Infectious rain? Evaluation of human pathogen concentrations in stormwater in separate sewer systems

Christiane Schreiber, Sophie-Bo Heinkel, Nicole Zacharias, Franz-Michael Mertens, Ekkehard Christoffels, Uta Gayer, Christoph Koch and Thomas Kistemann

ABSTRACT

Separate sewer systems collect and discharge rainwater directly into surface water bodies. In residential areas covering moderate traffic load these are alternative drainage routes to avoid combined sewer overflow discharge and to keep rivers clean as required by the EU Water Framework Directive. This overflow's microbial quality, however, needs to be evaluated, since stormwater run-offs are potential pathways for pathogens into river systems. Between 2010 and 2016, two separate sewer systems in North Rhine-Westphalia (Germany) were investigated. The stormwater outflow was sampled during discharge events and microbiologically analysed. The results showed high concentrations of *Escherichia coli* (1,100–1,100,000 CFU/100 mL) and *Clostridium perfringens* (20–13,000 CFU/100 mL). *Campylobacter* and *Salmonella* were detected in 97% and 43% of the samples. *Giardia* cysts were more often detected (31.6%) than *Cryptosporidium* oocysts (10.5%). The sources of human pathogens in rainwater run-off are heterogeneous. While roads have already been declared as chemical polluters via rainwater run-off, our study detected supplementary pollution of mainly faecal microorganisms. Presumably, failed connections in the sewer system itself are important sources of human pathogens. We suggest treatment of stormwater run-offs before being discharged into the river system.

Key words | bacteria, contamination, faulty connections, parasites, stormwater discharge, surface run-off

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INTRODUCTION

During heavy rainfall events, masses of stormwater would overload local wastewater treatment plants and are therefore stored in retention basins or directly discharged into the receiving river system. This is true even for advanced wastewater management systems such as those found in Germany. Therefore, stormwater run-off is often collected in separate sewer systems and discharged directly into the nearest surface water body, since this type of water is supposed to be hygienically and ecologically uncritical. Nevertheless, contact with sealed surfaces changes the water quality essentially, as analysis of the rainwater run-off showed for chemical pollution (Lye 2009; Mertens *et al.* 2018). Sealed surfaces also exacerbate the degree of contamination with microorganisms and pathogens in rainwater run-off: due to the lack of vegetation on sealed surfaces, microorganisms are less subjected to interception and adhesion and thus more easily enter the run-off (McLaughlin *et al.* 2013). In view of increasing urbanisation with accompanying sewer system development, there is a need to focus on the microbial quality of stormwater run-off which is discharged into such systems and finally dewatered into open water bodies and the environment. The German Federal State of North Rhine-Westphalia obliges urban planners to take the construction of separate sewer systems for sewage and rainwater into account (LWG 1998 §51a) (especially for housing projects in newly built residential areas) (Mertens *et al.* 2018).

Rainwater studies do not investigate the condition of the rainwater itself, collected when it is falling directly from the sky, but collected as run-off from different surfaces.

doi: 10.2166/wst.2019.340

Therefore, the rainwater under analysis is subjected to surface contaminants, e.g. leached chemical micropollutants, surface contaminants. While chemical contaminations of different run-offs are well investigated, microorganisms were formerly taken into account mainly in studies focusing on rooftop run-off. In most cases, the rainwater was stored and used for drinking water purposes, household usage or watering (Lye 2009; Ahmed *et al.* 2011; Sánchez *et al.* 2015).

Few studies focused on rainwater run-off from sealed surfaces collected in separated sewers. These included two-pipe drainage systems (separate sewer systems) which support the functional operability of sewage treatment plants (STPs) during rain events by collecting the rainwater run-off from sealed surfaces (e.g. streets, roofs) in pipes separated from those draining the wastewater. Usually, intermediate storage of rainwater takes place via retention basins within the separate sewer systems. Optionally, one-way side-connections from the rain basins to a STP can allow a buffered flow of rainwater into the STP where it will be treated together with the wastewater. This prevents typical combined sewer overflow (CSO) discharge events which occur in combined sewer systems where wastewater and rainwater are collected together within a one-pipe system connected to the STP. Therefore, separate sewer systems can form hydraulic reliefs for the receiving water body.

