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Health Risk from the Use of Roof-Harvested Rainwater in Southeast Queensland, Australia, as Potable or Nonpotable Water, Determined Using Quantitative Microbial Risk Assessment

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Running title: QMRA of roof-harvested rainwater

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ABSTRACT

A total of 214 rainwater samples from 82 tanks were collected in urban Southeast Queensland (SEQ) in Australia and analysed for the zoonotic bacterial and protozoan pathogen using real-time binary PCR and quantitative PCR (qPCR). Quantitative Microbial Risk Assessment (QMRA) analysis was used to quantify the risk of infection associated with the exposure to potential pathogens from potable and non-potable uses of roof-harvested rainwater. Of the 214 samples tested, 10.7%, 9.8%, and 5.6%, and 0.4% samples were positive for *Salmonella invA, Giardia lamblia* β-giardin , *Legionella pneumophila mip*, and *Campylobacter jejuni mapA* genes. *Cryptosporidium parvum* could not be detected. The estimated numbers of viable *Salmonella* spp., *G. lamblia* β-giradin, and *L. pneumophila* genes ranged from 1.6×10^{1} to 9.5×10^{1} cells, 1.4×10^{-1} to 9.0×10^{-1} cysts, and 1.5×10^{1} to 4.3×10^{1} per 1000 ml of water, respectively. Six risk scenarios were considered from exposure to *Salmonella* spp., *G. lamblia* and *L. pneumophila*. For *Salmonella* spp., and *G. lamblia*, these scenarios were: (1) liquid ingestion due to drinking of rainwater on a daily basis (2) accidental liquid ingestion due to garden hosing twice a week (3) aerosol ingestion due to showering on a daily basis, and (4) aerosol ingestion due to hosing twice a week. For *L. pneumophila*, these scenarios were: (5) aerosol inhalation due to showering on a daily basis, and (6) aerosol inhalation due to hosing twice a week. The risk of infection from *Salmonella* spp., *G. lamblia*, and *L. pneumophila* associated with the use of rainwater for showering and garden hosing was calculated to be well below the threshold value of one extra infection per 10,000 persons per year in urban SEQ. However, the risk of infection from ingesting *Salmonella* spp. and *G. lamblia* via drinking exceeds this threshold value, and indicates that if undisinfected rainwater were ingested by drinking, then the gastrointestinal diseases of Salmonellosis and Giardiasis is expected to range from 5.0×10^{0} to 2.8×10^{1} (Salmonellosis) and 1.0×10^{1} to 6.4×10^{1} (Giardiasis) cases per 10,000 persons per year, respectively. 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 examined. Nonetheless, it would seem prudent to disinfect rainwater for potable use.

Keywords: Roof-harvested rainwater; Fecal pollution Zoonotic pathogens, Quantitative PCR, QMRA

INTRODUCTION

Roof-harvested rainwater has received significant attention as a potential alternative source of potable and non-potable water supply in water-scarce regions (36). To encourage the use of roof-harvested rainwater, government bodies of many countries such as Australia, Denmark, Germany, India and New Zealand are providing subsidies to residents to encourage the use of 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 subsidies to Southeast Queensland (SEQ) residents who use rainwater for non-potable domestics purposes (51). Over 260,000 householders were granted subsidies up to December 2008, when the scheme was concluded.

There is a general community feeling that roof-harvested rainwater is safe to drink, and this is partially supported by limited epidemiological evidence (27). Some studies have reported that roof-harvested rainwater quality is generally acceptable for potable use (14, 26). In contrast, the presence of potential zoonotic pathogens such as *Aeromonas* spp. *Campylobacter* spp. *Salmonella* spp. *Giardia* spp., and *Cryptosporidium* spp. in roof-harvested rainwater samples has been reported (2, 10, 35, 46, 50). Such organisms can cause gastrointestinal illness in human, with nausea, vomiting and/or diarrhoea occurring within 12-72 hours (*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 fecal indicators, such as fecal coliforms, *Escherichia coli* and enterococci have long been used as fecal indicators to determine the presence of pathogens. Most studies assess the quality of roof-harvested rainwater based on the concentration of these fecal indicators (14, 29). However, the major limitation in using fecal bacteria as indicators is their poor correlation with the presence of pathogenic microorganisms in water (2, 29). An alternative is the measurement of pathogens using traditional culture-based methods. However, there are several limitations in such methods and include the underestimation of the bacterial concentration due to the presence of injured or stressed cells (11) whilst certain microorganisms in environmental waters can be viable but not cultivable (39). 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 real-time binary PCR (presence/absence) and qPCR (quantitative) based assays to detect and quantify respectively zoonotic pathogens in samples from roof-harvested rainwater in SEQ residential houses.

The aims of the research study were two-fold: (I) to quantify the concentration and frequency of occurrence of *Salmonella* spp. *G. lamblia* and *L. pneumophila* using qPCR based methods in a range of domestic water tanks in SEQ, and (II) to apply Quantitative Microbial Risk Assessment (QMRA) analysis in order to estimate the risk of infection from exposure to these pathogens found in roof-harvested rainwater. The uniqueness of this study stems from the fact that instead of measuring fecal indicators, the pathogens that are capable of causing illness were directly quantified and this information was combined with QMRA to assess human health risk for potable and non-potable uses of roof-harvested rainwater.

MATERIALS AND METHODS

Target pathogens. *C. jejuni*, *L. pneumophila*, *Salmonella* spp., *G. lamblia*, and *C. parvum* were selected because these pathogens could be present in the feces of birds, mammals and reptiles that have access to the roof. Therefore, following rain events, fecal matter could potentially be transported to the tank via roof run-off.

Sampling and analysis. In all, 214 samples were collected from 82 residential houses in Brisbane, Gold Coast and Sunshine Coast Regions. The size of the sampled tanks ranged between 500 to 20,000 L (i.e., polyethylene water tanks), and the end uses were: (1) outdoor use (65%) including gardening and car washing, and (2) indoor

use (35%) including drinking, showering and kitchen use. Water samples were collected in sterilized 10 L containers from the outlet taps located close to the base of the tanks. Before the tank was sampled, the tap was sterilised with 70% ethanol, and allowed to run for 30 to 60 s to flush out water from the tap. Samples were transported to the laboratory on ice, and processed within 6 h. Water samples were collected in two phases. In the first phase, a total of 100 water samples were collected from 82 tanks, and were screened for the presence/absence of *C. jejuni*, *L. pneumophila*, *Salmonella* spp., *G. lamblia*, and *C. parvum* using real-time binary PCR assays (1). In the second phase water samples were collected from a subset of tanks sampled in the first phase. Tank water samples which were PCR positive for the selected pathogens were further sampled in order to obtain information on the occurrence of these pathogens. Fortnightly samples were collected from these tanks over a period of three months (Apr-Jun 2009) commencing with a rainfall event and tested using real-time binary PCR. Finally, real-time qPCR methods were used to quantify these pathogens for all positively identified samples in the first and second phase.

