Best urban water management practices to prevent waterborne infectious diseases under current and future scenarios

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Best urban water management practices to prevent waterborne infectious diseases under current and future scenarios

Aanbevelingen voor stedelijk water management om wateroverdraagbare infectieziekten te voorkomen in huidige en toekomstige scenario's

(met een samenvatting in het Nederlands)

Proefschrift

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"There are far, far better things ahead than any we leave behind."

C.S. Lewis

Contents


REGENWATER
VUILWATER

Chapter 1

General introduction

1. Urban water, drainage and public health

Water in urban areas includes surface water, groundwater and drinking water, and has various type-related functions, e.g. discharge of water surplus, water storage, water supply, transport of pollutants, breakdown of pollution, separation of functions/ areas and water-related recreation (Van de Ven, 2011). In addition to above-ground water, cities have underground urban drainage systems that remove storm water and wastewater. Urban drainage systems aim to maintain public health by protecting against the spread of diseases mainly through removing human excreta from the immediate vicinity of habitation to prevent human exposure to pathogenic micro-organisms (Butler and Davies, 2004). Urban drainage systems also aim to avoid damage from flooding after rainfall (Butler and Davies, 2004). Urban drainage systems can be divided into two basic types: combined sewer systems (where wastewater and storm water become mixed) and separated sewer systems (where wastewater and storm water are drained separately). A combined sewer system transports its water to a wastewater treatment plant, whereas a separated sewer system transports wastewater to the wastewater treatment plant and storm water to surface water (Butler and Davies, 2004) (see Figure 1). From 1970 onwards, most sewer systems constructed in The Netherlands have been separated sewer systems. Improvement of separated sewer systems is achieved by diverting the first amount of storm water to the wastewater treatment plant. This reduces discharges to rivers and streams of the storm water that is expected to be most contaminated (Korving, 2004).

In the Netherlands, 69% of the households are connected to a combined sewer system, 26% are connected to a separated sewer system and 4% are connected to a pressure drainage system (Dutch Centre of Expertise in Sewer Management and Urban Drainage, 2010). In 2008, 99.6% of the households of the Netherlands were connected to a drainage system, the remaining 0.4% of the households were not connected to a drainage system and had an individual water treatment system, such as a septic tank (Dutch Centre of Expertise in Sewer Management and Urban Drainage, 2010). These percentages of connections to a drainage system are slightly higher than the figures for other European countries like Germany, United Kingdom and France, where, respectively, 96%, 95% and 98% of all inhabitants are connected to a drainage system (Dutch Centre of Expertise in Sewer Management and Urban Drainage, 2010).

2. Waterborne infectious diseases associated with exposure to urban water

Infectious diseases are caused by exposure to pathogenic organisms, such as bacteria, viruses, parasites, algae, helminths and fungi (World Health Organization, 2011). These pathogenic organisms can cause infections by ingestion, inhalation, dermal contact (McKone and Daniels, 1991). Many outbreaks of infectious diseases have occurred among people who were exposed to contaminated water (Craun, Calderon and Wade, 2006; Communicable Disease Surveillance Centre Public Health Wales, 2011; Curriero et al., 2001). Waterborne outbreaks include gastro-intestinal,

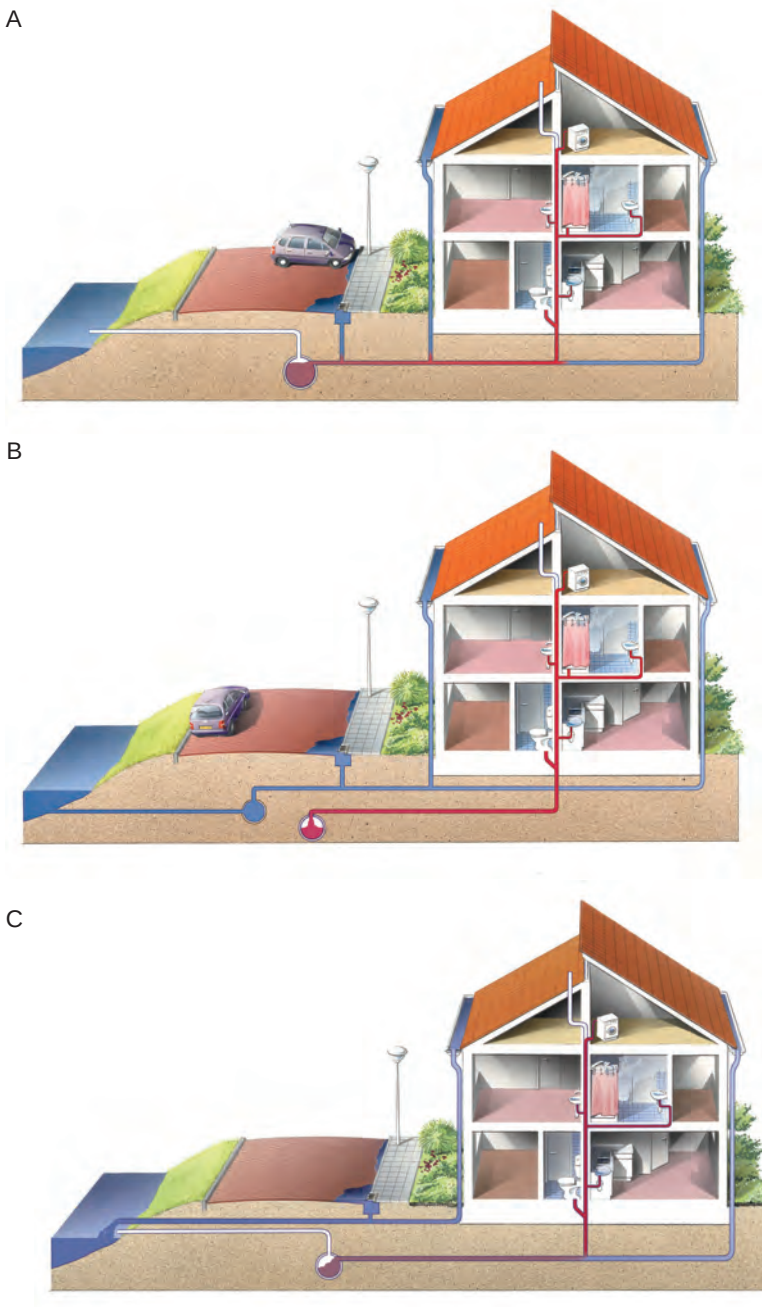

Figure 1. A) Combined urban drainage system, B) Separated urban drainage system, **C)** Combined sewer system where storm water is disconnected from the sewer system (Reproduced with permission from Stichting Rioned, Paul Maas)

respiratory and skin infections. These outbreaks occurred after exposure of people to pathogenic microorganisms while swimming in water influenced by discharges from sewer overflows, (Schets et al., 2008b), after consumption of contaminated tap water by sewage (Curriero et al., 2001), after contact with contaminated water originating from flooding (Schmid et al., 2005; Jablecki et al., 2005; Cann et al., 2013) and after exposure to water features where water treatment failed (Jones et al. ,2006; Kebabjian, 2003; Minshew et al., 2000; Hoebe et al., 2004; Den Boer et al., 2002; Hlady et al. ,1993; Cacciapuoti, Ciceroni and Maffei, 1987). These outbreaks demonstrate that urban water and urban drainage systems have a role in the transmission of waterborne infectious diseases, however, it is yet unknown to which extent.

In high income countries, such as the Netherlands, one of the main concerns in relation to waterborne infectious diseases is the economic burden of disease (de Wit, 2002; Friesema, Lugnér and Van Duynhoven, 2012). Costs are generated by patients seeking medical care, but also by persons missing work either due to their own illness or while taking care for an ill person (de Wit , 2002). Although the costs per patient are in general not particularly high, the total costs are substantial due to the high number of persons affected (Friesema, Lugnér and Van Duynhoven , 2012). For instance, each year 15.9 million episodes of an infectious intestinal disease (gastroenteritis, usually described as diarrhea or vomiting due to micro-organisms) occur in the Netherlands (Doorduyn, Van Pelt and Havelaar, 2012). The causes of these episodes have been suggested to be contamination from person to person (21%) and consumption of contaminated food or water (33%), while the cause of the remainder is unknown (Doorduyn, Van Pelt and Havelaar , 2012).

People may be exposed to contaminated water through inhalation at locations where aerosols are produced within the respirable size range. These contaminated aerosols enter the lungs and may cause infections due to the presence of pathogenic micro-organisms, such as adenoviruses and *Legionella* (World Health Organization, 2011), or inflammatory reactions due to endotoxins, possibly leading to symptoms like dry cough, shortness of breath and wheeze, fever, shivering, myalgia, malaise and/ or other influenza-like symptoms (Smit, Spaan and Heederik, 2005). Furthermore, ingestion of enteric pathogens originating from urban water may cause gastrointestinal infections (Leclerc, Schwartzbrod and Dei-Cas, 2002). Finally, dermal contact (skin and mucous membranes of nose, ears and eyes in contact with urban water) can result in wound infections including those due to *Aeromonas hydrophila* (Semel and Trenholme, 1990), otitis externa due to *Pseudomonas aeruginosa* (Van Asperen et al., 1995), conjunctivitis due to adenoviruses (Crabtree et al., 1997) or leptospirosis due to *Leptospira* (Cacciapuoti, Ciceroni and Maffei, 1987).

3. Waterborne pathogens

In the following paragraphs the most relevant pathogens associated with waterborne infectious diseases are discussed including *Campylobacter, Giardia, Cryptosporidium*, norovirus, enterovirus and *Legionella*. These pathogens were selected because they are bacterial, viral or protozoan pathogens (World Health Organization, 2011), that have been associated with waterborne disease

outbreaks (Craun, Calderon and Wade, 2006; Leclerc, Schwartzbrod and Dei-Cas, 2002; Craun et al., 2006) and were relevant for an exposure route (e.g. inhalation and ingestion) (Leclerc, Schwartzbrod and Dei-Cas, 2002). Furthermore, these pathogens may be present in high concentrations in the water system, combined with a high pathogenicity and environmental survival (Fewtrell and Smith, 2007), thus posing a potential health risk. The concentration of these pathogens in feces, waste water, storm water, surface water and runoff is shown in Table 1.
In the Netherlands, *Campylobacter* is the most commonly reported bacterial cause of acute gastroenteritis (Van Pelt et al., 2003). Diarrhoea, abdominal pain and fever are the most commonly reported symptoms, with vomiting and bloody diarrhea are reported less frequently; serious sequelae include neurological (such as Guillain-Barré syndrome) and rheumatological problems (McCarthy and Giesecke, 2001; Porter, Choi and Riddle, 2013). *C. jejuni* is the most commonly estimated *Campylobacter* spp. that causes human infections (Nauta et al., 2005). The infectious dose of *Campylobacter* is moderate (100-10000) (World Health Organization, 2011), and the risk of contracting Campylobacterios is greater for children under the age of five and adults over 60 years of age than for the general population (Louis et al., 2005).
Giardia spp. and *Cryptosporidium spp.* are protozoan pathogens capable of causing human infections, which may be asymptomatic. Both human pathogens cause diarrhea that is usually self-limiting, but chronic infections do occur specifically for Giardia (Thompson, 2004; Fayer, Morgan and Upton, 2000). Transmission occurs through the fecal-oral route, either directly from person-to person or from animal-to-human, but may also be waterborne (Thompson, 2004; Fayer, Morgan and Upton, 2000). Outbreaks have occurred at interactive water features (Eisenstein, Bodager and Ginzl, 2008; Anonymous, 1999; Anonymous, 2000). The infectious dose for *Giardia* and *Cryptosporidium* is very low (1-100 oocysts) (World Health Organization, 2011). The elderly may be more susceptible than the general population to cryptosporidiosis (Mor et al., 2009), while young children are more susceptible to *Giardia*, with as many as 20% of children in day care centers estimated to carry *Giardia* and excrete cysts without any symptoms (Pickering et al., 1984).
Norovirus infections cause acute gastroenteritis in approximately 40% of infected humans (World Health Organization, 2011). Typical symptoms include projectile vomiting, diarrhea with abdominal cramps and nausea within 24 to 48 hours after exposure (McCarthy, Estes and Hyams, 2000). The symptoms are usually relatively mild and illness lasts up to three days. The norovirus genus is divided into five genogroups (genogroup (GG)I to GGV) with genogroup GGI and GGII predominating over the past five years (Braks and de Roda Husman, 2013). The most common transmission routes for noroviruses are person-to-person contact, inhalation of contaminated aerosols, dust particles and airborne particles of vomitus. In relation to water, norovirus outbreaks have been traced to a contaminated interactive water fountain (Hoebe et al., 2004) as well as to flooding (Schmid et al., 2005) Because no robust cell culture system for the detection of infectious human noroviruses is available (Duizer et al., 2004), information on the persistence of infectious virus particles in the environment is limited. The infectious dose for norovirus is very low (1-10 particles) (World Health Organization, 2011).

Table 1. Concentration of indicator bacteria and pathogens in faeces, wastewater, surface water, storm water and runoff, modified from the guidelines for drinking water quality of the WHO (World Health Organization, 2011).

	Feces (N/gram)	Wastewater (N/L)	Surface water (N/L)	Storm water (N/L)	Runoff (N/L)	References
Indicator bacteria: *E. coli* and intestinal enterococci	10^{7} -10^{10}	10^{6}-10^{10}	10^{3} -10^{5}	10^{3} -10^{5}	10^{3} -10^{4}	(WHO, 2012; Schets et al., 2010; Marsalek and Rochfort, 2004)
Campylobacter	10^{n}	10^{2} -10^{6}	10^{2} -10^{4}	10^{-1} -10^{2}	0 -10^{2}	(Schets et al., 2010; Simmons et al., 2001; Koenraad et al., 1994; Lampard et al., 2012)
Cryptosporidium	10^{6} -10^{7}	10^{0} -10^{4}	10^{-1} -10^{2}	10^{-1} -10^{0}	0 -10^{0}	(Xiao and Herd, 1993; Medema, Ketelaars and Hoogeboezem, 2001; Schets et al., 2008a)
Giardia	10^{6} -10^{7}	10^{0} -10^{3}	10^{-1} -10^{3}	10^{-1} -10^{1}	0 -10^{1}	(Schets et al., 2010; Xiao and Herd, 1993; Medema Ketelaars and Hoogeboezem, 2001; Schets et al., 2008a; Danciger and Lopez, 1975; Shaber, Slifko and Marty Wanielista)
Norovirus	10^{5} -10^{9}	10^{0} -10^{4}	10^{1} -10^{3}	0 -10^{3}	-	(Chan et al., 2006; Ozawa et al., 2007; Lodder and De Roda Husman, 2005; Sauer et al., 2011)
Enterovirus	10^{6}	10^{0} -10^{4}	10^{-2} -10^{1}	0 -10^{4}	-	(Lodder and De Roda Husman, 2005; Lodder et al., 2013; Lodder et al., 2010; Sercu et al., 2009; Schijven et al., 2011; De Roda Husman et al., 2009)
Legionella	-	0-10^{5}	n.a.	0 -10^{4}	0 -10^{4}	(Simmons et al., 2001; Simmons et al., 2001; Van Heijnsbergen et al., submitted; Sakamoto et al., 2009; Palmer et al., 1993; Roll and Fujioka, 1995)

n.a.= not available
- = assumed to be absent

Enteroviruses are one of the most common causes of human infections and can cause a number of diseases, ranging from a mild febrile illness to poliomyelitis (polio), meningoencephalitis (inflammation of the brain) and myocarditis (inflammation of the heart muscle) (World Health Organization, 2011). Most infections, particularly those in children, are asymptomatic, but do lead to the excretion of large numbers of virus particles into the environment and are able to cause disease in other individuals. Person-to-person contact and inhalation of airborne viruses or viruses in respiratory droplets are considered to be the predominant routes of transmission of enteroviruses (World Health Organization, 2011), but waterborne transmission has been epidemiologically confirmed for outbreaks that were associated with children bathing in lake water (Sinclair, Jones and Gerba, 2009; Hauri et al., 2005). The infectious dose of enterovirus is low (1-100 viruses) (World Health Organization, 2011).

Legionella pneumophila is a bacterium that may cause either Legionella-pneumonia or Pontiac-fever, a mild flu-like condition. *Legionella spp.* proliferate at water temperatures of 25°C or above

and need amoebas or other protozoa for their replication. An abundance of *Legionella* species is not expected to occur in surface water, due to overgrowth of other microorganisms for which environmental growth conditions are more favorable (Braks and de Roda Husman, 2013). *Legionella* infections occur only through inhalation of contaminated aerosols; there is no evidence of person-to-person transmission and the *Legionella* cannot cause infections through ingestion. The concentration of *Legionella spp.* in different types of urban water is shown in Table 1. It should be noted that these concentrations may be underestimated, because the enumeration of *Legionella spp.* by culture has been largely hampered by the inability of viable-but-non-culturable *Legionella* to grow on plates, and growth of non-*Legionella* background flora outcompeting *Legionella* bacteria (Schalk et al., 2012).

4. Quantitative microbial risk assessment (QMRA)

Quantitative microbial risk assessment (QMRA) is a modeling tool to quantify health risks from exposure to pathogens (Teunis, Nagelkerke and Haas, 1999; Haas, 2000; Gibson III, Haas and Rose,1998). QMRA can provide an objective and scientific basis for risk management decisions (Medema and Ashbolt, 2006). In case a QMRA is carried out for microbial risk from exposure to water, information on the concentration of pathogens in the water, the exposure of people to these pathogens and dose-response relations for different pathogens are required. As shown in Table 1, source water specific data exist about pathogen concentrations in feces, wastewater, storm water, runoff and surface water; also dose-response relationships are available for several pathogens (Teunis, Nagelkerke and Haas, 1999; Teunis et al., 2008; Teunis et al., 2005; Teunis et al., 1997). However, only limited data are available in scientific literature about exposure volumes (Schijven and de Roda Husman, 2006). Existing data concerns exposure during swimming (Dufour et al., 2006; Schets, Schijven and de Roda Husman, 2011) and diving (Schijven and de Roda Husman, 2006). No data are available (U.S. EPA, 2011), to our knowledge, for exposure routes that include unintentional contact with water, such as exposure through inhalation of aerosols, ingestion of aerosols/ water droplets and exposure through hand-mouth contact.

5. Indicator organisms used to assess water quality and air quality

Measuring pathogens in water and air is labor- and knowledge-intensive, and thus expensive (Schets, 2011). Therefore, indicators are generally used to asses water quality and/or air quality. For example, endotoxin is a frequently used to assess air quality, while fecal indicator bacteria such as *E. coli* and intestinal enterococci are frequently used to monitor microbial water quality. Endotoxins are components of the cell walls of gram-negative bacteria (Smit et al., 2009). Gram-negative bacteria are commonly present in various environments e.g. on the surfaces of plants and in the intestinal tracts of humans and animals, resulting in highly variable endotoxin concentrations in air and water (Spaan et al., 2008). Inhalation of endotoxins may cause inflammatory reac-

tions or influenza-like symptoms (Smit, Spaan and Heederik, 2005). Endotoxin detection is used to assess air quality of occupational areas in the Netherlands such as livestock barns, harvesting situations and waste water treatment plants (Spaan et al., 2008).
The bacteria *E. coli* and intestinal enterococci are natural inhabitants of the gastrointestinal tracts of humans and warm-blooded animals (World Health Organization, 2011). These bacteria are released into the environment with faeces and may survive anywhere from a few hours up to several days in water (World Health Organization, 2011). *E. coli* and intestinal enterococci do not cause infections in humans (except for the human pathogenic variants such as *E. coli* O157), but point at a recent fecal contamination (World Health Organization, 2011) indicating the probability that enteric pathogens are present. The absence of fecal indicators does not exclude the presence of pathogens, because pathogenic viruses and protozoa may be more resistant to environmental conditions or treatment technologies, including filtration and disinfection (World Health Organization, 2011).

6. Climate change and the impact on waterborne infectious disease

The Intergovernmental Panel on Climate Change (IPCC) has published the fifth assessment report on the state of knowledge on climate change in 2013 (Intergovernmental Panel on Climate Change, 2013). IPCC concluded that as global warming continues, the global water cycle will be affected in ways that vary from area to area. In short, although regional exceptions may occur, the differences in precipitation between wet and dry areas and between wet and dry seasons will be enlarged. For the Netherlands, this means, according to the Royal Netherlands Meteorological Institute projections, that it can be assumed that Dutch summers will have fewer rainy days, while precipitation extremes will increase, and winters will become wetter (Hurk et al., 2008) as was established based on the fourth assessment report of the IPCC (personal communication Alexander Bakker). Furthermore, the average temperature in the Netherlands will increase 0.9 to 2.7 ˚C as compared with the 1990 averages (Hurk et al., 2008).

7. Alternative sanitation systems

Over the past years, interest in alternative sanitation systems has clearly increased. These sanitation alternatives consider wastewater as a resource instead of as waste. The alternative sanitation concepts are based on separation of grey water (water from sinks, showers and kitchens) and black water (faeces/water from toilets), and possibly yellow water (urine), and these source waters are as far as possible recovered and (re)used as sustainable sources of water or nutrients. The advantages of these sanitation systems in comparison with the conventional approach are less water use, less wastewater, and a contribution to the recycling of nutrients.

8. Water quality guidelines, regulations and policies

The World Health Organization has published guidelines for drinking water quality (World Health Organization, 2011) that suggest the use of Disability Adjusted Life Years (DALYs) to evaluate public health priorities and to assess the disease burden associated with environmental exposures, particularly microbial hazards. The WHO guideline defines an upper limit of 10^{-6} DALY per person per year as a tolerable burden of disease from exposure to drinking water. The Dutch government set a health based target of 10^{-4}, i.e. fewer than one infection with *Cryptosporidium*, *Giardia*, and enteroviruses should occur per 10,000 consumers of tap water per year (Anonymous, 2011).

The European Bathing Water Directive (CEC, 2006) imposes requirements on the monitoring, assessment and management of the quality of bathing water and for the provision of information on that quality. The aim of the directive is firstly to reduce and prevent the feacal pollution of bathing water and secondly to inform European citizens of the degree of pollution. This Directive concerns those surface waters in which bathing is authorized by the national authorities and regularly visited by a significant number of bathers. In addition, the Dutch government has defined regulations for swimming pools (Anonymous, 2009), which, whether indoor or outdoor, must use drinking water as their source water, and should be chlorine disinfected.

Local authorities have the responsibility to ensure the control of infectious diseases, as stated by law (Anonymous, 2008). The law is based on the International Health Regulations of the WHO (World Health Organization, 2005) which aims to provide guidance and support to countries to build strong national public health systems and to further develop and maintain an effective international system that is able to continuously assess the global context of public health risks. The law includes a reporting obligation for certain infectious diseases. For instance, a case of legionellosis must be reported by public health departments, who must supply data for the national surveillance system (National Institute for Public Health and the Environment, 2011).

Under Dutch law, local authorities have an obligation to collect and transport urban wastewater, rainwater and groundwater (Anonymous, 2007). The Dutch government has given a preferred sequence concerning the efficient and sustainable drainage of urban wastewater, rainwater and groundwater (Anonymous, 2012). It recommends keeping wastewater, rainwater and groundwater as separate as possible, under the condition that this can be performed efficiently. Further, it recommends transporting wastewater to a wastewater treatment plant, while groundwater and rainwater should be reused or discharged into the environment (if necessary after retention and purification). This preferred sequence has led to a policy by local authorities to disconnect rainwater piping from urban drainage systems (Figure 1C).

