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MICROBIAL RISKS FROM RAINWATER TANKS IN SOUTH EAST QUEENSLAND

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Abstract

Quantitative Microbial Risk Assessment (QMRA) analysis was used to quantify the risk of infection associated with the exposure to pathogens from potable and non-potable uses of roof-harvested rainwater in South East Queensland (SEQ). A total of 84 rainwater samples were analysed for the presence of faecal indicators (using culture based methods) and zoonotic bacterial and protozoan pathogens using binary and quantitative PCR (qPCR). The concentrations of *Salmonella invA*, and *Giardia lamblia* β–giradin genes ranged from 65-380 genomic units/1000 mL and 9-57 genomic units/1000 mL of water, respectively. After converting gene copies to cell/cyst number, the risk of infection from *G. lamblia* and *Salmonella* spp. associated with the use of rainwater for bi-weekly garden hosing was calculated to be below the threshold value of 1 extra infection per 10,000 persons per year. However, the estimated risk of infection from drinking the rainwater daily was 44-250 (for *G. lamblia)* and 85-520 (for *Salmonella* spp.) infections per 10,000 persons per year. Since this health risk seems higher than that expected from the reported incidences of gastroenteritis, the assumptions used to estimate these infection risks are critically discussed. Nevertheless, it would seem prudent to disinfect rainwater for potable use.

Introduction

Roof-harvested rainwater has received significant attention as a potential alternative source of potable water supply in water-scarce regions. To encourage the use of roof-harvested rainwater, government bodies of many countries such as Australia, Denmark, Germany, India and New Zealand are providing rebates to residents who use rainwater for domestic purposes. The use of rainwater is quite common in Australia, particularly in rural and remote areas, where reticulated mains or town water are not available. Recent water scarcity in several capital cities prompted the use of rainwater as an alternative source. For instance, the Queensland State Government initiated the 'Home Water Wise Rebate Scheme' that provides rebates to South East Queensland (SEQ) residents who use rainwater for non-potable domestic purposes (Spiller 2008). Over 260,000 tanks were granted rebates up to December 2008, when the scheme was concluded.

It would seem prudent to disinfect rainwater for potable use.

There is a general community sense that roof-harvested rainwater is safe to drink, and this is partially supported by limited epidemiological evidence (Heyworth *et al.* 2006). Some studies have reported that roof-harvested rainwater quality is generally acceptable for potable use (Dillaha and Zolan 1985). In contrast, the presence of potential zoonotic pathogens in rainwater samples has been reported (Lye 2002; Simmons *et al.* 2001; Ahmed *et al.* 2008). Such organisms can cause gastrointestinal illness in humans, with nausea, vomiting and/or diarrhoea occurring within 6 to 48 hours (e.g. *Salmonella* Typhimurium) to 9-15 days (*Giardia lamblia*) after ingestion of contaminated water.

Direct routine monitoring of microbiological quality of source water for all possible pathogens is neither economically, technologically nor practically feasible. Consequently, traditional faecal indicators, such as faecal coliforms, *Escherichia coli* and enterococci have long been used as surrogates to determine the presence of pathogens. Most studies assess the quality of roof-harvested rainwater based on the concentration of these faecal indicators (Dillaha and Zolan 1985; Sazakil *et al.* 2007). However, the major limitation in using faecal bacteria as indicators is their poor correlation with the presence of pathogenic microorganisms (Hörman *et al.* 2004).

An alternative is the measurement of pathogens using traditional cultural methods. However, there are several limitations of traditional culture based methods and include the underestimation of the bacterial concentration due to the presence of injured or stressed cells (Delgado-Viscogliosi *et al.* 2005) whilst certain microorganisms in environmental waters can be viable but not cultivable (Oliver 2000). Culture based methods are also generally laborious and costly. Recent advances in molecular techniques such as Polymerase Chain Reaction (PCR) technology enable rapid, specific and sensitive detection of many pathogens. Advances in PCR methodology also enable the quantification of potential pathogens in source waters that are otherwise difficult and/or laborious to culture using traditional microbiological methods. In view of this, we used binary PCR (presence/absence) and qPCR (quantitative) based assays to respectively detect and quantify potential zoonotic pathogens in samples from roof-harvested rainwater in SEQ domestic dwellings.

