Quantitative detection of pathogens in roof-harvested rainwater

*W Ahmed** [1, 2],
A Goonetilleke [2] &
T Gardner [1, 2]

1. Department of Natural Resources and Water, 80 Meiers Road, Indooroopilly QLD 4068
2. School of Urban Development Queensland University of Technology
GPO Box 2434, Brisbane QLD 4001
* Corresponding author
Tel (07) 3896 9522
Fax (07) 3896 9623
E-mail shuhat@yahoo.com

Roof-harvested rainwater is an alternative water source. Though generally considered acceptable for potable use, the presence of pathogens has been reported in research literature [1]. Various zoonotic pathogens are present in faeces of animals that have access to the roof and, following rain events, pathogens may be transported to rainwater tanks via roof runoff. The microbiological quality of water is traditionally assessed by enumerating faecal indicators such as *Escherichia coli* and enterococci [2]. Significant limitations in using faecal indicators include their poor correlation with pathogens and faecal indicator concentrations cannot be used to assess public health risk when compared to the direct monitoring of pathogens [3]. Polymerase chain reaction (PCR)-based techniques enable rapid and direct detection/quantification of pathogens in water that are otherwise laborious to culture using traditional microbiological methods.

In this study, the microbiological quality of roof-harvested rainwater was assessed by enumerating faecal indicators and detecting zoonotic pathogens in samples from rainwater tanks. The significance of this study stems from the fact that, instead of measuring faecal indicators, pathogens that are capable of causing illness were directly measured using quantitative PCR (qPCR) methods. The pathogen concentration data will be used to perform quantitative microbial risk assessment (QMRA). This work forms part of the development of a 'toolbox' of methodologies using qPCR-based methods which can be used to detect and quantify more than 35 microorganisms commonly found in water [more information on the qPCR 'toolbox' can be obtained from the corresponding author].

A total of 84 rainwater samples were collected from 66 residential houses in Brisbane and Gold Coast regions. Membrane filtration method was used for *E. coli,* and enterococci enumeration. For PCR/qPCR analysis, *Aeromonas hydrophila lip* gene, *Campylobacter jejuni mapA* gene, *Campylobacter. Coli ceuE gene, E. coli* O157 LPS, VT1, VT2 genes, *L. pneumophila mip* gene, *Salmonella invA and spvC* genes, *G. lamblia* β-giradin gene and *Cryptosporidium parvum* Cryptosporidium oocyst wall protein (COWP) gene were selected. Most of these genes were selected based on their virulent properties. In addition, priority was given to those genes which are single copy genes (where possible) so that gene copy numbers could be directly converted to cell counts. DNA extraction from rainwater samples, PCR amplification, the standards for qPCR and the primers used for this study are described elsewhere [4]. For each target pathogen, PCR reproducibility, limit of detection, detection efficiency and PCR inhibitory effects were evaluated.

For the samples tested, 57 (65%) were positive for *E. coli.* The concentrations were: 18 (20%) between 1-10 CFU/100ml, 16 (18%) between 11-100 CFU/100ml, 17 (19%) between 101- 1000 CFU/100ml, and 6 (7%) had >1001 CFU/100ml. For the 84 samples, 72 (82%) were positive for enterococci. The concentrations were: 16 (18%) between 1-10 CFU/100ml, 27 (31%) between 11-100 CFU/100ml, 20 (23%) between 101-1000 CFU/100ml, and 9 (10%) had >1001 CFU/100ml. The PCR positive results for potential pathogens are shown in Table 1.

Quantitative PCR assays were performed on selected pathogens considering their prevalence and infectious dose. Though *C. jejuni mapA* gene was detected in one sample, the concentration was below qPCR detection limit. *L. pneumophila*, Salmonella, and *Giardia lamblia* were detected in several samples (Table 1). *L. pneumophila mip* and *Salmonella invA* are single copy genes and were converted to cell numbers (i.e. 1 gene copy = 1 cell). *G. lamblia* β-giradin gene copy numbers were converted to cysts (16 gene copies = 1 cyst). Binary logistic regressions were also performed to identify the correlations between the concentrations of faecal indicator bacteria and the presence/absence of potential target pathogens (Table 2). The presence/absence of the potential pathogens did not correlate with any of the indicator bacteria concentrations.

Roof-harvested rainwater can be of poor microbiological quality.

Table 1. PCR positive results for potential pathogens.

Gene of target pathogen	**PCR positive results/ No. samples tested (% of sample positive)**	**Range of gene copies/100ml**
A. *hydrophila lip* gene	7/84 (8.3)	Not tested
Campylobacter coli ceuE gene	10/27 (37)	Not tested
C. jejuni mapA gene	1/84 (1.1)	Below qPCR detection limit
E. coli O157 LPS gene	0/84 (0)	Not tested
E. coli VT1 gene	0/84 (0)	Not tested
E. coli VT2 gene	0/84 (0)	Not tested
L. pneumophila mip gene	8/84 (9.5)	6-17
Salmonella invA gene	17/84 (20)	6.6-38
Salmonella spvC gene	0/27 (0)	Not tested
G. lamblia β-giradin gene	15/84 (18)	9-51
Cryptosporidium parvum COWP gene	0/84 (0)	Not tested

Table 2. The relationship between faecal indicators and the presence/absence of selected pathogens in samples from rainwater tanks.

Indicators vs. pathogenic microorganisms	**Nagelkerke's R square***	**P-value Δ**	**Odds ratio**
E. coli vs. *A. hydrophila*	0.055	0.460	1.00
E. coli vs. *C. jejun*	0.008	0.775	1.00
E. coli vs. *L. pneumophila*	0.006	0.640	1.00
E. coli vs. Salmonella	0.048	0.198	1.00
E. coli vs. *G. lamblia*	0.019	0.484	1.00
Ent vs. *A. hydrophila*	0.006	0.700	1.00
Ent vs. *C. jejuni*	0.001	0.943	1.00
Ent vs. *L. pneumophila*	0.007	0.555	1.00
Ent vs. Salmonella	0.016	0.388	1.00
Ent vs. *G. lamblia*	0.001	0.928	1.00

* Nagelkerke's *R* square, which can range from 0.0-1.0, denotes the effect size (the strength of the relationship); stronger associations have values closer to 1.0.

Δ *P*-value for the model chi square was <0.05 and the confidence interval for the odds ratio did not include 1.0. Greater odds ratios indicate a higher probability of change in the dependent variable with a change in the independent variable.

The presence of one or more pathogenic microorganisms along with faecal indicators represents a health risk to users. The pathogens had a poor correlation with faecal indicators. Currently we are performing QMRA using Monte Carlo analysis to determine the likely numbers of infections resulting from these exposures. These outcomes in terms of the impact of using roof-harvested rainwater on the disease burden of South East Queensland region of Australia will be interpreted.

Acknowledgements

This study was funded by Queensland Department of Natural Resources and Water. This was a collaborative project between DNRW and Queensland University of Technology.

References

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Dr Warish Ahmed is a water microbiologist at the Queensland Department of Natural Resources and Water and Queensland University of Technology. His area of expertise includes faecal pollution tracking and detection and quantification of pathogens in environmental waters.

Ashantha Goonetilleke is a professor in water/environmental engineering at Queensland University of Technology.

Ted Gardner is a principal scientist with the Queensland Department of Natural Resources and Water and an adjunct professor at Queensland University of Technology.

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