9. Aim of this Thesis

Since the extent to which exposure to urban water poses a risk for public health is unknown, this thesis aimed to investigate health risks associated with urban water systems and to determine ways to minimize those risks. This thesis is divided in two parts. Firstly, the health risks associated with exposure to urban water that is intended to beautify the urban area were discussed (chapter 2-4). These chapters focus especially on water features, because such features encourage exposure to urban water. Secondly, health risks were investigated for exposure to flooding in urban areas (chapter 5-6). Here, health risks are addressed for citizens who were exposed, whether intended or not, to urban floodwater at locations where the design capacity of the urban drainage system is exceeded by heavy rainfall.

Water features have been frequently associated with outbreaks of infectious diseases. To be able to indicate possible public health risks from exposure to water features, exposure to endotoxins in air and fecal indicator bacteria in water were measured at water features (chapter 2). Secondly, a quantitative microbial risk assessment was performed for water features that use rainwater as their source water. In this risk assessment, *Legionella* was used as a target organism to assess risks through inhalation and *Campylobacter* was used as a target organism to assess risks through ingestion (chapter 3). Thirdly, best water quality management practices were identified that may prevent fecal contamination of water features (chapter 4).

Exposure to floodwater may cause outbreaks of infectious diseases. To be able to quantify health risks for flooding from different urban drainage systems, samples of floodwater were analyzed for *Campylobacter, Cryptosporidium, Giardia,* norovirus, enterovirus (chapter 5) and *Legionella* (chapter 6). Subsequently, a quantitative microbial risk assessment was performed to calculate gastro-intestinal infection risks for flooding originating from combined sewer systems, separated sewer systems and rainfall generated surface runoff (chapter 5).

In addition, this thesis provides data to quantify exposure volumes that are required to perform a quantitative microbial risk assessment. Exposure volumes were quantified for inhalation of aerosols from water features (chapter 2) and ingestion of water through hand-mouth contact, ingestion of water droplets and ingestion of mouthfuls of water (chapter 3 and 5).

The research presented in this study provides data and tools concerning urban water management for current and future scenarios through its focus on both the estimation of microbial risks upon exposure to urban water by quantitative microbial risk assessment and best management practices for urban water.

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Chapter 2

Human exposure to endotoxins and fecal indicators originating from water features

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ABSTRACT

Exposure to contaminated aerosols and water originating from water features may pose public health risks. Endotoxins in air and water and fecal bacteria in water of water features were measured as markers for exposure to microbial cell debris and enteric pathogens, respectively. Information was collected about wind direction, wind force, distance to the water feature, the height of the water feature and the tangibility of water spray. The mean concentration of endotoxins in air nearby and in water of 31 water features was 10 endotoxin units (EU)/m^3 (Geometric Mean (GM), range 0-85.5 EU/m^3 air) and 773 EU/mL (GM, range 9-18 170 EU/mL water), respectively. Such mean concentrations may be associated with respiratory health effects. The water quality of 26 of 88 water features was poor when compared to requirements for recreational water in the Bathing Water Directive 2006/7/EC. Concentrations greater than 1000 colony forming units (cfu) *E. coli* per 100 mL and greater than 400 cfu intestinal enterococci per 100 mL increase the probability of acquiring gastrointestinal health complaints. Regression analyses showed that the endotoxin concentration in air was significantly influenced by the concentration of endotoxin in water, the distance to the water feature and the tangibility of water spray. Exposure to air and water near water features was shown to lead to exposure to endotoxins and fecal bacteria. The potential health risks resulting from such exposure to water features may be estimated by a quantitative microbial risk assessment (QMRA), however, such QMRA would require quantitative data on pathogen concentrations, exposure volumes and dose-response relationships. The present study provides estimates for aerosolisation ratios that can be used as input for QMRA to quantify exposure and to determine infection risks from exposure to water features.

1. INTRODUCTION

Water features may include decorative fountains, ornamental features and interactive fountains. These water features are often located in public areas such as shopping areas, hospitals, ponds, canals, parks or roundabouts. People may come into contact with water from the water features, which create aerosols to which people may be exposed e.g. by inhalation or ingestion.

Source water to fill water features may include local surface water, groundwater, rainwater or tap water determining the initial water quality. Subsequent contamination may occur through fecal bird droppings, runoff from paved surfaces (including e.g. dog feces), growth of micro-organisms in water (*Legionella* and algae), and in some cases discharges of combined sewer overflows (Schets et al., 2008). These contaminants may include a variety of chemical and biological contaminants, including pathogens and microbial cell debris, such as endotoxins.

A water feature sprays water, including its contaminants, into the air in the form of droplets and smaller water based aerosols. These aerosols may contaminate air, depending on feature specific factors such as water quality, flow rate, aerosolisation ratio (the fraction of the sprayed water that becomes an aerosol) and plume height (Environmental Protection Agency, 1982) and climatic factors such as temperature, rainfall, wind velocity and wind direction (Hunter, 2003). Contaminated aerosols potentially may have negative health effects for people who are exposed through contact, ingestion or inhalation (Carducci et al., 2000). Inhalation of aerosols will be the most likely route of exposure at water features if the spray device at the water feature produces aerosols within the respirable size range. At water features where produced aerosols are not in the respirable size range, ingestion of water may occur, whether intended (by swallowing mouthfuls of water) or unintended (through ingestion of aerosols or water droplets or through hand-mouth contact). Exposure to contaminated aerosols has been discussed in the context of many studies in agricultural and industrial environments, and is potentially associated with adverse health effects (Health Council of the Netherlands, 2010). For instance, exposure through inhalation of endotoxins causes an increased prevalence of (work-related) airway and flu-like symptoms (Pillai and Ricke, 2002), and gastrointestinal and neurological complaints and joint pain in sewage workers (Laitinen et al., 1994; Lundholm and Rylander, 1983). Furthermore, exposure through ingestion of aerosols with fecal pathogens can cause gastrointestinal diseases (Uhrbrand, Schultz and Madsen, 2011).

To be able to indicate possible public health risks from exposure to water features we measured endotoxins in air and water as well as fecal indicator bacteria in water. Endotoxins and fecal indicators were measured to provide insight into exposure through inhalation and ingestion of water(spray) from water features. Since, exposures near water features may be influenced by several climatic and feature-specific factors (i.e. wind direction, distance to the fountain, height of the plume of the fountain etc.), these factors were measured as well.

2. MATERIALS AND METHODS

2.1 Selection of markers to quantify exposure originating from water features

Endotoxin was measured as a marker for human exposure through inhalation of contaminated aerosols near water features. Endotoxins are lipopolysaccharides present in the outer membrane of gram-negative bacteria and some algae (Anderson, Slawson and Mayfield, 2002) and were often regarded as a marker of exposure through inhalation to gram-negative bacteria (Douwes et al., 2003). Furthermore, endotoxins are easy to measure, which is an advantage as compared with the detection of living micro-organisms in air, such as pathogens and algae. Concentrations of endotoxin were measured in water and in air.

The fecal indicator bacteria *E. coli* and intestinal enterococci were measured in water to indicate the presence of enteric pathogens (World Health Organization, 2011). It should be noted that there is no correlation between these fecal indicator bacteria and endotoxins (Anderson, Slawson and Mayfield, 2002), and that endotoxins do not equate to gram negative enteric pathogens.

2.2 Sampling sites

From March until June 2010, water samples were taken at 57 water features to determine the concentration of endotoxins, *E. coli* and intestinal enterococci. Based on the concentrations of endotoxin in water, the minimum duration to take air samples was predicted using a dilution factor from water to air of 10^8 (Stellacci et al., 2010). Subsequently, from June until November 2010, air and water samples were taken at 31 water features in the cities of Utrecht, Nijmegen, Rotterdam and Groningen. Samples were taken at locations where people potentially could be exposed to water from decorative and interactive water features. We installed a tripod at multiple wind directions from individual water features where possible. The sampling sites included 13 ornamental water features, 13 fountains in a pond or a canal and 5 interactive features. An interactive fountain was defined as a fountain where children were encouraged to play with water.

2.3 Sampling procedure

Air samples were collected using Gilian GilAir5 portable pumps at a flow of 3.5 liter per minute in combination with GSP inhalable dust sampling heads (JS Holdings) with 37 mm glass fiber filters (Whatman GF/A). The sampling device was placed on a tripod (height 1.5 m) at the nearest location to the fountain where people potentially could be exposed through inhalation. The measurement equipment was installed and collected on average 4.1 hour later (SD 1.1 h, range 3.1-8 h). If possible, the measurement equipment was installed at two sides of each fountain: one downwind and the other upwind of the location. On each sampling day, the equipment contained a control filter which was handled in the same way as the other samples, except for the active sampling. Information was collected about the height of the fountain, the distance from the fountain to the measurement equipment, the water and outside temperatures, and precipitation. Furthermore, the sample taker determined if water spray was tangible at the location of the tripod. The tangibility of the water spray was defined as water spray on skin of the sample taker during installation of the

measurement equipment. Subsequently, it was recorded whether the fountain was sheltered from the wind or was influenced by the wind, causing aerosols to be entrained in a certain direction. Furthermore, information was collected on the volume of the water system, whether it was a closed system and whether the water was disinfected by some kind of treatment.

2.4 Analytical procedures of water samples

Samples were collected directly into individual sterile 500 mL bottles and transported to the laboratory in a chilled cold box. Samples were analyzed for endotoxins as described previously by (Spaan et al., 2008). Samples of 50 mL were centrifuged at 1000g and the supernatant was stored at -20 °C. After collection of all samples, samples were analyzed by the quantitative kinetic chromogenic Limulus amebocyte lysate (LAL) method (Cambrex, Verviers, Belgium; lot no. lysate 1L676S, lot no. standard 2L20090 (RSE/CSE ratio 11.5 EU/ng)), in which the assay solution was pyrogen-free water +0.05% Tween-20. Samples were assayed at an initial dilution of 1:500 and, when the measured concentration was above the detection limit of the assay, retested at higher dilutions (up to 1:10,000). Additionally, water samples of 40 mL, 10 mL and 0.1 mL were analyzed within 24 hour of sampling for fecal indicator bacteria *E. coli* and intestinal enterococci. *E. coli* was enumerated using the Rapid Test on Tryptone Soy Agar (996292, Oxoid, Wesel, Germany) and Tryptone Bile Agar (806567, Oxoid) according to ISO9308-1 (Anonymous, 2000a). Colonies were confirmed with James Reagens (BioMerieux, Marcy ll'Etiole, France) according to the manufacturer's instructions. Intestinal enterococci were enumerated according to ISO7899-2 (Anonymous, 2000b) on Slanetz and Barley Agar (1005125, Oxoid) and Bile Esculin Azide Agar (726007, Remel).

2.5 Analytical procedures of air samples

Air filters were stored at -20 °C until all samples were collected. For extraction of endotoxin, each filter was immersed in 5 mL of pyrogen-free water with 0.05% Tween-20 in a glass tube and rocked vigorously for 1 hour at room temperature on a horizontal shaker. After 15 minutes of centrifugation at 1000·g, 1 mL aqueous supernatant per sample was collected and vortexed. The endotoxin concentration in the extract was assayed with the kinetic chromogenic Limulus Amoebocyte Lysate (LAL) method. Samples were assayed at an initial dilution of 1:50, and if the concentration was below detection limit, retested at lower dilutions (up to 1:25).

2.6 Computational and statistical methods

Data were analyzed with SPSS statistical software version 16. The concentration of endotoxins in air (EU/m^3) was calculated by dividing the number of endotoxin units (EU) on the filter by Q^*t, in which Q was the flow rate of the filtered air (m^3/min) and t was the sampling duration in minutes. Descriptive statistics for endotoxins, *E. coli* and intestinal enterococci were given as arithmetic mean (AM), standard deviation of AM (SD), geometric mean (GM), geometric standard deviation (GSD) and minimum and maximum per type of fountain. Because all meas-

ured concentrations were assumed to be log-normally distributed (Spaan et al., 2007), all calculations were performed with log-transformed concentrations to the base e. Regression analysis was used to explore which factors were associated with exposure, with exp(ß) representing the factor by which the estimated exposure was changed per unit change. For dummy variables, exp(ß) represented the difference in estimated exposure when the characteristic was present versus absent. In addition, a logistic regression analysis was conducted, because a high number of observations were below the detection limit. With a logistic regression analysis, an odds ratio (OR) was obtained that can be interpreted as the likelihood of observations above the detection limit when a certain factor was present compared to the likelihood of observations above the detection limit when a certain factor was absent. The significance of this association was shown by the p-value, where a p-value lower than 0.05 was assumed to be significant.

3. RESULTS

3.1 Air measurements

Endotoxin concentrations in air were measured at 31 locations, described in Table 1, yielding 79 air samples. Of these samples, 6 samples were excluded due to failure of equipment, 34 samples (taken at 19 locations) exhibited endotoxin concentrations above the detection limit of 0.8 EU/m^3 and the other 39 samples exhibited endotoxin concentrations below this detection limit.
The average distance between the fountain and the air measurement equipment was 8 meters (GM, range 0.5-49 m) with 18 measurements performed within a distance of 2 meters, 16 measurements performed at a distance between 2 and 10 meters and 39 measurements performed at a distance greater than 10 meters. According to the Royal Netherlands Meteorological Institute, all measurements took place at a wind speed below three Beaufort (5.5 m/s). Furthermore, all measurements took place on dry, cloudy days with temperatures below 20°C. Thirty measurements (41%) were carried out at locations where the water spray was tangible by the sample taker during installation of the measuring equipment. Twenty-two measurements (30%) were performed downwind, and during the other 51 measurements (70%) no main wind direction was observed. Forty-five measurements (62%) were executed at locations that were sheltered from wind; during the other 28 measurements (38%), the fountain was influenced by the wind causing aerosols to be entrained in a certain direction.
The concentrations of endotoxin in air ranged up to 85.5 EU/m^3, the average concentration (GM) was 10 EU/m^3 (Table 1). Statistical analysis showed that there were no differences between concentrations of endotoxin in air and type of fountain, such as fountains in a pond or a canal, ornamental features or interactive features (T- test: $p>0.2$). The concentration of endotoxin in air at water features was dependent on the water source from which water features were filled. The concentration of endotoxin in air at sites fed by surface water contaminated by a combined sewer overflow (CSO) was significantly higher than locations which received surface water or tap water as its source water (T-test: $p<0.05$). No statistical significant difference was found between the

Table 1. Description of 31 locations with water features where air measurements were performed and overview of measurement results.

Kind of fountain[a]	Source water	Closed Circulating System	Disinfection	Concentration fecal indicators in water		Endotoxin Concentration in water	n/N[d]	Concentration of endotoxin in air	Distance[e]	Height[f]
				E.C.[b]	I.E.[c]			Mean (range)		
		Yes/No	Yes/No	cfu/100mL	cfu/100mL	EU/mL		EU/m³	m	m
A	Surface water + CSO[i]	No	No	2 000	800	18 170	4/4	61.8 (33.1-85.5)	15	7
A	Surface water	No	No	300	80	3 000	2/2	20.0(6.4-34.1)	15	3
A	Surface water	No	No	80	550	1 370	3/3	12.7(3.0-17.8)	6-20	6
A	Surface water	No	No	800	70	916	1/1	5.9	5	2
A	Surface water	No	No	60	90	1 103	1/4	6.3	10-20	3
A	Surface water	No	No	50	20	1 017	0/2	-	30	7
A	Surface water	No	No	450	100	9 029	0/2	-	33	10
A	Surface water	No	No	180	40	6 976	0/2	-	23	6
A	n.a.[g]	n.a.	n.a.	100	23	3 063	1/2	16.8	10	2
A	n.a.	n.a.	n.a.	40	50	211	0/2	-	8	7
A	n.a.	n.a.	n.a.	6 000	500	2 385	2/2	2.6 (2.5-2.7)	22	7
A	Surface water	No	No	90	35	5 092	2/3	3.2 (2.9-3.5)	32	9
A	Surface water	No	No	1 700	500	1 603	1/2	2.9	33	6
B	Surface water	Yes	No	190	260	404	1/2	6.0	7	3
B	Tap water	Yes	Yes SF[h] and UV	<1	4	1 332	1/2	10.6	0.5	1
B	Tap water	Yes	Yes Chlorine	<1	<1	669	1/3	7.8	13-24	18
B	n.a.	n.a.	n.a.	1	10	75	0/2	-	2	1
B	n.a.	n.a.	n.a.	<1	<1	54	0/1	-	1	1
B	n.a.	n.a.	n.a.	640	150	161	0/2	-	2	1
B	Surface water	Yes	No	300	80	2 490	3/4	11.0 (3.7-25.5)	45	20
B	n.a.	n.a.	n.a.	30	20	1 415	2/2	5.8 (5.0-6.6)	8.5	3
B	n.a.	n.a.	n.a.	6	2	929	2/3	7.1 (5.3-8.9)	0.5-1	0.5
B	Tap water	Yes	Yes Chlorine	<1	<1	47	0/3	-	0.5-8	9
B	n.a.	n.a.	n.a.	200	3	1 540	2/3	16.7 (13.5-19.9)	4	6
B	Surface water	Yes	No	240	60	3 639	2/4	20.0 (18-22)	22	20
B	n.a.	n.a.	n.a.	10	20	1 619	1/2	-	17	5
C	Tap water	Yes	Yes SF and UV	40	32	9	0/2	-	1	1
C	Tap water	Yes	Yes SF and UV	12	26	11	0/2	-	1	0.5
C	Surface water	Yes	Yes Chlorine	3 000 000	1 000 000	2 799	1/1	19.0	4	2
C	Tap water	Yes	No	8	200	67	0/2	-	0.5	1
C	Tap water	Yes	Yes Chlorine	1	60	923	2/2	8.5 (7.2-9.8)	0.5	2

a) Kind of fountain is subdivided in Fountain in a pond or a canal (A), Ornamental fountain (B) and Interactive fountain (C) b) *E. coli* c) Intestinal Enterococci d) n/N represents the number of positive air samples divided by the number of total air samples e) Distance of air sampling device to fountain in meters f) Height of the plume of the fountain in meters g) n.a. = not available; h) SF= Sand Filtration UV= Ultraviolet i) Combined Sewer Overflow

Table 2. Concentrations of endotoxin, *E. coli* and intestinal enterococci in water of water features.

		Tap water	Surface water
Endotoxin in water (EU/mL)	n/N[a]	20/88	54/88
	AM[b]	$6.9*10^{2}$	$1.1*10^{3}$
	SD[c]	$7.9*10^{2}$	$1.7*10^{3}$
	GM[d]	$2.6*10^{2}$	$3.6*10^{2}$
	Min[e]	$9.0*10^{0}$	$1.3*10^{1}$
	Max[f]	$3.1*10^{3}$	$1.8*10^{4}$
E. coli in water cfu/100mL	n/N	11/88	40/88
	AM	$9.8*10^{1}$	$7.6*10^{4}$
	SD	$1.9*10^{2}$	$4.7*10^{5}$
	GM	$3.5*10^{1}$	$3.4*10^{2}$
	Min	$2.0*10^{0}$	$2.0*10^{0}$
	Max	$6.2*10^{3}$	$3.0*10^{6}$
Intestinal Enterococci in water Cfu/100mL	n/N	14/88	34/88
	AM	$4.9*10^{2}$	$3.0*10^{4}$
	SD	$1.6*10^{3}$	$1.7*10^{5}$
	GM	$2.5*10^{1}$	$7.2*10^{1}$
	Min	$1.0*10^{0}$	$6.0*10^{0}$
	Max	$6.4*10^{2}$	$1.0*10^{6}$

a) number of positive samples / number of total samples b) Arithmetic Mean c) Standard Deviation of AM d) Geometric Mean e) Minimum f) Maximum.

concentrations of endotoxin in air measured at water features fed by surface water as compared with tap water (T-test: $p>0.2$).

3.2 Water measurements

Endotoxin and fecal indicator bacteria concentrations were measured at 88 locations. All water samples contained detectable concentrations of endotoxin, with the majority containing both *E. coli* and intestinal enterococci (72%): The arithmetic means, geometric means (GM) and concentration ranges in all samples are shown in Table 2, organized to the origin of water of the fountain. The water samples showed concentrations of endotoxin ranging between 9 and 18,170 EU/mL, the geometric mean concentration being 773 EU/mL. Furthermore, at 26 of the 88 locations, the concentration of *E. coli* and/or intestinal enterococci in water exceeded the standards for fecal indicators in recreational waters according to European Bathing Water Directive 2006/7/EC. The mean temperature of all water samples taken from 88 water features was 18.1 °C (range 15.1 °C -19.7 °C; data not shown). The highest concentration of endotoxin, *E. coli* and intestinal enterococci in water has been detected in a pond that was contaminated by a discharge of a combined sewer overflow. Furthermore, one interactive fountain displayed very high concentrations of fecal indicator bacteria on the sampling day (Table 1).

3.3 Factors associated with exposure to endotoxin originating from water features

Table 3 shows the results of the multivariate logistic regression. Endotoxins in the air were detected more often when high concentrations of endotoxin were present in water. For each natural log increase of endotoxins in water, endotoxins in air were detected 3.0 times more frequently and

Table 3. Results of multi-variate logistic and linear regression analysis.

Monitoring parameters	Logistic Regression				Linear Regression		
	OR[b]	95%CI		P-value	Regression Coefficient ß [c]	S.E.	P-value
Concentration of endotoxin in water (EU/mL water) (lognormal transformed)	3.0	2.2	4	<0.01	0.47	0.12	<0.01
Distance of fountain to measurement location (m)	0.9	0.84	0.93	<0.01	-0.05	0.02	<0.01
Height of the plume of the fountain (m)	1.1	1.1	1.2	0.21	0 02	0.02	0.41
Tangibility of water spray (0/1)[a]	1.6	0.8	3.0	0.84	0.8	0.24	<0.01
Downstream wind (0/1)	1.2	0.5	5.7	0.52	0.07	0.15	0.65

a) (0/1) dummy variable: present versus absent b) Odd Ratios can be interpreted as the likelihood of the presence of endotoxin in air compared to a unit change or the presence or absence of a monitoring parameter. c) exp(ß) represented the factor by which the concentration of endotoxin (EU/m³ air) was changed per unit change or the presence or absence of a monitoring parameter

for each meter further away from the water feature, the likelihood to detect endotoxins decreased 0.9 times. The tangibility of water spray and the height of the plume of the water feature were also associated with the presence of endotoxin in air, however, this association was not significant. Other characteristics, such as the presence of a closed water system and use of disinfection procedures, were not included in regression models because this information was unavailable for too many water features. Also the wind direction was not included because it was impossible to observe the main wind direction for the majority of the measurements. Results from the multi-variate linear regression analyses are presented in table 3. Significant associations were also checked by use of scatter plots (not shown). For the concentration of endotoxins in water, the regression coefficient amounted to 0.47 per increase of a natural log unit in water, which implied that for the mean concentration of 773 EU/mL in water features, the concentration in air was 22.7 EU/m³ higher than when the concentration in water was 0 EU/m³. For the distance of the fountain to the measurement location, the regression coefficient amounted -0.05, which implied that for a distance of 10 m to the fountain, the concentration in air was 0.9 EU/m³ lower than at the location of the water feature. For the tangibility of water spray, the regression coefficient amounted to 0.8, which implied that when the aerosols were tangible, the concentration in air was 2.2 EU/m³ higher.

