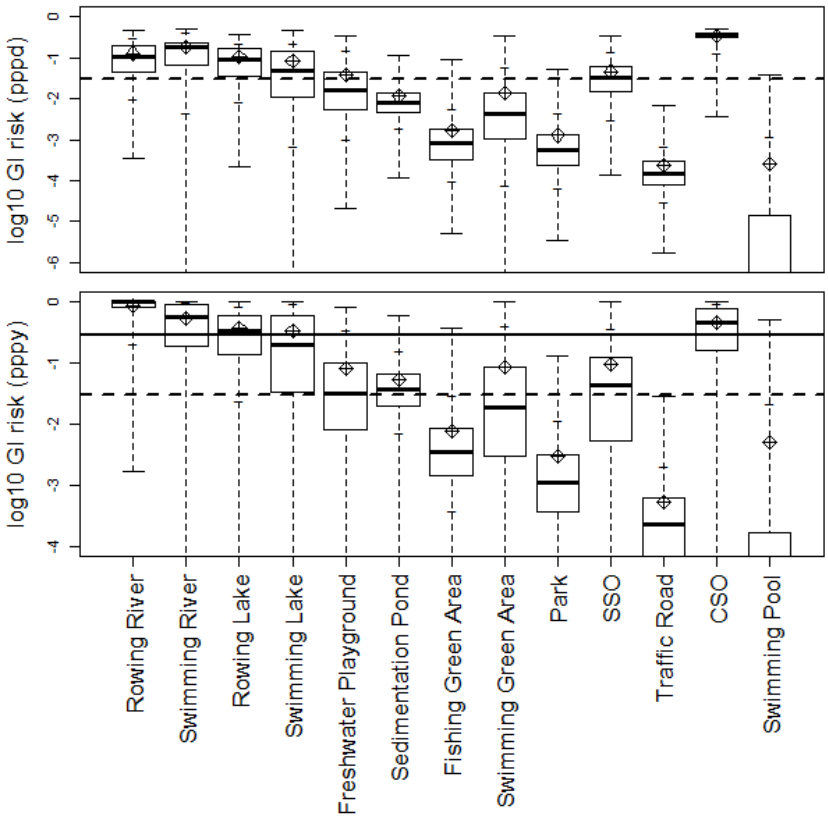

Figure 2−1:Event (upper) and annual (lower) probability of GI disease box and whiskers plots. The boxes show the interquartile range, solid lines in the boxes the median, diamonds the mean, upper and lower whiskers the maximum and minimum and hyphens the 90% CI of the risks. Horizontal lines show the 3% probability of illness that is associated with the excellent water quality in the EU Bathing Water Directive (dashed line), and the 29% annual incidence of GI disease in The Netherlands (solid line).

The annual risks for the subpopulation exposed followed the same pattern (Figure 2−2). All the annual risk medians were below the national annual incidence of legionellosis of 0.002% (average of the health surveillance data from 2009 and 2010) [61]. The mean probability for the rowers on the river and the lake were above the annual incidence when the *L. pneumophila* concentration data of all tested surface waters were included and below the annual incidence when the data from the lake impacted by the *L. pneumophila* rich wastewater treatment plant were excluded. The mean probability of LD for playing in pluvial floodwater from the CSO was above the annual incidence.

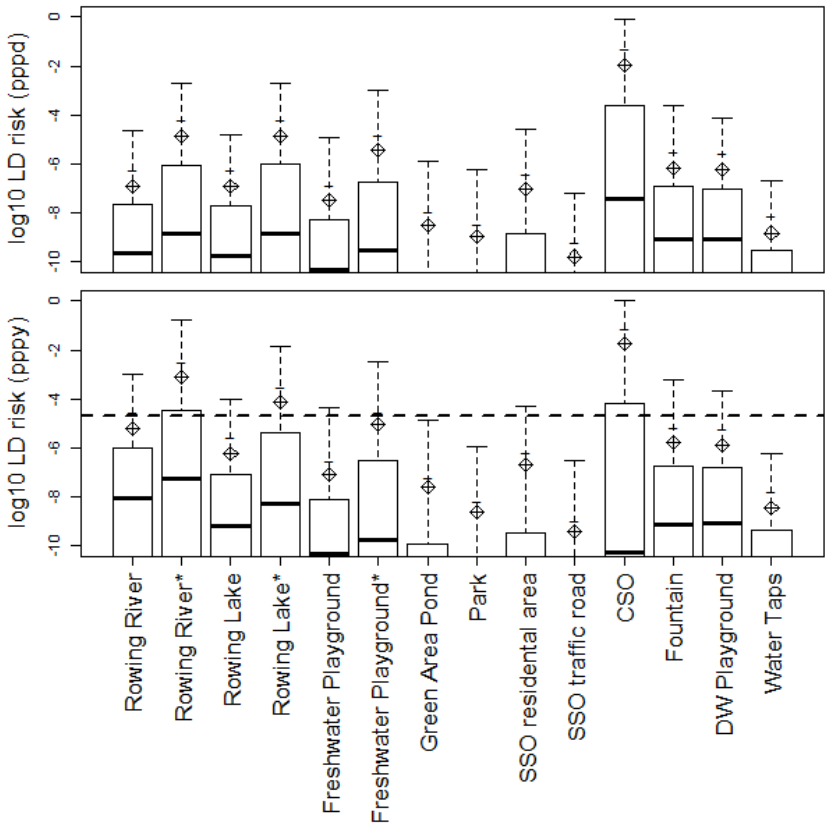

*Figure 2−2: Event (upper) and annual (lower) probability of LD box and whiskers plots. The boxes show the interquartile range, solid lines in the boxes the median, diamonds the mean, upper and lower whiskers the maximum and minimum, and hyphens the 90% CI of the probabilities. Horizontal line in the annual risk graph shows the 0.002% annual incidence of LD in The Netherlands (dashed line) *Risks estimated when data from an impacted lake are included.*

4.3. Sensitivity Analysis

In Figure 2−1 and Figure 2−2, the variability of the probabilities of illness at the different locations is shown by the extension of the whiskers. This variability reflects the variability of the model inputs. As indicated, the variability in the LD probabilities was higher than the variability in the GI probabilities.

Sensitivity analysis using SR (data on correlation coefficients and p-values can be found in Appendix A.3) shows that the variability of the GI per event is highly sensitive to the ingested volume and the pathogen concentrations. For rowing on the river and the lake, and playing in the freshwater playground, the concentration of *Campylobacter* shows very

strong significant correlations with the GI probability. For swimming in the river, the lake and the pond, playing in the flooded streets (both in the CSO and SSO), and playing in the wadi, the ingested volume has a strong to very strong correlation with the GI probability.

The concentration of norovirus is the predominant factor for the GI probability as a result of fishing in the sedimentation pond, and walking through the traffic road. However, the strength of the correlation is very close to the second factor, the ingestion volume. In the park and the green area pond, the effect of the pathogens dilution is the main responsible for the variability on the GI probability, followed by the concentration of norovirus. In the swimming pool, the concentration of *Cryptosporidium* has a very strong correlation with the probability of GI. The ingested volume is often either the first or the second factor influencing the output variability. Exposure time has only very weak to moderate correlations with the output.

At the annual level, the exposure frequency becomes the main factor for swimming in the river, playing in pluvial floodwater from the CSO and the SSO and in the wadi, crossing the traffic road, and swimming and sailing in the lake. It is the second factor for rowing on the river, swimming in the swimming pool, walking the dog at the park, and playing in the surface water playground.

In the LD models, the concentration of *L. pneumophila* in the water is always the factor with the highest influence on the variability of the LD probability, with strong to very strong correlations. Moderate correlations are found with the exposure frequency for walking at the traffic road, and playing in pluvial floodwater from the CSO, the SSO and in the wadi. The other inputs show very weak to no correlations with the LD probability.

5. Discussion

An integrated screening-level QMRA for multiple water bodies and features in an urban area, exposure types, pathogens, and illnesses was developed. This is the first time that such a holistic approach is taken in a QMRA study for waterborne illness. Previous studies focused on a single pathogen, disease or water system [7, 81, 136, 137, 151, 152, 165], and usually present the probability of infection as risk end point. In this study, the probability of GI and LD were assessed, to compare to the level of safety associated with excellent bathing water quality or the incidence of GI and LD in The Netherlands. This provided a relative risk context for the urban water managers to determine the priorities for risk management.

In the present study, the probabilities of illness were determined in a consistent and transparent approach for every water body, exposure type, and pathogen analysed. The results allow direct comparison between the water bodies. Risk management can be based on the probabilities of illness obtained, the level of variability and the source of this variability, and the parameter sensitivity of the models.

Model inputs were based on scientific evidence after a literature review on each QMRA step, and on assumptions, when no site-specific data were available. When assumptions were needed, a conservative approach was followed, as accorded with the risk managers involved in the study. A list of the assumptions used can be found in Appendix A.4. These assumptions influence the outputs of the model and should be considered by the risk managers. In the absence of site-specific information, site-specific research is needed to confirm (or disprove) their validity. The results of this assessment can be used to set priorities for site-specific data collection.

The GI risks derived from recreational exposure at several locations were not negligible. Highest annual probabilities of GI were obtained for playing in pluvial floodwater from a CSO and swimming and rowing in the river or lake, and lowest after walking through the traffic road and swimming in the swimming pool. The mean probabilities of the high risk exposure scenarios clearly exceeded the 3% GI level associated with excellent bathing water quality. The GI incidence in The Netherlands from all pathogens and all sources was 29% [32]. The annual probability of GI for the exposed population to the river and lake and the CSO are close to this annual incidence, indicating that these exposures could be a significant contribution to the annual incidence of GI in this exposed population.

In most locations, the calculated LD probabilities were low and below the mean incidence of LD in The Netherlands for 2009 and 2010 (0.002%) [61]. The calculated probability of LD was relatively high for the pluvial flood from the CSO and for rowing on the river and lake (high scenario), but the calculated probabilities were sensitive to the variable *L. pneumophila* input concentrations. At these locations, the LD probabilities were above the mean national incidence. The incidence data are based on diagnosed cases only, and unreported cases may occur, so it is likely that the 0.002% is underestimating the actual incidence of LD.

The estimated illness probabilities (particularly for LD) have a large variability, as shown by Figure 2−1 and Figure 2−2. This variability was due to the variability of the input parameters (Table 2−1, Table 2−2, Table 2−3, and Table 2−5). Concentrations of pathogens, which have a large effect on the variability of the disease probabilities, are variable, and this contributes to the variability of the illness probabilities. In addition, translation of data from other water bodies to those under study is a source of uncertainty. Site-specific data collection can be used to reduce this uncertainty. Ingestion volumes, however, are variable, but this variability will probably not be reduced by further data collection.

Comparing our risks estimates with those previously published is not straightforward, due to the variety of water sources, activities, and pathogens assessed in the present study. GI risk estimates from single exposure events at CSO and SSO are comparable to those found in other Dutch studies in CSO and SSO systems [81, 152]. Swimming in the swimming pool and in the river gave event risks comparable to those found by other authors in similar systems [80, 165]. Swimming risks were higher than those found

previously [136], but these did not incorporate *Campylobacter* and norovirus (our main risk drivers). Using a questionnaire survey, event GI probabilities of 1.4 – 1.5% for limited-contact water activities (rowing, fishing) in surface waters were obtained [178]. This is lower than the GI probability for rowing and higher than for fishing obtained in our study, but direct comparison is difficult since pathogen data were not reported.

LD probabilities were higher than those estimated from roof-harvested rainwater systems in Australia [137]. However, the concentrations of *L. pneumophila* in Australia were lower, the exposure was shorter, and the aerosol estimation method was different.

This study points to water bodies with a high probability of GI, even in the context of the high "background" incidence of GI in The Netherlands [32], and provides an evidence-base for water risk management. Data collection, specifically on concentrations of pathogens, at those sites with higher probability of disease, is recommended to reduce uncertainties and to plan the necessary actions for risk reduction. Measures to reduce exposure include advising people to swim only in designated areas (the river and the lake are not designated bathing areas), provide alternative bathing sites, inform about the risks of playing on flooded streets from CSO, or prevent flooding events. Increasing the residence time of the water at the sedimentation pond and/or adding other water treatment measures (e.g., filtration) would reduce the load of pathogens in the inner polder system. This could also be achieved at the surface water playground by treating the lake water before it enters the playground. Further treatment of the wastewater at the plants that discharge the effluent in the river would reduce the amount of pathogens in this water. In the wadis, the risk could be reduced by removal of faecal input, for example by not placing areas where dogs depositions are allowed in the surroundings, or filtering the water from the roofs' gutters, and, in frequently flooded areas, removing the CSO systems.

Chapter 3: Quantification of Waterborne Pathogens and Associated Health Risks in Urban Water

Abstract

People in urban areas are exposed to microbial hazards in urban waters. To quantify potential health risks associated with this exposure, pathogen concentrations in an urban river, lake, stormwater sedimentation pond, a pond in a park and a wadi (bioswale), all in the same urban area, were assessed. *E. coli* concentrations were variable in all locations during the studied period, mean values ranging between 1.2×10^2 (lake) and 1.7×10^4 (sedimentation pond) colony forming units (cfu)/100mL. High concentrations of *Campylobacter* were found at all locations, being the lowest in the lake (4.2×10^1 genomic copies (gc)/L) and the highest in the wadi (1.7×10^4 gc/L). *Cryptosporidium* was not found in any sample and low levels of adenovirus 40/41 were found in some samples in the river (1.8×10^1 gc/L) and lake (7.2×10^0 gc/L), indicating human faecal contamination at these sites. *L. pneumophila* was found in the sedimentation pond, with higher concentrations after rain events (1.3×10^2 gc/L). Cyanochlorophyll-a was found in the lake (7.0×10^{-1} µg/L), the sedimentation pond (1.1×10^0 µg/L) and the pond in the park (2.9×10^1 µg/L), where low levels of microcystin were found (2.1×10^0 µg/L). *Campylobacter* data, combined with published water exposure data, were used to estimate gastrointestinal risks from recreational exposure to these sites. This revealed risks above the annual disease incidence of campylobacteriosis in The Netherlands at all locations, being highest in the wadi and river. The sensitivity analysis showed that the *Campylobacter* concentration was the input with higher influence on risk variability for rowing in the river and lake, fishing in the pond and walking in the park, while the ingested volume was the main factor for swimming in the river and the lake and playing in the wadi. Measures are proposed to reduce the health risks at these locations.

1. Introduction

Water in urban areas is often perceived by citizens as a positive element because it provides aesthetic quality to the neighbourhood and offers recreational opportunities [5]. In The Netherlands, water bodies and features in metropolitan areas are abundant. As a consequence, human contact with water in urban public spaces happens very often during recreation in and around ponds in parks and other blue-green areas, urban rivers and lakes, water playgrounds, public swimming pools, street drinking water taps, or urban canals [179].

Exposure of humans to urban water concepts may lead to health risks when pathogenic microorganisms are present in these waters [150]. Microbial hazards may be present in water bodies due to input of faecal material such as sewage discharge containing human enteric pathogens (*Campylobacter*, *Cryptosporidium*, norovirus, rotavirus, etc.) or animal faecal input (from waterfowl, dogs, and other domestic and wild animals) containing zoonotic pathogens [14, 15], or growth of microorganisms in urban water bodies and features, such as toxic cyanobacteria in stagnant waters [16], or *Legionella pneumophila*, especially in warm water systems [17]. Microbial risks are also influenced by climate change. A higher frequency and strength of storms and draughts affects the concentration of pathogens [14, 18, 19], a temperature increase may promote formation of cyanobacterial blooms [20].

Previous research assessed the microbial quality and health risks of urban water, but focused on a single pathogen/disease and/or a single water feature [7, 81, 136, 137, 151-153, 180]. In a previous study, we characterized the health risks of multiple pathogens and multiple urban water features using literature data. The sensitivity analysis showed that site-specific microbial data is the main factor to decrease the uncertainties of the risk assessment [179]. The aim of the present study was to obtain site-specific data on microbial quality of several water locations in an urban area, to estimate the health risks derived from recreational exposure to those water locations. *Campylobacter spp.*, *Cryptosporidium*, adenovirus 40/41, *Legionella pneumophila* and cyanobacteria were the pathogens targeted during a ten-week monitoring campaign. Furthermore, two urban stormwater storage locations were studied during a rain event. Concentrations of microorganisms were correlated with weather parameters to evaluate the impact of future climate scenarios.

2. Materials and Methods

2.1. Sampling Locations

The studied urban area is a polder located in the southeast of the city of Amsterdam (The Netherlands). It is surrounded by the river Amstel, a lake, and a ring of canals fed from the river. The water that flows through the system is stormwater overflow from combined sewers in some areas and separated sewers in others. Stormwater is stored in reservoirs that

act as sedimentation ponds. When there is not enough rainfall, weirs are opened to allow the flow of surface water from the ring of canals.

The selected locations for this study were the river, the lake, a pond located in a park, and a sedimentation pond that receives stormwater from a separate sewer (Figure 3−1). These locations were sampled weekly for a period of 10 weeks. Furthermore, the sedimentation pond and a bioswale (wadi) were studied during a rain event (Figure 3−1). Activities that take place at these locations, bringing humans in contact with the water of those features, are swimming and rowing in the river and lake, walking in the park, fishing in the pond, and playing in the wadi.

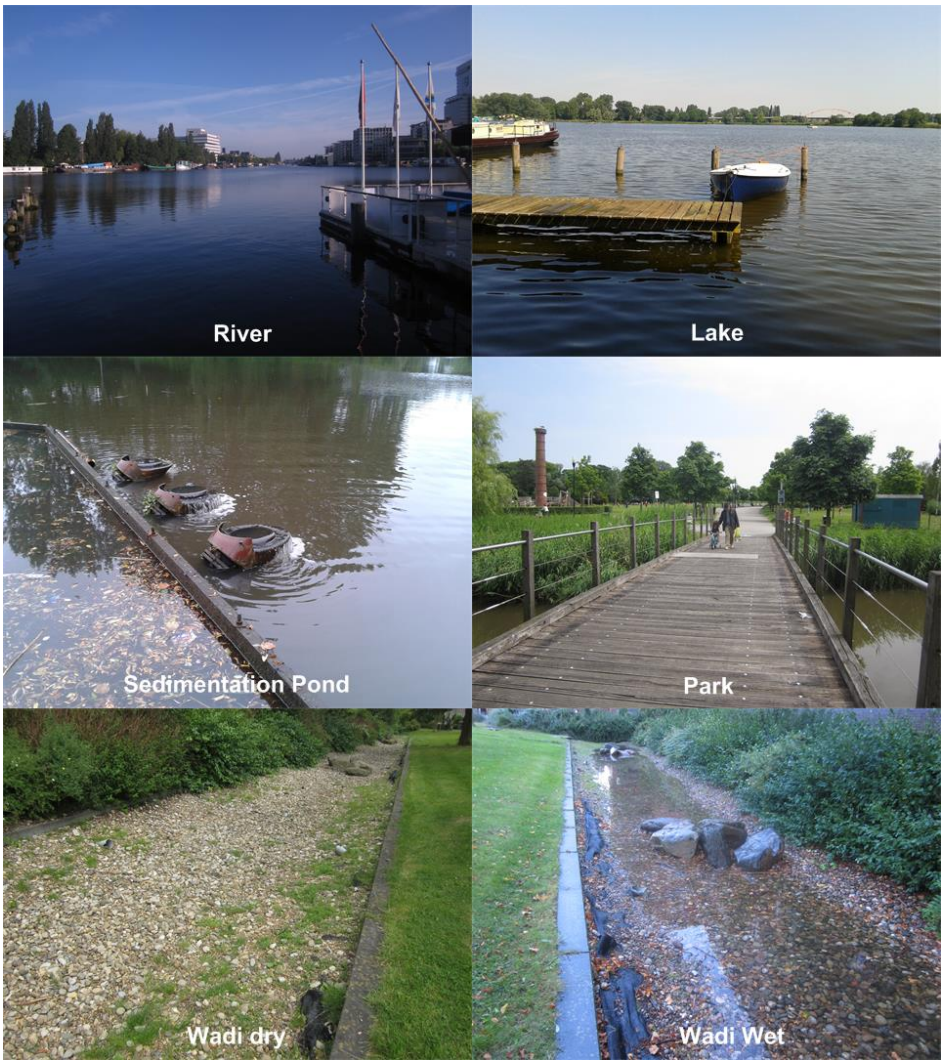

Figure 3−1: Selected sampling locations.

2.2. Target Pathogens

Waterborne pathogens can cause diseases of different nature in humans. *Escherichia coli* was selected as indicator of faecal contamination since the European Bathing Water directive relies on this indicator for classifying the water quality [117]. To account for different illnesses and pathogens types, *Campylobacter spp.*, *Cryptosporidium*, adenovirus 40/41, *L. pneumophila*, and cyanobacteria were selected for this study. This way, gastrointestinal, respiratory, and skin diseases are included, and bacteria, protozoa, viruses and toxins are studied. Furthermore, adenovirus 40/41 is not only a human pathogen but its presence in water indicates human-faecal contamination [135].

2.3. Sample Design

The monitoring study was conducted during ten weeks in 2012, from mid- July to the end of September. Approximately 100 L of water were collected from each location for concentration and further analysis, and 1 L samples for direct *E. coli* culture. Samples were pre-treated with a cloth filter (with 100 µm pore size) after the first sampling week to avoid sampling coarser particles that hampered sample processing. Water temperature was monitored at the moment of sampling.

During a rain event on September 19, 2012, seven 1 L samples were collected from the outlet (weekly monitoring location) and seven from the inlet of the sedimentation pond during the lapse of one week, in order to observe the evolution of water quality after a rainfall event. Furthermore, four samples were collected from a wadi on the same rain event day. The water in the wadi drained out in few hours, so further sampling was not possible. Water temperature was monitored at the moment of sampling.

2.4. Sample Processing

Samples were transported to the lab as soon as possible, stored at 4 ºC and analysed within 24 hours. 100 L samples were concentrated into 0.5 L (approximately) using a Hemoflow®. The Hemoflow® method had previously been studied at our lab and showed an average recovery efficiency of 93% for *E. coli*, 35% for *Campylobacter* (analysed with MPN method) and 67% for *Cryptosporidium* in surface water samples [181].

All samples were analysed for indicator *E. coli* using culture methods. *E. coli* was also cultured from 100 L samples after concentration, in order identify effects of the Hemoflow® concentration method. Briefly, decimal dilutions of duplicate aliquots were plated on Lauryl Sulfate Agar. Volumes of 0.1 µL or smaller were plated directly, after the corresponding decimal dilutions in sterile water. For higher volumes, the membrane filtration culture method was used. A positive control (PC), consisting on 1 mL of *E. coli* (stored under -80 °C) diluted in 250 mL of sterile water, and a negative control (NC), consisting on sterile water, were also processed. All plates were incubated for 14 hours at 44ºC, preceded by 5 hours at 25 ºC. *E. coli* characteristic colonies were counted, a

representative part was isolated in 100 µL of DNAse-RNAse-free water (Gibco, Life Technologies), and stored under -80 ºC for future PCR identification.

Five µL of solution containing the isolated colonies were mixed with 20 µL of PCR mix containing BioRad Powermix for multiplex PCR, and primers and probes targeting *E. coli* and Eubacteria. A PC, consisting of cultured *E. coli*, a DNA template NC consisting of cultured *Pseudomonas diminuta*, and a NC consisting of DNAse-RNA-se-free water were also analysed. Colonies were identified as *E. coli* when both reactions (eubacteria and *E. coli*) gave a positive signal. Primers, probes, and PCR conditions are shown in Table 3−1. The EC *uidA* primers are described in Heijnen and Medema [182], the EC probe and the sequences for *Eubacteria* have been designed by Dutch water laboratories.

Concentrates of the weekly monitoring samples and 1 L samples from the rain events were stored under -80 ºC for future further processing. Frozen concentrates and rain event samples were thawed at room temperature and centrifuged (Varifuge 3.0RS, Dijkstra Vereenigde) at 5 ºC for 30 min, at 4,000 rpm, and low deceleration speed to avoid pellet detachment. Centrifugation was repeated until all the sample was processed. Pellets were frozen at -80 ºC and supernatants were further filtered using Centricon® Plus-70 Centrifugal Filter units (Merk Millipore). Small volumes of the supernatant were loaded at a time, and filtered by centrifugation at 900 g for 10-30 min at 4 ºC. Concentrates recovered from the filter by centrifugation were stored at -80 ºC.

2.5. DNA Extraction

After slow thawing, DNA from pellets and supernatants' concentrates was extracted using the PowerMax® Soil DNA Isolation Kit (MO BIO Laboratories, Inc) following the manufacturer's instructions. Briefly, 10 mL of pellet or 5 mL of supernatant were added to a bead tube together with a series of buffers that aid in homogenization. Cell lysis and DNA extraction occurred by mechanical and chemical methods. Extracted genomic DNA was captured on a silica membrane in a spin column format, washed, and eluted from the membrane with 5 mL of elution buffer.

A PC, consisting of 9 mL of one of the extraction samples spiked with 1 mL of *L. pneumophila*, 200 µL of *Campylobacter coli* and 100 µL of *Cryptosporidium parvum* solutions, was treated as the rest of the samples in each extraction day. A NC was also prepared consisting on 10 mL of DNAse/RNAse-free water. Ten µL of internal control (IC), a fragment of the Dengue virus, were added in the first steps to all samples PC and NC in order to calculate the efficiency of the extraction method. The DNA in the 5 mL elutates was mixed with 1:10 of sodium acetate (3M, Sigma Aldrich) and ethanol of 99% purity (J.T. Baker), and centrifuged at 5,000 g for 45 min. The pellets were cleaned twice with 70% ethanol, centrifuged at 5,000 g for 15min, and eluted in 300 µL of DNAse/RNAse-free water.

2.6. Quantitative PCR

Ten µL of two and four-fold dilutions of each DNA sample (these dilutions showed absence of inhibition of the PCR reaction, see Appendix B.1) were analysed in duplicate by probe q-PCR. The primers and probes used to quantify the DNA of each microorganism are shown in Table 3−1. *Campylobacter* target genes used are found in several *Campylobacter* species, including the human pathogenic *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari*, and the sequences have been designed by Dutch water laboratories. *Cryptosporidium* primers target *C. hominis* and *C. parvum*, and the sequences have been modified from Guy, et al. [183]. Adenovirus sequences target the long fiber protein gene of adenovirus 40 and 41 and are based on Ko, et al. [184]. *L. pneumophila* primers and probes have been published by Wullings, et al. [185]. A standard curve, consisting on serial dilutions of the target DNA for *L. pneumophila*, *Campylobacter spp.*, adenovirus 40/41 and IC, was used to quantify the DNA in each PCR reaction. *Cryptosporidium* used a different standard curve, consisting of serial dilutions containing *Cryptosporidium* target sequences. *L. pneumophila* and the IC were quantified in a single multiplex q-PCR reaction, while the rest of the targets were quantified in simplex q-PCR reactions.

2.7. Cyanobacteria

Total chlorophyll and cyanochlorophyll-a (chlorophyll belonging to *Cyanobacteria*) were analysed with the Fluoroprobe method as described by Van der Oost, et al. [124] in weekly water samples from the same locations. Those samples containing cyanochlorophyll-a concentrations higher than 12.5 µg/L, were frozen for further ELISA (Enzyme-Linked Immuno Sorbent Assay) analysis of microcystin. The boundary of 12.5 µg/L was chosen for being the concentration above which the recreational water is considered to pose small health risks in The Netherlands [123].

Briefly, the microcystin was extracted with boiling water for 30 min after addition of methanol (1:1) and diluted with distilled water to obtain a toxin concentration of 0.1-1.6 µg/L. The microcystin content was measured with the Envirogard Microcystins Plate® kit (SDI) following the manufacturer's instructions. The kit uses polyclonal antibodies which bind microcystins. The optical density of the samples was measured at 460 nm with a Tecan Spectra Fluor Plus microplate reader and microcystin levels were determined with a standard calibration curve [124].

2.8. Weather Correlations

Hourly weather data from the closest weather station to the Watergraafsmeer (Schiphol) were downloaded from the Royal Dutch Meteorological Institute (KNMI) website [186]. Spearman rank correlation coefficients (rho) and p-values between microorganisms concentrations and several weather parameters were calculated with R version 3.0.1. Weather parameters studied were ambient temperature, rainfall amount, relative humidity,

solar radiation, cloud coverage, wind intensity, and dry period, the latter defined as the number of days between the sampling event and the last storm with rainfall of at least 1, 5 and 10 mm. Furthermore, water temperature, recorded at the moment of sampling, was also included in the analysis.

Table 3−1: Sequence of the primers and probes for the q-PCR analysis and the reaction parameters.

Target	Target Gene		Primer and Probe sequences (5'-3')	PCR Parameters
E. coli identification - *E. Coli*	*uid*A	F	ATGGAATTTCGCCGATTTTGC	3 min at 95°C followed by 30 cycles of 20 s at 95°C, 60 s at 60°C and 40 s at 72°C
		R	ATTGTTTGCCTCCCTGCTGC	
		P	AGCAGAAAAGCCGCYGACTTCG	
- Eubacteria		F	CACACTGGRACTGAGACACGG	
		R	CGCGGCATGGCTGSATCAG	
		P	HGCTGCCTCCCGTAGGAGT	
Campylo-bacter spp. (q-PCR method)	16S rRNA gene	F	TGAGGGAGAGGCAGATGG	3 min at 95°C followed by 45 cycles of 20 s at 95°C and 1 min at 60°C.
		R_1	CGCAATGGGTATTCCTGG	
		R_2	CGCAATGGGTATTCTTGG	
		P	TTGGTGGTGTAGGGGTAAAATCCG	
Cryptospo-ridium parvum and *hominis*	COWP	F	CAGGAGATGATTGTGTACTATATG	3 min at 94°C followed by 40 cycles of 15 s at 94°C and 1 min at 60°C.
		R	GACAGGTTGAGTTGGAGCAG	
		P	CCCACCAAATTTCATTTTACAAGGC CTCC	
Adenovirus	Adv40/ 41 (long fiber protein gene)	F	CTTTCTCTCTT(A/C)ATAGACGCCC	3 min at 95°C followed by 45 cycles of 20 s at 95°C and 1 min at 60°C
		R	GAGGGGGCTA(G/C)AAAACAAAA	
		P	CGGGCACTCTTCGCCTTCAAAGTGC	
Legionella pneumophila	*Mip*	F	CCGATGCCACATCATTAGC	5 min at 95°C followed by 43 cycles of 20 s at 95°C and 48 s at 60°C
		R	CCAATTGAGCGCCACTCATAG	
		P	TGCCTTTAGCCATTGCTTCCG	

**F, forward primer; R, reverse primer; P, probe.*

2.9. Health Impact Assessment

To estimate the health risks of recreational exposure to the different sites, distributions were fitted to the pathogens data and exposure models were built for each location and activity using literature data as in Sales-Ortells and Medema [179].

Because adenovirus 40/41 was found occasionally and in concentrations close to its limit of quantification (LOQ), it was not included in the risk assessment. *L. pneumophila* was not found in locations where activities result in aerosolization of water, and *Cryptosporidium* was not found in any location. Therefore, they were not included in the risk assessment. Finally, cyanobacteria was also not included because it was found in concentrations below the threshold for small health risks [123] (see results).

Gamma distributions were fitted to the measured concentrations of *Campylobacter spp.* (C_o) and beta distributions to the recovery efficiency of the samples (R). Then, and a new distribution was created by 10,000 combinations of the gamma and beta distributions to estimate the real concentration (C_r) as described in Pouillot, et al. [172] (equation 3.1), but adapted for continuous variables. The goodness of fit of the distributions to the data was analysed with the Kolmogorov-Smirnov test.

$$C_r = C_o + gamma(C_o + 1, (1 - R)/R\ ) \tag{3.1}$$

Human pathogenicity was based on the presence/absence of adenovirus 40/41 at the sampling site. When no adenovirus was found, all the *Campylobacter* was assumed to be from bird and/or dog faecal origin. The probability of finding human pathogenic *Campylobacter* in animal samples (zoonotic *Campylobacter*) was then estimated using data on different bird species droppings and dogs faeces positive in zoonotic *Campylobacter* (*C. jejuni*, and *C. coli*) relative to the *Campylobacter spp.* positive [187, 188]. This was introduced as a normal distribution (0.18, 0.05). When adenovirus 40/41 were present in the samples, the infectivity was assumed to be 100% because the pathogens were considered to have human-faecal origin.

The activities conducted at each site are: swimming and rowing at the river and lake, fishing at the sedimentation pond, walking the dog in the park (where dogs swim in the pond and splash water), and playing in the wadi. The distribution and parameters used for each step of the exposure assessment, and the literature source, are shown in Table 3−2.

The dose of *Campylobacter* (d) was estimated using equation 3.2:

$$d = C_r \times P_i \times v[\times t] \tag{3.2}$$

where Pi is the probability of *Campylobacter* of being human pathogenic, v the volume ingested, and t the time spent at the location (only used when v is given in mL/h).

The risk of *Campylobacter* infection per event (P_d) was calculated using the hypergeometric dose response model (equation 3.3) with parameters $\alpha = 0.024$ and $\beta = 0.011$ [24]. The disease risk was calculated multiplying the infection risk by a disease given infection factor of 0.33 [30]. These model and parameters were chosen because these are the most conservative ones and were derived from a data set that included *Campylobacter* outbreak data.

$$P_d = 1 - \ {}_1F_1\ (\alpha, \alpha + \beta, -d_i) \tag{3.3}$$

Table 3−2: Exposure assessment steps, distribution and parameters for the different activities.

Step	Activity	Distribution	Parameters	Units	References
Ingestion Volume	Swimming	Gamma	$\alpha = 0.64$ $\beta = 58$	mL/ event	[80] (children in surface water)
	Rowing	Triangular	Min = 0.1 Mode = 3.1 Max = 7	mL/h	[82, 165], min is an assumption
	Fishing	Triangular	Min = 0.1 Mode = 3.5 Max = 7	mL/ event	[82, 165], min is an assumption
	Walking	Triangular	Min = 0.1 Mode = 3.5 Max = 10.6	mL/h	[82] min is an assumption
	Playing in wadi	Triangular	Min = 0 Mode = 0.051 Max = 5	mL/event	Based on [81]
Exposure time	Rowing	Triangular	Min = 1 Mode = 2 Max =4	H	Assumption
	Walking	Triangular	Min = 15 Mode = 30 Max =60	Min	Assumption
Exposure frequency	Swimming	Negative binomial	$\mu = 8.0$ $k = 1.3$	days/year	Assumption
	Rowing in the river	Step Uniform	Min = 1 Max = 108	days/year	Assumption
	Rowing in the lake	Negative binomial	$\mu = 5.1$ $k = 12$	days/year	Assumption based on courses offered by the sailing school
	Fishing	Binomial	N = 12 P = 0.2	days/year	Assumption based on [165]
	Walking	Binomial	N = 12 P = 0.2	days/year	Assumption based on [165]
	Playing in wadi	Negative binomial	$\mu = 6.2$ $k = 3.2$	days/year	Assumption

The annual risk (P_y) was estimated for each location and exposure activity using equation 3.4:

$$P_y = 1 - (1 - P_d)^f \quad (3.4)$$

where f are the exposure events per year for each activity and location. The exposure days were based on exposure frequencies at each location, and varied for each location and activity (see table 3−2). Exposure frequencies for swimming in the river and lake, and playing in the wadi depend on weather conditions. The river and lake are not official swimming locations and, hence, swimming is not expected there, but still happens during hot weather conditions. Therefore, the number of hot weather events (defined as days when the mean temperature was at least 18 ºC and the maximum temperature was at least 25 ºC) in the past ten years in Amsterdam were recorded [186] and a negative binomial distribution was built assuming that the probability for a child swimming there during every hot event is very low, while the probability of swimming once in a year is very high. The same approach was followed for playing in the wadi, but in this case, the number of rain events with at least 5 mm of rainfall (amount of rain needed to fill the wadi, based on observation) during the summer period (May to September) in the past ten years were considered to build the exposure events distribution.

Risks were calculated using Monte Carlo simulations with random sampling of 10,000 values from each distribution input for each model. A sensitivity analysis was conducted by fixing one input at its 97.5 or 2.5 percentiles at a time, while maintaining the variability of the other inputs, and the health risks were recalculated. The Kolmogorov-Smirnov test was used to check if the differences between the output of the sensitivity analysis and the original model were statistically significant.

For swimming in the river and the lake, the results were compared with an alternative scenario, using the exposure frequency distribution from Schets, et al. [80] for children swimming in surface waters. In the wadi, an alternative scenario was built with exposure frequency data from de Man, et al. [81]. Statistical analysis, distribution fit, Monte Carlo simulation, and sensitivity analysis were implemented with R version 3.0.1 [177].

3. Results

3.1. Indicator and Pathogen Concentrations

Concentrations of *E. coli* were highly variable through the weekly monitoring study. Highest concentrations were found at the sedimentation pond, and lowest at the lake. *Campylobacter spp.* was found at all locations, with higher concentration in the sedimentation pond. *Cryptosporidium* was not found in any sample, and its mean LOQ was 5.8 genomic copies (gc)/L in the river, 7.9 gc/L in the lake, 4.5 gc/L in the sedimentation pond, and 6.7 gc/L in the pond. Adenovirus40/41 was found occasionally in the river and lake, in concentrations close to its LOQ, which was 13.8 gc/L in the river, 18.9 gc/L in the

lake, 5.2 gc/L in the sedimentation pond, and 16.0 gc/L in the pond (mean values) *L. pneumophila* was found in the sedimentation pond in six out of ten samples, while in the other locations all samples were negative (mean LOQ was 13.8 gc/L in the river, 18.9 gc/L in the lake, 10.9 gc/L in the sedimentation pond, and 16.0 gc/L in the pond).

Cyanochlorophyll-a concentrations were lower than the 12.5 µg/L threshold for safe recreational water in the river, the lake, and the sedimentation pond. It was higher than the safety benchmark for nine out of ten samples in the pond in the park. Concentrations of microcystin at the pond, were, however, lower than the benchmark value for safe recreational water (12.5 µg /L). The LOQ of the cyanochlorophyll-a was 0.5 µg /L and of microcystin 1 µg /L. Mean and 95% values of the weekly monitoring concentrations of indicators, pathogens and toxins for the four locations are summarized in Table 3−3.

Table 3−3: Concentration of microorganisms and microcystin in the weekly monitoring samples.

		E. coli	***Campy-lobacter spp.***	**AdV 40/41**	***L.pn***	**Total chloro-phyll-a**	**Cyano-chloro-phyll-a**	**Micro-cystin**
		cfu/ 100mL	gc/L	gc/L	gc/L	µg/L	µg/ L	µg/ L
River	Pos/tot	10/10	9/10	6/10	0/10	10/10	0/10	-
	Mean	4.6×10^{2}	13.8	18	-	17.6	-	-
	95%	8.1×10^{3}	250	45.2	-	37.0	-	-
Lake	Pos/tot	10/10	6/10	4/10	0/10	10/10	6/10	-
	Mean	1.2×10^{2}	42.0	7.2	-	19.1	0.7	-
	95%	2.8×10^{3}	653	14.3	-	47.7	1.4	-
Sedimen-tation pond	Pos/tot	10/10	10/10	0/10	6/10	9/10	1/10	-
	Mean	1.3×10^{3}	368	-	45.2	2.0	1.1	-
	95%	4.0×10^{4}	3842	-	194	3.5	0.6	-
Pond	Pos/tot	9/9	5/9	0/9	0/9	10/10	10/10	9/9
	Mean	6.4×10^{2}	173	-	-	110	29.3	2.1
	95%	3.3×10^{3}	333	-	-	202	57.3	3.6

** mean is geometric mean of positives; cfu is colony forming units, gc is genomic copies, Adv is adenovirus, L. pn is L. pneumophila, Pos/tot is positive out of total samples.*

The mean recovery efficiency of the molecular extraction methods was 50.4 % in the river, 46.6% in the lake, 73.1% in the sedimentation pond and 70.4% in the pond (analysed volumes and recovery efficiency for each sampling location are shown in Appendix B.2).

The concentration of indicators and pathogens in the sedimentation pond and the wadi during the rain event are shown in Table 3−4. Geometric means of *E. coli*, *Campylobacter spp*. and *L. pneumophila* in the sedimentation pond were higher than during the weekly monitoring period. *Cryptosporidium* and adenovirus 40/41 were not found in any sample. However, the LOQ was high (79.2 gc/Lfor *Cryptosporidium* and 190 gc/L for adenovirus

40/41) because of the low volume of sample. The mean recovery efficiency of the samples was 37.8%. No statistically significant differences were found on the concentration of *E. coli* and pathogens between the inlet and outlet of the sedimentation pond. A decay in time was observed in both inlet and outlet for the three bacteria.

In the wadi, the concentration of *E. coli* was comparable to the concentration found in the sedimentation pond during the rain event, while the mean concentration of *Campylobacter spp.* was higher, and *Cryptosporidium,* adenovirus40/41 and *L. pneumophila* were below their LOQ (90.3 gc/L for *Cryptosporidium* and 216.7 for adenovirus and *L. pneumophila*). The recovery efficiency was 42.2%.

Table 3−4: Concentration of microorganisms in the rain event samples.

		E. coli	***L.pn***	***Campylobacter spp.***
		cfu/ 100mL	gc/L	gc/L
Sedimentation pond	Pos/tot	7/7	6/7	7/7
Inlet	Mean	1.1×10^{4}	1.3×10^{2}	7.0×10^{3}
	95%	4.0×10^{4}	3.0×10^{2}	3.2×10^{4}
Sedimentation pond	Pos/tot	7/7	7/7	7/7
Outlet	Mean	1.7×10^{4}	1.2×10^{2}	6.5×10^{3}
	95%	4.0×10^{4}	3.7×10^{2}	2.4×10^{5}
Wadi	Pos/tot	4/4	0/4	4/4
	Mean	1.1×10^{4}	-	1.7×10^{4}
	95%	1.5×10^{4}	-	2.0×10^{4}

**two samples of the sedimentation pond outlet belong to the weekly monitoring as well; mean is geometric mean of positives; cfu is colony forming units, gc is genomic copies, L. pn is L. pneumophila, Pos/tot is positive out of total samples.*

3.2. Weather Correlations

Table 3−5 shows Spearman correlations (and p-values) between the different weather parameters and water temperature, and concentrations of *E. coli* and pathogens. Only significant correlations ($p < 0.05$) are shown. Example plots of the rank correlations are shown in Appendix B.3.

In the river, no significant correlation was found between *E. coli* and other parameters, but *Campylobacter spp.* was inversely correlated with ambient temperature and directly with the cloud coverage and RH. Adenovirus was directly correlated with the rain and inversely with water temperature and ambient temperature.

In the lake, *E. coli* and *Campylobacter spp.* were inversely correlated with ambient temperature and radiation; furthermore, *Campylobacter spp.* was directly correlated with the rain. Adenovirus was directly correlated with RH and inversely with water and ambient temperature, and with radiation.

Table 3−5: Spearman rank correlation coefficient (p-values) between the microorganisms in each weekly sampling location and weather parameters (only statistically significant correlations – p-value<0.05 – are shown).

Location	Microorganism	*E. coli*	Temperature (°C)	Rain	RH	Radiation	Cloud Coverage	Wind	Dry period
River	*Campylobacter*		-0.71 (0.03)		0.67 (0.04)		0.71 (0.02)		
	Adenovirus		-0.76 (0.01)	0.65 (0.04)		-0.70 (0.03)			
Lake	*E.coli*		-0.81 (0.008)			-0.70 (0.03)			
	Campylobacter		-0.64 (0.048)			-0.72 (0.02)			
	Adenovirus		-0.75 (0.01)		0.66 (0.04)	-(0.72(0.02)			
Sedimenta-tion pond*	*E.coli*		-0.73 (0.002)	0.85 (7.1×10^{-5})		-0.69 (0.004)		0.54 (0.04)	-0.85 (5.6×10^{-5})
	Campylobacter		-0.65 (0.009)						
	L. pn	0.787 (0.0007)	-0.61 (0.02)	0.92 (8.9×10^{-7})	0.68 (0.005)	-0.73 (0.002)			-0.85 (5.6×10^{-5})
Pond in park	*E.coli*			0.71 (0.03)					
	Campylobacter					-0.73 (0.03)			
	Cyanobacteria			-0.71 (0.03)					0.73 (0.02)

**15 sampling points are considered in the sedimentation point, including the weekly monitoring and rain event samples; L.pn is L. pneumophila.*

In the sedimentation pond, *E. coli* was directly correlated with *L. pneumophila*, the rain and the wind, and inversely correlated with temperature, radiation and elapsed time since the last 1 mm and 5 mm rainfall. *Campylobacter spp.* was not correlated with any parameter. This could mean that the origin of *Campylobacter spp.* are the birds present in the pond. This is strengthen by the absence of adenovirus at this location (indicating absence of human faecal contamination). *L. pneumophila* was directly correlated with rain and wind (and with *E. coli*) and inversely correlated with radiation and elapsed time since last 1mm and 5mm rainfall.

Pooling all the data from the sedimentation pond (weekly monitoring plus rain event), *E. coli* was, furthermore, correlated inversely with the water temperature, and *L. pneumophila* inversely correlated with the water temperature and directly with RH. Concentration of *L. pneumophila* was significantly different when less than 1mm of rain fell in the previous 24 h than when it rained more than 1mm. *Campylobacter spp.* was inversely correlated with ambient temperature and radiation. No correlation was found between *Campylobacter spp.* and intensity of rain or duration of dry period.

In the pond in the park, *E. coli* was correlated directly with the rain and *Campylobacter spp.* was inversely correlated with radiation. Cyanochlorophyll-a was correlated with the rain and duration of the dry period.

3.3. Health Impact Assessment

Gamma distributions were fitted to the observed *Campylobacter spp.* concentration (Table 3−6) and to the recovery of the pellets DNA extraction, and the real concentration was estimated using equation 3.1. The ANOVA test demonstrated that the recovery was not significantly different for the different locations. Therefore, all the recovery data were pooled together and the parameters of the beta distribution were estimated from the pooled data using the maximum likelihood estimation method ($\alpha = 2.68$, $\beta = 1.85$).

Table 3−6: Distribution of observed Campylobacter spp. concentration.

Location	Parameters
River	$\rho = 0.57$
	$\lambda = 60.6$
Lake	$\rho = 0.31$
	$\lambda = 148.6$
Sedimentation Pond	$\rho = 0.92$
	$\lambda = 1.46 \times 10^3$
Pond in Park	$\rho = 0.98$
	$\lambda = 56.88$
Wadi	$\rho = 80.45$
	$\lambda = 68.04$

Table 3−7 shows the mean (95% CL) of each step of the risk assessment. All the scenarios resulted in *Campylobacter* health risks above the national incidence of *Campylobacter* disease of 5.6×10^{-3} per person per year (pppy) [32], which includes all sources of *Campylobacter* (i.e. food and water).

Table 3−7: Results of the risk assessment steps.