DNA extraction. For real-time binary PCR and qPCR analysis of bacterial pathogens, 1 L of water sample from each tank was filtered through 0.45 µm pore size membrane (Advantec, Tokyo, Japan). Samples were processed according to the previously published method (2). DNA was extracted using DNeasy blood and tissue kit (Qiagen, Valencia, CA), and stored at -80°C until use. For real-time binary PCR and qPCR analysis of the pathogenic protozoans, a 2.5 L of water sample from each tank was filtered through a 3 µm pore size membrane (Advantec). Samples were processed according to a previously published method (22). DNA was extracted directly to the filter using DNeasy blood and tissue kit (Qiagen).

Real-time PCR positive controls. Strains and purified DNA were purchased from the American Type Culture Collection (ATCC), as follows: *Campylobacter jejuni* (ATCC 33560D, purified DNA), *L. pneumophila* (ATCC 33152, strain), and *Salmonella enterica* serovar Typhimurium (ATCC 14028, strain), *G. lamblia* (ATCC 30888D, purified DNA of Portland-1 strain), and *C. parvum* (PRA-67D, purified DNA). Bacterial DNA was extracted from the broth cultures of *L. pneumophila* and *S.* Typhimurium strains using DNeasy blood and tissue kit (Qiagen).

Primers, preparation of standard curves and PCR conditions. Real-time PCRs of pathogens were performed using previously published primers. The primer sequences and cycling parameters are shown in Table 1. Standards for real-time qPCR of *L. pneumophila mip*, *Salmonella invA*, and *G. lamblia* β-giardin genes were prepared from the genomic DNA of the selected pathogens. The concentration of genomic DNA was determined by measuring the absorbance at A_{260} using Beckman Coulter DU® 730 spectrophotometer. The genomic copies were calculated, and a tenfold dilution was prepared from the genomic DNA, ranging from 10^6 to 10^0 copies per µl of DNA extract using CAS-1200™ precision liquid handling system (Corbett Life Sciences, Brisbane, Australia), and stored at -20°C until use. For each standard, the concentration was plotted against the cycle number at which the fluorescence signal increased above the threshold value (C_T value). The amplification efficiency (E) was determined by running the standards, and was estimated from the slope of the standard curve by the formula $E = (10^{-1/slope}) - 1$. A reaction with 100% efficiency generates a slope of -3.32.
Amplification was performed in 25 µl reaction mixtures using Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA). The PCR mixture contained 12.5 µl SuperMix, 300 nM of each primer, 5.75 µl of DNase and RNase free deionised water and 5 µl of template DNA. For each PCR experiment, corresponding positive DNA and negative controls (i.e., sterile water) were included. The real-time PCR reactions were performed using the Rotor-Gene 6000 real-time cycler (Corbett Life Sciences). Real-time PCR set up was performed using the liquid handling system (Corbett Life Sciences).

PCR reproducibility and limit of detection. The reproducibility of the real-time qPCR was assessed by determining intra-assay repeatability and inter-assay reproducibility. The Coefficient of Variation (CV) was calculated using six dilutions (10^6 to 10^1 gene copies) of the *L. pneumophila*, *S.* Typhimurium, and *G. lamblia* genomic DNA. Each dilution was quantified in replicates. The CV for evaluation of intra-assay repeatability was calculated based on the C_T value by testing the six dilutions six times in the same experiment. The CV for inter-

assay reproducibility was calculated based on the C_T value of six dilutions on six different days. To determine the qPCR limit of the detection, known gene copies of the *L. pneumophila* (i.e., 5×10^3 to 5×10^0), *S.* Typhimurium (i.e., 5×10^3 to 5×10^0), and *G. lamblia* (i.e., 7×10^3 to 7×10^0), were measured from pure genomic DNA isolated from corresponding control strains, and were tested with the qPCR. The lowest concentration of gene copies that were detected consistently in replicate assays was considered as the qPCR limit of detection.

Recovery efficiency of the qPCR assays in rainwater samples. The recovery efficiencies were determined only for *Salmonella* and *G. lamblia* qPCR assays. The recovery efficiency of *L. pneumophila* was assumed to be similar to that of *Salmonella* qPCR assay. Deionised water (n=3) and rainwater samples (n=3) were spiked with known concentrations of *S.* Typhimurium cells and *G. lamblia* cysts (obtained from Biotechnology Frontiers, New South Wales, Australia). Initially, samples (n = 3) were collected from several rainwater tanks, and were tested for the presence of *Salmonella* spp. and *G. lamblia* using real-time binary PCR detection. Water samples from those tanks which showed the absence of *Salmonella* spp. and *G. lamblia* were selected for this experiment. The samples were autoclaved to destroy background microbial flora and kept under UV light to minimise any background DNA that could be present. The *S.* Typhimurium strain was grown overnight in LB broth, and cell concentrations were determined using microscopic counts. Ten-fold serial dilutions were made and spiked into 1 L of deionised and rainwater samples. Similarly, known concentrations of *G. lamblia* cysts were serially diluted and spiked into 2.5 L 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 (%) = (No. of cells after filtration/No. of cells before filtration) × 100. All results were corrected according to their relevant recovery ratios.

Quality control. To prevent false positive results for rainwater samples, a method blank was included for each batch (n = 10) of water samples. In brief, 1 L of distilled water sample was filtered through 0.45 μm pore size membrane (Advantec). The filter paper was washed with sterile sodium-Tris-EDTA (STE) buffer followed by centrifugation as described above. The supernatant was discarded, and the pellet was resuspended in sterile distilled water. DNA was extracted using DNeasy blood and tissue kit (Qiagen). To prevent false positive results during DNA extraction, a reagent blank was included in each batch (n = 10) of samples. For all PCR assays, the PCR conditions for annealing temperature were optimized by performing gradient analysis (i.e., temperature ranged from 53°C to 63°) for each target. The primer concentrations (100 nM to 500 nM) were also optimized to reduce the level of primer dimer for each target. To separate the specific product from non-specific products, DNA melting curve analysis was performed for each PCR experiment. During melting curve analysis, the temperature was increased from 57 to 95°C at approximately 2°C per min. Samples were considered to be positive when shown to have the same melting temperature as the positive control. To minimize PCR contamination, DNA extraction, PCR set up, and gel electrophoresis were performed in separate laboratories.

