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Assessing the Risk of *Legionella* Infection through Showering with Untreated Rain Cistern Water in a Tropical Environment

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Abstract: In September 2017, two category-5 hurricanes Irma and Maria swept through the Caribbean Sea in what is now known as the region's most active hurricane season on record, leaving disastrous effects on infrastructure and people's lives. In the U.S. Virgin Islands, rain cisterns are commonly used for harvesting roof-top rainwater for household water needs. High prevalence of *Legionella* spp. was found in the cistern water after the hurricanes. This study carried out a quantitative microbial risk assessment to estimate the health risks associated with *Legionella* through inhalation of aerosols from showering using water from cisterns after the hurricanes. *Legionella* concentrations were modeled based on the *Legionella* detected in post-hurricane water samples and reported total viable heterotrophic bacterial counts in cistern water. The inhalation dose was modeled using a Monte Carlo simulation of shower water aerosol concentrations according to shower water temperature, shower duration, inhalation rates, and shower flow rates. The risk of infection was calculated based on a previously established dose–response model from *Legionella* infection of guinea pigs. The results indicated median daily risk of 2.5×10^{-6} to 2.5×10^{-4} depending on shower temperature, and median annual risk of 9.1×10^{-4} to 1.4×10^{-2}. Results were discussed and compared with household survey results for a better understanding of local perceived risk versus objective risk surrounding local water supplies.

Keywords: quantitative microbial risk assessment (QMRA); roof-top harvested rainwater; hurricanes; aerosol; risk perception; risk management

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1. Introduction

Roof-top harvested rainwater (RHRW) is a major water resource to supplement both potable and non-potable water supplies in many parts of the world. Rainwater harvesting is beneficial in promoting water saving in water-stressed, semi-arid areas [1]. It is especially useful in tropical climates, such as in the Caribbean Virgin Islands, where rainfall is plentiful but there is limited surface and groundwater storage capacity. According to U.S. Virgin Island Code (Title 29 §308), all residential buildings constructed in the Virgin Islands are required to install rain tanks in order to alleviate demands on surface waters and desalinated water. In fact, rainwater is the only source of tap water piped to rural homes in the Virgin Islands. In these tropical islands, rain tanks serve multiple functions: (1) providing storage of water for daily use, (2) lessening the impact of runoff on stormwater systems, and (3) allowing low-cost water access to rural homes that are separated from the municipal water delivery systems. RHRW provides both potable and non-potable water for indoor household uses, while non-potable use is especially common in the U.S. Virgin Islands [2]. However, the quality of RHRW water is not well documented and is seldom tested.

Health risks associated with RHRW could attribute to the occurrence of microbial contamination from wild animals' feces [3]. Natural soil bacteria and microbes from decaying leaf litters carried in rainwater can form biofilms in both the rainwater storage and distribution systems, which often harbor opportunistic human pathogens [4]. Among diverse microorganisms, *Legionella* species, a bacterium commonly found in soil and plant litters in tropical regions, is of concern because they are the most documented causative agent of waterborne outbreaks [5]. The *Legionella* contamination is particularly problematic in warmer environments, such as tropical islands including the Virgin Islands. The daily temperature (22–32 °C) range of these tropical islands is ideal for *Legionella* growth (25–42 °C) [6]. *Legionella* infection in humans is primarily through inhalation of aerosols to the lungs from contaminated water sources. Shower water, the main application of rainwater for indoor non-potable use, can be a major vehicle for transmission of *Legionella* through water aerosols. The main symptoms of *Legionella* infection are respiratory illnesses, which are also known as Legionnaires' disease and Pontiac Fever [7].

In addition to the uncertainties of water quality associated with RHRW, severe storms and flooding can further exacerbate the contamination in the rain cisterns. In September 2017, two category-5 hurricanes, Irma and Maria, swept through the Caribbean Sea in what is now known as the region's most active hurricane season on record [8]. The wind, rain, and destruction delivered by the hurricanes impacted rain catchment systems and damaged many cisterns on the Virgin Islands. Excess loads of leaf litters, soil, and other organic debris that harbor opportunistic pathogens were washed from the rooftop to the underground cisterns. In addition, island-wide power outages halted treatment of human sewage, resulting in septic overflows that directly affected surface waters and possibly shallow groundwater. The underground cisterns compromised by fine cracks and poor seals may be impacted by sewage contamination through connection with surface and shallow groundwater [9]. A boil water advisory was issued by the VI Water and Power Authority on 27 September 2017 to curtail public health risk [10]. However, the lack of access to fuel and electricity long after the passing of the hurricanes made the advisory impractical [11].