	Units	River Rowing	River Swimming	Lake Rowing	Lake Swimming	Sedimentation Pond Fishing	Pond in Park Walking	Wadi Playing
Co	gc/L	34.7 (126.8)	35.2 (128.7)	45.6 (204.5)	44.5 (205.0)	1.4×10^{3} (4.2×10^{3})	60.9 (182.2)	5.5×10^{3} (6.5×10^{3})
Cr	gc/L	145.4 (552.0)	148.3 (530.2)	191.7 (779.4)	203.5 (769.7)	5.6×10^{3} (1.9×10^{4})	263.2 (870.7)	8.2×10^{3} (9.8×10^{3})
d	gc/ event	0.8 (3.1)	3.6 (14.9)	0.8 (3.2)	4.3 (13.3)	3.7 (12.3)	0.1 (0.5)	2.5 (6.2)
Pdill	pppd	0.06 (0.2)	0.1 (0.2)	0.05 (0.2)	0.08 (0.2)	0.1 (0.2)	0.02 (0.08)	0.2 (0.2)
Pyill	pppy	0.3 (0.3)	0.2 (0.3)	0.1 (0.3)	0.2 (0.3)	0.27 (0.3)	0.04 (0.2)	0.3 (0.3)

**Co is the observed concentration; Cr is the real concentration; d is the dose; Pdill is the envent probability of disease; Pyill is the annual probability of disease;gc is genomic copies; pppd is per person per event; pppy is per person per year*

The sensitivity analysis (Figure 3−2) shows different degree of input influence on the risk variability in each model. All differences were statistically significant. For the rowing models, the main factor affecting the risk variability is the observed *Campylobacter spp.* concentration, followed by the exposure events. For swimming, the volume is the factor with higher influence on the output, followed by the observed concentration. The observed concentration is again the main factor for fishing in the sedimentation pond, followed by the ingested volume, and walking in the park, followed by the recovery. In the wadi, the ingested volume is the main factor, followed by the exposure frequency.

The uncertainty regarding the exposure frequency for swimming in the river and lake and playing in the wadi was also assessed. The exposure frequencies, based on weather events (hot events for swimming, rain events for playing in the wadi) were compared with literature exposure frequencies from similar situations: swimming in surface water locations [80] and playing in flooded streets [81]. The differences were 10% for the river and the lake and 35% for the wadi.

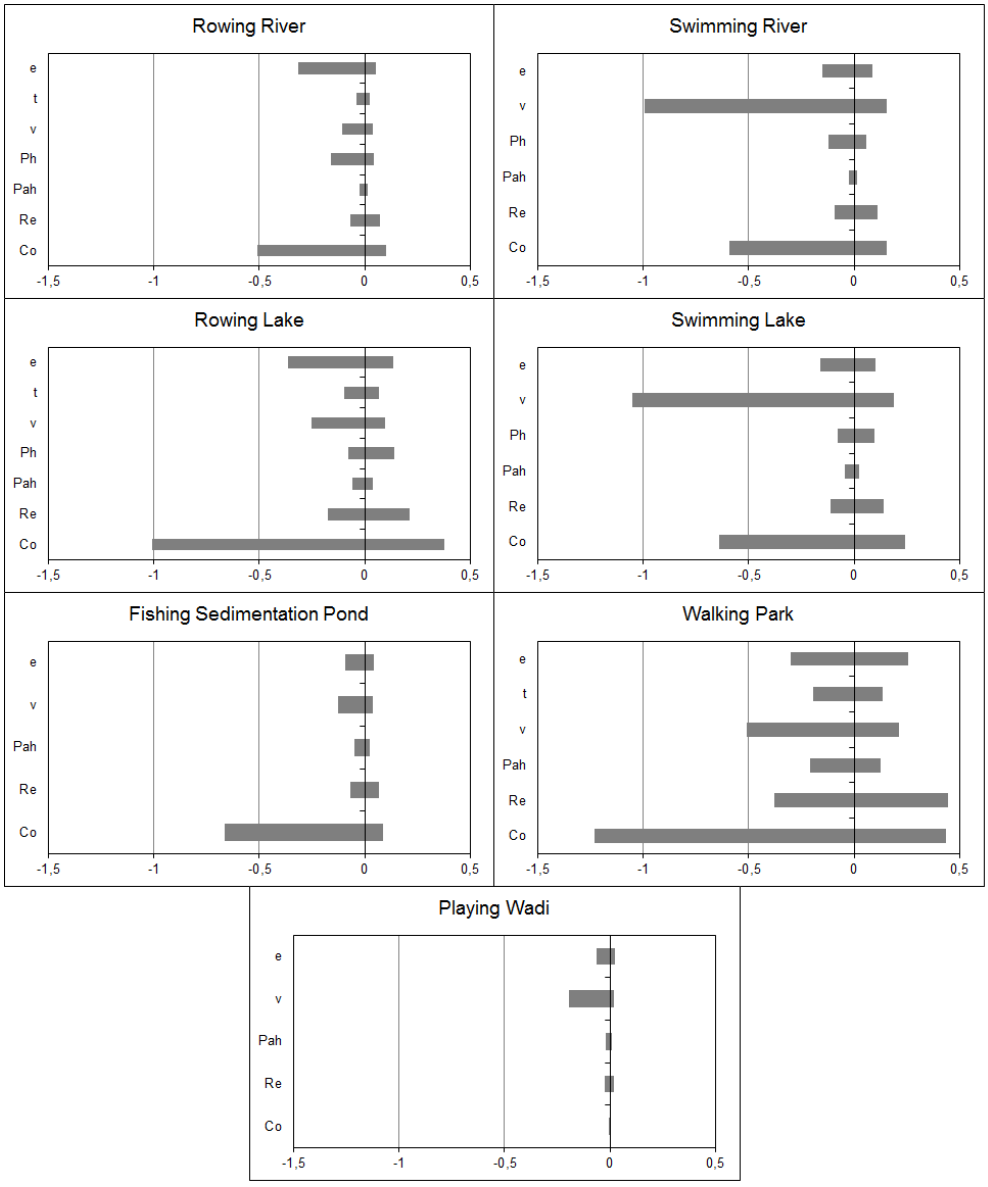

Figure 3−2: Sensitivity analysis showing the effect on the risk of changing each input parameter to its 95%CI limits while keeping the variability of the other inputs. Horizontal axis shows the log10(mean(Pyill(i))/mean(Pyill)). Co is the observed concentration, Re is the recovery efficiency, Pah is the fraction of human pathogenic Campylobacter among Campylobacter of animal origin, Ph is the fraction of human Campylobacter, v is the volume ingested, t the time spent at the location, e is the exposure frequency.

4. Discussion

4.1. Water Quality and Weather Correlations

Concentrations of *E. coli* in the river are in concordance with those found by Schets, et al. [136] during an 11 months study at the same location, but the concentrations in the lake were higher than those found in the same study. Seasonal variability could explain these differences in the lake, since we only monitored it during the summer season, while Schets, et al. [136] sampled throughout the year.

In the river and the lake, *Campylobacter spp.* concentrations were lower but in the same range as those found in a river and lake in Brabant (The Netherlands) [158]. However, de Roda Husman, et al. [158] used a MPN method, detecting only viable (and infective) bacteria, while with the q-PCR method used in the present study, no distinction is made between viable and dead *Campylobacter spp*. Therefore, the concentration of infective *Campylobacter spp.* in Amsterdam is probably lower than in Brabant. This could be due to higher avian faecal input in Brabant, since *Campylobacter* was associated with bird counts in the reservoirs [158].

In the sedimentation pond and the pond in the park, the concentration was in the range of the *Campylobacter spp.* concentration found in one (out of six) surface runoff sample and five (out of eight) of the storm sewer samples studied in The Netherlands by de Man, et al. [81] using an MPN method. In the rest of the surface runoff and storm sewer samples, the concentration was lower or below the LOQ. The method used by de Man, et al. [81] detects viable thermotolerant *Campylobacter*, while we are detecting *Campylobacter spp*. Therefore, the concentration found in the storm water study is probably higher than the one we found in the sedimentation pond and the pond in the park.

In the screening study, we assumed that the concentration of *Campylobacter spp.* in the pond was 1log lower than in the sedimentation pond due to dilution and natural effects (die-off, predation, etc). In this study, we observed that the concentration of *Campylobacter spp.* is indeed lower in the pond, but this difference was not statistically significant. This is probably due to avian faecal influence in the park, that introduce new *Campylobacter* (ducks, geese and other birds are commonly seen in the pond), obscuring the possible decrease of *Campylobacter* from the sedimentation pond.

In the wadi, the concentration was 1-4 logs higher than that found in similar locations [81] and used in the screening study. The four samples correspond to one rain event. The finding of such high concentrations already in one rain event indicates that temporary stormwater reservoirs can be faecally contaminated to a large extent, and further research should be conducted to better assess the water quality of urban wadi's.

Cryptosporidium was not found in any sample. The LOQ of the method was always several logs higher than the concentrations found in previous studies [81, 136, 151, 161]. Therefore, the possibility of presence of *Cryptosporidium* in lower concentrations than the

LOQ cannot be ignored. On the other hand, the other studies used microscopy methods and, therefore, quantified *Cryptosporidium spp*., while the method of our study targetted *C. parvum* and *C. hominis* only, so were more specific to human and cattle sources.

Adenovirus 40/41 was found occasionally in the river and lake, in concentrations close to its LOQ. This indicates human faecal contamination at those sites. Therefore, *Campylobacter* at those sites could be of human origin, while *Campylobacter* found at the sedimentation pond and the park has probably avian-faecal origin (ducks and geese are commonly found in the ponds) and/or canine-faecal origin (street runoff can transport dog faeces to surface waters [14]). Schets, et al. [136] detected other human viruses (norovirus, rotavirus, enterovirus) in the same river, indicating also human faecal contamination.

Concentrations of adenovirus in the river were 1log lower than those found in two rivers in the East of Spain [189] and in rivers and lakes from nine European countries, including The Netherlands [48]. The concentration in the lake was 2 logs lower than in the European study. However the frequency of contamination at European surface water locations was similar to the frequency in the lake when analysed by nested PCR (41.1% of samples contained adenovirus), and to the frequency in the river, for samples analysed with q-PCR (61.3%) [48]. The concentration was also lower than in another European study, where concentrations in a freshwater site in The Netherlands was 640 gc/L [190]. Higher concentrations of adenovirus, up to 10^6 gc/L, were found in rivers outside Europe [191-194], and much lower, 10^{-5} to 10^{-2} gc/L, in South African river water [195]. Differences in adenovirus can be due to the specific nature of the system – e.g. the amount of sewage water that receives [48] –, the infection status of the population, and quantification methods used.

The concentration of *L. pneumophila* in the sedimentation pond was 1 log lower than that found by van Heijnsbergen, et al. [196] in rainwater on roads and used in the screening study [7]. In the pond in the park, *L. pneumophila* was not found. Therefore, we overestimated the concentration of *L. pneumophila* in the screening study. The difference in concentration can be due to several factors. First of all, the method used by van Heijnsbergen, et al. [196] was amoebal co-culture. This method detects only viable *L. pneumophila*, indicating that the difference between the two is even higher. Furthermore, van Heijnsbergen, et al. [196] sampled water from street puddles, while our pond, which receives water from street runoff, contains a larger volume of water, probably diluting the *L. pneumophila* concentration. In the river and lake, the LOQ of the samples was below the concentration found by Wullings, et al. [159] in Dutch rivers and lakes and used in the screening, indicating that in the screening study, the concentration of *L. pneumophila* at those locations was overestimated. The q-PCR method used to quantify *L. pneumophila* is the same as in Wullings, et al. [159], so differences must be due to specific characteristics of each water system and location.

Frank [16] investigated the concentrations of cyanochlorophyll-a and microcystin in 155 lakes in southern Germany. They found concentrations of chlorophyll up to 290 µg /L,

cyanobacteria were dominant in 20.3% of the samples, as determined by microscopy, and microcystin, determined with ELISA, ranged from 20 to 566 µg/L (only samples with dominance of potentially toxic cyanobacteria were analysed). In our pond samples, cyanobacteria were dominant in only two samples; in one of them, the microcystin concentration was below the LOD, and in the other one it was found at 4 µg/L. Our results show, therefore, no relation between presence of cyanobacterial populations and microcystin production. However, different cyanobacteria species can produce different toxins that have not been monitored in this study [75]. Therefore, the possible presence of other cyanotoxins that cause pathology in humans cannot be excluded. However, the cyanochlorophyll-a concentrations in this study were low. Kardinaal, et al. [197] found peak concentrations (2.7 and 7 µg/L) of microcystins at the end of August/early September in two Dutch lakes. The seasonality in the pond in the park is similar (highest concentration found on mid-September), and the maximum concentration found (4 µg/L) is on the same range.

No statistically significant differences were found on the concentration of *E. coli* and pathogens between the inlet and outlet of the sedimentation pond. This indicates that the sedimentation pond does not settle microorganism efficiently during rain events. The inlet of the sedimentation pond was not investigated during the weekly monitoring period. Hence, it remains unknown if the microorganisms are efficiently settled in the sedimentation pond during dry periods.

Previous studies observed similar associations between bacteria or disease burden and weather parameters [33, 57, 180, 198-202]. The correlation between *E. coli* and *L. pneumophila* in the sedimentation pond is, however, a new finding. This might be due to the nature of the system, resulting in an increase of particles and bacteria in the pond during rain events due to stormwater runoff and overflow (both bacteria are strongly correlated with the rainfall). The origin of the *E. coli* is probably animal faecal (street dog depositions and bird droppings) because no adenovirus 40/41 was found. The origin of the *L. pneumophila* is probably in the water system and the rainwater itself, since it has been previously found in rainwater in street puddles [59, 60, 196]. This correlation was not found with *Campylobacter* because its origin is probably the direct faecal depositions from geese and ducks into the sedimentation pond, as stated earlier.

Climate predictions for The Netherlands indicate temperature raising in summer and, while longer periods of drought will occur, the intensity and frequency of extreme rain events will also augment [203]. The correlations found indicate that the temperature and/or solar radiation increase would result in a decrease in *E. coli*, *Campylobacter*, adenovirus, and *L. pneumophila* concentration. The higher frequency and intensity of storm events could result in higher (short peak) concentrations of *E. coli* and *L. pneumophila*, and lower cyanobacteria due to flushing. However, the nutrient load supplied by the rain events will help the growth of cyanobacteria afterwards, especially when dry periods become longer. This supports previous findings on pathogens climate change studies [202, 203].

4.2. Health Impact Assessment

The GI health risks in the river and the lake were lower, but in the same range, than those estimated earlier in the screening-level risk assessment [179]. This is due to the lower *Campylobacter* concentration found in the locations, as compared to the concentrations used in the screening study. *Cryptosporidium* was not found, but its effect on the risk in the screening study was negligible. Norovirus was not monitored, but, although it did have effect on the risk, its contribution was much lower than *Campylobacter*'s contribution to the total gastrointestinal risk. Furthermore, two factors were added to this study that contributed to lower GI risks and were not considered in the screening: the probability of finding human *Campylobacter*, based on adenovirus data, and the probability of finding zoonotic *Campylobacter* in the adenovirus negative samples.

In the sedimentation pond the GI risks estimated in this monitoring study were higher than in the screening study. The *Campylobacter* distribution used in the screening study was derived from a data set were several negative samples were found [81], while all our samples were positive. The risk in the pond in the park was also higher than that estimated in the screening study but it was the lowest of the studied scenarios, as expected. In the screening study, a triangular distribution based on *E. coli* data was used to estimate the log reduction of the concentration of pathogens due to dilution and other natural effects. However, the concentration of *Campylobacter* in the pond did not correspond with this log reduction, resulting in higher concentration and higher risks than those previously estimated, despite no information on norovirus, which was the pathogen contributing most to the GI risk in the screening study. Absence of adenovirus in the pond, however, indicates absence of human faecal contamination.

The wadi was studied on one rain event (consisting of four samples), resulting in the highest GI risks. In the screening study, lower concentrations of *Campylobacter* were used, resulting in lower health risks. Norovirus also contributed to the risk and had a higher effect on risk magnitude and variability than *Campylobacter* at this location. Although norovirus has not been monitored in this study, the unexpected high concentrations of *Campylobacter* found resulted in higher risks. Further investigation is needed, analysing more samples from different rain events, to better characterize the risks derived from recreational exposure to temporary stormwater storages.

Recommendations to reduce the gastrointestinal risks on the exposed population are: in the river and lake, which are non-designed bathing waters, advise the citizens on the risk associated with bathing in these waters. Furthermore, the water quality could be improved by additional wastewater treatment (e.g., UV-disinfection) of the effluents that discharge in the river, and by clearance of combined sewer overflows into the river. In the stormwater sedimentation ponds and receiving park water, inform the public that water may be extra contaminated after rainfall events and contact should be avoided. Also, the sedimentation pond could be re-designed to obtain improved particle settlement during rain events.

Finally, in the wadi, citizens should also be advised to avoid direct contact with the water, and to prevent animal contamination (e.g. by removing dogs' depositions in the wadi draining area).

5. Conclusions

- High concentrations of *Campylobacter spp.* found in all locations result in high gastrointestinal risks for the population exposed (above the annual incidence in The Netherlands). *Cryptosporidium* was not found at any site. *L. pneumophila* was found in the sedimentation pond.
- *E. coli* concentrations were variable through the weeks in all locations, and usually above the threshold for good bathing water quality. This, however, did not correlate with any gastrointestinal pathogen. Adenovirus was found occasionally in the river and the lake, indicating human faecal contamination at those sites.
- The sedimentation pond does not result in efficient settlement of particles during rain events (no significant differences in pathogens and indicators concentration between the inlet and outlet were found). Further research is needed to understand its particle settling efficiency during dry periods and changes to be made for better performance during rain events.
- Highest risks were found for rowing in the river, due to the high concentration of *Campylobacter spp.* and extent of exposure events for regular rowers, and playing in the wadi, due to the high concentration of *Campylobacter spp.* found already in one rain event, indicating the need for better characterization of temporary stormwater storages where recreation also takes place.

Chapter 4: Microbial Health Risks Associated with Exposure to Stormwater in a Water Plaza

Abstract

Climate change scenarios predict an increase of intense rainfall events in summer in Western Europe. Current urban drainage systems cannot cope with such intense precipitation events. Cities are constructing local stormwater storage facilities to prevent pluvial flooding. Combining storage with other functions, such as recreation, may lead to exposure to contaminants. This study assessed the microbial quality of stormwater collected in a water plaza and the health risks associated with recreational exposure. The water plaza collects street run-off, diverges first flush to the sewer system and stores the rest of the run-off in the plaza as open water. *Campylobacter*, *Cryptosporidium* and *Legionella pneumophila* were the pathogens investigated. Microbial source tracking tools were used to determine the origin (human, animal) of the intestinal pathogens. *Cryptosporidium* was not found in any sample. *Campylobacter* was found in all samples, with higher concentrations in samples that contained human *Bacteroides* than in samples with contamination from birds and dogs (15 vs 3.7 gc (genomic copies)/100 mL). In both cases, the estimated disease risk associated with *Campylobacter* and recreational exposure to the water plaza were higher than the Dutch national incidence. This indicates that the health risk associated with recreational exposure to the water plaza is significant. *L. pneumophila* was found only in two out of ten pond samples. Legionnaire's disease risks were lower than the Dutch national incidence. Presence of human *Bacteroides* indicates possible cross-connections with the combined sewer system that should be identified and removed.

This chapter is based on:
Sales-Ortells, H., and Medema, G. 2015. Microbial Health Risks Associated with Exposure to Stormwater in a Water Plaza. *Water Research* 74, 34-46.

1. Introduction

Climate change is expected to produce an increase in frequency and intensity of storm events. Consequently, urban sewage systems will be overwhelmed more often, and street flooding with stormwater will occur more frequently [149]. New urban water features are emerging in cities to deal with this problem. These features serve as temporary storage of stormwater, reducing pluvial flooding during intense rainstorms, and making stormwater available for other purposes, such as landscape irrigation or recreation. Water plazas are an example of these temporary storage of stormwater in which the water is used for urban recreation.

Rainwater is, in principle, of good microbiological quality, but gets contaminated through roof and soil input (e.g. surface runoff) [204]. Therefore, exposure of humans to urban stormwater may lead to health risks [150]. Microbial hazards may be present in water bodies that collect rainwater due to input of faecal material such as sewage discharge containing human enteric pathogens (*Campylobacter*, *Cryptosporidium*, norovirus, rotavirus, etc.) or animal faecal input (from waterfowl, dogs, and other domestic and wild animals) containing zoonotic pathogens [14, 15]. *Legionella pneumophila* has been found in rainwater on roads [59], roof rainwater harvesting systems [137, 180], and pluvial floods [60]. Microbial risks are also influenced by climate change. A higher frequency and strength of storms and draughts affects the concentration of pathogens present in (storm) water [14, 18].

The microbial quality and/or health risks in rainwater have been assessed in various features such as pluvial flooding and runoff [81, 152, 205], splash parks that use rainwater [7], or rainwater roof harvesting containers [137, 206, 207]. Water plazas that collect roof and street run-off from a larger urban area are relatively new engineering concepts that combine stormwater storage with water recreation and their water quality and microbial risks have not been studied previously.

Identifying the probable sources of faecal contamination may be important in estimating human health risks [169]. Faecal source tracking (FST) tools consist on identification of host-specific gut bacteria, host-specific viruses, detection of chemicals associated with human waste (sterols, caffeine, etc), or mitochondrial DNA from gut cells that are shed through the faeces [208]. FST has been used to identify faecal sources in roof harvested rainwater [209] and sewage impacted stormwater drains [210, 211], and in Quantitative Microbial Risk Assessment (QMRA) studies in bathing beaches [169, 170, 212] and other recreational waters [213].

The aim of this study was to investigate the microbial hazards and health risks associated with a newly built stormwater plaza in an urban environment in Rotterdam (The Netherlands). For this purpose, the water in the plaza was monitored for reference pathogens during a stormwater run-off simulation experiment. FST was also applied to

determine the origin of faecal contamination and relate faecal markers to pathogens presence and concentration.

2. Materials and Methods

2.1. Site Description

The water plaza Bellamyplein, located in the city of Rotterdam (The Netherlands), has a surface of 5,000 m^2 and is designed to collect rainwater from the streets and roofs from an area of 2 ha, although in the current situation, this area is only 0.8 ha. It can store up to 864 m^3, corresponding to 108 mm of rain, in the current situation, and 43.2 mm, in the future. The square has four platforms at different levels. When it rains, the water flows towards the plaza and into an underground drain. When this has filled up (60 m^3) the water flows on to the lowest terrace (at -2.10 m NAP or Amsterdam Ordenance Datum), and from there it flows up till the highest terrace is filled (at -1.40 m NAP), when it continues raining (Figure 4−1). 10.6 mm of rain will fill up the lowest terrace (4.3 mm in the future) where children can already play. The plaza is equipped with a first flush pump that pumps the first 60 m^3 of collected water into the combined sewer system.

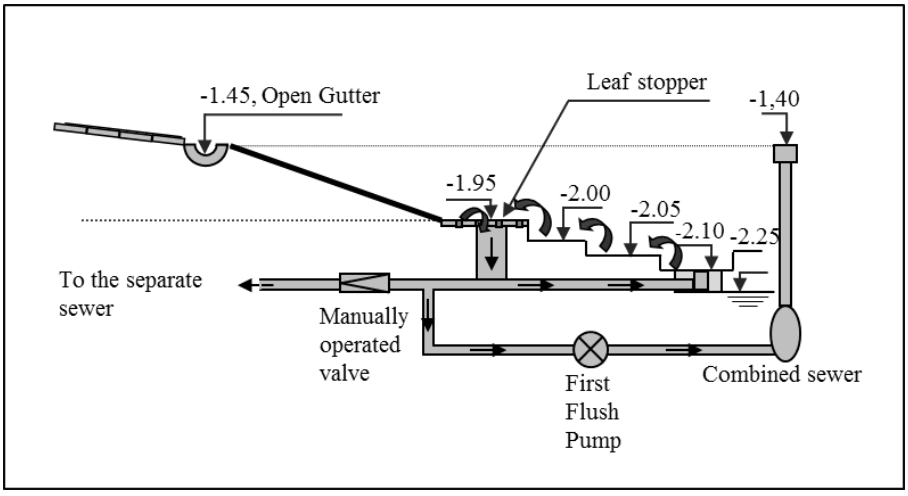

Figure 4−1: Description of the water flow in the water plaza. Numbers indicate the NAP level. (Modified from Rodenburg and Doelder [214].)

2.2. Microorganisms of Interest

E. coli (EC) was chosen as indicator for faecal contamination since the European Bathing Water directive relies on this indicator for classifying the water quality [117]. Three host-specific indicators were chosen: Human *Bacteroides* (HB), Avian *Helicobacter* (AH), and canine mitochondrial DNA (CD) as indicators of human, avian, and canine faecal contamination, respectively.

Campylobacter spp. and *Cryptosporidium* were the gastrointestinal pathogens selected because their presence is expected in locations where bird and dog droppings are present [187, 188]. *L. pneumophila* was selected because it has been shown to multiply in engineered water systems [56], cases of legionnaire's diseases (LD) have been related to increased rain conditions, and it has been found in pluvial floods [59, 60] and rainwater harvesting containers [137]. Viruses were not included in the study because human faecal material was not expected in street run-off [81].

2.3. Simulation Experiment and Sampling

A simulation experiment was conducted to study the functionality of the system. The square was cleaned with pressured drinking water the day before the event. This provided the unique opportunity to study the impact of fresh street deposits (without contribution of run-off from roofs) on microbial water quality. On the day of the experiment, two fire hydrants, located in two streets surrounding Bellamyplein, were opened and ran for three hours. The water flowed over the street pavement into the street gutters constructed to lead the water into the underground pipe system and then flow up into the square. The first flush pump operated to take the first flush to the sewer (Figure 4−2).

Figure 4−2: Rain simulation event in the water plaza.

Figure 4−3 indicates the time sequence of the experiment (start and stop of the plaza flushing with drinking water and the first flush pump, and start of draining). Approximately 180 m^3 of mains water was discharged and filled up the Bellamyplein to a maximum level of -1.80 m NAP, flooding all four terraces successively.

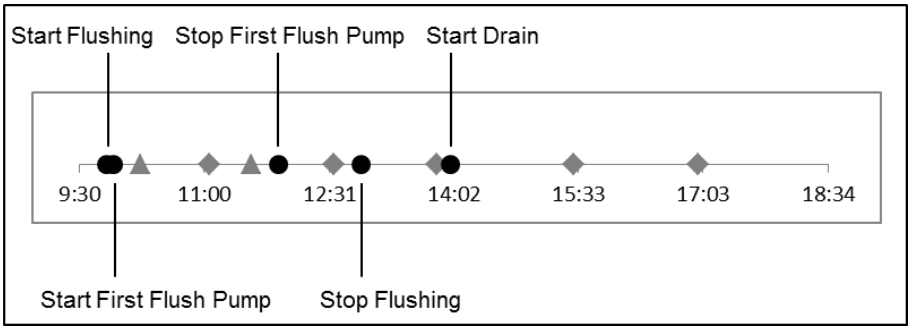

Figure 4−3: Simulation Experiment. Triangles represent the first flush samples, and diamonds the samples form the water plaza terraces.

Once the level of the water was high enough (-1.90 m NAP), two samples were taken every 90 min to look at temporal and spatial variation. In total, ten samples of 22 L each were collected from the water above two platforms (the lowest platform, at -2.10 m NAP, and the second highest platform, at -2 m NAP) and two samples of 12 L each from the first flush pump. The time at which the samples were collected is also indicated in Figure 4-3 (time difference between samples from the two terraces, approximately 5 min, is not represented in the figure). The samples were kept under 5 °C, and transported to the lab for analysis as soon as possible, but at least within 24 h.

2.4. Sample Processing

For EC analysis, decimal dilutions of duplicate aliquots were plated on Lauryl Sulphate Agar plates. Volumes of 0.1 µL or smaller were plated directly, after the corresponding decimal dilutions in sterile water. For higher volumes, the membrane filtration culture method was used, filtering different volumes of water onto 0.45 µm pore filters (Millipore). A negative (NC), consisting on sterile water, and a positive control (PC) were prepared. For the PC, 1 mL of EC solution (stored at -80 °C) was diluted in 250 mL of sterile water, and 100 mL of the solution were filtered. All plates were incubated for 14 hours at 44ºC, preceded by 5 hours at 25 ºC. EC characteristic colonies were counted, a representative part was isolated in 100 µL of DNAse-RNAse-free water (Gibco, Life Technologies), and stored at -80 ºC for future PCR confirmation.

Five µL of solution containing the isolated colonies were mixed with 20 µL of PCR mix containing BioRad Powermix for multiplex PCR and primers and probes targeting eubacteria and EC. A PC, consisting of cultured EC, a DNA template NC consisting of cultured *Pseudomonas diminuta*, and a NC consisting of DNAse-RNAse-free water were

also analysed. Colonies were identified as EC when both reactions (eubacteria and EC) gave a positive signal. Primers, probes, and PCR conditions are shown in Table 4−1. The EC *uidA* primers were first described by Heijnen and Medema [182], the EC probe and the sequences for eubacteria have been designed by Dutch water laboratories.

Approximately 20 L of each sample were concentrated into 0.5 L using a Hemoflow®. The Hemoflow® method had previously shown a recovery of 93% for *EC*, 35% for *Campylobacter* (analysed with MPN method) and 67% for *Cryptosporidium* in 50 L surface water samples [181]. The first flush samples were mixed and treated as one sample for the concentration and further processing and analysis.

Approximately 250 mL of the Hemoflow® concentrate was centrifuged at 4,000 rpm, for 30 min, with low deceleration, at 5 °C. The pellets were resuspended in a small volume of supernatant, and stored at -80 °C for further processing. After thawing at room temperature, DNA from pellets (4.7-10 mL) was extracted using the PowerMax® Soil DNA Isolation Kit (MO BIO Laboratories, Inc) following the manufacturer's instructions. Briefly, the pellets were added to a bead tube together with a series of buffers that aid in homogenization. Cell lysis and DNA extraction occurred by the combination of mechanical and chemical treatment. Extracted genomic DNA was captured on a silica membrane in a spin column format, washed, and eluted from the membrane with 5 mL of elution buffer.'

A PC, consisting of 4 mL of one of the extraction samples spiked with 200 µL of *C. coli* solution (stored at -80ºC) and 100 µL of *C. parvum* solution (stored at 4°C), was treated as the rest of the samples. A NC was also prepared consisting of 10 mL of DNAse-RNAse-free water. 10 µL of internal control (IC), a DNA fragment of the Dengue virus, were added in the first steps to all samples, PC, and NC in order to calculate the efficiency of the extraction method. The DNA in the 5 mL elutates was mixed with 1:10 of Sodium Acetate solution 3M (Sigma) and absolute ethanol (>99.9% purity, J.T. Baker), and centrifuged at 5,000 g for 45 min. The pellets were washed twice with 70% ethanol, centrifuged at 5,000 g for 15 min, and eluted in 200 µL of DNAse-RNAse-free water.

The concentration of *Campylobacter spp.* and *Cryptosporidium* in the water samples, and the IC, was determined through simplex q-PCR (CFX96 Real Time System, C1000 Thermal Cycler, Bio-Rad Laboratorium B.V.), using a regression line consisting on serial dilutions of DNA of each target. Primers and probes are shown in Tabel 4−1. *Campylobacter* target genes used are found in several *Campylobacter* species, including the human pathogenic *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari*, and the sequences have been designed by Dutch water laboratories. *Cryptosporidum* primers target *C. hominis* and *C. parvum*, and the sequences have been modified from Guy, et al. [183].

For *L. pneumophila* and FST targets, 1 L of water and 250 mL, respectively, were filtered on polycarbonate filters of 0.2 µm pore size (Sartorius Stedim Biotech) and the DNA was extracted with the PowerBiofilm DNA Isolation kit following the manufacturer's instructions. Two PC were prepared by filtering drinking water spiked with two different concentrations of *L. pneumophila* solutions stored at -80 °C and a NC was prepared through

filtration of 250 mL of DNase-RNase-free water. The filters were introduced into bead tubes and heated to activate lysis components. The lysis was enhanced by bead beating and a series of buffers were added to precipitate out humic substances, polyphenols and polysaccharides. Genomic DNA was then captured on a silica column, washed and eluted in 400 µL (*L. pneumophila*) or 100 µL (FST targets) of elution buffer. IC (10 µl) was added to the sample on the first steps of the extraction to subsequently calculate the recovery of the method.

The 400 µl of *L. pneumophila* DNA extraction solutions were concentrated in 100 µl with Na-Acetate/Ethanol. Sodium acetate 3M (1:10) and absolute ethanol were added to the extracts, mixed, incubated at -20°C for at least 1h, and centrifuged at 17,000 g for 15 min. The pellets were washed twice with 70% ethanol, centrifuged at 17,000 g for 5 min, and resuspended in 100 µl of DNAse-RNAse-free water.

The concentrations of *L. pneumophila*, HB, and CD in the water samples were determined through TaqMan Q-PCR (CFX96 Real Time System, C1000 Thermal Cycler, Bio-Rad Laboratorium B.V.), using a regression line consisting on serial dilutions of DNA of each target and the IC. AH concentration was determined with SYBR green q-PCR. The sequence of all primers and probes, and PCR conditions, are specified in Table 4−1. *L. pneumophila* primers and probes have been published by Wullings, et al. [185], the primers and probes for HB are in Krentz, et al. [215] and Staley, et al. [213]; CD sequences are published in Tambalo, et al. [134], and AH in Green, et al. [132].

2.5. Health Risks

The observed pathogen concentrations (C_o) and recovery (R) of the molecular extraction methods, were used to estimate the real concentration (C_r) of pathogens in the Bellamyplein, fitting distributions to the data. The methodology described by Pouillot, et al. [172] was followed, with some modifications. For *L. pneumophila*, a gamma distribution was fitted to the observed concentration data and a beta distribution to the recovery efficiency data. Then, the Cr was estimated as shown in equation 4.1:

$$C_r = C_o + gamma(C_o + 1, (1 - R)/R\ ) \quad (4.1)$$

A significant difference was found in *Campylobacter* concentrations between samples with HB and those without (see results). Therefore, two risk scenarios were built: one with animal *Campylobacter* data for water without HB and one with human *Campylobacter* data for water with HB. Lognormal distributions were fitted to the data and equation 1 was used to estimate the *Cr*. For the animal *Campylobacter* data, we estimated the probability of the presence of human pathogenic *Campylobacter* in animal faecal samples from different bird species and dogs. For this purpose, we used the percentage of faecal samples positive for *C. jejuni* and *C. coli* relative to the samples that were *Campylobacter spp.* positive reported by Waldenstrom, et al. [187] and Baker, et al. [188].

Table 4−1: Target genes, primers and probes sequences, and conditions of the PCR reaction.

Target	Target Gene		Primer and Probe sequences (5'-3')	PCR Parameters
-*E. coli*	*uid*A	F	ATGGAATTTCGCCGATTTTGC	3 min at 95°C
		R	ATTGTTTGCCTCCCTGCTGC	followed by 30
		P	AGCAGAAAAGCCGCYGACTTCG	cycles of
				20 s at 95°C,
-Eubacte-		F	CACACTGGRACTGAGACACGG	60 s at 60°C and 40
ria		R	CGCGGCATGGCTGSATCAG	s at 72°C
		P	GCTGCCTCCCGTAGGAGT	
Campylo-	16S rRNA	F	TGAGGGAGAGGCAGATGG	3 min at 95°C
bacter	gene	R_1	CGCAATGGGTATTCCTGG	followed by 45
spp.		R_2	CGCAATGGGTATTCTTGG	cycles of 20 s at
		P	TTGGTGGTGTAGGGGTAAAATCCG	95°C and 1 min at
				60°C.
Cryptos-	COWP	F	CAGGAGATGATTGTGTACTATATG	3 min at 94°C
poridium		R	GACAGGTTGAGTTGGAGCAG	followed by 40
parvum		P	CCCACCAAATTTCATTTTACAAGGCCT	cycles of 15 s at
and			CC	94°C and 1 min at
hominis				60°C.
Legio-	*mip*	F	CCGATGCCACATCATTAGC	5 min at 95°C
nella		R	CCAATTGAGCGCCACTCATAG	followed by 43
pneumo-		P	FAM-TGCCTTTAGCCATTGCTTCCG	cycles of
phila				20 s at 95°C
				and 48 s at 60°C
Human	HF183	F	ATCATGAGTTCACATGTCCG	3 min at 95°C
associated	16S rRNA	R	TACCCCGCCTACTATCTAATG	followed by 39
Bacteroi-		P	TTAAAGGTATTTTCCGGTAGACGATGG	cycles of
des 16S				15 s at 95°C and 1
				min at 60°C
Canine	Mito-	F	GCCTTTCCTTACAGGATTCTAC	3 min at 95°C
DNA	chondrial	R	GTGGCAACGAGTGTAATTAAG	followed by 40
(mito-	NADH	P	TCATCGAGTCCGCTAACACGTCGAAT-	cycles of
chondrial)	subunit 5			15 s at 95°C and 1
	marker			min at 60°C
Avian	16S rRNA	F	TCG GCT GAG CAC TCT AGG G	3 min at 95°C
Helico-		R	GCG TCT CTT TGT ACA TCC CA	followed by 39
bacter				cycles of
spp.				15 s at 95°C and 1
				min at 60°C

For human *Campylobacter* data we assumed that all *Campylobacter* detected were infective to humans. The concentration of *Campylobacter* in samples with HB were assumed to be partly from human origin and partly from animal origin. The fraction of animal *Campylobacter* in these mixed samples was modelled as a triangular distribution, based on the concentration of *Campylobacter* in samples with only animal contamination. Then, this fraction was subtracted from the total *Campylobacter* concentration found in mixed samples to obtain the concentration of *Campylobacter* from human faecal origin.

Children have been seen playing in the water plaza after intense rain events. The volume of water ingested (V) by children playing in the water plaza per event (Table 4−2) was assumed to be the same as that of children playing in urban flood water in The Netherlands [81]. The ingested dose (d_i) was calculated using equation 4.2.

$$d_i = C_r \times V \tag{4.2}$$

For the inhalation route, an aerosolization ratio (a) was used to estimate the concentration of *L.pneumophila* in the air, from a study conducted in decorative fountains [8]. The respiratory minute volume (RMV) for children playing was found in USUSEPA [84] and was assumed to follow a lognormal distribution. A deposition of 12.7% of inhaled aerosols was assumed [167]. The time spent playing at the water plaza was considered equivalent to the time spent by children playing with floodwater [81]. The inhaled dose (d_r) was calculated according to equation 4.3:

$$d_r = C_r \times a \times RMV \times I \times t \tag{4.3}$$

Table 4−2 shows the values and literature sources for each step of the QMRA. The probability of infection per exposure event of *Campylobacter* was calculated using equation 4.4:

$$P_d = 1 - {}_1F_1\ (\alpha, \alpha + \beta, -d_i) \tag{4.4}$$

where ${}_1F_1$ is the hypergeometric distribution and α and β are the parameters of the Beta-distribution (0.024 and 0.011, respectively) [24]. The disease probability per event was estimated using a disease given infection factor of 0.33 [30].

The probability of infection and disease per exposure event of *L. pneumophila* were calculated using equation 4.5:

$$P_d = 1 - exp(-r \times d_r) \tag{4.5}$$

where r is the probability of one cell to survive the host barriers and successfully initiate a response [26], and it is 0.06 for infection [106] and 1.7×10^{-4} for disease [175].

The annual number of rain events during the period of April to October with a volume of rainfall enough to fill the water plaza during the last 10 years was recorded [186]. A negative binomial distribution was built to describe the frequency of events (E) assuming that the minimum frequency of a child playing in the water plaza was one, and assigning

the maximum probability to it, while the probability of a child playing in the water plaza every time it contains water was assumed to be very low.

Table 4−2: Distributions and parameters of every step of the exposure assessment.

Step	Distribution (parameters)	Units	Source
Campylobacter			
Detected concentration from animal origin (Coa)	Lognormal (0.4, 1.0)	gc/100 mL	This article
Detected concentration from human origin (Coh)	Lognormal (2.4, 0.7)	gc/100 mL	This article
Fraction of animal *Campylobacter* in mixed samples (Fa)	Triangular (5.6, 9.1, 42.7)	%	This article
Recovery *Campylobacter* (Rc)	Beta (25.9, 79.5)	-	This article
Human pathogenic fraction of animal *Campylobacter* (Ia)	Normal (0.18, 0.05)	-	[187, 188]
Human pathogenic fraction of human *Campylobacter* (Ih)	P.E. (1)	-	Assumption
L. pneumophila			
Detected concentration *L. pneumophila* (Col)	Gamma (0.2, 5.02)	gc/100 mL	This article
Recovery *L. pneumophila* (Rl)	Beta (1.9, 10.8)		This article
Exposure			
Ingestion volume (V)	Triangular (0, 0.051, 5)	mL/event	Mean and 95%CL from [81]
Aerosolization ratio (a)	Normal (-8.07, 0.3)	Log10 (L water / L air)	[8]
Respiratory minute volume (RMV)	Normal (log10(22.67), 0.06)	L/min	[84]
Deposition in the lower respiratory tract (I)	Point Estimate (12.7)	%	[167]
Exposure duration (t)	Normal (21, 5)	min	Mean from [81]
Exposure frequency (10.625 mm rain) (E_{10})	Nbinom (2.7, 18.2) – trunc 1	Events/year	This article
Exposure frequency (4.25 mm rain) (E_4)	Nbinom (6.5, 2.3) – trunc 1	Events/year	This article

Parameters of the gamma distribution are shape and scale; parameters of the negative binomial distribution are the mean and dispersion; gc, genomic copies.

The annual probability of infection/disease (P_y) was calculated using equation 4.6.

$$P_y = 1 - (1 - P_d)^E \quad (4.6)$$

Monte Carlo simulations were run with random sampling of 10,000 values from each distribution input for each model, resulting in 10,000 random estimates of the risk. A sensitivity analysis was conducted by fixing one input at its 97.5 or 2.5 percentiles at a time, while maintaining the variability of the other inputs. The Kolmogorov-Smirnov test was used to check if the differences were statistically significant. Statistical analysis, distribution fitting, Monte Carlo simulation and sensitivity analysis where implemented with R version 3.0.1 [177].

3. Results

3.1. Water Quality

Figure 4−4 shows the concentrations of indicators and pathogens in the first flush and the water in the plaza at the lower and upper terrace over time. The water was fecally contaminated as indicated by the presence of EC in all samples. The EC concentration varied between 1.4×10^1 and 1.5×10^2 cfu (colony forming units)/100 mL. Unexpectedly, HB was detected in the two first flush samples and in 4 of the 10 water plaza samples, indicating that human faecal pollution was present in the water. CD was detected in all samples and AH was present in all but one sample, indicating dog and bird droppings contaminated the water in the plaza.

Campylobacter spp. was detected in the first flush pooled sample and in all water plaza samples in concentrations from 3.5×10^1 to 1.1×10^3 gc (genomic copies)/L. *L. pneumophila* was found in one sample in the first flush, and in 1/5 sample of each terrace in concentrations of 1.1×10^3, 1.5×10^2 and 3.1×10^2 gc/L. *Cryptosporidium* was not found in any sample. Table 4−3 shows the recovery of the DNA extraction for *Campylobacter spp.* and *Cryptosporidium*, *L. pneumophila*, and FST targets. The limit of quantification (LOQ) was 5-14 gc/L for *Campylobacter spp.*, 2-6 oocysts/L for *Cryptosporidium* and 30 gc/L for *L. pneumophila*.

No significant difference was found between the concentration of EC, HB, AV, CD, *Campylobacter*, and *L.pneumophila* at the two terraces of the Bellamyplein. Therefore, all samples were pooled to check for correlations between microbial parameters and for the risk assessment. Moderate to high correlations were found between EC and *Campylobacter*, and with HB for both bacteria, indicating human faecal material as possible source of contamination. However, HB were not present in all samples. In those samples where HB were found, the concentration of EC and *Campylobacter spp.* was significantly higher than in samples where HB were absent (p-value < 0.05) (Figure 4−5).

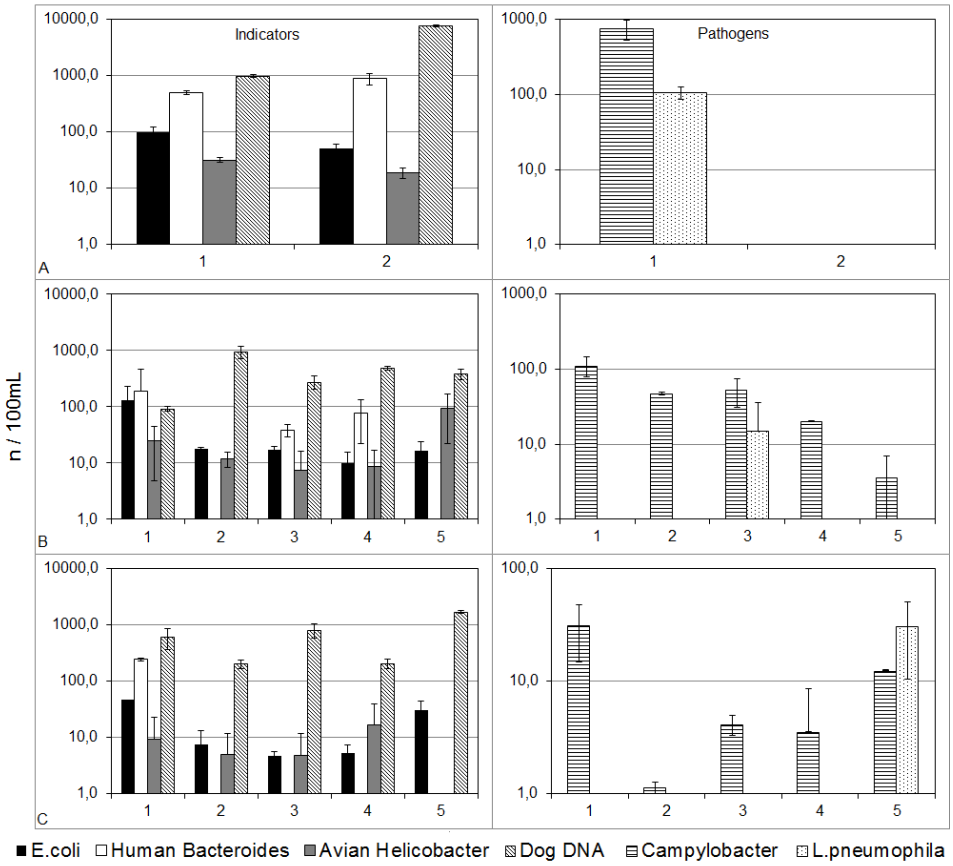

Figure 4−4: Concentration of indicators and pathogens in the first flush samples (A). in the lower terrace (B), and in the upper terrace (C). Bars represent the average of two aliquots, vertical lines are the standard deviation. Concentration of Campylobacter in the first flush sample is a pool of the two samples.

Table 4−3: Average (standard deviation) of the recovery efficiency (%) of the molecular extraction methods.