The aims of the research study were three-fold: (1) to compare the water quality in rainwater tanks using traditional faecal indicators *E. coli* and enterococci with the presence of bacterial and protozoan pathogens such as *Aeromonas hydrophila*, *Campylobacter coli*, *Campylobacter jejuni*, enterohaemorrhagic *E. coli*, *Legionella pneumophila*, *Salmonella* spp., *Giardia lamblia* and *Cryptosporidium parvum* measured using binary PCR based methods and (2) to quantify the concentration of selected pathogens using qPCR based methods and (3) to apply Quantitative Microbial Risk Assessment (QMRA) analysis to pathogen concentrations in order to quantify the risk of infection from potable and non-potable uses of roof-harvested rainwater. The uniqueness of this study stems from the fact that instead of measuring faecal indicators, the pathogens that are

capable of causing illness were measured and combined this information with QMRA to assess human health risk.

Materials and methods

Sources of samples. A total of 84 tank water samples were collected from 66 residential houses in Brisbane and Gold Coast regions in South East Queensland in 2008. Water samples were collected in sterilized 10 L containers from the outlet taps located close to the base of the tanks. Before sampling, the tap was allowed to run for 30-60 s to flush out water from the tap. Samples were transported to the laboratory on ice, and processed within 8-10 h.

Enumeration of *E. coli* and enterococci. The membrane filtration method was used to process the water samples for *E. coli* and enterococci enumeration. Sample serial dilutions were made, and filtered through 0.45-µm pore size nitrocellulose membranes (Advantec, Tokyo, Japan), and placed on modified mTEC agar (Difco, Detroit, MI, USA) and mEI agar (Difco) for the isolation of *E. coli* and enterococci, respectively.

DNA extraction from rainwater samples. For binary PCR and qPCR analysis, 1-2.5 L water sample from each tank was filtered through 0.45-µm pore size membrane (Advantec). DNA was extracted directly on the membrane using DNeasy blood and tissue kit (Qiagen, Valencia, CA), and stored at -80°C until further analysis.

Selected pathogens and target genes for binary PCR and qPCR analysis. *A. hydrophila lip, C. coli ceuE, C. jejuni mapA*, enterohaemorrhagic *E. coli* VT1, VT2 and O157 LPS, *L. pneumophila mip, Salmonella invA* and *spvC*, *G. lamblia* β-giradin and *C. parvum* COWP genes were selected for binary PCR analysis. Of these, *C. jejuni mapA, Salmonella invA*, and *G. lamblia* β-giradin genes, were selected for qPCR analysis.

Primers and qPCR standard curves for PCR analysis. Previously published primers were used for this study and the primer sequence have been described elsewhere (Ahmed *et al.* 2008). For qPCR assays, the standards were prepared from the genomic/plasmid DNA of selected pathogens. A tenfold serial dilution was prepared from the calculated genomic and plasmid gene copies, ranging from 10^6 to 10^1 gene copies/µL of DNA. For each standard, the concentration was plotted against the cycle number at which the fluorescence signal increased above the threshold value (C_T value). PCR amplification was performed in 25-µL reaction mixtures (described in Ahmed *et al.* 2008) using Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA). To separate the specific product from non-specific products, DNA melting curve analysis was performed for each PCR experiment.

PCR limit of detection. To determine the PCR limit of detection, known gene copies of the pathogens (5 x 10^3 to 5 x 10^0 gene copies) were tested with the PCR. The lowest concentration of gene copies that were detected consistently in replicate assays was considered as the PCR limit of detection. The test showed that the PCR limit of detection was as low as 5 gene copies for bacterial pathogens. For *G. lamblia* β-giradin gene, and *C. parvum* COWP gene, the detection limit was 7 gene copies. Lower levels (i.e., 1 copy) were tested for each target, but the results were not reproducible.

PCR inhibitors. An experiment was conducted to determine the potential presence of PCR inhibitory substances in rainwater samples collected from 3 different tanks. Each sample (i.e. 1 L) was concentrated using the membrane filtration technique as described above. DNA was extracted using DNeasy blood and tissue kit (Qiagen), and tested with the PCR. DNA was also extracted from ultra pure DNAse and RNase free sterile distilled water (Invitrogen) in the same manner for comparison with the tank water. All samples were spiked with 10^3 gene copies of *S.* Typhimurium DNA. The C_T values obtained for the DNA samples from spiked tank water were then compared to the DNA samples from distilled water. No significant differences were observed between the C_T values for spiked distilled water, undiluted DNA, and serially diluted rainwater thus indicating the tested rainwater samples were free of PCR inhibitor.