In case of an overflow of the rainwater retention basin as a result of exceeding the maximum retention capacity during heavy rain events, short-term increases in the hydraulic loading of the receiving surface water body occur. In those cases, rainwater will be discharged out of the basin directly into the river without any treatment. Thus, surface water body contamination by rainwater is possible if the collected run-off is loaded by chemicals or microorganisms.

Former studies of rainwater run-off collected in separate sewer systems mainly focused on chemicals. These studies detected pesticides, phthalates and biocides as well as pharmaceuticals (Birch *et al.* 2011; Zgheib *et al.* 2012; Mertens *et al.* 2018). Schreiber *et al.* (2016) found *Escherichia coli* median concentrations of 3.1×10^4 CFU/100 mL in stormwater discharges. The results show that rainwater from separate sewers poses a relevant risk to both human health and the environment.

Our study compares the outflow of two different separate sewer systems concerning microbial pathogens in order to assess the hygienic-microbiological quality of water which is directly discharged from separate sewers into the nearest water body. In two consecutive research projects (2009–2016), both separate sewer systems have been investigated and monitored concerning the run-off content of chemicals, biocides, pharmaceuticals, parasites and bacteria. Schreiber *et al.* (2016) used the *E. coli* concentrations of water from one stormwater retention basin to assess the importance of several point sources as well as diffuse pollution for hygienic-microbial surface water contamination within the investigated river catchment area. In this study, two stormwater retention basins of separate sewers are compared to each other concerning microbial contamination. Besides *E. coli* as a bacterial indicator of faecal pollution, there is an additional focus on *Clostridium perfringens* which is facultative pathogenic and more persistent than other bacteria because it can form spores. Furthermore, the prevalence of other pathogenic bacteria (*Campylobacter* spp. and *Salmonella* spp.) and of protozoal parasites (*Cryptosporidium* spp. and *Giardia* spp.) was examined.

METHODS

Study area

The studies were conducted within the catchment area of river Swist, a small sub-catchment of the river Rhine, Germany. Two rainwater storages from separate sewer systems (Table 1) were tested (2010–2012, 2015–2016). The area is located at 166 m altitude and has approximately 600 mm of total mean annual precipitation (data: HOWIS Erft, www.erftverband.de). During the study period, yearly rainfall peaks occurred in June and July during heavy convective rainfall. Also, the rain intense months of February

Table 1 | Technical details of the investigated stormwater retention basins of separate sewers

Stormwater retention basin of separate sewer	A	B
Location	North Rhine-Westphalia, Germany	
Receiving water body	Swist River	
Built-up area	Residential, few fruit farms, industry and commercial halls	Residential, few fruit farms, retail houses and commercial halls
Sealed run-off area [km^2]	0.74	0.24
Storage volume [m^3]	3,650	1,400
Maximum inflow [L/s]	1,100	450
Average outflow [L/s]	158	40
Amount of samples	26	14
Assessment phase	2010–2012	2015–2016

and March saw correspondingly high amounts of immediate run-off.

During the first project phase, separate sewer A was investigated with a view to the microbiological quality of its outflow. Separate sewer system A collects immediate rainwater run-offs from sealed surfaces from a run-off area of 0.74 km^2. The catchment serves a predominantly suburban residential area of about 8,000 inhabitants as well as some retail buildings. Additionally, there are few fruit farms located in the area which do mainly not contribute substantively to the surface run-off collected in the sewer system, as the rainwater seeps directly into the ground. The separate sewer system A is connected to a separate rainwater storage which is loaded with a maximum of 1,100 L/s. Whenever the retention volume of 3,650 m^3 is exhausted, this retention basin of separate sewer A dewaters with an average of 158 L/s.

Separate sewer system B serves a smaller, predominantly suburban, residential area, with few fruit farms, of 0.24 km^2 sealed run-off area. The sampled retention basin for separate stormwater storage has a storage capacity of 1,400 m^3 with a maximum inflow of 450 L/s. Both separate sewer systems A and B provide drainage for different parts of the same village. No commercial or industrial sites of significant size or facilities with special wastewater such as hospitals exist within the run-off catchments.