Location	*Campylobacter* and *Cryptosporidium*	*L. pneumophila*	FST targets
First Flush pump	30.3	25.8 (11)	26.7 (5.8)
Lower terrace	23.9 (5.1)	13.2 (9.2)	29.2 (15.9)
Upper terrace	25.2 (3.9)	17.2 (8.7)	37.4 (3.6)

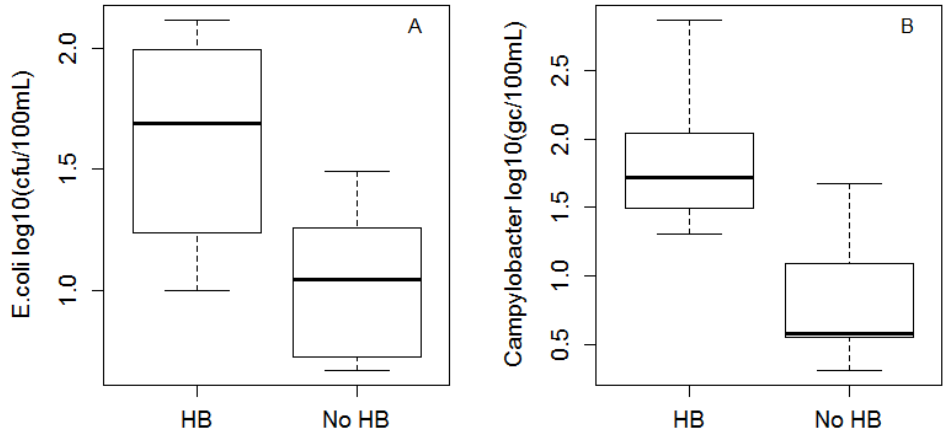

Figure 4-5: Concentration of EC (A) and Campylobacter spp. (B) in samples with and without HB.

3.2. Health Risks

Distributions were built to describe the *Campylobacter spp.* and *L. pneumophila* concentration in the water plaza using the q-PCR data and the recovery of the molecular extraction method. The pathogen concentration distributions were combined with literature data on frequency and duration of exposure of children to water and aerosols.

Approximately 10.6 mm of rain are needed for the Bellamyplein to fill up the lowest terrace with water in the current situation and 4.3 mm in the future, when stormwater from a bigger area will flow into the square. In the past 10 years (2003-2013), the average number of rain events equal to or higher than 10.6 mm and 4.3 mm during the April-October period, were 14.6 and 38.3, respectively [186]. A negative binomial distribution was constructed for these two scenarios, using the average as the maximum number of exposure events (Table 4−2).

Table 4−4 shows the results of every step of the risk assessment, from the concentration of pathogens in water to the event probability of disease. Figure 4−6 shows the annual probability of disease for the 10.6 mm of rain and the 4.3 mm of rain scenarios.

The sensitivity analysis showed that the factor with higher influence on the annual risks is the ingestion volume followed by the pathogen concentration in the *Campylobacter* models. In the *L. pneumophila* model, the measured pathogen concentration is the factor with higher influence on the risk, followed by the aerosolization ratio (Figure 4−7). The exposure frequency has a relatively high impact, when 4.3 mm of rain are needed to fill up the square. All differences were significant except for the recovery in the animal *Campylobacter* model.

Table 4−4: Results of the risk assessment in mean (95%).

Model Step	Units	Zoonotic *Campylobacter*	Human *Campylobacter*	*L. pneumophila*
Concentration in water	pdu/100 mL	3.7 (8.4)	15 (37)	1.4 (6.5)
Exposure per event	mL water (ingestion), L air (inhalation)	1.7 (3.9)	1.7 (3.9)	571 (952)
Dose	pppe	1.1×10^{-2} (4.1×10^{-2})	2.5×10^{-1} (8.0×10^{-1})	1.1×10^{-5} (5.210^{-5})
Event disease risk	pppe	2.5×10^{-3} (9.2×10^{-3})	4.5×10^{-2} (1.2×10^{-1})	1.2×10^{-9} (5.2×10^{-9})

pppe, per person per event.

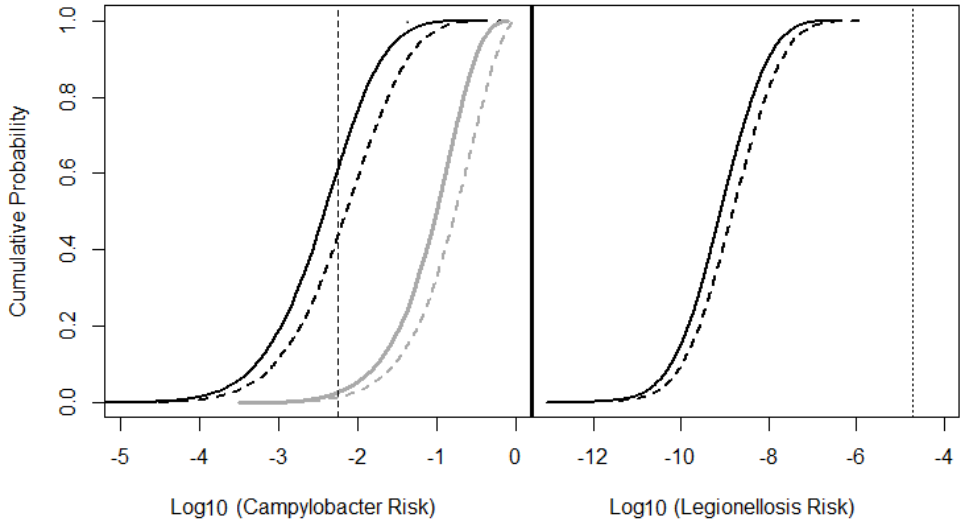

Figure 4-6: Cumulative distribution function of the annual disease risk per person per year (pppy) of Campylobacter (left) and LD (right), for the 10.6 mm of rain scenario (solid line) and the 4.3 mm of rain scenario (dashed line). In the Campylobacter plot, the black lines are the risks derived from Campylobacter of animal-faecal origin, and grey lines from human-faecal origin. Vertical dashed lines represent the national incidence of campylobacteriosis (5.6×10^{-3} pppy) and LD (2.0×10^{-5} pppy).

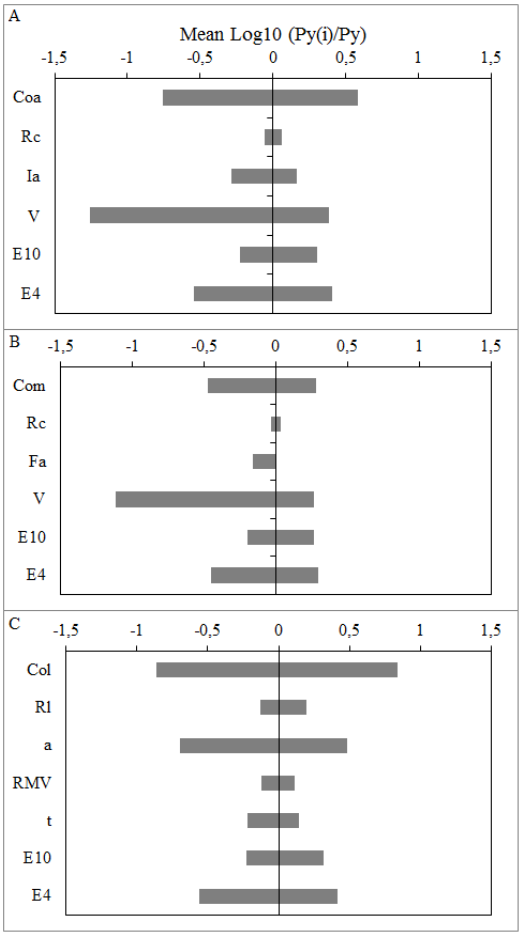

Figure 4-7: Sensitivity analysis of the zoonotic Campylobacter (A), human Campylobacter (B), and L. pneumophila (C) models. Coa, Observed concentration of animal Campylobacter; Rc, recovery efficiency of Campylobacter; Rl, recovery efficiency of L. pneumophila; Ia, probability of animal Campylobacter for being human pathogenic; V, volume ingested; E10, exposure events in the 10.6 mm scenario; E4, exposure events in the 4.3 mm scenario; Coh, observed concentration of human Campylobacter; Fa, fraction of animal Campylobacter in mixed samples; Col, observed concentration of L. pneumophila; a, aerosoliztion ratio; RMV, Respiratory minute volume; and t, exposure time.

4. Discussion

4.1. Water Quality

We measured the concentration of pathogens and faecal indicators in a water plaza during a rain simulation event. This water plaza was installed to collect and temporarily store

stormwater during heavy rainfall events and has also a recreative function. EC was found in all water samples, indicating the water was faecally contaminated despite cleaning of the water plaza the day before. The concentrations of EC were below the European Bathing Water directive threshold for excellent water quality, but higher concentrations are expected under real rain events, when the water plaza is not cleaned and the stormwater flows over a larger part of the street pavement and collects water from the roofs.

Finding of AH (in 11/12 samples) and CD (in 12/12 samples) indicates that birds and dogs are sources of the faecal contamination of the water. Unexpectedly, HB were found in 4/10 samples from the terraces and in the first flush samples, indicating human faecal contamination of the water also occurred. *Campylobacter spp.* was found in all samples, *L. pneumophila* was only found in one sample at each sampling location, and *Cryptosporidium* was not found in any sample, despite the low LOQ of the method. The origin of *Campylobacter* could be both human or animal. The concentration of *Campylobacter spp.* (and EC) was significantly higher when HB were found, indicating that *Campylobacter* was, at least partially, of human faecal origin. Other studies did not find correlations between *Campylobacter* and HB in recreational waters [216], or HB and culturable EC in separate sewer outfalls, but a weak correlation was found between HB and EC measured with q-PCR [210].

The presence of HB in the water plaza indicated the presence of human faecal material. This was unexpected, since the Bellamyplein is drained by a rainwater sewer (except for the first flush), therefore, sanitary sewage should not be in contact with the water plaza. Its presence could be due to unidentified cross-connections between rainwater and sanitary sewers [204] or to backflow from the combined sewer that is connected to the first flush pump [217]. Human viruses were not included in the study because their presence was not expected. The finding of HB, however, indicates that human faecal contamination was present and, therefore, further research during real rain events should be conducted to check for the presence of human viruses.

Water quality data were compared to data from similar water bodies (rainwater storages, sewer overflow, street runoff). Water quality varied substantially between water type, features and study. Differences in the system analysed are one of the causes of this variation. For instance, while in street runoff water and sewer system overflows the water flows over a large portion of the street and contains roof gutters contamination, in the rain simulation event the roof gutters were not connected with the water plaza, the water plaza was cleaned on the previous day, and the water from the mains flowed over a smaller portion on the street. The country where the study is conducted also impacts the pathogen concentrations due to the infection status of the population (and animals) that shed microorganisms in their faeces [217]. Furthermore, the season of the study influences microorganisms concentrations detected [218]. *L. pneumophila* can increase with warmer temperatures [17], while *Campylobacter* is usually found in higher concentrations in colder seasons [33].

EC concentrations in the water plaza were similar to those found in roof rainwater harvesting (RRH) tanks in The Netherlands, while *Campylobacter* concentrations were higher than in the RRH [180]. Water from RRH was analysed by a culture-MPN method that targets thermotolerant *Campylobacter* species. The water plaza samples were analysed with q-PCR, which quantifies DNA from both viable and dead bacteria, and targeting *Campylobacter spp*. Therefore, part of the *Campylobacter* detected may be dead or not infectious to humans. However, the high concentrations found in samples with HB suggest that at least part of it may be human pathogenic. Because the water plaza was cleaned the previous day, faecal contamination might be recent and, hence, *Campylobacter* could be still infective. On the other hand, the streets were not flushed before the simulation event, and older *Campylobacter* – probably non-infective because it survives in the environment for only short periods of time [33] – could be introduced with the water that flowed over the street pavement into the square.

In RRH tanks in Australia, similar concentrations of EC, with culture [206], and *Campylobacter* and *L. pneumophila*, with q-PCR, were found, while *Cryptosporidium* could not be isolated [137]. The target for *Campylobacter* was, however, the mapA gene specific for *C. jejuni*, while in the water plaza other species are being quantified together with *C. jejuni*. High concentrations of *C. jejuni* in RRH tanks could be explained by the faecal contamination found in the roofs (mostly bird droppings), while the water plaza was not receiving rainwater from roofs. The recovery efficiency of the method used for *Campylobacter* and *Cryptosporidium* was in the same range as that of the method by Ahmed, et al. [137] for *Giardia*, but lower than for *Salmonella* in the same study. The recovery of the *L. pneumophila* method was also lower. This could be due to higher presence of particles in the water plaza as compared to roof-rainwater collectors resulting in higher inhibition of the PCR reaction.

Concentration of *L. pneumophila* in positive samples (2/10) from the pond were in the same range as those found in RRH tanks in control households from a case control study in Australia, but lower than the concentrations found in case households by culture and molecular typing [219]. Higher concentrations could be reached under hot conditions [17] as those predicted in future climate scenarios in The Netherlands [220].

In RRH in Denmark, EC concentrations were in the range of the water plaza, no *L. pneumophila* was found, *C. jejuni* was detected in two out of 17 samples, and *Cryptosporidium* was found in six out of 17 samples in concentrations up to 50 oocysts/L [161]. The methods used were not specified. System and geographical differences discussed earlier can be the cause of the differences in water quality.

Birks, et al. [221] found, in RRH in England, higher concentrations of EC than in the water plaza. However, *Campylobacter*, *L. pneumophila*, and *Cryptosporidium* were not found. Pathogens were analysed in only two samples, and the methods used were not stated in the publication, making comparison difficult.

EC concentrations in the water plaza were lower than those in separate sewers and street runoff in The Netherlands [81] and in the US [222], while the concentration of *Campylobacter* was in the same range as in the separate sewer and the positive street runoff samples in The Netherlands [81]. However, de Man, et al. [81] used a MPN culture-based method targeting thermotolerant species only. The concentration of viable human-pathogenic *Campylobacter* in the water plaza is, hence, probably lower than in separate sewers in The Netherlands. As stated earlier, the limited area of the street overflow and the absence of roof connections in the simulation event, as compared to real rain events, could result in these lower concentrations.

In separate sewer outfalls in the US, Sauer, et al. [210] found higher EC (by culture) and HB (by qPCR) concentrations than those we found in the water plaza. System differences and the country of study could be the source of this. The recovery efficiency of the DNA extraction method for the sewer outfalls study was lower than the recovery of the water plaza, probably due to higher presence of debris in the sewer outfalls. Furthermore, regarding the HB, the different sequence of primers and probes, and q-PCR conditions can also affect the results [223].

4.2. Health Risks

We used the water quality data from the water plaza during the rain simulation event to estimate the health risks for children playing in it after extreme rain events. The results show that the mean *Campylobacter* disease risk for children playing in the water plaza after a single event is 2.5×10^{-3} in the presence of animal faecal contamination, and 4.5×10^{-2} pppe in the presence of human faecal contamination. The risk of playing in the water plaza increases if children play in the water after more than one rain event, to 1.8×10^{-2}, and 2.4×10^{-1} pppy for animal and human contamination, respectively, in the worst case scenario, when only 4.25 mm of rain are needed to fill the lowest terrace. Both are above the national incidence of *Campylobacter* disease of 5.6×10^{-3} pppy [32], which includes all sources of *Campylobacter* (i.e. food and water).

We have used concentrations of *Campylobacter* obtained with q-PCR, which targets DNA from viable and non-viable *Campylobacter* and includes *Campylobacter* species that are not human pathogens. Hence, we might be overestimating the *Campylobacter* health risks. However, correlation of *Campylobacter* concentrations with HB indicates that they are, at least partly, from human-faecal origin and potentially pathogenic for humans.

The risk of infection per event (data not shown) estimated with q-PCR *Campylobacter* concentrations of human origin are comparable to those found in infiltration fields with playgrounds filled up with surface runoff stormwater and storm sewer overflows in The Netherlands [81]. The risks estimated with *Campylobacter* of animal origin were lower, due to the lower concentration of *Campylobacter* in samples without HB than in those with, and to the low probability of the animal *Campylobacter* being human pathogenic. However, this probability is uncertain and was calculated using data from a study in wild migratory birds

conducted in an observatory in Sweden [187], and a study in dog faeces in South Australia [188]. Although some of the birds species studied by Waldenstrom, et al. [187] are found in cities in the Netherlands, the habitat of the birds also influences the microbial load in faeces. For instance, seagulls in harbour cities like Rotterdam, feed from human sewage, getting usually infected with human pathogenic microorganisms and transporting them to water supplies [224].

Furthermore, the only species considered to estimate the probability of human-pathogenic *Campylobacter* in animal sources were *C. jejuni* and *C. coli* because these are the responsible species for the majority of human GI cases. However, *C. lari* and *C. upsaliensis* – the first frequently isolated from bird droppings and the second from dog faeces – are also known to be human pathogens [30, 187, 188]. To reduce these uncertainties, *Campylobacter* should be identified to the species level in the water plaza.

The mean risks of LD from exposure to the water plaza are 1.2×10^{-9} pppe and 8.8×10^{-9} pppy, again for the worst case scenario, far below the annual national incidence of 2×10^{-5} pppy [61]. The *L. pneumophila* concentration was based on a data array where only two samples were positive out of 10. The risk per event was lower than that resulting from exposure to splash parks that use rainwater as source water [7]. The duration of exposure was assumed higher for the water plaza than that observed in the splash parks. The difference in infection risks is due to the lower concentration of *L. pneumophila* found in the water plaza. Also, aerosols are continuously generated in splash parks, while in the water plaza they are only generated by children when they splash.

Higher concentrations of pathogens in first flush samples compared to the terraces (approximately 1 log difference) indicate that the first flush is useful in removing microbial load from the water plaza. However, the QMRA results suggest that the first flush should eliminate a higher volume of water (by increasing the operating flow and/or time) to reduce the health risks. Future research should include deeper monitoring of the first flush water to estimate the necessary volume of water diverted in the first flush to decrease the health risks below the national incidence.

Climate change predictions for The Netherlands related to summer rain indicate a small increase of daily means (1-4%) with higher frequency and intensity of extreme rainfall events (10-40%) [220]. Therefore higher exposure frequencies can be expected in the future, resulting in higher health risks.

Recommendations for decreasing or removing the microbial load and health risks in the water plaza include: cleaning/disinfection of the water plaza after an extreme rain event (e.g. filtration, chlorination of the water); identification and removal of human faecal sources (cross-connection with combined sewers); increasing the capacity and/or the operating time of the first flush pump; regular cleaning of the catchment area and gutters; and informing the neighbours of the importance of keeping the streets clean (e.g., by collecting dogs' depositions). Furthermore, informing the public about the health risks derived from recreational uses of the water plaza after rain events may reduce exposure.

5. Conclusions

The microbial quality and health risks for children playing in a water plaza have been studied for the first time. The results show that the *Campylobacter* disease risks for children playing in a water plaza are higher than the annual average for the general population through all exposure pathways. LD risks were below the Dutch national incidence, but higher risks are expected in the future under hot conditions that promote growing of *L. pneumophila* in the system.

- Even though concentrations of EC were below the level for excellent bathing water in the EU Bathing Water Directive, concentrations of *Campylobacter spp.* (detected by q-PCR) were high in the water plaza.
- Presence of HB indicated the presence of human faecal contamination and, therefore, potential presence of human pathogenic viruses. Furthermore, correlation with *Campylobacter* concentrations, indicate that the *Campylobacter* species found could be human pathogens. Further research is needed to discover the human faecal source and, if possible, eliminate it, for instance, by identifying and cutting the connection with the sanitary sewer.

Chapter 5: Health Risks Derived from Consumption of Lettuces Irrigated with Tertiary Effluent Containing Norovirus

Abstract

Wastewater is a valuable resource for water-scarce regions, and is becoming increasingly important due to the rising frequency of droughts as a result of climate change. The health risks derived from ingestion of lettuce that has been irrigated with effluent from a wastewater treatment plant (WWTP) in Catalonia (Spain) were estimated following a quantitative microbial risk assessment (QMRA) approach using site-specific data. Norovirus was selected for this analysis, since it is the most common cause of acute gastroenteritis outbreaks in Catalonia. Two scenarios, irrigation with secondary and with tertiary effluent, were analysed. An uncertainty analysis was conducted to determine the impact of possible internalization of norovirus into edible parts of the lettuce. The mean disease burden for ingestion of lettuce irrigated with secondary and tertiary effluent was 7.8×10^{-4} Disability Adjusted Life Years (DALYs) per person per year (pppy) and 3.9×10^{-4} DALYs pppy, respectively. A sensitivity analysis revealed that the model parameters with higher influence on the probability of disease are the concentration of norovirus in the effluent and the consumption of lettuce. In order to decrease the disease burden to the guidance level of 10^{-6} DALYs pppy, the tertiary treatment should be able to achieve a 4.3 log reduction of the concentration of norovirus. If internalization of norovirus into lettuces occurs, this would require a reduction of 7.6 log. This is the first time that site-specific data and virus internalization in crops are incorporated in a QMRA of irrigation of lettuce and its impact is quantified.

This chapter is based on:

Sales-Ortells, H., Fernandez-Cassi, X., Timoneda, N., Dürig, W., Girones, R., Medema, G. 2015. Health Risks Derived from Consumption of Lettuces Irrigated with Tertiary Effluent Containing Norovirus. *Food Research International* 68, 70-77

1. Introduction

Wastewater has been widely used in the past for irrigation purposes. It is still in use in developing countries due to water scarcity, the associated nutrient value of these waters for crop growth, and economic limitations. In developed countries, the use of treated wastewater is increasingly seen as a way to deal with water scarcity (exacerbated by climate change), as a more economical alternative to inter-basin transfers, and as an environmentally sustainable practice [163]. Uses of reclaimed water include irrigation of landscapes, recreational fields, plants' nurseries, or agricultural lands for food crops, among others. In Spain, 362.2 Hm^3 of reclaimed water (42.39 Hm^3 in Catalonia) are used annually, corresponding to 10.6% of the total volume of treated wastewater. 71% of it is used for agricultural irrigation [225].

Although domestic wastewater is treated by secondary or tertiary wastewater treatment, reclaimed water can contain infectious pathogens, posing a risk for public health. Wastewater treatment methodologies are used to reduce concentrations of faecal indicators, e.g. faecal coliforms (FC) or *Escherichia coli* (EC), to below certain standards [119]. However, wastewater treatment can be considerably less effective in the elimination of enteric pathogens, such as enteric viruses (EV) and protozoa [226]. While concentrations of FC and EC are usually monitored at the wastewater treatment plants (WWTP), EV, which are relatively resistant to treatment technologies, are not [119], and concentrations of faecal indicators below the standards do not imply absence of EV hazards.

The health risks derived from irrigation of fresh produce with reclaimed water have been previously studied for EV [141, 143, 227-229]. Few studies focused on the Norovirus risks [79, 144, 230]. Mara and Sleigh [144], [230] found infection risks of norovirus to range between 10^{-5} and 1per person per year (pppy), depending on the initial concentration, and concluded that additional reduction of the norovirus concentration in wastewater is needed, but easily achievable by water treatment. Mok, et al. [79] found, for an estimated concentration of 6.03×10^7 virus/L in raw sewage, a 90% Confidence Interval (CI) of 4.66×10^{-4} to 4.4×10^{-3} Disability Adjusted Life Years (DALY) pppy in lettuce irrigated with wastewater treated by stabilization ponds. Other wastewater treatment methods (Actiflo, chlorination, ozone or UV) did not reduce the disease burden below the WHO recommendation of 10^{-6} DALY pppy [22] based on the previous guideline revision by Blumenthal, et al. [121], but this reduction could be achieved by a combination of the stabilization pond with any of the other treatment technologies.

Those studies, however, did not use site-specific data on norovirus concentrations in reclaimed water, and only considered the viruses deposited on lettuce surface (and not internalization of viruses through the roots). Furthermore, all QMRA studies have used a model derived from *Bacteroides fragilis* bacteriophage B40-8 [141, 231] to estimate the norovirus field-decay, while recent studies [232, 233] have provided more specific data to

estimate the inactivation of norovirus, not only in-field, but also during crops transport and storage.

The objective of this study was to quantify the health risks of lettuce irrigation with treated domestic wastewater in Catalonia (Spain) and the effect of secondary versus tertiary wastewater treatment on these health risks. This study followed a Quantitative Microbial Risk Assessment (QMRA) approach and norovirus was selected as reference pathogen, since it is the most common cause of acute gastroenteritis outbreaks in Catalonia [234]. Recent literature indicates the ability and extent of lettuces to internalize virus particles [235-237]. This is an important element that influences the outcome of the risk assessment and has not been considered in previous QMRA studies. We introduced this as an alternative scenario in the QMRA model and quantified the effect on the health risks.

2. Methods

2.1. Study-Site Description

The WWTP is located on the North-East coast of Spain and is designed to treat wastewater from 175,000 inhabitants with a flow capacity of 35,000 m^3/day. The conventional secondary treatment consists of sedimentation and activated sludge. The tertiary treatment, with a design capacity of 600 m^3/h, consists of flocculation by addition of iron chloride, followed by filtration (pulsed-bed sand filters), UV treatment (2 banks with 4 medium pressure lamps each, with a UV dose of 25-30 mJ/cm^2, according to the UV supplier) and chlorination (dosing of 3 to 6 mg/L of sodium hypochlorite with a contact time of 30 to 90 min). This tertiary effluent is used for irrigation and its production depends on the demand of the users, being higher from April to October, with a peak in July-August. Characteristics of the secondary and tertiary effluent measured by the WWTP system can be found in Appendix C.1.

The tertiary effluent is intended to irrigate several vegetable farms located in the vicinity of the WWTP. At the farms, different vegetables are irrigated through sprinkler, furrow, or drip irrigation. Most of them, however, with lettuce in particular, are irrigated with a sprinkler system every other day, in the evening. Lettuce is harvested, transported to the local market, and sold to the customers twice per week.

2.2. Hazard Identification

Norovirus are single stranded RNA virus that belong to the Caliciviridae family [53, 238]. They are found in water and food worldwide and are a leading cause of acute gastroenteritis [79], specially genogroups NoVGI and NoVGII [51]. A study on epidemiological data from a 10-year period revealed that norovirus was the most common cause of acute gastroenteritis outbreaks in Catalonia from 2004 to 2010 [234].

Concentrations of norovirus in wastewater range from 10^0 to 10^5 virus/L [238, 239], with higher concentrations usually found in winter. In secondary effluents, norovirus concentrations of the range of 10^1 to 10^3 have been found [239]. Norovirus has been linked to food outbreaks, including salad crops [240-244].

2.3. Exposure Assessment

A conceptual exposure model was designed to describe the virus fate and transport from the secondary effluent to the consumers fork (Appendix C.2).

Norovirus concentration in effluent

Data on the concentration of norovirus were obtained from the secondary (n=8) and tertiary effluents (n=8), and from a reservoir to store tertiary effluent (n=8). Monthly samples were gathered for a period of 8 months. Detailed methodology is described in Appendix C.3.

Briefly, viruses present in 10 L samples were concentrated using the skimmed milk organic flocculation method as described by [245]. A negative control was included in each sampling event using tap water as matrix, and neutralizing the free chlorine by adding 100 mL of 10% sodium thiosulfate solution. Viral extraction of RNA from 140 µl of concentrates was done with the QIAamp® Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) employing the automated system QIACube (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Extracts were stored at -80ºC until analyzed. A negative control of extraction was included in each extraction batch using free DNAse/RNAse molecular water. Samples were tested using specific real-time RT-qPCR for the viral pathogens NoVGI [246] and NoVGII [247]. Duplicate alliquots of undiluted and log10 diluted extracts were analysed.

More than one non-template control (NTC) were included in the RT-qPCRs. MX3000Pro sequence detector system (Stratagene, La Jolla, CA, USA) was used to quantify the samples. Detection limits (LOD) are 10 genome copies (gc) per reaction tube [247], equivalent to 570 gc/L. Plasmid DNA was used as a positive control and as a quantitative standard. RT-qPCR standards were generated as described by Calgua, et al. [248]. Recovery of the method can be found in Calgua, et al. [245].

Although most of the norovirus outbreaks and clinical cases in Catalonia are related to NoVGII, both genogroups were added up since NoVGI is also a human pathogen. An ANOVA test was run to check for significant differences between the norovirus combined concentrations in the three sampling points. Gamma and lognormal distributions were fitted to the data using the maximum likelihood estimation and the matching moments methods. These distributions were used because they have shown before to give a good fit to pathogen concentrations in water [156, 249]. Goodness of fit was analysed graphically and by the Kormogorov-Smirnov test.

Norovirus removal by tertiary treatment

The absence of a statistical difference between the concentration of norovirus in secondary and tertiary effluent (see Results) indicated little removal by the coagulation and sand filtration in the tertiary treatment. Also, UV and chlorination did not reduce the concentration, but this could at least partly be due to the fact that RT-qPCR detects both active and inactive (i.e. non- infective) viruses [250]. Concentrations of EC in secondary and tertiary effluent indicate that the tertiary treatment is reducing the EC concentrations very significantly throughout the year (Appendix C.1). To model the reduction of (infectious) norovirus concentration by the tertiary treatment adequately, we used data on surrogate viruses to determine the virus elimination during the tertiary treatment processes.

Studies have shown the disinfection efficiency of surrogates (MS2 virus, feline calicivirus, sapovirus, etc) through chlorine and UV treatment [251-253]. The efficiency of this particular WWTP was determined in a previous study, in which the reduction of the concentration of faecal indicator bacteria (EC, enterococci, etc.) and surrogate viruses (somatic, F-specific and *Bacteroides* phages) was determined for UV treatment, for chlorine disinfection, and for the combination of the two [226]. The UV dose was 25 mJ/cm^2 (according to UV supplier) when used alone or combined with chlorine, and the UV_{254nm} transmittance of the secondary effluent was 46+/-5 %. The chlorine dose was 10 ppm (alone), and 5 ppm (when combined with UV), and in combination with chlorine decay and contact times this obtained average Ct-values (concentration of disinfectant times the contact time) of 216 and 100 $mgCl_2$ min/L, respectively.

Results of this study showed that, while chlorine (with or without UV) was effective for disinfection of EC and enterococci, it had very little effect on the reduction of F-RNA and other bacteriophages. For the latter, UV treatment (with or without chlorine) managed 2 to 6 times higher inactivation than chlorination alone (Table 5−1) [226]. The data on norovirus concentration in the secondary effluent were combined with the data on inactivation of norovirus by UV to estimate the concentration of infectious norovirus in tertiary effluent. A PERT distribution was introduced, using the mean and 95% CI of the log reduction, to include the variability on the inactivation data.

Table 5−1: Mean (95% CI) of log10 reduction of E. coli and F-RNA bacteriophages by tertiary treatment processes (extracted from Montemayor, et al. [226])

Disinfection Method	EC	F-RNA bacteriophages
UV	1.80 (1.52-2.10)	0.94 (0.57-1.30)
Cl	5.00 (4.82-5.22)	0.30 (0-0.66)
UV + Cl	5.05 (4.82-5.40)	0.85 (0.63-0.93)

Transfer of Norovirus to lettuce by irrigation

The crop fields are located in the immediate surroundings of the WWTP and do not store the tertiary effluent. Therefore, the time between tertiary effluent production and use is very short and it is assumed that no inactivation of viruses occurs in this period. Irrigation of

lettuce is done with an overhead sprinkler system through which lettuce surfaces receive a considerable amount of water. Mok and Hamilton [254] studied the volume of water that clings to lettuces after irrigation by such a system and found that it was best described by a lognormal3 (-4.75, 0.50, 0.006) distribution. We assumed that all viruses in the water that retained the lettuce after irrigation are and remain attached to the lettuce.

Virus internalization

Several studies have indicated that enteric viruses can be internalized into crops. This can occur through the roots, where the viruses are transported via the lettuce vascular system to the leafs, or though the stomata and wounds present on the leafs [255, 256]. Few manuscripts have been published on the ability of norovirus and surrogates to be internalised by lettuce [235-237].

Only one study used human norovirus and obtained a high proportion of internalization in the leaves (0.24 to 0.72 virus/g) of lettuce grown in hydroponic solution [235]. The internalization rate of Murine norovirus (MNV) into edible parts of lettuce ranged from 4×10^{-6} to 2×10^{-3} virus/g [237] and of Sapovirus was 1×10^{-7} to 5×10^{-7} virus/g [236]. Different laboratory conditions (growth substrate, relative humidity (RH), initial virus titer, etc.) were associated with the differences in internalization [236]. No quantitative information has been found in the literature on internalization through the surface of lettuce leaves.

Virus inactivation in the field and during storage and transport

Sunlight and high temperatures influence virus inactivation in the field. During storage and transport, inactivation might also happen, but at a slower rate because of lower temperatures and absence of sunlight. Recently, studies have investigated the persistence of surrogates for human norovirus on crop surfaces.

MS2 virus on lettuce surface was inactivated by almost 3 logs after 25 hours at 30 °C and exposed to artificial sunlight. In the dark at 30 °C, the decrease after 25 hours was maximum 1 log, while at 4 °C in the dark no inactivation was found [232]. Hirneisen and Kniel [233] found no difference in survival between MNV, Tulane virus (TV) (with tissue culture and qPCR) and NoVGII (with RT-qPCR) on spinach surface. The decimal reduction times (D), i.e. the time needed for 1 log reduction in virus titer, for MNV and TV, ranged from 1.40 ± 0.14 to 5.73 ± 2.41 days at 18 °C, depending on the spinach leaf type, the inoculation location (abaxial, adaxial or whole plant) and the presence of UV-A and B light.

All these data suggest that viruses located on the surface of lettuces, which are exposed to sunlight and high temperatures, will be inactivated by 1 to 2 logs in the period between the last irrigation and harvesting at our study site: 12 to 36 hours total, with 6 to 18 hours of sunlight. During dark hours, inactivation ranges from 0 to 1 log [232]. Internalized viruses are only affected by high temperatures, therefore their inactivation during the field period is assumed to be the same as that of the virus on the lettuce surface during dark hours, hence 0

to 1 log for the 12 to 36 hours. During transport and storage (before selling, in the market, and in the households), lettuce is not exposed to sunlight, but is to different temperatures. Times between harvest and consumption are estimated to range from 13 to 55h. Therefore, the reduction during this period is also between 0 and 1 log.

Since no period appears to be most probable, a uniform distribution was used to define the degree of inactivation between last irrigation and consumption. As an alternative scenario, the model from Petterson et al [141, 231] from *B. fragilis* bacteriophage B40-8 has been used for inactivation in the field (only) as in previous studies, to quantify this uncertainty.

Virus removal by washing

Viruses on the plant surface will be partially removed by washing practices. Since usually no disinfection products are used for washing salad crops in Spain, a log reduction of the viruses on the surface is defined by PERT (0.1, 1, 2), based on Mok, et al. [79].

Consumption of lettuce

A survey of food consumption was conducted in Spain during 2009 and 2010 [257]. 3,000 people covering both sexes, different age ranges, geographic regions, and urban settlements were interviewed. The retrospective intake questionnaire consisted on a diet history (three days), a 24h recall, and a food-frequency survey. The Spanish Agency (AESAN) provided the daily lettuce ingestion data in percentiles, average and standard deviation (personal communication). Total population consumed an average of 20.7 (±26.4) g per person per day (pppd), with 95% UCL of 74.2 g pppd. Average and standard deviation were used to construct several distributions, and the lognormal distribution was chosen because it resulted in percentile values closest to the survey data (mean = 19.4, 95% UCL = 87.2 g pppd).

Dose

The daily dose of virus on lettuce surface (d_S) ingested by the consumers of the market where the reclaimed water irrigated lettuce are sold was calculated as shown by equation 5.1:

$$d_S = 10^{(log10(C_{eff} \times V_{surf}) - R_S - R_T - R_{wash})} \times I \quad (5.1)$$

where *Ceff* is the concentration of norovirus in secondary or tertiary effluent, *Rs* is the reduction of virus on the surface due to exposure to UV and high temperatures in the field, R_T is the reduction of viruses achieved during the lapsed time between harvest and consumption, *Rwash* is the reduction of surface viruses due to washing with water, and *I* the lettuce ingestion. The exposure model inputs are summarized in Table 5-2.

Uniform distributions were combined with the internalization ratios found in the literature [235-237] to define three different internalization scenarios with high (0.24-0.72), medium (4×10^{-6} to 2×10^{-3}) and low (1×10^{-7} to 5×10^{-7}) internalization ratios. In all three scenarios, the in-field reduction of internalized viruses (R_I) was considered uniform

(0,1) log 10 units [232]. The dose of internalised virus (d_I) was calculated with equation 5.2.

$$d_I = 10^{(log10(C_{eff} \times F_{int}) - R_I - R_T)} \times I \quad (5.2)$$

where *Fint* is the internalized fraction of viruses in the irrigation water that is found in the leaves (in viruses/g of lettuce).

Table 5–2: Exposure assessment inputs, units, distribution and parameter values, and references.

Model inputs	Units	Distribution (parameter values)	Literature references
C_{seff}: concentration of NV in secondary effluent	gc/L	Gamma(0.3, 1.2×10^{-6})	This chapter
C_{teff}: concentration of NV in tertiary effluent	gc/L	Gamma(0.3, 1.2×10^{-6})	This chapter
R_w: log reduction due to tertiary treatment (alternative scenario)	Log10 units	PERT(0.57, 0.94, 1.30)	[226]
V_{surf}: water that clings to lettuce surface through sprinkler irrigation	mL/g	Lognormal3 (-4.57, 0.50, 0.006)	[254]
R_s: in-field reduction of surface viruses	Log10 units	Uniform (1, 2)	[232, 233]
R_t: reduction of viruses during transport and storage	Log10 units	Uniform (0, 1)	[232]
R_{wash}: reduction of surface viruses due to washing	Log10 units	PERT (0.1, 1, 2)	[79]
I: daily consumption of lettuce	g pppd	Lognormal (20.72, 26.35, inf=0, sup=120)	[257]

**second parameter of the gamma distribution is the rate; gc, genome copies, pppd is per person per day*

2.4. Dose−Response Assessment

In order to estimate the individual risk of norovirus infection per event, the Beta-Poisson model defined by Teunis, et al. [54] was used (equation 5.3)

$$P_d = 1 - {}_1F_1(\alpha, \alpha + \beta, -d) \quad (5.3)$$

where ${}_1F_1$ is the Kummer confluent hypergeometric function, α and β are shape parameters with values 0.04 and 0.055, respectively, and d the dose (either d_S or d_S+d_I). The illness given infection risk (P_{dill}) is calculated by multiplying the infection risk by an illness given infection ratio, which is, for norovirus, 0.67 [258].

2.5. Risk Characterization

Farmers irrigate the crops with tertiary effluent during warm months, and not in winter. Therefore, frequency of ingestion of reclaimed water irrigated lettuce (*f*) happens from April to October, i.e. 214 days per year, assuming all the lettuce consumed comes from the street market. The annual probability is estimated using equation 5.4 [26].

$$Py = 1 - (1 - Pd)^f \quad (5.4)$$

Annual disease burden was calculated using DALYs. DALYs account for the years lived with disability (YLD) (equation 5.5) plus the years of life lost (YLL) (equation 5.6) due to the hazard, as compared to the average expected age of death in a community.

$$YLD = Pyill \times Dw \times Dt \quad (5.5)$$

$$YLL = Pyill \times Nd \times L \quad (5.6)$$

where P_{yill} is the estimated annual disease risk, *Dw* is the disability weight, *Dt* the duration of illness, *Nd* the number of deaths per illness and *L* the average years lost per fatality. The *Dw* for acute gastroenteritis is 0.0007 for cases who do not visit the general practitioner (GP), which constitute 83.1% of norovirus disease cases in Catalonia and 0.0062 for patients who did, which are 16.9% in Catalonia [259, 260]. The Dt was estimated, in The Netherlands, from 3 to 6 days, with average 3.8 days, in people who did not visit the GP, and from 5.73 to 7.23 in those who did [259]. We introduced this variability by defining a lognormal distribution (mean=log10(3.8), sd=0.1) and uniform (5.73-7.23) days, for non-visiting and visiting GP, respectively.

No information on norovirus mortality in Spain has been found. In The Netherlands, the annual mortality is 0.009% of norovirus cases and the years lost due to premature death was estimated to be 20.7 in The Netherlands [32].

Risks were calculated using Monte Carlo simulations with random sampling of 10,000 values from each distribution input. The Anderson-Darling test was run to see if the differences between the base scenario and the internalization scenarios were significant. Sensitivity analysis was conducted to know how sensitive the model outputs are to the inputs. The effect of the value of a model parameter on the probability of illness was calculated by varying a model parameter to the 95% CI limits of its variability, while keeping the variability of the other parameters. The effect of tertiary treatment on the DALYs was checked by running the model every time with a different log reduction of the norovirus concentration. Monte Carlo simulations, and uncertainty and sensitivity analysis, were performed using R version 3.0.1 [177].

3. Results

3.1. Concentration of Norovirus in Secondary and Tertiary Effluent

NoVGI was found in 71% and NoVGII in 100% of the samples. Concentrations of NoVGI and NoVGII were summed up because both genotypes are able to infect humans.

Concentrations in secondary effluent ranged from 2.0×10^4 to 1.9×10^6 , in tertiary effluent from 4.4×10^3 to 1.5×10^6, and in the reservoir from 1.8×10^3 to 3.1×10^5 gc/L. The norovirus combined concentrations from the three sampling points was pooled after an ANOVA analysis showed no significant differences between the log transformed data of the three locations. Both distributions (lognormal and gamma) gave a good fit to the pooled data and the gamma distribution was selected because it has previously described concentrations of norovirus in water [156]. Figure 5−1 shows the concentration of norovirus (NoVGI and NoVGII combined) and the fitted curves.

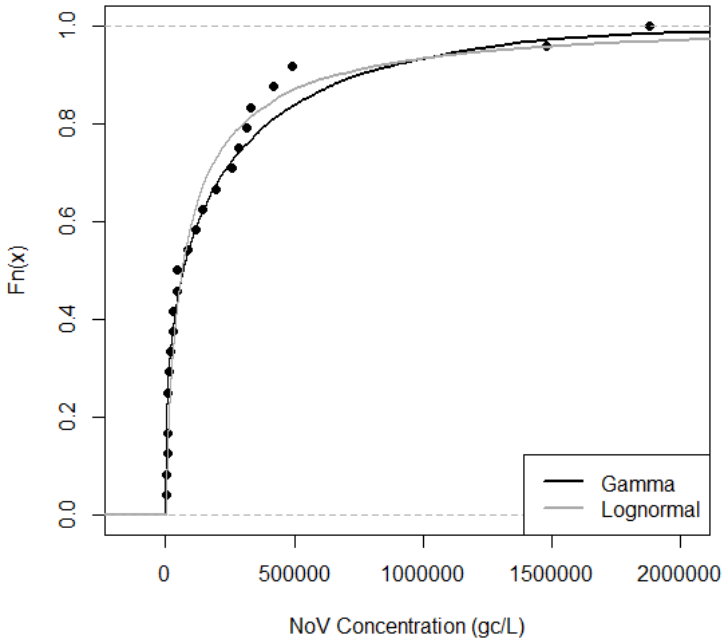

Figure 5-1: Empirical Cumulative Distribution Function (ecdf) graphs of the norovirus data and the gamma and lognormal distribution fitted to the data. Parameters of the gamma distribution are shape=3.2 × 10^{-1}, rate=1.2 × 10^{-6} (in virus/L). Parameters of the lognormal distribution are meanlog=11.1, sdlog=1.9 (in virus/L).

3.2. Impact of Tertiary Treatment on Burden of Disease

The virus concentration at the main steps of the exposure assessment, the dose, and the risk estimates are shown in Table 5−3. The mean individual probability of developing gastroenteritis after eating lettuce irrigated with secondary and tertiary water containing norovirus was 2.3×10^{-2} and 5.2×10^{-3} pppd (per person per day), respectively. The mean annual disease burden was 7.8×10^{-4} and 3.9×10^{-4} DALYs pppy. Due to the limited efficiency of virus removal by the tertiary treatment, the disease burden reduction by the use of tertiary effluent as compared to the use of secondary effluent, is very limited.

Table 5−3: Mean (95% UCL) of the QMRA results for irrigation of lettuces with secondary and tertiary effluent.

Results	Units	Irrigation with Secondary Effluent	Irrigation with Tertiary Effluent
Concentration in Water	Virus/mL	263.1 (1169)	31.7 (139.3)
Concentration on Lettuce after Irrigation	Virus/g	4.66 (20.3)	0.56 (2.47)
Concentration on Lettuce at Consumption	Virus/g	0.01 (0.04)	0.001 (0.005)
Dose	Pppd	0.19 (0.73)	0.02 (0.09)
Pd illness	Pppd	0.02 (0.15)	0.005 (0.02)
Py illness	Pppy	0.45 (1)	0.24 (0.99)
Disease Burden	DALYs/ year	7.8×10^{-4} (1.9×10^{-3})	3.9×10^{-4} (1.9×10^{-3})

3.3. Impact of Internalization

Internalization scenarios when using internalization rates derived from Dicaprio, et al. [235] (high internalization rate) and Wei, et al. [237] (medium internalization rate) resulted in higher disease burden, but not when using data from Esseili, et al. [236] (low internalization rate) (Figure 5−2). In the first two cases, the concentration of internalised viruses (49.3 and 0.03 gc/g, respectively) was higher than that on the lettuce surface (0.02 gc/g), and was, therefore, driving the probability of disease. In the latter case, the concentration was much lower (3.1×10^{-5} gc/g) and, therefore, the concentration on the lettuce surface was responsible for the probability of disease. In the scenarios that resulted in a higher disease burden, the highest variation was observed in the medians, while the variation in the 95 percentile was very low. The Anderson-Darling test showed that the disease burden of the base scenario was statistically different from all the internalization scenarios (p-value <0.0001), except from the low internalization scenario (p value = 0.8).

3.4. Sensitivity Analysis

The sensitivity analysis showed that the concentration of virus in the tertiary effluent and the consumption of lettuce were major factors influencing the variability of the risks (Figure 5−3). Washing the lettuce, in-field inactivation, inactivation during transport and storage, and the volume of water clinging on the lettuce surface had little effect on the variability of the probability of disease.

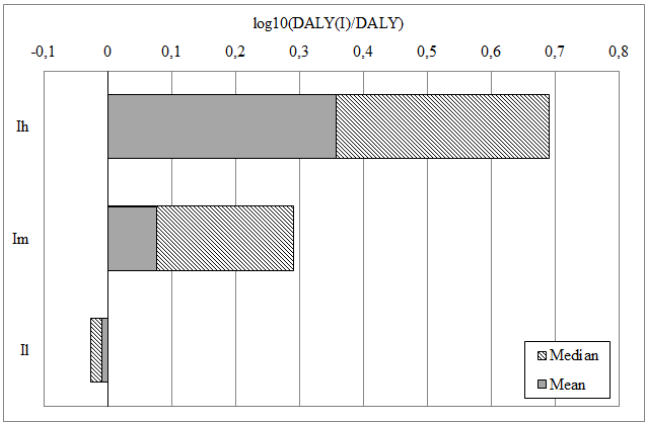

Figure 5-2: Impact of the internalization scenarios on the burden of disease. Ih, Im and Il stand for high, medium and low internalization rates, respectively.