Quantitative Microbial Risk Assessment. QMRA is a four-step process for estimating the human health risk associated with defined scenarios from exposure to specified pathogens (38). The four steps are: (I) hazard identification, (II) exposure assessment, (III) dose-response assessment, and (IV) risk characterisation. The first step of QMRA is hazard identification which was achieved by collating research literature reporting the presence of specific pathogens such as *C. jejuni*, *L. pneumophila*, *Salmonella* spp., *G. lamblia* and *C. parvum* in roof-harvested rainwater tanks (2, 6, 10, 35, 46, 49). These pathogens were then detected (i.e., positive/negative) in a number of water samples using real-time binary PCR.

The second step is exposure assessment where the pathogen concentration of source water (i.e., tank rainwater) and the volume ingested/inhaled by a person are estimated. For the estimation of pathogen concentration, the concentration of genomic copies (determined by qPCR) of each pathogen was converted to bacterial cells and protozoan cysts. *L. pneumophila mip* (16) and *Salmonella invA* (17) are single copy genes and therefore, allow the estimation of cells (i.e., one gene copy of *L. pneumophila* or *Salmonella* = one cell of *L. pneumophila* or *Salmonella*). *G. lamblia* ß-giardin gene is expressed as single-copy gene within the nucleus of each trophozoite

(25). Cysts of *Giardia* contain two trophozoites that have undergone multiple steps of nuclear division, resulting in 16 copies of total genetic information within each cyst (5). Therefore, there are 16 copies of the ß-giardin gene per *Giardia* cyst (22). However, only a proportion of the PCR quantified cells and cysts may be viable and infectious (53). Therefore, further assumptions were made from an examination of literature. It has been suggested that the percentage of *Giardia* cysts that are viable and infectious is around 25% (33). For *L. pneumophila* and *Salmonella*, it was conservatively assumed that at least 25% of the cells would be viable and infective based on research literature (54).

To estimate the possible pathogen dose received by an individual, the likely infection routes appropriate to each pathogen must be considered. Infection may occur by ingesting (accidentally during garden hosing or deliberately via drinking) water containing *Salmonella* spp. or *G. lamblia*. Another possible route is to swallow aerosols containing these organisms. For *L. pneumophila* cells to cause infection, they must be inhaled deep into the lungs. Given these possible routes, the infection risk associated with each of a total of six scenarios (four risk scenarios for *Salmonella* spp. and *G. lamblia* and two risk scenarios for *L. pneumophila*) were estimated.

For Salmonellosis and Giardiasis risk, the scenarios were: (1) liquid ingestion due to drinking of rainwater on a daily basis (2) accidental liquid ingestion due to garden hosing twice a week (3) aerosol ingestion due to showering on a daily basis, and (4) aerosol ingestion due to hosing twice a week. For Legionellosis risk, the scenarios were (1) aerosol inhalation due to showering on a daily basis, and (2) aerosol inhalation due to hosing twice a week. For liquid ingestion, volumes were assumed to be 1000 ml per day due to drinking (56), and 1 ml per event for accidental liquid ingestion due to hosing (52). For aerosol inhalation during showering, to estimate the volume of shower water that is deposited in the alveoli of an adult requires knowledge of aerosol size distribution at the receptor and the proportion of inhaled aerosols that are deposited in the alveoli of the receptor. This information is difficult to obtain. However several studies which have estimated the aerosol size distributions measured next to a shower rose (31, 41, 59). Schlesinger (48) provided estimates of alveolar depositional efficiencies across aerosol size classes. This information was used to adjust the data of O'Toole et al. (41), and Keating and McKone (31) to estimate total alveoli deposition. Zhou et al. (59) estimated deposition using a lung model. Based on these estimates, the volume of shower water inhaled was calculated for an adult breathing 20 L per min during a 7 min hot shower. The volume of shower water inhaled represented by the 0.3 - 6.0 m (respirable) aerosol size class was calculated to range from 0.02 μl to 0.84 μl for a 7 min hot shower, across the different experimental conditions, shower heads, and flow rates used in the studies. For the scenario of aerosol inhalation by showering, the worst case volume of 0.84 μl was chosen for infectious risk calculations.

For aerosol inhalation during hosing, it was also assumed that exposure would only occur during that portion of time that the recipient was actually downwind of the hose nozzle. Hence exposure to aerosols during hosing was assumed to take place for 7 min with the user breathing 20 L per min as for showering. The volume of hosing water that would be deposited in the alveoli of the user was calculated from the aerosol size distributions measured for a hose spraying against a car door. O'Toole et al. (41), adjusted for differences in alveolar depositional efficiencies across aerosol size classes as according to Schlesinger (48). The volume of hose water represented by the 0.3 - 6.0 m aerosol size class was calculated to be 0.008 - 0.04 μl for a high pressure hose under spray and jet settings, and 0.09 - 0.5 μl for a garden hose with a trigger nozzle using spray or jet settings. For the scenario of aerosol inhalation by hosing, the worst case volume of 0.5 μl volume was chosen for infection risk calculations. It has been suggested that aerosols above 6 μm tend to be deposited in the upper respiratory tract where they would be swallowed (41). Accordingly, the volume of shower or hose water ingested represented by the > 6.0 μm aerosol size class was calculated to range from 58 μl to 1.9 ml for showering and 0.002 μl to 1.9 μl for hosing, for the same time of exposure and inhalation rate described above.

The third step is dose-response assessment which describes the relationship between administered dose and the probability of infection in the exposed population. The dose-response relationships used for this study were obtained from the literature. For *L. pneumophila* (3) and *G. lamblia* (44) an exponential dose-response model was used, whilst for *Salmonella* (24), a Beta-Poisson dose response relationship was used (Fig. 1).

The fourth and final step of QMRA is risk characterisation where exposure and dose-response assessment are combined to estimate the probability of infection (expressed as likely numbers of infections per 10,000 persons per year) for the urban SEQ community, and to compare with an arbitrary but commonly accepted risk level of one extra infection per 10,000 persons per year (55). The number of infections by a specific pathogen per 10,000 persons in SEQ was determined as the number of infections per 10,000 exposed persons × the proportion of SEQ persons that were exposed to the specific pathogen through drinking, showering or hosing of rainwater. To estimate the latter, market survey data was used to establish the number of households in Brisbane that use roof-harvested rainwater for potable purposes (20). From the total 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 frequently use the rainwater for cooking and drinking purposes. This suggests that almost 30% of urban SEQ households possess a rainwater tank, and that 6.3% of urban SEQ households use the rainwater for potable purposes, and are therefore are at risk of exposure to each pathogen identified in the tank water samples. It was conservatively assumed that all of the urban SEQ households with rainwater tanks also use the water for their gardens. It was also assumed that only those using rainwater for potable purposes also used the tank water for showering.