Recovery efficiency of the qPCR assays. The recovery efficiency was determined by spiking distilled water (n=3) and tank water samples (n=3) with known concentration of *S.* Typhimurium cells. Initially, samples were collected from several rainwater tanks and were tested for the presence of *Salmonella* spp. using binary PCR. Water samples that were PCR negative for *Salmonella* spp. were selected for this experiment. The samples were autoclaved to destroy background microbial flora. The *S.* Typhimurium strain was grown overnight in LB broth and cell concentrations were determined using membrane filtration method. Ten-fold serial dilutions were made and spiked into 250 mL of deionised and rainwater samples. The samples were filtered through membranes, and DNA extraction was performed according to the method described above. Samples were tested in triplicate for each concentration, and the recovery efficiency (%) was calculated using the following equation: Recovery Efficiency (%) = (No. of cells after filtration/No. of cells before filtration) x 100.

The recovery efficiency in autoclaved distilled water samples ranged from 68% to 93% with the greatest variability occurring at lower cell counts. The mean recovery efficiency was 80% ± 10%. The recovery efficiency in autoclaved *rainwater* samples ranged from 58% to 91% with the greatest variability occurring at lower cell counts. The mean recovery efficiency was 75% ± 12%. All results presented in this paper were corrected according to their relevant recovery ratios.

Quantitative Microbial Risk Assessment (QMRA). QMRA, as described by Gerba *et al.* (1996), is a four-step process for assessing the human health risk from exposure to specified pathogens. The first step, Hazard Assessment, identifies the pathogen present in the rainwater which we achieved using binary-PCR analysis. Step 2 is Exposure Assessment where the pathogens present in the environment and the amount of pathogen ingested by a person active in the environment are quantified.

The number of infective units ingested by a person active in the environment was calculated as:

Ingestion Dose (no. of infective units) = C x V ……. (Eqn. 1)

Where

C = concentration of infective units (number per mL of roof-harvested rainwater). Although qPCR analysis quantified the pathogens detected in the roof-harvested rainwater, it was necessary to first convert the genomic units of the pathogen genes to cell numbers. Assumptions were then made concerning the proportion of the cells that were viable and infective since PCR cannot distinguish between viable and nonviable cells and does not provide information on the infectivity of the target pathogenic microorganisms.

V = volume of rainwater ingested (mL). Two possible scenarios were considered, (1) ingestion occurred deliberately due to

drinking of rainwater daily, and (2) accidental ingestion of rainwater occurs whilst watering the garden with a hose twice a week. Volumes were assumed to be 1000 mL per day for drinking (US EPA 2004), and 1 mL per event for hosing (Tanaka *et al*. 1998 assumed 1 mL ingested for golf-course irrigation exposure whilst Olivieri and Seto (2007) suggested that water intake due to park irrigation could amount to 0.01 to 1% of the daily total water intake, with a median ingestion value of 6 mL).

The third step of QMRA involves establishing the dose response model to use to describe the relationship between ingestion dose and level of infection that results from a single exposure. The dose response relationships used for this study were obtained from the literature. The fourth and final step was to combine the exposure data and dose-response relationship to estimate the risk probability (expressed as likely numbers of infections per 10,000 persons per year) for the urban SEQ community, and compare this with the arbitrary but commonly accepted risk level of one extra infection per 10,000 persons per year (US EPA 1992). To convert the risk probability per single exposure to the risk probability per year, the following equation was used.

No. infections per 10,000 persons per year $= 1 - (1 - P_i)^E$ …….. (Eqn. 2)

where

E = the number of exposure events per year.

P_i = the infection risk from a single exposure.

For this final step, it was assumed that the pathogen distribution indicated by the sampled roof-harvested rainwater tanks was representative of the tanks in urban SEQ. However, it was necessary to know how many people within the urban SEQ community would be exposed to rainwater through drinking or hosing.

Market survey data from Gardiner (2009) was used to establish the number of households in Brisbane that have a rainwater tank and use it for potable purposes. Out of all the households in urban SEQ (807,555), the survey estimated that 208,100 had tanks retrofitted to existing dwellings and 5,876 were new dwellings with mandated tanks with internal connections. Within each of these groups, 22% and 19% respectively used the rainwater for cooking/drinking purposes frequently. This suggests that almost 30% of urban SEQ households possessed a rainwater tank, and that 6.3% of urban SEQ households use the rainwater for potable purposes and therefore could be at risk of exposure to the each pathogen identified in the tank water samples.