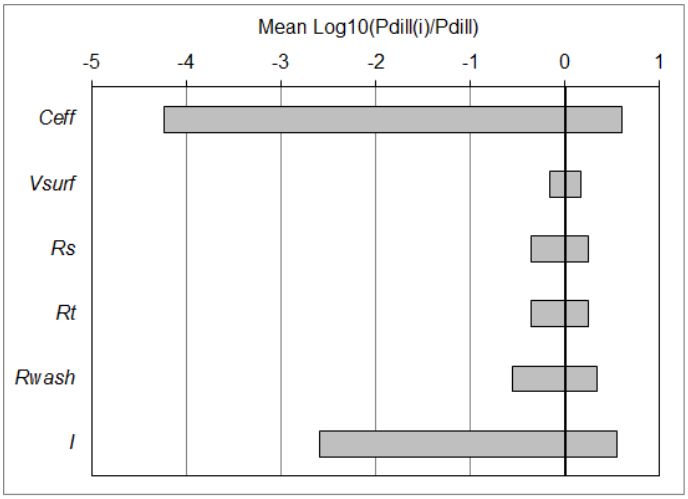

Figure 5-3: Sensitivity of P_{dill} (event probability of disease) by varying each parameter to its extreme values.

An alternative scenario was build using the virus decay of Petterson, et al. [141], [231], as done in other norovirus QMRA in crops [78, 79]. This resulted in lower inactivation of norovirus and, hence, higher disease burden (mean of 1.4×10^{-3} DALYs pppy), than when using norovirus and surrogate inactivation data under several temperature and sunlight conditions (Appendix C.4).

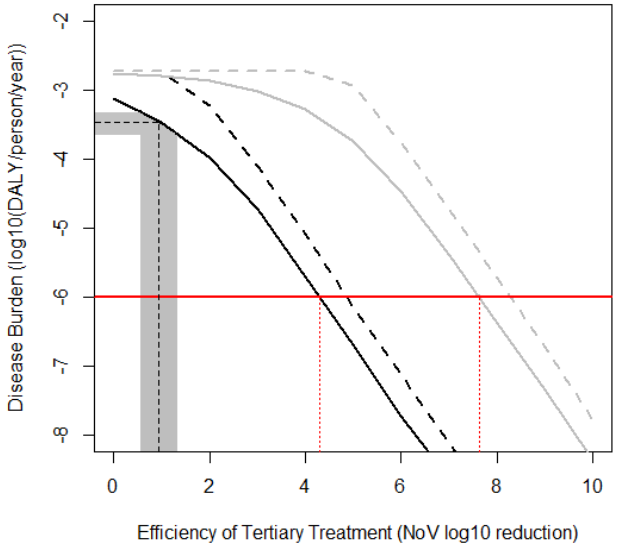

Figure 5-4: Annual disease burden plotted against the efficiency of the tertiary treatment. The black solid line is the mean disease burden considering only surface deposition of virus on lettuce, with the dashed black line representing the 95% CL. The grey solid line is the mean disease burden when high internalization of viruses into lettuce (using data from Dicaprio, et al. [235]) is included, with the dashed grey line representing the 95% CL. Red horizontal line is the recommended risk threshold of 10^{-6} DALY pppy, with dotted vertical lines showing the needed treatment efficiency to achieve the threshold disease burden (4.3 and 7.6 decimal log reduction for surface only and internalized virus in lettuces, respectively). Dashed black vertical line correspond to the known efficiency of the treatment plant and the corresponding disease burden (when virus are not internalized in lettuces), the grey area being the 95% CI.

The disease burden was plotted against the efficiency of the tertiary treatment to show the impact of additional virus removal by the tertiary treatment on the burden of disease, both for the scenario without internalization and the scenario with the highest internalization (Figure 5−4). The shoulder in this figure is the result of the high norovirus doses and the dose-response function. The graph shows that 4.3 decimal logarithms reduction of the norovirus concentration (approx.) is required to achieve a burden of disease of 10^{-6} DALYs pppy, if no internalization of viruses into lettuce is considered, and 7.6 decimal logarithms reduction (approx.) if internalization as described by Dicaprio, et al. [235] is incorporated. The graph also shows that the reduction currently achieved by the tertiary treatment plant is not enough to reduce the DALYs under the WHO guideline value, even when no internalization is happening.

4. Discussion

The health risks associated with consumption of lettuce irrigated with secondary and tertiary treated effluent containing norovirus in the north-east of Spain were estimated, based on norovirus data collected at this site. Although the tertiary treatment was efficient enough to reduce the concentration of EC below the regulatory threshold for reclaimed water uses for crops irrigation (table C.1 and [119]), additional removal is needed in the system in order to meet the 10^{-6} DALYs pppy recommended by the WHO [22]. If this is to be achieved by the tertiary treatment alone, then a 4.3 decimal logarithmic reduction of the norovirus concentration must be ensured.

The concentrations of norovirus in secondary and tertiary effluent were not significantly different. Concentrations were measured with RT-qPCR, detecting, specifically, NoVGI and NoVGII genes. No infectivity assays are currently available for norovirus due to the lack of specific cell culture lines. The infectivity of norovirus in secondary effluent is, therefore, not known. However, the norovirus dose-response model was derived from a study where a solution of Norovirus with unknown infective particles was administered to human volunteers. Since the technologies used for secondary treatment might remove virus particles but do not result in further inactivation, we consider the ratio of genomic copies to infectious norovirus particles in secondary effluent comparable to the ratio in the (aged) samples used for the dose response studies.

However, during tertiary treatment it is likely that norovirus is affected by the UV and chlorination. We used site-specific data on bacteriophage inactivation to estimate norovirus inactivation. Montemayor, et al. [226] used three different disinfection methods (UV, chlorine, and a combination of the two) to study the reduction efficiency of the WWTP for different indicators. When using chlorine alone, the dose applied was 10 ppm, which is higher than the one used in the studied tertiary treatment. This, however, did not result in a high inactivation of bacteriophages, while it reduced the concentration of EC by 5 logs. UV yielded higher inactivation of bacteriophages, either when used alone or in combination with chlorine doses of 5 ppm. Unfortunately, no validated UV dose was given. The WWTP uses a combination of UV and chlorine, at doses between 3 and 6 ppm, although sometimes the UV treatment is by-passed, because the chlorine treatment is effective to reduce the concentration of EC to below the legal requirements. Therefore, tertiary treatment without UV may occasionally occur. This may result in periods of higher risk, given that the chlorination was not very effective against bacteriophages.

Recently, the study of virus internalization into vegetables has experienced increasing attention. However, different studies show very different outputs for internalization of norovirus and its surrogates into edible parts of lettuces [235-237]. Sources of this variability can be the growth media (soil vs hydroponic solution), the RH of the environment, the applied virus dose, and species of lettuce and virus used in the experiments [237, 256]. Overall, results show that lettuces are able to internalize norovirus

and surrogates, and these can reach edible parts of the crops, under laboratory conditions. It is not so clear to what extend this happens under field-conditions, with different kind of soils, lower soil saturation, lower concentration of norovirus in irrigation water, and different climatic conditions influencing internalization. Specifically, the use of lower concentrations of viruses in irrigation water compared to the ones used in laboratory studies could result in internalized concentrations below the LOD of the methods used. This risk assessment showed that virus internalization into lettuces can have a large impact on the risk estimates (if high and medium internalization rates are considered) or no influence at all (if low internalization rates are considered), demonstrating the need for further research on the ability of lettuce on internalizing norovirus under field conditions. This is the first time that virus internalization into crops has been incorporated in a QMRA study.

The amount of virus attached to the lettuce surface through overhead sprinkler irrigation was estimated by Mok and Hamilton [254]. This is a conservative approach because it was assumed that all pathogens in the wastewater captured on the lettuce attach to its surface, and might lead to an overestimation of the risks. However, norovirus have been shown to bind specifically to the carbohydrates of the cell wall of lettuce leaves surface [261]. Virus attachment and survival differs on virus type, plants properties, and weather conditions [233, 262]. Furthermore, differences might exist between the experimental conditions of the water retention study, and our field conditions, for instance, the volume of water used for irrigation, the position of the overhead sprinklers (distance from the irrigated vegetables and height), or environmental conditions (wind direction and speed, temperature, etc.).

Other QMRA studies have estimated the in-field decay of norovirus on lettuce surface with the *B. fragilis* bacteriophage B40-8 model and assumed post-harvest decay as negligible [78, 79]. This is a conservative assumption because the bacteriophage is very resistant to environmental conditions [141] and because viruses can undergo post-harvest degradation, depending on temperature conditions and time [232]. In an attempt to use a more specific approach, we have used data derived from studies on the decay of norovirus surrogates [232, 233]. Although human norovirus data are not available, we believe that this is more appropriate, since MS2, TV, and MNV have been shown to be good norovirus surrogates [233, 263] and because virus inactivation is dependent on different temperature and solar radiation conditions [232]. However, specific norovirus data would provide the best assessment, but would require human volunteer studies [264].

Other approaches to understand norovirus inactivation are being studied, such as the combination of enzymatic treatment with real-time nucleic acid sequence-based amplification [265], the combination of RT-qPCR with RNAse treatment [266, 267], or the quantitative evaluation of oxidative damages on viral capsid protein [268]. However, more research is needed in this field, for instance, to know if these methods are able to identify loss of infectivity due to different causes (heat, UV, chlorine, pH, etc.) to the same extent.

Our results show that the annual disease burden of consuming lettuce irrigated with reclaimed water exceeds the recommended threshold of 10^{-6} DALYs pppy. The risks are

even higher when internalization is considered. Further measures should be applied in the system to reduce the virus load in the irrigation water, prevent lettuce contamination, or inactivate or remove the virus after contamination. To reduce the virus load in reclaimed water, improving of the UV system should be considered, e.g., adding a pre-treatment step that increases the transmittance of the water, as done in other WWTP [226]. Measures to prevent contamination include using an irrigation method that results in lower surface deposition (such as subsurface drip irrigation), although this would not reduce the virus internalization. To inactivate the viruses after contamination, farmers could be advised to irrigate with a different source water on the last irrigation event, or increase the time between the last irrigation and the harvest, increasing the inactivation of viruses already deposited on the surface.

5. Conclusions

We conducted a stochastic QMRA to quantify the disease burden of norovirus through ingestion of lettuce that has been irrigated with reclaimed water. This is the first time that site-specific data on human NoVGI and NoVGII in sewage effluent were used in a QMRA of norovirus on lettuce. Decay data of norovirus and surrogates have been used to describe the virus inactivation in the field and during transport and storage of lettuce, in contrast with the more conservative commonly used decay model derived from Bacteriophage B40−8.

- The recently discovered internalization of viruses in crops can have a significant impact on the disease burden if internalization occurs in the field. More research is needed to better understand and quantify virus internalization into lettuce under field conditions.
- Although the tertiary effluent of the target WWTP meets the EC requirements of national guidelines, additional barriers (either in the treatment or in irrigation practices) would be needed to meet the WHO recommendation for gastrointestinal disease burden.

Chapter 6: Screening-Level Risk Assessment of *Coxiella burnetii* (Q Fever) Transmission via Aeration of Drinking Water

Abstract

A screening-level risk assessment of Q fever transmission through drinking water produced from groundwater in the vicinity of infected goat barnyards that employed aeration of the water was performed. Quantitative data from scientific literature were collected and a Quantitative Microbial Risk Assessment approach was followed. An exposure model was developed to calculate the dose to which consumers of aerated groundwater are exposed through aerosols inhalation during showering. The exposure assessment and hazard characterization were integrated in a screening-level risk characterization using a dose-response model for inhalation to determine the risk of Q fever through tap water. A nominal range sensitivity analysis was performed. The estimated risk of disease was lower than 10^{-4} per person per year (pppy), hence the risk of transmission of *C. burnetii* through inhalation of drinking water aerosols is very low. The sensitivity analysis shows that the most uncertain parameters are the aeration process, the transport of *C. burnetii* in bioaerosols via the air, the aerosolization of *C. burnetii* in the shower, and the air filtration efficiency. The risk was compared to direct airborne exposure of persons in the vicinity of infected goat farms; the relative risk of exposure through inhalation of drinking water aerosols was 0.002%.

This chapter is based on:

Sales-Ortells, H., Medema, G. 2012. Screening-Level Risk Assessment of Coxiella burnetii (Q Fever) Transmission via Aeration of Drinking Water. *Environmental Science and Technology* 46 (7), 4125-4133.

1. Introduction

Q fever (QF) is a worldwide zoonosis caused by the bacteria *Coxiella burnetii*. Infected domestic animals secrete it in high concentrations in placentas, birthing/abortion fluids, feces, and urine and it is also transmitted by ticks [64, 269]. Once in the environment, *Coxiella* can travel long distances in the air [65, 68].

In the past four years, four outbreaks of QF in humans have been reported in The Netherlands following the kidding season (KS, goat's birthing period). The extent of the outbreaks, the case history, and the spread of the cases suggest that a wide-scale environmental contamination or multiple point-source contamination sites are more probable as *C. burnetii* sources than direct contact with animals, consumption of contaminated unpasteurized milk, or contact with parturient pet animal [270]. Hence, the occurrence of *Coxiella* in the air is considered as a probable route of transmission.

The Netherlands is an urbanized country that employs intensive farming practices with high numbers of animals per barnyard. Barnyards are located in close proximity to urban areas, facilitating the spread of a disease like QF. Goats have been found to be the primary source of QF infection in this country. The bacteria have been found in the air 5 km and even 10 km away from infected farms [72].

Groundwater is commonly used in The Netherlands as a source for drinking water. A high number of groundwater treatment plants (GTP) are located in the vicinity of infected barnyards (IB), 41 inside the 5-km radius zone and three of them less than 1 km from an IB (Figure 6−1). An aeration step is necessary for many groundwaters to increase the oxygen concentration and remove undesirable volatile compounds present in anaerobic groundwater. Depending on the quality of the groundwater, i.e., the gases that have to be removed, a different system is used to achieve the desired level of air-water contact: spraying, tower aerators, cascades, deep well aerators, plate aerators, or compressor aerators [271]. If the air is contaminated with *C. burnetii*, bacteria potentially may be transferred to the water, survive treatment and distribution, and reach consumers' taps, where they may be ingested and/or inhaled.

We performed a screening-level risk assessment (RA) to evaluate the magnitude of the risk of developing QF through municipal water supply. The RA targets the general population in areas where goat farms are infected with *C. burnetii*, anaerobic groundwater is used for drinking water production, and the groundwater is aerated. The exposure scenario is inhalation of tap waterborne aerosols while taking a shower. In an alternative scenario the air that is used for aeration of anaerobic groundwater is passed through air filters that remove a large fraction of the aerosols that contain *Coxiella.* The result was compared with the risk of direct inhalation of contaminated air in goat farm areas.

The study was performed following a deterministic and conservative approach. In each step, the literature value that was associated with a higher probability of illness was selected. When the highest value was considered far from reality or not representative of

the Dutch population, a lower value was used. A nominal-range sensitivity analysis [115] (SA) was conducted using alternative data for each step.

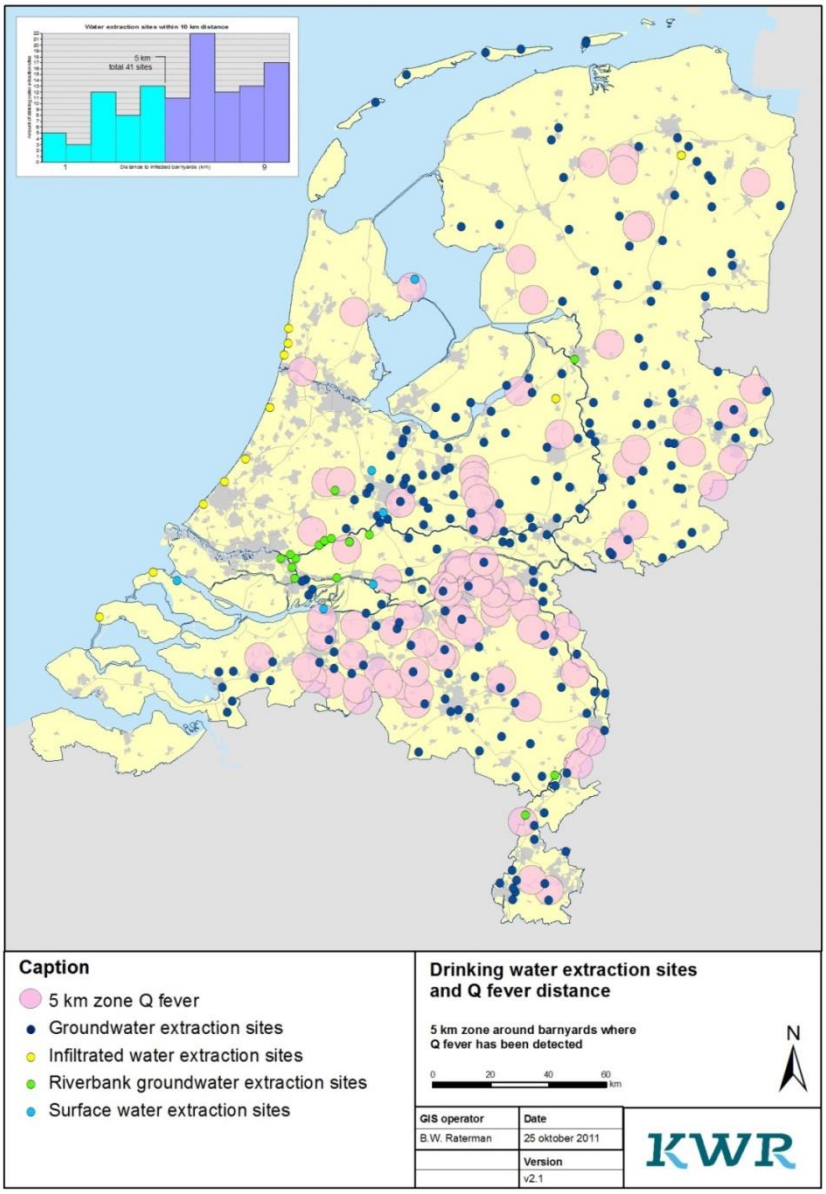

Figure 6-1: Drinking water extraction sites and distance from 5km zone of Q fever infected barnyards in 2009.

2. Hazard Identification

Livestock and pets are major reservoirs of *C. burnetii*. The bacteria are found inside the host cells in two forms: an inactive spore-like small cell variant (0.2-0.4 µm) and a large cell variant (up to 2 µm). The small cell variant is very resistant to drying, UV irradiation, acid or alkaline pH, disinfectants, and other chemicals. This small cell variant appears to be a resistant stage adapted to survive in the environment and be transmitted to new hosts [63].

Microorganisms are excreted in milk, urine, and feces of infected animals. Most importantly, during birthing the organisms are shed in high numbers within the amniotic fluids and the placenta [64, 65, 67, 272]. Infection of humans usually occurs by inhalation of these organisms from air that contains airborne barnyard dust contaminated by dried placental material, birth fluids, and excreta of infected herd animals. Humans are very susceptible to the disease by the inhalation route, and one single microorganism may be sufficient to cause infection [69]. QF becomes symptomatic in 40% of infected people, causing acute or chronic syndromes such as flu-like symptoms, pneumonia, endocarditis, and hepatitis. Twenty-five percent of patients require hospitalization, with 1-3% of fatalities [70, 71].

The number of QF cases in The Netherlands has increased since 2007, starting with an outbreak in Noord-Brabant. Between 1997 and 2006 the average number of QF cases per year was 11 [273], while 168, 1,000, 2,354, and 504 cases were identified in The Netherlands in 2007, 2008, 2009, and 2010, respectively, with 19 fatalities in total. In 2011, only 67 cases have been detected as of August 17. The reduction of cases observed in 2010 and 2011 is probably due to the measures undertaken by the Dutch government preventing the emission of high amounts of bacteria [72, 274].

Once the bacteria that have emerged from the IB in aerosols reach the water treatment plant and enter the water as a result of the aeration process, they may be removed by subsequent treatment steps. If these are not present or not effective, the bacteria may enter the distribution mains and be transported to the homes of the consumers who may either ingest the bacteria with the water or inhale it via aerosols generated, for instance in the shower. Both pathways have been reported to transmit the disease. However, inhalation appears to be the dominant pathway; ingestion of *Coxiella* would be unlikely to produce clinical symptoms [64] and it has been reported that drinking milk contaminated with *C. burnetii* has caused seroconversion in human volunteers, without clinical disease [275].

3. Problem Formulation

The Ministry of the Environment set 10^{-4} per person per year (pppy) risk of infection as the acceptable annual risk in the RA guidelines for enteric pathogens in drinking water in The Netherlands [120]. The risk of infection with *C. burnetii* via ingestion or inhalation of tap water from GTP that use aeration and operate downwind from animal barnyards should not

exceed 10^{-4} pppy. The risk through tap water should also be negligible (lower than 10^{-4} pppy) compared to other routes of exposure (e.g., direct inhalation of contaminated air).

4. Exposure Assessment

The concentration of bacteria in tap water is unknown, therefore, the dose (*d*) has to be estimated, starting from the concentration of bacteria in the IB air and calculating the changes in concentration produced by the subsequent steps.

The case study was based on a hypothetic GTP located 1 km northeast (NE) from an infected goat barnyard. This was selected because in The Netherlands the wind blows predominantly from the southwest (SW), considering SW any wind blowing between south and west (46% of the days in 2009) [276]. *C. burnetii* shedding from goats is higher during the KS and the concentration in the air is higher afterwards. Two different periods were defined: (A) wind blowing from the SW after the KS; (B) wind blowing from the SW during the rest of the year.

Period A was estimated to be from March 8 to May 30. The estimation was based on the date of symptom onset [72] (Appendix D.1) and considering an incubation period between 1 and 3 weeks [277]. This period does not coincide with the KS (from January/February to March/April) observed in the literature [67, 278, 279], because once the bacteria are secreted in the placentas, it takes time for the placentas to degrade and the bacteria to become airborne. The same method has been used in another study to calculate the likely period of human infection for 2008 obtaining similar results [280].

4.1. *Coxiella* Concentration in Barnyard Air

Only two studies were found that provide data to calculate the concentration of *C. burnetii* in the air of IB. Air from a barnyard, which had previously tested positive for *C.burnetii*, was sampled with the windows opened during sheep shearing. Air (675 L) was passed through polycarbonate filters and the bacteria were recovered for PCR analysis in 25 µL of buffer. Aliquots of 5 µL of sample were analyzed by PCR giving positive results in all samples and negative results in the control. A lower detection limit of 120 *C. burnetii* to obtain a positive PCR response was reported [281]. Hence, a concentration of at least 880 cells/m^3 was present in the barnyard air at the time of sampling.

In another study, dust was sampled from approximately 800 L of air in a sheep barn where employees had developed QF, cattle had tested positive for complement fixing antibodies, and the bacteria were found in milk. The air was bubbled through 35 mL of beef extract broth. After 45-90 min of sampling, 10-25 mL of broth remained. Guinea pigs that were inoculated with 2.5 mL of broth developed an immune response to *C. burnetii*, and the bacteria was isolated from guinea pigs that were inoculated with 5 mL of broth [282]. Assuming the transfer rate of the bacteria from the air to the broth to be one and one bacterium to be enough to cause the response in guinea pigs, the concentration of bacteria

in the barnyard was 8.35 cells/m^3 air. The former value (880 cells/m^3) was used as the point estimate (PE) in period A, while the latter was used as the PE in period B and as the alternative value (AV) in the SA.

4.2. *Coxiella* Air Transport and Dilution

Several models describe the transport, dilution, and inactivation of bacteria through the air. The downwind concentration of viable microbes has been previously estimated using a modified Pasquill inert particle dispersion model, which is an empirical plume model based on observations of the dispersion of tracers in the atmosphere (equation 6.1):

$$\frac{X}{Q} \times \frac{\bar{U}}{exp(-\lambda x \sqrt{U})} = \frac{1}{2 \prod \sigma_y \sigma_z} \times exp\left[-\left(\frac{H^2}{2\sigma_z^2}\right)\right] \equiv g \quad (6.1)$$

where X is the number of particles per cubic meter in the air inlet at the GTP, Q is the number of particles emitted from the source per second (emission rate), $\bar{U}$ is the mean air speed in meters per second, λ is the microbial inactivation rate, x is the downwind distance from the source, σ_y and σ_z are the diffusion factors in the y and z directions in meters (crosswind and vertical direction respectively) and are functions of meteorological conditions (stability class and wind speed) and downwind distance from the source, and H is the source height in meters [283].

The source (the air outlet of a closed barnyard) is located at (x, y, z) = (0, 0, 10). The source is selected to be at a height of 10 m, since it results in a higher estimation of bacteria in the air with the distance than using the ground level source (0 m), which is used in the SA. The stability class (A-F, in order of increasing stability; higher stability means less lateral spread of particles in the plume and increased deposition of particles at the downwind GTP site) depends on insolation and surface wind speed at 10 m above the surface and can be obtained from Table 2 in Lighthart and Frisch [283] (Appendix D.2). The second part of the equation can be expressed as g, which was interpolated from the graphs given by Lighthart and Frisch [283] (Appendix D.2). These graphs give the value of g with respect to the distance of the point of interest (air inlet of the GTP) from the source for different heights and stability classes.

To calculate Q, published data about ventilation rate (VR) in cattle in The Netherlands were used. The highest number for dairy cows in cubicles in The Netherlands in winter was selected as a PE (938 m^3/h/LU) [284]. The VR is expressed in livestock units (LU) and one LU is equivalent to 500 kg. Assuming a mean weight of 100 kg for goats, we recalculated the VR to adapt it to goat barnyards. As an AV, the VR obtained for dairy cows housed in litter was used (268 m^3/h/LU). 900 goats were assumed to be in the IB and the value of 5,000 goats was used in the SA [280, 285]. Q was estimated as the product of the concentration of bacteria in the barnyard air and the VR. The death rate of the microorganism during air transport was assumed to be 0 because *C. burnetii* is very resistant to adverse environmental conditions [64, 286].

The meteorological conditions for the year 2009 were obtained from the KNMI website [276]. The wind was blowing from the SW a total of 34 days from March 8 to May 30, 2009 (9.3% of the year), while during the rest of the year it was blowing from the SW 137 days (37.5% of the year). A mean wind speed of 4.2 m/s was obtained for period A, and 3.9 m/s was obtained for period B. The insolation was considered slight and the atmospheric stability class was C (slightly unstable) in both periods. For the SA, a wind speed of 5.7 m/s and stability class D (neutral) were used. Considering a more specific wind direction, i.e., wind blowing from between the south-southwest (SSW) and the west-southwest (WSW), only 17 and 80 days correspond to period A and B, respectively, and the mean wind speeds change slightly. This scenario was also considered in the SA.

An alternative approach is to use empirical data from an experimental study on the downwind dispersion of microorganisms from a biosolids disposal site [287]. Data referring to sulfite-reducing *Clostridia* were extracted from the graph in Páez-Rubio and Peccia [287], a regression line was fitted and adapted to the *C. burnetii* data available, and the log reduction of *Coxiella* cells after 1 km was calculated.

Instead of 1 km, the values of 360 m and 5 km were used in the SA. The former as being the closest a GTP has been found from an IB in The Netherlands; the latter as being the radius around IB where high concentrations of the bacteria had been found [72]. The 5-km zone has also been reported as a higher risk of infection zone [280].

4.3. Air Filtration

Data from a questionnaire from water utilities in the south of The Netherlands showed that several air filters with different particle removal efficiency are used by some GTP, but others do not use air filtration. We chose absence of air filtration (0% removal) as the PE value, and the removal by HEPA filters (99.95%) as AV.

4.4. *Coxiella* Transfer to Water During Aeration

The questionnaire also showed that several different types of aeration exist, with forced and nonforced airflow. Nonforced systems work with natural ventilation. In forced air systems the RQ (air flow to water flow ratio) depends on the type of aeration and ranges from 0.5 to 5 L air/L water [271]. Data from the water utilities showed that a RQ of 20 is not uncommon, and this is the value used as PE for being the most conservative, while a RQ of 0.5 has been used as an alternative.

The transfer of *Coxiella* bacteria from air to water in these situations is unknown. A liquid impingement sampling method for *Legionella* showed a recovery rate of 10% [88]. We assumed a 100% transfer of the bacteria due to the high RQ applied and used a 10% transfer in the SA.

4.5. *Coxiella* Removal by Water Treatment

Groundwater that is aerated is usually also treated by rapid sand filtration (RSF) [271]. No data are available for removal of *C. burnetii* bacteria through RSF. We assume that the removal by RSF is similar to the removal of *E. coli* and *Clostridium* spores. *E. coli* are removed through RSF with 0.5 ± 0.3 log and *Clostridium* spores with 0.7 ± 0.5 log [288]. A 0.5 log removal was used as PE, while 0.7 log was used in the SA.

4.6. *Coxiella* Inactivation in Water

C. burnetii is able to survive in the environment for long periods. Survival times of 20 – 30 days in soil or barn litter and 30 months in tap water are reported [289]. Because of the short residence time of treated water in the distribution network (hours – days) and because no disinfectant is used for the treatment of drinking water in GTP in The Netherlands [271], we assume that no significant inactivation occurs during the transport from the GTP to the consumers' home.

4.7. *Coxiella* in Shower Aerosols

Bacteria are transferred from water to aerosols generated from the shower heads. The bacteria air/water ratio can be calculated from the data from several *Legionella* studies and a study on exposure to endotoxin in aerosols generated from showers and humidifiers [85-89] (Appendix D.3). The study from Deloge-Abarkan, et al. [88] is the only study calculating the recovery efficiency of the methods used. Therefore, we used their data on the culturable *Legionella spp.* and calculated a ratio of 2×10^{-6}. This value was used as PE, while in the SA 3×10^{-8} was used, calculated from two other studies [85, 87].

4.8. *Coxiella* Dose through Inhalation

The average shower duration among the Dutch population during 2007 was between 7.7 and 7.9 min and every person took 0.8 showers per day. The shower duration was longer during the week days (8.1 min) than during the weekends [99]. We chose a duration of 8.1 min and a frequency of 1 shower per day to cover a wide range of the population. Alternatively, we used the values of 7.7 min per shower and 0.8 showers per day.
Breathing rates and tidal volumes in healthy people during rest are 12 breaths/min and 500 mL, respectively [86, 290]. These data are used as PE, while in the SA 31 breaths/min and 1,549 mL for people after exercise are used [291].

Not all the aerosols inhaled reach the lower respiratory tract (LRT). Zhou, et al. [97] calculated the particle deposition fraction during showering. In our study, we used their deposition data in the alveolar and bronchiolar region for hot water and mouth breathing and calculated an average for the three flow rates studied. This resulted in a 12.7% deposition in the LRT of the total aerosols inhaled. Alternatively, data for cold water and nose breathing were used. It has been reported that 90% of *L. pneumophila* cells that are

aerosolized from shower faucets are trapped in aerosol particles between 1 and 5 µm in diameter [87]. Aerosols in this diameter range are able to reach the alveolar region [292]. The 90% factor has been used as an alternative to the 12.7% deposition.

Table 6−1: Exposure assessment assumptions (point estimate values and alternative values).

Assumptions	Point estimate	Reference	Alternative value	Reference
Coxiella concentration at the barnyard air (cells/m^3)	880/8.35*	[281, 282]	8.35	[282]
Air Model	Plume model	[283]	Graphical extrapolation	[287]
Ventilation rate (m^3/h/LU)	938	[284]	268	[284]
Number of goats in the farm (n)	900	[285]	5000	[280]
Goat's weight (kg)	100	Assumption	-	
Inactivation of *C. burnetii* in the air	0	Assumption [289]	-	
Distance between barnyard and groundwater treatment plant (m)	1000	Assumption	360/5000	Closest distance/ [72, 280]
Source height (m)	10	Assumption	0	Assumption
Wind speed (m/s)	4.17/3.92	[276]	5.7	[276]
Insolation	Slight	[276]	-	
Air filtration efficiency (%)	0	Questionnaire	99.95	Questionnaire
RQ ratio (L air/L water)	20	Questionnaire	0.5	[271]
Transfer rate of *C. burnetii*	1	Assumption	0.1	[88]
Removal by water treatment (log)	0.5	[293]	0.7	[293]
Inactivation of *C. burnetii* in water	0	Assumption [271, 289]	-	
Aerosolization Ratio (Cair/Cwater)	2×10^{-6}	[88]	3×10^{-8}	[85, 87]
Shower frequency (pppd)	1	Assumption [99]	0.8	[99]
Shower duration (min)	8.1	[99]	7.7	[99]
Breathing rate (breaths/min)	12	[290]	31	[291]
Breathing volume (mL)	500	[290]	1,549	[291]
Deposition in the LRT (%)	12.7	[97]	90/27** / 1.74***	[87] / [97]

**880 cells/m^3 used for period A; 8.35 cells/m^3 used in period B and as alternative value in the sensitivity analysis ** Cold water ***nose breathing. LU, Livestock units; pppd, per person per day.*

All the parameters used for each step of the exposure assessment (PE and AV), are shown in Table 6−1. The calculated PE of the dose is 1.2×10^{-7} *C. burnetii* pppd (per person per day). Appendix D.4 shows the PE of season A and the results after each step.

5. Hazard Characterization

It has been suggested that *C.burnetii* infection in animals and humans follows a one-hit model and the probability of a single organism to initiate an infection in guinea pigs is 0.9 [69]. Data suggest that the probability of *C. burnetii* of initiating a disease given infection is 100%, since in six out of seven human experimental groups, 80 – 100% of the subjects developed symptoms of QF disease. In the lowest exposure dose group, none of the subjects presented symptoms [69, 294]. However, in a recent study in the Nehterlands, it was found that the incidence of Q fever in the Dutch population was less than 10% lower than the incidence of seroconversion [295]. Based on the arguments above, the factor 0.9 was used as the dose – response parameter (r) to calculate the probability of infection (Pi) of *C. burnetii* through inhalation of shower aerosols following an exponential model (equation 6.2). A morbidity factor (s) of one was assumed to calculate the risk of developing QF disease given infection (P_d), and 0.1 [295] as alternative value.

$$P_i = 1 - exp(-d \times r) \tag{6.2}$$

6. Risk Characterization

Exposure and dose–response assessment are combined to estimate the P_d of the population exposed. To calculate the annual risk (P_y), equation 6.3 was used [26].

$$P_y = 1 - [(1 - P_{dA})^{E_A} \times (1 - P_{dB})^{E_B} \times (1 - P_{dR})^{E_R}] \tag{6.3}$$

where R is the rest of the year, when there is no exposure via water, and E is the number of exposure events per year (34 days in period A, 137 in period B, and 194 in the rest of the year).

The *C. burnetii* concentration calculated at every exposure step, the dose, and the risk of developing illness for periods A and B are shown in Table 6−2. The total annual risk of QF, including both periods, is 3.7×10^{-6} pppy. The risk after the KS is, as expected, higher than during the rest of the year. Indeed, the total risk depends on the risk during period A.

A SA was conducted. For every run only one model input was changed while holding all other inputs at their nominal values in order to see the effect of each AV on the risk of QF. The results of the SA are shown in Figure 6−2. Some uncertainties are not included because of their negligible effect on the output, e.g., the goats' weight; others because no information about their magnitude is available, e.g., true value of *C. burnetii* concentration in the barnyard air. It was found that the steps that affect disease risk most are the air model, the RQ value in the aeration process, the efficiency of the air filters, and the water to air ratio during shower aerosolization.

Table 6−2: Results for every step of the exposure assessment and the risk characterization.

Steps	Period A (34 days)	Period B (137 days)	Units
Coxiella concentration in the barnyard air	880	8.35	*Coxiella*/m^3 air
Emission of *Coxiella* from the barnyard	4.1×10^4	3.9×10^2	*Coxiella*/s
Coxiella concentration at the water treatment plant air inlet	1.5	0.02	*Coxiella*/m^3 air
Coxiella concentration in raw water	30	0.3	*Coxiella*/m^3 water
Coxiella concentration in treated water	9.4	0.1	*Coxiella*/m^3
Coxiella concentration in tap water	9.4	0.1	*Coxiella*/m^3
Coxiella concentration in shower aerosols	1.9×10^{-5}	1.9×10^{-7}	*Coxiella*/m^3 air
Respiratory minute volume	6	6	L/min
Air inhaled during showering	0.05	0.05	m^3
Coxiella inhaled during showering	9.1×10^{-7}	9.1×10^{-9}	*Coxiella*
D	1.2×10^{-7}	1.2×10^{-9}	*Coxiella* pppd
P_d	1.0×10^{-7}	1.1×10^{-9}	pppd
P_y	3.5×10^{-6}	1.4×10^{-7}	pppy

d, dose; pppd, per person per day; Pd, daily probability of disease; Py, annual probability of disease; pppy, per person per year.

Figure 6−3 illustrates the effect of the distance between a GTP and an IB on the risk. The risk shows a decay defined by $y = 1.15 \times x^{-1.83}$. A GTP located 163 m downwind from an IB poses a risk of 10^{-4} pppy through inhalation of shower aerosols. This distance is shorter than the closest distance a GTP has been found from an IB to date in The Netherlands.

6.1. Risk of Q Fever through the Air

To compare the waterborne risk of developing QF with the risk after direct exposure to the air, the risk for a person standing 1 km downwind from an IB (direct inhalation) for 8.1 min

per day was determined. The same air model and assumptions were applied for the exposure assessment steps. The *d* in this case is 9.2×10^{-3} *Coxiella* pppd and the risk of QF disease is 2×10^{-1} pppy. Figure 6−3 shows the decrease of the risk with the distance.

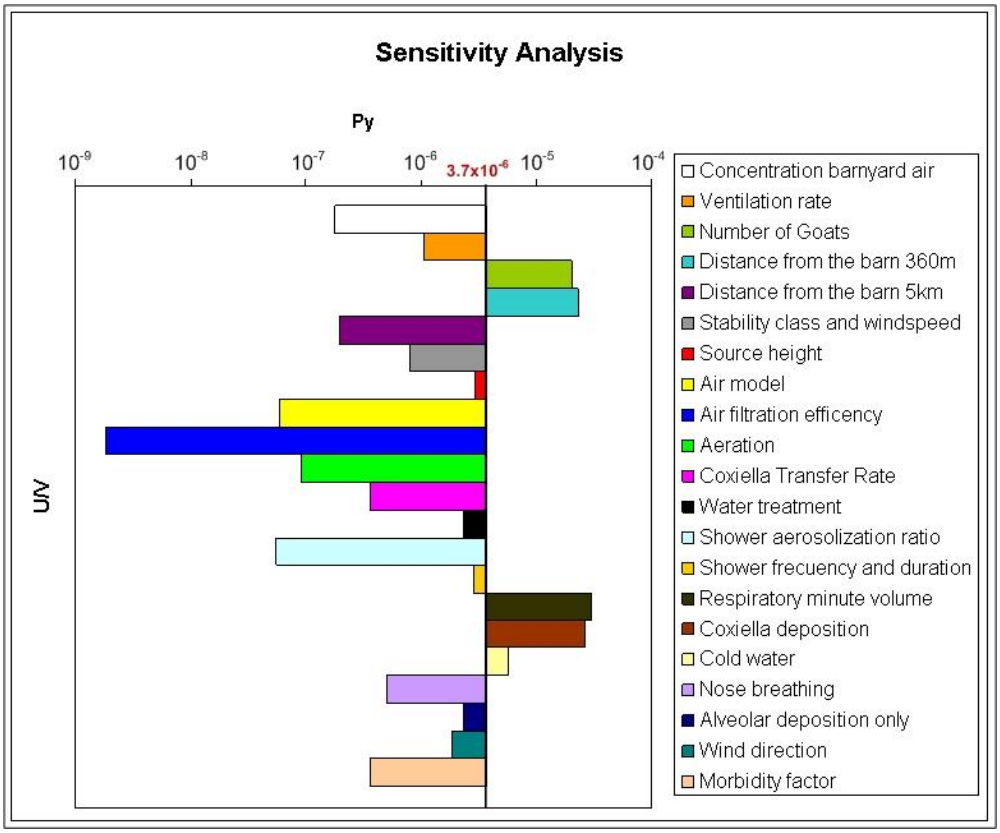

Figure 6−2: Effect of the alternative values on the annual risk of Q fever through inhalation of shower aerosols.

7. Discussion

We described a screening-level RA of QF through inhalation of drinking water aerosols in The Netherlands following a conservative approach. The results indicate that the use of air contaminated with *C. burnetii* to aerate groundwater poses a very low risk of QF disease to the population through inhalation of aerosols during showering (3.7×10^{-6} pppy risk of disease compared to the acceptable 10^{-4} pppy risk of infection [120]). The average risk of disease of *C. burnetii* in 2009 in The Netherlands by any transmission route was 1.4×10^{-4} pppy (2,357 cases in a population of 16.5 million). For people living in a 5-km radius around infected goat farms, the risk of disease in 2009 was 7×10^{-4} pppy. These averages have been calculated from symptomatic reported cases [72].

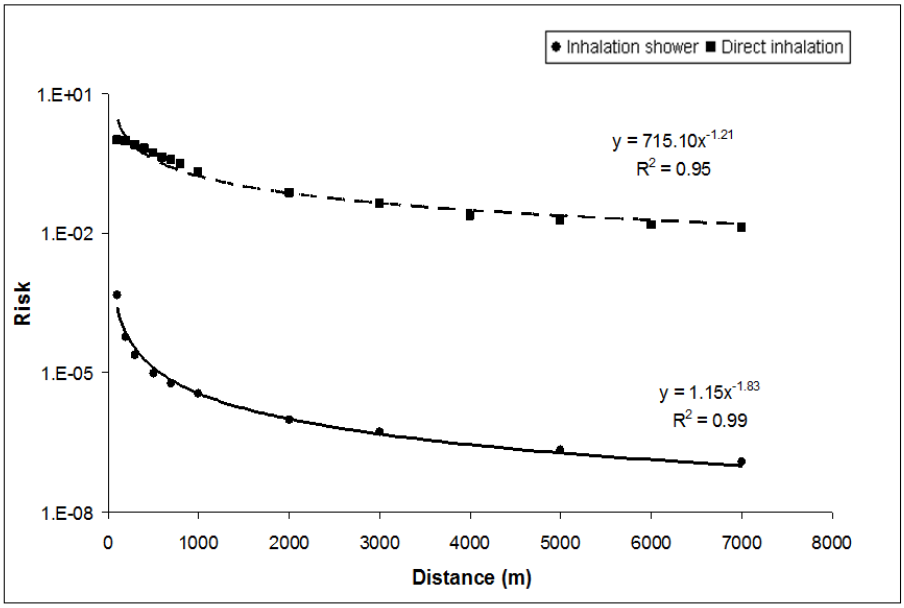

Figure 6−3: Risk of developing Q fever versus downwind distance of the GTP from the IB, period A only.

No data are available about the concentration of *C. burnetii* in barnyard air in The Netherlands. To estimate it, it is necessary to know the number of goats in that specific barnyard (900 goats [285]), the prevalence of *C. burnetii* among them [71, 296], the number of parturient/aborting goats (90% of pregnant goats may abort [65]), the concentration of bacteria in the placenta, birthing fluids, feces, and urine [66, 297] (in the placenta, more than 10^9 HID (Hamster Infectious Dose)/g [298]), the amount of feces, vaginal mucus, and urine produced daily per goat; the rate of transmission from the infected materials to the air, the rate of decomposition of the placentas, the barnyard cleaning practices and frequency, the frequency of straw changing, the barnyard soil moisture, etc. Also, the volume of the barnyard and the rate of air exchange with the outside air should be considered. As some of these data are not available and this calculation would generate numerous uncertainties, literature data about the concentration of the bacteria in the air of a barnyard have been used in this study.

Only two studies were found that showed data which allowed the estimation of the bacteria concentration. However, both studied sheep, not goat, barns. Moreover, the highest concentration was found when sampling the air during sheep shearing and this value was used here for the KS emission, when the secretion of the bacteria to the environment is probably higher. Despite the uncertainties generated and due to the lack of data available, we considered this the best and simplest way to estimate the concentration of bacteria in air. Future research can contribute new data to improve the model. Data on (viable) *Coxiella* bacteria in the air are essential to understand airborne transmission.

Interspecies extrapolations have been applied throughout the study due to the lack of data available for *C. burnetii*, e.g., the removal efficiency of water sand filtration or the ratio of bacterial transference from air to water. The effect of these substitutions on the model output cannot be quantified.

It has been suggested that *C. burnetii* is emitted to the environment attached to dust particles [270]. Therefore, the emission of dust affects the emission of bacterial cells and it should be included in the model, as well as factors that affect dust emission and transport. Farms with low vegetation in the surroundings have a higher probability of transmitting *C. burnetii* to humans. This has been related to the effect of vegetation in reducing the amount of dust available for dispersion of the bacteria. Further definition of the model would include local environmental conditions.

A plume model has been used to estimate the transport and dilution of the bacteria in the air. It allows calculating the concentration of particles in the air solely at the ground level, while the air inlet of a GTP is located some meters above the ground. A verification of the concentration of *C. burnetii* in the air at the inlet of the GTP would be useful to validate the model. Air samples should be taken with an air sampling method with a high known recovery rate (e.g., liquid impingement [88]) and analyzed with quantitative methods that determine viability [299, 300].

The method used to determine the parameter g in the model is not accurate. The interpolation from the graph is imprecise and can be subjective, generating errors in the result. Another way to solve the model has been tried, consisting in interpolating σ_z and σ_y from graphs, but this poses the same problem of imprecision.

During the air transport, rain can drag particles from the air and deposit them on the ground. When the rain stops, the soil is wet so it can take some days until the aerosols can be suspended again on the air, and this generates late delivery of cells into the GTP. This is not included in the model.

For short distances (1,000 m) between the air inlet of the GTP and the barnyard, the decrease of the concentration of bacteria at the inlet is similar when using the Lighthart and Frisch [283] model or the Páez-Rubio and Peccia [287] data. However, for long distances, the concentration decreases exponentially with the Páez-Rubio and Peccia data, and the distance becomes the dominant factor (Appendix D.5).

At a distance below 1 km, the risk rapidly increases. Most of the GTPs are >5 km from an IB. Thirty-one GTPs are within the 5-km radius and three are within a 1-km radius. At 360 m (the closest distance observed between an IB and GTP) the annual risk is 2.3×10^{-5}. At 163 m the estimated annual risk would become 10^{-4}, the risk of infection level that is considered acceptable for enteric pathogens in drinking water, with the notion that this study estimates the risk of Q fever, with a higher rate of hospitalization and mortality than enteric diseases. This means that the probability of infection of *C. burnetii* through ihalation of shower water aerosols might be closer to the acceptable risk.

The exponential inhalation dose – response model with the *r* of 0.9 was used in this study, based on the human dose – response study [69]. The dose – response modeling in mice exposed intraperitoneally to *C. burnetii* suggested that the Beta-Poisson model gave the best fit in two out of three studies [301]. Nonetheless, the results from mice cannot be extrapolated to humans and intraperitoneal exposure does not equal inhalation nor ingestion exposure. Following ingestion the microorganism encounters several host barriers (e.g., oral antibodies, stomach acid, intestinal wall) that reduce its probability of surviving and causing an illness, and which are not present in the intraperitoneal route. More research should be done about the clinical disease produced through ingestion of *C. burnetii* and the dose – response relationship.