Assuming that the pathogen distribution indicated by the sampled roof-harvested rainwater tanks was representative of the tanks in urban SEQ, as the percentage of the urban SEQ population that was exposed to each pathogen could be estimated for each risk scenario as:
% of rainwater tanks in which the pathogen was detected by real-time binary PCR × % of urban SEQ households that use the rainwater as specified in the risk scenario.

The probability of infection per single exposure was converted to the probability of infection per year using the equation

$$\text{No. infections per 10,000 urban SEQ persons per year} = 1 - (1 - P_i)^E$$

where

E = the number of exposure events per year.

P_i = No. infections per 10,000 urban SEQ persons from a single exposure.

The number of exposure events per year was determined as the number of events per year adjusted by the proportion of the year that pathogens were present in the tanks. The latter was estimated from the fortnightly sampling conducted in the second phase of the study described in the Materials and Methods.

RESULTS

Quantitative PCR standards, reproducibility and limit of detection. DNA from 10 fold dilutions of quantified *L. pneumophila*, *S.* Typhimurium and *G. lamblia* strains were analysed in order to determine the reaction efficiencies. The standard curves had a linear range of quantification from 10^6 to 10^1 genomic copies per µl of DNA extracts. The amplification efficiencies were > 95% for all qPCR assays and the correlation coefficient (r^2) was > 0.98 for all three assays. The reproducibility of each qPCR assay was determined by assessing intra-assay and inter-assay Coefficient of Variation (CV) of the standards. The mean CV values for intra-assay and inter-assay were: 3.4% ± 0.8% and 1.9% ± 1.1% (for *L. pneumophila mip*), 1.9% ± 0.8% and 1.9% ± 1.3% (for *Salmonella invA*), and 3.2% ± 1.2% and 4.5% ± 2.1% (for *G. lamblia* β-giradin) genes indicating high reproducibility. The qPCR limit of detection was as low as 5 gene copies for *L. pneumophila mip,* and *Salmonella invA* genes. For, *G. lamblia* β-giradin gene, the limit of detection was 7 gene copies.

Recovery efficiency. The estimated recovery efficiency in autoclaved distilled water samples ranged between 93% to 48% (for *Salmonella*) and 43% and 23% (for *G. lamblia*) with the greatest variability occurring at lower cell and cyst counts. The mean recovery efficiencies were 72% ± 16% (for *Salmonella*) and 35% ± 11% (for *G. lamblia*). The estimated recovery efficiency in autoclaved rainwater samples ranged between 91% and 45% (for *Salmonella*) and 41% and 19% (for *G. lamblia*) with the greatest variability occurring at lower cell and cyst counts. The mean recovery efficiencies were 66% ± 17% (for *Salmonella*) and 33% ± 12% (for *G. lamblia*).

Concentration of pathogens in roof-harvested rainwater. Of the 214 samples tested during the phase I and II, *Salmonella invA, G. lamblia* β-giardin genes, and *L. pneumophila mip* genes were also detected in 23 (10.7%), 21 (9.8%), and 12 (5.6%) rainwater samples respectively, using real-time binary PCR. However, certain samples were non-quantifiable (Table 2). *C. jejuni mapA* gene was detected in one sample by real-time binary PCR but was non-quantifiable. None of the samples were positive for *C. parvum* COWP genes. The concentration of *Salmonella invA*, *G. lamblia* β-giradin, and *L. pneumophila mip* genes in quantifiable samples ranged from 6.5×10^1 to 3.8×10^2, 0.9×10^1 to 5.7×10^1 and 6.0×10^1 to 1.7×10^2, genomic copies per 1000 ml of water, respectively (Table 3). After conversion of genomic copies, the **c**oncentrations of *Salmonella* spp. and *G. lamblia* and *L. pneumophila* in water samples ranged from 6.5×10^1 and 3.8×10^2 cells (for *Salmonella* spp.), 0.6×10^0 and 3.6×10^0 cysts, and 6.0×10^1 and 1.7×10^2 cells (for *L. pneumophila*) per 1000 ml of water. Assuming that at least 25% of the quantified pathogens could be viable and infective, the range of viable and infective cells and cysts were calculated as shown in Table 4.

Occurrence of pathogens in roof-harvested rainwater. During phase II, 114 samples were collected from 19 tanks (i.e., subset of 82 tanks) to determine the occurrence of pathogens over time. Pathogens were found to be present in the tanks between 0 and 32% of the time in the three months, with an average of 4.2% of time (for *Salmonella* spp.), 5.1% of time (for *G. lamblia*), and 3.4% of time (for *L. pneumophila*) (Table 5). The results suggest that the pathogens are present approximately 5% of the time.

Likely dose received by exposed persons. Estimates of the ingestion dose (range and geometric mean) of each pathogen by person exposed according to the six scenarios are shown in Table 6. For liquid ingestion via drinking, 1.6×10^1 to 9.5×10^1 *Salmonella* cells and 1.4×10^{-1} to 8.9×10^{-1} *G. lamblia* cysts may be ingested, whilst for hosing, the dose is three orders of magnitude less. The dose ranges for the remaining scenarios are given in Table 6, and are several orders of magnitude lower than the scenario of liquid ingestion via drinking.

Infection risk for exposed persons. Infection risk per 10,000 exposed persons indicated by dose-response relationships ranged from low (4.5×10^{-3} to 1.3×10^{-2} for aerosol inhalation via hosing) to high (2.8×10^1 to 1.8×10^2 for liquid ingestion via drinking) for each event (Table 7).

Infection risk for SEQ population. The fraction of the urban SEQ population that is potentially exposed to each pathogen was calculated to be below 5% in all the scenario tested (Table 7), assuming that the proportion of the 82 tanks sampled containing these pathogens (7.3% for *L. pneumophila*, 12.2% for *Salmonella*, and 15.9% for *G. lamblia*) is representative of the tanks in urban SEQ. By multiplying the infection risks per 10,000 exposed persons with the fraction of the population that was exposed to each pathogen, the infection risk from *Salmonella* spp., *G. lamblia* and *L. pneumophila*, per 10,000 urban SEQ people per event was found to range from 2.1×10^{-5} to 5.9×10^{-5} infections from aerosol inhalation via hosing, and 2.8×10^{-1} to 1.8×10^0 infections from drinking (Table 7). Finally, the risk of infection per 10,000 people per year was calculated to range from 2.2×10^{-4} to 6.2×10^{-4} for aerosol inhalation via hosing, and 1.0×10^1 to 6.4×10^1 for drinking (Table 7). The risk of infection from ingestion *Salmonella* spp. and *G. lamblia* via drinking far exceeds the threshold value of one 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 5.0×10^0 to 2.8×10^1 (Salmonellosis) and 1.0×10^1 to 6.4×10^1 (Giardiasis) cases per 10,000 urban SEQ persons per year, respectively.