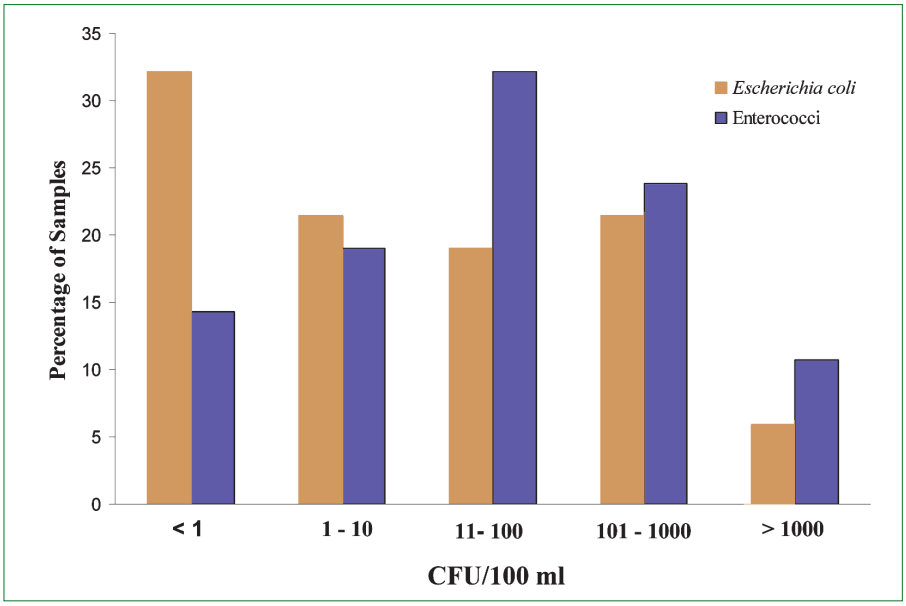

Figure 1. Concentrations of faecal indicators in water samples collected from roof-harvested rainwater tanks.

Table 1. PCR positive results for potential pathogens.

Target pathogens	PCR positive results/ Number of samples tested	% of samples positive
Aeromonas hydrophila lip gene	7/84	8
Campylobacter coli ceuE gene	10/27	37
Campylobacter jejuni mapA gene	1/84	1
Escherichia coli O157 LPS gene	0/84	0
Escherichia coli VT1 gene	0/84	0
Escherichia coli VT2 gene	0/84	0
Legionella pneumophila mip gene	8/84	10
Salmonella invA gene	17/84	20
Salmonella spvC gene	0/27	0
Giardia lamblia β-giradin gene	15/84	18
Giardia parvum COWP gene	0/84	0

Results and Discussion

Roof-harvested rainwater quality determined by faecal indicators using traditional culture based methods, and direct pathogen measurement using binary PCR. Of the 84 roof-harvested rainwater samples tested for faecal indicators, 57 (65%) and 72 (82%) were found to be positive for *E. coli* and enterococci, respectively. The concentrations of *E. coli* and enterococci in these positive samples are shown in Figure 1. In the 84 samples tested, 56 (64%) samples exceeded the Australia and New Zealand Environment and Conservation Council (ANZECC) recreational water quality guidelines for fresh and marine waters of 35 enterococci/100 mL for primary contact (ANZECC 2000). In the 84 samples tested, 54 (61%) were positive for both indicators, and 75 (89%) were positive for at least one indicator.

Using binary PCR, *C. coli* were the most prevalent among all the bacterial pathogens tested in this study (37% of samples, Table1). However, only one sample was positive for *C. jejuni mapA* gene. The *C. coli* and *C. jejuni* detected could potentially have originated from bird faeces (Kapperud and Rosef 1983), although other potential sources such as possums or lizards cannot be ruled out. The presence of *Campylobacter* spp. in roof-harvested rainwater samples has also been reported in New Zealand (Savill *et al.* 2001) and "campylobacteriosis" was found to be epidemiologically associated with the consumption of roof-harvested rainwater (Eberhart-Phillips *et*

al. 1987). *A. hydrophila lip* gene, *L. pneumophila mip* gene, and *Salmonella invA* gene were also detected in rainwater samples from a number of tanks. *A. hydrophila* has been reported as one of the most common *Aeromonas* spp. associated with human intestinal disease (Kühn *et al.* 1997). The *Salmonella invA* genes enable the bacteria to invade human cells and are reported to be present in all 2,000 *Salmonella* serovars. *Aeromonas* spp., *Legionella* spp., and *Salmonella* spp. have previously been detected using culture-based methods in the United States and New Zealand and in the tropics in roof-collected rainwater cisterns and/or from tanks (Broadhead *et al.* 1998; Simmons *et al.* 2001).