During showering the P_d increases slightly when using cold water instead of warm water (Figure 6−2). This is because the aerosols generated with cold water are smaller and, therefore, a higher amount can reach the bronchiolar and alveolar regions compared to the aerosols generated with warm water, which are bigger. In this study, the aerosolization of bacteria has been estimated using a ratio calculated from data obtained at 20 ºC (±1ºC) and 30 – 35% relative humidity (RH) [88]. However, during showering with warm water (38 – 42 ºC), the air in the shower stall can reach a RH of 80% or even 100% and under these conditions the aerosol formation is higher than under lower water temperatures, reaching a higher concentration in the shower stall [88, 90, 97]. Furthermore, when using warm water the concentration of aerosols might be higher in the upper half of the shower stall, including the breathing area, due to the chimney-like convection flow originated by the hot water heating the air [97]. Hence, we may have underestimated the aerosol inhalation during warm water showering.

The model considers only one farm located SW from the GTP, and not the presence of several farms around the GTP, which agrees more with the reality. However, the air model indicates that it is the closest farm that governs the estimated risk. The model allows for changes in the characteristics of the GTP, the year, or the location.

As expected, the risk derived from direct exposure (2.1×10^{-1} pppy) is higher than the showering risk (3.7×10^{-6} pppy), as the reduction of the bacteria concentration due to the aeration, the water treatment, and the shower aerosolization steps are not present in the direct exposure. The health surveillance data showed an attack rate of 3.8×10^{-3} for residents in the 1 – 2 km area for 2008 [280]. Our direct exposure risk estimate is higher, but was calculated for daily open-air exposure at 1 km. Also, the overall attack rate of QF in The Netherlands in 2009 was 2.3 fold higher [72].

Only the risk of QF in the healthy population has been assessed in the present study. Children, elderly, immunosuppressed patients, people with heart valve problems or dysphagia, and pregnant women should be addressed differently. Higher prevalence of *C. burnetii* seropositivity in HIV positive patients compared to healthy blood donors and 20% of immunocompromised among QF patients have been reported [302]. In The Netherlands, 15% of the population is 65 years old or older (old population). 1.8% of the population and

6.8% of the old population suffered from severe heart disease in 2009. 1.4% of the population and 4.5% of the old population had cancer in 2009, and, hence, were immunosuppressed [303]. The median age of the QF patients in The Netherlands in 2009 was 49 years. Six deaths among QF patients were reported the same year, all in patients with other underlying diseases [304].

In conclusion, the contribution of the drinking water aerosols inhalation pathway to the occurrence of QF in the Dutch population is considered negligible. This is based on the following:

- the low annual risk of 3.7×10^{-6} pppy estimated from this screening-level RA for aerosol exposure during showering;
- an assumption of negligible risk from ingestion of the drinking water supply based on available evidence;
- the relatively high attack rate of the disease through other pathways that has resulted in the QF cases in The Netherlands ($1.4 – 7 \times 10^{-4}$);
- the conservative approach taken in this screening-level RA;
- the distance between IB and GTP that is in all cases higher than the safe distance estimated in the study.

The actual concentration of bacteria in the air could increase the estimated risk of *C. burnetii* transmission through water. This uncertainty could not be quantified due to the lack of published data.

Chapter 7: General Discussion

The aim of this dissertation was to understand and evaluate the microbial health risks derived from human exposure to new urban water features and new uses of urban-related water systems. A specific Quantitative Microbial Risk Assessment (QMRA) model was built for each scenario, using the characteristic exposure elements of the scenario. The models differed in the waterborne pathogens addressed, the exposure model (depending on the uses and activities conducted at each site), and the degree of complexity. The latter was selected depending on the information available and the objectives of the study.

The risk-outcome of the QMRA studies were the estimated number of infections or diseases amongst the exposed or total population. To give meaning/perspective to these risk outcomes for risk management, the outcomes are presented against tolerable infection or disease risk levels for water exposure or against actual levels of disease burden in the community to evaluate the significance of the calculated health risks. The value of these studies for the management of these waterborne health risks is not only this relative risk outcome, but certainly also the understanding that was created about the processes that govern the health risks. This is valuable for selecting appropriate prevention and control measures for urban water features by water authorities and risk managers.

1. Risk Assessment Approach

The method of choice for analysing microbial health risks in this dissertation is QMRA, complemented with microbial and epidemiological data. In the risk assessment process, firstly, target pathogens have been selected based on criteria specified in the introduction. Pathogen concentrations data have been either collected from published literature on several locations (when opportune) or collected on-site.

Exposure models have been built for each water feature, pathogens and activity that resulted in human-water contact, directly or indirectly. Data for the exposure models have been selected from literature, from national surveys/questionnaires, published datasets (e.g., weather data) and, when no other options were available, assumptions have been made based on expert judgment or in evidence from similar locations/activities/pathogens. Subsequently, dose-response models were used to translate the exposure dose (through ingestion or inhalation) to a measurable host response (infection/illness).

Finally, all the data have been combined to estimate annual health risks derived from direct/indirect exposure to the water features. The estimated risks have been compared, to understand their significance, either with health targets in existing water guidelines and/or national disease incidence data from epidemiological studies. Furthermore, a sensitivity analysis was conducted for every QMRA model to understand the effect of inputs variability on the estimated risk variability, and identify those with a higher effect.

This method provides information on the safety of the water concepts, allowing water managers and urban planners to undertake opportune actions where necessary. The sensitivity analysis, furthermore, provides information on where these actions should be taken (reduction of pathogen load or limitation of human-water contact).

Several urban water locations have been studied (in a deeper or shallower manner), that lead to different exposure types, in this thesis. Waters studied include:

- Natural surface waters: a river and a lake where different recreation activities take place, including swimming and rowing.
- Engineered surface waters: canals, ponds and playgrounds, used for recreation (swimming, fishing, walking, rowing).
- Stormwater: a stormwater sedimentation pond, streets flooded with combined (CSO) and separate sewer overflows (SSO), and a wadi and a water plaza connected to separate sewers. Recreation activities at these locations include playing in the water and fishing.
- Chlorinated water: an outdoors swimming pool.
- Drinking water: an ornamental fountain where children play, municipal water taps, and household water produced from groundwater that is aerated with potentially *C. burnetii* contaminated air (people are exposed through showering).
- Wastewater: secondary and tertiary effluent are used to irrigated crops that are consumed raw.

Other urban water features could be of interest, and new water features in the future can be assessed with the approach presented in this thesis. A rationale, the QMRA methodology, exposure data and health-based targets are provided in this thesis that can be used in future health risks assessments. The studied features are discussed in the next paragraphs, and conclusions and recommendations are proposed.

2. Health Risks

2.1. Screening-Level Microbial Risk Assessment of Urban Water Locations: a Tool for Prioritization

In chapter 2, an integrated screening-level QMRA for multiple water bodies and features in an urban area, exposure types, pathogens, and illnesses was developed. This is the first time that such a holistic approach is taken in a QMRA study for waterborne illnesses. The probability of gastrointestinal illness (GI) and legionnaires' disease (LD) were assessed, and compared to the level of safety associated with excellent bathing water quality or the disease incidence in The Netherlands. This provided a relative risk context for the urban water managers to determine the priorities for risk management. The probabilities of illness were determined following a consistent and transparent approach for every water body, exposure type, and pathogen analysed. The results allow direct comparison of disease risk

between the water bodies, and risk management can be based on the probabilities of illness obtained, the level of variability and the source of this variability, and the parameter sensitivity of the models.

The GI risks derived from recreational exposure at several locations were certainly not negligible. Highest annual probabilities of GI were obtained for playing in pluvial floodwater from a CSO and swimming and rowing in the river or lake, clearly exceeding the 3% GI level associated with excellent bathing water quality. The annual probability of GI for the exposed population to the river and lake and the CSO were close to the annual national incidence from all pathogens and all sources (29%) [32], indicating that these exposures could be a significant contribution to the annual incidence of GI in the exposed population.

In most locations, the calculated LD probabilities were low and below the mean incidence of LD in The Netherlands for 2009 and 2010 (0.002%) [61]. The calculated probability of LD was relatively high for the pluvial flood from the CSO and for rowing on the river and lake (high scenario), but the calculated probabilities were sensitive to the variable *L. pneumophila* input concentrations. At these locations, the LD probabilities were above the mean national incidence. The incidence data are based on diagnosed cases only, and unreported cases may occur, so it is likely that the 0.002% is underestimating the actual incidence of LD.

The estimated illness probabilities contain both variability and uncertainty due to variability and uncertainty of the input parameters. Concentrations of pathogens in water bodies, which have a large effect on the variability of the disease probabilities, are variable, and this contributes to the variability of the illness probabilities. This variability is embedded in the risk assessment. In addition, translation of pathogen concentration data from other water bodies to those under study leads to uncertainty. This uncertainty lead us to take a conservative approach, selecting the higher pathogen concentrations in our models. Site-specific data collection can be used to reduce this uncertainty. Ingestion volumes are also variable, and we considered that the variability will not be reduced by further data collection.

Because of the large number of water sites included in the study (fifteen) it was considered more opportune to start with a screening-level risk assessment based on literature data and not gather site-specific pathogen data at this level, in order to reduce time, economic, and manpower efforts. Therefore, model inputs were based on scientific evidence after a literature review on each QMRA step, and on assumptions, when no site-specific data were available. Site-specific research is needed to confirm (or disprove) the validity of the assumptions. The results of this assessment was used to set priorities for site-specific data collection (Chapter 3).

Measures to reduce health risks at these locations include advising people to swim only in designated areas (the river and the lake are not designated bathing areas), provide alternative bathing sites, inform about the risks of playing on flooded streets from CSO, or

prevent flooding events. Increasing the residence time of the water at the sedimentation pond and/or adding other water treatment measures (e.g., filtration) would reduce the load of pathogens in the inner polder system. This could also be achieved at the surface water playground by treating the lake water before it enters the playground. Further treatment of the wastewater at the plants that discharge the effluent in the river would reduce the amount of pathogens in this water. In the wadis, the risk could be reduced by removal of faecal input, for example by not placing areas where dogs depositions are allowed in the surroundings, or filtering the water from the roofs' gutters, and, in frequently flooded areas, removing the CSO systems.

2.2. Quantification of Waterborne Pathogens and Associated Health Risks in Urban Water

One of the main conclusions in Chapter 2 was that site-specific data were needed to reduce uncertainties in and confirm the outcome of the screening-level QMRA. Pathogens data were one of the main uncertainties in the models, together with exposure frequency and ingested volume. We considered the uncertainty in the pathogen data larger than the uncertainty in exposure frequency and volume, so collection of pathogen data was the focus of chapter 3. Pathogen site-specific data also help in the risk management process since it provides a higher degree of certainty of the estimated risks and gives information about concentration and sources of pathogens in these waters. Microbial site-specific data were collected in two phases: 1) a weekly monitoring study in the river, lake, sedimentation pond, and pond in the park. 2) a stormwater sampling event in the sedimentation pond and the wadi.

Pathogens analysed were *Campylobacter spp*., *Cryptosporidium*, adenovirus 40/41, *L. pneumophila* and cyanobacteria (cyanochlorophyll-a and microcystin). Adenovirus was selected instead of norovirus for being more prevalent in recreational waters in Europe [48]. *Campylobacter spp.* was found in high concentrations at all locations, being highest at the wadi. *Cryptosporidium* was not found at any location and *L. pneumophila* was found in the sedimentation pond, with higher concentrations in rain event samples. Adenovirus was found only occasionally in two locations and in concentrations close to its limit of quantification (LOQ). Therefore, samples were not further processed for norovirus quantification.

The method used in Chapter 3 for DNA extraction from sample concentrates for molecular quantification of pathogens (q-PCR) showed good recovery efficiency (up to 73 ± 15%) for large volumes of water samples (100 L, resulting in analysis of up to 1.2 L ± 0.4). In the rain water samples, which consisted of lower volumes of water (1 L), and therefore lower volume of sample analysed (up to 43.9 ± 2.5 mL), the recovery was also good (up to 42.2 ± 6.1%). Both the volumes and recovery efficiency showed a considerable variability, depending most likely on the observed turbidity of the samples, although this was not monitored. Furthermore, the recovery of the concentration steps was not assessed

individually for every sample during the experiments and for specific microorganisms, but it had been assessed earlier in our lab [181]. Therefore, further research is recommended to characterize the recovery efficiency for samples for each specific microorganism, and on method improvements to reduce the variability between samples.

Adenovirus data were not used for risk assessment for several reasons: it was found occasionally in two locations (in six samples in the river and four samples in the lake), the concentrations were very close to its LOQ, and the dose-response model available is for adenovirus 4, while we were targeting adenovirus 40/41. However, the data on adenovirus presence in water were used to determine the origin of faecal contamination. Human adenovirus has been proposed as an indicator of human faecal contamination, especially in bathing waters because they are more prevalent than other enteric viruses (e.g., noroviruses) [48, 135].

In samples where adenovirus was found, the *Campylobacter* present was assumed to be from human origin, while in those where it was not found, *Campylobacter* was assumed to be from animal origin. For the animal *Campylobacter*, the fraction of zoonotic bacteria was estimated using literature data [187, 188]. This approach is a source of uncertainty, because the data were gathered from different countries and animals (Sweden for birds and Australia for dogs), and, in the birds study, the samples came from a natural (and not urban) environment. The presence of certain pathogens in humans and animals faeces differs between geographical areas [305]. Furthermore, birds in urban areas might be more infected with human pathogens due to sewage contact (e.g. seagulls in harbour cities) [224]. To overcome this uncertainty, the development of a specific method that detects only human-pathogenic *Campylobacter* (i.e., therrmophilic *Campylobacter*: *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*) [306] or is able to detect specific species [307] is recommended .

Site-specific pathogens data were collected in this chapter to reduce uncertainties from Chapter 2. *Cryptosporidium* was not found at any studied location, but the LOQ of the method was always higher than the concentration assumed in the screening study, so we cannot conclude that the water features were free of the parasite. However, *Cryptosporidium* concentration did not show a high influence on the gastrointestinal risk in any of the studied locations in Chapter 3, so no further research was conducted. *Campylobacter spp.* was found in the river and lake in lower concentrations than expected, while in the sedimentation pond, the pond in the park and the wadi, the concentrations were higher. Norovirus was not investigated in Chapter 3 because adenovirus was found only occasionally and in concentrations close to its LOQ.

Despite the absence of *Cryptosporidium* and norovirus data, the GI risks in the studied locations were higher than in the screening study, due to the high concentrations of *Campylobacter spp.* found. However, data used in the screening study were derived from either culture methods and/or targeting *C. jejuni*, while in the monitoring study we targeted *Campylobacter spp.* and used a q-PCR method. Some of the priorities for site selection were confirmed in the monitoring study: the highest GI risks were found for rowing in the

river and the lowest for walking in the park. The results for playing in the wadi were, on the other hand, unexpectedly high (being the highest together with the river) due to the unexpected high concentrations of *Campylobacter spp.* found.

L. pneumophila was only found in the sedimentation pond in lower concentrations than those used in the screening study. Because at this location aerosolization is not expected (fishing is the only known activity conducted here), the health risks were not investigated. At the other locations, the LOQ of the method was lower than the expected concentration and, hence, the LD risks can be considered negligible. Overall, the monitoring study demonstrated that the *L. pneumophila* data selection conducted in Chapter 2 was too conservative.

Exposure frequency for activities depending on weather conditions (rain for playing in the wadi, high temperatures for swimming in the river and lake) was based on location weather data, instead of on literature from similar locations, increasing the specificity of these studies. The main variability in the models was then either derived from site-specific data (*Campylobacter* for rowing in the river and lake, fishing in the sedimentation pond and walking in the park) or on data gathered from similar locations in the same country of study (volume of water ingestion for swimming in the river and lake and playing in the wadi). The variability regarding the volume of water ingested is inherent to the population and cannot be reduced by further sampling.

In Chapter 2, the concentration of pathogens in the ponds receiving water from the sedimentation pond was considered lower than in the source water due to natural processes (dilution, settlement, inactivation, predation), based on *E. coli* concentrations. In Chapter 3, site-specific data were collected, and different results were obtained regarding this lower concentration. On the one hand, *L. pneumophila* was not present in the pond, while it was in the sedimentation pond. Considering the concentration in the sedimentation pond positive samples and the LOQ in the pond, the difference in concentration would be of, at least, 28%. On the other hand, the difference in *Campylobacter spp.* concentration between the two locations was not statistically significant, indicating that the source of the bacteria were probably the birds located in both ponds (the sedimentation pond and the pond in the park), obscuring any decrease in its concentration (and this was supported by the absence of adenovirus, indicating absence human-faecal contamination).

The wadi sensitivity analysis shows low effect of inputs variability on risk variability. Highest effect is assigned to the ingested volume, but the magnitude of the variation is lower than for the other models (river, lake, sedimentation pond and pond). The concentration of *Campylobacter spp.* used in the wadi was based on four samples belonging to a single rain event, all showing very high concentrations. The fact that only one rain event was investigated results in very low variability in the *Campylobacter spp.* concentration. Further sample collection and analysis would be more representative of the variability between rain events and probably increase the variability on the *Campylobacter spp.* concentration in the wadi.

Recommendations to reduce the gastrointestinal risks on the exposed population are: in the river and lake, which are non-designed bathing waters, advise the citizens on the risk associated with bathing in these waters. Furthermore, the water quality could be improved by additional wastewater treatment (e.g., UV-disinfection) of the effluents that discharge in the river, and by clearance of combined sewer overflows into the river. In the stormwater sedimentation ponds and receiving park water, inform the public that water may be extra contaminated after rainfall events and contact should be avoided. Also, the sedimentation pond could be re-designed to obtain improved particle settlement during rain events. Finally, in the wadi, citizens should also be advised to avoid direct contact with the water, and to prevent animal contamination (e.g., by removing dogs' depositions in the wadi draining area).

2.3. Microbial Health Risks Associated with Exposure to Stormwater in a Water Plaza

In Chapter 3, high GI health risks were found in the wadi, suggesting the need for further study of temporary stormwater storage features. In Chapter 4, the microbial quality and health risks of a waterplaza were studied. *Campylobacter spp.*, *Cryptosporidium*, and *L. pneumophila* were monitored, together with the faecal indicator *E. coli* and FST tools: human *Bacteroides*, avian *Helicobacter*, and canine mitochondrial DNA, to determine the source of contamination.

The exposure assessment model was built using distributions fitted to the pathogens' concentrations and literature data on the different steps. Exposure frequency was based on the amount of rainfall needed to fill up the water plaza to a minimum level, where children can already play in the water. Weather data on rain events in Rotterdam in the past ten years were used for this purpose. In future climate scenarios, however, a higher frequency of extreme rain events is expected [220], and so is the frequency of exposure.

The results show that the *Campylobacter* disease risks for children playing in a water plaza are higher than the annual average for the general population through all exposure pathways. Even though concentrations of *E. coil* were below the level for excellent bathing water in the EU Bathing Water Directive, concentrations of *Campylobacter spp.* (detected by q-PCR) were high in the water plaza. Human, birds and dogs were all identified as faecal contamination sources, but a significant statistical difference was found between the concentration of *Campylobacter spp.* in samples with human *Bacteroides* and those without, indicating human faecal contamination as the major source of *Campylobacter*. The same approach described in Chapter 3 was followed to estimate the amount of zoonotic *Campylobacter* in samples without human *Bacteroides* (from animal origin), resulting in the same uncertainties.

L. pneumophila was found in two samples (and 1 of the first flush samples) resulting in low health risks (below the national incidence). However, the risk could increase under

future climate conditions, when the weather is warmer and multiplication of *L. pneumophila* can occur [17].

Recommendations for decreasing or removing the microbial load and health risks in the water plaza include: cleaning/disinfection of the water plaza after an extreme rain event (e.g. filtration, chlorination of the water); identification and removal of human faecal sources (cross-connection with combined sewers); increasing the capacity and/or the operating time of the first flush pump; regular cleaning of the catchment area and gutters; and informing the neighbours of the importance of keeping the streets clean (e.g., by collecting dogs' depositions). Furthermore, informing the public about the health risks derived from recreational uses of the water plaza after rain events may reduce exposure.

2.4. Health Risks Derived from Consumption of Lettuces Irrigated with Tertiary Effluent Containing Norovirus

The risks derived from consumption of lettuces that have been irrigated with reclaimed water have been assessed in Chapter 5. In Spain, 71% of reclaimed water is used for agricultural irrigation [225], hence consumption of crops irrigated with wastewater can have important public health consequences. While regulation on water quality determines the upper limit of *E. coli* concentration for its use [119], viruses, which are less resistant to water treatment [226], are not monitored. Norovirus is the first cause of gastroenteritis in Spain, and risk assessment studies on norovirus transmission through this pathway are limited for several reasons, including the inability to grow norovirus in culture. This study was the first to use norovirus site-specific data in a risk assessment of crops irrigated with reclaimed water, and the first one to assess the effects of virus internalization into lettuce crops.

The occurrence of internalization under field conditions is still an uncertain factor, but if it is proven, it could have a significant impact on the disease burden and on the control options. More research is needed to better understand and quantify virus internalization into lettuce under field conditions. Decay data of norovirus and surrogates were used to describe the virus inactivation in the field and during transport and storage of lettuce, in contrast with the more commonly used conservative decay model derived from *Bacteroides fragilis* Bacteriophage B40-8. The results showed that, although the tertiary effluent of the target wastewater treatment plant (WWTP) met the *E. coli* requirements of national guidelines, additional norovirus reduction is needed to protect public health. The WHO guideline was used to compute the required level of additional treatment.

No statistical significant difference was found between samples from secondary effluent and tertiary effluent. This could be because the RT-q-PCR technique is not able to differentiate between dead and alive pathogens. Norovirus does not grow in culture, making the detection of infective norovirus particles not possible. Methods to quantify infective norovirus have been investigated and include: the combination of enzymatic treatment with real-time nucleic acid sequence-based amplification [265], the combination of RT-q-PCR

with RNAse treatment [266, 267], the quantitative evaluation of oxidative damages on viral capsid protein [268], or the use of a physiologically relevant three-dimensional organoid model of human small intestinal epithelium [308] and a large intestine tissue model [309]. Richardson, et al. [11] argues that human volunteer studies are necessary to understand norovirus inactivation. However, because the norovirus dose-response model developed by Teunis, et al. [54] used data derived from a challenging study where the doses of norovirus were determined by RT-q-PCR, using this methodology for quantification is appropriate in QMRA.

Several options exist to reduce the norovirus content on lettuces. This reduction can be introduced at different points of the system: the water treatment (changing the water treatment method, adding water treatment steps or improving the existing method by modifying physico-chemical parameters of the wastewater influent), the irrigation practices (subsurface drip irrigation, increasing the time between last irrigation and harvest), the consumers practices (giving advice to disinfect the lettuces with chlorine, to rinse them thoroughly, etc.), or at several steps at a time (a combination of several options). If internalization of norovirus in the field occurs, control options after irrigation will become less effective and more effort will need to be put on pre-irrigation control measures.

At the water treatment level, changing physico-chemical parameters of the influent (e.g., decreasing the water turbidity/UV absorption) would help improving the efficiency of the tertiary treatment. This is a cost-effective measure recommended at any WWTP. If modifying water parameters is not possible, adding another water treatment step to the already existing might be useful.

Another option at the WWTP level would be to use an alternative treatment method. Membrane technologies have been incorporated recently in the wastewater treatment field. Different membrane technologies exist that differ in the pores size of the filter. Reverse osmosis (RO) consist on filtration of effluent through membranes with very small pore size. Compared to other treatment technologies, RO has the main advantages of low energy consumption and high rate of contaminant removal [310]. These methods, however, require pre-treatment, such as ultra-filtration, to minimize membrane fouling.

Reclaimed wastewater treated with reverse osmosis is already in use, for instance, for drinking water production in California. Nanofiltration membranes have shown to reduce FRNA bacteriophages and norovirus effectively. FRNA bacteriophages were reduced in five logs, and norovirus to below their limit of detection (2-4logs) but further research is needed to better characterize norovirus reduction through this method [311].

Adapting the irrigation practices can also reduce the virus load on the lettuces. To begin with, reclaimed water should not be used when the tertiary system is under maintenance (for instance, when the UV-lamps are being cleaned). Increasing the time between last irrigation and harvesting has previously been suggested as an effective measure [163]. If this is not desired by the farmers (because it might change the appearance of the crops), then the irrigation water source could be substituted for a different source during this final

growth period. This will help reducing the virus load because virus on the surface will die due to solar radiation and high midday temperatures, and crops will stop receiving virus load. Using a different irrigation system, e.g. subsurface drip irrigation, will reduce the amount of viruses attached to the lettuce surface, but may not reduce the internalised dose.

At the consumer level, measures include advise to store the lettuce at room temperature (and not in the fridge), to use disinfectant to clean the lettuce, and to rinse it thoroughly with water. These measures are, however, not easy to control (use of disinfectant by consumers might not reach the necessary Ct values to reduce the virus load efficiently). Therefore, managers should not relay on them for health risks reduction.

A combination of measures might be the best, and most cost-effective, way of reducing the virus load. The WHO recommends a combination of measures considering the log reduction on DALYs that each measure achieves [22]. In Chapter 5, results showed that an extra 4.3 log reduction of the virus concentration would be necessary to decrease the DALYs below the guidelines threshold of 10^{-6} pppy. Then, a combination of drip irrigation (reduction of 2logs) with increasing the time between last irrigation and harvesting to one extra day (1 log reduction) and advise of washing salad with disinfectant (2 logs) would be enough to decrease the DALYs below the guidelines threshold [22]. However, if we consider the worst case scenario of high rates of virus internalization, these measures would not be enough, because an extra 2.6 log reduction is needed. A tertiary treatment step should then be added to these measures to reach the safety benchmark.

The studied treatment plant complied with the Spanish regulations for quality of reclaimed water that is used to irrigate crops that are eaten raw with a method that allows direct contact of water with the crop (*E. coli* in tertiary effluent were below 100 cfu/100 mL). Presence of high concentrations of norovirus, however, could not be avoided by implementation of this regulation. European regulations should include virus monitoring and specify virus levels, at least for reclaimed effluents used for irrigation of food crops that are eaten raw.

Because quantifying all possible human virus would not be a cost-effective measure, a virus indicator should be defined. This virus indicator should be representative of the worst case scenario (resistant to environment and water treatment practices), and, for better assessment, would depend on the treatment method. For instance, if tertiary treatment consist of nanofiltration (filtration with membranes of 0.01-0.001 μm pore size), the use of MS2 for monitoring would be a good surrogate, since it is smaller than norovirus (25 nm vs 28-30 nm) [311]. However, it would not be such a good surrogate if chlorine is the disinfection method, since MS2 would die off faster than norovirus.

2.5. Screening-Level Risk Assessment of *Coxiella burnetii* (Q Fever) Transmission via Aeration of Drinking Water

A Q fever outbreak was ongoing in The Netherlands at the beginning of this Thesis work. Because of the magnitude of the outbreak (known as the largest human Q fever outbreak

reported to date), it was thought that the concentration of *C. burnetii* in the air (its main transmission pathway) could be very high. Therefore, it is plausible that the bacteria could be transmitted to groundwater during aeration for drinking water production in drinking water treatment plants located close to contaminated farms. For this reason, the risk of developing Q fever after showering with drinking water that has been aerated with *C. burnetii* contaminated air was assessed in Chapter 6.

A screening-level risk assessment was conducted using literature data, survey data and assumptions, and following a conservative approach. The results indicate that the use of air contaminated with *C. burnetii* to aerate groundwater poses a very low risk of Q fever disease to the population through inhalation of aerosols during showering (3.7×10^{-6} pppy risk of disease compared to the acceptable 10^{-4} pppy risk of infection [120]). The average risk of disease of *C. burnetii* in 2009 in The Netherlands by any transmission route was 1.4×10^{-4} pppy (2,357 cases in a population of 16.5 million). For people living in a 5-km radius around infected goat farms, the risk of disease in 2009 was 7×10^{-4} pppy. These averages have been calculated from symptomatic reported cases [72], and hence might be underestimating the disease incidence.

This study was conducted because of the Q fever outbreak that occurred in the 2007-2012 period in The Netherlands. Limited literature was available at the time, and we had to extrapolate data from other countries, other seasons, and other bacteria. The Q fever outbreak, however, lead to a body of new research on the topic, providing new data that can be used to improve the model.

To estimate the *C. burnetii* concentrations in infected barnyards, we used data derived from the shearing season, and not from the kidding season, when higher *C. burnetti* cells are shed into the environment through the placentas and birthing fluids [65]. Furthermore, qualitative PCR, indicating presence/absence and not quantity, was used in that study [281], and we made a rough estimation on the probable concentration of *C. burnetii* in the barnyard air, knowing the detection limit of the method. At that time, the most used PCR assay for *C. burnetii* detection was targeting the multi-copy gene IS111, which is repeated between 20 to 200 times in a single *C. burnetii* cell and, hence, did not allow for quantification of the pathogen. Recent research has led to the design of a new q-PCR assay targeting the gene *com1*, a single copy gene in *C. burnetii*'s genome [312]. The same authors used this method to estimate the concentrations of *C. burnetii* in the environment and in ruminant farms [313, 314].

Briefly, barnyard air (500 L) was sampled with Sartorius MD8 Airport Device with cellulose nitrate filters of 8 µm pore size. Filters were transferred to sterile petri dishes and stored at -20 ºC. 10 mL of lysis buffer were added to the filter, and the sampled cells were extracted from it by shaking during 6 h at 50 rpm. DNA extraction was done with Nuclisens magnetic extraction kit. According to manufacturer's instructions, the DNA is extracted into 10 µL of extraction buffer/water. Three µL of the DNA solution and 10 fold dilutions were used for analysis with q-PCR.

The results are not expressed in genomic copies, but on the amplification cycle at which the PCR emitted enough fluorescence to be detected (Cq). Because the publication does not provide a regression line, it is not possible to extrapolate the number of genomic copies. However, a rough estimate has been done using the limit of detection of the method (10.6 copies/reaction) and the highest Cq reported (38.7 cycles) and assuming that they are equivalent. Then, we extrapolated the highest and the lowest Cq found in the barnyard (highest Cq represent the lowest concentration, and lowest Cq the highest concentration). To calculate the higher and lower extremes of possible concentration of *C .burnetii* in the air, we assumed that the highest concentration had been derived from a 10 fold dilution of the sample used for the q-PCR reaction, and the lowest concentration from the use of the direct sample.

Consequently, we roughly estimated a maximum concentration of *C. burnetii* in barnyard air of 10,320 cells/m^3 (1.1 log higher than the 880 cells/m^3 used in our study) and a minimum of 745 cells/m^3 (close to our best estimate and 2logs higher than our alternative scenario value of 8.35 cells/m^3). The actual measured concentrations will probably fall between this range. Our best estimate in Chapter 6 falls between these two values, although it is closer to the lowest one. This supports our hypothesis that the concentration of the pathogen in the air in Dutch barnyards was probably higher due to the magnitude of the outbreak and because the used data in the best case scenario were derived from the shearing season and not from the kidding season, when higher *C. burnetii* cells are shed into the environment through the placentas and birthing fluids [65]. The use of the highest estimated concentration into the model in chapter 6 would result in a one log higher probability of disease respect to the previously estimated, still below the 10^{-4} probability of infection level that is considered tolerable (for enteric pathogens via drinking water), but again, with the distinction that we are estimating disease, and not infection, and that Q fever is a much serious disease than gastroenteritis.

Another interesting outcome of the Q fever research is the new available human dose-response relationship for *C. burnetii* infection and a dose-dependent illness curve [105]. The new infection model follows a hypergeometric dose-response curve, with parameters $\alpha = 0.23$ and $\beta = 0.18$. The parameters of the dose-dependent illness curve are $\eta = 0.88$ and $\rho = 6.88$. These models are less conservative than the exponential model used in our study, leading to lower estimates of risk.

Despite the low estimates of risk obtained in this assessment, groundwater treatment plants are recommended to filter the air with HEPA filters to avoid potential contamination of drinking water with airborne pathogens.

3. General Discussion, Conclusions and Recommendations for Future Research

The microbial health risks of several urban water concepts have been assessed. Specifically, exposure through recreation at several water features from an urban area (including a river, lake, a sedimentation pond, a swimming pool, canals and ponds, a wadi, a decorative fountain, flooded streets in combined and separate overflows…), and in a water plaza. Furthermore, the risks derived from consumption of lettuces irrigated with reclaimed wastewater, and showering in water that is aerated with *C. burnetii* contaminated air, have also been assessed.

A QMRA approach has been used, which is very versatile. It allows for different levels of detail, depending on the scope, objectives, and available data. Chapter 6 used a deterministic approach, following a conservative approach and using an alternative value in each step of the model to determine the effect of uncertainties. In the rest of the Thesis, a stochastic approach was followed. In chapter 2, a qualitative assessment was conducted as a previous step to the screening-level risk assessment (see appendix A.1), which was then improved with site-specific data in chapter 3.

The versatility of the QMRA process also resides on its ability for assessing various water features and various microbial hazards. In Chapters 4, 5 and 6, we estimated the risks derived from exposure to one water system and/or one microbial hazard, as done previously in other QMRA studies. In Chapters 2 and 3, we conducted an holistic research, analysing the risks derived from exposure to various water features, and different pathogens and diseases.

3.1. Hazard Identification

Hazard identification is usually the first step of QMRA and consists on identifying the potential hazards in the studied systems. The ideal in health impact assessment would be to estimate the risks posed by every single waterborne pathogen. This is not feasible because it would be very costly, and so a selection has to be made. The rationale for selection in this Thesis included the coverage of different pathogens classes and diseases, and epidemiological evidence on their prevalence/incidence among the population of the studied area.

The selection was also specific for each water feature. In Chapters 2 and 3, pathogens from the three main different groups were selected. In the screening-level risk assessment, while five different pathogens were targeted, the swimming pool was assumed to contain only *Cryptosporidium* (because it can resist residual chlorine in the swimming pool if reintroduced by bathers) and drinking water features were assumed to contain only *L. pneumophila* (because it can grow in engineered water systems). In Chapter 3, cyanobacteria were not analysed in the wadi, since it is a temporary reservoir of

stormwater, and norovirus was not considered in any location after adenovirus was found only occasionally and in low concentrations.

In some instances, the assumptions made for pathogen selection, based on specific characteristics of the studied system, turned out to be incorrect. This was the case in the water plaza (Chapter 4), where human pathogenic viruses were not included because their presence was not expected in separate sewers (and they were not found in rain water features in chapter 3). However, after finding human *Bacteroides* in several samples, indicating human faecal contamination of the water plaza, recommendations for future research include analysis of adenovirus and/or norovius.

Cryptosporidium was not found in any location in Chapters 3 and 4. *Cryptosporidium* was preferred for monitoring over *Giardia* because the former is associated more frequently with recreational water outbreaks [42], because, due to its size, *Cryptosporidium* is more difficult to remove from water by physical treatment, it is resistant to oxidizing disinfectants, and it survives longer than *Giardia* in environmental waters [31]. *Cryptosporidium* and *Giardia* were monitored in urban floodwater [81] and in urban surface waters [136] in The Netherlands. In urban floodwater, the concentration and frequency of positive samples was usually similar for both pathogens [81]. This indicates that monitoring *Giardia* instead of *Cryptosporidium* in our stormwater locations would have probably not resulted in different findings.

In surface waters, however, *Giardia* was isolated more frequently than *Cryptosporidium* in the river and lake, and the concentration was around 1 log higher [136]. This indicates that *Giardia* could have been present in our samples. However, it is unlikely that this would have affected the estimated health risks, which were dominated by *Campylobacter*. This is supported by the high *Campylobacter* concentrations found and by the *Giardia* dose-response model [315], resulting in lower health risks, at low dose, than the *Campylobacter* model [24].

Norovirus was the only pathogen selected to estimate the health risks from crop irrigation with reclaimed water in Chapter 5. The rationale for this was that viruses are human specific pathogens, they are found in sewage water in high concentrations [51, 238, 239, 316], and previous studies showed low removal of phages by tertiary treatment [226]. Adenovirus was not selected, although it is found in high concentrations in sewage [189, 317] and it is less sensitive to UV treatment [251], because it shows higher sensitivity to chlorine treatment [318] and because a specific dose-response model for enteric adenovirus does not exist.

Other pathogens could be selected for QMRA studies based on the same rationale, for instance, if the studies are conducted in a different country or geographical area where other pathogens have a high prevalence in water (e.g Hepatitis A in South-East Asia), if an outbreak of a specific pathogen occurs in the study area (e.g. the 2011 *E. coli* O104:H4 outbreak in lettuces in Germany), if epidemiological or microbiological data indicate a high prevalence of emerging pathogens or diseases (e.g. human polyomavirus), or new drug

resistant strains of a pathogen are increasingly reported in waters (e.g. multidrug resistant pathogens in hospitals can be transmitted through the water). The methodology used in this thesis can then be adapted to these circumstances to estimate the health risks derived from other pathogens.

3.2. Exposure Assessment

In the exposure assessment process, data are needed to estimate the exposure dose. This includes: 1. pathogen concentrations in source water. 2. processes that have an effect (positive or negative) on this concentration. 3. human behaviour that results in contact with water and the magnitude of this contact (ingested volume, inhaled air).

Data collection

Regarding the concentration of pathogens in water, it has been either collected from literature on similar locations or it has been collected on site. In Chapters 2 and 6, concentration of pathogens in water and of *C. burnetii* in the air of a barnyard was based on literature data. For the urban water sites considered in Chapter 2, data were selected following prioritization steps, with increasing uncertainty of the data: 1. data collected from the same feature investigated, 2. data collected from similar features in The Netherlands, and 3. data collected from similar features in other countries. The latter, and more uncertain, was only used for *L. pneumophila* concentration in the CSO. Few data were obtained from the very same features, and these were *Crytposporidium* concentrations in the studied river and lake. For the rest of pathogens and sites, national data were used, with consequent uncertainties.

In Chapters 3, 4 and 5, site-specific pathogen data collection was conducted. In Chapter 3, sampling selected sites helped improving the risk assessment and reducing the uncertainties. Differences in concentration were found between the monitoring and screening study that have been discussed earlier. The differences resulted, sometimes, in higher gastrointestinal risks than those derived from the screening study. The LD risks were, however, negligible. There are several reasons that explain these differences: the method used, the studied locations, the geographical area of the study, the underlying population disease, the season, the natural variability of the samples, etc. Anyway, this study demonstrated the need of site-specific data collection for more accurate risk assessment.

Although data collection from site-specific water samples helped improving the models and reducing uncertainties, fitting distributions to the data demonstrated that a higher number of samples was needed. Usually, the fit of two commonly used distributions for fitting pathogen concentrations in water (gamma and lognormal) was compared, but the goodness-of-fit methods failed to determine which of the two gave a better fit. This indicates that the amount of data points is not enough to distinguish between the two distribution shapes and more data should be gathered. Because this was not feasible, the

distribution with slightly better fit was selected, and the risk estimate was compared with the outcome when using the rejected distribution. This resulted in statistical significant differences that were, however, small and did not affect the conclusions of the study. Using a higher number of samples is recommended for future research.

Molecular methods

When site-specific data have been gathered, in Chapters 3, 4 and 5, molecular methods have been used to quantify pathogens. These methods do not discern between dead and alive (infective) pathogens, which can result in an overestimation of the concentration of pathogens and, hence, the resulting health risks. This is an important factor specially for *Campylobacter* risk, since *Campylobacter* are very sensitive to adverse environmental conditions (UV light, high temperatures) and die rapidly after being excreted [33]. However, the alternative culture methods do not take into consideration the viable but non culturable (VBNC) microorganisms, underestimating the pathogen concentrations and health risks. Therefore, under these circumstances, the choice was to follow the most conservative approach and q-PCR techniques were used. Furthermore, for some pathogens (norovirus), culture techniques do not exist at the moment. Future research should be conducted using viable q-PCR [266, 319, 320].

As stated earlier, the method used for *Campylobacter* quantification is not species specific. Although FST tools have been used to determine the origin of contamination, assumptions based on data from other countries and geographical areas have been made to estimate the concentration of pathogenic *Campylobacter* in the water, and this is a source of uncertainty. In Chapter 5, norovirus was determined with RT-q-PCR, quantifying NoVGI and NoVGII separately. Data from both genogroups were used in the QMRA model because, although NoVGII is the most common genogroup found in infected humans, NoVGI has also been isolated from infected humans. However, further attention should be given to this matter, to determine if NoVGI has the same infectivity and virulence as NoVGII.

Pathogen concentrations

As previously stated, site-specific quantification of pathogens reduces uncertainties in QMRA and allows for a more specific evaluation of the health risks. Concentrations of pathogens are variable between and within water systems. The following has been observed in the studied systems:

- *Campylobacter spp.* was always found in all studied systems (the river, the lake, the sedimentation pond, the pond in the park, the wadi, and the water plaza) and the concentration was, usually, high.
- *Cryptosporidium* was not found in any of the analysed locations, but the LOQ of the method was above the expected concentration, and the method was specific for *C. parvum* and *C. hominis*.

- Norovirus was found in high concentrations in reclaimed water in both secondary and tertiary effluents.
- Adenovirus was found occasionally and in low concentrations in two of the five investigated locations (the river and the lake).
- *L. pneumophila* was found only in stormwater harvesting features (the sedimentation pond and the water plaza, but not in the wadi) and always in low concentrations.

Exposure assumptions

Other information needed for exposure assessment, after the concentrations of pathogens in water source, are the exposure pattern of the population (volumes of water ingested accidentally, breathing rates, time spent at the location, etc.), and the changes in pathogen load in the water (due to dilution, predation, disinfection, multiplication, etc.) or pathogen aerosolization. Literature data (Chapters 2, 3, 4) or data from surveys, questionnaires and measurements conducted by official institutes (Chapters 5 and 6), were used to gather information on the population exposure patterns. However, sometimes data were not available at some points of the exposure models and then assumptions were made, following a conservative approach. These assumptions influence the outputs of the model and should be considered by the risk managers.

For the changes in pathogen load or aerosolization, the literature was reviewed. Concentrations of *L. pneumophila* have been measured in the water and a water to air ratio has been used to determine the inhaled dose. An alternative (and more direct) approach would be to measure the bacteria concentration in the air. However, *L. pneumophila* is very sensitive to airborne sampling methods, and its recovery tends to be low with the long sampling times required at locations where low concentrations of the bacteria are expected in the air [321], as is the case of many urban water features.

For norovirus in reclaimed water, the concentration in both secondary and tertiary effluent was measured. However, no significant difference between the two was found, probably because the RT-q-PCR method used for quantification is not able to distinguish between active and inactive virus particles. Therefore, literature data were used to estimate the virus load reduction by tertiary treatment. Since techniques to determine norovirus infective particles are very recent, data from surrogate viruses had to be used for this purpose. Surrogate viruses were also used to estimate the inactivation of virus in the field.

3.3. Hazard Characterization

To determine the magnitude of the effect of a certain microbial dose on the host, dose-response models have been used. When different models were available in the literature, the choice was for the most conservative one. We could be, therefore, overestimating risks. Assumptions an limitations of the dose-response models are listed here:

- The models based on human challenging studies (*Cryptosporidium*, norovirus and *C. burnetii*) use healthy adult volunteers and, hence, do not cover the immunocompromised population, children and elderly (this is not the case of the *Campylobacter* study, where outbreak data from mostly children were used to derive the dose-response).
- Low numbers of subjects often participate in the volunteer experiments the dose-response models are derived from.
- When using the dose-response models, it is assumed that the microorganisms used in the dose-challenging studies have the same virulence as the ones found in the studied water features.
- Development of immunity and thus protection against re-infection, is not considered. Studies on *Campylobacter* and *Cryptsoporidium* suggest immune protection of adults to infection [24, 41].
- The *Campylobacter* dose-response model was derived from a combination of a feeding study with human volunteers and outbreak data from two studies where most of affected people were children and the dose was unknown (assumptions were made to estimate the dose). Because of the children involved in the outbreak studies, and because it was derived from an outbreak, this is a conservative model.
- The dose in the *Campylobacter* and *L. pneumophila* dose-response studies was given in cells and cfu, respectively, but we have used q-PCR data. We made the assumption that one genomic copy is equivalent to one *Campylobacter* or one *L. pneumophila* cfu. This assumption has been previously made in other studies [137] and is widely accepted. However, we do not know the fraction of infective units in our samples, which are probably lower than the total amount of bacteria, and are, therefore, overestimating the dose.
- The *Campylobacter* model has been derived from a combination of *C. jejuni* strains, while we are detecting *Campylobacter spp*. Human pathogenic *Campylobacter spp.* other than *C. jejuni* might have a different dose-response relationship, but no studies have been done in species different than *C. jejuni*.
- The L. *pneumophila* model has been derived from dose-challenging studies in guinea pig models. The extrapolation from guinea pig to humans was assumed straight-forward because no evidence was found supporting a greater or lower susceptibility of humans compared to guinea pigs [106]. Specifically, similar growth rates at similar dose levels and similar protease productions were observed in isolated guinea pigs and human alveolar macrophages, and *Legionella* counts increased similarly in both species' macrophages. Furthermore, similar deposition patterns in pulmonary regions for 5 μm particles in guinea pigs versus human systems have been observed. The particle clearance half-lives are also in the same range for both species. The animal model was validated with human spa outbreak

data (from one whirlpool spa and two natural hot springs). The dose in the outbreaks, however, was not known and was estimated using general range of air concentrations reported for *Legionella* in air near showers and aerated faucets supplied by *Legionella* contaminated water.

- Also in the *L. pneumophila* dose-response model, the assumption was made that the infection probability is not dependent on the total lung surface area or inhalation volume, and thus does not scale with body weight, or lung volume. This is based on the *L. pneumophila* mechanism of action for macrophages infection and disease production.
- In the norovirus dose-response model, we are assuming no aggregation of the virus inocula. However, the difference between using aggregated and non-aggregated model was not significant.
- The norovirus dose-response study used challenging data on human volunteers. Those volunteers belonging to the ABH histoblood non-secretor group, which are not susceptible to the virus because the virus cannot bind to their cells and infect them, were excluded from the study [54]. When applying the dose-response model to the general population, we are not considering the fraction of the population that are resistant to the virus and, hence, we are overestimating infection and disease.
- At the moment of the *C. burnetii* study, no dose-response had been published for this pathogen that used human data. The use of a conservative dose-response based on Jones, et al. [69] data resulted in overestimation of the results, as argued in section 2.5.

3.4. Risk Characterization

The risk characterization is the last step of the QMRA process and consists on combining the information gathered in the previous steps to derive an estimate of risk.

Deterministic versus stochastic approach

As stated earlier, the different scenarios studied in this dissertation differ on the level of complexity. QMRA can be both stochastic or deterministic. Deterministic QMRA is performed when not much data is available, or as a preliminary step, i.e. screening-level QMRA (since it is less demanding in terms of data input and computational effort), to determine if it is necessary to conduct more complex QMRA.