In an effort to assess the impact of hurricanes on water quality in the disaster-stricken region, water samples from 22 households' rain cisterns on the island of St. Thomas, Virgin Islands, were collected as soon as the island became accessible [9]. Water samples were analyzed for microbial composition and contamination. Among 22 cisterns sampled, 86% were positive for *Legionella* spp. A household survey was also carried out alongside the water sample collection to understand the primary use of the rain cistern water and the public perception of water quality. Based on the survey outcomes, a quantitative microbial risk assessment (QMRA) was carried out using the *Legionella* contamination data and human exposure through showering water to understand the risk of *Legionella*-related disease in the post-disaster region. The outcomes of this study contribute to the decision of water quality management and disaster relief strategies.

2. Materials and Methods

2.1. Household Survey

This study was approved by the University of California, Irvine Institutional Review Board (IRB #2017-4032). Household surveys were conducted in St. Thomas, Virgin Islands, in November 2017, three months after the island was struck by Hurricane Maria. Verbal consent was collected from participants before survey questions were recorded. The purpose of the survey was to understand the island residents' perception of water quality and water use behavior. Household characteristics were also obtained, such as income and education, to test the correlation between risk perception and socioeconomic status. Surveys were collected from all residents who had given permission to sample their cisterns. Additional surveys were also collected from neighboring residents at nearby grocery stores, community gathering places, bars, and restaurants while water samplings were taking place in the neighborhood. Responses from a total of 107 complete surveys were included in this analysis. A copy of the survey questionnaires is included in supplementary information. The survey data were coded and binned, and the outcomes were plotted in Excel (Microsoft). The relationships between income level and awareness of water use, water quality, water safety, and perception of risk were assessed using χ^2 tests in RStudio [12]. The χ^2 test compares categorical survey responses with the null hypothesis that there is no association between income level and water quality awareness, water use, and water safety perception. A $p < 0.10$ was considered as statistically significant.

2.2. Risk Assessment

QMRA was conducted based on the framework outlined by the U.S. National Academy of Sciences [13]. The four main components are hazard identification, exposure assessment, dose–response assessment, and risk characterization. A fifth supplementary component, risk management, was also included to provide management recommendations based on the simulated risk outcomes. A Monte Carlo simulation was used to analyze the range of the data and estimations of parameters, while providing randomization and variability in the selections. All calculations were performed using MATLAB version R2019b (MathWorks) and RStudio [12].

2.2.1. Hazard Identification

According to CDC Waterborne Disease & Outbreak Surveillance Reports, *Legionella* has emerged as the most frequently reported etiology among drinking water-associated outbreaks. All waterborne outbreak-associated deaths reported in the most current surveillance period (2013–2014), including the outbreaks reported in hospital/health care settings or long-term care facilities, were caused by *Legionella*. *Legionella* can cause a serious type of pneumonia called Legionnaires' disease [14]. Legionnaires' disease is remarkably similar to other types of pneumonia, with symptoms that include cough, shortness of breath, fever, muscle aches, and headaches. The bacteria can also cause a less serious illness called Pontiac fever. Pontiac fever symptoms are primarily fever and muscle aches; it is a milder infection than Legionnaires' disease. Symptoms begin between a few hours to 3 days after being exposed to the bacteria and usually last less than a week.

Legionella spp. in rainwater cisterns, especially in tropical environments, has been previously reported [15,16]. *Legionella* is known to grow and persist within biofilms in engineered water storage and distribution systems such as cisterns [4], and spread by showerhead, sink faucets, and other water handling devices that generate aerosols and water droplets. Inhalation of aerosols that harbor *Legionella* into the lungs is the major route of human infection [17]. There are no vaccines that can prevent Legionnaires' disease.