Water sampling

In 2010–2012, a total of 26 event-based water samples were taken and microbiologically analysed from sewer system A. In 2015–2016, 14 event-based water samples from separate sewer B were taken and microbiologically analysed. An automatic water sampler was installed at the outflow of each separate sewer. In case of a discharging event, an automatic water sampling procedure was initiated at the outflow of each rainwater retention basin in response to inputs from an additionally installed ultrasound altimeter recognising water level changes. 200 L of the first flush were pumped into barrels (Christoffels *et al.* 2014). After mixing the water volume in the barrels using a disinfected machine mixer, a 1-L sample was filled into sterile glass bottles for bacteriological analyses. Onsite, the samples were characterized by organoleptic criteria (odour, colour, visual turbidity, appearance) in addition to measurement of pH-values, temperature, electrical conductivity and turbidity.

Depending on turbidity and filter clogging, an amount of up to 100 L water was pumped at a flow rate of 2 L per minute through a parasitological filter system consisting of a pre-filter against coarse particles, a filter case containing the filter module and a water meter. *Giardia* cysts and *Cryptosporidium* oocysts stick in the filter module consisting of compressed, reticulated open-pore polyurethane foam. The nominal pore size is 1.0 μm.

The filter cartridges and bacteriological water samples were transported to the laboratory in transportable coolers at 4 °C. An automatically generated SMS and email alert informing the sample taker when sampling started guaranteed a microbiological analysis within a maximum of 24 h after discharge into the river system.

Microbiological analysis

C. perfringens, *Campylobacter* spp., *E. coli*, as well as *Salmonella* spp. prevalence, was determined in the laboratory with routine methods (Table 2). From one research phase to another, the analysis methods were slightly enhanced and updated according to new ISO and DIN EN methods. In the case of *C. perfringens* detection, DIN EN 26461-2

Table 2 | Applied detection methods for the microbial analysis of the stormwater discharge of the separate sewers A and B

Separate sewer Parameter	A Method guideline	B Parameter – Method guideline	Unit
C. perfringens spores	Following DIN EN 26461-2 (1993)	Following ISO 14189 (2013)	CFU/100 mL
Campylobacter spp.	Following (Schulze 1996), semi-quantitative analysis was qualitatively evaluated		CFU/100 mL
E. coli	Membrane filtration on ChromoCULT coliforms agar	DIN EN ISO 9308-1 (2012) modified by application of antibiotic supplement	CFU/100 mL
Salmonella spp.	Following ISO 6579 (1993), qualitative	ISO 19250 (2010), qualitative	CFU/100 mL
Cryptosporidium spp.	ISO 15553 (2006), EPA Method 1623	ISO 15553 (2006), EPA Method 1623	Oocysts/100 L
Giardia spp.	ISO 15553 (2006), EPA Method 1623	ISO 15553 (2006), EPA Method 1623	Cysts/100 L

CFU, colony-forming units.

(1993) and ISO 14189 (2013) are equivalent, ensuring the comparability of both results. Additionally, both detection procedures for *Salmonella* spp. are essentially similar and thus comparable. The detection procedure for *E. coli* DIN EN ISO 9308-1 (2012) is based on membrane filtration on ChromoCULT® coliforms agar (Merck). Its modification by application of an antibiotic supplement to inhibit background flora is currently in the status of a draft as ISO 9308-4, and already in the scientific discourse since 2014. Both results of *E. coli* detection are therefore also comparable.

Other detection procedures applied in both projects, such as the *E. coli* detection on the basis of the most probable number (MPN) as well as the detection of enterococci according to DIN EN ISO 7899-1 (1999) and DIN EN ISO 7899-2 (2000), respectively, were evaluated as not being directly comparable and therefore not included into the results of this study.

Cysts of *Giardia* spp. and oocysts of *Cryptosporidium* spp. were detected according to ISO 15553:2006 and EPA Method 1623 by immunomagnetic separation (Dynal®) and fluorescence microscopy. Double-staining was done with DAPI for DNA identification in cells, indicating that cells are intact and therefore possibly may be still alive, and specific FITC-labelled surface antibodies for parasite detection (*Waterborne*™, *Inc*.). The antibodies used mark human pathogens like *Giardia lamblia* or *Cryptosporidium parvum* and *C. hominis* as well as other, not human relevant species like *G. muris* or *C. baileyi*.