4. DISCUSSION

4.1 Air Quality

In this study, we explored whether human exposure to microbial markers can occur from inhalation near water features. The geometric mean of all air samples of 10 EU/m³ air (GSD=2.1, range 0-85.5 EU/m³ air) was high compared to the study of Mueller-Annelling et al., 2004) who reported a weekly average with a geometric mean of 0.44 EU/m³ in outdoor air (GSD 3.1, range 0.03-5.5

EU/m^3 air). Since similar data on endotoxin levels in outdoor air were limited (Health Council of the Netherlands, 2010), further comparisons cannot be made. Endotoxin concentrations in air have also been measured in situations where risks of exposure to endotoxins were expected, such as around livestock facilities and wastewater treatment plants. In a major survey in air near and at Dutch wastewater treatment plants, endotoxin concentrations were measured with a geometric mean of 27-64 EU/m^3, which were associated with work-related health effects in sewage workers (Spaan et al., 2008; Smit, Spaan and Heederik, 2005). Furthermore, in the USA, endotoxin concentrations of 30 EU/m^3 air were measured downwind of swine livestock facilities (Thorne, Ansley and Perry, 2009). Such exposure levels in air were associated with respiratory health effects for neighboring residents (Radon et al., 2007; Schinasi et al., 2011). It should however be noted that, in our study, for 12 of the 31 water features (39%), the endotoxin measurements were below the detection limit, indicating that respiratory health effects were unlikely.
The maximum measured exposure concentration of 85.5 EU/m^3 air in this study approaches the exposure limit set by the Health Council of The Netherlands for the work environment of 90 EU/m^3 air over an eight-hour period (Health Council of the Netherlands, 2010). Exposure time at water features may range from several seconds/minutes to several hours, but is unlikely to reach an eight-hour period. However, the measured exposure concentration near water features may be a health risk for people with atopic diseases including rhinitis and asthma, since they have an increased susceptibility to endotoxins (Smit et al., 2009), which may require shorter exposure times.

4.2 Water quality

No requirements for water quality of water features exist. However, at one-third of the water features, the concentrations *E. coli* and intestinal enterococci in water exceeded the standards for fecal indicator bacteria according to the European Bathing Water Directive (2006), indicating that pathogens might be present. Such pathogens may originate from specific sources, such as feces of animals (e.g. birds) or people (in case of combined sewer overflows). Ingestion of such pathogens may cause gastrointestinal infections. Ingestion may occur due to ingestion of aerosols or due to ingestion of water through hand-mouth contact, through ingestion of water droplets during splashing or through drinking of mouthfuls of water. Therefore, especially at interactive water features, infection risks through ingestion may be larger than risks through inhalation.
Pathogens may also originate from the environment (such as *Legionella spp.*). Several outbreaks are known to have been caused by *Legionella spp.* at water features (Haupt et al., 2012; Hlady et al., 1993; O'Loughlin et al., 2007; Palmore et al., 2009; Correia et al., 2001). This, together with the fact that for many cases of infection with *Legionella spp.* the source of the pathogen is unknown, indicates a possible role for water features as a transmission route/source of this pathogen (Schalk et al., 2012). Furthermore, *Legionella* is gram-negative and, as a result, a source of endotoxins. Therefore, exposure to pathogens such as *Legionella*, as well as to endotoxin near water features cannot be excluded as posing a potential health risk.
The concentrations of endotoxins found in water samples in this study are in line with those found

in other studies (Anderson, Slawson and Mayfield, 2002; Watson et al., 1977), except for the concentration of endotoxin in waters influenced by discharges of combined sewer overflows. As reviewed by Anderson et al. 2002, the concentration of endotoxins in raw untreated surface water ranged from >10 to 10 500 EU/mL, with concentrations in general below 500 EU/mL water. These concentrations were determined at locations including those where a combined sewer overflow (CSO) discharged on surface water. Furthermore, a study near showers and humidifiers (Anderson, Dixon and Mayfield, 2007) indicated that humidifiers filled with drinking water with concentrations of endotoxin above 1,000 EU/mL were likely to induce symptoms like chills and fever, often referred to as Organic Dust Toxic Syndrome (Basinas et al., 2011). In this study, the endotoxin concentrations ranged up to 18,170 EU/mL in water of water features that were influenced by a combined sewer overflow. Therefore, water features in surface water influenced by a combined sewer overflow should be avoided to prevent human exposure to contaminated aerosols.

4.3 Factors that determine exposure near water features

Possible health effects from exposure to micro-organisms in air are determined by the density of micro-organisms in the aerosolized liquid, the height of the plume of aerosols and the distance between an exposed person and the source of aerosolisation (Environmental Protection Agency, 1982). In addition, possible health effects from exposure to bioaerosols are likely to be affected by the particle distribution of the aerosols. The size of aerosols influences deposition efficiency in the respiratory tract (Heyder et al., 1986). Smaller aerosols (<5mm) have a higher deposition in the alveolar tract. Larger particulates (<15mm and >5mm) predominantly deposit in the higher airways and will be ingested. In this study, particulates smaller than approximately 15μm were measured by GSP inhalable dust sampling heads; those particulates can enter the respiratory tract during inhalation. Furthermore, the concentration of aerosols in air is determined by the flow rate of the water (i.e. the sprayed volume per time unit) and the aerosolisation factor (i.e. the fraction of water which becomes an aerosol) (Environmental Protection Agency, 1982). These factors differ for different types of water features, for instance, a decorative fountain regularly aerosolizes more water than an interactive feature. The flow rate of the sprayed water and the aerosolisation factor were not taken into account in this study due to difficulties in gathering this information for all water features; however, these factors are critical to determine how closely a person may interact with a fountain. Moreover, although these factors may differ for the different types of water features, in this study no differences in concentrations of endotoxin in air were found between ornamental features, interactive features and fountains in surface waters. The concentration of endotoxin in air was found to be associated with the concentration of endotoxin in water, the distance to the water feature and the tangibility of water spray. As a result, possible health effects are mainly determined by these factors and by the susceptibility of exposed people for waterborne disease (immunocompromised or not), the level of exercise and the duration of exposure. For instance, the duration of exposure for those playing with an interactive water fountain may be longer than the duration of exposure for those walking next to a fountain. Health effects due to exposure through

inhalation of endotoxins may include respiratory symptoms such as dry cough or shortness of breath, accompanied by a decrease in lung function (Health Council of the Netherlands, 2010). Exposure through inhalation of pathogens may cause infectious diseases and allergies (Douwes et al., 2003). Furthermore, exposure through ingestion of aerosols with pathogens originating from feces may cause gastrointestinal diseases (Uhrbrand, Schultz and Madsen, 2011).

4.4 The value of this study for quantitative microbial risk assessment (QMRA)

Public health risks associated with water features may be quantified by means of microbial risk assessment (World Health Organization, 2011). A QMRA requires data about pathogen concentrations, exposure volumes and dose-response relationships. To perform a QMRA, a characterization of spray devices (i.e. their flow rate and aerosolisation ratio) is required to transform the sprayed volume of water into the volume of water that is inhaled or ingested by people exposed to it (Environmental Protection Agency, 1982). However, in absence of these data, an aerosolisation ratio can be estimated based on the concentrations of endotoxin in air and water. In this study, the aerosolisation ratio (expressed as EUm^{-3} aerosol / EUm^{-3} water) ranged from $2.0 \cdot 10^{-10}$ to $1.1 \cdot 10^{-7}$ with on average $8.6 \cdot 10^{-9}$ for all water features. These aerosolisation ratios represent the inhalable fraction of aerosols and can be used, as was done by Stellacci et al. (2010), to calculate infection risks. QMRA may also provide insight into possible intervention measures. To achieve this, information is required about faecal and other inputs to source water(s), the performance (effectiveness and reliability) of water treatment applied to water supplying fountains, as well as properties of spray devices. While all of this data were not collected as part of this study, our data can be used to inform preliminary risk assessments and as a basis for designing future studies to determine the most appropriate intervention measures to reduce health risks associated with water features.

5. CONCLUSION

The present study has demonstrated the presence of endotoxins in air and water and fecal indicator bacteria in water of water features. Exposure to microbial content near water features may give rise to respiratory and gastrointestinal complaints. The extent to which exposure to water features causes these complaints should be investigated, probably through an epidemiological study. Exposure can be minimized by precautionary measures that improve the water quality or decrease the contact with contaminated aerosols. Water quality can be improved by water filtration (to remove endotoxins and pathogens) and disinfection (to inactivate pathogens) and in case of combined sewer overflows no water features should be installed or otherwise CSO storage and treatment facilities should be built. The contact with aerosols can be minimized by choosing locations for water features well away from people or by changing the water spray to spray fewer aerosols. A quantitative microbial risk assessment (QMRA) could be used to estimate the potential risks of infection (World Health Organization, 2011) resulting from exposure to water and aerosols from water features.

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Chapter 3

Health risk assessment for splash parks that use rainwater as source water

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ABSTRACT

In the Netherlands, rainwater becomes more and more popular as an economic and environmentally sustainable water source for splash parks, however, the associated public health risk and underlying risk factors are unknown. Since splash parks have been associated with outbreaks of infectious diseases, a quantitative microbial risk assessment was performed using *Legionella pneumophila* as a target pathogen to quantify the risk of infection for exposure due to inhalation and *Campylobacter jejuni* for ingestion. Data for *L. pneumophila* and *C. jejuni* concentrations in rainfall generated surface runoff from streets were extracted from literature. Data for exposure were obtained by observing 604 people at splash parks, of whom 259 were children. Exposure volumes were estimated using data from literature to determine the volume of exposure through inhalation at 0.394 µL/min (95% CI-range 0.0446-1.27 µL/min), hand-to-mouth contact at 22.6 µL/min, (95% CI-range 2.02-81.0 µL/min), ingestion of water droplets at 94.4 µL/min (95% CI-range 5.1-279 µL/min) and ingestion of mouthfuls of water at $21.5 \cdot 10^{3}$ µL/min (95% CI-range $1.17 \cdot 10^{3}$ - $67.0 \cdot 10^{3}$ µL/min). The corresponding risk of infection for the mean exposure duration of 3.5 minutes was $9.3 \cdot 10^{-5}$ (95% CI-range $0\text{-}2.4 \cdot 10^{-4}$) for inhalation of *L. pneumophila* and $3.6 \cdot 10^{-2}$ (95% CI-range $0\text{-}5.3 \cdot 10^{-1}$) for ingestion of *C. jejuni*. This study provided a methodology to quantify exposure volumes using observations on site. We estimated that using rainwater as source water for splash parks may pose a health risk, however, further detailed quantitative microbial analysis is required to confirm this finding. Furthermore we give insight into the effect of water quality standards, which may limit infection risks from exposure at splash parks.

1. INTRODUCTION

Splash parks are often located in shopping areas or play grounds. They are popular features that encourage many children to play with water. Splash parks use water that is typically stored in an underground reservoir or surge chamber and is sprayed into the air; after it hits the ground, it flows back to the reservoir through floor drains. Although almost all splash parks incorporate some form of disinfection, many show poor water quality through poor design or poor maintenance (Kebabjian, 2003). Thus, splash parks have been associated with outbreaks of infectious diseases, including *Legionella* (Hlady et al., 1993; Palmore et al., 2009; Haupt et al., 2012; Correia et al., 2001), *Cryptosporidium* and *Giardia* (Eisenstein, Bodager and Ginzl, 2008b; Anonymous, 2000; Anonymous, 1999), *Shigella* (Fleming et al., 2000; Bancroft, Keifer and Keene, 2010), *Salmonella* (Andión Campos, 1995; Molinero et al., 1998; Usera et al., 1995), *Leptospira* (Cacciapuoti, Ciceroni and Maffei, 1987), and noroviruses (Hoebe et al., 2004).

People may be exposed to waterborne pathogens in splash park through inhalation, ingestion and dermal contact. Inhalation of aerosols causes deposition of water in the respiratory tract (Heyder et al., 1986) and may cause allergic reactions (Douwes et al., 2003). If pathogens are present in water of splash parks, inhalation may cause infectious diseases such as pneumonia due to *Legionella pneumophila* (Fields, Benson and Besser, 2002). Ingestion of water, whether intended (by swallowing mouthfuls of water) or unintended (through getting water droplets in the mouth or through hand-to-mouth contact) can cause gastroenteritis though infection with enteric pathogens such as norovirus, rotavirus, *Campylobacter*, *Giardia* or *Cryptosporidium,* and may cause other severe illnesses such as hemolytic uremic syndrome (Keene et al., 1994) or Gullain-Barré syndrome (McCarthy and Giesecke, 2001). Finally, dermal contact (skin and mucous membranes of nose, ears and eyes in contact with the water) can result in infections such as wound infections due to *Aeromonas hydrophila* (Semel and Trenholme, 1990), otitis externa due to *Pseudomonas aeruginosa* (Van Asperen et al., 1995), or conjunctivitis due to adenoviruses (Crabtree et al., 1997).

Quantitative microbial risk assessment (QMRA) is a tool to quantify health risks and to get insight into measures that can prevent outbreaks (World Health Organization, 2011). A QMRA requires information on the concentration of pathogens in the matrix, the fate and behaviour of pathogens, the volume of water to which people were exposed, and the dose-response relation for the pathogen. Because harvested rainwater has been widely regarded as a sustainable source for water (re)use in urban areas and for recreational purposes, it is often used as a source water of splash parks (De Man et al., 2014b). Pathogens may be present in rainwater dependent on weather conditions such as rainfall intensity and temperature (Schets et al., 2010; Kaushik, Balasubramanian and de la Cruz, 2012). Furthermore, the atmospheric deposition of airborne microorganisms (Evans, Coombes and Dunstan, 2006), the (rooftop) runoff of fecal depositions of birds and other mammals (Ahmed et al., 2012; Fewtrell and Kay, 2007), and the growth or decay of micro-organisms in collected rainwater may influence the presence of pathogens in rainwater (Ahmed et al., 2013). Pathogens may also be introduced into water of splash parks by people, dogs, birds and other animals upon contact with the water (Hoebe et al., 2004)

To be able to quantify the public health risk of splash parks that use rainwater as their source water, an exposure assessment was performed using field observations. The generated exposure data were used to determine exposure volumes and infection risks for inhalation and ingestion by a QMRA approach. The QMRA was performed with Monte Carlo simulations to provide a range of uncertainties in infection risks.

2. METHODS

2.1 Hazard Identification

People are exposed to the water of urban splash parks through ingestion, inhalation and dermal contact. To estimate the public health risks, these exposure routes were used to choose model organisms that (I) were pathogens of concern in situations where people were exposed to water, (II) were present in rainwater and (III) showed a dose-response relation. Based on these criteria, *Legionella pneumophila* was chosen to model inhalation and *Campylobacter jejuni* for ingestion. These pathogens were preferred above other pathogens such as *Giardia, Cryptosporidium* or *Salmonella* because *Legionella pneumophila* and *Campylobacter jejuni* may be present in high concentrations in rainwater, combined with a high pathogenicity and environmental survival, thus posing a potential health risk. For dermal contact, no dose-response relationship is available and therefore this exposure route could not be considered.

Legionella spp. are found in a wide range of water environments and can proliferate at temperatures above 25 °C (World Health Organization, 2011). *Legionella* was assumed not to proliferate in the water of splash parks because the water temperature is generally below 25 °C in the Netherlands (Zuurman, personal communication). Data on *Legionella* concentrations in splash parks are lacking, and therefore we assumed that its concentration was equal to concentrations found in rainwater samples on roads. Reservoirs of splash parks were filled with such rainwater runoff at several locations in the Netherlands (Zuurman, personal communication). *L. pneumophila* was found in rainwater by several studies using PCR (Ahmed et al., 2008; Ahmed et al., 2010) and data about cultured *L. pneumophila* in rainwater on roads were previously reported by Sakamoto et al. (2009) and recently by Van Heijnsbergen et al. (submitted) who used the method described by Schalk et al. (2012). Counts and tested volumes of these data were used to fit a gamma distribution for the concentration of *L. pneumophila* in water of splash parks using the method of Schijven et al. (2011).

Campylobacter is an enteric pathogen that occurs in a variety of environments, such as food and water (World Health Organization, 2011). The presence of *C. jejuni* in rainwater was confirmed by several studies (Fewtrell and Kay, 2007) and also by using PCR (Ahmed et al., 2010). A study by De Man et al. (2014a) showed that culturable *C. jejuni* was present in rainfall generated overland flow; these data were used to fit a gamma distribution for the concentration of *C. jejuni* in water of splash parks. *C. jejuni* may also contaminate the source water of splash parks through the activities of people, birds and other animals while a water feature is in operation. Because there is

no information about such contaminations, these were not included as part of the current study. Almost all splash parks incorporated some form of disinfection. Nevertheless, many splash parks exhibited poor water quality resulting from poor design and/or poor maintenance (Kebabjian, 2003). Furthermore, as De Man et al. (2014) showed, disinfection of rainwater at splash parks is ineffective in reducing pathogen concentrations. Therefore disinfection was not included in this study.

2.2 Exposure Assessment

Exposure volumes (µL/min) were quantified for inhalation and for ingestion. The volume of ingestion due to hand-to-mouth-contact with wet hands per minute (Q_{HM}) was calculated using:

$$Q_{HM} = h \, x \, A \, x \, f_{HM} \qquad [1]$$

where h represented the film thickness of water on hands (mm), A the skin-surface area of the hand that touched the mouth (mm^2) and f_{HM} the frequency of hand-to- mouth contact (n per min). The volume of ingestion of droplets of water in the mouth (Q_D) was determined using:

$$Q_D = V_D \, x \, f_D \qquad [2]$$

where V_D represented the volume of water droplets (µL) and f_D the frequency of splashing water droplets in someone mouth (n per min). The volume of ingestion due to drinking mouthfuls of water per minute (Q_m) was determined using:

$$Q_M = V_M \, x \, f_M \qquad [3]$$

where V_M represented the volume of a mouthful of water (µL) and f_M the frequency that people take a mouthful of water (n per min). The inhaled volume of water per minute during the visit of the splash park was determined using:

$$Q_I = IR \, x \, VIWS \qquad [4]$$

where IR represented the inhalation rate of air (m^3/min) and $VIWS$ the fraction of inhalable water spray (µL water /m^{3}air).

Values of model parameters were considered to be uncertain, therefore an uncertainty analysis was carried out through Monte Carlo simulations. Model parameters were explained by various types of distributions. To determine the volume distributions according to equation 1-4, from each of these distributions, 10^6 values were randomly drawn using Mathematica version 9.0 (Wolfram Research).

Film thickness of water on hands, h [mm]. The film thickness of liquids on skin was represented by the amount of material that remains on the skin after contact with a liquid. A value for h was estimated in an experiment by U.S. EPA (2011) as the amount of liquid retained on the skin

(g/mm^2) divided by the density of the liquid (g/mm^3) used. This showed a range of 2.34×10^{-2} to 1.97×10^{-2} mm for retention of water on the skin after initial contact with water. The uncertainty of h was considered to be uniformly distributed within this range.

Skin-surface area of the hand that was mouthed, A [mm^2]. Most frequently, a finger or a part of a finger is mouthed by children (U.S. EPA, 2011). The average surface area of child's finger is reported to be 2000 mm^2 (U.S. EPA, 2011). Therefore, the uncertainty of A was assumed to be uniformly distributed between 100 and 2000 mm^2 of a hand for children (U.S. EPA, 2011)

The frequency of hand-to-mouth contact f_{HM} [min^{-1}]**.** Freeman et al. (2001) gathered hand-to-mouth frequency data of 102 children. Boys were observed to have hand- to-mouth contact of 1.7 (0-5.6) times per hour, girls 2.3 (0-6.2) times per hour. Because data on the variability of hand-to-mouth contact for boys and girls and its uncertainty have not been reported and were unavailable upon request, we assumed the uncertainty of f_{HM} could be described by a Gamma Distribution. The gamma(α,b) distribution models the time required for α events to occur, given that the events occur randomly in a Poisson process with a mean time between events of β (Vose, 2008). Therefore, the uncertainty of f_{HM} was assumed to be gamma distributed with α=2 and β=0.5.

Volume of a droplet, V_D [µL]. Children who make each other wet cause droplets of water to be ingested by themselves or other children. These water droplets were assumed to be spherical and to have a diameter that varied between 1 and 10 mm, to reflect the range in inhalable droplet sizes. The volume of a water droplet was determined by $4/3\pi r^3$, thus the volume of water of one droplet varied between 0.5 µL and 524 µL, for which a uniform distribution was assumed in absence of data on a more specific distribution.

Frequency of getting droplets of water in the mouth f_D [min^{-1}]**.** The frequency of getting a splash of water in the mouth was determined using onsite observations. The number of times that an individual received a splash of water in their face was counted, assuming that per moment of splashing one droplet was ingested by that person. Using the total duration of exposure of a person, a gamma distribution was fitted for the frequency of getting droplets of water in the mouth as described previously in this paragraph.