We have used the stochastic approach in all models except for the Q fever model, in Chapter 6. The objective of this study was to perform a screening-level QMRA as a preliminary step to building a more complex model if the evidence suggested so. Furthermore, the lack of available data at the point of that study supported the use of a less complex approach. Although new evidence has emerged since the performance of that study, the results obtained suggest that the risk of Q fever through drinking water is very low. Moreover, since a conservative approach was followed, conducting a stochastic

analysis would only result in even lower estimates of risk (see sensitivity analysis, figure 6-2).

Gastrointestinal versus respiratory risk

In general, the results presented here show that gastrointestinal pathogens (*Campylobacter* and norovirus) are found in urban waters in high concentrations resulting in high risks (above the guidelines thresholds and/or annual incidence) for the people exposed through recreation or through consumption of fresh produce. On the other hand, exposure to water contaminated with respiratory pathogens (*L. pneumophila* and *C. burnetii*) resulted in low risks, either through recreation or household exposure. Lower risks of respiratory diseases are the result of low pathogens doses in source water, as compared to gastrointestinal pathogens, and due to the aerosolization step, that reduces considerably the pathogens dose. However, effects of respiratory pathogens are more serious than the effects of gastrointestinal pathogens, at least when considering the acute symptoms. To better compare the magnitude of the health risks, DALYs should be used, as has been done for norovirus in Chapter 5. This was not possible for *L. pneumophila* and *C. burnetii* because of lack of data that are necessary to calculate the DALYs. Further research is needed to be able to conduct this assessment.

The high campylobacteriosis risks can also be due to the dose-response model used, derived from a combination of a human challenging study and children outbreak data [24]. Another dose-response model is available, derived from a volunteer feeding study [104]. The use of this dose-response model for adults, would result in risks 1log lower than those estimated. However, for those activities were children are the main exposed group, the use of the hypergeometric model from Teunis, et al. [24] is recommended.

Sensitivity Analysis

A sensitivity analysis was conducted in each chapter to assess the influence of each model input on the model output (i.e., the health risks). The sensitivity analysis showed the following: pathogen concentrations were the main responsible input factor for risk variability for rowing in the river (in the screening and water quality study) and lake (in the water quality study), fishing in the sedimentation pond, walking in the park, swimming in the swimming pool, playing in surface water playgrounds, all aerosol exposure pathways in the *L. pneumophila* models, and ingestion of crops irrigated with reclaimed water. Ingestion volume was the main factor for swimming in the river and lake (in the water monitoring study), playing in the wadi (in the water monitoring study), swimming in the pond, and playing in the water plaza. Exposure frequency was the main factor for rowing in the lake (in the screening study), swimming in the river and lake (in the screening study), walking along the traffic road, playing in flooded streets in CSO and SSO systems and in the wadi (in the screening study). Finally, the air filtration efficiency was the uncertainty with higher effect on the risk of Q fever through drinking water.

To reduce uncertainties regarding the concentration of pathogens in Chapter 2, site-specific data collection was conducted in Chapter 3. Furthermore, the exposure frequency for swimming in the river and the lake and playing in the wadi, were the main responsible factors for risk variability in these models and were retrieved from literature on similar locations in Chapter 2. In Chapter 3, a different approach was followed consisting on collecting information on weather factors that influence exposure at those locations. Assumptions were made to determine the shape of the probability distributions.

QMRA model outputs are always susceptible of variability and uncertainty. Risk managers need to consider not only the risk output, but the variability and uncertainty associated to it, in order to undertake opportune measures to reduce the risks. Variability and uncertainty can be reduced when new information is available, or new methods are developed that allow for reduction of uncertainty in (site-specific) data collection. Therefore, QMRA models need to be flexible enough to be modified with new information and adapted to up-to-date knowledge. This work has shown the flexibility of these models in Chapter 3, using site-specific data for describing pathogen concentrations and weather information for exposure events. Furthermore, the Q fever risk assessment model shown in Chapter 6 has been analysed with new data derived from the outbreak studies, including measured air concentration of *C. burnetii* in barnyard air and a new dose-response model.

3.5. Risk Management

Reference level of health risk

The health risks estimated in this thesis have been compared with reference values (10^{-4} infections pppy, 10^{-6} DALYs pppy), and/or with national incidence data. For annual probability of disease, no references exist, except for the DALYs, but these are not always possible/easy to estimate. Bathing waters rely on levels of indicators established in the bathing water directive, to determine if the water quality is excellent, good, or enough for bathing. However, no guidelines exist for recreational activities other than bathing and, in this thesis, correlations were not found between *E. coli* and *Campylobacter* or adenovirus in urban waters, and concentrations of *E. coli* below the safety guidelines did not result in negligible gastrointestinal disease risks (chapter 4 and 5). Other works also did not find an association between faecal indicators and pathogens [33, 322, 323].

Directives should not rely on faecal indicators but, rather, on actual pathogens or on FST tools. Furthermore, the quantification of several pathogens/indicators is recommended. Adenovirus has been shown to be a good indicator of human faecal contamination and so is human *Bacteroides*. But because not only human faecal contamination is source of human pathogens, other microorganisms should be included.

Campylobacter is a good indicator of recent faecal contamination because it dies rapidly. *Campylobacter* quantification at the species level or, at least, quantification of thermotolerant groups, would be a good indicator of human-pathogenic faecal bacteria, but a method should be used that distinguish dead from alive (infective) bacteria, such as those

discussed earlier. On the other hand, *Cryptosporidium* is a good indicator of less recent contamination because it can survive for long periods in the environment and can resist adverse environmental conditions. Furthermore, if risk of aerosolization exist at the studied urban water location, concentration of *L. pneumophila* should also be monitored.

Use of assumptions and transparency

The use of assumptions in QMRA studies is often necessary in specific steps of the risk assessment process where no (site-specific) scientific evidence exists. Many of the assumptions made in this dissertation are very common and their use is wide spread in the scientific literature, although not always explicitly identified. In Chapter 2, an effort was made to state them explicitly (see annex A.4), to create transparency and clarity in the study, and the recommendations for "good QMRA practice" of the US Environmental Protection Agency [154, 155] were followed. In other chapters, the assumptions are identified in the methods section, and their validity is discussed if considered necessary.

When assumptions were needed, a conservative approach was followed, in order to ensure public health safety, unless evidence indicated otherwise. These assumptions influence the risk estimates and should be considered by risk managers. In the absence of site-specific information, site-specific research is needed, and recommended, to confirm (or disprove) their validity.

3.6. Recommendations

Recommendations for risk management have been discussed for each specific water feature. Recommendations for future research include:

- Use of site-specific data is recommended for any QMRA study because it reduces uncertainties and it helps in the risk management process increasing the certainty of the results.
- Furthermore, conduct site-specific sampling for, at least, two (consecutive) summer periods to account for temporal variability. This will also result in a higher number of sampling points that might help in distribution fitting. Expand the study in the wadi and in the water plaza during real rain events.
- In the sedimentation pond, study the inlet also during non-rain events to determine its particle settlement efficiency.
- Use of a quantitative method that allows for distinction between different *Campylobacter* species in order to have more specific information on human pathogenic *Campylobacter*.
- Use of a quantitative method able to distinguish between dead and alive pathogens (e.g. viable q-PCR).
- Quantification of *L. pneumophila* (and other airborne pathogens) concentration in the air at those water features where exposure to aerosols exist.

- Study the population behaviour for those activities and locations where ingestion volume and/or exposure frequency have been identified as important factors for risk variability and where it has not been previously investigated, such as wadis and water plazas. For this purpose, questionnaire's and/or observations have been successfully used in the past. For the water plaza, observation studies can be done remotely when live-streams are available.

Appendix A: Screening-level Microbial Risk Assessment of Urban Water Locations: a Tool for Prioritization

A.1. Expert Judgment

Several locations were initially proposed for a HIA (Table A.1). For a first-step selection, the health risks considered are gastrointestinal illnesses (caused by gastrointestinal bacteria, virus, and protozoa), respiratory illnesses (caused by bacteria), and skin diseases (caused by cyanotoxins). Gastrointestinal illness is developed after colonization and infection of the gastrointestinal tract by microorganisms swallowed during high-contact activities or low-contact activities with droplet/aerosol generation. Respiratory illness can be acquired during activities in which aerosol generation is involved, and the activity is conducted close to the aerosol source. Skin diseases are derived from skin contact with toxins that are present in the water, and can be also due to high-contact recreation or contact with droplets/aerosols.

A semi-quantitative expert judgement assessment was made on the microbial water quality and the degree of human exposure. The expert team was composed of professionals of the water quality sector, people with knowledge on the water uses in the area, and members of health authorities with knowledge in microbial health effects. A score was given describing the water quality and degree of exposure at every location (Table A.1). Selection of locations for the second stage of the assessment was not only based on the total score but also on the relation to climate change. The selected water features for the second stage of the study were:

- Local storage of stormwater run-off in wadi's
- Urban green/blue area, with temporary storage of stormwater from separated sewers in ponds and ditches (Julianabak, Julianavijver, Frankendael)
- Urban water recreation areas: water playground (Jeugland), water fountain (Hogeweg) and local surface water used for recreation (Amstel, Nieuwe Diep, etc.)
- Water on the street during rain events (Galilei Plantsoen, Tuindorp, Mr. Treublaan).

Those locations with same or similar water quality and same or similar activities, were analysed as one. For example, the river was considered representative of the Weespertrekvaart and the separate sewer overflow (SSO) flooded street of the wadi. For the LD inhalation models, playing in the freshwater playground was considered representative of swimming in the river and the lake.

Table A−1: Water sources and exposure scenarios from the locations included in the screening-level risk assessment study.

Location	Water quality			Exposure routes		Total Score	Risk Level
	Description	Hazards	Score	Description	Score		
Wespertreekvaart (canal)	Surface water	GI, LD, CT	3	Swimming hot days	3	9	High
River Amstel (river)	Surface water	GI, LD, CT	3	Rowing. Swimming hot days	2-3	6-9	High
Nieuwe Diep Lake (lake)	Surface water	GI, LD, CT	3	Rowing. Swimming in hot days.	2-3	6-9	High
Galileiplantsoen (CSO)	Combined sewer system overflow (CSO)	GI, LD	3	Children playing	2	6	High
Julianapond (green area pond)	Sedimentated rain water + Surface water	GI, LD, CT	2	Fishing. Swimming in hot days.	1-3	2-6	Low-high
Jeugdland 2 (surface water playground)	Surface water + Drinking water manly-influenced	GI, LD	3	Children playing	2	6	High
Tuindorp (SSO in residential area)	Rain water overflow (separate system)	GI, LD	2	Children playing	2	4	Moderate
Wadi	Rain water overflow (separate system)	GI, LD	2	Children playing in dry and wet wadi	2	4	Moderate
Badbuiten (Swimming Pool)	Chlorinated water	GI	1	Swimming	3	3	Moderate
Public water taps	Drinking water	LD	1	Drinking	3	3	Moderate
Julianabak (sedimentation pond)	Rain water overflow (separate system)	GI, LD	2	Fishing.	1	2	Low
Hogeweg (ornamental fountain)	Drinking water	LD	1	Children playing	2	2	Low
Jeugdland 1 (drinking water playground)	Drinking water	LD	1	Young children playing	2	2	Low
Mr Treublaan street (SSO in a traffic road)	Rain water overflow (separate system)	GI, LD	2	Pedestrian and cyclists splashed by cars	1	2	Low

Table A−1:Continued.

Location	Water quality			Exposure routes		Total Score	Risk Level
	Description	Hazards	Score	Description	Score		
Pond around Park Frankendael (pond)	Sedimented rain water + Surface water	GI, LD, CT	2	Aerosols from dogs shaking water after swimming	1	2	Low
Ring of canals (ring)	Canal receiving water from AM	GI, LD	3	No direct contact, no eaerosols	0	0	Low
Water playground Radioweg	No information	?		No information		?	?
Ice skating court	No information	?		Ice skating, insignificant water exposure, no climate link	0	0	Low
City vegetable gardens	No information on the water source	?		Irrigation of plants and crops by the neighbours	1	?	Low

GI: Gastrointestinal illness; LD: Legionnair's disease; CT: Cyanotoxicity; CSO: combined sewer overflow; SSO, separate sewer overflow

Figure A−1: The Watergraafsmeer polder with the locations selected for the screening-level QMRA. CSO, combined sewer overflow; SSO, separate sewer overflow. Source: Waternet.

A.2. Results

Table A−2:Mean (95th percentile) probability of gastrointestinal infection and disease per event and per year.

	Infection (%)		Disease (%)	
	Event	**Annual**	**Event**	**Annual**
Rowing River	35 (71)	94 (100)	13 (32)	84 (100)
Swimming River	46 (82)	71 (100)	18 (44)	52 (100)
Swimming pool	0.06 (0.2)	1.0 (4.1)	0.03 (0.1)	0.5 (2.1)
Park	0.2 (0.8)	0.5 (2.1)	0.1 (0.5)	0.3 (1.2)
CSO	55 (75)	61 (100)	34 (44)	47 (95)
Sedimentation pond	2.1 (5.2)	9.1 (24)	1.2 (3.1)	5.3 (15)
Surface water playground	11 (47)	19 (77)	3.7 (15)	8.0 (34)
Fishing green area pond	0.3 (1.0)	1.4 (4.9)	0.2 (0.6)	0.8 (2.7)
Swimming green area pond	2.7 (12)	13 (65)	1.5 (6.6)	8.6 (43)
Traffic road	0.04 (0.1)	0.1 (0.4)	0.02 (0.07)	0.05 (0.2)
Rowing lake	13 (52)	35 (98)	4.5 (18)	18 (64)
Swimming lake	24 (70)	51 (100)	8.7 (24)	33 (97)
SSO	8.6 (24)	16 (58)	4.7 (14)	9.4 (37)

Table A–3: Mean (95th percentile) probability of L. pneumophila infection and disease per event and per year at the subpopulation level.

	Infection (%)		Disease (%)	
	Event	**Annual**	**Event**	**Annual**
Rowing River	0.01 (0.04)	0.4 (1.9)	1.3×10^{-5} (6.6×10^{-5})	7.2×10^{-4} (3.4×10^{-3})
Rowing River Influenced	0.7 (3.4)	9.7 (83)	1.4×10^{-3} (0.01)	0.07 (0.3)
Park	5.2×10^{-5} (1.6×10^{-4})	1.2×10^{-4} (3.3×10^{-4})	9.2×10^{-8} (2.9×10^{-7})	2.1×10^{-7} (5.9×10^{-7})
CSO	20 (100)	18 (100)	1.0 (4.7)	1.7 (8.2)
Ornamental Fountain	0.04 (0.2)	0.09 (0.4)	6.3×10^{-5} (3.2×10^{-4})	1.5×10^{-4} (6.6×10^{-4})
Drinking water playground	0.03 (0.2)	0.08 (0.3)	5.9×10^{-5} (2.7×10^{-4})	1.4×10^{-4} (6×10^{-4})
Surface water playground	1.8×10^{-3} (8.2×10^{-3})	4.2×10^{-3} (0.02)	3.1×10^{-6} (1.5×10^{-5})	7.6×10^{-6} (3.5×10^{-5})
Surface water playground influenced	0.2 (0.9)	0.5 (1.8)	3.4×10^{-4} (1.6×10^{-3})	9.4×10^{-4} (3.2×10^{-3})
Traffic road	9.2×10^{-6} (4.3×10^{-5})	2.2×10^{-5} (6.7×10^{-5})	1.7×10^{-8} (7.6×10^{-8})	3.9×10^{-8} (1.2×10^{-7})
Rowing lake	0.01 (0.03)	0.03 (0.2)	1.2×10^{-5} (5.8×10^{-5})	6×10^{-5} (2.6×10^{-4})
Rowing lake influenced	0.7 (3.7)	2.7 (17)	1.4×10^{-3} (0.01)	0.01 (0.03)
SSO	5.9×10^{-3} (0.03)	0.01 (0.03)	1.1×10^{-5} (4.4×10^{-5})	2.6×10^{-5} (6×10^{-7})
Public taps	8.7×10^{-5} (4.3×10^{-4})	2.1×10^{-4} (9.7×10^{-4})	1.6×10^{-7} (7.6×10^{-7})	3.7×10^{-7} (1.7×10^{-6})

A.3. Sensitivity Analysis

Table A−4: Sensitivity analysis: Spearman rank correlation coefficients (p-value) for GI event risks.

	μ_w *crypto-sporidium*	μ_w *campy-lobacter*	μ_w *norovirus*	**Dilution**	**V**	**T**
River (rowing)	0.02 (0.1256)	0.86 (<0.0001)	0.20 (<0.0001)	-	0.31 (<0.0001)	0.17 (<0.0001)
River (swimming)	-0.01 (0.6527)	0.57 (<0001)	0.20 (<0.0001)	-	0.72 (<0.0001)	-
Swimming pool	0.95 (<0.0001)	-	-	-	0.25 (<0.0001)	-
Park	-0.005 (0.6205)	0.19 (<0.0001)	0.41 (<0.0001)	-0.71 (<0.0001)	0.40 (<0.0001)	0.21 (<0.0001)
CSO	-0.001 (0.8681)	0.33 (<0.0001)	0.26 (<0.0001)	-	0.87 (<0.0001)	-
Sedimentation pond	0.01 (0.2011)	0.30 (<0.0001)	0.67 (<0.0001)	-	0.59 (<0.0001)	-
Surface water playground	0.003 (0.8019)	0.88 (<0.0001)	0.08 (<0.0001)	-	0.37 (<0.0001)	0.20 (<0.0001)
Pond in green area (fishing)	0.01 (0.5072)	0.19 (<0.0001)	0.43 (<0.0001)	-0.75 (<0.0001)	0.38 (<0.0001)	-
Pond in green area (swimming)	-0.01 (0.5006)	0.13 (<0.0001)	0.28 (<0.0001)	-0.47 (<0.0001)	0.80 (<0.0001)	-
Traffic road	-0.01 (0.6078)	0.26 (<0.0001)	0.59 (<0.0001)	-	0.57 (<0.0001)	0.42 (<0.0001)
Lake (rowing)	0.01 (0.6078)	0.91 (<0.0001)	0.08 (<0.0001)	-	0.29 (<0.0001)	0.18 (<0.0001)
Lake (swimming)	0.02 (0.1298)	0.64 (<0.0001)	0.07 (<0.0001)	-	0.71 (<0.0001)	-
SSO	-0.003 (0.7696)	0.23 (<0.0001)	0.48 (<0.0001)	-	0.81 (<0.0001)	-
Wadi	-0.02 (0.0959)	0.22 (<0.0001)	0.47 (<0.0001)	-	0.822 (<0.0001)	-

Table A−5: Sensitivity analysis: Spearman rank correlation coefficients (p-value) for GI annual risks.

	μ_w *crypto-sporidium*	μ_w *campy-lobacter*	μ_w *norovirus*	Dilution	V	T	f
River (rowing)	-0.01 (0.3055)	0.66 (<0.0001)	0.16 (<0.0001)	-	0.24 (<0.0001)	0.13 (<0.0001)	0.51 (<0.0001)
River (swim-ming)	-0.01 (0.4827)	0.36 (<0.0001)	0.11 (<0.0001)	-	0.48 (<0.0001)	–	0.67 (<0.0001)
Swim-ming pool	0.89 (<0.0001)	–	–	-	0.17 (<0.0001)	–	0.26 (<0.0001)
Park	-0.01 (0.4279)	0.14 (<0.0001)	0.32 (<0.0001)	-0.56 (<0.0001)	0.32 (<0.0001)	0.17 (<0.0001)	0.51 (<0.0001)
CSO	-0.01 (0.595)	0.07 (<0.0001)	0.11 (<0.0001)	-	0.27 (<0.0001)	0.22 (<0.0001)	0.92 (<0.0001)
Sedimen-tation pond	-0.01 (0.2142)	0.27 (<0.0001)	0.58 (<0.0001)	-	0.52 (<0.0001)	–	0.43 (<0.0001)
Surface water play-ground	-0.01 (0.5240)	0.73 (<0.0001)	0.06 (<0.0001)	-	0.27 (<0.0001)	0.17 (<0.0001)	0.45 (<0.0001)
Pond in green area (fishing)	0.004 (0.6467)	0.17 (<0.0001)	0.40 (<0.0001)	-0.72 (<0.0001)	0.36 (<0.0001)	–	0.30 (<0.0001)
Pond in green area (swim-ming)	-0.01 (0.5799)	0.09 (<0.0001)	0.22 (<0.0001)	-0.37 (<0.0001)	0.62 (<0.0001)	–	0.54 (<0.0001)
Traffic road	0.002 (0.8276)	0.12 (<0.0001)	0.29 (<0.0001)	-	0.27 (<0.0001)	0.21 (<0.0001)	0.81 (<0.0001)
Lake (rowing)	-0.001 (0.9288)	0.49 (<0.0001)	0.05 (<0.0001)	-	0.53 (<0.0001)	–	0.54 (<0.0001)
Lake (swim-ming)	0.01 (0.3741)	0.08 (<0.0001)	0.01 (0.2593)	-	0.55 (<0.0001)	–	0.74 (<0.0001)
SSO	0.01 (0.5260)	0.09 (<0.0001)	0.23 (<0.0001)	-	0.40 (<0.0001)	–	0.78 (<0.0001)
Wadi	-0.001 (0.7873)	0.11 (<0.0001)	0.24 (<0.0001)	-	0.43 (<0.0001)	–	0.77 (<0.0001)

Table A−6: Sensitivity analysis: Spearman rank correlation coefficients (p-values) for LD risks.

	μ_w *L. pneumo-phila*	Dilution	Aerosoliza-tion Ratio	Breathing rate	Duration of exposure	Exposure Frequency
River (rowing)	0.99 (<0.0001)	-	0.06 (<0.0001)	0.01 (0.3099)	0.01 (0.1978)	0.08 (<0.0001)
Park	0.89 (<0.0001)	-0.07 (<0.0001)	0.04 (<0.0001)	0.0001 (0.9902)	-0.01 (0.5327)	0.17 (<0.0001)
CSO	0.69 (<0.0001)	-	0.03 (0.0011)	0.01 (0.1780)	0.001 (0.9280)	0.47 (0.9208)
Ornamental fountain	0.87 (<0.0001)	-	0.07 (<0.0001)	0.01 (0.2174)	0.07 (<0.0001)	0.23 (<0.0001)
Drinking water play-ground	0.88 (<0.0001)	-	0.06 (<0.0001)	0.02 (0.0247)	0.06 (<0.0001)	0.22 (<0.0001)
Surface water play-ground	0.88 (<0.0001)	-	0.07 (<0.0001)	0.03 (0.0075)	0.08 (<0.0001)	0.21 (<0.0001)
Traffic road	0.74 (<0.0001)	-	0.02 (0.0139)	-0.01 (0.6349)	0.002 (0.8140)	0.39 (<0.0001)
Lake (sailing)	0.88 (<0.0001)	-	0.02 (0.0244)	0.01 (0.1645)	0.03 (0.0049)	0.03 (0.0007)
SSO	0.72 (<0.0001)	-	0.04 (<0.0001)	0.04 (<0.0001)	0.01 (0.3250)	0.44 (<0.0001)
Wadi	0.71 (<0.0001)	-	0.04 (<0.0001)	0.004 (0.6709)	-0.004 (0.6915)	0.45 (<0.0001)
Public water taps	0.88 (<0.0001)	-	0.06 (<0.0001)	0.03 (0.0012)	0.03 (0.0046)	0.24 (<0.0001)

A.4. Assumptions and Rationale

Assumption 1. Concentrations of pathogens from other locations (similar in water source and population) are representative for the concentrations in our specific locations. Because no specific pathogen concentrations on the study locations is available, this approach is considered to be the most appropriate to evaluate the priorities, more appropriate at this risk prioritizing stage than embarking on an area-wide pathogen monitoring campaign.

Assumption 2. For some locations, pathogens distributions are selected arbitrarily (based on distributions that are fitted in similar studies, i.e., gamma and lognormal) and fitted to statistics of data (mean and quantiles). Environmental pathogen studies do not commonly report the statistical distributions fitted to the data. We studied the literature on statistical distributions that fit to pathogen data in water bodies and used these distributions as most appropriate means to reflect the variability in pathogen concentrations at our study sites.

Assumption 3. At the sedimentation pond, the concentration of pathogens is estimated assuming a 1 log reduction in pathogens load due to the sedimentation process. Effects of extreme rain events on pathogen concentrations are not included. This is based on reductions of pathogens in sedimentation ponds from water treatment systems [324].

Assumption 4. At the locations that receive water from the sedimentation pond (the green area pond and the pond at the park), concentrations of pathogens are estimated by assigning a 1log reduction to the concentration in the sedimentation pond. This is based on the average reduction in the *E. coli* concentrations, that was the best available information to assess the reduction in concentration of enteric microbes entering from the sedimentation pond. Concentration during dry periods events (when surface water is used to fill in the ponds) is not considered.

Assumption 5. Aerosolization ratio is derived from experiments conducted on ornamental fountains with endotoxins. This is a worst case assumption, but does use scientific data on the spread of bacteria (l-compounds) via aerosols from ornamental fountains under Dutch conditions.

Assumption 6. Volumes of water ingested are, for some activities where data were absent, extrapolated from similar activities. In some exposure time assessments, distributions are selected (usually triangular) and fitted to published summary statistics.

Assumption 7. For inhalation, it is assumed that all bacteria in aerosols that reach the lower respiratory tract are susceptible of initiating and infection. Without more specific information on viability and infectivity, this was used as a conservative assumption.

Assumption 8. Exposure times and frequencies are sometimes based on expert assessment, and not on quantitative observational studies (e.g., walking the dog).

Assumption 9. The exposure frequencies in CSO, SSO and wadis are based on survey studies, and depend on the frequency of extreme rain events in the survey year. An

increasing frequency of extreme precipitation, as prognosed in climate change scenarios for the Netherlands, would probably increase the exposure frequency.

Assumption 10. The pathogens found in the different water sources have the same virulence as those used to derive the dose-response curves. This is a common assumption in all QMRA studies; where possible (i.e., *Campylobacter*) we opted for the most conservative dose-response curves to urge on the safe side.

Assumption 11. The population exposed has the same vulnerability of infection as that used to derive the dose-response model. Also this is a common assumption in all QMRA studies. The dose-response models are usually derived from feeding trials with healthy young adults. They may not represent people with compromised immune systems, who may be more prone to develop infection and disease symptoms.

Appendix B: Quantification of Waterborne Pathogens and Associated Health Risks in Urban Water

B.1. Inhibition Test

An inhibition test was performed with PC from the different weekly monitoring locations and one sample from the river (N=6) to select those dilutions with higher probability of finding DNA. Original extractions and two, four, six, eight, and ten times dilutions were analysed by q-PCR in duplicate. DNA targets used in the inhibition test were the IC and *L. pneumophila*. Results were analysed with ANOVA techniques using the R software version 3.0.1 [177]. It was considered that the lowest dilution that was not statistically different from higher dilutions but statistically different ($p<0.05$) from the dilutions bellow it, was the lowest dilution with no significant inhibition effects.

The log-transformed data of the recovery efficiency (RE, calculated with the IC results) were analysed with the Kruskal-Wallis test for non-parametric data followed by one way ANOVA of the ranked data and Tukey HSD post-hoc analysis. To analyse the results of the *L. pneumophila* data, only the PC were used (N=5) because *L. pneumophila* was not found in the river sample, and the log-transformed data were analysed by one way ANOVA followed by the Tukey HSD test. The RE results showed that four times dilution samples were enough to eliminate inhibition effects, while the *L.pneumophila* results indicated that two times dilutions were enough (Figure B−1). Therefore, two fold and four fold dilutions were used to analyse the samples from the four locations.

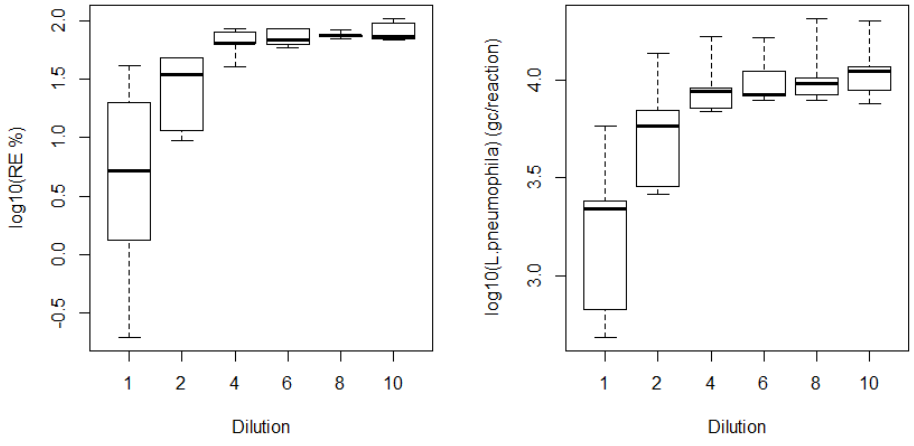

Figure B−1: Results of the inhibition test with the internal control data (left) and L. pneumophila data (right).

B.2. Analysed Volumes and Recovery Efficiency

Table B−1: Analysed volumes and recovery efficiency of the weekly monitoring samples.

Location		Dilution	Volume Analysed (mL)[1]	RE (%)[2]
River	Pellets	2	720 (480-1250)	30.1 (12.6-46.8)
		4	340 (150-620)	50.4 (32.0-76.5)
	Supernatants	2	270(120-1000)	40.3 (12.4-80.1)
		4	210 (60-700)	49.2 (22.7-63.6)
Lake	Pellets	2	580 (580-620)	27.3 (13.5-60.3)
		4	310 (250-490)	46.6 (25.5-78.7)
	Supernatants	2	340 (220-490)	15.8 (12.9-19.3)
		4	170 (110-250)	19.8 (16.3-27.4)
Sedimentation pond	Pellets	2	430 (330-550)	59.2 (11.5-96.1)
		4	210 (170-270)	70.7 (50.5-99.7)
	Supernatants	1	1180 (740-1840)	49.1 (33.1-56.6)
		2	590 (370-920)	45.1 (30.7-51.5)
Pond	Pellets	2	300 (230-540)	47.3 (25.8-84.8)
		4	180 (120-270)	63.6 (47.7-85.9)

[1]Volume analysed in each PCR assay; [2]Samples with RE below 10% are not included and where not used in the study.

Table B−2: Analysed volumes and recovery efficiency of the rain event samples.

Location	Dilution	Volume Analysed (mL)	RE (%)
Sedimentation pond inlet	1	41.7 (36.9-45.1)	38.2 (26.9-43.5)
	10	5.1 (4.6-5.3)	31.6 (20.5-36.3)
Sedimentation pond outlet	1	43.9 (39.3-46.6)	38.1 (29.9-48.5)
	10	5.4 (4.3-5.9)	34.0 (26.9-45.2)
Wadi	1	33.8 (30.5-35.3)	42.2 (33.3-46.6)
	10	3.4 (3.1-3.5)	32.9 (29.6-36.5)

Only the pellets were processed and analysed

B.3. Correlations

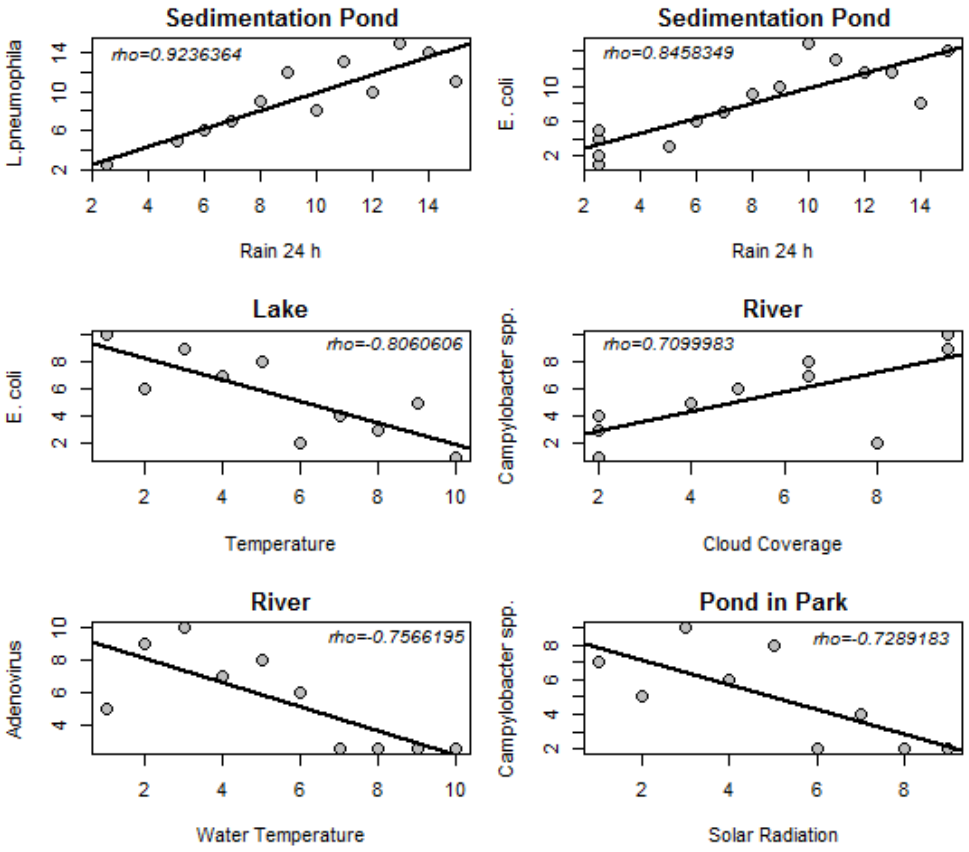

Figure B−2: Examples of Spearman rank correlation coefficients (rho). The axes show the ranks of the variables. The red line is the linear regression of the ranks.

Appendix C: Health Risks Derived from Consumption of Lettuces Irrigated with Tertiary Effluent Containing Norovirus

C.1. Study-Site Description

Table C−1: Characteristics of the secondary and tertiary effluent (monthly averages of 2013 weekly samples).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Secondary effluent												
Suspended Solids (mg/L)	6.4	5.9	11.4	7.0	8.5	7.8	8.3	12.2	10.5	8.6	5.3	3.0
Turbidity (NTU)	4.3	4.2	5.0	5.3	4.9	4.9	4.7	5.6	5.8	4.5	3.5	1.8
E. coli (10^4 cfu/100mL)[a]	3.5	2.3	4.0	0.97	5.9	12	12	27	8.0	6.5	3.0	0.82
Tertiary effluent												
Vol (1000 m^3)	3.7	3.0	4.4	36.3	44.6	123.4	189.9	157.7	58.5	33.0	18.0	15.5
Suspended Solids (mg/L)	3.7	4.7	3.1	3.8	5.1	5.8	5.2	7.7	6.8	6.3	3.8	2.8
Turbidity (NTU)	3.0	2.1	2.3	2.3	2.7	2.7	2.54	3.4	3.9	3.3	2.3	1.6
E. coli (cfu/100mL)[a]	1	1	2	1	2	1	1	<1	<1	1	1	<1
Total Residual Chlorine (mg Cl_2/L)	0.3	0.5	0.4	1.2	1.1	2.0	2.3	2.3	2.3	2.8	1.6	1.6
Conductivity (dS/m)	1.2	1.2	0.8	1.0	1.1	1.2	1.4	1.4	1.3	1.3	1.2	1.4
pH	7.8	7.8	7.8	7.8	7.6	7.5	7.6	7.8	8.1	8.0	7.8	7.7

[a]*Geometric mean; cfu: colony forming units*

C.2. Exposure Assessment

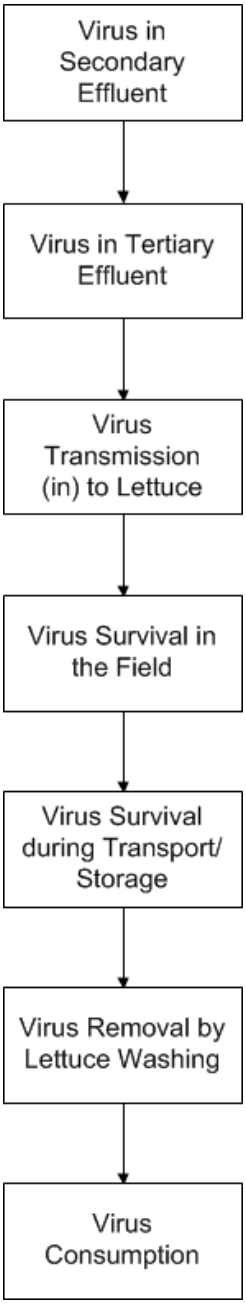

Figure C−1: Conceptual exposure model (from wastewater to fork).

C.3. Norovirus Sample Treatment and qPCR

C.3.1. Viral Concentration and Nucleic Acid Extraction

Viruses present in 10 L samples were concentrated using the skimmed milk organic flocculation method [245]. All samples were carefully adjusted to a conductivity of 1.5 mS/cm^2, and acidified to pH 3.5 using HCl 1N. 10 g of skimmed milk powder (Difco, Detroit, MI, USA) were dissolved in 1 L of artificial seawater (Sigma, Aldrich Chemie GMBH, Steinheim, Germany), and adjusted to pH 3.5 using HCl 1N to obtain a pre-flocculated 1% (w/v) skimmed milk solution (PSM). Then, 100 mL of PSM were added to all previously conditioned samples to obtain a final concentration of 0.01% of skimmed milk. Samples were stirred for 8 h at room temperature and flocks were allowed to settle by gravity during 8 h. Carefully, the supernatant was removed and the remaining 500 mL of solution were centrifuged at 8,000 g for 30 min at 4 °C. The pellets were suspended using 10 mL of phosphate buffer at pH 7.5 and were stored at -20 °C until nucleic acid (NA) extractions were performed. A negative concentration control was also included in each sampling event using tap water as a matrix and neutralizing the free chlorine adding 100 mL of 10% sodium thiosulfate solution.

C.3.2. Extraction of Nucleic Acids from Viral Concentrates

Viral extraction of NA was performed using 140 µl of viral concentrates in the QIAamp® Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) employing the automated system QIACube (Qiagen, Valencia, CA, USA) following the manufacturer's procedure. NA were stored at -80 ºC until analyzed. A negative control of extraction was included in each extraction batch using free DNAse/RNAse molecular water.

C.3.3. Quantitative RT-PCR

Samples were tested using specific RT-q-PCR for the viral pathogens NoVGI [246] and NoVGII [247]. All samples were analyzed in duplicate using undiluted and log10 dilutions of the NA. To demostrate that there was not basal fluorescence produced by the mix, more than one non-template control (NTC) were included in the qPCRs. MX3000Pro sequence detector system (Stratagene, La Jolla, CA, USA) was used to quantify the samples. Detection limits are 10 gc per reaction tube, according to Kageyama, et al. [247].

C.3.4. Plasmid DNA for the Viral qPCR Assays

Plasmid DNA was used as a positive control and as a quantitative standard. The capsid proteins regions of the NoVGI.4 (2931bp) and NoVGII.13 (3004bp) were cloned into pTrueBlue®-Pvu II vector (donated by Dr. J. Vinjé of the CDC, Atlanta) and were used as qRT-PCR standard.

To reduce the possibility of DNA contamination in the laboratory, 10 µg of each plasmid DNA were linearized using SacI for NoVGI and XhoI for NoVGII (Promega, Madison, WI) and subsequently the reaction products were purified and quantified.
Serial dilutions in TE buffer were performed using the linearized standards ranging from 10^0 to 10^5 molecules per 5 µl. Aliquots of standard dilutions were stored at -80 °C until use. UltraPure™ DNase/RNase-Free distilled water was used as negative control of the NA extraction and q-PCR assays.

C.4. Uncertainty Analysis

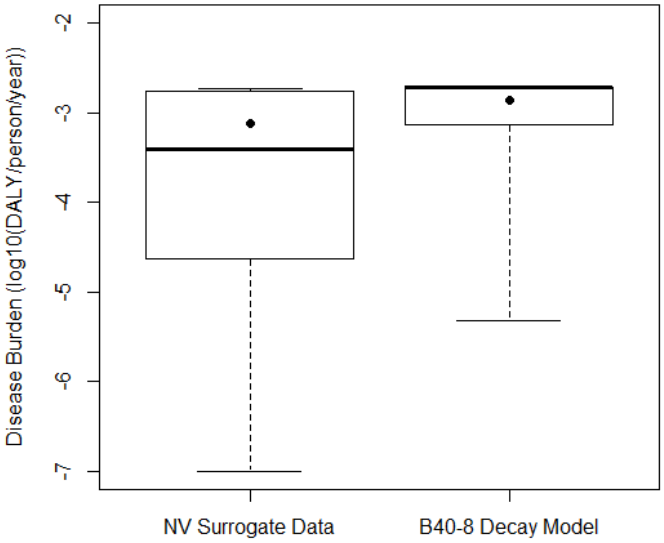

Figure C−2: Annual disease burden of the base scenario (using norovirus (NV) surrogate data) vs the alternative scenario using the Bacteriophage B40-8 decay model. The boxes show the interquartile range, solid lines in the boxes the median, dots the mean, and upper and lower whiskers the 90% CI of the disease burden.

Appendix D: Screening-level Risk Assessment of *Coxiella burnetii* (Q fever) Transmission via Aeration of Drinking Water.

D.1. Q fever Onset of Symptoms in The Netherlands

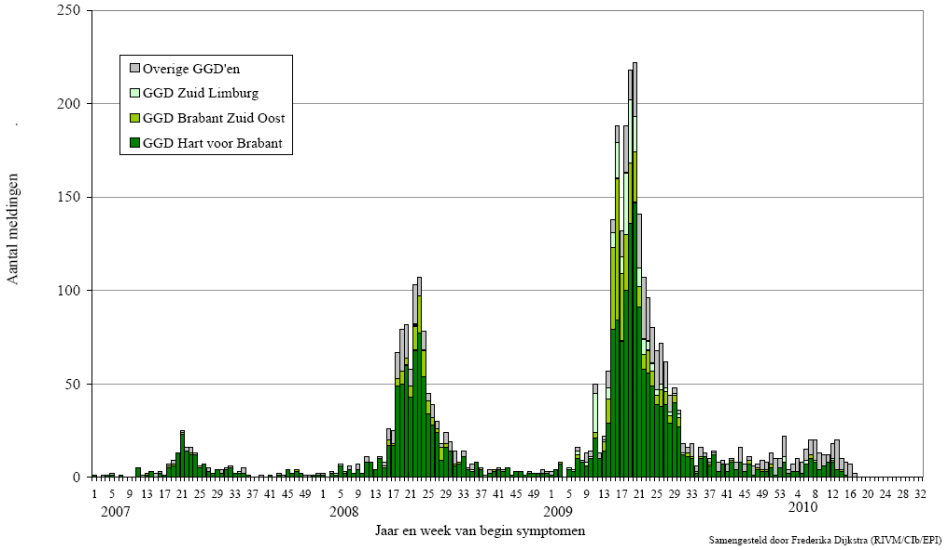

Figure D−1 Number of Q fever cases in The Netherlands per year and week of the onset of symptoms [72].

D.2. *Coxiella* Air Transport and Dilution

Table D−1: Relation of turbulent types to Meteorological conditions [283].

Surface windspeed (m/s)	Day time insolation			Nigh time conditions	
	Strong	Moderate	Slight	Thin overcast or ≥ 4/8 cloudiness	≤ 3/8 cloudiness
<2	A	A-B	B		
2	A-B	B	C	E	F
4	B	B-C	C	D	E
6	C	C-D	D	D	D
>6	C	D	D	D	D

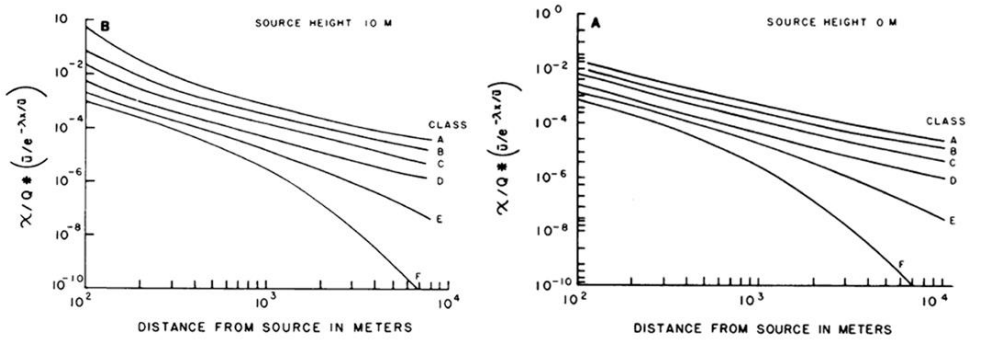

Figure D−2: Function of $X/Q*\left(\overline{U}/e^{-\lambda x/\overline{U}}\right)$ versus distance from the source in meters for the indicated stability classes and 2 source heights: 0m and 10m [283]

Table D−2: Calculation of the aerosolization ratio.

Air sampling method	Analytical method	*Legionella* in water (cells or cfu/m^3)*	*Legionella* in air (cells or cfu/m^3)*	Recovery rate	C. air / C. water ratio	Reference
Agar impaction	Culture	4.0×10^{4}	2.0×10^{-3}	40	2.0×10^{-6}	[88]
Filtration	FISH	4.7×10^{5}	1.4×10^{-2}	7,000	2.1×10^{-4}	[88]
Agar impaction	Culture	2.0×10^{5}	6.0×10^{-3}	-	3.0×10^{-8}	[87]
Liquid impingement	Microscopy	2.2×10^{7}	3.4×10^{1}	-	1.5×10^{-6}	[89]
All Glass Cyclone	Culture	1.0×10^{3}	3.3×10^{-4}	-	3.3×10^{-7}	[86]

**Cells/m^3 for FISH and microscopy methods; CFU/m^3 for culture methods*

D.4. Exposure Assessment Steps

Table D−3: Exposure assessment: Air model.

Variable	Symbol	Formula	Value P.E. (period A)	Units	Source
Concentration *C. burnetii* at barnyard air	Cb		880	*Coxiella*/ m^3 air	[281]
Ventilation rate	v		938	m^3/h/LU	[284]
Goats in farm	Ng		900	#	[285]
Mean weight goats	Mg		100	kg	Assumption
Livestock Unit	LU		500	Kg	[284]
LU goats per farm	LUg	$LUg = \frac{Mg \times Ng}{LU}$	180		
Emission rate	Q	$Q = Cb \times v \times LUg$	4.13×10^{4}	*Coxiella*/ sec	
Coxiella inactivation in the air	λ		0		Assumption
Distance from the source	x		1000	m	Model scenario
Source height	H		10	m	Assumption
Mean air speed	$\overline{U}$	Average period A	4.17	m/s	[276]
Stability class	SC	Table extrapolation	C		[276, 283]
G	g	Graph extrapolation	1.5×10^{-4}	m^{-2}	[283]
Concentration at water treatment plant air inlet	χ	$\chi = \frac{g \times Q}{\overline{\overline{U}}}$	1.48	*Coxiella*/ m^3 air	

Table D−4: Exposure assessment: Groundwater aeration, treatment and distribution.