In the 84 samples we tested, 15 (18%) were positive for the *G. lamblia* β-giardin gene. *Giardia* could be derived from bird faecal matters deposited on the roof. To our knowledge, this is the first study in Australia that reports the presence of *G. lamblia* in water samples from domestic rainwater tanks. Although *L. pneumophila* was found in 10% of the rainwater samples, the results are neither presented nor discussed further in this paper because of the need to quantify aerosol size distribution that can lodge in the human lung which is a necessary precursor to undertake QMRA of this respiratory pathogen. These issues are discussed elsewhere (Ahmed *et al.* 2009). Importantly, none of the samples tested were positive for enterohaemorrhagic *E. coli* O157 LPS, VT1, VT2, *Salmonella spvC* or *C. parvum* COWP genes.

Binary logistic regressions were also performed to identify the correlations between the concentrations of faecal indicator bacteria and the presence/absence of potential pathogens. The presence/absence of the potential pathogens did not correlate with any of the indicator bacteria concentrations. The poor correlation between faecal indicators and pathogens, especially viral and protozoans, has been reported previously (Hörman *et al.* 2004). Of the 84 samples tested, 52% were found to be positive for at least one pathogen, compared with 89% of samples positive for at least one indicator and hence unsuitable for primary contact. However, such values do not indicate the magnitude of the infection risk.

Table 2. Concentrations of pathogens in tank water sample.

Target pathogens	Range of genomic units/1000 mL of tank water sample	Range of cells/ 1000 mL of tank water sample	Range of viable and infective cells*/1000 mL of tank water sample
Salmonella spp.	65 – 380	65 – 380	16 – 95
G. lamblia	9 – 57	0.6 – 3.6 cysts	0.1 – 0.9 cysts

** Assumes 25% of the cells were both viable and infective*

Concentrations of *C. jejuni mapA*, *Salmonella invA* and *G. lamblia* β-giardin genes using qPCR. *C. jejuni mapA* gene, though detected in one sample by binary PCR analysis, could not be quantified due to its concentration being below the qPCR detection limit. However, *Salmonella invA*, and *G. lamblia* β-giradin gene were detected in 11 and 13% of samples, respectively, and their concentrations ranged from 65-380 genomic units/1000 mL and 9-57 genomic units/1000 mL of water respectively (Table 2). *Salmonella invA* are single copy genes such that 1 gene copy = 1 cell. *G. lamblia* β-giradin gene copies were converted to cysts number assuming that 16 gene copies = 1 cyst (Guy *et al*. 2003).

Only a proportion of the cells/cysts may be both viable and infectious. It has been suggested from a cell culture-PCR study that the percentage of *Cryptosporidium* spp. that were both viable and infective may be 37% (LeChevallier *et al.* 2003). In the absence of similar published information for *Salmonella* and *G. lamblia*, it was conservatively assumed that at least 25% of the cells were both viable and infective. Hence, the concentration of viable and infective *Salmonella* spp. cells and *Giardia lamblia* cysts in the rainwater were estimated to be 16-95 infective units per 1000 mL, and 0.1-0.9 infective units per 1000 mL respectively (Table 2).

Determining the human health risk from potable and non-potable uses of roof-harvested rainwater. Estimates of the ingestion dose of each pathogen by people exposed according to the two scenarios are shown in Table 3. For drinking, 16 - 95 *Salmonella* cells and 0.14 - 0.9 *G. lamblia* cysts may be ingested, whilst for garden hosing 0.02 - 0.1 *Salmonella* cells and 0.0001 - 0.0009 *G. lamblia* cysts may be ingested.

Dose response relationships used for non-typhoid *Salmonella* spp. and *G. lamblia* are shown in Figure 1. Infection risk per 10,000 exposed persons indicated by these relationships ranged from low (0.02-0.18 for garden hosing) to high (18-176 for drinking) for each event

