Quantitative PCR Detection of Zoonotic Pathogens in Roof-Harvested Rainwater for Quantitative Microbial Risk Assessment

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Although, roof-harvested rainwater is generally considered acceptable for potable use, the presence of pathogens has been reported in research literature (1). 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 indicating the need for direct monitoring of pathogens to assess public health risk (3).

In this study, the microbiological quality of roof-harvested rainwater was assessed by quantifying zoonotic pathogens using quantitative PCR (qPCR). The significance of this study stems from the fact that instead of measuring faecal indicators, pathogens that are capable of causing illness were measured and combined with quantitative microbial risk assessment (QMRA) to assess human health risk.

Eighty-four rainwater samples were collected from 66 residential properties in Brisbane and Gold Coast regions, of Queensland, Australia. *Campylobacter jejuni mapA, Salmonella invA*, and *G. lamblia* β-giradin genes were selected for qPCR analysis. DNA extraction from rainwater samples, PCR amplification, 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.

C. jejuni mapA gene was detected in only one sample, and its concentration was below qPCR detection limit. The concentrations of *Salmonella*, and *G. lamblia* are shown in Table 1. *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) for QMRA analysis.

Table 1: Quantitative PCR results for potential pathogens

Gene of target pathogen	PCR positive results/Number of samples tested (% of sample positive)	Range of units/100 ml for contaminated tank samples
C. jejuni mapA gene	1/84 (1)	Below qPCR detection limit of 10 gene copies
Salmonella invA gene	9/84 (11)	6.5 – 38 cells
G. lamblia β-giradin gene	11/84 (13)	0.6 – 3.6 cysts

About one third of the households in Brisbane have a rainwater tank (roughly equates to one third of the population) and about 10% of these households use the tank for potable purposes, the percentage of population exposed to each pathogen can be determined with a relatively high degree of certainty.

A major drawback in PCR is that it cannot distinguish between viable and nonviable cells. In addition, these methods do not provide information on the infectivity of the target strains. LeChevallier et al (6) suggests the percentage of CC-PCR detected *Cryptosporidium spp* that were both viable and infective may be 37%. In the absence of similar information concerning *Giardia*, we have assumed that 25% of the cells were both viable and infective. Therefore, the numbers of infective units per 100 mL in the tank water samples could be ranged from 1.6 – 9.5 (for *S.* Typhimurium) and 0.1 – 0.9 (for *G. lamblia*). *Salmonella* and *Giardia* infection route would be via ingestion of tank water by daily drinking or accidental ingestion of aerosols during bi-weekly hosing.

Table 2 - Exposure and calculation of possible dose for individuals exposed to contaminated tank water

Risk scenario	Pathogens exposure	Volume per event [a]	Range of Dose (infective units per event)	No of events per year
Ingestion via drinking	*Salmonella* spp.	1000 ml	16 – 95	365
	G. lamblia		1 – 9	
Ingestion via hosing aerosols	*Salmonella*	1 ml	0.02 – 0.1	104
	G.lamblia		0.001 – 0.01	

[a] Volume per event data was extracted from research literature.

A Beta Poisson dose response relationship (α=-0.3126, β=2884) was used for *Salmonella enteric* serovar Typhimurium, and an Exponential dose-response relationship (r = 0.01991) was used for *G. lamblia* (5).

Table 3 - The infection risk for individuals exposed to contaminated tank water for risk scenarios.

Risk Scenario	Pathogens	Infection risk per event (No. per 10000 exposed persons)	% of population exposed to pathogens	Infection risk per event (No. per 10000 persons)	No. of events/yr	Infection risk per year (No. per 10000 persons)
Ingestion via drinking	*Salmonella* spp. *G. lamblia*	18 – 101 276 – 1625	0.3 0.4	0.06 – 0.35 1.2 – 6.8	365 365	22- 125 414 - 2200
Ingestion via hosing aerosols	*Salmonella* *G.lamblia*	0.02 – 0.1 0.28 – 1.8	3.2 3.9	0.0006 – 0.003 0.01 – 0.07	104 104	0.06 – 0.34 1.1 – 7.2

The risk of infection of salmonella appears to be higher compared to Notifiable Diseases Surveillance System Database (Salmonellosis 5.7 cases/10000 in Queensland). However, Giardiasis is not notifiable disease in Queensland, and no data is available on the background illness rates. There are several factors need to be considered. In this study **untreated** samples were collected from the tank. However, a number of households will use UV disinfection or boil the water before potable use. In this case, the risk of infection would be lower than the calculated value. A significant number of individual may acquire immunity to certain pathogens due to frequent exposure, and therefore, they may not be infected. In addition, not every individual will seek medical attention if the illness is mild in nature and lasts for few days. Also, pathogen contamination within a tank may not persist for a whole year, as assumed here. Finally, the percentage of viable and infective cells could be lower than what we assumed. Nonetheless, roof-harvested rainwater could represent a health risks to users. Based on our data, we recommend using disinfection methods before the tank water is used for potable purpose.

References

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