Discussion

Sources of contamination. Rainwater tanks may become contaminated due to material being washed into the tank from the roof and gutters following rain events. The primary sources of pathogens are likely to be from fecal materials from birds, lizards, possums which have access to the roof. Indeed, an anecdote from the study was tank water contamination was evident from the observation of obvious accumulation of bird feces under a TV antenna located on the roof area connected to the tank. One rainwater sample in this study was positive for *C. jejuni mapA* genes. *C. jejuni* is recognised as one of the etiologic agent of acute diarrheal disease (rather than general *Campylobacter* spp) and could potentially be from bird faeces (30, 57). However, other potential sources

such as possums or lizards cannot be ruled out, although, *Campylobacter* spp. could not be isolated from the possum feces in New Zealand (12) while the presence of *Campylobacter* spp. in roof-harvested rainwater samples has been reported in New Zealand (46). The *salmonella* spp. detected in rainwater samples could potentially be from bird feces (21). Both *Legionella* spp., and *Salmonella* spp. have previously been detected in roof-harvested rainwater cisterns and/or from tanks, using culture-based methods in the United States, New Zealand and in the tropics (6, 49). *Giardia lamblia* β-giardin gene which was detected in 21 (9.8%) out of 214 samples tested in phase I and II in this study, has also been reported to have high prevalence (45%) in rainwater cisterns in the U.S. Virgin Islands (10). However, no *Cryptosporidium parvum* was detected in this study, unlike the study by Crabtree et al. (10) with 23% of rainwater cisterns in the U.S. Virgin Islands contaminated with this pathogen. This could be due to the fact that, in this study, we used a small volume of water for the detection of protozoan pathogens compared to Virgin Islands study where 100 L of water samples were used for the detection.

Study approach. In this study, qPCR methods were used to quantify bacterial and protozoan pathogens in water samples from roof-harvested rainwater tanks. One advantage of PCR methods is that these could be used to detect and quantify specific pathogens with greater specificity compared to traditional culture based methods for the detection of pathogens in water (8, 53). The PCR methods used in this study were rigorously evaluated prior to being used to detect and quantify these pathogens in tank rainwater samples. The specificities of primers were determined against known microbial genomes and sequence by Basic Local Alignment Search Tool (BLAST) program to ensure no homology was detected with known gene sequences of other pathogenic microorganisms commonly found in water. The cross reactivity of each primer set was also evaluated by testing DNA isolated from other non-target species of microorganisms commonly found in water (2). The primers used in this study did not amplify any PCR products other than those products that were expected. An experiment was conducted to determine the potential presence of PCR inhibitory substances in rainwater samples collected from three different tanks. The results indicated that the tested rainwater samples were free of PCR inhibitory substances (2).

Viability and infectivity % assumptions. One major limitation of the qPCR method is that PCR cannot be used to differentiate between viable and non-viable cells which is essential information for QMRA. It has been suggested that the percentage of *Cryptosporidium* spp. and *Giardia* spp. that are viable and infective could be 37% and 25% respectively as determined by qPCR integrated with cell culture method (33, 34). A recent study reported that the ethidium monoazide-qPCR were able to detect 17-31% and 16-28% viable *Candidatus* Liberibacter asiaticus in citrus and periwinkle, respectively (54). In our study, it was assumed that 25% of the qPCR detected cells and cysts could be viable and infective. It has to be noted that we may be underestimating the percentage of viable and infective cells and cysts, and warrants more rigorous investigation in environmental water samples where pathogens might inactivate rapidly due to environmental factors such as temperature, predation and sunlight. To overcome this problem, qPCR could be integrated with cell culture or dyes (i.e., ethidium monoazide, propidium iodide and propidium monoazide which penetrates only dead cells) in order to obtain information regarding the viability of bacterial and protozoan pathogens (13, 43, 45).

Persistence assumption and implication for QMRA numbers. Another critical piece of information required for QMRA was the proportion of time that the pathogens are present in rainwater tanks. The occurrence of pathogens in roof-harvested rainwater is not well documented in research literature. Most studies collected one-off samples from rainwater tanks, and screened for a number of pathogens using traditional culture and PCR based methods (2, 49). However, the results of the three month sampling study suggests that it is unlikely that pathogens will be present in tanks all the time, with pathogens occurring only up to 5% of the time. Fewtrell and Kay (18) who undertook a recent risk assessment analysis with respect to *Campylobacter* spp. in toilets flushed with roof-harvested rainwater assumed the presence of *C. jejuni* in tank water from 0-10% of time. It must be noted that the presence of pathogens in the tank water is likely to be strongly related to rainfall events. This is because pathogens in the feces deposited on the roof are generally washed off to the tanks after rainfall events. Rainwater samples in the first phase were collected immediately after rainfall events (data not shown), whilst only the first samples in the second (three-month survey) phase were collected following rainfall. Dry conditions accompanied sample collection throughout the remainder of the survey period. The highest levels of contamination were indeed shown by samples that were taken immediately following rainfall events (data not

shown). This suggests that for areas with frequent light rains throughout the year, pathogens may be present for a larger proportion of the time than what we have assumed for South East Queensland in our calculations (10%). **QMRA results compared to other data.** The results of QMRA based on the assumptions discussed above indicate that the only likely risk encountered from the roof-harvested rainwater samples was from *drinking* water contaminated with *Salmonella* spp. and *G. lamblia*. The number of infections per 10,000 urban SEQ people per year ranged from 5.0×10^0 to 2.8×10^1 for *Salmonella* spp. and 1.0×10^1 to 6.4×10^1 for *G. lamblia*. *L. pneumophila*, at the levels detected in the roof-harvested rainwater samples and did not present as a threat for potable uses of tank water. Non-potable uses of the tank water also presented no threat to human health at the pathogen concentrations detected.

