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

H. de Man [a,c,*], H.H.J.L. van den Berg [c], E.J.T.M. Leenen [b], J.F. Schijven [c], F.M. Schets [c], J.C. van der Vliet [a], F. van Knapen [a], A.M. de Roda Husman [a,c]

[a] Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
[b] Grontmij Water & Energy, De Bilt, The Netherlands
[c] National Institute for Public Health and the Environment, Bilthoven, The Netherlands

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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 floodwaters 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,

* *Corresponding author*. Institute for Risk Assessment Sciences, Division of Veterinary Public Health (VPH), P.O. Box 80175, 3508 TD Utrecht, The Netherlands. Tel.: +31 30 2539688.
E-mail address: h.deman@uu.nl (H. de Man).
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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 et al., 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 L was collected per sampling location according to ISO 5667-2 (Anonymous, 2006a) and analyzed within 24 h 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, 0.1 ml, 0.01 ml and 0.001 ml were analyzed for fecal indicator bacteria *Escherichia 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 et al., 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 to 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 et al., 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 et al., 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

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

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 et al., 2011). The rate of ingestion due to hand-mouth contact with wet hands Q_{HM} was calculated using

$$Q_{HM} = h \times a \times f \quad [2]$$

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

$$D = C \times V_{total} \quad [3]$$

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

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

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

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

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

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

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

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

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 2.6 and 2.8. 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 can be found in the supplemental contents. Samples were taken within 30–240 min (average 90 min) 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 1. *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 and 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,

Table 1 – Estimated concentration (95% confidence interval) of waterborne pathogens in samples of urban floodwater.

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	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.
generated	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)
surface	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)
runoff	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 (<10^3)	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.

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 can be found in the supplemental contents. 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 2 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,

Table 2 – 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 and the mean duration of an exposure event.

	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

Table 3 – 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 P_{inf_event} for children and adults.

	Site no.	Children						Adults
		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.

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 min for children and 18 min 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.

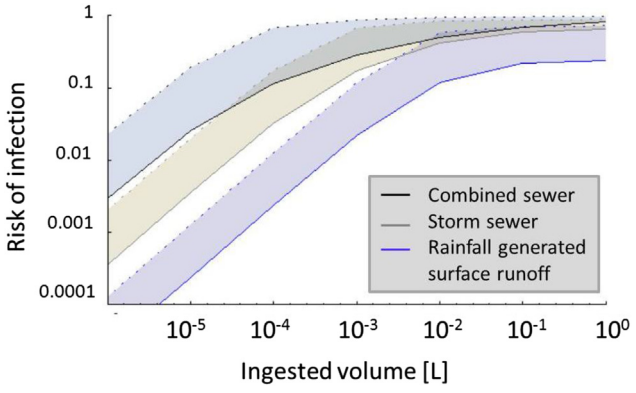

Fig. 1 – Mean risk of infection for exposure to floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff, as a function of the ingested volume per exposure event (95th percentiles were shown by dotted lines).

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 3). These risks were mainly caused by the presence of *C. jejuni*, noroviruses and enteroviruses in floodwater.

Fig. 1 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, Fig. 2 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

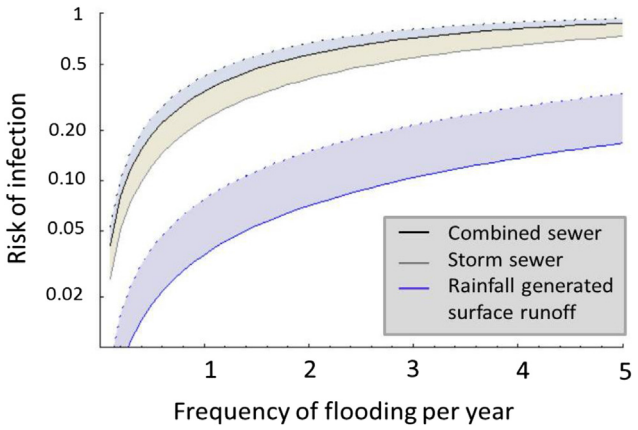

Fig. 2 – Mean risk of infection for exposure to floodwater originating from combined sewers, storm sewers and rainfall generated surface runoff, as a function of the frequency of exposure to flooding (95th percentiles were shown by dotted lines).

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 et al., 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 et al., 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

Table 4 – List of assumptions to calculate infection risks.

Parameter	Assumption
Concentration 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 typelocation (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.

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 Fig. 2). 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 Fig. 1. 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 4). 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 3. 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; 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. Fig. 2 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) overground 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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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2013.09.022.

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