RESULTS AND DISCUSSION

The outflow of both separate sewers contained *E. coli* in a concentration range of 10^3–10^6 CFU/100 mL with a tendency of higher contamination in sewer B (see Figure 1). Median concentrations were calculated as 3.7×10^4 (A) and 8.6×10^4 (B) CFU/100 mL (see Table 3).

These concentrations of *E. coli* indicate significant faecal contamination of the rainwater run-off. Other studies have shown similar median concentrations of about 10^4 CFU/100 mL for low, medium and high dense built-up areas (McCarthy 2009; Hathaway & Hunt 2011).

Although rainwater is often perceived as 'save' or 'clean', the detected *E. coli* median concentrations show that the rainwater run-off has not even bathing water quality according to Directive 2006/7/EC (900 CFU/100 mL based upon a 90–percentile evaluation to be 'sufficient') or to the surface bathing water regulations of North Rhine-Westphalia

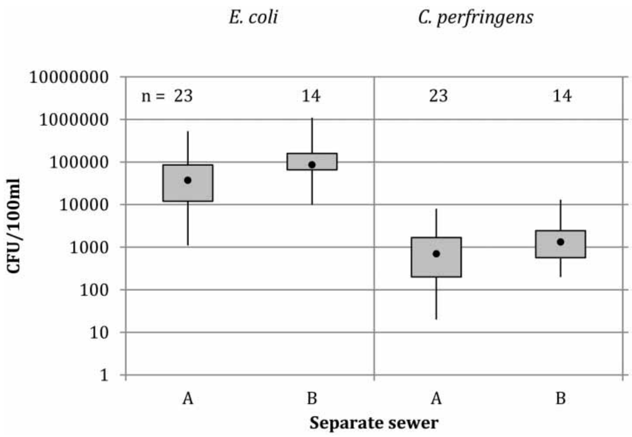

Figure 1 | Concentration of indicator bacteria in water samples of separate sewers A and B in a box-whisker plot; point marks the median, box marks the 25 and 75 percentiles, and vertical stroke marks minimum and maximum.

(Germany) with a single maximum threshold concentration of 1,800 CFU/100 mL (SGV.NRW 11.12.2007 §7 Fn3). This comparison illustrates the poor quality of the separate sewers' outflow and its contribution to the contamination of the receiving surface water.

The study shows *C. perfringens* spore concentrations of 2×10^1–2.3×10^4 CFU/100 mL in both separate sewers. While the median concentration in sewer A was 0.7×10^3 CFU/100 mL, the median concentration in sewer B was slightly higher, with 1.3×10^3 CFU/100 mL (see Table 3). Overall, Sewer B showed a tendency to contain higher concentrations of *C. perfringens* spores (see Figure 1). The concentrations are higher than found in the upper reaches of the connected river Swist, which are free of any settlement or wastewater influence (Schreiber *et al.* 2015). The presence of *C. perfringens* spores in all water samples poses a human health risk, since *C. perfringens* can cause intestinal infections as well as gas gangrene. It might be an indicator for the presence of other disinfectant-resistant microorganisms. Clostridia spore persistence in water is more similar to protozoal oocysts and cysts than to non-sporulating enteric bacteria cells. Additionally, similar partitioning behaviour may exhibit similar transport behaviour in the environment (Cizek *et al.* 2008). Accordingly, the occurrence of human pathogenic protozoal parasites in stormwater should be investigated if clostridia are present.

Campylobacter spp. was detected in 97% of all stormwater samples (see Table 3). Rechenburg & Kistemann (2009) detected a correlation between *Campylobacter* concentrations in rivers and heavy rainfalls. Our results suggest that rooftops as well as other surfaces exposed to