Table 2. Model parameters used in the exposure assessment

Parameter	Value or Distribution of values	Source
IR, Inhalation Rate (m^3/min)		
Children	Uniform [$1.11\cdot10^{-2}$, $4.36\cdot10^{-2}$]	(U.S. EPA, 2011)
Adults	Uniform [$1.03\cdot10^{-2}$, $7.77\cdot10^{-2}$]	
VIWS, Volume of inhalable water spray , (µL/m^3)	Average: 10.8 95% Confidence Interval: 1.76-36.3	(De Man et al., 2014b)
h, Film Thickness of water on hands, (mm)	Uniform[$1.97*10^{-2}$, $2.34*10^{-2}$]	(U.S. EPA, 2011)
A, Surface area of the hand that is mouthed, (mm^2)	Uniform[100,2000]	(U.S. EPA, 2011)
f_{HM}, Frequency of hand-to-mouth contact, (n/min)	Gamma[2,0.5]	(Freeman et al. , 2001)
f_D, Frequency of getting water droplets in the mouth (n/min)	Gamma[2.1,0.17]	Field observations
V_D, Volume of a droplet (µL)	Uniform[0.5,524]	Estimate
V_M, Volume of a mouthful of water (µL)	Gamma[4.72,5300]	(Schets, Schijven and de Roda Husman, 2011)
f_M, Frequency of taking a mouthful of water (n/min)	Gamma[1.2,0.76]	Field observations

Volume of a mouthful of water V_M [μL]. The volume of a mouthful of water V_M was estimated by Schets, Schijven and de Roda Husman (2011). The mean volume of a mouthful of water for a child was described by a Gamma distribution with α=4.72 and β=5300 and used as such in this study.
Frequency of taking a mouthful of water f_M [min^{-1}]. The frequency of taking a mouthful of water was also determined using observations on site. The number of times that a person leans over a fountain to take a mouthful of water was counted assuming that each time, one mouthful was ingested. Using the total duration of exposure of a person, a gamma distribution for the frequency of taking a mouthful of water was fitted as described previously.
Inhalation Rate, *IR* [m^3/min]. The inhalation rate (*IR*) is dependent on the intensity of people's activity. *IR* for children varied between $1.01 \cdot 10^{-2}$ m^3/min for light activities and $6.24 \cdot 10^{-2}$ m^3/min for high intensity activities. For adults, it varied between $1.03 \cdot 10^{-2}$ and $7.77 \cdot 10^{-2}$ m^3/min (U.S. EPA, 2011).
Volume of inhalable water spray, *VIWS* [μL water/ m^3 air]. The fraction of inhalable water spray (*VIWS*) per m^3 was extracted from a study of de Man et al. (2014b). The concentration of inhalable endotoxin in air near splash parks varied from 7.2 to 19 endotoxin units (EU)/m^3, while the concentration of endotoxins in water varied from 9 to 2799 EU/mL. That study showed a significant linear relation between the EU in water and air (R^2 =0.645). The volume of inhalable aerosols per cubic meter *(VIWS)* was estimated by maximum likelihood using beta regression (Espinheira, Ferrari and Cribari-Neto, 2008; Ferrari and Cribari-Neto, 2004). The parameters α and β for a Beta (α, β) distribution were re-parameterized with a mean dilution factor μ and a precision parameter ϕ according to $\alpha = \mu\phi$ and $\beta = (1-\mu)\phi$. The values for μ and ϕ were estimated by maximum likelihood. The uncertainty distribution for μ was obtained by Markov Chain Monte Carlo sampling from the likelihood function with the Metropolis-Hästings algorithm (Gilks, Richardson and Spiegelhalter, 1996).
Field observations to collect quantitative data on the behaviour of splash park visitors were performed at two splash parks in urban centers on five days in June 2010 from 12:00AM until 4:00PM. During these observations, distinction was made between the different routes of exposure: 1) having wet hands, 2) having a wet face, 3) drinking mouthfuls of water and, 4) being present within 2 meters of the water spray. Field observations were used to quantify several exposure model parameters (see Table 2).
Exposure through ingestion was mainly the case for children who interacted with water. Therefore, exposure through ingestion was only calculated for children. This choice was also made because there was a lack of information for model parameters A, f_{HM}, f_D and f_M for adults. Exposure through inhalation was calculated for children as well as for adults, because adults were exposed through inhalation while chaperoning their children.

2.3 Dose Response

The dose D of exposure to the model organisms *L. pneumophila* and *C. jejuni* was calculated by multiplication of the concentration distribution C (numbers of pathogens per liter) and total exposure volume V (liter). The distribution for V for ingestion was calculated by:

$$V_{Ingestion} = (Q_{HM} + Q_D + Q_M)\, t \qquad [5]$$

where t was the duration of exposure according to the field observations and Q_{HM}, Q_D and Q_M were summed when relevant according to the observed routes of exposure during the field observations. The risk of infection for inhalation due to exposure to *Legionella* was analyzed using the exponential dose-response relation

$$P_{inf} = 1 - e^{-rD} \qquad [6]$$

where r represents an infectivity of 0.06 (Armstrong and Haas, 2007). The risk of infection for ingestion due to exposure to *Campylobacter* was analyzed using the hypergeometric dose-response relation

$$P_{inf} = (D; \alpha,\beta) = 1 - {}_1F_1\,(\alpha, \alpha + \beta, -D) \qquad [7]$$

where ${}_1F_1$ was a Kummer confluent hypergeometric function and α and β represented the beta distributed dose response parameters for *Campylobacter*. In the case of *C. jejuni*, the values for parameters α and β were 0.024 and 0.011, respectively (Teunis et al., 2005).

2.4 Risk Characterization

The risk of infection for inhalation and ingestion was calculated as a function of the duration of exposure. Given the large range of possible concentrations of *L. pneumophila* and *C. jejuni,* a scenario analysis was performed for five scenarios with different concentrations, from 1-10^4 cfu/L water. Subsequently a sensitivity analysis was carried out to determine the effect of the model parameters on the risk of infection. It was assumed that the model parameters were independent. The effect of the value of a model parameter was calculated by varying a model parameter (within its range of uncertainty), including the uncertainties of the other parameters.

3. RESULTS

A QMRA was performed to calculate the risk of infection inherent to exposure to splash parks using rainwater as their source. To quantify the risk of infection, *L. pneumophila* and *C. jejuni* were selected as target pathogens. The concentration of *L. pneumophila* in rainwater was described by a Gamma distribution with r=0.045 and λ=26000 with an average concentration of *L. pneumophila* of 1200 cfu/l. The concentration of *C. jejuni* in rainwater was described by a Gamma distribution with r=0.76 and λ=330, the average concentration of *C. jejuni* was 250 cfu/l.
Exposure was investigated during outdoor temperatures between 20-23 degrees. Six hundred and four people were observed, of whom 259 were children estimated to be below 13 years old. Significant differences ($p<0.05$) in behavior (*i.e.* in exposure) were observed between people younger and older than 13 years (Table 1). The mean duration of a visit at an interactive water

Table 1. Results of observations: Number of people observed per category of exposure

	Having wet hands	Having wet face	Drinking mouthfuls of water	Being present within 2 meters of water spray
Children	198	65	8	257
Adults	192	31	2	347

fountain was 3.5 minutes (range 1-120 min). No significant differences (p>0.05) were observed between the duration of a visit for people younger and older than 13 years. Observations on site were used to quantify the frequency that droplets of water reached children's mouths (f_D), these data (N=12 children) were fitted to a gamma distribution, resulting in parameters r=2.1 and λ=0.17. Also, the frequency with which people take a mouthful of water (f_M) was quantified (N=8 children) and fitted to a gamma distribution, resulting in r=1.2 and λ=0.76 (Table 1).
The estimated mean volume of inhalation of water aerosols for children was 0.394 µL/min, (95% CI-range 0.0446-1.27 µL/min) and for adults 0.489 µL/min (95% CI-range 0.0494-1.55 µL/min). The estimated mean volume of ingestion due to hand-to-mouth-contact with wet hands for children was 22.6 µL/min, (95% CI-range 2.02-81.0 µL/min). The estimated mean volume of ingestion of water droplets that splash into children's mouths was 94.4 µL/min (95% CI-range 5.1-279 µL/min) and the mean volume of ingestion through drinking mouthfuls of water amounted $21.5 \cdot 10^3$ µL/min (95% CI-range $1.17 \cdot 10^3$ - $67 \cdot 10^3$ µL/min) for children.
The estimated infection risk due to inhalation of *L. pneumophila* for a child was $9.3 \cdot 10^{-5}$ (95% CI-range 0-$2.4 \cdot 10^{-4}$) and for adults $1.1 \cdot 10^{-4}$ (95% CI-range 0-$82.8 \cdot 10^{-4}$) for the mean exposure duration of 3.5 minutes (Figure 1). The estimated risk of infection due to ingestion of *Campylobacter* was $1.3 \cdot 10^{-2}$ (95% CI-range 0-$5.3 \cdot 10^{-2}$) for ingestion due to hand-to-mouth contact with wet

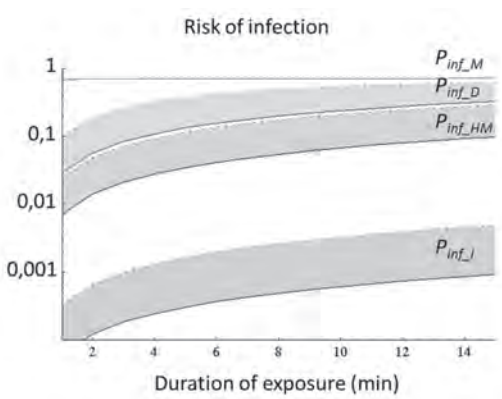

Figure 1. Risk of infection (mean and 95% percentile) for ingestion of mouthfuls of water P_{inf_M}, ingestion of water droplets P_{inf_D}, ingestion due to hand-to-mouth contact P_{inf_HM} and inhalation P_{inf_I} for mean concentrations of *C.jejuni* and *L.pneumophila* in rainwater that is used as source water for splash parks. (95th percentiles were shown by dotted lines)

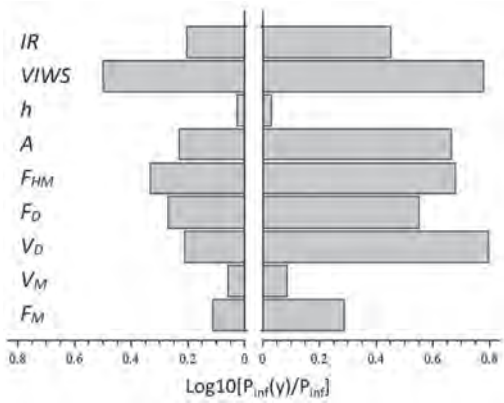

Figure 2. Sensitivity of P_{inf} by varying a model parameter within its range of uncertainty.

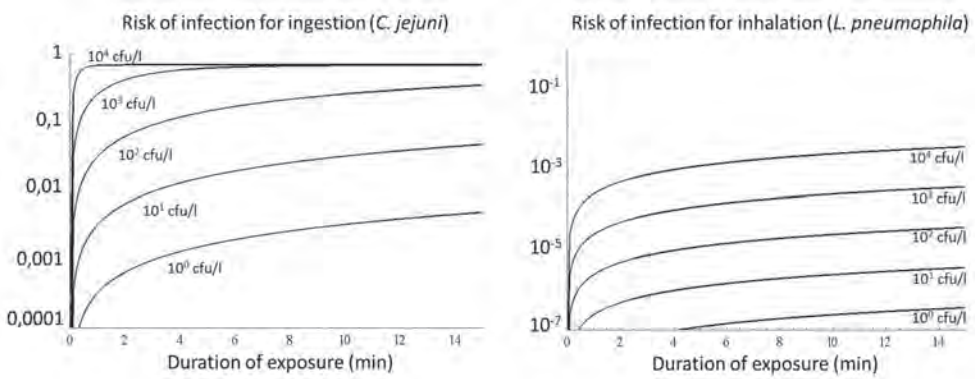

Figure 3. Risk of infection for inhalation and ingestion for different scenarios with respect to concentrations of pathogens in water as a function of the duration of exposure to water of a splash park.

hands, $4.5 \cdot 10^{-2}$ (95% CI-range 0-$1.9 \cdot 10^{-1}$) for ingestion of water droplets in the mouth and $4.7 \cdot 10^{-1}$ (95% CI-range $2.3 \cdot 10^{-2}$-$7.1 \cdot 10^{-1}$) for ingestion of mouthfuls of water . Based on the observational data, the estimated mean total risk of infection for children after ingestion of *Campylobacter* amounted to $3.6 \cdot 10^{-2}$ (95% CI-range 0-$5.3 \cdot 10^{-1}$) for the mean exposure duration of 3.5 minutes. The sensitivity analysis showed that the risk of infection was most affected by the volume of inhalable water spray *VIWS* and the volume of a water droplet V_D and least affected by the volume of a mouthful of water V_m and the film thickness of water on hands h (Figure 2). Figure 3 shows the results of the scenario-analyses, here the risks of infection are presented for different concentrations of pathogens in the water of splash parks. It demonstrates that concentrations of *L. pneumophila* and *C. jejuni* of less than 10 cfu/l (which is equal to absence in 100 ml) would lead to a decrease in the risk of infection for both *L. pneumophila* and *C. jejuni*.

4. DISCUSSION

4.1 Hazard Identification

This study estimated the public health risk of splash parks that use rainwater as their source water. *L. pneumophila* and *C. jejuni* have been chosen to determine the risk of infection for inhalation and ingestion at splash parks. The target pathogen *L. pneumophila* that is used to quantify health risks after inhalation, can grow and become more virulent at water temperatures above 25˚C. The growth of *Legionella* was not incorporated into our model, because water temperatures above 25˚C require prolonged outside temperatures of 30-35˚C (Zuurman, personal communication), which is uncommon in The Netherlands. Under optimal conditions, the concentration of *L. pneumophila* may double in eight hours (Cooling Tower Institute, 1990) and increase 3 to 4 orders of magnitude in 48 to 72 hours (Holden et al., 1984). This is in contrast to *C. jejuni* which will not multiply at temperatures between 25 and 30 ˚C (Jones, 2001). Besides the investigated model parameter *C. jejuni,* other pathogens at splash parks may also cause gastro-intestinal diseases. For instance, norovirus may be introduced in water of splash parks by people who interact with the water (Hoebe et al., 2004) or bird and other animals may introduce pathogens like *Giardia* and *Cryptosporidium* (Eisenstein, Bodager and Ginzl, 2008a). Given the poor water quality at splash parks (de Man et al., 2014b; de Man et al., 2014), together with the increase of water temperature on warm days also gives rise to risks of non-fecal pathogens such as *A. hydrophila* and *P. aeruginosa*. While risks posed by these pathogens deserve evaluation, the necessary dose-response data is currently lacking. And health risk from contact exposure cannot be assessed.

4.2 Exposure Assessment

The present study quantified exposure volumes through inhalation and ingestion, by providing insight into volumes of exposure due to inhalation, hand-to-mouth contact, splash of droplets in someone's mouth and drinking of water. The observational component of the study yielded important data that was previously missing. The methodology used in this study can be used to inform QMRA in other situations where people are exposed to water in the studied ways.

The exposure assessment for ingestion was not performed for adults, because information was missing for several model parameters *(A, F_{HM}, F_D, F_M)*. Based on the uncertain assumption that exposure volumes of ingestion of adults were equal to children and using the data of the field observations (i.e. 55% of the people got wet hands and 9% got water droplets in their mouths) gives an exposure volume of 20 µL/min. This exposure volume was approximately one order of magnitude lower than the volume of exposure of children (707 µL/min), which supports the conclusion that children were more at risk at splash parks than adults.

In our study, we quantified infection risk as a function of exposure duration. The importance of exposure is endorsed by the study of Hoebe et al. (2004), who reported that the attack rate for gastrointestinal illnesses was higher for children who interacted for a longer duration with a splash park. This assumption is also supported by the outbreak of Legionellosis at the 1999 West Frisian Flower Show in The Netherlands, where a relation was found between the duration of exposure

and the concentration of antibodies in titers of exposed individuals (Den Boer et al., 2002). It should be noted that the average exposure duration of 3.5 minutes reported in this study was short because these observations took place in an urban environment. At playgrounds and other recreational water parks, the exposure duration may increase to 0.5 hours or possibly up to 2 hours (Hoebe et al., 2004), which would increase the ingested or inhaled dose of pathogens and therefore the subsequent health risk. According to our model, the risk of infection would increase to $2.3 \cdot 10^{-1}$ for ingestion of *C. jejuni* and $2.8 \cdot 10^{-3}$ for inhalation of *L. pneumophila* for an exposure duration of 2 hours.

4.3 Risk assessment

A standard for exposure to enteric pathogens by consumption of unboiled tap water in set by the Dutch Ministry of Infrastructure and Environment, stating that less than one infection per 10,000 persons per year should occur. Relating this standard to the current study results showed exceedence for *C. jejuni* and *L. pneumophila* (for the latter one, in case of exposure of adults and for exposures of children longer than 5 minutes). The risk of infection for ingestion of water of a splash park also exceeded the value of 0.001 pppy, which was recommended as an infection risk benchmark for the reuse of rooftop-harvested rainwater (Lim and Jiang, 2013). Furthermore, the estimated risk of infection exceeded the value of 0.01, being at the threshold at which epidemiologic studies can identify health risks (Wade et al., 2006; Ashbolt et al., 2010).
Thus, the present study shows that exposure to splash parks may pose a public health risk. This, together with the fact that outbreaks were associated with malfunctioning of disinfection systems (Kebabjian, 2003; Hoebe et al., 2004) indicates that legislation is required to minimize health risks. Water quality standards, we believe, can provide a tool for operators to monitor water quality and to make interventions when necessary. The scenario analyses (Figure 3) provides information about the estimated effect of a reduction of *C. jejuni* or *L. pneumophila* (i.e. the effect of water quality standards) on the risk of infection determined for splash parks that use rainwater as their source water. Concentrations of *L. pneumophila* and *C.jejuni* of less than 10 cfu/l (which is equal to absence in 100 ml) would lead to a decrease in the risk of infection of 10 to 100 times for *C. jejuni* and *L. pneumophila*, respectively.

5. CONCLUSION

This study showed that exposure to splash parks that use rainwater as their source water can cause a infection risk. While at this moment, splash parks do not have to meet any criteria for water quality and/or design, this study should give rise to debates concerning the need for such guidelines. The scenario analyses gives information about infection risks for specific concentrations of pathogens in their source water. This information is valuable in terms of the insight it provides into the effect of water quality standards on public health. Furthermore, this study uses a new methodology making

it possible to quantify exposure volumes using onsite observations, a methodology both practical and useful when assessing quantitative microbial risk wherever people are exposed to water.

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Chapter 4

Risk factors and monitoring for water quality to determine best management practices for splash parks

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ABSTRACT

Splash parks have been associated with infectious diseases outbreaks as a result of exposure to poor water quality. To be able to protect public health, risk factors were identified that determine poor water quality.

Samples were taken at 7 splash parks where operators were willing to participate in the study. Higher concentrations of *E. coli* were measured in water of splash parks filled with rainwater or surface water as compared with sites filled with tap water, independent of routine inspection intervals and employed disinfection. Management practices to prevent fecal contamination and guarantee maintaining good water quality at splash parks should include selection of acceptable source water quality.

1. INTRODUCTION

No consistent requirements for water treatment in splash parks exist. In the majority of the cases, splash parks are unregulated and subject neither to construction review nor to routine inspection by public health officials. Splash parks have been associated with outbreaks of bacterial, parasitic and viral diseases (Eisenstein et al. 2008; Cacciapuoti et al. 1987; Hoebe et al. 2004). Splash parks may comprise of water sprays, dancing water jets, waterfalls, dumping buckets, shooting water cannons, or similar features that encourage children to play with water. Typically, a splash park makes use of a small amount of water that is recirculated, while the water may come into contact with many children when bather densities are high.

The water in the reservoir may be contaminated by contaminants originating from the source water itself, or from people using the splash park for bathing purposes (Hoebe et al. 2004), or from runoff flowing into its reservoir possibly including feces of animals (De Man et al. in press). These contaminants may include human pathogenic microorganisms, such as enteric bacteria, parasites and viruses. Pathogens can be removed or inactivated by disinfection, provided that the disinfection systems are well designed, operated and maintained. Disinfection technology at splash parks usually include high-flow sand filtration, combined with ultraviolet disinfection or chlorination. To be able to protect public health, risk factors associated with fecal contamination were identified for splash parks. *E. coli* was measured in water during routine inspections at seven splash parks as a proxy for fecal contamination. Characteristics and management practices were recorded for each splash park. In addition, dynamics in fecal contamination were monitored during four weeks to evaluate the efficacy of the proposed best water quality management practices for splash parks.

2. METHODS

2.1 Description of sampling locations

Seven splash parks in The Netherlands were sampled from May until September 2011. These locations were selected based on information from local authorities about operators who are willing to participate. The splash parks differed in specific characteristics, including source water, disinfection system, the runoff of rainwater into their reservoir, routine inspection intervals, actions performed during routine inspection and the size of their reservoirs (Table 1). The routine inspection interval was defined as the regular period after which the operator performed some actions to maintain good water quality at the splash park. The routine inspection intervals varied from one week up to six months, see table 1.

2.2 Assessment of water quality

E. coli indicates fecal contamination and the possible presence of enteric pathogens in the water (World Health Organization 2011). Therefore, this indicator was measured to determine water quality. Water samples were taken during two routine inspection intervals at each splash park, yielding 5-10 samples per water feature. Water samples of 40 ml, 10 ml, 1 ml and 0.1 ml were

Table 1. Description of splash parks

	Source water	Disinfection	Does rainwater runoff fill the reservoir?	Routine inspection interval	Actions performed during routine inspection by operator	Size of reservoir (m3)
1	Tap water	Manually dosing of Chlorine	No	1 week	Dosing of Chlorine	2
2	Tap water	Manually dosing of Chlorine	No	1 week	Dosing of Chlorine	2
3	Tap water	High-flow sand filtration + UV	No	2 weeks	Backwashing SF	4
4	Tap water	High-flow sand filtration + UV	No	4 weeks	Backwashing SF	4
5	Rainwater	High-flow sand filtration + Chlorine dosing by pump	Yes	2 weeks	Backwashing SF Checking the functioning of the pump that doses chlorine	3
6	Local surface water	Manually dosing of Chlorine	Yes	4 weeks	Dosing chlorine	16
7	Local surface water	UV	Yes	6 months	Change of the UV-lamp	30

analyzed in duplicate within 24 hours of sampling for *E. coli*. *E. coli* was enumerated using membrane filtration followed by the Rapid Test on Tryptone Soy Agar (996292, Oxoid, Wesel, Germany) and Tryptone Bile Agar (806567, Oxoid) according to ISO9308-1 (Anonymous, 2000a). The measurements were assessed according to standards for *E. coli* given in the European Bathing water Directive 2006/7/EC, because there are no requirements for the water quality of splash parks and because exposure volumes through ingestion may be similar as compared to swimming (De Man et al. in press) . According to Directive 2006/7/EC good water quality should not exceed 1,000 colony forming units (cfu) *E. coli* per 100ml (CEC 2006). At locations where the water quality of the splash parks was poor, the operator of the splash park was asked to drain the reservoir, to clean it using a pressure washer and to disinfect it with chlorine. Subsequently, measurements were repeated to determine the fecal contamination during 4 weeks.

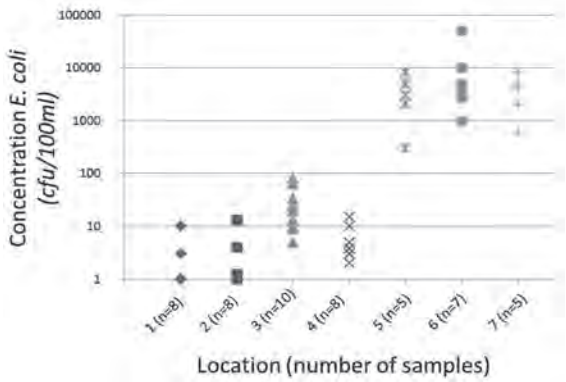
2.3 Statistical Analyses

The maximum likelihood method was used to estimate the concentration of *E. coli* in the undiluted sample, according to the method of Schijven et al. (2011).

