Rainwater harvesting: quality assessment and utilization in The Netherlands

F. M. Schets, R. Italiaander, H. H. J. L. van den Berg and A. M. de Roda Husman

ABSTRACT

The use of roof-collected rainwater as a freely available and sustainable alternative to drinking water produced by drinking water companies increases worldwide. Initially, rainwater is free of microbial contamination, but it may become contaminated by animals and humans or, alternatively, human pathogens may grow in stored rainwater resulting in a significant human health risk from infectious diseases. This three-year study demonstrated that rainwater stored in different reservoirs in The Netherlands was frequently faecally contaminated and incidentally contained potential human pathogens such as *Campylobacter*, *Cryptosporidium*, *Giardia*, *Aeromonas hydrophila* and *Legionella*. Analysis of samples during a period with variable weather conditions showed a correlation between rainfall intensity and faecal indicator counts and increased detection of pathogens after heavy rainfall incidents. Outside temperature had a limited effect on both the temperature and the microbiological quality of the water in the reservoirs, which did not comply with Dutch drinking water legislation and should thus not be consumed without treatment. In general, a health risk may arise from exposure to pathogens when contaminated droplets are inhaled, ingested or come into contact with the skin. Health risks may be reduced by regular cleaning of the collection, storage and transport means, but to assess their efficacy field intervention studies are required.

Key words | rainwater harvesting, roof-collected rainwater, water quality

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ABBREVIATIONS AND NOTATIONS

DNA	deoxyribonucleic acid
HPC	heterotrophic plate count
MPN	most probable number
PCR	polymerase chain reaction
PVC	polyvinylchloride
RNA	ribonucleic acid
rpm	revolutions per minute
TSA	trypton soy agar

INTRODUCTION

In remote and rural areas where access to tap water is limited, rainwater harvesting is common practice. However, also in areas that have easy access to tap water, as almost everywhere in The Netherlands, rainwater harvesting increases. People use rainwater for toilet flushing or watering the garden or crops because it is available for free and its use reduces their expenses for purified drinking water that they obtain from drinking water companies (Schets *et al.* 2007). Moreover, more people are becoming aware of the necessity to take care of the Earth's natural resources, including drinking water sources and energy. Due to the increased demand for purified drinking water groundwater levels decrease, with negative effects on various ecosystems as a result (Lieste *et al.* 2007). Therefore, the use of collected rainwater as an alternative to the use of purified drinking water fits into a policy of sustainable management and environmentally friendly behaviour.

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Initially, rainwater is free of human pathogenic microorganisms, but it may become contaminated at surface run-off and during storage in containers (WHO 2006). Contamination may occur when animal faeces runs off roofs or animals have access to the rainwater reservoirs or open mains, but also during maintenance or repair of the rainwater storage and distribution system (WHO 2006). The microbiological quality of stored rainwater may depend on storage conditions such as temperature and time, but also on the materials of which the storage containers are made, maintenance of the system and hygiene practice at the tap (WHO 2006). Presence of faecal indicator bacteria suggests contamination of the water with faeces of human or animal origin, indicating that pathogens which may be present in these faeces, such as *Campylobacter*, *Salmonella*, *Vibrio*, *Cryptosporidium*, *Giardia* and enteric viruses, may also be present in the water (WHO 2006). Due to different persistence in the environment, a quantitative relation between faecal indicators and pathogens such as *Cryptosporidium* and *Giardia* is not to be expected; these organisms may be present in the absence of faecal indicators (WHO 2006; Schets *et al.* 2008). Depending on materials used, maintenance status and storage time, e.g. *Aeromonas* may grow in the reservoirs (Havelaar *et al.* 1990) and when biofilms are formed, e.g. *Legionella* may colonize the system (Berry *et al.* 2006; Simmons *et al.* 2008). Biofilms formed in water distribution systems are known to cause public health problems such as protecting and supporting pathogenic microorganisms, bacterial regrowth and depletion of disinfection agents (Martiny *et al.* 2003; September *et al.* 2007). Several studies in various countries have demonstrated the presence of faecal indicator bacteria and pathogens in rainwater storage reservoirs. Faecal indicators, *Pseudomonas aeruginosa* and *Salmonella* were detected in German reservoirs (Holländer *et al.* 1996), whereas faecal indicators, *Aeromonas*, *Salmonella* and *Cryptosporidium* were found in New-Zealand reservoirs (Simmons *et al.* 2001). In Denmark, reservoirs were contaminated with *Escherichia coli*, *P. aeruginosa*, *Aeromonas*, *Legionella*, *Campylobacter* and *Cryptosporidium* (Albrechtsen 2002). The chemical composition of collected rainwater varies due to the influence of roof material such as galvanized lead or concrete tiles and surrounding environmental conditions like air pollution in urban and industrial areas (Thomas & Greene 1993).