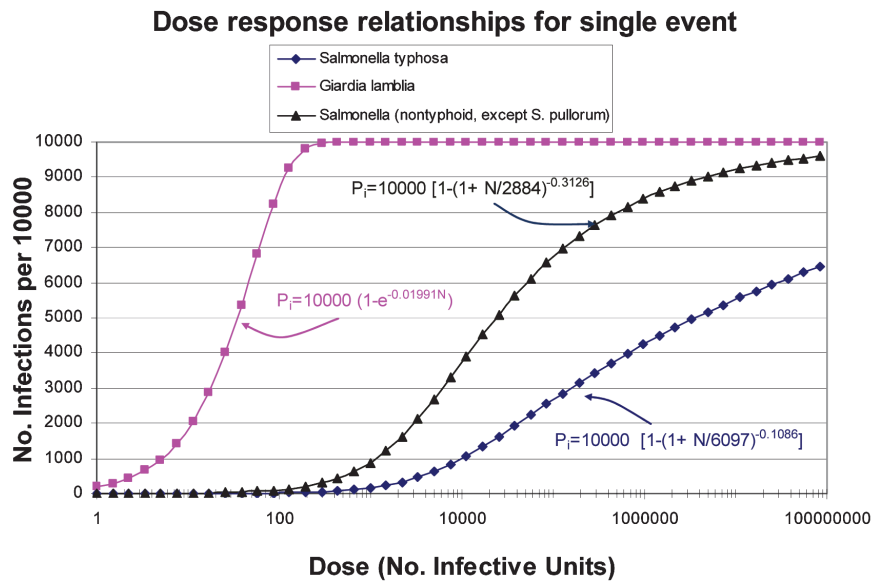

Figure 2. An exponential dose response relationship was used for *Giardia lamblia* (Rose et al. 1991) and a beta Poisson dose response relationship for nontyphoid *Salmonella* was used for *Salmonella* Typhimurium) (Haas 1999). The less infectious relationship for *Salmonella typhosa* (causing typhoid disease) is shown for comparison. All dose response relationships relate N, the number of infective units ingested to Pi, the expected infections per 10,000 people.

(Table 4). The fraction of the urban SEQ population that was potentially exposed to each pathogen was then calculated to be at least 3.2-3.9% from garden hosing, and fewer (0.68-0.83%) from drinking rainwater (Table 4), assuming that the proportion of tank samples containing these pathogens (18% for *G. lamblia* and 20% for *Salmonella* spp.) are representative of the tanks in urban SEQ.

By multiplying these infection risks with the fraction of the population that was exposed to each pathogen, the infection risk from *Salmonella* spp. or *G. lamblia* per 10,000 urban SEQ persons for each event was found to range from 0.0005 to 0.007 infections from garden hosing and 0.12 to 1.5 infections from drinking (Table 4). Using Equation 2, the risk of infection per 10,000 people per year was calculated to range from 0.06 to 0.72 for garden hosing, but from 44 – 520 for drinking (Table 4). The exposure risk to *Salmonella* spp. and *G. lamblia* from drinking far exceeds the threshold value of 1 extra infection per 10,000 persons per year and indicates that if undisinfected rainwater were ingested by drinking, then the gastrointestinal diseases of Salmonellosis and Giardiasis is expected to be high with infection incidence ranging from 44-250 (for salmonellosis), and 85-520 (for giardiasis) cases per 10,000 people per year.

These predictions were not supported by the incidence of these diseases reported in the Notifiable Diseases Surveillance System Database (http://www9.health.gov.au/cda/Source/CDA-index.cfm) where cases of Salmonellosis is reported at an incidence of 5.7 cases/10,000 in Queensland. A number of explanations are possible including the naturally high incidence of gastroenteritis in the community e.g. 8,000 cases per 10,000 people per year (Hellard *et al.* 2001) masking the actual diseases; the fact that Giardiasis is not a notifiable disease in Queensland; the fact that not every individual will seek medical attention if the illness is mild in nature and lasts for few days; the fact that a number of households will use UV disinfection or boil the water before potable use; the possibility of individuals acquiring immunity to certain pathogens due to frequent exposure; a possibly much lower ratio of viable organisms to gene copies that was assumed in the analysis; a possibly lower percentage (than the assumed 25%) of measurable pathogens which are both viable and infective; or more likely, that pathogens do not occur at concentrations reported in Table 3 for 365 days of the year, as was assumed in the risk model calculations. This latter hypothesis is being pursued by a longitudinal study using fortnightly sampling over three months.

Table 3. Exposure and calculation of possible dose for individuals exposed to contaminated tank water.

Risk scenario	Pathogens exposure	Volume per event (mL)	Range of Dose (infective units per event)	No of events per year
Ingestion via drinking	*Salmonella* spp.	1000	16 – 95	365
	G. lamblia		0.14 – 0.9	
Ingestion via hosing	*Salmonella* spp.	1	0.02 – 0.1	104
	G. lamblia		0.0001 – 0.0009	

Conclusions

This study adds to the growing body of evidence that traditional faecal indicators *E. coli* and enterococci are not satisfactory surrogates for the presence of human enteric pathogens, showing no significant correlations with any of the pathogens measured.

The use of qPCR or similar techniques which permit direct quantification of pathogens and QMRA to interpret the pathogen concentrations in terms of infection risks will greatly enhance both the understanding and control of pathogen risks in potable water supplies such as roof-harvested rainwater tanks. Of all the 84 samples of roof-harvested rainwater collected in SEQ, 37% containing *C. coli*, 20% with *Salmonella* (non-typhoid), 18% with *G. lamblia*, 10% with *L. pneumophila*, 8% with *A. hydrophila* and 1% with *C. jejuni*. No *C. parvum* nor enterohaemorrhagic *E. coli* were found. The QMRA analysis indicated that **potable** use could present a significant health risk due to infection from *G. lamblia* and *Salmonella* spp.