Legionella spp. was detected in 86% of rain cistern samples in 2017 post-hurricane Maria water quality study in St. Thomas, Virgin Islands [9], indicating a possible health risk through water aerosol exposure. Although the direct source of the *Legionella* was not clear, it is necessary to assess the health risk of such hazard because rain cistern water was the primary water used by the island residents for showering, the most common form of personal hygiene practice.

2.2.2. Exposure Assessment

The *Legionella* in rain cistern water in the 2017 post-hurricane season was collected from the study of Jiang et al. (2020) [9]. The study collected water samples from 22 households' rain cisterns on the island of St. Thomas and detected *Legionella* using NextGen sequencing of 16S rRNA gene [9]. The concentrations of *Legionella* were reported as the fraction of total microbial population [9] in each cistern water sample. To convert the fraction of *Legionella* to a range of concentrations that may be encountered in cistern water, the total viable heterotrophic bacteria determined by heterotrophic plate counts (HPC) collected from rain cisterns in St. Thomas from an earlier study were used to represent a baseline distribution of cultivable bacteria present in cistern water from RHRW [18]. The HPC was determined by SMEWW 9215C on R2A media using 0.1 mL of diluted sample [18]. For HPC that was reported as a tabulated concentration in Crabtree et al. (1996) [19], the detection limit was used as the upper bound. The HPC data were fitted with a non-parametric cumulative distribution function (CDF) curve. A second CDF curve was also created based on the reported *Legionella* data in the 2017 study. Then, the concentration of *Legionella* (C_{Leg}) in cistern water was estimated as follows:

$$C_{Leg} = C_{bac} \times \%_{Leg} \tag{1}$$

where C_{bac} is the total viable bacterial concentration that is generated by randomly sampling from the CDF of HPC using the Monte Carlo sampling procedure; and $\%_{Leg}$ is the percent of *Legionella* in a cistern that is obtained by randomly sampling from the CDF of the fraction of *Legionella* detected in cisterns. The CDF for *Legionella* percentage values was left truncated at 0.

The human exposure to *Legionella* in this study was assumed to be through showering using cistern water only. Other aerosol exposures, such as through toilet flushing or water faucets, are also possible [20]. However, shower risk is considerably higher and is assumed to be a single daily exposure event. Since the data for *Legionella* are from water samples taken directly at the cistern, the additional growth of *Legionella* in indoor plumbing and showerheads was not considered (see Section 4 for additional details). In addition, no reduction in bacteria through physical chemical water treatment was included. The treatments of the cistern water on St. Thomas vary significantly from household to household ranging from a simple screen filter for litter removal to installation of reverse osmosis membrane filters. Since the use of high-end technologies for treating rainwater is rare based on our field obversions, such treatment removal of *Legionella* was not considered in this estimation. Only thermal inactivation through a conventional water heater used for heating shower water to 60 °C for a warm shower was included as an exposure scenario. According to Rogers et al., (1994) [21], *Legionella* inactivation at temperatures below 50 °C is negligible.

Two separate scenarios were considered in the exposure assessment: cold shower and hot (warm) shower. Cold shower assumes water drawn directly from the household cistern, at a temperature of 24–25°C. No mixing with water from heater and thus no thermal inactivation of *Legionella* was included. The warm shower scenario assumes a shower water temperature of 43.5 °C, which includes mixing a portion of cold water from the cistern directly and a portion of hot water from the water heater (60 °C). For this warm water shower scenario, the $\%_{hot}$ was calculated as follows:

$$T = \frac{(m_1 c_1 T_1 + m_2 c_2 T_2)}{m_1 c_1 + m_2 c_2} \tag{2}$$

$$\%_{hot} = \frac{m_1}{m_1 + m_2} \times 100\% \tag{3}$$

where m is the mass of water, c is the water heat capacity, and T is the temperature of each respective water stream.