Table 3 | Occurrence of microbial parameters in the separate sewer effluent

			Separate sewer	
	Unit	**Value**	**A**	**B**
E. coli	Total samples	N	23	14
	Positive samples	n	23	14
		%	100.0	100.0
	CFU/100 mL	Median (n)	37,265	85,909
		Min (n)	1,100	10,000
		Max (n)	527,300	1,100,000
C. perfringens spores	Total samples	N	23	14
	Positive samples	n	23	14
		%	100.0	100.0
	CFU/100 mL	Median (n)	700	1,318.5
		Min (n)	20	200
		Max (n)	8,000	13,000
Campylobacter spp.	Total samples	N	23	12
	Positive samples	n	22	12
		%	95.7	100.0
Salmonella spp.	Total samples	N	23	14
	Positive samples	n	4	12
		%	17.4	85.7
Cryptosporidium spp.	Total samples	N	26	12
oocysts	Samples countable	N_c	4	6
	Positive samples	n	4	0
		%	15.4	0
	Oocysts/100 L	Median (n)	11.88	0
		Min (n)	10.3	0
		Max (n)	22.47	0
Giardia spp. cysts	Total samples	N	26	12
	Samples countable	N_c	5	7
	Positive samples	n	5	7
		%	19.2	58.3
	Cysts/100 L	Median (n)	39.2	37.5
		Min (n)	10.1	2.3
		Max (n)	3,666	880

N_c = number of samples where positive or negative results can be extrapolated as a defined number of concentration per 100 L, because either volume filtered is ≥100 L or parasites were found in sample volumes <100 L.

excreta from birds and mammalians (wildlife and pets) contaminate the rainwater run-off. Therefore, besides STPs and CSOs, separate sewers also need to be considered as sources of *Campylobacter* spp. in rivers. *Campylobacter* contaminations pose a health risk, since the intake may cause diarrhoea and colic. Surveillance data from Germany and from Europe (EFSA & ECDC 2016; Robert Koch-Institut 2017) show, that the incidence of reported food- and water-borne infections caused by *Campylobacter jejunii* increased from 2009 to 2016 on a regional as well as on a continental scale. These infections are mainly, but not all, attributed to be food-borne. However, there is also an infection risk resulting from contaminated surface waters, e.g. during recreational purposes like bathing (Rechenburg & Kistemann 2009).

Salmonella is the second most important bacterial agent causing diarrhoea in Europe (EFSA & ECDC 2016). Its occurrence is not only associated with food, but also with livestock and wild animals faeces. *Salmonella* was detected in 43% of the samples in total. While in sewer A only 17% of the samples tested positively, sewer B had 86% positive samples (see Table 3). The water- and food-transmitted pathogen is regarded as one of the main threats for consumers. Poultry, pigs and cattle are kept under surveillance in the EU (EFSA & ECDC 2016). *Salmonella* bacteria cause severe enteric infections; its occurrence in surface waters positively correlates with rainfall events (Wilkes *et al.* 2009). Since the catchment areas of both separate sewers, A and B, are neither strongly influenced by wild animals nor by livestock farms, the role of human beings and pets as the main contributors to the surface water contamination with *Salmonella* by CSO and stormwater discharge may be deduced.

Cryptosporidium spp. and *Giardia* spp. are relatively disinfection-resistant intestinal protozoa. Both parasites can be found in water bodies (Budu-Amoako *et al.* 2012; Kistemann *et al.* 2012). In River Swist tributaries free of any settlement or wastewater influence, the concentrations (maxima: *Cryptosporidium* spp. 161.5 oocysts/100 L, *Giardia* spp. 4.8 cysts/100 L) and detection frequencies (in total of 62 samples 18% positive for oocysts and 16% for cysts, respectively) were very low (Schreiber *et al.* 2015). *Giardia* cysts can often be found in raw sewage of municipal STPs (Kistemann *et al.* 2008), whereas *Cryptosporidium* shows lower association with sewage of human faeces but higher association with sewage contaminated with faecal matter from livestock and other mammals (Carey *et al.* 2004). Since 2010, the number of cases of diarrhoeal diseases caused by *Cryptosporidium* has increased in Germany (Robert Koch-Institut 2017).