These predictions were then compared with the incidence of these diseases reported in the Notifiable Diseases Surveillance System Database (http://www9.health.gov.au/cda/Source/CDA-index.cfm). Cases of Salmonellosis is reported at an incidence of 5.7 cases per 10,000 in Queensland, whilst Giardiasis, which is not a notifiable disease in Queensland State, has been reported at up to 5 cases per 10,000 in other states over the past ten years. Hence, the QMRA suggests that the additional use of rainwater tanks in urban SEQ may in fact substantially increase the incidence of Salmonellosis and Giardiarsis. No such rise in reported Salmonellosis in Queensland over recent years is apparent.

A number of explanations are possible for this discrepancy. There is a naturally high incidence of gastroenteritis in the community e.g. 8,000 cases per 10,000 people per year (28) which may mask the actual disease. Before the disease can be reported in the Notifiable Diseases Surveillance System Database, it must first be identified and not every individual will seek medical attention if the illness is mild in nature and lasts only for a few days. Consequently, the incidence of disease indicated in the Notifiable Diseases Surveillance System Database is at best a minimum value, and may be substantially underestimating actual disease incidence. Hall et al. (23) estimated that between 8-11% of *Campylobacter* and *Salmonella* related illnesses are reported.

Similarly, the methodology used to estimate health risk, apart for the assumptions of 25 % of pathogen being viable and infective, and pathogens occurring in the tank for 10% of the time, did not take into account households that used effective disinfection treatment of rainwater before potable use. The use of such treatments such as UV disinfection or boiling the water before potable use would eliminate/reduce exposure of individuals to pathogens and hence infection. Another factor is the possibility of individuals acquiring immunity to certain pathogens due to frequent exposure. However, to counterbalance this, no attempt was made to include the greater infection risk to the elderly or immunocompromised for a given dose since the dose response relationships used in the QMRA was based on healthy adults and these relationships were applied uniformly across the population.
Water borne disease outbreaks. Have rainwater consumption been shown to cause disease elsewhere? A literature search indicated that to-date, several disease outbreaks associated with the rainwater consumption have been reported worldwide (4, 7, 19, 32, 37, 50). However, epidemiological studies in Melbourne (28) and in South Australia (27) have both indicated that the consumption of tank rainwater did not significantly contribute to gastroenteritis disease in either city. However, it should be noted that such studies cannot be practically sized to allow the high sensitivity needed to detect increased rates of infection of the order of magnitude suggested in current study. Eisenberg et al. (16) estimated that to detect illness at an annual risk of 100 cases per 10,000 people per year, a sample of 416,000 participants would be required while the Melbourne and South Australian studies had less than 10,000 participants.

Conclusions

Recent water restrictions in several capital cities in Australia and drought conditions have resulted in the installation of rainwater tanks at rates not seen before. The increasing role being played by rainwater tanks in water security in SEQ, including the mandating of rainwater tanks for all new developments in SEQ means that tank and roof hygiene will assume greater importance in the future. Therefore, the development of a robust methodology for the assessment of possible health risk from roof-harvested rainwater is essential. We believe that the methodology developed so far provides a step towards achieving this objective but further refinements

will be needed to provide a better estimate of health risk. It is evident that further information is needed relating to the occurrence of pathogens throughout the year, and the viability of pathogens in roof-harvested rainwater tanks. Currently, a study is being designed in which a number of rainwater tanks will be surveyed for a year for the presence of pathogens in order to obtain information regarding their seasonal persistency and variability. Culture based methods and qPCR methods incorporating dyes such as propidium iodide, and propidium monoazide will be incorporated into the methodology to provide information on the viability of the detected cells in water samples.

Current estimates of health risk suggests that it would be prudent to disinfect roof-harvested rainwater such as the installation of a UV disinfection unit, boiling or other forms of disinfectants before using the water for potable uses, especially drinking. This would be especially prudent for the elderly and immunocompromised. Maintenance of good roof and gutter hygiene, and elimination of overhanging tree branches and other structures where possible to prevent the congregation of animals is also recommended. Consideration should be given to include Giardiasis to the notifiable disease list in Queensland, given that Giardia was found in rainwater tank samples.

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TABLE 1 - Target genes, primers and cycling parameters used for pathogen detection

Target	Primer sequence (5΄- 3΄)	Cycling parameters	Amplicon size (bp)	Reference
C. jejuni mapA gene	GCT AGA GGA ATA GTT GTG CTT GAC AA [a] TTA CTC ACA TAA GGT GAA TTT TGA TCG [b]	2 min at 50ºC, 10 min at 95ºC for initial denaturation, and 40 cycles of 95ºC for 15 s, 59ºC for 30 s for annealing	72	**Price et al. 2006**
L. pneumophila mip gene	GCA ATG TCA ACA GCAA [a] CAT AGC GTC TTG CATG [b]	2 min at 50ºC, 15 min at 95ºC for initial denaturation, and 45 cycles of 94ºC for 30 s, 56ºC for 1 min for annealing and 72ºC for 1 min, followed by a final extension step of 72ºC for 10 min	159	**Wilson et al. 2003**
Salmonella invA gene	ACA GTG CTC GTT TAC GAC CTG AAT [a] AGA CGA CTG GTA CTG ATC GAT AAT [b]	2 min at 50ºC, 5 min at 94ºC for initial denaturation, and 45 cycles of 94ºC for 30 s, 59ºC for 35 s for annealing and 72ºC for 2 min, followed by a final extension step of 72ºC for 10 min	244	**Chiu and Ou 1996**
G. lamblia β-giardin gene	CCT CAA GAG CCT GAA CGA TCTC [a] AGC TGG TCG TAC ATC TTC TTC CTT [b]	2 min at 50 ºC, 10 min at 95ºC for initial denaturation, and 45 cycles of 95ºC for 15 s, 59ºC for 1 min for annealing, followed by a final extension step of 72 ºC for 5 min	74	**Guy et al. 2003**
Cryptosporidium oocyst wall protein (COWP) gene	CAA ATT GAT ACC GTT TGT CCT TCTG [a] GGC ATG TCG ATT CTA ATT CAG CT [b]	2 min at 50 ºC, 10 min at 95ºC for initial denaturation, and 45 cycles of 95ºC for 15 s, 59ºC for 1 min for annealing, followed by a final extension step of 72 ºC for 5 min	150	**Guy et al. 2003**

[a:] Forward primer [b:] Reverse primer.