Worldwide, collected rainwater is used for domestic and drinking purposes. In The Netherlands, however, drinking water legislation (Anonymous 2001) determines that rainwater may only be used as grey water in the household, moreover it is recommended explicitly for toilet flushing only (Ministry of Housing, Spatial Planning and the Environment 2003). As for drinking water provided by the Dutch drinking water companies, the infection risk at exposure to grey water in the household must be below the generally accepted level of less than one infection per 10,000 persons per year, which is outlined for drinking water in the Dutch Drinking Water Act (Anonymous 2001).

The objective of this three-year study was to gain insight into the microbiological quality of rainwater stored in reservoirs in The Netherlands and the effect of environmental conditions and storage container material on the survival of microorganisms in these reservoirs to substantiate recommendations on safe collection and storage of rainwater.

METHODS

Monitoring in 2005 and 2006

In 2005, a pilot study was done in which the microbiological quality of collected rainwater at four different sites (Table 1; Schets *et al.* 2005, 2007) in The Netherlands was examined. The collected rainwater at the studied sites was used for toilet flushing, cleaning floors and watering plants. Reservoir water samples were taken weekly in June 2005 and were tested for the presence of total coliforms (Anonymous 2000*a*), *E. coli* (Anonymous 2000*a*; Rapid Test), intestinal enterococci (Anonymous 2000*b*), *Campylobacter* (Anonymous 2005), *Salmonella* (Anonymous 2002), *Vibrio* (Anonymous 2007*a*), *Legionella* (Anonymous 2007*b*), *Aeromonas* (Havelaar *et al.* 1987; Havelaar & Vonk 1988), *Clostridium perfringens* (Bisson & Cabelli 1979) and heterotrophic plate count at 22°C (Anonymous 1999). In 2006, a follow-up study was carried out, dealing specifically with the effect of environmental conditions on the microbiological quality of rainwater stored in reservoirs. From May until August 2006 samples

Table 1 | Characteristics of the examined rainwater reservoirs

Characteristic	Rainwater reservoir code WVE	ECO	GD	RT
Placing of reservoir	Underground, outside building	Underground, underneath building	Cellar, underneath building	Outside, adjacent to house
In use since	1998	1996	1993	2001
Rainwater harvesting from	Roof with vegetation	Rubber roof without vegetation	Roof, balconies	Roof
Application of rainwater for	Toilet flushing, cleaning floors	Toilet flushing, cleaning floors	Toilet flushing	Watering garden
Water treatment	None	Leaf catcher, sand filter	Filter for large particles	None

were taken biweekly from reservoirs WVE, ECO and GD (Table 1). The sampling plan was flexible to enable (extra) sampling during normal and heavy rainfall, whether or not after a period of drought. Samples were tested for the parameters indicated above, supplemented with analysis for the presence of *Cryptosporidium* and *Giardia* (Anonymous 2006*a*) and enteroviruses (Rutjes & de Roda Husman 2004).

Samples were taken and handled according to ISO 19458 (Anonymous 2006*b*); samples for bacteriological analyses (5–10 l) were kept on melting ice while being transported to the laboratory whilst samples for virus (10 l) and parasite analyses (10 l) were transported at ambient temperatures. All samples were analysed within 24 hours of sampling. Analyses were done according to ISO and Dutch national standards as indicated in brackets for each parameter. Of all samples, water temperature was measured on site, whereas pH and turbidity were recorded in the laboratory by using standard laboratory equipment according to the instruction manual.

Rainfall data and information on other weather conditions were obtained from the Royal Dutch Meteorological Institute (www.knmi.nl). Correlation coefficients r were calculated for microbiological and meteorological parameters by using the CORREL function in Excel 2003.

Storage experiments in 2007

In 2007 laboratory experiments were conducted to study the effect of storage temperature and storage container material on the survival of naturally occurring and seeded *E. coli* and *Aeromonas* in rainwater collected from reservoirs WVE and ECO (Table 1). Water samples (30 l) were taken from the reservoirs according to ISO 19458 (Anonymous 2006*b*) and transported to the laboratory at ambient temperatures. Upon sampling from the reservoirs, temperature, pH, turbidity and conductivity were determined as indicated above. Volumes of 3–5 l were stored in polyethylene, galvanized iron and concrete containers in the dark at 15, 25 and 35°C in standard laboratory incubators. Water was stored at each temperature in each type of container in duplicate; one container was stored without any additives and the other was seeded with overnight cultures of *A. hydrophila* M800 and *E. coli* WR1 (NCTC 13167).