Human infection occurs through inhalation of aerosols containing *Legionella* into the lungs, where the bacteria can replicate in the alveolar macrophages of the lungs [22]. Aerosols produced by common household shower heads have been found to contain *Legionella* when shower water is contaminated by the bacteria [23]. The concentration of *Legionella* in water aerosols was assumed to equal the concentration in the water as used in a previous study [3]. Preferential aerosolization of *Legionella* from bulk water may occur as indicated in previous reports [24]. However, the partition rate is highly variable and was not included here to reduce the uncertainty of the simulation. Inhalation of aerosols per minute of shower duration (mg/min), M_{AB}, is based on the volumetric flow rate of water from the shower head and aerosol deposition of mass in the bronchial and alveolar region according to previous experiments by Zhou et al., (2007) [25]. This mass deposition varies with shower temperatures, showerhead flow rates, and human breathing habits (oral inhalation or nasal inhalation). A uniformed distribution was adopted to include the range of water aerosol mass deposition to human lungs for different showerhead water flow rates and human breathing habits. The detailed data for deposition rates from

Zhou et al. are summarized in Supplementary Table S1. The randomly selected mass deposition rates from the uniformed distribution for warm shower U(0.036, 0.364) and cold shower U(0.001, 0.008) were used in the Monte Carlo simulation. The input parameters for exposure assessments are listed and defined in Table 1.

The total dose of *Legionella* inhaled and deposited in the bronchial and alveolar region of a person's lungs during exposure in a single shower event (CFU) was adapted from the model for showering established by Lim et al. (2015) [3] and was estimated as

$$Dose_{Leg} = \left(C_{Leg} \times \frac{(100 - \%_{hot}) + \%_{hot} \times 10^{-logT}}{100} \right) \times \frac{M_{AB}}{\rho_w} \times t_{sho} \quad (4)$$

where t is the duration of a single shower event (min), ρ_w is the density of water at a temperature T, $10^{-\log T}$ is the log-reduction of *Legionella* at temperature T, and $\%_{hot}$ is the amount of water from the water heater at 60 °C used for mixing to heat the shower water to a temperature of 43.5 °C.

Table 1. Input parameters for the Monte Carlo simulation to calculate daily and annual risk of *Legionella* infection.

Parameter Definition	Symbol	Point Estimate or Distribution	Unit	Source
Concentration of total heterotrophic bacteria measured in an untreated rainwater cistern	C_{bac}	Empirical distribution	CFU/mL	[19]
Percent of total bacteria DNA represented by *Legionella*	$\%_{Leg}$	Empirical distribution	unitless	[9]
Shower water temperature	T_{cold} T_{hot}	24.5 43.5	°C °C	This study
Percent of shower water that is heated to 60 °C by a conventional water heater	$\%_{hot}$	54	unitless	This study Equation (3)
Density of water	ρ_{hot} ρ_{cold}	991 997	g/cm^3 g/cm^3	This study
Thermal inactivation of *Legionella* at temperature T = 60°C	$logT$	3	unitless	[26]
Shower duration	t	Normal distribution (μ = 7.8, α = 0.02, left-truncated at zero)	min	[27]
Aerosol mass inhaled and deposited in the alveolar-bronchiolar region, hot shower	$M_{AB\text{-}h}$	Uniform distribution U(0.036, 0.364)	mg/min	[25]
Aerosol mass inhaled and deposited in the alveolar-bronchiolar region, cold shower	$M_{AB\text{-}c}$	Uniform distribution U(0.001, 0.008)	mg/min	[25]
Dose–response curve constant	k	0.0599	unitless	[28]
Number of exposures per year	n	365	per year	This study

2.2.3. Dose–Response Assessment

We adopted a *Legionella* dose–response model established through clinical trials on guinea pigs [29]. The endpoint of response is infection due to exposure to a known dose of *Legionella* through inhalation. An exponential model, shown in Equation (5), is the best fitted model to the clinical data based on the dose inhaled.

$$P_{inf} = 1 - exp(-k \times Dose) \quad (5)$$

where k is 0.0599 as determined by Armstrong and Haas based on the guinea pig trial data [28].