3. RESULTS AND DISCUSSION

Fifty-one samples were taken at seven locations. *E. coli* was detected at all locations (Figure 1). Water quality at three splash parks (splash parks 5, 6 and 7) exceeded 1000 *E.coli* cfu/100 ml, as required for official bathing waters according to the European Bathing Water Directive in order to protect human health. These splash parks, filled with rainwater or surface water, were found to have substantially higher concentrations *E. coli* in the water than sites that were filled using tap water and where runoff not drains into the reservoir (splash parks 1, 2, 3 and 4). These higher concentrations could be expected, since rainwater and surface water usually contain *E. coli* (Ahmed et al. 2011), while the absence of *E. coli* from 100 ml tap water in the Netherlands is required by

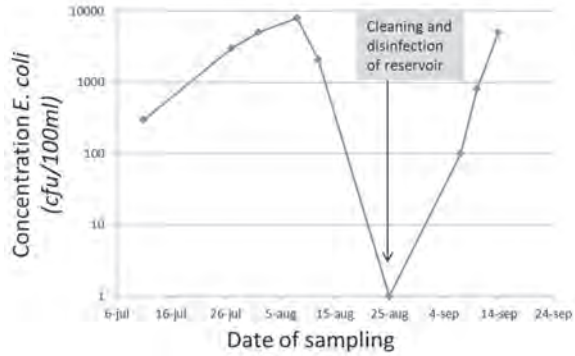
Figure 1. Estimated concentrations of *E.coli* [colony forming units (cfu)/ 100 ml] in water of splash parks.



law (Anonymous 2011). Our measurements showed that, in contrast with splash parks replenished with tap water, splash parks using local surface water or allowing rain water runoff cannot be disinfected. This is consistent with Johnson et al. (1991), who stated that decontamination is more effective when applied to a product of good microbial quality. This means that the trend to use rainwater or surface water as source water in fountains, splash parks or other water features is undesirable from the perspective of public health, under the assumption that *E. coli* is a good indicator for the presence of human pathogens (World Health Organization 2011).

Irrespective of the type of disinfection applied at a location (UltraViolet radiation (UV)/ Chlorination/ high flow sand filtration), the type of disinfection did not improve water quality adequately. Apparently, none of the employed disinfection systems removed or inactivated bacteria such as *E. coli* from the water below the detection method of our analyses Possible explanations for the failure to inactivate *E. coli* include that 1) the dose of UV fluence was too low, 2) the turbidity of the water absorbed the UV fluence, 3) the dose of chlorine was insufficient or 4) chlorine reacted with organic and inorganic compounds and was not available to inactivate *E. coli* (Deborde and von Gunten 2008). Even though *E. coli* was absent, in 5 of the 51 samples, the water quality may be unacceptable because the employed disinfection methods may be able to efficiently remove indicator bacteria, but not pathogenic viruses, parasites, spores and viable but nonculturable bacteria (Koivunen and Heinonen-Tanski 2005), such as noroviruses, *Cryptosporidium, Clostridium* and *Legionella pneumophila.* On the other hand, assuming 1 gram of feces contaminating a reservoir filled with tap water of 4m^3 would lead to a concentration of (10^8 cfu/gram / 4m^3 =) 2500 cfu *E. coli* per 100ml in water. The measured concentrations (below 10 cfu *E. coli* per 100ml at splash parks 1, 2 and 4) were low compared to this value, and showed that the employed disinfection may have inactivated the bacteria present as a result of the hypothetical fecal contamination event. Further comparison of locations showed that the size of the reservoir, the interval between routine inspections, and the actions performed during those inspections, did not influence the levels of fecal contamination. The results did not show any substantial increase or decrease in the fecal

Figure 2. Estimated concentrations *E.coli* [cfu/100ml] at location 5, before and after cleaning and disinfection of the reservoir.



contamination of splash parks (data not shown). This implicated that, despite all efforts of an operator of a splash park to prevent contaminations of the water, the design of a splash park (i.e. the choice of source water and prevention of runoff flowing into the reservoir) influenced the fecal contamination of a splash park the most.

The water quality at locations 5, 6 and 7 was poor and the operators of these sites were asked to clean and disinfect the reservoirs. The operator of location 5 consented and as Figure 2 shows, water quality measurements before and after cleaning and disinfecting the reservoir showed that fecal contamination, absent directly after the cleaning and the refilling of the reservoir with tap water, returned within 4 weeks. One explanation for this might be contamination brought in by people using the fountains for bathing. However, this was also the case for splash parks 1, 2, 3 and 4. A more likely explanation is that the runoff of rainwater in the reservoir increased the fecal contamination of the water and exceeded the capacity of the disinfection technology.

Three of seven splash parks showed a poor water quality, however, our study results may have been biased by the fact that samples were only taken at splash parks where operators were willing to participate. The three splash parks were found to be fecally contaminated by *E. coli* in similar concentrations detected at splash parks where disease outbreaks have occurred (Slater et al. 2006; Jones et al., 2006). A recent study has shown that signage and hygiene attendants did not adequately limit non-hygienic behaviors at splash parks (Nett et al. 2010). Furthermore, it is likely that especially vulnerable subpopulations (children, pregnant women or elderly) (Gerba et al. 1996) are exposed to splash parks and may be at greater risks. Therefore, to prevent outbreaks, local governments, together with public health departments and engineers, should consider the risk of exposure to water in splash parks. To guarantee good water quality, three best management practices could be employed : 1) the use of tap water as source water, 2) avoidance of rainwater runoff onto the reservoir and 3) the use disinfection technology to prevent recontamination. These best management practices would greatly improve the water quality of splash parks and could prevent outbreaks of infectious diseases.

4. CONCLUSION

The study showed that splash parks using tap water as source water have better water quality than splash parks using rainwater or surface water as source water. The disinfection systems in use are able to disinfect fecal contaminations in tap water, but are unable to disinfect rainwater or surface water. This strongly suggest that, from the perspective of public health, neither rainwater nor surface water should be recycled, given the disinfection systems in use, as source water for fountains, splash parks or other water features. This needs to be taken into account by policy makers in the preparation of legislation for splash parks and should inform architects and engineers designing splash parks.

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Eurocam Media

Chapter 5

Quantitative assessment of infection risk from exposure to waterborne pathogens in urban floodwater

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ABSTRACT

Flooding and heavy rainfall have been associated with waterborne infectious disease outbreaks, however, it is unclear to which extent they pose a risk for public health. Here, risks of infection from exposure to urban floodwater were assessed using quantitative microbial risk assessment (QMRA). To that aim, urban flood waters were sampled in the Netherlands during 23 events in 2011 and 2012. The water contained *Campylobacter jejuni* (prevalence 61%, range 14- >10^3 MPN/l), *Giardia spp.* (35%, 0.1-142 cysts/l), *Cryptosporidium* (30%, 0.1-9.8 oocysts/l), noroviruses (29%, 10^2-10^4 pdu/l) and enteroviruses (35%, 10^3-10^4 pdu/l). Exposure data collected by questionnaire, revealed that children swallowed 1.7 ml (mean, 95% Confidence Interval 0-4.6 ml) per exposure event and adults swallowed 0.016 ml (mean, 95% CI 0 – 0.068 ml) due to hand-mouth contact. The mean risk of infection per event for children, who were exposed to floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff was 33%, 23% and 3.5%, respectively, and for adults it was 3.9%, 0.58% and 0.039%. The annual risk of infection was calculated to compare flooding from different urban drainage systems. An exposure frequency of once every 10 years to flooding originating from combined sewers resulted in an annual risk of infection of 8%, which was equal to the risk of infection of flooding originating from rainfall generated surface runoff 2.3 times per year. However, these annual infection risks will increase with a higher frequency of urban flooding due to heavy rainfall as foreseen in climate change projections.

1. INTRODUCTION

One of the major global concerns in climate change is the increased frequency of extreme events. Extreme rainfall events may occur more often and may cause flooding to occur more often (Easterling et al., 2000). In addition, the risk of flooding may increase by ongoing urbanization and increased imperviousness of urban areas (Ten Veldhuis et al., 2010).

To prevent flooding, urban drainage systems are expanded with semi-natural devices, such as infiltration trenches, swales and ponds. These locations are often multi-functional, also operating as recreational areas and only filling up during exceptional storms (Butler and Davies, 2004). In The Netherlands, such locations are often combined with playgrounds, with the intention that children can play, swim or boat when these locations are filled.

Exposure to urban floodwater or water from sites that are intended to store or infiltrate rainwater may pose a health risk in humans. Such water may contain a variety of contaminants depending on the origin of the floodwater. Floodwater originating from rainfall-generated surface runoff may be contaminated by dirt from paved surfaces (including dog feces and bird droppings), while floodwater originating from flooded storm sewers may be contaminated by illicit connections to sanitary sewers (Marsalek and Rochfort, 2004) and floodwater originating from backflow from a combined sewer system will be contaminated with wastewater (Smith, Kay and Fewtrell, 2007). As a result, floodwater may contain human enteric pathogens such as norovirus and enterovirus, which are prevalent in urban wastewater (Lodder and De Roda Husman, 2005), or *Campylobacter, Giardia and Cryptosporidium*, which have been frequently reported in both animal feces and human wastewater (Schets et al., 2008; Koenraad et al., 1994). These enteric pathogens account for a large proportion of all gastrointestinal illnesses in the Netherlands and the US (De Wit et al., 2001; Mead et al., 1999) and may cause outbreaks when people are exposed to floodwater. The waterborne pathogens *Campylobacter, Cryptosporidium, Giardia,* norovirus and enterovirus can be seen as representative of the fate and transport of other pathogens potentially of concern from the waterborne route of exposure (Ferguson et al., 2003).

According to a systematic review (Cann et al., 2013), the most common waterborne pathogens that were identified during outbreaks after extreme water events, such as flooding and heavy rainfall were *Vibrio spp*. (24%), *Leptospira spp*.(19%), *Campylobacter* 9%), *Cryptosporidium spp*. (9%) and norovirus (6%). However, it is unclear to which extent flooding pose a risk for public health. Health risks from exposure to water from the flooding of different urban drainage systems such as combined sewers, storm sewers and infiltration fields can be quantified using the quantitative microbial risk assessment (QMRA) framework. QMRA requires information on the concentration of pathogens in the water or on the correlation between indicator bacteria and pathogens in the water, the exposure of people to these pathogens and dose-response relations for different pathogens.

In the present study, we aimed to assess health risks due to ingestion of urban floodwater by determining the risk of infection for a set of waterborne pathogens that can cause gastrointestinal diseases.

The waterborne pathogens *Campylobacter, Cryptosporidium, Giardia,* norovirus and enterovirus were quantified in urban floodwater. Questionnaires were used to gather data to be able to estimate the volume of floodwater ingested by people during exposure. The generated pathogen data and exposure data were used to calculate the risk of infection for flooding originating from combined sewers, storm sewers and rainfall generated surface runoff. As a result, this study provides insight into health risks resulting from the flooding of different urban drainage systems.

2. MATERIAL AND METHODS

2.1 Sampling

Samples were taken from June 2011 until May 2012 by a sampling team that drove to locations where flooding was expected according to a Dutch meteorological website (www.weerplaza.nl). At location, they checked their smartphone for emergency calls to the fire brigade about flooding (www.112meldingen.nl) and went to those addresses. Samples were taken where buildings were flooded, infiltration fields had filled or at least 100 m^2 of the street was flooded (in order to prevent sampling of small rainwater puddles). One grab sample of approximately 20 liters was collected per sampling location according to ISO 5667-2 (Anonymous, 2006a) and analyzed within 24 hours after sampling. On the site, the duration of flooding until sampling was estimated by collecting information from residents. Furthermore, a distinction was made between three different origins of floodwater, using the following classification system:

1. Flooding originating from overflowing combined sewers;
a) The floodwater had a typical smell of sewage;
b) Toilets in houses were flooded;
c) Manhole covers of the combined sewer system were floated or displaced;
2. Flooding originating from overflowing storm sewers;
a) A storm sewer drained into a rainwater infiltration field;
b) Manhole covers of the storm sewer system were floated or displaced;
3. Flooding originating from rainfall generated surface runoff
a) A connection to an urban drainage system was absent;
b) Rainfall generated surface runoff drained into a rainwater infiltration field.

2.2 Fecal indicator bacteria

Volumes of 40 ml, 10 ml, 1 ml, 0.1ml, 0.01 ml and 0.001 ml were analyzed for fecal indicator bacteria *E. coli* was enumerated using the Rapid Test on Tryptone Soy Agar (996292, Oxoid, Wesel, Germany) and Tryptone Bile Agar (806567, Oxoid) according to ISO9308-1 (Anonymous, 2000a). Colonies were confirmed with James Reagens (BioMerieux, Marcy ll'Etiole, France) according to the manufacturer's instructions. Intestinal enterococci were enumerated according to ISO7899-2 (Anonymous, 2000b) on Slanetz and Bartley Agar (1005125, Oxoid) and confirmation on Bile Esculin Azide Agar (726007, Remel).

2.3 Campylobacter

The presence of Campylobacter in volumes of 50 ml, 5 ml and 0.5 ml volumes was determined using the method described in ISO 17995 (Anonymous, 2005). This method was extended by PCR on the Preston Broth and the typical colonies to be able to score overgrown samples positive for *Campylobacter.* PCR-detection of *Campylobacter jejuni* was performed according to the method of the Water Laboratory Northern Netherlands at Glimmen (Wubbels, Wiel and Douma, 2013).

2.4 Cryptosporidium and Giardia

For enumeration of *Cryptosporidium* and *Giardia*, water samples (450 ml and 10 l) were concentrated as described in ISO 15553 (Anonymous, 2006b), these two volumes were analysed to account for possible low recoveries due to the high amount of dirt in the samples. Slides for microscopy were performed using Easystain (TCS Biosciences Ltd, Buckingham, United Kingdom) according to the manufacturer's instructions. Slides were examined at x250 magnification using epifluorescence microscopy (Zeiss Axioskop; Carl Zeiss, Jena, Germany). On each sampling day, one sample was spiked with *Cryptosporidium* and *Giardia* to determine the recovery.

2.5 Enteric Viruses

Water samples were stored at -20˚C and were analysed after collection of all samples. RNA was extracted from 1 ml and 5 ml water samples by binding to silica beads (bioMerieux, Boxtel, The Netherlands) according to the instructions of the manufacturer. Subsequently, for removal of PCR inhibitors the RNA was cleaned up by the RNeasy MinElute Cleanup Kit (Qiagen, Venlo, The Netherlands) and stored at -20°C. A Lightcycler 480 (Roche Diagnostics, Almere, The Netherlands) was used for real time PCR, using TaqMan hydrolysis probes. Norovirus GI, GII were amplified using the UltraSense One-Step Quantitative RT-PCR System (Invitrogen) (Verhaelen et al., 2012) and enterovirus was done as described by (Benschop et al., 2010), with slight modifications (Lodder et al., 2013). Each sample was included in the PCR run undiluted and by a 10fold dilution to prevent that possible inhibition repressed the molecular detection of viruses in the floodwater sample. Furthermore, mengovirus was used as a process control to get insight into inhibition (Verhaelen et al., 2012).

2.6 Calculation of the concentrations of indicator bacteria and pathogens.

The maximum likelihood method was used to estimate the concentration of pathogens in the undiluted sample. The 95% confidence interval was estimated for each concentration. The concentrations of *E. coli,* intestinal enterococci, *Giardia and Cryptosporidium* were calculated assuming that they were Poisson distributed in the water, using information about the counted pathogen data and the tested volumes. The concentrations of *Campylobacter,* norovirus and enterovirus were estimated as most probable numbers using information about the presence or absence of the pathogen in the diluted sample, under the assumption that negative samples do not contain pathogens (Mood, Graybill and Boes, 1974).

2.7 Determination of exposure volumes by questionnaires.

Data on exposure to floodwater were collected using questionnaires. Questionnaires were sent in September and October 2012 to a group of 715 residents who were possibly exposed to floodwater. The questionnaires were sent to sites that were intended to store/infiltrate rainwater after rainfall and to sites that were flooded (i.e. sites where the capacity of the urban drainage system was too small). The questionnaires included questions about whether people got wet hands or swallowed water after flood incidents. They were asked to report the volume of water they swallowed in five classes: 1) no water, 2) a few drops, 3) one or two mouthfuls, 4) three to five mouthfuls, and 5) six to eight mouthfuls (Schets, Schijven and de Roda Husman, 2011). The participants were also asked for the purpose of the contact: 1) to clear away floodwater, 2) to play and splash, 3) to swim (wearing swimsuits) or 4) other reasons. Furthermore, participants were asked to report the duration of exposure to floodwater in classes of minutes of water contact (0-15, 15-30, 30-60, 60-120, more than 120 min) and to report the frequency of exposure in classes of number of times (0, 1, 2, 3, 5-10, >10). Participants were also asked to answer the questions for children younger than 14 years old.

2.8 Calculation of ingested volumes.

The data from the questionnaires were subdivided into datasets for children and adults. Subsequently, the volume of ingestion was estimated using the method of de Man (unpublished results). Briefly, the volume of ingestion per person V_{total} was calculated using

$$V_{total} = V_d + V_m + Q_{HM}\ x\ t \qquad [1]$$

where V_d was the volume of ingestion of a few drops [ml], V_m was the volume of ingestion of a mouthful of water [ml], Q_{HM} was the ingestion rate [ml/min] due to hand-mouth-contact with wet hands and t was the duration of the hand-to-mouth contact according to the questionnaire [min]. The volume of ingestion of droplets, $V_{d,}$ was estimated to be uniformly distributed between 0.5 and 5 ml (Schijven and de Roda Husman, 2006). The volume of ingestion of a children's mouthful of water was estimated to be gamma distributed with a mean of 25 ml (95% CI 7.8-52.2 ml) (Schets, Schijven and de Roda Husman, 2011). The rate of ingestion due to hand-mouth contact with wet hands Q_{HM} was calculated using

$$Q_{HM} = h\ x\ a\ x\ f \qquad [2]$$

in which h represented the film thickness of water on hands, a the skin-surface area of the hand that was mouthed and f the frequency of hand-mouth contact; h was assumed to be uniformly distributed between $2.34*10^{-2}$ to $1.97*10^{-2}$ mm, a was assumed to be uniformly distributed between 100 and 2000 mm^2 (U.S. EPA, 2011) and f was assumed to be Poisson distributed with an average value of 2 times per hour (Freeman et al., 2001) for children. Because data of a and f was missing

for adults, the volume of ingestion through hand-mouth contact of adults was assumed to be equal to children's ingestion volume, although we realize that this is uncertain.

2.9 Risk assessment

The risks of infection with waterborne pathogens *C. jejuni, Cryptosporidium spp., Giardia spp.,* noroviruses and enteroviruses were estimated for three different scenarios: 1) exposure to floodwater originating from sewers, 2) exposure to floodwater originating from storm sewers and 3) exposure to floodwater originating from rainfall generated surface runoff. Risk of infection were calculated using dose-response relationships, in which the ingested dose of pathogens D was calculated using

$$D = C \, x \, V_{total} \qquad [3]$$

where C is the pathogen concentration and V_{total} is the individual consumption of water according to the questionnaires. The risk of infection per exposure event of *C. jejuni* enteroviruses and noroviruses was calculated using

$$P_{event} = 1 - {}_1F_1 \, (\alpha, \alpha + \beta, -D) \qquad [4]$$

where ${}_1F_1$ is the hypergeometric distribution and α and β are the parameters of the Beta-distribution. In the case of *C. jejuni*, the best estimates of parameters α and β are 0.024 and 0.011, respectively (Teunis et al., 2005). In the case of enteroviruses, the best estimates of α and β are 0.167 and 0.191, respectively (Teunis and Havelaar, 2000), and in the case of noroviruses, the best estimates of α and β are 0.04 and 0.055 (Teunis et al., 2008). The risk of infection per exposure event of *Cryptosporidium spp.* and *Giardia spp.* was calculated using

$$P_{event} = 1 - e^{-rD} \qquad [5]$$

where r is the infectivity parameter of the exponential dose-response model. In the case of *Cryptosporidium spp.*, the best estimate of r is 0.0040 and in the case of *Giardia spp.* the best estimate of r is 0.0199 (Teunis et al., 1996). It was assumed that all measured pathogens were infectious, except for enteroviruses for which the estimated ratio between infectious and defective particles was assumed to be 1:100 (De Roda Husman et al., 2009).
In order to compare the health risks from the different origins of floodwater, the overall risk of infection per exposure event was calculated using

$$P_{inf\_Event} = 1 - (1-P_{inf\_Ca})(1-P_{inf\_Cr})(1-P_{inf\_G})(1-P_{inf\_N})(1-P_{inf\_E}) \qquad [6]$$

where P_{inf_Ca} represented the risk of infection with *Campylobacter*, P_{inf_Cr} represented the risk of infection with *Cryptosporidium*, P_{inf_G} represented the risk of infection with *Giardia*, P_{inf_N} represented the risk of infection with norovirus and P_{inf_E} represented the risk of infection with enterovirus. This summation was firstly justified by the fact that, from the view of public health, the cause of an infection is not important because these pathogens caused similar complaints. Secondly, one pathogen was likely to prevail to cause a gastrointestinal infection (Friesema et al., 2012). In order to compare health risks from different origins of floodwater the frequency of flooding of a drainage system needs to be accounted, because it differs between drainage system (Butler and Davies, 2004). Therefore, we chose to calculate the annual risk of infection using

$$P_{inf\_Year} = 1 - \sum_{i=1}^{N} (1 - P_{inf\_Event}) \qquad [7]$$

in which N was the frequency of exposure events to flooding per year.

2.10 Computational and statistical methods

Data were analyzed using Mathematica (version 8.0; Wolfram Research, Champaign, IL). Firstly, volume distributions and pathogen distributions were calculated based on information of section 2.5 and 2.7. Secondly, the infection risks per pathogen were calculated using Monte Carlo simulations with random sampling of 10000 values from the volume distributions and the generated pathogen data. These infection risks were calculated for children and adults and summed according to equation 6 to get the overall risk of infection per exposure event per location.
Subsequently, scenario analyses were performed to calculate the risk of infection as a function of the ingested volume per exposure event and as function of the frequency of flooding per location.

3. RESULTS

3.1 Indicator bacteria and waterborne pathogens in samples of urban floodwater

Twenty-three samples were taken during urban flooding events in the Netherlands at 18 locations. A description of the sampling sites, the origin of the floodwater, and the concentrations of fecal indicator bacteria is shown in Table 1. Samples were taken within 30 to 240 minutes (average 90 minutes) after the start of flooding. All samples were muddy, indicating that sludge was present. Urban floodwaters were tested for the presence of the waterborne pathogens *Campylobacter, Giardia, Cryptosporidium*, norovirus and enterovirus. The results of the pathogen measurements are shown in Table 2. *Campylobacter* was present in 14 of 23 samples (61%) with concentrations from 7 to more than 1500 MPN (Most Probable Number)/l. It should be noted that for 8 of the 23 samples the culture method failed due to overgrowth by other bacteria on the Karmali agar plates. *Cryptosporidium species* were found in 7 of 23 samples (30%) and *Giardia species* in 8 of 23 samples (35%). Concentrations ranged from 0.1 to 9.8 oocysts per liter for *Cryptosporidium* and from 0.1 to 142 cysts per liter for *Giardia.* The slides were difficult to examine due to the

high degree of dirt on the slide. The recovery of the analyses varied between 1.1-35% for *Cryptosporidium* and 0.9-17% for *Giardia*. Enteric viruses were tested in 17 samples of floodwater: 6 samples (35%) were positive for enterovirus, 2 samples (12%) were positive for norovirus genotype I (HNoV GI) and 4 samples (24%) were positive for human norovirus genotype II (HNoV GII). Concentrations of enterovirus ranged from 1600 pdu (PCR detection unit)/l to 40,000 pdu/l, HNoV GI ranged from 610 pdu /l to 3300 pdu/l and HNoV GII ranged from 530 pdu/l to 40,000 pdu/l. The positive control, mengovirus, was detected in 65% of all dilutions. For 16 of the 17 samples at least one dilution was positive for mengovirus.