Overnight cultures were obtained by inoculation of a colony of *E. coli* WR1 and *A. hydrophila* M800 freshly grown (24 h, 37°C and 30°C, respectively) on Trypton Soy Agar (TSA) into 150 ml Buffered Peptone Water in a 500-ml Erlenmeyer flask followed by incubation at 37°C and 30°C, respectively, rotating at 100 rpm, for 18–24 h. The concentration of culturable *E. coli* and *A. hydrophila* in the overnight cultures was determined by using plate counts on TSA (24 h, 37°C and 30°C, respectively). Based on these counts, containers with rainwater were seeded with a volume of (a dilution of) the overnight cultures to obtain a final concentration of approximately 10,000 bacteria per litre. This concentration was chosen because it was in the range of concentrations detected in the samples from the studied rainwater reservoirs and it enabled the detection of at least a 4-log reduction of the number of seeded bacteria.

Sampling from both the seeded and non-seeded containers was done with a sterile pipette, withdrawing water from the upper layers after careful mixing of the contents of the containers. Sampling started immediately after inoculation and was done at one, two, five days and subsequently

weekly after inoculation. Enumeration of culturable *E. coli* and *Aeromonas* was done by using the culture methods indicated above.

RESULTS

Monitoring 2005

All (faecal) indicator bacteria as well as *Aeromonas* and *Clostridium perfringens* were found in all rainwater reservoirs, although not all samples were positive and detected numbers varied considerably among reservoirs (Table 2). Examination of 16 samples from 4 reservoirs demonstrated the presence of total coliforms in 16 (100%), *E. coli* in 15 (94%) and intestinal enterococci in 16 (100%) samples. *Aeromonas* and *C. perfringens* were detected in 11 (69%) and 14 (88%) of these 16 samples, respectively. *Campylobacter* sp. and *Legionella pneumophila* (serogroup 2–14) were detected once in samples taken on different days from reservoir GD. Detection of *Legionella* in rainwater samples on Buffered Charcoal Yeast Extract Agar (Anonymous 2007*b*) was largely hampered by growth of non-*Legionella* background flora; as a result *Legionella* detection failed in seven samples (47%). *Salmonella* and *Vibrio* were not found in any of the samples. In each reservoir there were minor variations in pH, turbidity and temperature of the water, except for reservoir RT, in which water temperature increased from 19°C to 33°C (Table 3). During the sampling period the weather was fair and stable (average temperature 12–21°C; maximum temperature 19–33°C) and there was no significant rainfall (0–5.2 mm per week).

Monitoring 2006

As in 2005, all (faecal) indicator bacteria and *Aeromonas* and *C. perfringens* were found in all rainwater reservoirs. Again, the number of positive samples and the bacterial counts varied among reservoirs (Table 4). Total coliforms were present in 22 of 24 (92%) samples from three reservoirs, *E. coli* and intestinal enterococci were detected in 19 (79%) and 20 (83%) of these samples, respectively. *Aeromonas* was found in all the samples, with *A. hydrophila* being the most prevalent species (84% of 32 isolated *Aeromonas* strains). *C. perfringens*-analysis yielded reliable results in 15 samples only (5 for each reservoir); the bacterium was found in 9 of these samples (60%).

The human pathogenic microorganisms *Campylobacter* and *Legionella* were found in some samples. *Campylobacter* was detected in three samples from reservoir GD and one sample from reservoir ECO. In water from reservoir GD Most Probable Numbers (MPN) were 240/l or >240/l;

Table 2 | The microbiological quality of rainwater stored in reservoirs, monitored in June 2005

Parameter	Rainwater reservoir code WVE	ECO	GD	RT
Total coliforms*	20	15	3,818	480
	(12–37)	(6–18)	(160–10,900)	(45–1,004)
*E. coli**	5	4	125	2
	(4–10)	(0–7)	(17–330)	(0–6)
*Enterococci**	3	2	1,720	10
	(0–4)	(1–6)	(130–1,590)	(2–20)
HPC 22°C*	1,022	1,894	9,100	131,383
	(610–1,960)	(95–5,455)	(3,100–15,100)	(62,150–183,500)
*Aeromonas**	1,162	31	2,848	77
	(320–2,150)	(0–124)	(1,200–3,925)	(0–242)
*C. perfringens**	3	2	5	8
	(0–5)	(1–3)	(2–10)	(0–18)

*The displayed numbers (n/100 ml) are the average of analysis results of four samples per reservoir, and the observed range in brackets.