TABLE 2 – Real-time binary PCR and qPCR results for potential pathogens

Target pathogens (genes)	Real-time binary PCR positive samples/No. of samples tested (% of samples positive)	qPCR quantifiable samples/No. of samples tested (% of samples quantifiable)
Salmonella (*invA* gene)	23/214 (10.7)	14/214 (6.5%)
G. lamblia (β-giardin gene)	21/214 (9.8)	17/214 (7.9%)
L. pneumophila (*mip* gene)	12/214 (5.6)	9/214 (4.2%)

TABLE 3 – Concentrations of genomic copies of pathogens in roof-harvested rain water samples. Samples showing no detectable pathogens are not listed

Tank ID (Sampling occasion)	Genomic copies of pathogens per 1000 ml of water		
	Salmonella invA gene	*G. lamblia* β-giradin gene	*L. pneumophila mip* gene
T1 (O1)	7.5×10^1	1.6×10^1	-
T1 (O4)	1.1×10^2	-	-
T2 (O1)	1.5×10^2	-	-
T3 (O1)	-	-	1.4×10^2
T3 (O2)	6.5×10^1	-	-
T3 (O3)	-	-	1.7×10^2
T3 (O4)	1.8×10^2	-	1.1×10^2
T3 (O5)	1.0×10^2	1.4×10^1	-
T7 (O1)	1.8×10^2	5.7×10^1	1.0×10^2
T8 (O1)	-	-	1.5×10^2
T11 (O1)	2.7×10^2	-	-
T11 (O3)	-	-	9.0×10^1
T11 (O4)	-	-	1.4×10^2
T14 (O1)	-	1.4×10^1	-
T15 (O1)	-	-	6.0×10^1
T15 (O1)	-	2.8×10^1	-
T15 (O1)	-	1.3×10^1	-
T18 (O1)	-	1.9×10^1	-
T18 (O2)	2.1×10^2	1.1×10^1	-
T18 (O3)	1.1×10^2	-	-
T20 (O1)	-	5.1×10^1	-
T28 (O1)	7.0×10^1	-	-
T32 (O1)	-	2.1×10^1	-
T32 (O2)	-	1.8×10^1	8.0×10^1
T 38 (O1)	3.8×10^2	0.9×10^1	-
T 38 (O2)	-	1.6×10^1	-
T 39 (O1)	3.0×10^2	4.8×10^1	-
T 40 (O1)	-	3.6×10^1	-
T 44 (O1)	3.3×10^2	5.6×10^1	-
T 45 (O1)	-	2.1×10^1	-
Minimum	6.5×10^1	0.9×10^1	6.0×10^1
Geometric mean	1.5×10^2	2.2×10^1	1.1×10^2
Maximum	3.8×10^2	5.7×10^1	1.7×10^2

TABLE 4 – Concentrations of viable and infective pathogens in roof-harvested rain water samples

Pathogens	Range and geometric mean of genomic copies per 1000 ml of water	Range and geometric mean of cells and cysts per 1000 ml of water [a]	Range and geometric mean of viable and infective cells and cysts per 1000 ml of water [b]
Salmonella spp.	6.5×10^1 - 3.8×10^2 (1.5×10^2)	6.5×10^1 - 3.8×10^2 (1.5×10^2)	1.6×10^1 - 9.5×10^1 (3.8×10^1)
G. lamblia	9.0×10^0 - 5.7×10^1 (2.2×10^1)	0.6×10^0 - 3.6×10^0 (1.4×10^0)	1.4×10^{-1} - 9.0×10^{-1} (3.5×10^{-1})
L. pneumophila	6.0×10^1 - 1.7×10^2 (1.1×10^2)	6.0×10^1 - 1.7×10^2 (1.1×10^2)	1.5×10^1 - 4.3×10^1 (2.8×10^1)

[a] Genomic copies were converted to cells and cysts; [b] Assumes 25% of the cells and cysts are viable and infective

TABLE 5 – Occurrence of pathogens in selected rainwater tanks sampled at fortnightly intervals over three months

Tank ID	Real-time binary PCR results/number of sampling occasions					
	Salmonella invA gene	Occurrence (%)	*G. lamblia* β-giradin gene	Occurrence (%)	*L. pneumophila* mip gene	Occurrence (%)
T1	1/6	16%	0/6	0%	0/6	0%
T2	0/6	0%	0/6	0%	0/6	0%
T3	2/6 [a]	32%	1/6	16%	1/6	16%
T7	0/6	0%	0/6	0%	0/6	0%
T8	0/6	0%	0/6	0%	0/6	0%
T11	0/6	0%	0/6	0%	2/6 [a]	32%
T12	0/6	0%	0/6	0%	0/6	0%
T14	0/6	0%	0/6	0%	0/6	0%
T15	0/6	0%	2/6 [a]	32%	0/6	0%
T18	2/6 [a]	32%	1/6	16%	0/6	0%
T 20	0/6	0%	0/6	0%	0/6	0%
T 28	0/6	0%	0/6	16%	0/6	0%
T 32	0/6	0%	1/6	0%	0/6	16%
T 33	0/6	0%	0/6	16%	1/6	0%
T 38	0/6	0%	1/6	0%	0/6	0%
T 39	0/6	0%	0/6	0%	0/6	0%
T 40	0/6	0%	0/6	0%	0/6	0%
T 44	0/6	0%	0/6	0%	0/6	0%
T 45	0/6	0%	0/6	0%	0/6	0%
Average % of occurrence		4.2%		5.1%		3.4%

[a] Two consecutive occasions were PCR positive

TABLE 6 – Exposure pathway, ingested/inhaled volumes and calculated ingested/inhaled pathogen dose for individuals exposed to tank water containing pathogens

Pathogens exposure and risk scenarios	Volume per day or event	Range and geometric mean of dose
Salmonella spp.		
Liquid ingestion via drinking	1000 ml	1.6×10^{1} - 9.5×10^{1} (3.8×10^{1})
Liquid ingestion via hosing	1 ml	1.6×10^{-2} - 9.5×10^{-2} (3.8×10^{-2})
Aerosol ingestion via showering	1.9 ml	3.1×10^{-2} - 1.8×10^{-1} (7.2×10^{-2})
Aerosol ingestion via hosing	1.9 µl	3.1×10^{-5} - 1.8×10^{-4} (7.4×10^{-5})
G. lamblia		
Liquid ingestion via drinking	1000 ml	1.4×10^{-1} - 8.9×10^{-1} (3.5×10^{-1})
Liquid ingestion via hosing	1 ml	1.4×10^{-4} - 8.9×10^{-4} (3.5×10^{-4})
Aerosol ingestion via showering	1.9 ml	2.6×10^{-4} - 1.7×10^{-3} (6.5×10^{-4})
Aerosol ingestion via hosing	1.9 µl	2.7×10^{-7} - 1.7×10^{-6} (6.7×10^{-7})
L. pneumophila		
Aerosol inhalation via showering	0.84 µl	1.3×10^{-5} - 3.6×10^{-5} (2.3×10^{-5})
Aerosol inhalation via hosing	0.5 µl	7.5×10^{-6} - 2.1×10^{-5} (1.4×10^{-5})