However, the overall health risk indicated by the qPCR analysis (44-250 infections per 10,000 persons per year by *Salmonella* spp. and 85-520 infections per 10,000 persons per year by *G. lamblia* seems higher than expected from reported incidences of gastroenteritis. This suggests that further work is needed to improve the assumptions made in the analysis. One critical assumption is the proportion of gene copies that represent both viable and infective organisms since qPCR does not provide information regarding viability or infectivity. To overcome this limitation, qPCR could be integrated with cell culture to obtain information regarding the viable and infective proportion of the target pathogen, and this will be pursued in further work. The assumption concerning the occurrence of pathogens in rainwater throughout the whole year is being tested in a longitudinal study.

Until these issues concerning the assumptions are resolved, it would seem prudent to disinfect rainwater for potable use. This could involve filtration using under-sink units, ultra violet disinfection units or more simply, boiling the water.

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Table 4. The infection risk for individuals exposed to contaminated tank water for two risk scenarios.

Risk Scenario	Pathogens	Infection per 10,000 people with rainwater tanks from single event	% of population exposed to pathogens	Infection risk per event per 10,000 people	No. of events/yr	Infection risk per year (No. per 10,000 persons)
Ingestion via drinking	*Salmonella* spp.	18 – 101	0.68	0.12 – 0.69	365	44 - 250
	G. lamblia	28 – 176	0.83	0.23 – 1.50	365	85 - 520
Ingestion via hosing	*Salmonella* spp.	0.02 – 0.10	3.2	0.0005 – 0.0033	104	0.06 – 0.34
	G. lamblia	0.03 – 0.18	3.9	0.001 – 0.007	104	0.11 – 0.72

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References

Ahmed,W., Vieritz, A., Goonetilleke, A. & Gardner, T. 2009, 'The risk of infection associated with the use of roof-harvested rainwater in Southeast Queensland, Australia', (in preparation).

Ahmed, W., Huygens, F., Goonetilleke, A. & Gardner, T. 2008, 'Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia', *Applied and Environmental Microbiology*, 74, pp. 5490-6.

Australian and New Zealand Environment and Conservations Council with the Agriculture and Resource Management Council of Australia and New Zealand. 2000. The Australian and New Zealand guidelines for fresh and marine water quality. National Water Quality Management Strategy. ANZECC and ARMCANZ, Canberra, Australia.

Broadhead, A.N., Negron-Alvira, A., Baez, L.A., Hazen, T.C. & Canoy, M.J. 1998, 'Occurrence of *Legionella* species in tropical rain water cisterns', *Caribbean Journal of Science*, 24, pp. 71–3.

Delgado-Viscogliosi, P., Simonart, T., Parent, V., Marchand, G., Pierlot, P. E. et al. 2005, 'Rapid method for enumeration of viable *Legionella pneumophila* and other *Legionella* spp. in water', *Applied and Environmental Microbiology*, 71, pp. 4086–96.

Dillaha, T.A. & Zolan, W.J. 1985, 'Rainwater catchment water quality in Micronesia', *Water Research*, 19, pp. 741–6.

Gardiner, A. (2009) Domestic Rainwater Tanks: Usage and Maintenance Patterns in South East Queensland. *AWA Water Journal* 36:1, 151-156.

Gerba C. P., Rose J. B., and Haas C. N. 1996, Quantitative microbial risk assessment for reclaimed wastewater. *Water Tech Sydney AWWA*, pp. 254-60.

Guy, R.A., Payment, P., Krull, U.J. & Horgen, P.A. 2003, 'Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage. *Applied and Environmental Microbiology*, 69, pp. 5178-85.

Haas, C.N., Rose, J.B., & Gerba, C.P. 1999, 'Quantitative Microbial Risk Assessment', John Wiley & Sons, Inc (Pub). New York.

Hellard, M.E., Sinclair, M.I., Forbes, A.B. & Fairley, C. K. 2001, 'A randomised, blinded, controlled trial investigating the gastrointestinal health effects of drinking water quality', *Environmental Health Perspectives*, 109, pp. 773-7.

Heyworth, J.S., Glonek, G., Maynard, E.J., Baghurst, P.A. & Finlay Jones, J. 2006, 'Consumption of untreated tank rainwater and gastroenteritis among young children in South Australia', *International Journal of Epidemiology*, 35, pp. 1051-8.