In separate sewer A, both parasites were found in a number of samples, while in sewer B only cysts of *Giardia* could be detected in the given sample volumes (see Figure 2). The lower detection limit depends on the filterable volume, the recovery rate and the analysed portion of the sample, and thus it will differ from one sample to another. Often less than the intended 100 L could be filtrated, due to the samples' turbidity and organic load. In those samples, positive parasites detected could only be extrapolated to a result

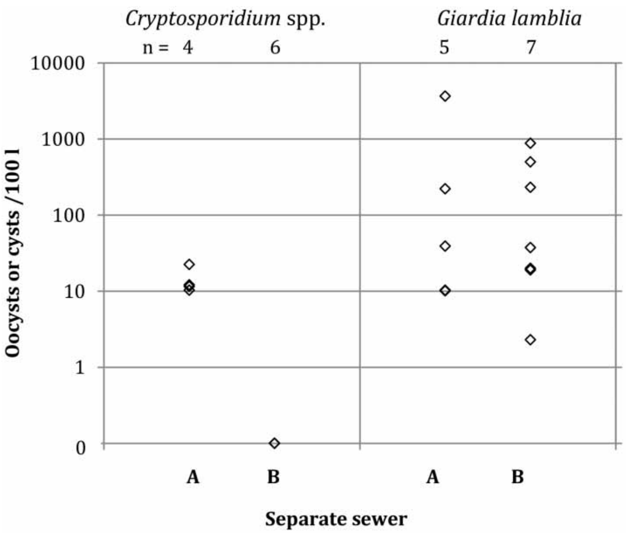

Figure 2 | Concentration of parasites in numerically evaluable water samples of separate sewers A and B.

for 100 L reference volume. A negative detection in less than 100 L, however, provides only the information that fewer than the detection limit were present in the analysed volume, corresponding to '< x' cysts/oocysts are present in projected 100 L. For example, in the case of 50 L filtered and investigation of the whole sample material, a negative detection at an average recovery rate of 20% would give a result as <10 cysts/100 L. Consequently, in contrast to 0/100 L, a remaining risk of stormwater contamination with parasites cannot be excluded if less than 100 L had been investigated.

In sewer A, *Cryptosporidium* spp. could be found in 15.4% (4/26) of all samples. Thereof, a median value of 11.9 *Cryptosporidium* spp. oocysts in 100 L were detected with a range of 10.3–22.5 oocysts per 100 L. For the remaining 84.6% of samples, presence or absence of oocysts in 100 L reference volume is not clear. In contrast, none of the 50% (6/12) countable samples of sewer B contained *Cryptosporidium* spp. (N_c, meaning positive or negative results can be extrapolated as a defined number per 100 L, because either volume filtered is ≥100 L or parasites were found in sample volumes <100 L). Uncertainty of contamination is given for the other 50% of samples.

Cryptosporidium has a wide range of animal hosts (Carey *et al.* 2004). Open-air enclosures of a small animal husbandry with rabbits and domestic fowl in the neighbourhood of the retention basin ostensibly contribute to the given *Cryptosporidium* concentration in sewer A. However, both sewers collect rainwater run-off from rooftops and streets, potential contamination sources of excreta from wild and domestic animals. Cryptosporidiosis outbreaks were found to be associated with severe rain events (reviewed in Semenza *et al.* 2012). Limit values for parasites in different water types, e.g. recreational waters or wastewater, do not exist yet in Germany.

Giardia spp. was found in 31.6% of all samples. In the remaining two thirds of samples, contamination cannot be definitively excluded due to sample volumes less than 100 L. Positive samples of sewer A showed a median concentration of 39.2 and a range of 10.1–3,666 cysts per 100 L. Sewer B had a median concentration of 37.5 cysts per 100 L with a range of 2.3–880 cysts per 100 L. The two-core trophozoite *G. lamblia* takes particularly human beings as hosts, as well as other mammals, and is able to cause intestinal infections by a low dose of 10 cysts (Exner & Gornik 2004). *Giardia* is common as an enteric parasite in pets, easily transmissible to human beings (Thompson 2004). By the discharges of their hosts, the parasites contaminate water and enter into the environment. Sewer A's catchment contains a place in close vicinity which is not sealed where people exercise their dogs; this suggests that in the catchment area, people tend to have companion animals. Pets and domestic animals defecating on sealed surfaces can contribute to *Giardia* spp. contamination in the separate sewers. However, it is not clear how much excreta from cats and dogs on sealed surfaces contaminate run-off water with *Giardia*. It needs to be assessed to which extent the concentration of this parasite in rainwater run-off can be reduced by the use of animal excrement bags by pet owners.