3.2 Calculation of ingested volumes.

A questionnaire was sent to 6 locations, 4 of which were intended to store and infiltrate water after heavy rainfall, while 2 locations were flooded. The response to the questionnaires was 28% (204 of 715 questionnaires were sent back). In total, 114 women and 90 men responded, also responding on behalf of 189 children. The average age of adults who took part in this study was 42 years and for children it was 6.2 years, a figure depicting the age ranges among respondents is shown in figure 1. At locations that were not intended to store rainwater, the adults reported that they had to clear away floodwater. At locations that were intended to store rainwater, adults and children played in the water, 10% of the children (n=21) reported that they wore swimsuits when playing. Table 3 shows the distributions of the volumes of swallowed water per exposure event per location: 82% of the adults reported no contact with water, while for children this was 47%. When people had contact with water, adults reported that their hands became wet, whereas for children it was also reported that they ingested a few droplets or a mouthful of water. Only the children that swam reported that they had ingested a mouthful of water. Data of all locations was pooled because the number of data per location were too small.

The mean volume of ingestion for children was 1.7 ml (95% Confidence Interval (CI) 0-4.6 ml) and for adults it was 0.016 ml (95% CI 0 – 0.068 ml). The mean duration of exposure was 21 minutes for children and 18 minutes for adults. The frequency of exposure at locations that were intended to store rainwater was 2.3 times per year, for both adults and children.

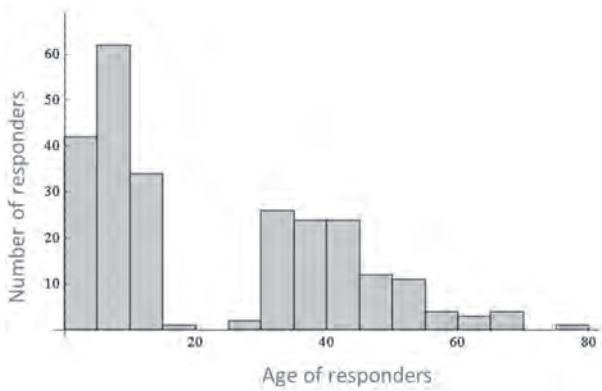

Figure 1. Age of responders

Table 1. Description of sampling sites and concentrations of fecal indicator bacteria (95% confidence interval) in samples of urban floodwater.

Origin	Site no.	Description of sampling sites	Location intended to store rainwater	Date of sampling (dd-mm-yyyy)	*E. coli* cfu/l	Intestinal Enterococci cfu/l
Rainfall generated surface runoff	1	Tunnel in a main road	no	06-06-2011	$6.3 \cdot 10^5$ ($5.3 \cdot 10^5$ - $7.5 \cdot 10^5$)	$7.0 \cdot 10^5$ ($5.9 \cdot 10^5$ - $8.2 \cdot 10^5$)
	2	Festival tent in urban area	no	28-06-2011	$5.0 \cdot 10^5$ ($4.1 \cdot 10^5$ - $6.0 \cdot 10^5$)	$9.5 \cdot 10^4$ ($8.2 \cdot 10^4$ - $1.1 \cdot 10^5$)
	3	Congress center	no	28-06-2011	$4.0 \cdot 10^5$ ($3.2 \cdot 10^5$ - $4.9 \cdot 10^5$)	$2.0 \cdot 10^5$ ($1.4 \cdot 10^5$ - $2.7 \cdot 10^5$)
	4a	Infiltration field with playground	yes	23-08-2011	$2.0 \cdot 10^5$ ($1.4 \cdot 10^5$ - $2.7 \cdot 10^5$)	$1.5 \cdot 10^5$ ($1.0 \cdot 10^5$ - $2.1 \cdot 10^5$)
	4b			11-05-2012	$2.7 \cdot 10^4$ ($2.0 \cdot 10^4$ - $3.4 \cdot 10^4$)	$2.3 \cdot 10^4$ ($1.7 \cdot 10^4$ - $3.0 \cdot 10^4$)
	5	Private industrial area	no	23-05-2012	$1.6 \cdot 10^4$ ($1.1 \cdot 10^4$ - $2.2 \cdot 10^4$)	$1.7 \cdot 10^3$ ($1.2 \cdot 10^3$ - $2.3 \cdot 10^3$)
Storm sewer	6	Street in residential area	no	28-06-2011	$5.0 \cdot 10^5$ ($4.1 \cdot 10^5$ - $6.0 \cdot 10^5$)	$9.5 \cdot 10^4$ ($8.2 \cdot 10^4$ - $1.1 \cdot 10^5$)
	7a	Infiltration field with playground	yes	23-08-2011	$4.8 \cdot 10^5$ ($3.9 \cdot 10^5$ - $5.8 \cdot 10^5$)	$3.3 \cdot 10^5$ ($2.6 \cdot 10^5$ - $4.2 \cdot 10^5$)
	7b			11-05-2012	$6.6 \cdot 10^5$ ($5.6 \cdot 10^5$ - $7.8 \cdot 10^5$)	$7.1 \cdot 10^5$ ($6.1 \cdot 10^5$ - $8.3 \cdot 10^5$)
	8a	Infiltration field with playground	yes	23-05-2012	$2.0 \cdot 10^3$ ($1.4 \cdot 10^3$ - $2.7 \cdot 10^3$)	$2.1 \cdot 10^3$ ($1.5 \cdot 10^3$ - $2.8 \cdot 10^3$)
	8b			23-08-2011	$4.6 \cdot 10^4$ ($3.8 \cdot 10^4$ - $5.5 \cdot 10^4$)	$2.7 \cdot 10^4$ ($2.1 \cdot 10^4$ - $3.5 \cdot 10^4$)
	9a	Infiltration field with playground	yes	11-05-2012	$3.0 \cdot 10^5$ ($2.3 \cdot 10^5$ - $3.8 \cdot 10^5$)	$2.9 \cdot 10^5$ ($2.2 \cdot 10^5$ - $3.7 \cdot 10^5$)
	9b			23-08-2011	$3.9 \cdot 10^5$ ($3.1 \cdot 10^5$ - $4.7 \cdot 10^5$)	$5.3 \cdot 10^5$ ($4.4 \cdot 10^5$ - $6.3 \cdot 10^5$)
	10	Street in residential area	yes	11-05-2012	$4.6 \cdot 10^5$ ($3.8 \cdot 10^5$ - $5.5 \cdot 10^5$)	$9.2 \cdot 10^5$ ($8.0 \cdot 10^5$ - $1.1 \cdot 10^6$)
Combined sewer	11	Street in residential area	no	28-06-2011	$1.5 \cdot 10^6$ ($1.3 \cdot 10^6$ - $1.7 \cdot 10^6$)	$1.5 \cdot 10^5$ ($1.1 \cdot 10^5$ - $2.2 \cdot 10^5$)
	12	Street in residential area	no	28-06-2011	$1.5 \cdot 10^7$ ($1.3 \cdot 10^7$ - $1.7 \cdot 10^7$)	$3.3 \cdot 10^5$ ($2.6 \cdot 10^5$ - $4.2 \cdot 10^5$)
	13	Street in residential area	no	28-06-2011	$1.0 \cdot 10^6$ ($8.7 \cdot 10^5$ - $1.1 \cdot 10^6$)	$4.1 \cdot 10^5$ ($3.3 \cdot 10^5$ - $5.0 \cdot 10^5$)
	14	Street in residential area	no	28-06-2011	$1.1 \cdot 10^7$ ($9.8 \cdot 10^6$ - $1.3 \cdot 10^7$)	$4.2 \cdot 10^5$ ($3.4 \cdot 10^5$ - $5.2 \cdot 10^5$)
	15	Street in industrial area	no	28-06-2011	$8.0 \cdot 10^6$ ($6.8 \cdot 10^6$ - $9.3 \cdot 10^6$)	$4.5 \cdot 10^5$ ($3.7 \cdot 10^5$ - $5.5 \cdot 10^5$)
	16a	Park with deep pit used as combined sewer overflow	yes	23-08-2011	$1.2 \cdot 10^7$ ($1.1 \cdot 10^7$ - $1.4 \cdot 10^7$)	$7.1 \cdot 10^5$ ($6.0 \cdot 10^5$ - $8.3 \cdot 10^5$)
	16b			11-05-2012	$1.9 \cdot 10^7$ ($1.7 \cdot 10^7$ - $2.1 \cdot 10^7$)	$4.8 \cdot 10^5$ ($3.9 \cdot 10^5$ - $5.7 \cdot 10^5$)
	17	Flooding inside a house in a small street	no	23-05-2012	$2.0 \cdot 10^5$ ($1.5 \cdot 10^5$ - $2.7 \cdot 10^5$)	$8.6 \cdot 10^4$ ($5.3 \cdot 10^4$ - $1.3 \cdot 10^5$)
	18	Parking area nearby residential area	no	23-05-2012	$1.0 \cdot 10^6$ ($8.7 \cdot 10^5$ - $1.1 \cdot 10^6$)	$2.1 \cdot 10^5$ ($1.5 \cdot 10^5$ - $2.8 \cdot 10^5$)
	19	Street in residential area	no	15-09-2012	n.d.	n.d.
	20	Street in residential area	no	08-09-2012	n.d.	n.d.

n.d.= not determined

Table 2. Estimated concentration (95% confidence interval) of waterborne pathogens in samples of urban flood water.

Origin	Site no.	*Campylobacter*(mpn/l)	*C. Jejuni* (mpn/l)	*Cryptosporidium* (n/l)	*Giardia* (n/l)	NoVGIvirus (pdu/l	NoVGIIvirus (pdu/l)	Enterovirus (pdu/l)
		ISO	PCR			PCR	PCR	PCR
Rainfall generated surface runoff	1	21 (6.2 - 54)	42.1 (12.3 - 108)	0.0 (0 - 0.5)	0.0 (0 - 0.5)	n.d.	n.d.	n.d.
	2	0 (<10^3)	n.d.	0.0 (0 - 3.9)	0.0 (0 - 3.9)	0 (<10^3)	0 (<10^3)	0 (<10^3)
	3	0 (<10^3)	n.d.	0.0 (0 - 4.1)	0.0 (0 - 4.1)	0 (<10^3)	0 (<10^3)	0 (<10^3)
	4a	187 (37.1 - 621)	>38	0.0 (0 - 0.2)	0.0 (0 - 0.2)	n.d.	n.d.	n.d.
	4b	0 (0 - 11)	85.5, 19.6 - 329)	0.0 (0 - 0.6)	0.0 (0 - 0.6)	0 (<10^3)	0 (<10^3)	0 (<10^3)
	5	0 (0 - 11)	0 (0 - 11)	0.0 (0 - 0.8)	0.0 (0 - 0.8)	0 (<10^3)	0 (<10^3)	0 (<10^3)
Storm sewer	6	0 (<10^2)	n.d.	0.0 (0 - 0.2)	0.0 (0 - 0.2)	0 (<10^3)	0 (<10^3)	n.d.
	7a	480 (102 - 1950)	> 317	0.3 (0 - 0.8)	0.4 (0 - 0.9)	n.d.	n.d.	n.d.
	7b	9244, 205 - 3697)	2198 (437.5 - 7568)	0.6 (0 - 1.4)	0.0 (0 - 0.3)	3321 (551 - 1.9·10^4)	0 (<10^3)	0 (<10^3)
	8a	18 (3.0 - 55)	>96.5	0.0 (0 - 0.2)	0.0 (0 - 0.2)	n.d.	n.d.	n.d.
	8b	480 (102 - 1950)	480 (102 - 1950)	0.0 (0 - 0.4)	0.0 (0 - 0.4)	0 (<10^3)	0 (<10^3)	0 (<10^3)
	9a	52.8 (10.8 - 239)	>15	0.0 (0 - 0.2)	0.0 (0 - 0.2)	n.d.	n.d.	n.d.
	9b	462 (98 - 182)	2198 (437.5 - 7568)	0.0 (0 - 1.1)	0.0 (0 - 1.1)	0 (<10^3)	0 (<10^3)	0 (<10^3)
	10	7 (0.4 - 32)	85, 20 - 325)	0.6 (0 - 1.6)	2.1 (1 - 3.7)	0 (<10^3)	0 (<10^3)	0 (<10^3)
Combined sewer	11	0 (<10^3)	n.d.	0.6 (0 - 1.2)	0.3 (0 - 0.8)	0 (<10^3)	0 (<10^3)	10148 (1247 - 4.9·10^4)
	12	0 (<10^3)	n.d.	0.0 (0 - 0.2)	0.0 (0 - 0.2)	0 ((<10^3)	741 (42 - 3265)	0 (<10^3)
	13	0 (<10^3)	n.d.	0.0 (0 - 0.2)	0.0 (0 - 0.2)	0 (<10^3)	529 (30 - 2329)	1643 (94 - 7262)
	14	0 (<10^3)	n.d.	0.0 (0 - 0.2)	0.1 (0 - 0.4)	0 (<10^3)	0 (<10^3)	39528 (5263 - 2.4·10^5)
	15	0 (<10^3)	n.d.	0.1 (0 - 0.6)	1.0 (0 - 2.0)	610 (35 - 2725)	4008 (1211 - 9738)	10148 (1247 - 4.9·10^4)
	16a	> 965	> 965	0.2 (0 - 0.6)	0.4 (0 - 0.9)	n.d.	n.d.	n.d.
	16b	> 1500	> 1500	0.0 (0 - 1.2)	3.7 (1 - 7.5)	0 (<10^3)	39528 (5263 - 2.4·10^5)	39528 (5263 - 2.4·10^5)
	17	7 (0.4 - 32)	84.8, 19.6 - 325)	0.0 (0 - 5.6)	0.0 (0 - 5.6)	0 (<10^3)	0 (<10^3)	10148 (1247 - 4.9·10^4)
	18	18 (3.0 - 61)	84.8, 20 - 325)	9.8 (5 - 16)	142 (122 - 164)	0 (<10^3)	0 (<10^3)	0 (<10^3)

n.d. = not determined

Table 3. Results from questionnaires: Number (%) of adults and children who were exposed by hand-mouth contact, ingestion of a few drops or a mouthful of water.

	Site no.	None	Hand-mouth contact	Few Drops	Mouthful	Total number of responders
Children	4	12(50)	12(50)	1(4)	1(4)	24
	9	22(71)	9(29)	0(0)	0(0)	31
	10	14(64)	8(36)	2(9)	1(5)	22
	11	32(33)	65(67)	16(16)	2(2)	97
	19	5(71)	2(29)	0(0)	0(0)	7
	20	4(80)	1(20)	0(0)	0(0)	5
Adults	4	36(82)	8(18)	0(0)	0(0)	44
	9	37(90)	4(10)	0(0)	0(0)	41
	10	28(90)	3(10)	0(0)	0(0)	31
	11	43(86)	7(14)	0(0)	0(0)	50
	19	8(73)	3(27)	0(0)	0(0)	11
	20	12(52)	11(48)	0(0)	0(0)	23

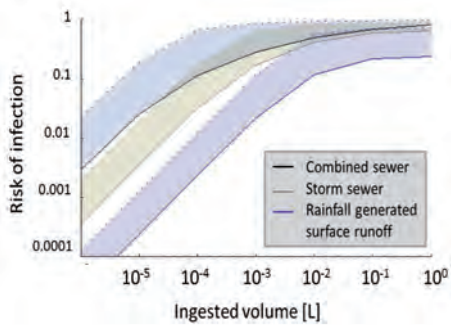

Figure 2. Mean risk of infection for exposure to floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff, as a function of the ingested volume per exposure event. (95th percentiles were shown by dotted lines)

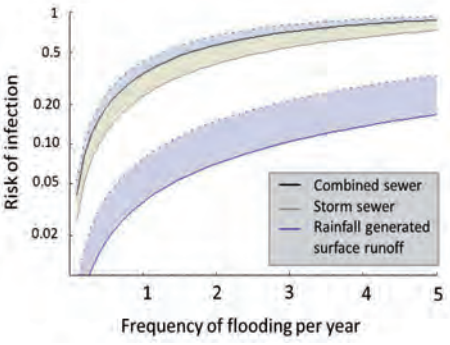

Figure 3. Mean risk of infection for exposure to floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff, as a function of the frequency of exposure to flooding. (95th percentiles were shown by dotted lines)

Table 4. Arithmetic mean percent (95th percentile) risk of infection for children's exposure to waterborne pathogens, and the mean percent risk of infection per exposure event Pinf_event for children and adults.

		Children						Adults
Origin	Site no.	*Campylobacter jejuni*	*Cryptosporidium spp.*	*Giardia spp.*	Noroviruses	Enteroviruses	P_{inf_event}	P_{inf_event}
Rainfall generated surface runoff	1	2.5 (6.3)	0 (0)	0 (0)	n.d.	n.d.	2.5 (6.3)	0.02 (0.1)
	2	n.d.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	3	n.d.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	4a	19 (40)	0 (0)	0 (0)	n.d.	n.d.	19 (40)	0.21 (0.88)
	4b	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Storm sewer	6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	7a	39 (62)	0.00028 (0.00073)	0.00103 (0.0027)	n.d.	n.d.	39 (62)	0.54 (2.2)
	7b	55 (69)	0 (0)	0.00345 (0.0091)	46 (49)	0 (0)	76 (84)	3.3 (13)
	8a	19 (39)	0 (0)	0 (0)	n.d.	n.d.	19 (39)	0.21 (0.88)
	8b	39 (62)	0 (0)	0 (0)	0 (0)	0 (0)	39 (62)	0.54 (2.2)
	9a	5.3 (13)	0 (0)	0 (0)	n.d.	n.d.	5.3 (13)	0.05 (0.22)
	9b	5.3 (13)	0 (0)	0 (0)	0 (0)	0 (0)	5.3 (13)	0.05 (0.22)
	10	0.84 (2.2)	0.0014 (0.0037)	0.0077 (0.02)	0 (0)	0 (0)	0.85 (2.2)	0.01 (0.03)
Combined sewer	11	n.d.	0.00021 (0.00055)	0.0021 (0.0055)	0 (0)	7 (18)	7 (18)	0.08 (0.32)
	12	n.d.	0 (0)	0 (0)	31 (44)	0 (0)	31 (44)	0.51 (2.1)
	13	n.d.	0.00007 (0.00018)	0 (0)	15 (30)	1 (3)	16 (32)	0.19 (0.8)
	14	n.d.	0.00007 (0.00018)	0 (0)	0 (0)	24 (44)	24 (44)	0.3 (1.24)
	15	n.d.	0.00071 (0.0019)	0 (0)	47 (49)	7 (18)	51 (58)	2.8 (11)
	16a	68 (71)	0.00057 (0.0015)	0.0014 (0.0037)	n.d.	n.d.	68 (71)	2.5 (9.6)
	16b	71 (72)	0.0046 (0.0122)	0 (0)	52 (54)	24 (44)	89 (93)	28 (61)
	17	0.84 (2.2)	0 (0)	0 (0)	0 (0)	7 (18)	8.2, 20)	0.09 (0.36)
	18	0.84 (2.2)	0.12 (0.31)	0.04 (0.1)	0 (0)	0 (0)	1 (2.6)	0.01 (0.04)

n.d. = not determined

3.3 Risk of infection

The mean risk of infection for children who were exposed to floodwater originating from combined sewers was 33%, from storm sewers 23% and from rainfall generated surface runoff 3.5%. For adults, the corresponding mean risks of infection were respectively 3.9%, 0.58% and 0.039% (Table 4). These risks were mainly caused by the presence of *C. jejuni*, noroviruses and enteroviruses in floodwater.

Figure 2 displays the overall risk of infection due to exposure to floodwater as a function of ingested volume for the different origins of floodwater. It shows that the risk of infection resulting from exposure to flooding originating from combined sewers was higher than the risks of infection resulting from flooding of storm sewers or rainfall generated surface runoff. Moreover, Figure 3 shows the overall risk of infection as a function of the frequency of exposure to flooding for children. An exposure frequency of once every 10 years to flooding originating from combined sewers resulted in an annual infection risk of 8%, which equaled the risk of infection of flooding originating from storm sewers once every 5 years, and flooding originating from rainfall generated surface runoff 2.3 times per year.

4. DISCUSSION

4.1 Indicator bacteria and waterborne pathogens in samples of urban floodwater

All samples of urban floodwater were found to be fecally contaminated, as demonstrated by the occurrence of the fecal indicator bacteria *E. coli* and intestinal enterococci. The concentrations of fecal indicator bacteria observed in floodwater were similar to those determined during flooding after heavy rainfall in the Netherlands (Ten Veldhuis et al., 2010) and to those found in the US in surface water after flooding due to the hurricanes Katrina and Rita (Sinigalliano et al., 2007). The presence of fecal indicators suggest that enteric waterborne pathogens are possibly present. Our study showed no correlation between fecal indicator bacteria and pathogens, as previously described (World Health Organization, 2011).

The present study showed that floodwater originating from flooding of combined sewers was frequently contaminated with waterborne pathogens. The concentrations of enterovirus and norovirus were found to be similar to concentrations found in floodwater in Jakarta (Phanuwan et al., 2006) and all pathogen concentrations were lower than generally detected in raw sewage in the Netherlands (Lodder and De Roda Husman, 2005; Koenraad et al., 1994; Medema, Ketelaars and Hoogeboezem, 2001). One explanation of the lower pathogen concentrations could be inactivation; however, these are limited over such a short time lapse. Most likely the diluting effect of rainwater contributes most to the pathogen reduction. Furthermore, the prevalence and concentration of pathogens in floodwater originating from a main combined sewer were higher than in floodwater originating from a combined sewer of a few houses (see location 16 versus 17). This is consistent with literature (Smith, Kay and Fewtrell, 2007; World Health Organization, 2011), because the prevalence of pathogens in sewers will vary according to the illnesses circulating in the source

population. As a result, the prevalence of pathogens in floodwater is dependent on the location of flooding (the prevalence of pathogens in flooding of a main sewer with sewage of many people will be higher than flooding of a small sewer with sewage of some people) and on the seasonality of the pathogen, because *Campylobacter, Cryptosporidium, Giardia* and norovirus have their own seasonal variability (Fisman, 2012).
Floodwater originating from storm sewers was found to be contaminated by the pathogens *Campylobacter, Cryptospodidium and Giardia* and norovirus. The presence of norovirus at one of the locations (no. 7) indicated that an illicit cross-connection between the storm sewer and the sanitary sewer was likely, because norovirus originate especially from human fecal contamination sources (Lodder and De Roda Husman, 2005). Such illicit cross-connections between storm sewers and sanitary sewers occur frequently (Marsalek and Rochfort, 2004).