TABLE 7 – Infection risks for individuals exposed to contaminated tank water for six risk scenarios

Pathogens exposure and risk scenarios	Range and geometric mean of infection risk per 10,000 exposed people with rainwater tanks from single event	% of SEQ population exposed to pathogens	Range and geometric mean of infection risk per 10,000 people in SEQ from single event	No of events per year	Range and geometric mean of infection risk per year (No. per 10,000 people in SEQ)
Salmonella spp.					
Liquid ingestion via drinking	1.8×10^{1} - 1.0×10^{2} (4.1×10^{1})	0.77	1.4×10^{-1} - 7.8×10^{-1} (3.2×10^{-1})	36.5	5.0×10^{0} - 2.8×10^{1} (1.2×10^{1})
Liquid ingestion via hosing	1.8×10^{-2} - 1.0×10^{-1} (4.2×10^{-2})	3.66	1.4×10^{-4} - 8.0×10^{-4} (3.2×10^{-4})	10.4	1.4×10^{-3} - 8.3×10^{-3} (3.3×10^{-3})
Aerosol ingestion via showering	3.3×10^{-2} - 1.9×10^{-1} (7.8×10^{-2})	0.77	2.6×10^{-4} - 1.5×10^{-3} (6.1×10^{-4})	36.5	9.4×10^{-3} - 5.5×10^{-2} (2.2×10^{-2})
Aerosol ingestion via hosing	3.4×10^{-5} - 2.0×10^{-4} (8.0×10^{-5})	3.66	2.6×10^{-7} - 1.5×10^{-6} (6.2×10^{-7})	10.4	2.7×10^{-6} - 1.6×10^{-5} (6.4×10^{-6})
G. lamblia					
Liquid ingestion via drinking	2.8×10^{1} - 1.8×10^{2} (6.9×10^{1})	1.01	2.8×10^{-1} - 1.8×10^{0} (6.9×10^{-1})	36.5	1.0×10^{1} - 6.4×10^{1} (2.5×10^{1})
Liquid ingestion via hosing	2.8×10^{-2} - 1.8×10^{-1} (6.9×10^{-2})	4.75	2.8×10^{-4} - 1.8×10^{-3} (6.9×10^{-4})	10.4	2.9×10^{-3} - 1.9×10^{-2} (7.2×10^{-3})
Aerosol ingestion via showering	5.3×10^{-2} - 3.3×10^{-1} (1.3×10^{-1})	1.01	5.3×10^{-4} - 3.4×10^{-3} (1.3×10^{-3})	36.5	1.9×10^{-2} - 1.2×10^{-1} (4.8×10^{-2})
Aerosol ingestion via hosing	5.4×10^{-5} - 3.4×10^{-4} (1.3×10^{-4})	4.76	5.4×10^{-7} - 3.4×10^{-6} (1.3×10^{-6})	10.4	5.6×10^{-6} - 3.6×10^{-5} (1.4×10^{-5})
L. pneumophila					
Aerosol inhalation via showering	7.6×10^{-3} - 2.1×10^{-2} (1.4×10^{-2})	0.46	3.5×10^{-5} - 9.9×10^{-5} (6.4×10^{-5})	36.5	1.3×10^{-3} - 3.6×10^{-3} (2.4×10^{-3})
Aerosol inhalation via hosing	4.5×10^{-3} - 1.3×10^{-2} (8.3×10^{-3})	2.20	2.1×10^{-5} - 5.9×10^{-5} (3.8×10^{-5})	10.4	2.2×10^{-4} - 6.2×10^{-4} (4.0×10^{-4})

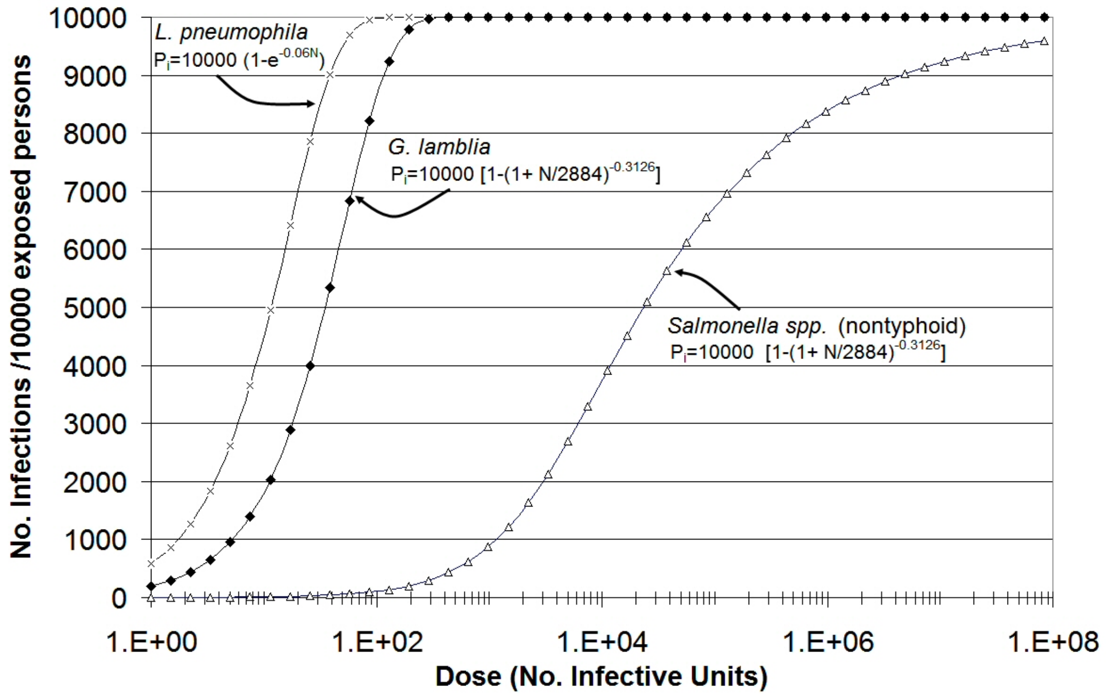

FIGURE 1 – Exponential dose response relationships were used for *Giardia lamblia* (Rose *et al.* 1991) and *Legionella pneumophila* (Armstrong and Hass 2008), and a beta Poisson dose response relationship for nontyphoid *Salmonella* was used for *Salmonella* Typhimurium) (Haas 1999). All dose response relationships relate N, the number of infective units ingested to P_i, the expected infections per 10,000 exposed persons.