Table 3 | Physical characteristics of rainwater stored in reservoirs, in 2005 and 2006

Parameter	Rainwater reservoir code WVE	ECO	GD	RT
Water temperature 2005*	14.7	17.9	13.2	25.7
	(13.4–16.3)	(17.4–18.4)	(12.5–14.0)	(19.9–32.9)
Water temperature 2006*	15.7	17.8	14.6	ND
	(13.7–19.1)	(17.1–18.9)	(12.4–16.4)	
pH 2005*	6.9	7.2	7.0	6.1
	(6.8–7.2)	(6.8–7.8)	(6.5–7.5)	(5.6–6.5)
pH 2006*	6.9	7.0	7.2	ND
	(6.8–7.1)	(6.6–7.8)	(5.9–8.2)	
Turbidity 2005*	1.6	1.1	2.3	1.8
	(1.2–2.2)	(0.6–1.8)	(1.8–3.7)	(1.1–2.8)
Turbidity 2006*	0.5	0.1	0.9	ND
	(0.0–2.2)	(0.0–1.0)	(0.0–2.3)	

*The displayed figures are the average of measurements in four (2005) or eight (2006) samples per reservoir, and the observed range in brackets.

the MPN in the ECO reservoir sample was 26/l. All *Campylobacter*-isolates were identified as *C. jejuni* subspecies *jejuni*. *Legionella* non-*pneumophila* was found in one sample from reservoir ECO, whereas 14 samples were negative. *Legionella* detection was again hampered by growth of non-*Legionella* background flora, which led to indefinite results in nine samples (38%).

One sample from reservoir GD contained one *Cryptosporidium* oocyst and one sample from reservoir ECO contained one *Giardia* cyst; both the oocyst and the cyst met the morphological criteria outlined in ISO 15553 (Anonymous 2006*a*). Additional species identification by PCR of these low numbers of (oo)cysts was not done. Analysis of sample volumes of 1–21 did not yield any positive findings for *Salmonella* and *Vibrio*. Neither culturable enteroviruses nor enterovirus-RNA was detected in any of the samples. In each reservoir there was some variation in pH, turbidity and water temperature (Table 3).

Table 4 | The microbiological quality of rainwater stored in reservoirs, monitored June–August 2006

Parameter	Rainwater reservoir code WVE	ECO	GD
Total coliforms*	104	54	2,783
	(24–314)	(0–314)	(0–15,500)
*E. coli**	20	33	1,934
	(0–53)	(0–175)	(0–10,000)
*Entercocci**	22	167	1,555
	(2–100)	(0–1,255)	(0–9,546)
HPC 22°C*	5,946	5,685	394,673
	(505–15,950)	(536–18,636)	(392–3,045,000)
*Aeromonas**	4,193	733	15,642
	(70–16,818)	(7–3,182)	(18–85,000)
*C. perfringens**	1	2	13
	(0–4)	(0–11)	(3–31)

*The displayed numbers (n/100 ml) are the average of analysis results of eight (five for *C. perfringens*) samples per reservoir, and the observed range in brackets.

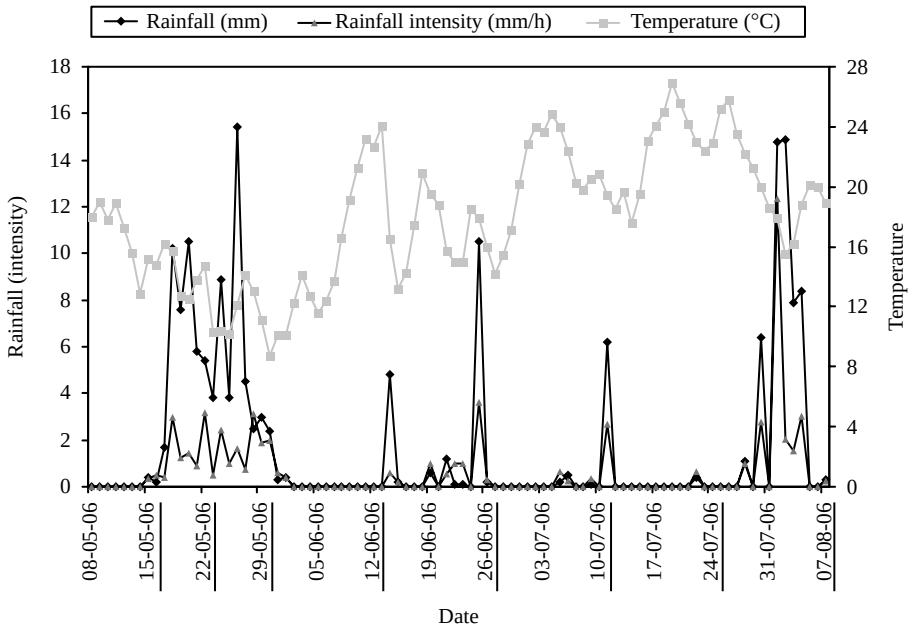

Figure 1 | Outside temperature, rainfall and rainfall intensity during the 2006 study period. Samples from rainwater storage reservoirs were taken on the underlined dates.

During the study period the weather conditions varied strongly (Figure 1). Periods of drought alternated with wet episodes and periods in which precipitation intensity was high. Outside temperature fluctuated as well. High concentrations of *E. coli* and intestinal enterococci were found in all reservoirs on sampling days that were preceded by days with heavy rainfall (Schets *et al.* 2007) and, likewise, *Campylobacter*-positive samples were only found on these sampling days (Schets *et al.* 2005, 2007). A moderate to strong correlation was observed between rainfall intensity and counts of all microbiological parameters except *C. perfringens*. *C. perfringens* counts were strongly correlated with rainfall amount and wind speed (Table 5).