4.2 Risk of infection

The estimation of the overall risk of infection was performed to compare the risks of infection for floodwater originating from combined sewers, with those for storm sewers and with rainfall generated surface runoff. As expected, the risk of infection per exposure event for floodwater originating from combined sewers was higher than the risk of infection per exposure event for floodwater originating from storm sewers or rainfall generated surface runoff. Our study showed that an exposure frequency of once every 10 years to floodwater originating from a combined sewer system led to an annual risk of infection of 8%, which was equal to the annual risk of infection of an exposure frequency once every 5 years for floodwater originating from storm sewers and an exposure frequency of 2.3 times a year for floodwater originating from rainfall generated surface runoff (see Figure 3). This figure provides a quantitative base to assess the functioning of urban drainage systems and to evaluate measures that can minimize health risks resulting from flooding. Measures should aim firstly to prevent from flooding, and secondly, if flooding occurred, to ensure that the water is contaminated by pathogens as little as possible. From that perspective, the trend to drain storm water and human wastewater separately can be an effective development, provided that illicit cross-connections are absent. However, the trend to combine aboveground rainwater storage sites with playgrounds, which encourage that children expose themselves to rainfall generated surface runoff or water originating from a storm sewer, is not desirable from the perspective of public health protection.
The resulting risk of infection from exposure to floodwater originating from sewers was higher than the risk of infection for exposure to floodwater originating from storm sewers or from rainfall generated surface runoff. The estimated infection risks, as a function of the ingested volume and the origin of the floodwater, are displayed in Figure 2 and 3. This figure may be used to approximate the risk of infection for a particular exposure volume and a specific origin of floodwater. Assuming exposure volumes greater than 5 µl for floodwater originating from combined sewers, greater than 50 µl from floodwater originating from storm sewers and greater than 800 µl from floodwater originating from rainfall generated surface runoff would lead to a risk of infection

higher than 0.01, being at the threshold at which epidemiologic studies can identify health risks (Wade et al., 2006; Ashbolt et al., 2010).
The present study quantified the risk of infection by quantitative microbial risk assessment. A QMRA involves three parts: The concentration of pathogens, exposure volumes and dose response parameters. Our QMRA had to rely partly on assumptions that are uncertain (Table 5). These uncertainties in input parameters had a large influence on the output parameters. For instance the recovery of the analyses of *Giardia* en *Cryptosporidium* was low (as described previously by Rochelle et al., 1999). Correction of this low recovery would lead to infection risks that would become 10-1000 times higher than in Table 4. On the other hand, the employed methods to detect pathogens were not indicative for the infectivity of the detected pathogens (De Roda Husman et al., 2009), which may have led to an overestimation of the infection risks. As a result of these uncertainties, the outcome of our QMRA should be regarded as an indication, rather than an absolute assessment of health risk. The outcome can be used to guide risk managers, and to select the most appropriate control measures (Medema and Ashbolt, 2006). Reduction of the uncertainties to quantify the risk of infection requires better detection methods that take into account pathogen viability and infectivity, however, these methods are not available to date. Furthermore, more samples could be taken from floodwater to further reduce uncertainties, however, it is difficult to sample floodwaters timely and safely. Therefore, to determine the extent to which exposure to urban floodwater causes intestinal diseases should be epidemiologically investigated through a retrospective cohort study.
Urban drainage systems in high income countries, such as the Netherlands, are designed to cope with rainfall having a return period of one or two years, knowing that flooding will occur every 5-10 years (Butler and Davies, 2004). Climate change predictions (Lenderink et al., 2011;

Table 5. List of assumptions to calculate infection risks

Parameter	Assumption
Concentration of pathogens	- Waterborne pathogens were homogeneously distributed in floodwater. - The 23 samples of floodwater were representative for floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff. - The measured concentrations of waterborne pathogens were representative for that type location (although there were uncertainties due to measurement difficulties such as low detection-limits and low recoveries). - All detected waterborne pathogens were infectious (regardless if they were detected by culture, microscopy or PCR), except for enterovirus for which a ratio between infectious and defective particles was assumed to be 1:100 (De Roda Husman et al., 2009).
Volume of ingestion	- Data obtained by questionnaire were representative for children and adults. - Exposure behavior can be described using questionnaire outcomes. - Exposure volumes can be quantified using equations 1 and 2.
Dose response	- Each host is equally susceptible to a waterborne infection - Each waterborne pathogen gives an equal probability of infection, which was described by its specific dose-response parameters. - The risk of infection for different waterborne pathogens could be summed to calculate the total risk of infection for the pathogens included.

Hurk et al., 2008) indicate that the return period in which the design capacity of the urban drainage system may be exceeded in the Netherlands is expected to be halved. Figure 3 can be used to determine the effect of climate change on the risk of infection caused by flooding.
This study focused only on the risk of infection for a few waterborne pathogens that can cause gastrointestinal diseases. However, heavy rainfall was also associated with an increased incidence of Legionellosis (Fisman et al., 2005; Hicks et al., 2007) and other infectious diseases, including wound infections, respiratory diseases (upper respiratory diseases, tuberculosis) and conjunctivitis (Jablecki et al., 2005). Currently, such risk evaluations are limited by the lack of dose-response data. Literature has shown that outbreaks after flooding occur among fire-fighters and rescue-workers (Jablecki et al., 2005; Schmid et al., 2005). These people should be made aware of the health risks and should wear protective clothing to minimize exposure as much as possible. Furthermore, it could be prudent to consider health risks in the vaccination programs to protect them against certain infections that may lead to severe illness and sequelae.

5. CONCLUSION

The present study has given quantitative insight into the risks of infection due to exposure to floodwater originating from different urban drainage systems. The results of this study demonstrate that floodwater contains enteric pathogens and may therefore pose a health risk. Health risks caused by flooding can be minimized by:

- Preventing exposure: People should be made aware that floodwater is contaminated by pathogens and that they therefore should avoid exposure to floodwater and should use good hygiene. This can also be made clear by engineers who should advice against combining rainwater infiltration fields with recreational areas.
- Elimination of the hazard: Flooding should be avoided and, if flooding occurs frequently in a certain area, contamination of floodwater with pathogens should be avoided as much as possible. This would involve 1) free surface drainage of rainwater or 2) changing combined sewer systems into separated sewer systems. These interventions would lower the prevalence and concentrations of pathogens in floodwater during urban flooding after heavy rainfall.

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Chapter 6

Legionella pneumophila isolated from pluvial floods by amoebal co-culture

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ABSTRACT

Viable *L. pneumophila* bacteria were isolated by amoebal co-culture from pluvial floods after intense rainfall and from water collected at sewage treatment plants. Several isolated *L. pneumophila* strains belonged to sequence types that have been previously identified in patients.

1. INTRODUCTION

The specific *Legionella* strains that are found most often in patients are rarely isolated from (patient-related) environmental samples (Den Boer et al. 2008; Doleans et al., 2004; Harrison et al., 2009) and the sources of the infecting bacteria often remain unknown (Den Boer et al., 2007). A possible explanation for this discrepancy may be that exposure to pathogenic *Legionella* strains has occurred through yet unknown sources. Alternatively, pathogenic *Legionella* bacteria are missed because of failure of methods used for the detection of *Legionella* in the environment. The standard culture method for detection of *Legionella* is plating of samples on Buffered Charcoal Yeast Extract (BCYE)-plates. However, plates are easily overgrown by other bacteria present in the samples. Furthermore, it is known that *Legionella* bacteria can enter a Viable-But-Not-Culturable (VBNC) state (Steinert et al., 1997; Wingender and Flemming, 2011). Amoebal co-culture has been applied as a method to isolate *Legionella* bacteria that could not be recovered by plating on BCYE-plates (La Scola et al., 2001; Barbaree et al., 1986; Fallon and Rowbotham, 1990). In this study, an amoebal co-culture procedure (La Scola et al., 2001) was applied in order to identify yet unknown sources for *Legionella*.

2. MATERIALS AND METHODS

For the co-culture procedure, *Acanthamoeba castellanii* cells (ATCC #30234 American Type Culture Collection, Rockville, Md., USA) were used. After co-cultivation of the sample with amoebae, the amoebal suspension was plated on BCYE-plates (Oxoid Ltd., Hampshire, UK) which were inspected for *Legionella* colonies, after incubation at 32°C for seven days.
The ability of several *Legionella* strains to be co-cultured with *A. castellanii* was investigated. Eleven *L. pneumophila* strains that tested positive with the Dresden Mab 3/1 monoclonal antibody (Mab 3/1 positive, 16), ten *L. pneumophila* strains that tested negative (Mab 3/1 negative) and eight *L. anisa* strains, which were collected from clinical and environmental sources, and a *L. pneumophila* Philadelphia strain that was retrieved from the ATCC (# 33152) were tested with amoebal co-culture. The Mab 3/1 positive strains, which are considered to be pathogenic for humans (Lück et al., 1992; Helbig et al., 1995), replicated equally well as Mab 3/1 negative strains, which are considered non-pathogenic. The enrichment of all tested *Legionella* strains was at least 5-6 $\log_{10}$-units after six days of co-cultivation. The *L. pneumophila* Philadelphia strain retrieved from the ATCC, could not be co-cultured on *A. castellanii* cells. The eight *L. anisa* strains replicated well. This is in contrast with findings of Neumeister et al. (1997) who described that *L. anisa* does not replicate in the presence of *A. castellanii* cells. Possibly, between strains of the same *Legionella* species, a difference in sensitivity for co-cultivation with *A. castellanii* exists. As a consequence, some *Legionella* strains will be missed by amoebal co-culture (Neumeister et al., 1997; Gao et al., 1999), which is a limitation of the method.
Thirteen samples were taken from water of pluvial flooding incidents in residential areas at locations where more than 100 m^2 of the residential area was flooded or at locations where buildings

were flooded. The floodwater originated from surface runoff or from flooded sewers. Twenty-four water samples from five sewage treatment plants (STPs) were taken, both from influent and from the aeration ponds. Surface water samples were taken from six locations on a small river at locations where combined sewer overflows enter that river. In addition, four fountains that were fed by surface water were sampled. Since most samples contained soil or sludge, these were not concentrated by filtration. Of each sample, 100µl was investigated in duplicate with the amoebal co-culture procedure (La Scola et al., 2001).
Legionella strains that were isolated from the environmental samples were identified by sequence analysis of the *mip*-gene with degenerated primers: Mip-FP1 (5'-GAASARCAAATGAAA-GAYGTTC-3') and Mip-RP8 (5'-CCAGGRATAACTTGYGAWAC-3'). Sequences were analysed with Bionumerics software, version 6.6 (Applied Maths, Kortrijk, Belgium) and compared to *Legionella* sequences present in the NCBI database. *L. pneumophila* strains were further typed by serotyping (Den Boer et al., 2007) and by sequence-based typing (SBT) (Gaia et al., 2005; Ratzow et al., 2007).

3. RESULTS

In 50% of pluvial flood water samples from surface runoff (three out of six), *Legionella* was detected (see table 1). One sample contained a mixture of a *L. pneumophila* serogroup (SG) 1 and a SG8 strain, one sample contained a mixture of *L. fallonii* and *L. wadsworthii* and from one sample only *L. pneumophila* SG8 was isolated. For two *L. pneumophila* stains a sequence type (ST) could be assigned by SBT, namely ST 46 and ST 1079. ST46 has been found in 20 out of 471 patients from whom *Legionella* has been isolated and typed in The Netherlands in the period 2002-2011. In one out of 7 pluvial flood samples (14%) originating from flooding of the sewerage, *Legionella* was detected, which was typed as *L. wadsworthii*.
In 38% of the STP samples (nine out of twenty-four) *Legionella* was found (table 2). One plant contained *L. pneumophila* on all three sampling days in the aeration pond and once in the influent pond. Of the five *L. pneumophila* strains that were isolated from STPs, two strains could be typed by SBT. Both types, ST 93 and ST 625, have been found in 3 out of 471 patients in The Netherlands in the period 2002-2011. For comparison with amoebal co-culture, sixteen STP samples were also tested directly on BCYE-agar plates with antibiotics (Oxoid). However, *Legionella* could not be isolated due to overgrowth of the plates with other bacteria. The presence of *L. pneumophila* in water samples from the aeration pond of STPs is of importance for workers at the plant since aerosols are generated and can be dispersed into the environment (Blatny et al., 2011; Kusnetsov et al., 2010; Olsen et al., 2010). Only one surface water sample contained *Legionella (L. gormanii)*. No *L. pneumophila* was detected in surface water samples.

Table 1. Isolation and typing of *Legionella* from pluvial floods

Origin of flooding	Sample no.	Sampling date	*Mip*-gene sequence analysis	Sero-typing	ST
Runoff	1	6-7-11	*L. pneumophila (12)* *L. pneumophila (8)*	SG1 SG8	ST46
	2	6-28-11	-		
	3	6-28-11	*L. falloni(1)* *L. wadsworthii(2)*		
	4	8-23-11	*L. pneumophila(3)*	SG8	ST1079
	5	8-23-11	-		
	6	8-23-11	-		
Sewer	7	6-28-11	-		
	8	6-28-11	-		
	9	6-28-11	*L. wadsworthii(1)*		
	10	6-28-11	-		
	11	6-28-11	-		
	12	8-23-11	-		
	13	8-23-11	-		

- = negative, SG=serogroup, ST=sequence type. In parentheses the number of *Legionella* isolates that were identified by *mip*-gene sequencing is indicated.

Table 2. Isolation and typing of *Legionella* from sewage treatment plants

STP #	I/A	3-23-11	5-23-11	6-8-11
1	I	*L. spp*	n.d.	n.d.
	A	-	n.d.	n.d.
2	I	-	-	-
	A	-	-	*L. lytica(1), L. fallonii(2)*
3	I	-	*L. pneumophila (6)*,SG3, ST93	-
	A	*L. pneumophila (2)* SG4, SG9, *L. lytica(1)*	*L. pneumophila (6)* SG3	*L. pneumophila(3)*
4	I	*L. lytica(1)*	-	-
	A	-	-	-
5	I	nd	-	*L. pneumophila (2)*,SG2, ST625
	A	nd	*L. sainthelensi(1)*	-

I=Influent, A=aeration pond, nd = not done, - = negative, SG=serogroup, ST=sequence type. In parentheses the number of *Legionella* isolates that were identified by *mip*-gene sequencing is indicated.

4. DISCUSSION

This is the first publication showing that potentially pathogenic *Legionella* strains can be detected in a very unsuspicious environmental source such as water from pluvial floods after intense rainfall. The implication for public health is not clear. An association between increased rainfall and patients with Legionnaires' disease exists (Hicks et al., 2007; Fisman et al., 2005; Karagiannis, Brandsema and Van der Sande, 2009). Also, viable *L. pneumophila* bacteria have been found in rainwater on the road (Sakamoto et al., 2009). Air sampling is required to investigate whether *Legionella* bacteria in pluvial floods are aerosolized and if a risk of exposure during and shortly after periods of heavy rainfall exists. And soil and air samples could be analysed to determine the origin of pathogenic *Legionella* bacteria.

Through the selective enrichment of intracellular bacteria in the amoebal co-culture step there is a higher probability of isolating *Legionella* bacteria from samples with a high background of microbial flora. This method can contribute to the tracing of yet unidentified sources of *Legionella* exposure and disease.

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Chapter 7

General discussion

1. General discussion

Since the 19th century, when John Snow discovered that a large cholera outbreak in London was caused by a contaminated drinking-water well, it is common knowledge that exposure to contaminated water can cause infectious diseases (Butler and Davies, 2004). From that moment on, open sewers in urban areas, including canals, started to be closed, and safe tap water and sanitation were gradually developed and introduced in our society. In The Netherlands, this means that safe tap water and sanitation are today available for every inhabitant, a development that has improved the health of people substantially (World Health Organization, 2011).
Now, in the beginning of the 21st century, the integration of the various flows of water in urban areas is again being allowed to happen. The vicinity of water is seen as an ideal location to provide recreation (Schets, 2011) and water is regarded as increasing the economic value of houses and their surroundings (Luttik, 2000). Together with functions of water such as water storage and prevention of heat stress (Van de Ven, 2011), trends arise towards (re)integration of water into the urban area, either by re-digging canals that were formerly covered for reasons of public health and bad smells, or by installing water features like splash parks or ornamental features. The question is whether this integration of water into urban areas has an effect on public health. This thesis provides information to assess public health risks from exposure to urban water and floodwater originating from urban drainage systems.

2. Risk assessment and acceptable risks

Although the original main aim of urban drainage was to maintain public health by protecting against the spread of diseases (Butler and Davies, 2004), unlike systems for drinking water, this is not a leading criteria for the current design of urban drainage systems. Present day urban drainage systems are designed to collect superfluous water, be it wastewater or rainwater. These systems run full at relatively modest storm periods of once per 2 years, assuming that substantial flooding of the sewer systems will occur only once per 5 to 10 years (Butler and Davies, 2004). In the Netherlands, the majority of the inhabitants are connected to a combined sewer system (which collects wastewater and storm water in one pipe). For such systems, a frequency of flooding of once per 5 years leads to an overall risk of infection for enteric pathogens (*Campylobacter, Giardia, Cryptosporidium*, norovirus, enterovirus) of 0.08 per child per year for flooding originating from a combined sewer overflow (chapter 5), while for an adult this yearly infection risk is 0.0077 per person per year (pppy) (see figure 1).
Ideally, this yearly infection risk should be used by engineers to assess these systems. The combined sewer system risk can be compared to the risk of infection for exposure to floodwater originating from storm sewers or from rainfall generated surface runoff. For instance, the yearly infection risk of 0.08 for children is comparable to playing 2.3 times per year in an infiltration field that is filled by rainfall generated surface runoff. The yearly infection risk can also be used to assess other water systems, such as splash parks that are filled with rainwater (chapter 3). For such systems, the yearly

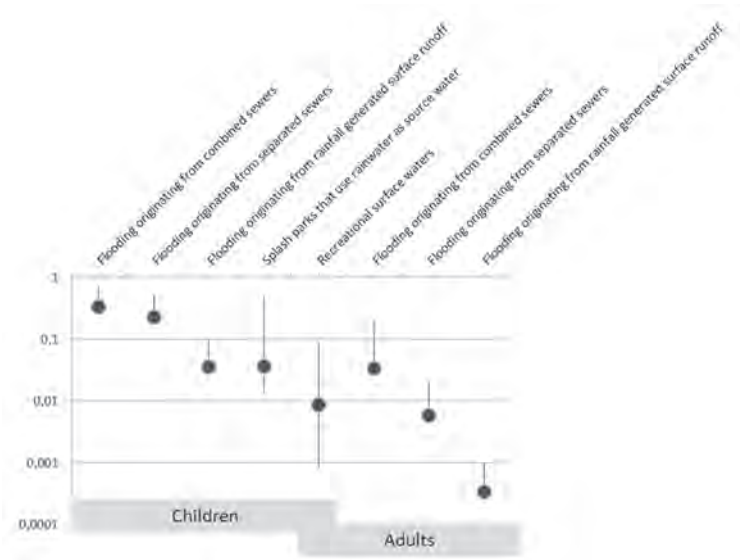

Figure 1. Risk of infection per exposure event for exposure to flooding, splash parks that use rainwater as source water and recreational surface water.

infection risk of 0.08 for children was exceeded at an exposure frequency of more than 3 times per year, assuming a mean risk of infection for *Campylobacter* of 0.036 per exposure event (chapter 3). However, the risk of infection for splash parks was only calculated for one model organism, namely *Campylobacter,* whereas the yearly infection risk of flooding was calculated more realistically by a summation of the infection risks from the pathogens *Campylobacter, Giardia, Cryptosporidium*, norovirus and enterovirus. In contrast with combined sewer overflows, splash parks may also be additionally contaminated with these pathogens by people interacting with the splash parks. Therefore, splash parks filled with rainwater may cause greater yearly infection risks than flooding originating from combined sewer overflows.

Risks from exposure to water in urban areas can also be compared to risks related to swimming in recreational surface waters in the Netherlands. Because, to our knowledge, the general infection risks for such bathing waters are unknown, we assessed these risks by a point estimation for a scenario in which recreational surface water is contaminated by a discharge of a combined sewer overflow, and has a concentration of approximately 1000 cfu *E. coli* per 100 ml, which is the limit for good water quality according to the European Bathing Water Directive. Although, according to policy in the Netherlands, such combined sewer overflows should not discharge to recreational bathing waters, these overflows occur and therefore the infection risks were estimated for such recreational surface waters by a quantitative microbial risk assessment. This assessment was based on the methodology described in chapter 5, pathogen concentrations mentioned in chapter 1, and exposure volumes for ingestion during swimming (Schets, Schijven and de Roda Husman, 2011;

Dufour et al., 2006). Assuming that human wastewater originating from a discharge of a combined sewer overflow is diluted 2 logs by rainfall and 4 logs due to mixing with bathing water (Leenen, 2013), and that 37 ml of this water is ingested by a child (Schets, Schijven and de Roda Husman, 2011; Dufour et al., 2006), the estimated risk of infection is 0.0086 per exposure event. This implies that the risk of infection for children that are exposed to floodwater, or water from splash parks that use rainwater as its source water, are 10 to 100 times larger than risks for swimming in bathing water that is contaminated by a combined sewer overflow.

However, in The Netherlands, children swim on average 8 times per year in fresh water (Schets, Schijven and de Roda Husman, 2011) which leads to an infection risk of 0.07 per child per year and this figure is comparable to the risk of infection of 0.08 per child per year for flooding (chapter 5). No health-based or health-related design criteria are formulated for urban water management. It is obvious that the yearly infection risks of respectively 0.08 for children and 0.0077 for adults are orders of magnitude higher than the limit of 1 in 10,000 infections per person per year used by the Dutch government (Anonymous, 2011) for exposure to enteric pathogens through the consumption of tap water. To achieve lower risks, immense underground urban drainage systems should be built to prevent flooding. Such immense drainage systems would not function properly during dry weather flow and would be very expensive.

It is interesting to ask which level of infection risk would be acceptable in urban water management. For this question, the approach of the USA's Environmental Protection Agency (EPA) is noteworthy. The EPA stated that "any risk that is currently tolerated can be considered to be acceptable" and used this approach to set allowable bacterial indicator densities for bathing waters (Hunter and Fewtrell, 2001). Based on scientific literature (Cabelli et al., 1983; Cabelli et al., 1982; Cabelli et al., 1979; Dufour, 1984), they estimated by epidemiological studies that their previous standards had resulted in 8 gastrointestinal illness cases per 1000 bathers (0.008) at freshwater sites and they assume that these levels were tolerated as people still used the bathing areas (Hunter and Fewtrell, 2001). Standards based on this level are used as a threshold level in quantitative microbial risk assessment (personal communication J. Rose). It is difficult to compare this illness rate of 0.008 for swimming in bathing waters with the yearly infection risk for children (0.08) and adults (0.0077) for exposure to flooding, because the latter infection risks were calculated using a quantitative microbial risk assessment, which typically tends to overestimate disease incidence because it does not incorporate protective effects of immunity (Swart et al., 2012).

Another way to assess whether risks from exposure to urban water are acceptable is to calculate Disability Adjusted Life Years (DALYs) (World Health Organization, 2011). One DALY can be defined of as one lost year of "healthy" life. The sum of these DALYs across the population, or the burden of disease, can be used to measure the gap between current health status and an ideal health situation where the entire population is free of disease and disability (World Health Organization, 2011). The costs of these DALYs can be balanced against the costs of extra investments to prevent from illness associated with urban water. It should be noted that investment costs for changes in urban drainage systems are so high (Dutch Centre of Expertise in Sewer Manage-

ment and Urban Drainage, 2010) that public health issues are today not an incentive to make such investments. However, at locations where urban drainage systems have to be renovated, health issues may be a factor in the design of a replacement drainage system that prevents exposure to human pathogens.