Hörman, A., Rimhannen-Finne, R., Maunula, L., von Bonsdorff, C.-H., Torvela, N., Heikinheimo A., & Ha¨nninen, M.-L. 2004, '*Campylobacter* spp., *Giardia* spp., *Cryptosporidum* spp., noroviruses, and indicator organisms in surface water in southwestern Finland, 2000–2001', *Applied and Environmental Microbiology*, 70, pp. 87–95.

Kapperud, G. & Rosef, O. 1983, 'Avian wildlife reservoir of *Campylobacter fetus* sub spp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway', *Applied and Environmental Microbiology*, 45, pp. 375–80.

Kühn, I., Albert, M.J., Ansaruzzaman, M., Bhuiyan, N.A., Alabi, S.A., Sirajul Islam, M., Neogi, P. K.B., Huys, G., Janssen, P., Kersters, K., & Mollby. R. 1997, 'Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls, and from surface water in Bangladesh', *Journal of Clinical Microbiology*, 35, pp. 369–373.

LeChevallier, M.W., Di Giovanni, G.D., Clancy, J.L., Bukhari, Z., Bukhari, S., Rosen, J.S., Sobrinho, J. & Frey, M.M. 2003, 'Comparison of method 1623 and cell culture-PCR for detection of *Cryptosporidium* spp. in source waters, '*Applied and Environmental Microbiology*, 69, pp. 971-9.

Lye, D. J. 2002, 'Health risks associated with consumption of untreated water from household roof catchment system', *Journal of American Water Research Association*, 38, pp. 1301–6.

Oliver, J. D. 2000, 'The public health significance of viable but nonculturable bacteria', p. 277–300. *In* R. R. Colwell and D. J. Grimes (ed.), Nonculturable microorganisms in the environment. ASM Press, Washington, DC.

Olivieri A.W. & Seto, E. 2007, 'Application of microbial risk assessment techniques to estimate risk due to exposure to reclaimed waters', Watereuse Foundation. Alexandria VA, 84pp.

Rose JB; Haas CN, and Regli S. 1991, 'Risk Assessment and Control of Waterborne Giardiasis', *American Journal of Public Health*, 81, pp. 709-13.

Savill, M.G., Hudson, J.A., Ball, A., Klena, J.D., Scholes, P., White, R.J. McCormack, R.E., & Jankovic. D. 2001, 'Enumeration of *Campylobacter* in New Zealand recreational and drinking waters', *Journal of Applied Microbiology,* 91, pp.38–46.

Sazakli, E., Alexopoulos, A. & Leotsinidis, M. 2007, 'Rainwater harvesting, quality assessment and utilization in Kefalonia Island, Greece', *Water Research,* 41, pp.2039–47.

Simmons, G., Hope, V., Lewis, G., Whitmore, J. & Wanzhen, G. 2001, 'Contamination of potable roof-collected rainwater in Auckland, New Zealand', *Water Research*, 35, pp. 1518–24.

Spiller D. 2008, 'Policy forum: Urban water pricing and supply. Water for today, Water for tomorrow: Establishment and operation of the SEQ Water Grid. *The Australian Economic Review,* 41, pp. 420-7.

Tanaka, H., Asano, T., Schroeder, E.D., & Tchobanoglous, G. 1998, 'Estimating the safety of wastewater reclamation and reuse using enteric virus monitoring data', *Water Environment Research*, 28, pp. 39-51.

USEPA. 2004, 'Estimated per capita water ingestion and body weight in the United States – An update. Based on data collected by the United States Department of Agriculture's 1994-1996 and 1998 continuing survey of food intakes by individuals'. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. 20460. EPA-822-R-00-001. October 2004. http://www.epa.gov/waterscience/criteria/drinking/percapita/2004.pdf. Accessed Sept 2009.

Editor's Note

The issue of relative safety of potable use of roof-harvested rainwater has been debated for some years. A recent epidemiological study by the WQRA (Karin Leder) suggests that there was little benefit in filtering such water.

The issue is perhaps clouded (pun not intended) by the re-suspension of sediment by recent rainfall events, as thoroughly discussed by Magyar (2008), in relation to risks from lead and other metals. During the recent water efficiency conference, a delegate from India, S. Vishwanath, spoke to me. He is head of an institute to encourage use of roof water, primarily in Bangladesh (in preference to the arsenic-laden borewater), but also throughout India. He quoted that microbial analysis

immediately after rain showed high readings, but two days of settlement reduced numbers to ‘safe’ levels. None the less, the web-site www.rainwaterclub.org, recommends some form of disinfection. Ahmed’s word ‘prudent’ seems appropriate.