Storage 2007

Both *E. coli* and *A. hydrophila* survived longer in PVC containers than in galvanized iron containers (Figure 2). Although a limited number of data were obtained, survival in clay containers appeared to mimic survival in PVC containers (Figure 3). The numbers of culturable bacteria gradually declined in all containers, but the decline was more rapid at elevated temperatures (Figure 4). Apart from storage temperature and storage container material, the water type influenced survival of *E. coli* and *A. hydrophila*.

When seeded in water from reservoir WVE and stored in PVC containers at 15°C, *E. coli* and *A. hydrophila* could

Table 5 | Correlation coefficients for bacterial counts in rainwater reservoirs and meteorological parameters

Microbiological parameter	Meteorological parameter														
	Rainfall amount			Rainfall intensity			Wind speed			Outside temperature			Reservoir temperature		
Reservoir	WVE	ECO	GD	WVE	ECO	GD	WVE	ECO	GD	WVE	ECO	GD	WVE	ECO	GD
Total coliforms	−0.2	0.2	0.2	0.3	**0.9**	**0.9**	−0.3	−0.2	−0.1	0.2	−0.2	−0.2	**0.5**	−0.2	0.3
E. coli	−0.2	0.2	0.3	0.3	**0.8**	**0.7**	−0.2	−0.1	−0.2	0.2	−0.2	−0.4	**0.6**	−0.2	0.2
Intestinal enterococci	0.1	0.2	0.2	**0.7**	**0.7**	**0.8**	−0.2	−0.2	−0.1	−0.1	−0.1	−0.3	0.1	−0.2	0.3
HPC 22°C	−0.1	0.3	0.1	0.2	**1.0**	**0.7**	−0.5	0.0	−0.2	0.3	−0.2	−0.1	**0.6**	−0.2	0.4
Aeromonas	−0.1	0.3	0.3	**0.6**	**0.7**	**0.8**	−0.4	0.1	0.3	0.1	−0.3	−0.3	**0.5**	−0.2	0.2
C. perfringens	**0.6**	**0.6**	**0.8**	0.2	0.2	0.4	0.1	**0.9**	**1.0**	**−0.6**	−0.4	**−0.5**	**−0.5**	**−0.7**	−0.1

Correlation coefficients in bold display moderate to strong correlations.

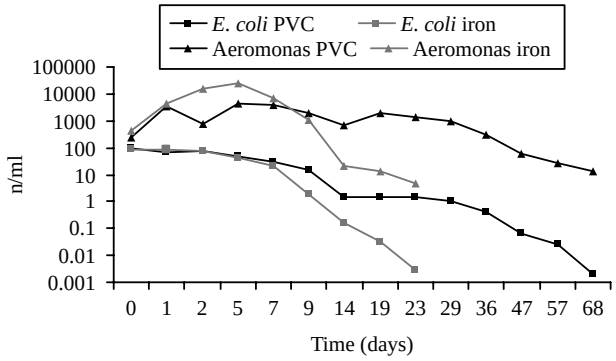

Figure 2 | Survival of *E. coli* WR1 and *A. hydrophila* M800 in rainwater from reservoir WVE, stored in PVC and galvanized iron containers at 15°C.

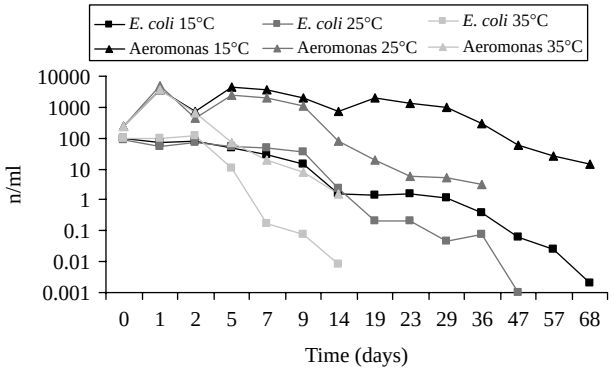

Figure 4 | Survival of *E. coli* WR1 and *A. hydrophila* M800 in rainwater from reservoir WVE, stored in PVC containers at 15, 25 and 35°C.

be detected for up to 68 days after seeding. However, when seeded in water from reservoir ECO and stored in PVC containers at 15°C, *E. coli* was detectable for up to 34 days, whereas *A. hydrophila* could no longer be detected on day 27.

Naturally occurring *E. coli* and *Aeromonas* were present in water from both reservoirs at low numbers that gradually declined and went undetectable within one (reservoir ECO) to two (reservoir WVE) weeks from the start of the experiments.