The separate sewer systems differ concerning their microbial concentrations (Table 3). There is a general trend of higher concentration of bacteria in separate sewer system B, even though in comparison to sewer A, sewer B receives rainwater run-off from only 85% of the area. The median concentration of *Giardia* spp. is similar in both sewer systems, with only *Cryptosporidium* posing an exception. While in the catchment of separate sewer A, retail properties slightly dominate the run-off area, the catchment of sewer B contains more private households. Consequently, the number of failed connections from private settlements might be higher in sewer B; failed connections from private households facilitate transmission of parasites like *Giardia lamblia* and bacteria associated with humans into separate sewers. *Cryptosporidium*, in contrast, seems more likely to originate from polluted diffuse surface run-off.

The high number of non-evaluable samples for parasites indicates that the amount of parasites in water with a high amount of suspended material is hardly accessible by the methods used in the present study. Nevertheless, the results prove that there is a significant risk of parasite contamination in the water discharged by separate sewer systems; this underscores the need for better assessment methods. Considering the extremely low concentrations of parasites necessary to cause severe infections (Exner & Gornik 2004), reference volumes below 100 L are required.

For this reason, two scenarios were considered for evaluating the potential remaining risk given by the absent detection in less than 100 L filtered water volume supplementary to the absolutely countable samples described above: in a worst-case scenario, all projected numbers formed the maximum amount of parasites possibly being in the water volume. This was done by replacing the sample specific detection limit into pretending that one parasite was found within every specific water volume ($<x = x$). In contrast, the best-case scenario considered all results $<x$ as parasites being not existent in 100 L ($<x = 0$).

The 38 combined samples from separate sewers A and B used in the calculated worst- and best-case scenarios show for both parasites comparable median values (Figure 3). Median and maximum best-case scenario concentrations of *Cryptosporidium* spp. (0 and 22.47 oocysts/100 L, respectively) are identical with those of the countable samples only, while the worst case shows much higher potential contamination (median 11.3 oocysts/100 L, maximum 333 oocysts/100 L). Conceivably, risk calculations on the basis of the few countable samples could underestimate the potential risk to human health posed by *Cryptosporidium* oocysts in stormwater.

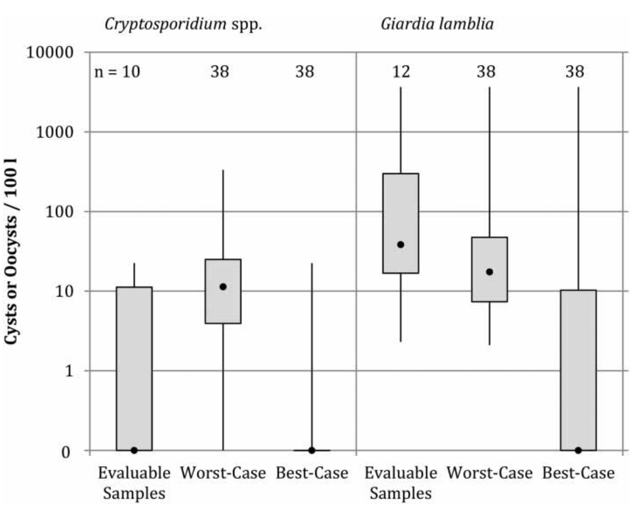

Figure 3 | Combined parasitological contamination of both separate sewers with $<x = x$ as worst-case scenario and $<x = 0$ as best-case scenario compared to the few evaluable samples.

For *Giardia* spp. the countable samples show higher amounts of *Giardia* cysts/100 L than the calculated concentrations of both best-case (Median 0 cysts/100 L) and worst-case (17.4 cysts/100 L) scenarios, although the maximum concentrations stay stable at 3,666 cysts/100 L for all scenarios. Accordingly, to take only the few countable samples into account would clearly overestimate the potential risk to human health posed by *Giardia lamblia* contamination of stormwater.

The results show that settlements can be sources of microbial pollution of rainwater in separate sewers. Regulatory and water management institutions need to be alerted to the potential need for treatment of rainwater before it is discharged into the river system, specifically depending on the catchment area of the stormwater run-off.