Variable	Symbol	Formula	Value P.E. (period A)	Units	Source
Aeration ratio	a		20	L air/ L water	Assumption (based on questionnaire)
C. burnetii Transfer rate from air to water	t		1	-	Assumption
Air filtration removal	f		0	-	Assumption (based on questionnaire)
Transfer to water during aeration	t_a	$t_a = \frac{t \times a}{1 - f}$	20	-	
Concentration in raw water	Cr	$Cr = \chi \times t_a$	29.69	*Coxiella*/ m^3 water	
Removal by water treatment	I		0.5	log	[288]
Concentration in treated water	C_T	$C_T = 10^{(\log Cr - I)}$	9.39	*Coxiella*/ m^3 water	
Inactivation in water	i		1	-	Assumption
Concentration in tap water	C_W	$C_W = C_T \times i$	9.39	*Coxiella*/ m^3 water	

Table D−5: Exposure assessment: Aerosolization in the shower, inhalation and deposition of aerosols in the lower respiratory tract.

Variable	Symbol	Calculation	Value P.E. (period A)	Units	Source
Ratio Cair/Cwater	c		1.99×10^{-6}	-	[88]
Concentration in shower aerosols	C_A	$C_A = C_W \times c$	1.86×10^{-5}	*Coxiella*/m^3 water	
Shower frequency	fs		1	pppd	[99]
Shower duration	ts		8.1	min	[99]
Breathing rate	b		12	Breathings/ min	[290]
Breathing volume	V_T		500	mL	[290]
Respiratory minute volume	V_R	$V_R = V_T \times b$	6	L/min	
Air inhaled during a shower	A_S	$A_S = t_S \times V_R$	4.86×10^{-2}	m^3	
Coxiella inhaled during a shower	C_S	$C_S = C_A \times A_S$	9.06×10^{-7}	*Coxiella* pppd	
Bronquiolar deposition for mouth breathing	Db		4.6	%	Average [97]
Alveolar deposition for mouth breathing	Da		8.13	%	Average [97]
Dose (*Coxiella* deposition in the lower respiratory tract)	d	$d = C_S \times (Db + Da)$	1.15×10^{-7}	*Coxiella* pppd	

D.5. Air Transport Model Uncertainty

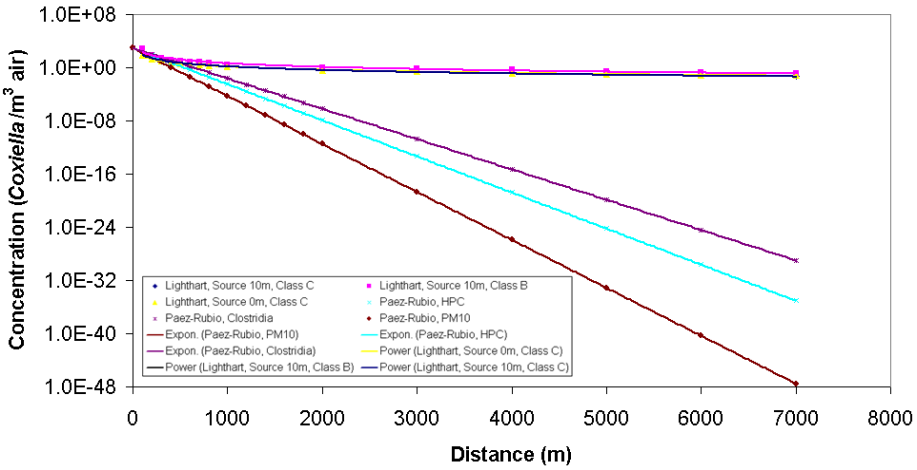

Figure D−3: Decay of C. burnetii concentration in the air with the distance downwind from the source. Comparision of Lighthart's model and Paez-Rubio's adapted data.

Bibliography

[1] Eurostat. Population Density. http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&init=1&plugin=1&language=en&pcode=tps00003.
[2] World Bank Group. The World Bank. http://databank.worldbank.org/data/views/reports/tableview.aspx.
[3] Wong, T.; Brown, R. The water sensitive city: principles for practice. *Water Science & Technology* **2009**, *60* (3), 673-892.
[4] Hamin, E. M.; Gurran, N. Urban form and climate change: Balancing adaptation and mitigation in the U.S. and Australia. *Habitat International* **2009**, *33* (3), 238-245.
[5] White, M.; Smith, A.; Humphryes, K.; Pahl, S.; Snelling, D.; Depledge, M. Blue space: The importance of water for preference, affect, and restorativeness ratings of natural and built scenes. *Journal of Environmental Psychology* **2010**, *30* (4), 482-493.
[6] Luttik, J. The value of trees, water and open space as reflected by house prices in the Netherlands. *Landscape and Urban Planning* **2000**, *48* (3–4), 161-167.
[7] de Man, H.; Bouwknegt, M.; van Heijnsbergen, E.; Leenen, E. J. T. M.; van Knapen, F.; de Roda Husman, A. M. Health risk assessment for splash parks that use rainwater as source water. *Water Research* **2014**, *54*, 254-261.
[8] de Man, H.; Heederik, D. D. J.; Leenen, E. J. T. M.; de Roda Husman, A. M.; Spithoven, J. J. G.; van Knapen, F. Human exposure to endotoxins and fecal indicators originating from water features. *Water Research* **2014**, *51*, 198-205.
[9] Schets, F. M.; AM, D. E. R. H.; Havelaar, A. H. Disease outbreaks associated with untreated recreational water use. *Epidemiology of Infection* **2010**, 1-12.
[10] Fewtrell, L.; Butler, D.; Memon, F. a.; Ashley, R.; Saul, A. An overview of UK-based HIA research on water management and flooding. In *Health Impact Assessment for Sustainable Water Management*, Fewtrell, L.; Kay, D., Eds. IWA Publishing: London, **2008**.
[11] Richardson, S. D.; DeMarini, D. M.; Kogevinas, M.; Fernandez, P.; Marco, E.; Lourencetti, C.; Ballesté, C.; Heederik, D.; Meliefste, K.; McKague, A. B. What's in the Pool? A Comprehensive Identification of Disinfection By-products and Assessment of Mutagenicity of Chlorinated and Brominated Swimming Pool Water. *Environmental Health Perspectives* **2010**, *118* (11), 1523.
[12] Percival, S. L.; Chalmers, C. M.; Embrey, M.; Hunter, P. R.; Sellwood, J.; Wyn-Jones, P. *Microbiology of Waterborne Diseases*. Elsevier Academic Press: London, **2004**.
[13] WHO *Guidelines for safe recreational water environments. Volume 1: coastal and Fresh waters*; World Health Organization: Geneva, Switzerland, **2003**.
[14] Hofstra, N. Quantifying the impact of climate change on enteric waterborne pathogen concentrations in surface water. *Current Opinion in Environmental Sustainability* **2011**, *3* (6), 471-479.
[15] Arnone, R. D.; Walling, J. P. Waterborne pathogens in urban watersheds. *Journal of Water and Health* **2007**, *5* (1), 149-162.
[16] Frank, C. A. P. Microcystin-producing cyanobacteria in recreational waters in southwestern Germany. *Environmental Toxicology* **2002**, *17* (4), 361-366.

[17] Wullings, B. A.; van der Kooij, D. Occurrence and Genetic Diversity of Uncultured Legionella spp. in Drinking Water Treated at Temperatures below 15°C. *Applied and Environmental Microbiology* **2006**, *72* (1), 157-166.
[18] Hunter, P. R. Climate change and waterborne and vector-borne disease. *Journal of Applied Microbiology* **2003**, *94*, 37-46.
[19] Schijven, J.; Bouwknegt, M.; de Roda Husman, A. M.; Rutjes, S.; Sudre, B.; Suk, J. E.; Semenza, J. C. A Decision Support Tool to Compare Waterborne and Foodborne Infection and/or Illness Risks Associated with Climate Change. *Risk Analysis* **2013**, *33* (12), 2154-2167.
[20] O'Neil, J. M.; Davis, T. W.; Burford, M. A.; Gobler, C. J. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* **2012**, *14* (0), 313-334.
[21] WHO. Health Impact Assessment. http://www.who.int/hia/en/.
[22] WHO. Guidelines for the Safe Use of Wastewater, Excreta and Greywater. In *Wastewater Use in Agriculture*, Organization, W. H., Ed. WHO: Geneva, **2006**; Vol. II.
[23] Fewtrell, L.; Bartram, J. Water quality: guidelines, standards and health: assessment of risk and risk management for water-related infectious diseases/edited by Lorna Fewtrell and Jamie Bartram. **2001**.
[24] Teunis, P.; Van den Brandhof, W.; Nauta, M.; Wagenaar, J.; Van den Kerkhof, H.; Van Pelt, W. A reconsideration of the Campylobacter dose-response relation. *Epidemiology of Infection* **2005**, *133* (4), 583-92.
[25] WHO/FAO *Hazard Characterization for Pathogens in Food and Water*; FAO/WHO: **2003**.
[26] Haas, C. N.; Rose, J. B.; Gerba, C. P. *Quantitative Microbial Risk Assessment*. John Willey & Sons, Inc (Pub): New York, **1999**.
[27] Medema, G.; Ashbolt, N. J. *QMRA: its Value for Risk Management*; **2006**; p 34.
[28] Rusin, P.; Rose, J.; Haas, C.; Gerba, C. Risk Assessment of Opportunistic Bacterial Pathogens in Drinking Water. In *Rev Environ Contam Toxicol*, Ware, G., Ed. Springer New York: **1997**; Vol. 152, pp 57-83.
[29] Vellema, P.; van den Brom, R. The rise and control of the 2007–2012 human Q fever outbreaks in the Netherlands. *Small Ruminant Research* **2014**, *118* (1–3), 69-78.
[30] FAO/WHO *Risk assessment of Campylobacter spp. in broiler chickens: technical report*; Geneva, **2009**; p 132.
[31] WHO. *Guidelines for Drinking-Water Quality*. 4th ed.; WHO: Geneva, **2011**; p 564.
[32] Havelaar, A. H.; Haagsma, J. A.; Mangen, M.-J. J.; Kemmeren, J. M.; Verhoef, L. P. B.; Vijgen, S. M. C.; Wilson, M.; Friesema, I. H. M.; Kortbeek, L. M.; van Duynhoven, Y. T. H. P.; van Pelt, W. Disease burden of foodborne pathogens in the Netherlands, 2009. *International Journal of Food Microbiology* **2012**, *156* (3), 231-238.
[33] Jones, K. Campylobacters in water, sewage and the environment. *Journal of Applied Microbiology* **2001**, *90* (S6), 68S-79S.
[34] Blaser, M. J.; Smith, P. F.; Wang, W. L.; Hoff, J. C. Inactivation of Campylobacter jejuni by chlorine and monochloramine. *Applied and Environmental Microbiology* **1986**, *51* (2), 307-311.
[35] Keener, K. M.; Bashor, M. P.; Curtis, P. A.; Sheldon, B. W.; Kathariou, S. Comprehensive Review of Campylobacter and Poultry Processing. *Comprehensive Reviews in Food Science and Food Safety* **2004**, *3* (2), 105-116.

[36] de Wit, M. A. S.; Koopmans, M. P. G.; Kortbeek, L. M.; Wannet, W. J. B.; Vinjé, J.; van Leusden, F.; Bartelds, A. I. M.; van Duynhoven, Y. T. H. P. Sensor, a Population-based Cohort Study on Gastroenteritis in the Netherlands: Incidence and Etiology. *American Journal of Epidemiology* **2001**, *154* (7), 666-674.
[37] van Pelt, W.; de Wit, M. A.; Wannet, W. J.; Ligtvoet, E. J.; Widdowson, M. A.; van Duynhoven, Y. T. Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991-2001. *Epidemiology and Infection* **2003**, *130* (3), 431-41.
[38] Smith, H. V.; Rose, J. B. Waterborne Cryptosporidiosis: Current Status. *Parasitology Today* **1998**, *14* (1), 14-22.
[39] Fayer, R. Cryptosporidium: a water-borne zoonotic parasite. *Veterinary Parasitology* **2004**, *126* (1-2), 37-56.
[40] Jiang, J.; Xiao, L. An Evaluation of Molecular Diagnostic Tools for the Detection and Differentiation of Human-Pathogenic Cryptosporidium spp. *Journal of Eukaryotic Microbiology* **2003**, *50*, 542-547.
[41] U.S.EPA *Economic analysis for the final long term 2 enhanced surface water treatment rule*; U.S. E.P.A.: Washington DC, **2005**.
[42] Karanis, P.; Kourenti, C.; Smith, H. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health* **2007**, *5* (1), 1-38.
[43] WHO. *Guidelines for safe recreational water environments. Volume 2: Swimming Pools and Similar Environments*. WHO: Geneva, **2006**; Vol. 2.
[44] Mena, K. D.; Gerba, C. P. Waterborne adenovirus. *Rev Environ Contam Toxicol* **2009**, *198*, 133-67.
[45] Jiang, S. C. Human Adenoviruses in Water: Occurrence and Health Implications: A Critical Review. *Environmental Science & Technology* **2006**, *40* (23), 7132-7140.
[46] Kundu, A.; McBride, G.; Wuertz, S. Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment. *Water Research* **2013**, *47* (16), 6309-6325.
[47] Viau, E. J.; Lee, D.; Boehm, A. B. Swimmer Risk of Gastrointestinal Illness from Exposure to Tropical Coastal Waters Impacted by Terrestrial Dry-Weather Runoff. *Environmental Science & Technology* **2011**, *45* (17), 7158-7165.
[48] Wyn-Jones, A. P.; Carducci, A.; Cook, N.; D'Agostino, M.; Divizia, M.; Fleischer, J.; Gantzer, C.; Gawler, A.; Girones, R.; Höller, C.; de Roda Husman, A. M.; Kay, D.; Kozyra, I.; López-Pila, J.; Muscillo, M.; José Nascimento, M. S.; Papageorgiou, G.; Rutjes, S.; Sellwood, J.; Szewzyk, R.; Wyer, M. Surveillance of adenoviruses and noroviruses in European recreational waters. *Water Research* **2011**, *45* (3), 1025-1038.
[49] Bofill-Mas, S.; Albinana-Gimenez, N.; Clemente-Casares, P.; Hundesa, A.; Rodriguez-Manzano, J.; Allard, A.; Calvo, M.; Girones, R. Quantification and Stability of Human Adenoviruses and Polyomavirus JCPyV in Wastewater Matrices. *Applied and Environmental Microbiology* **2006**, *72* (12), 7894-7896.
[50] Sinclair, R. G.; Jones, E. L.; Gerba, C. P. Viruses in recreational water-borne disease outbreaks: a review. *Journal of Applied Microbiology* **2009**, *107* (6), 1769-1780.
[51] Rajko-Nenow, P.; Waters, A.; Keaveney, S.; Flannery, J.; Tuite, G.; Coughlan, S.; O'Flaherty, V.; Doré, W. Norovirus Genotypes Present in Oysters and in Effluent from a Wastewater Treatment Plant during the Seasonal Peak of Infections in Ireland in 2010. *Applied and Environmental Microbiology* **2013**, *79* (8), 2578-2587.
[52] Atmar, R. Noroviruses: State of the Art. *Food and Environmental Virology* **2010**, *2* (3), 117-126.

[53] Patel, M. M.; Hall, A. J.; Vinjé, J.; Parashar, U. D. Noroviruses: A comprehensive review. *Journal of Clinical Virology* **2009**, *44* (1), 1-8.
[54] Teunis, P. F.; Moe, C. L.; Liu, P.; E. Miller, S.; Lindesmith, L.; Baric, R. S.; Le Pendu, J.; Calderon, R. L. Norwalk virus: How infectious is it? *Journal of Medical Virology* **2008**, *80* (8), 1468-1476.
[55] Hoebe, Christian J. P. A.; Vennema, H.; de Roda Husman, Ana M.; van Duynhoven, Yvonne T. H. P. Norovirus Outbreak among Primary Schoolchildren Who Had Played in a Recreational Water Fountain. *The journal of Infectious Diseases* **2004**, *189* (4), 699-705.
[56] Diederen, B. M. W. Legionella spp. and Legionnaires' disease. *Journal of Infection* **2008**, *56* (1), 1-12.
[57] Fisman, D. N.; Lim, S.; Wellenius, G. A.; Johnson, C.; Britz, P.; Gaskins, M.; Maher, J.; Mittleman, M. A.; Spain, C. V.; Haas, C. N.; Newbern, C. It's not the heat, it's the humidity: wet weather increases legionellosis risk in the greater Philadelphia metropolitan area. *The journal of Infectious Diseases* **2005**, *192* (12), 2066-2073.
[58] Hicks, L. A.; Rose, C. E.; Fields, B. S.; Drees, M. L.; Engel, J. P.; Jenkins, P. R.; Rouse, B. S.; Blythe, D.; Khalifah, A. P.; Feikin, D. R.; Whitney, C. G. Increased rainfall is associated with increased risk for legionellosis. *Epidemiology and Infection* **2007**, *135* (5), 811-817.
[59] Sakamoto, R.; Ohno, A.; Nakahara, T.; Satomura, K.; Iwanaga, S.; Kouyama, Y.; Kura, F.; Kato, N.; Matsubayashi, K.; Okumiya, K.; Yamaguchi, K. Legionella pneumophila in rainwater on roads. *Emerging Infectious Diseases* **2009**, *15* (8), 1295-1297.
[60] Schalk, J. A.; Docters van Leeuwen, A. E.; Lodder, W. J.; de Man, H.; Euser, S.; den Boer, J. W.; de Roda Husman, A. M. Isolation of Legionella pneumophila from pluvial floods by amoebal coculture. *Applied and Environmental Microbiology* **2012**, *78* (12), 4519-21.
[61] Beauté, J.; Zucs, P.; de Jong, B.; null. Legionnaires disease in Europe, 2009-2010. *Eurosurveillance* **2013**, *18* (10).
[62] Gurtler, L.; Bauerfeind, U.; Blumel, J.; Burger, R.; Drosten, C.; Groner, A.; Heiden, M.; Hildebrandt, M.; Jansen, B.; Offergeld, R.; Pauli, G.; Seitz, R.; Schlenkrich, U.; Schottstedt, V.; Strobel, J.; Willkommen, H. Coxiella burnetii - Pathogenic Agent of Q (Query) Fever. *Transfusion medicine and hemotherapy* **2014**, *41* (1), 60-72.
[63] McCaul, T. F.; Williams, J. C. Developmental Cycle of *Coxiella burnetii*: Structure and Morphogenesis of Vegetative and Sporogenic Differentiations. *Journal of Bacteriology* **1981**, *147* (3), 1063-1076.
[64] Madariaga, M. G.; Rezai, K.; Trenholme, G. M.; Weinstein, R. A. Q fever: a biological weapon in your backyard. *The Lancet Infectious Diseases* **2003**, *3*, 709-721.
[65] Van den Brom, R.; Vellema, P. Q fever outbreaks in small ruminants and people in the Netherlands. *Small Ruminant Research* **2009**, *86* (1-3), 74-79.
[66] Arricau-Bouvery, N.; Soriau, A.; Lechopier, P.; Rodolakis, A. Experimental Coxiella burnetii infection in pregnant goats: excretion routes. *Veterinary Research* **2003**, *34* (4), 423-433.
[67] Hatchette, T. F.; Hudson, R. C.; Schlech, W. F.; Campbell, N. A.; Hatchette, J. E.; Ratnam, S.; Raoult, D.; Donovan, C.; Marrie, T. J. Goat-associated Q fever: a new disease in Newfoundland. *Emerging Infectious Diseases* **2001**, *7* (3), 413-9.
[68] Hawker, J.; Ayres, J.; Blair, I.; Evans, M.; Smith, D.; Smith, E.; Burge, P.; Carpenter, M.; Caul, E.; Coupland, B.; Desselberger, U.; Farrell, I.; Saunders, P.; Wood, M.

A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? *Communicable Disease and Public Health* **1998**, *1* (3), 180-187.
[69] Jones, R. M.; Nicas, M.; Hubbard, A. E.; Reingold, A. L. The Infectious Dose of *Coxiella burnetii* (Q Fever). *Applied Biosafety* **2006**, *11* (1), 32-41.
[70] Maurin, M.; Raoult, D. Q Fever. *Clinical Microbiology Reviews* **1999**, *12* (4), 518-553.
[71] Muskens, J.; van Engelen, E.; van Maanen, C.; Bartels, C.; Lam, T. J. G. M. Prevalence of Coxiella burnetii infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. *Veterinary Record* **2011**, *168* (3), 79.
[72] RIVM. Infectieziekten Q-koorts. http://www.rivm.nl/cib/themas/Q-koorts/q-koorts-professionals.jsp#index_3.
[73] Q Fever. http://www.cdc.gov/ncidod/dvrd/qfever/.
[74] Marrie, T. J. Coxiella burnetii Pneumonia. *European Respiratory Journal* **2003**, *21*, 713-719.
[75] Funari, E.; Testai, E. Human health risk assessment related to cyanotoxins exposure. *Critical reviews in toxicology* **2008**, *38* (2), 97-125.
[76] Codd, G. A.; Morrison, L. F.; Metcalf, J. S. Cyanobacterial toxins: risk management for health protection. *Toxicology and Applied Pharmacology* **2005**, *203* (3), 264-272.
[77] Wood, S. A.; Dietrich, D. R. Quantitative assessment of aerosolized cyanobacterial toxins at two New Zealand lakes. *Journal of Environmental Monitoring* **2011**, *13* (6), 1617-24.
[78] Barker, S. F.; O'Toole, J.; Sinclair, M. I.; Leder, K.; Malawaraarachchi, M.; Hamilton, A. J. A probabilistic model of norovirus disease burden associated with greywater irrigation of home-produced lettuce in Melbourne, Australia. *Water Research* **2013**, *47* (3), 1421-1432.
[79] Mok, H.-F.; Barker, S. F.; Hamilton, A. J. A probabilistic quantitative microbial risk assessment model of norovirus disease burden from wastewater irrigation of vegetables in Shepparton, Australia. *Water Research* **2014**, (54), 347-362.
[80] Schets, F. M.; Schijven, J. F.; de Roda Husman, A. M. Exposure assessment for swimmers in bathing waters and swimming pools. *Water Research* **2011**, *45* (7), 2392-2400.
[81] de Man, H.; van den Berg, H. H. J. L.; Leenen, E. J. T. M.; Schijven, J. F.; Schets, F. M.; van der Vliet, J. C.; van Knapen, F.; de Roda Husman, A. M. Quantitative assessment of infection risk from exposure to waterborne pathogens in urban floodwater. *Water Research* **2014**, *48*, 90-99.
[82] Dorevitch, S.; Panthi, S.; Huang, Y.; Li, H.; Michalek, A. M.; Pratap, P.; Wroblewski, M.; Liu, L.; Scheff, P. A.; Li, A. Water ingestion during water recreation. *Water Research* **2011**, *45* (5), 2020-2028.
[83] Rijal, G.; Tolson, J.; Petropoulou, C.; Granato, T.; Glymph, A.; Gerba, C.; Deflaun, M.; O'Connor, C.; Kollias, L.; Lanyon, R. Microbial risk assessment for recreational use of the Chicago Area Waterway System. *Journal of Water and Health* **2011**, *9* (1), 169-186.
[84] USEPA *Exposure Factors Handbook 2011*; U.S. Environmental Protection Agency: Washington, DC, **2011**.
[85] Anderson, W. B.; Dixon, D. G.; Mayfield, C. I. Estimation of endotoxin inhalation from shower and humidifier exposure reveals potential risk to human health. *Journal of Water and Health* **2007**, *5* (4), 553-572.

[86] Dennis, P. J. L.; Wright, A. E.; Rutter, D. A.; Death, J. E.; Jones, B. P. C. Legionella pneumophila in aerosols from shower baths. *The Journal of Hygiene* **1984**, *93* (2), 349-353.
[87] Bollin, G. E.; Plouffe, J. F.; Para, M. F.; Hackman, B. Aerosols Containing Legionella pneumophila Generated by Shower Heads and Hot-Water Faucets. *Applied and Enviornmental Microbiology* **1985**, *50* (5), 1128-1131.
[88] Deloge-Abarkan, M.; Ha, T.-L.; Robine, E.; Zmirou-Naviera, D.; Mathieu, L. Detection of airborne Legionella while showering using liquid impingement and fluorescent in situ hybridization (FISH). *Journal of Environmental Monitoring* **2007**, *9*, 91-97.
[89] Perkins, S. D.; Mayfield, J.; Fraser, V.; Angenent, L. T. Potentially Pathogenic Bacteria in Shower Water and Air of a Stem Cell Transplant Unit. *Applied and Enviornmental Microbiology* **2009**, *75* (16), 5363-5372.
[90] O'Toole, J.; Keywood, M.; Sinclair, M.; Leder, K. Risk in the mist? Deriving data to quantify microbial health risks associated with aerosol generation by water-efficient devices during typical domestic water-using activities. *Water Science and Technology* **2009**, *60* (11), 2913-2920.
[91] Baron, P. A.; Willeke, K. Respirable droplets from whirlpools: Measurements of size distribution and estimation of disease potential. *Environmental Research* **1986**, *39* (1), 8-18.
[92] Ishimatsu, S.; Miyamoto, H.; Hori, H.; Tanaka, I.; Yoshida, S.-I. Sampling and detection of Legionella pneumophila aerosols generated from an industrial cooling tower. *Annals of Occupational Hygiene* **2001**, *45* (6), 421-427.
[93] Moore, G.; Hewitt, M.; Stevenson, D.; Walker, J. T.; Bennett, A. M. Aerosolisation of respirable droplets from a domestic spa pool: the use of MS-2 coliphage and Pseudomonas aeruginosa as markers for Legionella pneumophila. *Applied and Environmental Microbiology* **2014**.
[94] Kritana, P.; Taehyeung, K.; Soyoung, K.; Hyeontae, K.; Ki Youn, K.; Wongeun, S.; Chatchawan, V. Review of Air Dispersion Modelling Approaches to Assess the Risk of Wind-Borne Spread of Foot-and-Mouth Disease Virus. *Journal of Environmental Protection* **2012**, *2012*.
[95] Holmes, N. S.; Morawska, L. A review of dispersion modelling and its application to the dispersion of particles: An overview of different dispersion models available. *Atmospheric Environment* **2006**, *40* (30), 5902-5928.
[96] Holmberg, S.; Li, Y. Modelling of the Indoor Environment – Particle Dispersion and Deposition. *Indoor Air* **1998**, *8* (2), 113-122.
[97] Zhou, Y.; Benson, J. M.; Irvin, C.; Irshad, H.; Cheng, Y.-S. Particle Size Distribution and Inhalation Dose of Shower Water under Selected Operating Conditions. *Inhalation Toxicology* **2007**, *19* (4), 333-342.
[98] Sunger, N.; Teske, S. S.; Nappier, S.; Haas, C. N. Recreational use assessment of water-based activities, using time-lapse construction cameras. *J Expo Sci Environ Epidemiol* **2012**, *22* (3), 281-90.
[99] Foekema, H.; Thiel, L. v.; Lettinga, B. *Watergebruik thuis 2007*; TNS NIPO: Amsterdam, **2008**.
[100] Buchanan, R. L.; Smith, J. L.; Long, W. Microbial risk assessment: dose-response relations and risk characterization. *International Journal of Food Microbiology* **2000**, *58* (3), 159-172.

[101] Haas, C. N. Estimation of Risk due to Low Doses of Microorganisms: A Comparison of Alternative Methodologies. *American Journal of Epidemiology* **1983**, *118* (4), 573-582.
[102] Couch, R. B.; Cate, T. R.; Douglas, R. G., Jr.; Gerone, P. J.; Knight, V. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriological Reviews* **1966**, *30* (3), 517-29.
[103] Crabtree, K. D.; Gerba, C. P.; Rose, J. B.; Haas, C. N. Waterborne adenovirus: a risk assessment. *Water Science and Technology* **1997**, *35* (11-12), 1-6.
[104] Medema, G. J.; Teunis, P. F. M.; Havelaar, A. H.; Haas, C. N. Assessment of the dose-response relationship of Campylobacter jejuni. *International Journal of Food Microbiology* **1996**, *30* (1-2), 101-111.
[105] Brooke, R. J.; Kretzschmar, M. E.; Mutters, N. T.; Teunis, P. F. Human dose response relation for airborne exposure to Coxiella burnetii. *BMC Infect Dis* **2013**, *13*, 488.
[106] Armstrong, T. W.; Haas, C. N. A Quantitative Microbial Risk Assessment Model for Legionnaires' Disease: Animal Model Selection and Dose-Response Modeling. *Risk Analysis* **2007**, *27* (6), 1581-1596.
[107] Teunis, P. F. M.; Chappell, C. L.; Okhuysen, P. C. Cryptosporidium Dose Response Studies: Variation Between Isolates. *Risk Analysis* **2002**, *22* (1), 175-185.
[108] Haas, C. N.; Rose, J. B.; Gerba, C. P.; Regli, S. Risk Assessment of Virus in Drinking Water. *Risk Analysis* **1993**, *13* (5), 545-552.
[109] Lammerding, A. M.; Fazil, A. Hazard identification and exposure assessment for microbial food safety risk assessment. *International Journal of Food Microbiology* **2000**, *58* (3), 147-157.
[110] Mara, D. D.; Sleigh, P. A.; Blumenthal, U. J.; Carr, R. M. Health risks in wastewater irrigation: comparing estimates from quantitative microbial risk analyses and epidemiological studies. *Journal of Water and Health* **2007**, *5* (1), 39-50.
[111] Karavarsamis, N.; Hamilton, A. J. Estimators of annual probability of infection for quantitative microbial risk assessment. *Journal of Water and Health* **2010**, *8* (2), 365-373.
[112] WHO. Global Burden of Disease 2004 Update: Disability Weights for Diseases and Conditions. **2004**.
[113] Nauta, M. J. Separation of uncertainty and variability in quantitative microbial risk assessment models. *International Journal of Food Microbiology* **2000**, *57* (1-2), 9-18.
[114] Frey, H. C.; Mokhtari, A.; Zheng, J. *Recommended Practice Regarding Selection, Application and Interpretation of Sensitivity Analysis Methods Applied to Food Safety Process Risk Models*; Office of Risk Assessment and Cost-Benefit Analysis, U.S. Department of Agriculture: Washington, DC, January 30, **2004**.
[115] Frey, H. C.; Patil, S. R. Identification and Review of Sensitivity Analysis Methods. *Risk Analysis* **2002**, *22* (3), 553-578.
[116] Anonymous. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. The Council of the European Union. Official Journal of the European Communities: Brussels, **1998**; 98/83/EC.
[117] Anonymous. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC as amended by Regulation 596/2009/EC. *2006/7/EC*, **2006**.
[118] Briesch, R. Opinion of the European Economic and Social Committee on the 'Proposal for a Directive of the European Parliament and of the Council concerning the quality of bathing water'. *Brussels*, Official Journal of the European Union: **2003**; 2003/C 220/09. pp 39-43.

[119] BOE. Real Decreto 1620/2007, de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas. BOE: Madrid, **2007**; 1620/2007. pp 50639-50661.
[120] Anonymous. Besluit van 23 mei 2011, houdende bepalingen inzake de productie en distributie van drinkwater en de organisatie van de openbare drinkwatervoorziening (Drinkwaterbesluit). Staatssecretaris van Infrastructuur en Milieu: **2011**; BJZ2011043266.
[121] Blumenthal, U. J.; Peasey, A.; Ruiz-Palacios, G.; Mara, D. *Guidelines for Wastewater Reuse in Agriculture and Aquaculture: Recommended Revisions Based on New Research Evidence*; Water and Environmental Health, London and Loughborogh: **2000**.
[122] Pilotto, L. S.; Douglas, R. M.; Burch, M. D.; Cameron, S.; Beers, M.; Rouch, G. J.; Robinson, P.; Kirk, M.; Cowie, C. T.; Hardiman, S.; Moore, C.; Attewell, R. G. Health effects of exposure to cyanobacteria (blue–green algae) during recreational water–related activities. *Australian and New Zealand Journal of Public Health* **1997**, *21* (6), 562-566.
[123] Stowa. Blauwalgen Protocol. **2012**.
[124] Van der Oost, R.; Ibelings, B. W.; Lurling, M. Feasibility of Fluorescence Analysis for Risk Assessment of Toxic Cyanobacteria in Recreational Waters. *Harmful Algae* **Submitted**.
[125] Edberg, S. C.; Rice, E. W.; Karlin, R. J.; Allen, M. J. Escherichia coli: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology* **2000**, *88* (S1), 106S-116S.
[126] Field, K. G.; Samadpour, M. Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research* **2007**, *41* (16), 3517-3538.
[127] Caldwell, J.; Payment, P.; Villemur, R. Mitochondrial DNA as Source Tracking Markers of Fecal Contamination. In *Microbial source tracking: methods, applications, and case studies*, Hagedorn, C.; Blanch, A. R.; Harwood, V. J., Eds. Springer: New York, **2011**; pp 229-250.
[128] Harwood, V. J.; Staley, C.; Badgley, B. D.; Borges, K.; Korajkic, A. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews* **2014**, *38* (1), 1-40.
[129] Bakir, M. A.; Sakamoto, M.; Kitahara, M.; Matsumoto, M.; Benno, Y. Bacteroides dorei sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **2006**, *56* (7), 1639-1643.
[130] Ahmed, W.; Stewart, J.; Powell, D.; Gardner, T. Evaluation of Bacteroides markers for the detection of human faecal pollution. *Letters in Applied Microbiology* **2008**, *46* (2), 237-242.
[131] Hua, J. S.; Zheng, P. Y.; Bow, H. Species differentiation and identification in the genus of Helicobacter. *World journal of gastroenterology* **1999**, *5* (1), 7-9.
[132] Green, H. C.; Dick, L. K.; Gilpin, B.; Samadpour, M.; Field, K. G. Genetic markers for rapid PCR-based identification of gull, Canada goose, duck, and chicken fecal contamination in water. *Applied and Enviornmental Microbiology* **2012**, *78* (2), 503-10.
[133] Heijnen, L.; Learbuch, K.; Kardinaal, E.; Rotteveel, S.; Ruiter, H.; Leenen, I. Fecale verontreiniging in zwemwater identificeren met DNA-merkers. *H2O* **2014**.
[134] Tambalo, D. D.; Boa, T.; Liljebjelke, K.; Yost, C. K. Evaluation of two quantitative PCR assays using Bacteroidales and mitochondrial DNA markers for tracking dog fecal contamination in waterbodies. *Journal of Microbiological Methods* **2012**, *91* (3), 459-467.

[135] Rusiñol, M.; Fernandez-Cassi, X.; Hundesa, A.; Vieira, C.; Kern, A.; Eriksson, I.; Ziros, P.; Kay, D.; Miagostovich, M.; Vargha, M.; Allard, A.; Vantarakis, A.; Wyn-Jones, P.; Bofill-Mas, S.; Girones, R. Application of human and animal viral microbial source tracking tools in fresh and marine waters from five different geographical areas. *Water Research* **2014**, *59* (0), 119-129.
[136] Schets, F. M.; van Wijnen, J. H.; Schijven, J. F.; Schoon, H.; de Roda Husman, A. M. Monitoring of Waterborne Pathogens in Surface Waters in Amsterdam, The Netherlands, and the Potential Health Risk Associated with Exposure to Cryptosporidium and Giardia in These Waters. *Applied and Enviornmental Microbiology* **2008**, *74* (7), 2069-2078.
[137] Ahmed, W.; Vieritz, A.; Goonetilleke, A.; Gardner, T. Health Risk from the Use of Roof-Harvested Rainwater in Southeast Queensland, Australia, as Potable or Nonpotable Water, Determined Using Quantitative Microbial Risk Assessment. *Applied and Enviornmental Microbiology* **2010**, *76* (22), 7382-7391.
[138] Fewtrell, L.; Kay, D.; Watkins, J.; Davies, C.; Francis, C. The microbiology of urban UK floodwaters and a quantitative microbial risk assessment of flooding and gastrointestinal illness. *Journal of Flood Risk Management* **2011**, *4* (2), 77-87.
[139] McBride, G. B.; Stott, R.; Miller, W.; Bambic, D.; Wuertz, S. Discharge-based QMRA for estimation of public health risks from exposure to stormwater-borne pathogens in recreational waters in the United States. *Water Research* **2013**, *47* (14), 5282-5297.
[140] Ottoson, J.; Stenström, T. A. Faecal contamination of greywater and associated microbial risks. *Water Research* **2003**, *37* (3), 645-655.
[141] Petterson, S. R.; Ashbolt, N. J.; Sharma, A. Microbial Risks from Wastewater Irrigation of Salad Crops: A Screening-Level Risk Assessment. *Water Environment Research* **2001**, *73* (6), 667-672.
[142] Westrell, T.; Schonning, C.; Stenstrom, T. A.; Ashbolt, N. J. QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis and critical control points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Science & Technology* **2004**, *50* (2), 23-30.
[143] Hamilton, A. J.; Stagnitti, F.; Premier, R.; Boland, A.-M.; Hale, G. Quantitative Microbial Risk Assessment Models for Consumption of Raw Vegetables Irrigated with Reclaimed Water. *Applied and Environmental Microbiology* **2006**, *72* (5), 3284-3290.
[144] Mara, D.; Sleigh, A. Estimation of norovirus infection risks to consumers of wastewater-irrigated food crops eaten raw. *Journal of Water and Health* **2010**, *8* (1), 39-43.
[145] Ayuso-Gabella, N.; Page, D.; Masciopinto, C.; Aharoni, A.; Salgot, M.; Wintgens, T. Quantifying the effect of Managed Aquifer Recharge on the microbiological human health risks of irrigating crops with recycled water. *Agricultural Water Management* **2011**, *99* (1), 93-102.
[146] Katukiza, A. Y.; Ronteltap, M.; van der Steen, P.; Foppen, J. W. A.; Lens, P. N. L. Quantification of microbial risks to human health caused by waterborne viruses and bacteria in an urban slum. *Journal of Applied Microbiology* **2014**, *116* (2), 447-463.
[147] Zaneti, R.; Etchepare, R.; Rubio, J. More environmentally friendly vehicle washes: water reclamation. *Journal of Cleaner Production* **2012**, *37* (0), 115-124.
[148] Irvine, K.; Fuller, R.; Devine-Wright, P.; Tratalos, J.; Payne, S.; Warren, P.; Lomas, K.; Gaston, K. Ecological and Psychological Value of Urban Green Space. In *Dimensions of the Sustainable City*, Jenks, M.; Jones, C., Eds. Springer Netherlands: **2010**; Vol. 2, pp 215-237.

[149] Ashley, R. M.; Balmforth, D. J.; Saul, A. J.; Blanskby, J. D. Flooding in the future - predicting climate change, risks and responses in urban areas. *Water Science and Technology* **2005**, *52* (5), 265-273.
[150] Fewtrell, L.; Kay, D. *Health Impact Assessment for Sustainable Water Management*. IWA Publishing: London, **2008**.
[151] Schets, F. M.; Engels, G. B.; Evers, E. G. Cryptosporidium and Giardia in swimming pools in the Netherlands. *Journal of Water and Health* **2004**, *2* (3), 191-200.
[152] ten Veldhuis, J. A. E.; Clemens, F. H. L. R.; Sterk, G.; Berends, B. R. Microbial risks associated with exposure to pathogens in contaminated urban flood water. *Water Research* **2010**, *44* (9), 2910-2918.
[153] Oesterholt, F.; Martijnse, G.; Medema, G.; Kooij, D. v. d. Health risk assessment of non-potable domestic water supplies in the Netherlands. *Journal of Water Supply: Research and Technology-AQUA* **2007**, *56* (3), 171-179.
[154] USEPA; USDA *Microbial Risk Assessment Guideline. Pathogenic Microorganisms with Focus on Food and Water*; US EPA: **2012**.
[155] USEPA *Framework for Human Health Risk Assessment to Inform Decision Making*; USEPA: **2014**.
[156] Westrell, T.; Teunis, P.; van den Berg, H.; Lodder, W.; Ketelaars, H.; Stenström, T. A.; de Roda Husman, A. M. Short- and long-term variations of norovirus concentrations in the Meuse river during a 2-year study period. *Water Research* **2006**, *40* (14), 2613-2620.
[157] Schijven, J. F.; Teunis, P. F. M.; Rutjes, S. A.; Bouwknegt, M.; de Roda Husman, A. M. QMRAspot: A tool for Quantitative Microbial Risk Assessment from surface water to potable water. *Water Research* **2011**, *45* (17), 5564-5576.
[158] de Roda Husman, A. M.; Ketelaars, H.; Lodder, W. J.; Medema, G.; Schets, F. M. *De microbiologische kwaliteit van het ingenomen en afgeleverde water van Waterwinningbedrijf Brabantse Biesbosch in 2001*; RIVM: Bilthoven, **2006**; p 60.
[159] Wullings, B.; Veenendaal, H.; Kooij, D. v. d. Legionella pneumophila komt sporadisch voor in Nederlands oppervlaktewater. *H2O* **2009**, *42* (7), 44-46.
[160] van der Wielen, P. W. J. J.; van der Kooij, D. Nontuberculous Mycobacteria, Fungi, and Opportunistic Pathogens in Unchlorinated Drinking Water in the Netherlands. *Applied and Environmental Microbiology* **2013**, *79* (3), 825-834.
[161] Albrechtsen, H.-J. Microbiological investigations of rainwater and graywater collected for toilet flushing. *Water Science and Technology* **2002**, *46* (6-7), 311-316.
[162] Olsen, J. S.; Aarskaug, T.; Thrane, I.; Pourcel, C.; Ask, E.; Johansen, G.; Waagen, V.; Blatny, J. M. Alternative Routes for Dissemination of Legionella pneumophila Causing Three Outbreaks in Norway. *Environmental Science & Technology* **2010**, *44* (22), 8712-8717.
[163] Drechsel, P.; Scott, C. A.; Raschid-Sally, L.; Redwood, M.; Bahri, A. *Wastewater Iirrigation and Health. Assessing and Mitigating Risk in Low-income Countries*. Earthscan: London, **2010**.
[164] Diederen, B. M.; de Jong, C. M.; Aarts, I.; Peeters, M. F.; van der Zee, A. Molecular evidence for the ubiquitous presence of Legionella species in Dutch tap water installations. *Journal of Water and Health* **2007**, *5* (3), 375-383.
[165] Donovan, E.; Unice, K.; Roberts, J. D.; Harris, M.; Finley, B. Risk of Gastrointestinal Disease Associated with Exposure to Pathogens in the Water of the Lower Passaic River. *Applied and Enviornmental Microbiology* **2008**, *74* (4), 994-1003.

[166] Simon, X.; Duquenne, P.; Koehler, V.; Piernot, C.; Coulais, C.; Faure, M. Aerosolisation of Escherichia coli and associated endotoxin using an improved bubbling bioaerosol generator. *Journal of Aerosol Science* **2011**, *42* (8), 517-531.
[167] Sales-Ortells, H.; Medema, G. Screening-level risk assessment of Coxiella burnetii (Q fever) transmission via aeration of drinking water. *Environmental Science & Technology* **2012**, *46*, 4125-4133.
[168] Soller, J. A.; Bartrand, T.; Ashbolt, N. J.; Ravenscroft, J.; Wade, T. J. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Research* **2010**, *44* (16), 4736-4747.
[169] Soller, J. A.; Schoen, M. E.; Bartrand, T.; Ravenscroft, J. E.; Ashbolt, N. J. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research* **2010**, *44* (16), 4674-4691.
[170] Schoen, M. E.; Ashbolt, N. J. Assessing Pathogen Risk to Swimmers at Non-Sewage Impacted Recreational Beaches. *Environmental Science & Technology* **2010**, *44* (7), 2286-2291.
[171] Teunis, P.; Havelaar, A. H. *Cryptosporidium in Drinking Water: Evaluation of the ILSI/RSI Quantitative Risk Assessment Framework*; RIVM: Bilthoven, **1999**; p 108.
[172] Pouillot, R.; Beaudeau, P.; Denis, J.-B.; Derouin, F.; Group, A. C. S. A Quantitative Risk Assessment of Waterborne Cryptosporidiosis in France Using Second-Order Monte Carlo Simulation. *Risk Analysis* **2004**, *24* (1), 1-17.
[173] Makri, A.; Modarres, R.; Parkin, R. Cryptosporidiosis Susceptibility and Risk: A Case Study. *Risk Analysis* **2004**, *24* (1), 209-220.
[174] Atmar, R. L.; Opekun, A. R.; Gilger, M. A.; Estes, M. K.; Crawford, S. E.; Neill, F. H.; Ramani, S.; Hill, H.; Ferreira, J.; Graham, D. Y. Determination of the Human Infectious Dose-50% for Norwalk Virus. *Journal of Infectious Diseases* **2013**.
[175] Armstrong, T. W.; Haas, C. N. Legionnaires' disease: evaluation of a quantitative microbial risk assessment model. *Journal of Water and Health* **2008**, *6* (2), 149-66.
[176] Zwietering, M. H.; Gerwen, S. J. C. v. Sensitivity Analysis in Quantitative Microbial Risk Assessment. *International Journal of Food Microbiology* **2000**, *58*, 213-221.
[177] *R: A language and environment for statistical computing.*, 3.0.1; R Foundation for Statistical Computing: **2013**.
[178] Dorevitch, S.; Pratap, P.; Wroblewski, M.; Hryhorczuk, D. O.; Li, H.; Liu, L. C.; Scheff, P. A. Health Risks of Limited-Contact Water Recreation. *Environ Health Perspect* **2012**, *120* (2).
[179] Sales-Ortells, H.; Medema, G. Screening-Level Microbial Risk Assessment of Urban Water Locations: A Tool for Prioritization. *Environmental Science & Technology* **2014**, *48* (16), 9780-9789.
[180] Schets, F. M.; Italiaander, R.; van den Berg, H. H.; de Roda Husman, A. M. Rainwater harvesting: quality assessment and utilization in The Netherlands. *Journal of Water and Health* **2010**, *8* (2), 224-35.
[181] Veenendaal, H. R.; Brouwer-Hanzens, A. J. *A Method for the Concentration of Microbes in Large Volumes of Water*; European Comission: **2007**.
[182] Heijnen, L.; Medema, G. Quantitative detection of E. coli, E. coli O157 and other shiga toxin producing E. coli in water samples using a culture method combined with real-time PCR. *Journal of Water and Health* **2006**, *04.4*, 487-496.