DISCUSSION

This three-year study demonstrated that roof-collected rainwater stored in four different reservoirs in The Netherlands and used for toilet flushing, cleaning floors and watering plants was faecally contaminated and incidentally contained potential human pathogenic microorganisms such as *Campylobacter*, *Legionella*, *Cryptosporidium* and *Giardia*. The presence of total coliforms indicated the potential presence of biofilms in the reservoirs; these organisms can survive and grow in water systems, particularly in the presence of biofilms (WHO 2006). The microbiological quality of the stored rainwater did not comply with Dutch drinking water legislation (Anonymous 2001) and WHO drinking water guidelines (WHO 2006) and thus the water should not be consumed without treatment.

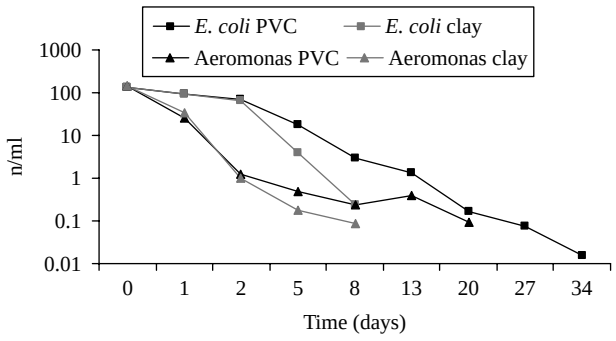

Figure 3 | Survival of *E. coli* WR1 and *A. hydrophila* M800 in rainwater from reservoir ECO, stored in PVC and clay containers at 15°C.

Although different studies on rainwater quality are difficult to compare in a quantitative manner because e.g. environmental conditions, detection methods used and sample volumes analysed vary, our major findings largely match previously published results from other countries. Total coliforms and *E. coli* were found in approximately 60–100% of the samples from rainwater reservoirs examined in Germany (Holländer *et al.* 1996), New Zealand (Simmons *et al.* 2001), Denmark (Albrechtsen 2002), Australia (Ahmed *et al.* 2008) and Bermuda (Lévesque *et al.* 2008). Pathogens such as *Campylobacter*, *Salmonella*, *Legionella*, *Cryptosporidium* and *Giardia* were detected by culture or microscopic methods in a few per cent of the samples or not at all (Holländer *et al.* 1996; Simmons *et al.* 2001; Albrechtsen 2002; Abo-Shehada *et al.* 2004), with an occasional exception, like the detection of *Cryptosporidium* in 35% of the samples from seven Danish reservoirs (Albrechtsen 2002). Ahmed *et al.* (2008) used real-time PCR for the detection of pathogens in roof-collected rainwater and found more samples positive, e.g. *Campylobacter coli ceuE* gene in 41%, *L. pneumophila mip* gene in 26%, *Salmonella invA* gene in 11% and *G. lamblia* β-giardin

gene in 16% of the samples. This higher percentage of positive samples may be the result of a higher sensitivity of real-time PCR compared to the other methods, the ability of PCR to detect injured, stressed or viable but non-culturable bacteria which are hard to detect by culture methods, or it may arise from the detection of DNA of non-viable organisms. Nevertheless, all studies indicate that the microbiological quality of roof-collected rainwater is generally poor compared to tap water quality in most developed countries and use of this water may pose a health risk, particularly when consumed without any treatment.

The observed faecal contamination in the Dutch reservoirs is most likely of animal origin and entered the reservoirs through roof run-off during rainfall. Since managers responsible for the rainwater systems studied reported that small mammals such as rodents had no direct access to any of the reservoirs as a result of the underground cellar-like construction of the reservoirs (Table 1; Schets *et al.* 2005, 2007) and they frequently observed large groups of birds on the roofs, it is conceivable that bird faeces are the main source of contamination. However, other animal sources cannot be fully excluded although animals other than birds, that may dwell on roofs in numbers that are of expected influence, are not part of the Dutch fauna. Pathogens such as *Campylobacter* have been detected in the faeces of gulls (*Larus* spp) (Broman *et al.* 2002; Moore *et al.* 2002; Waldenström *et al.* 2002), which are common dwellers of roofs in Dutch cities. There are limited data on the occurrence of *Cryptosporidium* and *Giardia* in faeces from birds that are likely to be present on roofs in an urban environment (Smith *et al.* 1993); however, it is possible that they have been the source of the protozoa. Both *Salmonella* and *Vibrio* have not been detected, although they are known to be present in the faeces of various birds (Ogg *et al.* 1989; Buck 1990; Refsum *et al.* 2002). Probably *Salmonella* was present in low concentrations and detection in rainwater reservoirs would have required the analysis of larger samples than was done, whereas birds that live in an inland urban environment may not be infected with *Vibrio* in contrast to coastal birds and reports from other countries (Ogg *et al.* 1989; Buck 1990).