STPs in the Swist catchment area have already been identified as a source of faecal bacteria in river systems. In the effluent of differently equipped STPs, median contaminations of *E. coli* of $0.3–2.2 \times 10^4$ CFU/100 mL and of *C. perfringens* of $0.4–1.6 \times 10^3$ CFU/100 mL as well as 4-226 cysts/100 L of *G. lamblia* were measured (Kistemann *et al.* 2008). Landscape run-off from unsealed surfaces such as forests and grassland contains <10 CFU/100 mL to 1.2×10^3 CFU/100 mL *E. coli*, while high concentrations have been detected in surface water surrounded by farmland and fruit growing drain-pipes with 7.4×10^3 and 5.9×10^3 CFU/100 mL within the catchment of River Swist (Schreiber *et al.* 2015). As shown in this study, separate sewers discharge higher concentrations from sealed surfaces to the river system than contained in diffuse discharge from unsealed surfaces.

In case of rainfall events, the occurrence of microbial pathogens in STPs and CSOs increases significantly (Rechenburg & Kistemann 2009; Kistemann *et al.* 2012). The authors concluded that those findings result from discharge of untreated sewage by CSOs. Our study shows that the rainwater run-off from sealed surfaces also contributes significantly to the concentration of pathogens.

The sources of pathogens in stormwater run-offs are various. As a drawback of our study, we did not specify the contamination routes by, e.g. source tracking markers additionally. Therefore, we cannot definitively identify single contamination sources of the rainwater collected. The occurrence of *Cryptosporidium* in only one of the two separate sewers suggests a high impact of husbandry on stormwater run-off, even in built-up areas where it is not expected.

Besides wildlife, birds and pets, street-cleaning trucks contain the potential of spreading pathogenic microbes from the contaminations place of origin among streets. Furthermore, the separate sewer systems themselves act as a source of pathogens due to failed connections, an occurrence that is openly known but little discussed in water management.

CSO additionally treated by retention soil filters (RSF) have been proved to discharge essentially lower rates of faecal bacteria (Scheurer *et al.* 2014) and pathogens to the river system than separate sewers (Schreiber *et al.* 2016). In a longterm study, a median reduction potential of 3.1 $\log_{10}$ stages have been estimated for RSF (Christoffels *et al.* 2014). This current study suggests RSF as suitable treatment step for rainwater run-offs.

CONCLUSIONS

This study shows that rainwater run-off discharged from separate sewer systems into open water bodies contains high concentrations of microbial pathogens. Some of the pathogens appear to run-off from sealed surfaces in settlements, while other pathogens seem to run-off from agriculturally used built-up areas such as sealed farm yard surfaces. Faulty connections of private households discharging untreated sewage directly to the separate sewer systems present a suspected important source of pathogens. For further unequivocal pathway identification, microbial source tracking methods would be helpful. In order to improve microbial surface water quality, our study shows that rainwater run-off requires treatment before being discharged into open river systems. The actual hygienic microbiological impact of the discharges is dependent on the specific load and the nature of the water body. The current practice of separate rainwater storage alone is not a suitable treatment. Compared to CSO effluents treated by RSFs, separate sewer system discharges are associated with pollution of river streams rather than dilution of contamination. Separate sewer systems pose an environmental and potential health risk as a source of human pathogens. Accordingly, they need to be scrutinized within the regulatory framework; this is important, as adequate rainwater treatment is needed to support the goals of the EU Water Framework Directive. RSFs may be a good alternative for the treatment of rainwater run-offs, in complementation or replacement of rainwater retention tanks in separate sewers, to achieve an efficient reduction of microbial loads.

Diffuse sources of run-off rainwater contamination need to be investigated more thoroughly, while innovative measures to reduce the contamination of run-off rainwater by bacteria and parasites need to be evaluated. Animal husbandry, as well as handling of pet waste, may play a significant role in this respect. The reduction of fault connections as point sources needs to be implemented through intensified controls and political intervention.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGEMENT

The research was funded by the Ministry for Environment, Agriculture, Conservation and Consumer Protection (MULNV) of the State of North Rhine-Westphalia, Germany (Az 54.2.-3.3-1800.Wt and Az 54.2.-3.3-1892.Wt).

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First received 26 March 2019; accepted in revised form 26 September 2019. Available online 15 October 2019