3. Outbreaks

Although some outbreaks are known to be associated with flooding and water features, data on outbreaks resulting from direct exposure to contaminated floodwater or water features are scarce. Underreporting of cases with water associated illness may result from dispersion of people to other parts of the country after their exposure to contaminated water (Schets, 2011). Finally, neither patient nor physician may even associate health complaints with exposure to contaminated water (Schmid et al., 2005). Consequently, a case is likely to be treated as a single case, because of lacking a link to a shared source of exposure and as thus not meeting outbreak criteria, although it may indeed be part of a diffuse outbreak (Schets, 2011).
The risks for gastrointestinal illnesses from flooding and splash parks have been determined to be above the threshold (0.01) at which epidemiologic studies can identify health risks (Wade et al., 2006; Ashbolt et al., 2010) and could therefore contribute to the estimation of disease burden from exposure to contaminated water. Concerning flooding, it would be interesting to compare historical data from patients with infectious diseases with data about extensive rainfall. Such an analysis would give insight into the contribution of heavy rainfall to infectious diseases. It could be used to quantify risks from exposure to splash parks and from exposure to floodwater. Furthermore, a prospective epidemiological study could examine illness rates after exposure to contaminated urban water, which could quantify gastrointestinal, airborne and dermal complaints. As literature has shown, all three types of complaints may follow exposure to contaminated water (Jablecki et al., 2005).
For many cases of legionellosis the source of the pathogen is unknown (Sakamoto et al., 2009). Outbreaks of legionellosis were associated with warm weather followed by wet periods with intensive rainfall (Karagiannis, Brandsema and Van der Sande, 2009). This thesis showed that samples of floodwater contained *Legionella pneumophila* (chapter 6). Flooding may therefore serve as a source/transmission route for *Legionella*. In addition, *Legionella* infections are relatively more frequent among professional drivers (Wallensten et al., 2010; Boer, Nijhof and Friesema, 2006), who are exposed to aerosols due to splash water from the road. A quantitative microbial risk assessment, as was previously performed for whirlpools (Bouwknegt et al., 2013; Armstrong and Haas, 2007), could be used to estimate the risk from exposure to *Legionella* by aerosolisation of floodwater from the street. Such an assessment should be based on data on *Legionella* in floodwater (chapter 6), and also on data of *Legionella* in rainwater puddles (Van Heijnsbergen et al., submitted).

4. Exposure Assessment

Exposure volumes, required to calculate the risk of infection, are difficult to determine because methods to quantify them are limited. As a result, the available data about exposure are biased by assumptions. In this thesis, methods to quantify exposure volumes through inhalation and ingestion (chapter 2 and 3) were developed and used to gather data and estimate volumes for exposure to floodwater (chapter 5) and for exposure to splash parks (chapter 3). Combining these data with pathogen concentrations in different kinds of water (see table 1 in chapter 1) and with dose-response relationships, makes it possible to calculate the risk of infection in for exposure to water, for instance for canoeing in urban water, or walking or cycling along a fountain. Still, information is lacking to quantify exposure through ingestion of aerosols larger than the respirable size range (Stellacci et al., 2010). Although this volume of exposure would be small, it could cause infections, for instance linked to locations where fountains were installed to increase the oxygen concentration in urban ponds after combined sewer overflows. Fountains at such locations may form contaminated aerosols containing norovirus and other enteric pathogens (Uhrbrand, Schultz and Madsen, 2011).

To determine more accurately the risk of infection from exposure through inhalation e.g. for legionellosis and other respiratory diseases, pathogen concentrations must be measured in air. However, methodologies to measure pathogens in air are under development, and to date they have many drawbacks that make it difficult to calibrate and validate an air sampling procedure. As a result, it is difficult to integrate those measurements in QMRA. Information is also lacking that would allow an assessment of infection risks from dermal contact. Although it is known that contaminated water may cause infections through dermal contact and contact with mucous membranes (Cacciapuoti, Ciceroni and Maffei, 1987; Semel and Trenholme, 1990), no assessments have been performed to calculate such risks, and significant hurdles need to be overcome to apply the process of quantitative microbial risk assessment to dermal exposure. Methods are needed to measure the concentration of pathogens deposited on the skin and the duration of exposure, as well as to determine the dose of pathogens required for infection. Dermal pathogen exposure assessment may learn from chemical exposure assessment. However, chemical dermal exposure assessments assume that chemicals are transferred across the skin (Nieuwenhuijsen, 2004), while infections from dermal contact are thought to be caused at wounds and mucous membranes where pathogens may penetrate more easily (Semel and Trenholme, 1990; Van Asperen et al., 1995). Ideally, the exposure routes of ingestion, inhalation and dermal contact should be integrated in the design and management of urban water. Such an integrated approach has the advantage that the risk for infectious diseases can be considered in relation to the function of water. For instance, at locations where people swim, the exposure routes of ingestion and dermal contact are key, while at locations with decorative water features inhalation and ingestion of aerosols are important. For exposure to interactive water features and flooding it appeared that all mentioned exposure routes are relevant, because outbreaks occurred with gastrointestinal, respiratory and dermal infections (Jablecki et al., 2005; Cacciapuoti, Ciceroni and Maffei, 1987; O'Loughlin et al., 2007;

Hoebe et al., 2004). Therefore, for the first time such integrated risk assessment was performed for exposure to water of splash parks (chaper 2) and exposure to flooding (chapter 5).

5. Climate change

Climate change may lead to an increase in exposure events to contaminated water, whether by droughts or by flooding, as was shown in a review concerning extreme water-related weather events associated with infectious diseases (Cann et al., 2013). Firstly, as a result of climate change, more frequent heavy rainfall events are expected during summers, giving rise to more frequent flooding because the urban drainage system is not designed to cope with such rainfall. Exposure to floodwater may lead to more cases of gastro-enteritis (chapter 5), and also to more cases of legionellosis (Karagiannis, Brandsema and Van der Sande, 2009) since *L. pneumophila* is frequently found in rainfall generated surface runoff (chapter 6). However, the association of legionellosis incidence and flooding should be investigated by QMRA. Secondly, the frequency of exposure events to contaminated water may be increased by measures to prevent heat stress. These measures typically introduce more water bodies in urban areas, such as canals and water features. Thirdly, people may be exposed more intensively during warm weather by ingestion of mouthfuls of water from water features during recreation (Hoebe et al., 2004), while during moderate temperatures such exposure is fewer (chapter 3). Finally, pathogens that proliferate during warm weather, such as *Legionella spp., Pseudomonas spp., Aeromonas spp. or Vibrio spp.,* may grow and cause outbreaks at artificial water systems like water features (Schets, 2011).

6. Alternative sanitation systems

Interest in alternative sanitation systems has clearly increased over the past years, and some alternatives are used in the Netherlands (Petersens et al., 2012). Alternative sanitation systems that use vacuum transport for black water (Petersens et al., 2012) may decrease disease risks arising from flooding in that they may prevent floodwater becoming contaminated by human wastewater. Alternative sanitation systems, which are mostly based on the concept of differentiating between types of waste water, require multiple piping systems. The construction of such piping systems increases the probability of illicit connections, as are also often seen in separated sewer systems (Clemens, 2001). These may lead to outbreaks of illness, as occurred in a Dutch residential area where drinking water was contaminated by gray water (Anonymous, 2003). Further, the use of water other than drinking water for purposes like recreation or irrigation may introduce health risks (Albrechtsen, 2002), as was shown in chapters 3 and 4. Therefore, health risks for sanitation alternatives, including risks from reuse of this water and nutrients, should be estimated and compared to the infection risks associated with conventional urban drainage systems and other water exposure, such as swimming in surface water.

7. General conclusions and recommendations for best urban water management practices to prevent waterborne infectious diseases under current scenarios

As shown in this thesis by QMRA, exposure to contaminated urban water may cause a public health risk. This paragraph discusses the recommendations of chapter 2 – 6, in the context of Dutch and European legislation and the current developments in urban water management.
In chapter 2, recommendations are given to prevent the spread of diseases through water features that form aerosols in the air. It is recommended to ensure good water quality and to prevent sewer overflows at locations where surface water is used as source water of water features. While this may look like a truism, water features are often installed in surface waters with combined sewer overflows. This may happen because water features are thought to increase the oxygen concentration after a sewer overflow, neglecting the fact that such water features may spread human pathogens. As chapter 2 recommends, planners need to choose locations for water features that are away from people in order to prevent inhalation of aerosols, rather than near people to refresh them or as a setting for recreation. Instead, many locations are known in the Netherlands where more than 20 000 people per day are exposed to aerosols from a fountain (personal communication with funding organizations of this study). Contamination of locations where large numbers of people are exposed to pathogens could lead to large outbreaks, as was already illustrated by the outbreak at the West Frisian Flower Show in 1999 (Den Boer et al., 2002).
Concerning splash parks, chapter 3 cautions against the use of rainwater as source water because it introduces health risks for people who interact with it. Three methods to reduce such risks are explained in chapter 4, which recommends using tap water as source water, preventing rainwater runoff in the reservoir and using a simple form of disinfection. These recommended measures for splash parks are comparable to swimming water legislation, where swimming pools, whether indoor or outdoor, are compelled to use tap water as source water and to have a minimum concentration of free chlorine (Schets, Schijven and de Roda Husman, 2011; Anonymous, 2009)
In chapter 5, measures are recommended to prevent infection risks from exposure to flooding. Firstly, infection risks can be minimized by raising awareness and informing the public that floodwater is contaminated with enteric pathogens and that therefore exposure to floodwater should be avoided or, if that is not possible, they should take hygienic measures. This applies specifically to children in residential areas, more than fifty percent of whom, in our study, played in floodwater and were exposed through hand-mouth contact, ingestion of water droplets and/ or mouthfuls of water (chapter 5). These figures imply that risks from flooding can decrease substantially through education that focuses on preventing exposure. Secondly, infection risks can be minimized by avoiding flooding and if flooding occurs frequently in a certain area, avoiding (as much as possible) contamination of floodwater with pathogens. This could involve disconnecting rainwater piping from urban drainage systems and using over-ground drainage such as an infiltration field. Infiltration fields, where water infiltrates directly into the ground, are an asset to their environment as people do not expose themselves to the water and as a result the risk of infection is low.

Conversely, infiltration fields that not function properly, where rainwater remains aboveground and that are used by children and adults to play, may lead to higher risks. Another option is to change a combined sewer system into a separate sewer system. However, illicit connections are estimated to be 5% of the individual connections into a separated sewer system (Clemens, 2001), and these may cause contamination of storm water by human enteric pathogens to the same extent as combined sewer systems. Together with other options such as green roofs, more water storage and water retention for irrigation purposes, these recommendations are in line with the preferred sequence of drainage by the Dutch Government (Anonymous, 2012) and may prevent sewer overflows from contaminating surface water and prevent human wastewater, including enteric pathogens, contaminating storm water. Both, enhanced education and the decoupling of storm drainage from sewers, would reduce human exposure to enteric pathogens and are therefore sustainable options from the view of public health.

In chapter 6, samples from urban water were analyzed for the presence of *Legionella*. *Legionella* was found in 50% of the samples of rainfall generated surface runoff; however, its implication for urban water management has to be investigated by a quantitative microbial risk assessment. In addition, such an assessment may evaluate the best management practices that can minimize risks from exposure to *Legionella*.

Under Dutch law (Anonymous, 2008), local authorities have the responsibility of ensuring the control of infectious diseases. Because they are also responsible for the collection and transport of human wastewater and storm water and the regulation of groundwater (Anonymous, 2007), they have both the opportunity and also the responsibility to invest in good urban water quality. Local governments, together with public health departments and engineers, should be aware that their policy in urban water management influences public health. To design urban water systems and urban drainage systems that, from a public health perspective, are sustainable, they need to investigate the locations people are exposed to urban water and to quantify the water quality at these locations. This process can be guided and enhanced by the results presented in this thesis.

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Chapter 8

Summary

Samenvatting

Dankwoord

Curriculum vitae

Summary

Water in urban areas may pose a public health risk when people are exposed to urban water, because it may contain pathogens. These pathogens may originate from fecal bird droppings, runoff from paved surfaces (including e.g. dog feces), growth of micro-organisms in water and in some cases discharges of combined sewer overflows.
Since the extent to which exposure to urban water poses a risk for public health was unknown, this thesis aimed to investigate health risks associated with urban water systems and to determine ways to minimize those risks.
Chapter 1 provides an overview of pathogens that have caused outbreaks of waterborne illness. (The lack of) Water quality guidelines, regulations and policies for urban water management are also addressed.
Exposure to contaminated aerosols and water originating from water features may pose public health risks. In **chapter 2**, endotoxins (in air and water) and fecal bacteria (in water) of water features were measured as markers for exposure to microbial cell debris and enteric pathogens. Exposure to air and water near water features was shown to result in exposure to endotoxins and fecal bacteria, which may lead to respiratory health effects and gastrointestinal health complaints. Regression analyses showed that the endotoxin concentration in air was significantly influenced by the concentration of endotoxin in water, the distance to the water feature and the tangibility of water spray. This study provides estimates for aerosolisation ratios that were used as input for a quantitative microbial risk assessment in **chapter 3** to quantify infection risks for exposure to splash parks.
In the Netherlands, rainwater becomes more and more popular as an economic and environmentally sustainable water source for splash parks.The associated public health risk, however, and underlying risk factors were unknown. Therefore, in **chapter 3**, a quantitative microbial risk assessment was performed using *Legionella pneumophila* as a target pathogen to quantify the risk of infection for exposure due to inhalation and *Campylobacter jejuni* by ingestion. The risk of infection for a mean exposure duration of 3.5 minutes was $9.3*10^{-5}$ for inhalation of *L. pneumophila* and $3.6*10^{-2}$ for ingestion of *C. jejuni*. The results of the QMRA showed that using rainwater as source water for splash parks may pose a health risk. This study provided a methodology to quantify exposure volumes using observations on site. Furthermore, it gives insight into the effect of setting water quality standards, which may limit infection risks from exposure at splash parks.
Splash parks have been associated with infectious disease outbreaks as a result of human exposure to poor water quality. To be able to protect public health, in **chapter 4**, risk factors were identified that determine poor water quality. Samples were taken at seven splash parks and were analyzed for *E.coli*. Higher concentrations of *E. coli* were measured in water of splash parks filled with rainwater or surface water as compared with sites filled with tap water. Inspection intervals and employed disinfection has no significant additional effect on the fecal contamination of the water. Management practices to prevent fecal contamination and guarantee maintaining good water quality at splash parks should include selection of acceptable source water quality and application of disinfection.

Flooding and heavy rainfall have been associated with waterborne infectious disease outbreaks. It is unclear to which extent they pose a risk for public health upon exposure. In **chapter 5**, risks of infection from exposure to urban floodwater were assessed using quantitative microbial risk assessment. To that aim, urban flood waters were sampled in the Netherlands. The water contained *Campylobacter jejuni*, *Giardia* spp., *Cryptosporidium* spp.,noroviruses and enteroviruses. Exposure data were collected at flood sites by questionnaires. The mean risk of infection for children who were exposed to floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff was 33%, 23% and 3.5% per event, respectively, and for adults it was 3.9%, 0.58% and 0.039% per event.

Warm, wet weather has been associated with cases of Legionellosis, the source of the majority of the infections, however, remains unknown. Therefore, in **chapter 6**, urban waters and water of wastewater treatment plants were analyzed for *Legionella*. *Legionella* was present in 3 of 6 samples of urban floodwater originating from rainfall generated surface runoff and in 5 of 24 samples originating from wastewater treatment plants. Several isolated *Legionella* strains belonged to sequence types that have been previously identified in patients. The presence of *Legionella* in urban floodwater indicates a possible transmission route for this pathogen.

In **chapter 7** the results of the study are discussed. The risks of infection from exposure to splash parks and floodwater were compared with the risk of infection after swimming in bathing water of *good* water quality according to the European Bathing Water Directive. This comparison showed that the risk of infection per case of exposure was higher for exposure to floodwater and splash parks than for swimming. The yearly infection risks are, however, dependent on the presence of pathogens in water and the frequency and the extent of exposure per year.

Furthermore, new scenario's for urban water management, like climate change and new sanitation, which may change the health risks associated with urban watermanagement were discussed. Subsequently, recommendations for the prevention of health risks associated with water features, splash parks and urban floodwater were discussed.

Samenvatting

Water wordt steeds vaker geïntegreerd in het stedelijk gebied. De nabijheid van water wordt gezien als een ideale plaats om te recreëren en daarnaast functioneert water in het stedelijk gebied als waterberging na hevige regenval en als verkoeling bij tropische temperaturen.
Water in het stedelijk gebied kan een risico vormen voor de gezondheid van mensen. In water kunnen namelijk ziekteverwekkende micro-organismen voorkomen die infectieziekten bij mensen kunnen veroorzaken (**hoofdstuk 1**). Deze ziekteverwekkers komen in stedelijk water terecht door afstroming van hondenpoep, vogelpoep en soms vanuit de riolering. Daarnaast kunnen sommige ziekteverwekkers, zoals Legionella, groeien in het milieu. De mate waarin blootstelling aan stedelijk water een risico vormt voor de volksgezondheid is onbekend. Dit proefschrift beschrijft het onderzoek naar gezondheidsrisico's nabij stedelijke watersystemen én naar manieren om deze risico's te minimaliseren.
Fonteinen vernevelen water tot aerosolen die kunnen worden ingeademd en ingeslikt door mensen. In **hoofdstuk 2** wordt het onderzoek beschreven naar lucht- en waterkwaliteit bij fonteinen. Uit het onderzoek blijkt dat blootstelling aan lucht en water in de buurt van fonteinen leidt tot blootstelling aan endotoxinen en fecale bacteriën: Deze blootstelling kan leiden tot respiratoire gezondheidsklachten en/of maag-darmklachten. Regressieanalyses toonden aan dat de endotoxine concentratie in de lucht sterk werd beïnvloed door de concentratie van endotoxine in het water, de afstand tot de fontein en de tastbaarheid van de waternevel. In deze studie wordt een schatting gegeven voor aerosolisatie-ratio's van waternevel. Deze ratio's zijn gebruikt als input voor de risico-analyse bij bedriegertjes (**hoofdstuk 3**).
Regenwater wordt in Nederland steeds vaker gezien als een duurzame waterbron die gebruikt kan worden voor recreatiedoeleinden. Echter, de bijbehorende risico's voor de volksgezondheid waren onbekend. In **hoofdstuk 3** is een kwantitatieve microbiologische risicoanalyse uitgevoerd voor bedriegertjes die gevuld worden met regenwater. Het risico op infectie bij een gemiddelde blootstellingsduur van 3,5 minuut was $9{,}3{\cdot}10^{-5}$ voor inhalatie van *L. pneumophila* en $3{,}6{\cdot}10^{-2}$ voor de inname van *C. jejuni*. Het onderzoek toonde aan dat het gebruik van regenwater als bron voor bedriegertjes een gevaar voor de gezondheid van mensen kan opleveren. Daarnaast is inzicht gegeven in het effect van de toepassing van waterkwaliteitsnormen waardoor gezondheidsrisico's bij bedriegertjes beperkt kunnen worden.
Bedriegertjes zijn vaak in verband gebracht met uitbraken van infectieziekten. **Hoofdstuk 4** beschrijft het onderzoek naar risicofactoren voor slechte waterkwaliteit bij bedriegertjes. Op zeven locaties met bedriegertjes werden monsters genomen. Hoewel op alle locaties het water gedesinfecteerd werd, werden significant hogere concentraties *E.coli* gemeten bij bedriegertjes waarvan het reservoir gevuld werd met regenwater of oppervlaktewater. Bij bedriegertjes die gevuld werden met drinkwater werden, onafhankelijk van de aanwezige vorm van desinfectie en het onderhoudsinterval, lagere concentraties *E.coli* gemeten. Om goede waterkwaliteit bij bedriegertjes te garanderen wordt daarom aanbevolen om het reservoir te vullen met drinkwater én een vorm van desinfectie te gebruiken.

Door hevige regenval ontstaat in Nederland regelmatig 'water op straat'. In literatuur is wateroverlast veelvuldig geassocieerd met uitbraken van infectieziekten, maar het is onduidelijk in welke mate 'water op straat' een risico vormt voor de volksgezondheid. In **hoofdstuk 5** is het risico op infectie gekwantificeerd met behulp van een kwantitatieve microbiologische risicoanalyse. In het onderzoek werden 23 'water op straat'-situaties bemonsterd. Het water werd geanalyseerd op de ziekteverwekkers *Campylobacter, Giardia, Cryptosporidium*, norovirus, en enterovirus. Daarnaast werden gegevens verzameld via enquêtes over de mate waarin mensen met het water in contact kwamen tijdens of na wateroverlast. Het risico op infectie is in kaart gebracht voor 'water op straat' afkomstig uit gemengde riolering, gescheiden riolering en afstromend regenwater. De gemiddelde kans op infectie voor kinderen die werden blootgesteld aan 'water op straat' bedroeg respectievelijk 33 % , 23% en 3,5 % per gebeurtenis en voor volwassenen was dit 3,9% , 0,58% en 0.039% per gebeurtenis.

Warm, vochtig weer wordt geassocieerd met een verhoging van het aantal Legionella-gevallen, de bron van besmetting is echter vaak onbekend. In **hoofdstuk 6** wordt het onderzoek beschreven naar de aanwezigheid van *Legionella* in stedelijk water en water van rioolwaterzuiverings-installaties. *Legionella* was aanwezig in 3 van de 6 monsters van 'water op straat' afkomstig vanuit afstromend regenwater en in 5 van 24 monsters afkomstig van een rioolwaterzuiverings-installatie. Sommige Legionella-stammen kwamen overeen met soorten die ook gevonden zijn bij patiënten met een Legionella infectie . Of de aanwezigheid van *Legionella* in afstromend regenwater daadwerkelijk een risico vormt voor de gezondheid van mensen moet in kaart gebracht worden met behulp van een kwantitatieve microbiologische risicoanalyse.

In **hoofdstuk 7** worden de resultaten van het onderzoek bediscussieerd. Het risico op infectie na blootstelling aan bedriegertjes en 'water op straat' wordt vergeleken met het risico op infectie na zwemmen in goedgekeurd open zwemwater. Hieruit blijkt dat de infectierisico's voor eenmalige blootstelling aan bedriegertjes of 'water op straat' hoger zijn dan voor zwemmen. De jaarlijkse infectierisico's zijn echter afhankelijk van de frequentie van blootstelling. Ook wordt ingegaan op nieuwe ontwikkelingen zoals klimaatverandering en de zogenaamde nieuwe sanitatie die de risico's rondom blootstelling aan water in het stedelijk gebied kunnen veranderen. Vervolgens worden aanbevelingen gegeven voor stedelijk water management waarmee gezondheidsrisico's van fonteinen, bedriegertjes en 'water op straat' voorkomen of verminderd kunnen worden.

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Curriculum Vitae

Heleen de Man- van der Vliet was born on September 24th 1985 in Nieuwer Ter Aa in the Netherlands, where she grew up. She graduated from secondary school at the Driestar College in Gouda in 2003. She started the study Civil Engineering at Delft University of Technology. Here, she specialized in Sanitary Engineering and Urban Drainage. During her study she advised two food plants of Unilever Nederland BV to improve the efficiency of their wastewater treatment plants.
In 2008, she received her MSc degree and started to work at Grontmij Company in Houten. December 2009, she started working at the Institute for Risk Assessment Sciences (IRAS) at Utrecht University on the research described in this thesis.