All *Campylobacter* isolates from the Dutch rainwater reservoirs were *C. jejuni* subspecies *jejuni*, which is an important cause of gastro-enteritis in humans (Allos 2001), but may also cause more serious disease such as bacteraemia in immune-incompetent individuals (Monselise *et al.* 2004). Considering the high *Campylobacter* counts observed in reservoir GD (>240 cfu/l) and assuming a high infectivity of *Campylobacter* and an estimated 1,000 toilet visits per person per year, exposure to a volume of 4 μl may lead to a risk of one infection in 10,000 persons per year (Schets *et al.* 2007). These are rough estimates and the exact route of exposure is unknown, but may be through swallowing of droplets via aerosols or through contact with contaminated surfaces (Gerba *et al.* 1975; Barker & Jones 2005). Exposure to a volume of approximately 4 μl per year during toilet visits is not unlikely to occur and therefore the requirement of less than one infection in 10,000 persons per year, as outlined in Dutch drinking water legislation (Anonymous 2001) and also applicable for rainwater used for toilet flushing (Ministry of Housing, Spatial Planning and the Environment 2003), will not always be met.

In Dutch drinking water legislation (Anonymous 2001), both *Aeromonas* and *C. perfringens* are included as technical parameters providing information on the hygiene status of the distribution system and performance of drinking water treatment, respectively. However, *Aeromonas* species and *C. perfringens* (Van Immerseel *et al.* 2004) may also be human pathogenic. *C. perfringens* was found in very low numbers and may be of limited relevance, but *A. hydrophila* was found in very high numbers in several samples from reservoirs GD and WVE. *A. hydrophila* can cause gastro-enteritis in humans (Havelaar *et al.* 1992) and infections of the human skin (Valley *et al.* 2004; Hiransuthikul *et al.* 2005). The presence of high numbers of *Aeromonas* in stored rainwater may therefore be of health concern and is unwanted from a hygienic point of view. *Aeromonas* is known to be able to adjust to the poor nutrient conditions in drinking water and can grow in distribution systems (Havelaar *et al.* 1990). It has also been found in biofilms in drinking water distribution systems (Chauret *et al.* 2001; Berry *et al.* 2006; September *et al.* 2007). The high *Aeromonas* concentration in reservoirs GD and WVE suggest that *Aeromonas* is also capable of growth in rainwater storage reservoirs and that *Aeromonas* bacteria potentially form biofilms. However, the much lower concentration in reservoir ECO and the

outcome of laboratory survival experiments indicate that conditions in reservoirs and composition of stored rainwater do not always support growth of *Aeromonas* to the same extent. Therefore, colonization of rainwater reservoirs by *Aeromonas* may be considered as a reservoir-specific problem.

Maintenance of the rainwater harvesting, storage and distribution system may play an important role in the microbiological quality of stored rainwater. Both Lévesque *et al.* (2008) and Simmons *et al.* (2008) noticed that only a limited number of users clean their reservoirs on a regular basis. Lévesque *et al.* (2008) found that reservoir water quality was significantly related to the frequency at which the reservoir was emptied and cleaned. Simmons *et al.* (2008) concluded from their study that conditions in rainwater reservoirs that do not undergo regular cleaning may support proliferation of *Legionella* in biofilms; aerosols containing *Legionella* discharged to air may seed roof-collected rainwater systems and thus cause cases of Legionnaires' disease. Schlech *et al.* (1985) reported an outbreak of Legionnaires' disease in the United States caused by a hotel roof-collected rainwater supply which was contaminated with *L. pneumophila*. With respect to the above, the observed presence of *Legionella* in rainwater used for toilet flushing in rainwater reservoirs in The Netherlands may pose a health risk when aerosols are formed during toilet flushing and particles with infectious *Legionella* are inhaled. Due to the observed hampering of the detection of *Legionella* in reservoir samples, the number of *Legionella* positive samples may even have been underestimated. Rapidly growing non-*Legionella* bacteria present in water samples are known to cause difficulties in *Legionella* detection and it has been observed that the addition of inhibitory agents to the culture medium or pre-treatment of the samples with acid or heat (procedures that are included in the Dutch standard NEN 6265 (Anonymous 2007*b*)) do not always sufficiently solve this source-specific problem (Roberts *et al.* 1987).