[183] Guy, R. A.; Payment, P.; Krull, U. J.; Horgen, P. A. Real-Time PCR for Quantification of Giardia and Cryptosporidium in Environmental Water Samples and Sewage. *Applied and Environmental Microbiology* **2003**, *69* (9), 5178-5185.
[184] Ko, G.; Jothikumar, N.; Hill, V. R.; Sobsey, M. D. Rapid detection of infectious adenoviruses by mRNA real-time RT-PCR. *Journal of Virological Methods* **2005**, *127* (2), 148-153.
[185] Wullings, B. A.; Bakker, G.; van der Kooij, D. Concentration and Diversity of Uncultured Legionella spp. in Two Unchlorinated Drinking Water Supplies with Different Concentrations of Natural Organic Matter. *Applied and Environmental Microbiology* **2011**, *77* (2), 634-641.
[186] KNMI. Daily Weather Data of The Netherlands. http://www.knmi.nl/climatology/daily_data/download.html.
[187] Waldenstrom, J.; Broman, T.; Carlsson, I.; Hasselquist, D.; Achterberg, R. P.; Wagenaar, J. A.; Olsen, B. Prevalence of Campylobacter jejuni, Campylobacter lari, and Campylobacter coli in different ecological guilds and taxa of migrating birds. *Applied and Environmental Microbiology* **2002**, *68* (12), 5911-7.
[188] Baker, J.; Barton, M. D.; Lanser, J. Campylobacter species in cats and dogs in South Australia. *Australian Veterinary Journal* **1999**, *77* (10), 662-666.
[189] Albinana-Gimenez, N.; Clemente-Casares, P.; Bofill-Mas, S.; Hundesa, A.; Ribas, F.; Girones, R. Distribution of Human Polyoma- viruses, Adenoviruses, and Hepatitis E Virus in the Environment and in a Drinking-Water Treatment Plant†. *Environmental Science & Technology* **2006**, *40* (23), 7416-7422.
[190] Bofill-Mas, S.; Calgua, B.; Clemente-Casares, P.; La Rosa, G.; Iaconelli, M.; Muscillo, M.; Rutjes, S.; de Roda Husman, A.; Grunert, A.; Gräber, I.; Verani, M.; Carducci, A.; Calvo, M.; Wyn-Jones, P.; Girones, R. Quantification of Human Adenoviruses in European Recreational Waters. *Food and Environmental Virology* **2010**, *2* (2), 101-109.
[191] Ye, X.; Ming, X.; Zhang, Y.; Xiao, W.; Huang, X.; Cao, Y.; Gu, K. Real-Time PCR Detection of Enteric Viruses in Source Water and Treated Drinking Water in Wuhan, China. *Current Microbiology* **2012**, *65* (3), 244-253.
[192] Haramoto, E.; Kitajima, M.; Katayama, H.; Ohgaki, S. Real-time PCR detection of adenoviruses, polyomaviruses, and torque teno viruses in river water in Japan. *Water Research* **2010**, *44* (6), 1747-1752.
[193] Fong, T.-T.; Phanikumar, M. S.; Xagoraraki, I.; Rose, J. B. Quantitative Detection of Human Adenoviruses in Wastewater and Combined Sewer Overflows Influencing a Michigan River. *Applied and Environmental Microbiology* **2010**, *76* (3), 715-723.
[194] Choi, S.; Jiang, S. C. Real-Time PCR Quantification of Human Adenoviruses in Urban Rivers Indicates Genome Prevalence but Low Infectivity. *Applied and Environmental Microbiology* **2005**, *71* (11), 7426-7433.
[195] van Heerden, J.; Ehlers, M. M.; Heim, A.; Grabow, W. O. K. Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. *Journal of Applied Microbiology* **2005**, *99* (2), 234-242.
[196] van Heijnsbergen, E.; de Roda Husman, A. M.; Lodder, W. J.; Bouwknegt, M.; Docters van Leeuwen, A. E.; Bruin, J. P.; Euser, S. M.; den Boer, J. W.; Schalk, J. A. C. Viable Legionella pneumophila bacteria in natural soil and rainwater puddles. *Journal of Applied Microbiology* **2014**, *117* (3), 882-890.
[197] Kardinaal, W. E. A.; Janse, I.; Kamst-van Agterveld, M.; Meima, M.; Snoek, J.; Mur, L. R.; Huisman, J.; Zwart, G.; Visser, P. M. Microcystis genotype succession in

relation to microcystin concentrations in freshwater lakes. *Aquatic microbial ecology* **2007**, *48* (1), 1-12.
[198] McCarthy, D. T.; Mitchell, V. G.; Deletic, A.; Diaper, C. Escherichia coli in urban stormwater: explaining their variability. *Water Sci Technol* **2007**, *56* (11), 27-34.
[199] White, A. J.; Kinlin, L.; Johnson, C.; Spain, C. V.; Ng, V.; Fisman, D. Environmental Determinants of Campylobacteriosis Risk in Philadelphia from 1994 to 2007. *EcoHealth* **2009**, *6* (2), 200-208.
[200] Karagiannis, I.; Brandsema, P.; van der Sande, M. Warm, wet weather associated with increased Legionnaires' disease incidence in The Netherlands. *Epidemiology & Infection* **2009**, *137* (02), 181-187.
[201] Gerba, C. P. Virus occurrence and survival in the environmental waters. In *Human viruses in water*, Bosch, A., Ed. **2007**; Vol. 17, pp 91-108.
[202] Paerl, H. W.; Paul, V. J. Climate change: Links to global expansion of harmful cyanobacteria. *Water Research* **2012**, *46* (5), 1349-1363.
[203] De Roda Husman, A. M.; Schets, F. M. *Climate change and recreational water-related infectious diseases*; 330400002/2010; RIVM: Bilthoven, **2010**.
[204] Marsalek, J.; Rochfort, Q. Urban wet-weather flows: sources of fecal contamination impacting on recreational waters and threatening drinking-water sources. *Journal of Toxicology and Environmental Health, Part A: Current Issues* **2004**, *67* (20-22), 1765-77.
[205] Sidhu, J. P. S.; Hodgers, L.; Ahmed, W.; Chong, M. N.; Toze, S. Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia. *Water Research* **2012**, *46*, 6652-6660.
[206] Ahmed, W.; Huygens, F.; Goonetilleke, A.; Gardner, T. Real-Time PCR Detection of Pathogenic Microorganisms in Roof-Harvested Rainwater in Southeast Queensland, Australia. *Applied and Environmental Microbiology* **2008**, *74* (17), 5490-5496.
[207] Ahmed, W.; Hodgers, L.; Sidhu, J. P. S.; Toze, S. Fecal Indicators and Zoonotic Pathogens in Household Drinking Water Taps Fed from Rainwater Tanks in Southeast Queensland, Australia. *Applied and Environmental Microbiology* **2012**, *78* (1), 219-226.
[208] Hagedorn, C.; Blanch, A. R.; Harwood, V. J. *Microbial source tracking: methods, applications, and case studies*. Springer: New York, **2011**; p 642.
[209] Ahmed, W.; Sidhu, J. P. S.; Toze, S. An Attempt to Identify the Likely Sources of Escherichia coli Harboring Toxin Genes in Rainwater Tanks. *Environmental Science & Technology* **2012**, *46* (9), 5193-5197.
[210] Sauer, E. P.; VandeWalle, J. L.; Bootsma, M. J.; McLellan, S. L. Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Research* **2011**, *45* (14), 4081-4091.
[211] Van De Werfhorst, L. C.; Murray, J. L.; Reynolds, S.; Reynolds, K.; Holden, P. A. Canine Scent Detection and Microbial Source Tracking of Human Waste Contamination in Storm Drains. **2014**.
[212] Colford, J. M. J.; Wade, T. J.; Schiff, K. C.; Wright, C. C.; Griffith, J. F.; Sandhu, S. K.; Burns, S.; Sobsey, M.; Lovelace, G.; Weisberg, S. B. Water Quality Indicators and the Risk of Illness at Beaches With Nonpoint Sources of Fecal Contamination. *Epidemiology* **2007**, *18* (1), 27-35.
[213] Staley, C.; Gordon, K. V.; Schoen, M. E.; Harwood, V. J. Performance of two quantitative PCR methods for microbial source tracking of human sewage and implications

for microbial risk assessment in recreational waters. *Applied and Enviornmental Microbiology* **2012**, *78* (20), 7317-26.
[214] Rodenburg, A.; Doelder, B. d. Soil and land. Water plaza Bellamyplein; innovation and sustainable water storage (Bodem en ruimtelijke ordeling. Waterplein Bellamyplein; innovatie en duurzame waterberging). *Bodem* August 2013, **2013**, pp 12-13.
[215] Krentz, C. A.; Prystajecky, N.; Isaac-Renton, J. Identification of fecal contamination sources in water using host-associated markers. *Canadidan Journal of Microbiology* **2013**, *59* (3), 210-20.
[216] Hellein, K. N.; Battie, C.; Tauchman, E.; Lund, D.; Oyarzabal, O. A.; Lepo, J. E. Culture-based indicators of fecal contamination and molecular microbial indicators rarely correlate with Campylobacter spp. in recreational waters. *Journal of Water and Health* **2011**, *9* (4), 695-707.
[217] Smith, K.; Fewtrell, L.; Kay, D. *Examination of the microbiology of urban flood water components*; University of Wales: Aberystwyth, **2007**.
[218] Fisman, D. Seasonality of viral infections: mechanisms and unknowns. *Clinical Microbiology and Infection* **2012**, *18* (10), 946-954.
[219] Simmons, G.; Jury, S.; Thornley, C.; Harte, D.; Mohiuddin, J.; Taylor, M. A Legionnaires' disease outbreak: A water blaster and roof-collected rainwater systems. *Water Research* **2008**, *42* (6–7), 1449-1458.
[220] Mooij, W.; Hülsmann, S.; De Senerpont Domis, L.; Nolet, B.; Bodelier, P.; Boers, P.; Pires, L.; Gons, H.; Ibelings, B.; Noordhuis, R.; Portielje, R.; Wolfstein, K.; Lammens, E. The impact of climate change on lakes in the Netherlands: a review. *Aquatic Ecology* **2005**, *39* (4), 381-400.
[221] Birks, R.; Colbourne, J.; Hills, S.; Hobson, R. Microbiological water quality in a large in-building, water recycling facility. *Water Science & Technology* **2004**, *50* (2), 165-172.
[222] Selvakumar, A.; Borst, M. Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *Journal of Water and Health* **2006**, *4* (1), 109-24.
[223] Girones, R.; Ferrús, M. A.; Alonso, J. L.; Rodriguez-Manzano, J.; Calgua, B.; de Abreu Corrêa, A.; Hundesa, A.; Carratala, A.; Bofill-Mas, S. Molecular detection of pathogens in water – The pros and cons of molecular techniques. *Water Research* **2010**, *44* (15), 4325-4339.
[224] Benskin, C. M.; Wilson, K.; Jones, K.; Hartley, I. R. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews* **2009**, *84* (3), 349-373.
[225] Iglesias, R.; Ortega, E.; Batanero, G.; Quintas, L. Water reuse in Spain: Data overview and costs estimation of suitable treatment trains. *Desalination* **2010**, *263* (1–3), 1-10.
[226] Montemayor, M.; Costan, A.; Lucena, F.; Jofre, J.; Munoz, J.; Dalmau, E.; Mujeriego, R.; Sala, L. The combined performance of UV light and chlorine during reclaimed water disinfection. *Water Science & Technology* **2008**, *57* (6), 935-40.
[227] Shuval, H.; Lampert, Y.; Fattal, B. Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Water Science and Technology* **1997**, *35* (11–12), 15-20.
[228] Stine, S. W.; Song, I.; Choi, C. Y.; Gerba, C. P. Application of Microbial Risk Assessment to the Development of Standards for Enteric Pathogens in Water Used To Irrigate Fresh Produce. *Journal of Food Protection* **2005**, *68* (5), 913-918.

[229] Seidu, R.; Heistad, A.; Amoah, P.; Drechsel, P.; Jenssen, P. D.; Stenstrom, T. A. Quantification of the health risk associated with wastewater reuse in Accra, Ghana: a contribution toward local guidelines. *Journal of Water and Health* **2008**, *6* (4), 461-71.
[230] Mara, D.; Sleigh, A. Estimation of norovirus and Ascaris infection risks to urban farmers in developing countries using wastewater for crop irrigation. *Journal of Water and Health* **2010**, *8* (3), 572-6.
[231] Petterson, S. R.; Ashbolt, N. J.; Sharma, A. Of: Microbial risks from wastewater irrigation of salad crops: a screening-level risk assessment. *Water Environment Research* **2002**, *74* (4), 411-411.
[232] Carratalà, A.; Rodriguez-Manzano, J.; Hundesa, A.; Rusiñol, M.; Fresno, S.; Cook, N.; Girones, R. Effect of temperature and sunlight on the stability of human adenoviruses and MS2 as fecal contaminants on fresh produce surfaces. *International Journal of Food Microbiology* **2013**, *164* (2–3), 128-134.
[233] Hirneisen, K. A.; Kniel, K. E. Norovirus Surrogate Survival on Spinach During Preharvest Growth. *Phytopathology* **2013**, *103* (4), 389-394.
[234] Martínez, A.; Torner, N.; Broner, S.; Bartolomé, R.; Guix, S.; de Simón, M.; Godoy, P.; Moreno, A.; Company, M.; Balanyà, P. J.; Sala, M. R.; Torra, R.; Ferrús, G.; Parrón, I.; Barrabeig, I.; Domínguez, A. Norovirus: a growing cause of gastroenteritis in catalonia (Spain)? *Journal of Food Protection* **2013**, *76* (10), 1810-1816.
[235] Dicaprio, E.; Ma, Y.; Purgianto, A.; Hughes, J.; Li, J. Internalization and dissemination of human norovirus and animal caliciviruses in hydroponically grown romaine lettuce. *Applied and Enviornmental Microbiology* **2012**, *78* (17), 6143-52.
[236] Esseili, M. A.; Wang, Q.; Zhang, Z.; Saif, L. J. Internalization of sapovirus, a surrogate for norovirus, in romaine lettuce and the effect of lettuce latex on virus infectivity. *Applied and Enviornmental Microbiology* **2012**, *78* (17), 6271-9.
[237] Wei, J.; Jin, Y.; Sims, T.; Kniel, K. E. Internalization of Murine Norovirus 1 by Lactuca sativa during Irrigation. *Applied and Environmental Microbiology* **2011**, *77* (7), 2508-2512.
[238] Lodder, W. J.; de Roda Husman, A. M. Presence of Noroviruses and Other Enteric Viruses in Sewage and Surface Waters in The Netherlands. *Applied and Environmental Microbiology* **2005**, *71* (3), 1453-1461.
[239] Katayama, H.; Haramoto, E.; Oguma, K.; Yamashita, H.; Tajima, A.; Nakajima, H.; Ohgaki, S. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. *Water Research* **2008**, *42* (6–7), 1441-1448.
[240] Ethelberg, S.; Lisby, M.; Bottiger, B.; Schultz, A. C.; Villif, A.; Jensen, T.; Olsen, K. E.; Scheutz, F.; Kjelso, C.; Muller, L. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. *Eurosurveillance* **2010**, *15* (6).
[241] Gallimore, C. I.; Pipkin, C.; Shrimpton, H.; Green, A. D.; Pickford, Y.; McCartney, C.; Sutherland, G.; Brown, D. W.; Gray, J. J. Detection of multiple enteric virus strains within a foodborne outbreak of gastroenteritis: an indication of the source of contamination. *Epidemiology and Infection* **2005**, *133* (1), 41-7.
[242] Makary, P.; Maunula, L.; Niskanen, T.; Kuusi, M.; Virtanen, M.; Pajunen, S.; Ollgren, J.; Tran Minh, N. N. Multiple norovirus outbreaks among workplace canteen users in Finland, July 2006. *Epidemiology and Infection* **2009**, *137* (3), 402-7.
[243] Wadl, M.; Scherer, K.; Nielsen, S.; Diedrich, S.; Ellerbroek, L.; Frank, C.; Gatzer, R.; Hoehne, M.; Johne, R.; Klein, G.; Koch, J.; Schulenburg, J.; Thielbein, U.; Stark, K.;

Bernard, H. Food-borne norovirus-outbreak at a military base, Germany, 2009. *BMC Infectious Diseases* **2010**, *10*, 30.
[244] Rutjes, S. A.; van den Berg, H. H. J. L.; Lodder, W. J.; de Roda Husman, A. M. Real-Time Detection of Noroviruses in Surface Water by Use of a Broadly Reactive Nucleic Acid Sequence-Based Amplification Assay. *Applied and Environmental Microbiology* **2006**, *72* (8), 5349-5358.
[245] Calgua, B.; Fumian, T.; Rusiñol, M.; Rodriguez-Manzano, J.; Mbayed, V. A.; Bofill-Mas, S.; Miagostovich, M.; Girones, R. Detection and quantification of classic and emerging viruses by skimmed-milk flocculation and PCR in river water from two geographical areas. *Water Research* **2013**, *47* (8), 2797-2810.
[246] Loisy, F.; Atmar, R. L.; Guillon, P.; Le Cann, P.; Pommpepuy, M.; Le Guyader, F. S. Real-time RT-PCR for norovirus screening in shellfish. *Journal of Virological Methods* **2005**, *123*, 1-7.
[247] Kageyama, T.; Kojima, S.; Shinohara, M.; Uchida, K.; Fukushi, S.; Hoshino, F. B.; Takeda, N.; Katayama, K. Broadly Reactive and Highly Sensitive Assay for Norwalk-Like Viruses Based on Real-Time Quantitative Reverse Transcription-PCR. *Journal of Clinical Microbiology* **2003**, *41* (4), 1548-1557.
[248] Calgua, B.; Rodriguez-Manzano, J.; Hundesa, A.; Suñen, E.; Calvo, M.; Bofill-Mas, S.; Girones, R. New methods for the concentration of viruses from urban sewage using quantitative PCR. *Journal of Virological Methods* **2013**, *187* (2), 215-221.
[249] Tanaka, H.; Asano, T.; Schroeder, E. D.; Tchobanoglous, G. Estimating the safety of wastewater reclamation and reuse using enteric virus monitoring data. *Water Environment Research* **1998**, *70* (1), 39-51.
[250] Sobsey, M. D.; Battigelli, D. A.; Shin, G. A.; Newland, S. RT-PCR amplification detects inactivated viruses in water and wastewater. *Water Science and Technology* **1998**, *38* (12), 91-94.
[251] Hijnen, W. A. M.; Beerendonk, E. F.; Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research* **2006**, *40* (1), 3-22.
[252] Tree, J. A.; Adams, M. R.; Lees, D. N. Virus inactivation during disinfection of wastewater by chlorination and UV irradiation and the efficacy of F+ bacteriophage as a 'viral indicator'. *Water Science and Technology* **1997**, *35* (11–12), 227-232.
[253] Tree, J. A.; Adams, M. R.; Lees, D. N. Chlorination of Indicator Bacteria and Viruses in Primary Sewage Effluent. *Applied and Environmental Microbiology* **2003**, *69* (4), 2038-2043.
[254] Mok, H.-F.; Hamilton, A. J. Exposure Factors for Wastewater-Irrigated Asian Vegetables and a Probabilistic Rotavirus Disease Burden Model for Their Consumption. *Risk Analysis* **2014**, *34* (4), 602-6013.
[255] Warriner, K.; Ibrahim, F.; Dickinson, M.; Wright, C.; Waites, W. M. Internalization of human pathogens within growing salad vegetables. *Biotechnology & genetic engineering reviews* **2003**, *20*, 117-134.
[256] Hirneisen, K. A.; Sharma, M.; Kniel, K. E. Human enteric pathogen internalization by root uptake into food crops. *Foodborne Pathogens and Disease* **2012**, *9* (5), 396-405.
[257] AESAN. *Encuesta Nacional de Ingesta Dietética Española (ENIDE)*. **2011**.
[258] Atmar, R. L.; Opekun, A. R.; Gilger, M. A.; Estes, M. K.; Crawford, S. E.; Neill, F. H.; Ramani, S.; Hill, H.; Ferreira, J.; Graham, D. Y. Determination of the 50% Human Infectious Dose for Norwalk Virus. *Journal of Infectious Diseases* **2014**, *209* (7), 1016-1022.

[259] Kemmeren, J. M.; Mangen, M.-J. J.; Duynhoven, Y. v.; Havelaar, A. H. *Priority setting of foodborne pathogens: Disease burden and costs of selected enteric pathogens*; RIVM: Bilthoven (The Netherlands), **2006**.
[260] Domínguez, A.; Torner, N.; Ruíz, L.; Martínez, A.; Barrabeig, I.; Camps, N.; Godoy, P.; Minguell, S.; Parrón, I.; Pumarés, A.; Sala, M. R.; Bartolomé, R.; Pérez, U.; de Simón, M.; Montava, R.; Buesa, J. Aetiology and epidemiology of viral gastroenteritis outbreaks in Catalonia (Spain) in 2004–2005. *Journal of Clinical Virology* **2008**, *43* (1), 126-131.
[261] Esseili, M. A.; Wang, Q.; Saif, L. J. Binding of human GII.4 norovirus virus-like particles to carbohydrates of romaine lettuce leaf cell wall materials. *Applied and Enviornmental Microbiology* **2012**, *78* (3), 786-94.
[262] Vega, E.; Smith, J.; Garland, J.; Matos, A.; Pillaii, S. D. Variability of virus attachment patterns to butterhead lettuce. *Journal of Food Protection* **2005**, *68* (10), 2112-7.
[263] Bae, J.; Schwab, K. J. Evaluation of Murine Norovirus, Feline Calicivirus, Poliovirus, and MS2 as Surrogates for Human Norovirus in a Model of Viral Persistence in Surface Water and Groundwater. *Applied and Environmental Microbiology* **2008**, *74* (2), 477-484.
[264] Richards, G. Critical Review of Norovirus Surrogates in Food Safety Research: Rationale for Considering Volunteer Studies. *Food and Environmental Virology* **2012**, *4* (1), 6-13.
[265] Lamhoujeb, S.; Fliss, I.; Ngazoa, S. E.; Jean, J. Evaluation of the Persistence of Infectious Human Noroviruses on Food Surfaces by Using Real-Time Nucleic Acid Sequence-Based Amplification. *Applied and Environmental Microbiology* **2008**, *74* (11), 3349-3355.
[266] Topping, J. R.; Schnerr, H.; Haines, J.; Scott, M.; Carter, M. J.; Willcocks, M. M.; Bellamy, K.; Brown, D. W.; Gray, J. J.; Gallimore, C. I.; Knight, A. I. Temperature inactivation of Feline calicivirus vaccine strain FCV F-9 in comparison with human noroviruses using an RNA exposure assay and reverse transcribed quantitative real-time polymerase chain reaction—A novel method for predicting virus infectivity. *Journal of Virological Methods* **2009**, *156* (1–2), 89-95.
[267] Mormann, S.; Dabisch, M.; Becker, B. Effects of Technological Processes on the Tenacity and Inactivation of Norovirus Genogroup II in Experimentally Contaminated Foods. *Applied and Environmental Microbiology* **2010**, *76* (2), 536-545.
[268] Sano, D.; Pintó, R. M.; Omura, T.; Bosch, A. Detection of Oxidative Damages on Viral Capsid Protein for Evaluating Structural Integrity and Infectivity of Human Norovirus. *Environmental Science & Technology* **2009**, *44* (2), 808-812.
[269] Marrie, T. J. Q fever - a review. *The Canadian Veterinary Journal* **1990**, *31* (8), 555-63.
[270] Hunink, J. E.; Veenstra, T.; Hoek, W. v. d.; Droogers, P. *Q fever transmission to humans and local environmental conditions*; Future Water, RIVM: **2010**.
[271] Moel, P. J. d.; Verberk, J. Q. J. C.; Dijk, J. C. v. *Drinking Water: Principles and Practices*. **2006**; p 413.
[272] Arricau-Bouvery, N.; Souriau, A.; Lechopier, P.; Rodolakis, A. Experimental Coxiella burnetii infection in pregnant goats: excretion routes. *Veterinary Research* **2003**, *34* (4), 423-433.

[273] Schimmer, B.; Morroy, G.; Dijkstra, F.; Schneeberger, P. M.; Weers-Pothoff, G.; Timen, A.; Wijkmans, C.; van der Hoek, W. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. *Euro surveillance* **2008**, *13* (31).
[274] Hogerwerf, L.; van den Brom, R.; Roest, H. I.; Bouma, A.; Vellema, P.; Pieterse, M.; Dercksen, D.; Nielen, M. Reduction of Coxiella burnetii prevalence by vaccination of goats and sheep, The Netherlands. *Emerging Infectious Diseases* **2011**, *17* (3), 379-86.
[275] Benson, W. W.; Brock, D. W.; Mather, J. Serologic Analysis of a Penitentiary Group Using Raw Milk from a Q Fever Infected Herd. *Public Health Reports* **1963**, *78*, 707-710.
[276] KNMI. Daily Weather Data of the Netherlands. http://www.knmi.nl/climatology/daily_data/download.html.
[277] Miller, J. D.; Shaw, E. I.; Thomson, H. A. Coxiella burnetii, Q Fever and Bioterrorism. In *Microorganisms and Bioterrorism*, Anderson, B.; Friedman, H.; Bendinelli, M., Eds. Springer US: **2006**; pp 181-208.
[278] Sanford, S. E.; Josephson, G. K.; MacDonald, A. Coxiella burnetii (Q fever) abortion storms in goat herds after attendance at an annual fair. *The Canadian Veterinary Journal* **1994**, *35* (6), 376-378.
[279] Berri, M.; Rousset, E.; Champion, J. L.; Russo, P.; Rodolakis, A. Goats may experience reproductive failures and shed Coxiella burnetii at two successive parturitions after a Q fever infection. *Research in Veterinary Science* **2007**, *83* (1), 47-52.
[280] Schimmer, B.; ter Schegget, R.; Wegdam, M.; Zuchner, L.; de Bruin, A.; Schneeberger, P.; Veenstra, T.; Vellema, P.; van der Hoek, W. The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases* **2010**, *10* (1), 69.
[281] Schulz, J.; Runge, M.; Schroder, C.; Ganter, M.; Hartung, J. Coxiella burnetii isolated from the air of a sheep barn when sheared. *Deutsche tierärztliche Wochenschrift* **2005**, *112* (12), 470-472.
[282] DeLay, P. E.; Lennette, E. H.; DeOmme, K. Q Fever in California II: Recovery of *Coxiella burnetii* from Naturally-Infected Air-Borne Dust. *The Journal of Immunology* **1950**, *65*, 211-220.
[283] Lighthart, B.; Frisch, A. S. Estimation of Viable Airborne Microbes Downwind from a Point Source. *Applied and Enviornmental Microbiology* **1976**, *31* (5), 700-704.
[284] Seedorf, J.; Hartung, J.; Schröder, M.; Linkert, K. H.; Pedersen, S.; Takai, H.; Johnsen, J. O.; Metz, J. H. M.; Groot Koerkamp, P. W. G.; Uenk, G. H.; Phillips, V. R.; Holden, M. R.; Sneath, R. W.; Short, J. L. L.; White, R. P.; Wathes, C. M. A Survey of Ventilation Rates in Livestock Buildings in Northern Europe. *Journal of Agricultural Engineering Research* **1998**, *70* (1), 39-47.
[285] Schimmer, B.; Dijkstra, F.; Vellema, P.; Schneeberger, P. M.; Hackert, V.; Schegget, R. t.; Wijkmans, C.; Duynhoven, Y. v.; Hoek, W. Sustained Intensive Transmission of Q Fever in the South of the Netherlands, 2009. *Euro surveillance* **2009**, *14* (19).
[286] Angelakis, E.; Raoult, D. Q fever. *Veterinary Microbiology* **2010**, *140* (3-4), 297-309.
[287] Páez-Rubio, T.; Peccia, J. In *The emission rate, biological characterization and transport of aerosols emitted during the disk incorporation of class B biosolids*, Annual Water Environment Federation® Technical Exhibition and Conference (WEFTEC®) **2006**; Water Environment Federation: 2006.

[288] Hijnen, W. A. M.; Medema, G. *Elimination of Microorganisms by Drinking Water Treatment Processes.* IWA Publishing: **2010**.
[289] Woerden, H. C. v.; Mason, B. W.; Nehaul, L. K.; Smith, R.; Salmon, R. L.; Healy, B.; Valappil, M.; Westmoreland, D.; Martin, S.; Evans, M. R.; Lloyd, G.; Hamilton-Kirkwood, M.; Williams, N. S. Q Fever Outbreak in Industrial Setting. *Emerging Infectious Diseases* **2004**, *10* (7), 1282-1289.
[290] Guyton, A. C.; Hall, J. E. Ventilación Pulmonar. In *Fisiología Médica*, 11 ed.; Elsevier, Ed. Madrid **2006**; pp 475-476.
[291] Benchetrit, G. Breathing pattern in humans: diversity and individuality. *Respiration Physiology* **2000**, *122*, 123-129.
[292] Thomas, R. J.; Webber, D.; Sellors, W.; Collinge, A.; Frost, A.; Stagg, A. J.; Bailey, S. C.; Jayasekera, P. N.; Taylor, R. R.; Eley, S.; Titball, R. W. Characterization and Deposition of Respirable Large- and Small-Particle Bioaerosols. *Applied and Enviornmental Microbiology* **2008**, *74* (20), 6437-6443.
[293] Hijnen, W. A. M.; Schijven, J. F.; Bonné, P.; Visser, A.; Medema, G. Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration. *Water Science and Technology* **2004**, *50* (1), 147-154.
[294] Tigertt, W. D.; Benenson, A. S.; Gochenour, W. S. Airborne Q fever. *Bacteriological Reviews* **1961**, *25* (3), 285-293.
[295] Hogema, B. M.; Slot, E.; Molier, M.; Schneeberger, P. M.; Hermans, M. H.; van Hannen, E. J.; van der Hoek, W.; Cuijpers, H. T.; Zaaijer, H. L. Coxiella burnetii infection among blood donors during the 2009 Q-fever outbreak in the Netherlands. *Transfusion* **2012**, *52* (1), 144-150.
[296] Guatteo, R.; Seegers, H.; Taurel, A.-F.; Joly, A.; Beaudeau, F. Prevalence of Coxiella burnetii infection in domestic ruminants: A critical review. *Veterinary Microbiology* **2011**, *149* (1-2), 1-16.
[297] Rousset, E.; Berri, M.; Durand, B.; Dufour, P.; Prigent, M.; Delcroix, T.; Touratier, A.; Rodolakis, A. Coxiella burnetii Shedding Routes and Antibody Response after Outbreaks of Q Fever-Induced Abortion in Dairy Goat Herds. *Applied and Enviornmental Microbiology* **2009**, *75* (2), 428-433.
[298] Welsh, H. H.; Lennette, E. H.; Abinanti, F. R.; Winn, J. F. Air-borne Transmission of Q Fever: the Role of Parturition in the Generation of Infective Aerosols. *Annals of the New York Academy of Sciences* **1958**, *70*, 528-540.
[299] Klee, S.; Tyczka, J.; Ellerbrok, H.; Franz, T.; Linke, S.; Baljer, G.; Appel, B. Highly sensitive real-time PCR for specific detection and quantification of Coxiella burnetii. *BMC Microbiology* **2006**, *6* (1), 2.
[300] Fritz, E.; Thiele, D.; Willems, H.; Wittenbrink, M. M. Quantification of *Coxiella burnetii* by polymerase chain reaction (PCR) and a colorimetric microtiter plate hybridization assay (CMHA). *European Journal of Epidemiology* **1995**, *11* (5), 549-557.
[301] Tamrakar, S. B.; Haluska, A.; Haas, C. N.; Bartrand, T. A. Dose-Response Model of Coxiella burnetii (Q Fever). *Risk Analysis* **2011**, *31* (1), 120-128.
[302] Marrie, T. J.; Raoult, D. Q fever - a review and isuues for the next century. *International Journal of Antimicrobial Agents* **1997**, *8*, 145-161.
[303] Central Bureau voor de Statistiek. CBS Statline. www.statline.cbs.nl.
[304] van der Hoek, W.; Dijkstra, F.; Schimmer, B.; Schneeberger, P. M.; Vellema, P.; Wijkmans, C.; Schegget, R. t.; Hackert, V.; Duynhoven, Y. v. Q Fever in the Netherlands: an Update on the Epidemiology and Control Measures. *Euro surveillance* **2010**, *15* (12), 1-4.

[305] Robertson, I. D.; Irwin, P. J.; Lymbery, A. J.; Thompson, R. C. A. The role of companion animals in the emergence of parasitic zoonoses. *International Journal for Parasitology* **2000**, *30* (12–13), 1369-1377.
[306] Perelle, S.; Josefsen, M.; Hoorfar, J.; Dilasser, F.; Grout, J.; Fach, P. A LightCycler real-time PCR hybridization probe assay for detecting food-borne thermophilic Campylobacter. *Molecular and Cellular Probes* **2004**, *18* (5), 321-327.
[307] Chaban, B.; Musil, K. M.; Himsworth, C. G.; Hill, J. E. Development of cpn60-Based Real-Time Quantitative PCR Assays for the Detection of 14 Campylobacter Species and Application to Screening of Canine Fecal Samples. *Applied and Environmental Microbiology* **2009**, *75* (10), 3055-3061.
[308] Straub, T. M.; Höner zu Bentrup, K.; Coghlan, P. O.; Dohnalkova, A.; Mayer, B. K.; Bartholomew, R. A.; Valdez, C. O.; Bruckner-Lea, C. J.; Gerba, C. P.; Abbaszadegan, M. A.; Nickerson, C. A. In Vitro Cell Culture Infectivity Assay for Human Noroviruses. *Emerging Infectious Diseases* **2007**, *13* (3), 396-403.
[309] Straub, T. M.; Bartholomew, R. A.; Valdez, C. O.; Valentine, N. B.; Dohnalkova, A.; Ozanich, R. M.; Bruckner-Lea, C. J.; Call, D. R. Human Norovirus Infection of Caco-2 Cells Grown as a 3-Dimensional Tissue Structure. *Journal of Water and Health* **2011**, *9* (2), 225-240.
[310] Hamoda, M. F.; Attia, N. F.; Al-Ghusain, I. A. Performance evaluation of a wastewater reclamation plant using ultrafiltration and reverse osmosis. *Desalination and Water Treatment* **2014**, (ahead-of-print), 1-11.
[311] Fitzhenry, K.; Barrett, M.; O'Flaherty, V.; Dore, W.; Keaveney, S.; Cormican, M.; Clifford, E. Detection and removal of pathogenic norovirus employing tertiary treatment in wastewater and water treatment facilities. *Water Practice & Technology* **2014**, *9* (3), 370-376.
[312] de Bruin, A.; de Groot, A.; de Heer, L.; Bok, J.; Wielinga, P. R.; Hamans, M.; van Rotterdam, B. J.; Janse, I. Detection of Coxiella burnetii in Complex Matrices by Using Multiplex Quantitative PCR during a Major Q Fever Outbreak in The Netherlands. *Applied and Environmental Microbiology* **2011**, *77* (18), 6516-6523.
[313] de Bruin, A.; Janse, I.; Koning, M.; de Heer, L.; van der Plaats, R. Q.; van Leuken, J. P.; van Rotterdam, B. J. Detection of Coxiella burnetii DNA in the environment during and after a large Q fever epidemic in the Netherlands. *Journal of Applied Microbiology* **2013**, *114* (5), 1395-404.
[314] de Bruin, A.; van der Plaats, R. Q. J.; de Heer, L.; Paauwe, R.; Schimmer, B.; Vellema, P.; van Rotterdam, B. J.; van Duynhoven, Y. T. H. P. Detection of Coxiella burnetii DNA on Small-Ruminant Farms during a Q Fever Outbreak in the Netherlands. *Applied and Environmental Microbiology* **2012**, *78* (6), 1652-1657.
[315] Teunis, P.; Heijden, O. G. v. d.; Giessen, J. W. B. v. d.; Havelaar, A. H. *The dose-response relation in human volunteers for gastro-intestinal pathogens*; Rijksinstituut voor Volksgezondheid en Milieu RIVM: 1996-04-30, **1996**.
[316] van den Berg, H.; Lodder, W.; van der Poel, W.; Vennema, H.; de Roda Husman, A. M. Genetic diversity of noroviruses in raw and treated sewage water. *Research in Microbiology* **2005**, *156* (4), 532-540.
[317] He, J.-W.; Jiang, S. Quantification of Enterococci and Human Adenoviruses in Environmental Samples by Real-Time PCR. *Applied and Environmental Microbiology* **2005**, *71* (5), 2250-2255.

[318] Thurston-Enriquez, J. A.; Haas, C. N.; Jacangelo, J.; Gerba, C. P. Chlorine Inactivation of Adenovirus Type 40 and Feline Calicivirus. *Applied and Environmental Microbiology* **2003**, *69* (7), 3979-3985.
[319] Banihashemi, A.; Van Dyke, M. I.; Huck, P. M. Detection of viable bacterial pathogens in a drinking water source using propidium monoazide-quantitative PCR. **2014**.
[320] Delgado-Viscogliosi, P.; Solignac, L.; Delattre, J.-M. Viability PCR, a Culture-Independent Method for Rapid and Selective Quantification of Viable Legionella pneumophila Cells in Environmental Water Samples. *Applied and Enviornmental Microbiology* **2009**, *75* (11), 3502-3512.
[321] Chang, C.-W.; Chou, F.-C.; Hung, P.-Y. Evaluation of bioaerosol sampling techniques for Legionella pneumophila coupled with culture assay and quantitative PCR. *Journal of Aerosol Science* **2010**, *41* (12), 1055-1065.
[322] Ferguson, C. M.; Coote, B. G.; Ashbolt, N. J.; Stevenson, I. M. Relationships between indicators, pathogens and water quality in an estuarine system. *Water Research* **1996**, *30* (9), 2045-2054.
[323] Savill, M. G.; Hudson, J. A.; Ball, A.; Klena, J. D.; Scholes, P.; Whyte, R. J.; McCormick, R. E.; Jankovic, D. Enumeration of Campylobacter in New Zealand recreational and drinking waters. *Journal of Applied Microbiology* **2001**, *91* (1), 38-46.
[324] Payment, P.; Plante, R.; Cejka, P. Removal of indicator bacteria, human enteric viruses, Giardia cysts, and Cryptosporidium oocysts at a large wastewater primary treatment facility. *Canadian Journal of Microbiology* **2001**, *47* (3), 188-193.

List of Publications

Journal Publications

- Sales-Ortells, H., Medema, G. "Microbial Health Risks Associated with Exposure to Stormwater in a Water Plaza". *Water Research*. **2015**. 74, 34-46. **DOI**: 10.1016/j.watres.2015.01.044
  URL: http://www.sciencedirect.com/science/article/pii/S0043135415000731
- Sales-Ortells, H., Fernandez-Cassi, X., Timoneda, N., Dürig, W., Girones, R., Medema, G. "Health Risks Derived from Consumption of Lettuces Irrigated with Tertiary Effluent Containing Norovirus". *Food Research International.* **2015**. 68, 70-77. **DOI**: 10.1016/j.foodres.2014.08.018
  URL: http://www.sciencedirect.com/science/article/pii/S0963996914005560
- Sales-Ortells, H. and Medema, G. "Screening-Level Microbial Risk Assessment of Urban Water Locations: a Tool for Prioritization". *Environmental Science and Technology.* **2014**. 48 (16), 9780-9789. **DOI**: 10.1021/es203744g **URL**: http://pubs.acs.org/doi/abs/10.1021/es5020407
- Sales-Ortells, H. and Medema, G. "Screening-Level Risk Assessment of *Coxiella burnetii* (Q Fever) Transmission via Aeration of Drinking Water". *Environmental Science and Technology*. **2012**. 46 (7), 4125-4133. **DOI**: 10.1021/es203744g **URL**: http://pubs.acs.org/doi/abs/10.1021/es203744g
- Sales-Ortells, H, Agostini, G., Medema, G. "Quantification of Waterborne Pathogens and Associated Health Risks in Urban Water". Submitted to *Environmental Science and Technology*.

Conference Proceedings

- Sales-Ortells, H., Medema, G. "Health Impact Assessment of Urban Water Related to Weather Parameters in The Netherlands".3rd SCARCE International Conference – Bridging Toxicants, Stressors and Risk-Based Management Under Water Scarcity. Valencia, Spain. November 2012.
- Sales-Ortells, H., Medema, G. "Screening-level Assessment of the Risk of *Coxiella burnetii* (Q fever) Related to Aeration of Drinking Water in The Netherlands". International Q fever symposium – An update on research findings and lessons learned from the epidemic in The Netherlands. RIVM. Amsterdam, The Netherlands. June 2012.
- Sales-Ortells, H., Medema, G. "Screening-level Assessment of the Risk of *Coxiella burnetii* (Q fever) Related to Aeration of Drinking Water in The Netherlands". Vakantiecursus. TU Delft. Delft, The Netherlands. January 2012.
- Sales-Ortells, H., Medema, G "Screening-level Assessment of the Risk of *Coxiella burnetii* (Q fever) Related to Aeration of Drinking Water in The Netherlands"Health Related Water Microbiolgy (HRWM) WaterMicro 2011, Rotorua, New Zealand. September 2011.

Acknowledgements

I would like to thank all the people who directly, or indirectly, contributed to this work:

First of all, I would like to express my gratitude to my promotor, Gertjan Medema, for giving me this opportunity, for the marvellous guidance and help during these 5 years, and for believing in me, even, and specially, when I didn't. Also, it was very nice to talk to you.

Secondly, I would like to thank the committee members: Prof. Rosina Gironés, Prof. Ana Maria de Roda Husman, Prof Frans van Knapen, Prof. Jan Peter van der Hoek, Dr. Frans van de Ven, and Prof. Luuk Rietveld for reading my thesis, helping in improving it with their comments, and for being part of my defence. Ana Maria, thank you also for all the support and tips you gave me during these years.

Rosina, I am deeply thankful to you and Rosa Araujo, Silvia Bofill Mas, Xavier Fernàndez Casi and Natàlia Timoneda, for welcoming me in Barcelona and sharing with me the work on the water reclamation project. Xavier, thank you also for coming with me to the field, explaining the methodology and revising the norovirus manuscript. Natàlia, thanks for coming to the field as well, and for your *R* tips. I would also like to include here my acknowledgements to the wastewater treatment plant manager, the farmers, and the golf course managers in Platja d'Aro for their help and patience answering all my questions. And of course, thank you all for the nice time I spent in Barcelona.

I am also thankful to the Watergraafsmeer expert group for sharing information on the Watergraafsmeer and for the constructive meetings: Paulien Hartog, Kasper Spaan, Alice Fermont, Marcel Zandvoort, Ron van der oost, Ben Rozema and Kees Broks. Thanks Ron also for the information on the Fluoroprobe and ELISA protocols.

I am deeply grateful to the team in Rotterdam for sharing with me their information on the "waterplein" and for inviting me to the simulation event: Daniël Goedbloed, Johan Verlinde, and Bert Doelder. Johan, thanks also for always answering my doubts.

I would also like to express my gratitude to Arno Zwart for his help with the Q fever chapter, especially with the air dispersion model, and to Joanne O'Toole, for reading the Q fever manuscript and giving me her impression.

Anke Brower, thank you for all the help you provided me in the lab, including working some weekends, and your tips for conducting, planning and organizing the lab work.

Edwin Kardinaal, thanks for being a good team leader and showing your interest on my research. And Ronald Italiander, thank you also for your help in organizing the lab work. Ana Marie van Wezel and Idsart Dijkstra, thanks for your manager tasks. Also Ana, thank you for caring about me when you heard about the New Zealand passport episode.

I would also like to thank my colleagues from the KWR microbiology team for their constructive comments on my research, for the nice time and for all those *lekker* Monday's homemade cakes: Gemma, Patrick, Leo, Bart, Paul, Wim, Kimberly, Luc, Ludmila, Michiel, Eveline, Rinske, and Dick. Special thanks to Bart and Leo for helping me with my

technical and scientific doubts and for providing me data, Paul for sharing your knowledge on Legionella, and Kimberly for your help in the lab.

To my colleagues from the KWR microbiology lab for helping me so many times with my doubts, giving a hand when I needed extra hands, staying sometimes late, and making the lab work more fun: Danielle, Carola, Anita, Lonneke, Gaby, Marijan, Ton, Goffe, and Meindert.

I would like to thank my colleagues from the department of Water Management of TU Delft for the constructive discussions, especially during the colloquium. Also, the secretaries for all the administrative help provided during these years.

My paranymphs, Sara and Bea: Thank you for accepting this role in my dissertation, but specially thank you for being my friends from the first moment I saw you stepping in my office at KWR. It's been great to have you around all these years.

I am also grateful to my students: Giulia and Wiebke for the work you did, for the constructive discussions and for the nice time. I also learned a lot from these supervisor experiences and it was great to have you as students.

The lunch-expats group: thanks André, Roberto, Andreas, Kerusha, Yuki, Jorge, Diego, Ana and Vicky for sharing so many laughs, gossips, and our friendship.

All my colleagues and friends at TU Delft and specially Diana, Sam, Laia, Annelies, Maria, David, and Angela for making my visits to (TU) Delft awesome.

To the Amsterdamers, for all the nice time, parties and adventures. I really miss you: Victor, Marta, Sebas, Dani, Merel, Sara, Oscar, David, Tinden, Kriss and Cveti. Hope you all move soon to the European Caribbean ;). And my friends in Utrecht, specially Laura (I wouldn't be here if it wasn't for you) and Jonathan.

To all my Desori friends in Vila-real, for always being there and because I miss you so much, with special attention to those who ever came to visit: Alba, Paloma, Anna S., Laura P., Jose, Laura B., Lluís, Reyes and Imma. A special mention as well to the new and the future little Desori members ☺. To my friends from the València times: Amparo, Isa, Núria, Leti, Alfonso, Jérémy and Xavier, for all the nice time together, for coming to visit and for receiving me in your houses all around Europe.

To my mother and my brother, for always being there, all your support and your unconditional love (and thanks mum for correcting some parts of the Thesis, and Guillem for your tips on the cover design). To my father, who, I'm sure, would have been very proud of his daughter. I also thank the rest of my family, aunts, uncles and cousins for your constant love and support, and specially to Piedad, Conxita, Manuel and Dolores.

And finally, I would like to thank Javi, for so many things I don't know where to start from. For always being by my side, loving me, helping me, believing in me more than I will ever do, cheering me up whenever I need it, adopting our cat Rita, pushing me to finish, and understanding my stress. Also thank you for coming with me to grab samples on a Sunday, helping with the PCR, the statistics and *R*, and for all your research tips and advice. Without you I wouldn't have made it.

Curriculum Vitae

Helena Sales-Ortells was born in Vila−real, Spain, in 1984. She coursed the first for years of Medicine in the Universitat de València (Spain) and the Universidade Nova de Lisboa (Portugal), under the Erasmus European program, and graduated in Food Science and Technology by the Universitat de València in 2008. Her research project consisted on evaluating the evolution of the Maillard Reaction and fat acid oxidation in dairy products for infants during storage, and the relation between the two reactions, and she obtained a cooperation grant from the Spanish Ministry of Education. In 2009, she moved to The Netherlands to conduct research in water migration in (monocomponent) starch films, with applications in the bread industry, in TIFN (Top Institute Food and Nutrition, Wageningen). This research was part of a six months internship under the Leonardo da Vinci European program. During her studies, she developed interest for microbiology and health. Coming from Valencia, a region with water problems (water scarcity, flooding risks and pollution), she was also interested in water research. Therefore, she started her PhD in water microbiology and health in 2009, in KWR Watercycle Research Institute and Delft University of Technology. During this period, she has attended several courses on microbiology and risk assessment. In her free time, she loves running and reading books, and she enjoys discovering the underwater world with her boyfriend during their diving holidays.