This study demonstrated that rainfall influenced the level of faecal contamination of the reservoir water with both the number of faecal indicator bacteria and the number of pathogenic microorganisms in the reservoirs increasing with high rainfall intensity, especially after a period of drought. The observed differences between the studied reservoirs suggest that roof material and roof slope may play a role in reservoir contamination, which has also been observed by Yaziz *et al.* (1989). Faecal material and dirt runs off steep roofs of smooth material (ECO and GD) more rapidly than off roofs with a gentle gradient and a vegetation layer on top (WVE). Retention of faecal material in vegetation delays run-off during rainfall and results in die-off of bacteria in the faeces during drought. Annual cleaning and repainting of roofs, as suggested by the Bermuda Department of Health, may reduce the amount of faecal material that is washed into reservoirs; however, in Bermuda these preventive measures were not related to *E. coli* contamination of the reservoir water (Lévesque *et al.* 2008). Evans *et al.* (2006) studied the effect of weather on the microbial composition of rainwater but focused on the influence of wind. They found a strong influence of both wind speed and wind direction on the heterotrophic plate count (HPC) and indicated that atmospheric disposition of microorganisms played an important role in contamination of roof-collected rainwater. A strong correlation between wind and HPC in Dutch rainwater reservoirs was not observed, but there was a strong correlation between wind speed and *C. perfringens* counts in samples from reservoirs GD ($r = 1.0$) and ECO ($r = 0.9$), which may suggest an increased deposition of *C. perfringens* (spores) at increased wind speed. HPC in Dutch reservoirs was higher in reservoirs with a higher water temperature and laboratory experiments showed that HPC increased with increasing water temperature and prolonged storage time, but was constant during storage at 15°C.

All reservoirs included in this study were well maintained and three of the four reservoirs were well isolated. Thus, outside temperature only slightly influenced water temperature in the reservoirs and had a minor effect on the microbiological quality of the stored rainwater. Laboratory experiments showed that die-off of both naturally occurring and seeded *Aeromonas* and *E. coli* was more rapid at 25 and 35°C as compared to 15°C. Also, die-off was more rapid in galvanized iron containers than in PVC containers, which may be due to dissolved toxic components from the galvanized iron container.

The microbiological risks of the use of roof-collected rainwater are largely unknown; however, disease outbreaks in developed countries attributed to consumption of

untreated rainwater have been reported (Lye 2002). Assessment of the health risk associated with the use of stored rainwater based on faecal indicator bacteria counts may not be suitable because of the large variety of microorganisms, including human pathogens, detected in rainwater collection systems. Faecal indicators provide information on the possible presence of pathogens; however, due to different properties, there may not be a quantitative relation between faecal indicators and pathogens, thus making faecal indicators not the appropriate indicators to quantify microbial risk (WHO 2006). When intended for drinking purposes, stored rainwater should be treated such that drinking water quality is obtained, the efficacy of treatment depending on the level of contamination. However, for other domestic purposes, such as toilet flushing, watering indoor plants or ornamental garden plants and cleaning floors, proper preventive and maintenance procedures may guard the microbiological quality and safe use of stored rainwater. To assess the efficacy of cleaning and flushing regime, evaluation of measures e.g. in field intervention studies is required. In the developing world, collected rainwater may be the only available drinking water source or may be a safer alternative to the use of heavily contaminated surface water or groundwater sources (Garrett *et al.* 2008). It has been demonstrated that point-of-use water quality interventions, including rainwater harvesting, reduce the risk of diarrhoea in rural communities (Clasen *et al.* 2006; Garrett *et al.* 2008). For safe collection, storage and use of roof-collected rainwater preventive and maintenance measures may largely be the same as in developed countries, but in this part of the world open reservoirs or ponds may, however, serve as breeding pools for insects that are vectors of diseases such as malaria (Kassahun Waktola 2008) and dengue (Mariappan *et al.* 2008). Therefore specific attention should be paid to insect-proof coverage of rainwater reservoirs.

CONCLUSIONS AND RECOMMENDATIONS

The faecal contamination of roof-collected rainwater stored in reservoirs in The Netherlands and the occasional presence of potential human pathogens indicates that the water is unsuitable for direct consumption without further treatment. Since there is a potential for biofilm formation in the reservoirs, public health problems may arise from protection and support of pathogenic microorganisms in these biofilms, bacterial regrowth and depletion of disinfection agents, when used.

The microbiological quality of rainwater stored in Dutch reservoirs largely depends on the amount of faecal material that is washed into the reservoirs during rainfall events. Laboratory experiments have shown that water quality is also influenced by storage temperature, the material of storage reservoirs and water type which in its turn may be influenced by the material of the harvesting roofs.

To reduce possible health risks associated with use of stored rainwater appropriate efforts are needed. Measures to ensure safe collection of rainwater may consist of preventing animals from having direct access to the reservoirs and regular cleaning of collection means such as roofs and roof-gutters to prevent washing in of animal faecal droppings deposited on roofs. Safe storage is done in well-isolated reservoirs to control temperature of the collected water and storage reservoirs should be cleaned or disinfected on a regular basis to prevent biofilm formation and growth of bacteria such as *Aeromonas* and *Legionella*. Care should also be taken to ensure hygienic transport and tapping.

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