2.2.4. Risk Characterization

The dose calculations represent a dose inhaled for a single, daily shower event. Therefore, the response, P_{inf}, represents a daily risk in this case. Annual risk represents the risk of a single infection for the duration of one year, or in this case, 365 consecutive daily exposure events. This annual risk, P_{annual}, is calculated as

$$P_{annual} = 1 - \prod_{i=1}^{365} \left(1 - P_{inf}\right) \quad (6)$$

A sensitivity analysis was conducted in order to identify the model parameters that had the greatest contribution to uncertainty and variability in the results. The analysis was based on the warm or cold shower scenarios. Using 10,000 iterations of the input parameters, the Spearman rank correlation coefficient was computed in MATLAB in order to determine the strength and direction of a presumed monotonic relationship between input parameters and model output, where a coefficient of 0 indicates no influence of the variable on the results, and a value of + or − 1 indicates a positive or negative influence on the output. The sensitivity analysis was conducted for the following model input parameters: *Legionella* concentration (C_{Leg}), the total viable bacterial concentration (C_{bac}), the fraction of *Legionella* ($\%_{Leg}$) in the cistern water, exposure time (t), and mass inhalation rates (M_{AB}) for each of the scenarios.

3. Results

3.1. Household Survey

The household survey results showed that bottled water was the primary source of drinking water for the island residents after the hurricanes. Tap/rain cistern water was mainly used for non-potable purposes including washing hands, dishes, and food (Figure 1). Approximately 80% of the survey participants reported using tap/cistern water for showering. The survey did not differentiate between municipal piped tap water and cistern tap water. However, it is important to mention that most of the rural homes in St. Thomas were not connected to the municipal piped water system. Rain cistern water was plumbed into houses during home construction. Municipal tap water from desalination of seawater was piped to downtown commercial area (hotels, shops and restaurants) and residential communities near the city center. However, municipal water was unavailable for an extensive period after the 2017 hurricanes hit. Cisterns became the only source of tap water. The survey results confirmed the priority of conducting risk assessment of daily *Legionella* exposure through shower water.

Moreover, survey results also showed that most island residents perceived their water to be safe or somewhat safe (Figure 2), yet this perception differed by income group. Lower-income households (<$40K per annual) overwhelmingly felt that their water was only somewhat safe, while higher-income households (>$40K per annual) tended to be more confident about water safety. This difference in perception of water safety between the two income categories is statistically significant at the 10 percent level (p = 0.058) based on χ^2 tests. It should be noted that the median household income in the U.S. Virgin Islands is $37,254 based on the 2010 census, which is significantly lower than the median household income of $57,617 in the United States [30].

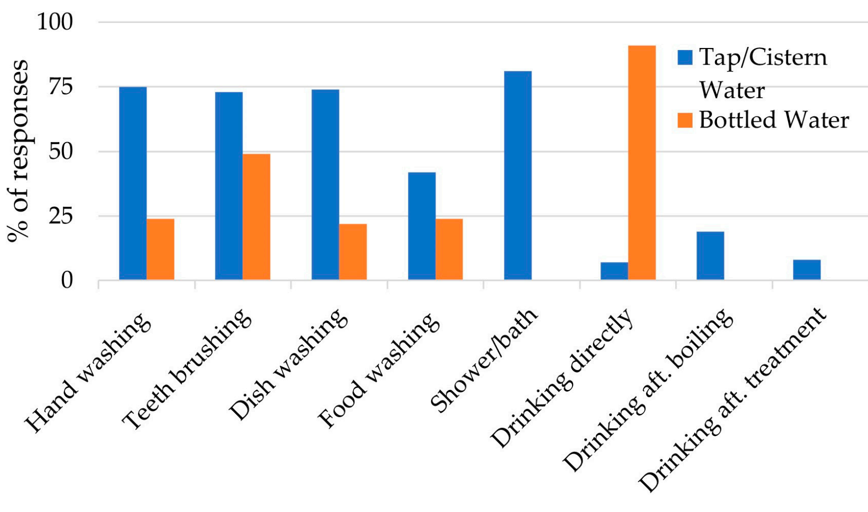

Figure 1. Survey responses (n = 107) of water usage in St. Thomas, Virgin Islands, after the hurricanes in 2017.

Survey results also indicate that the majority of the local residents (55%) were aware of the governmental advisory for boiling water (Figure 2), but less than half of residents (41%) believed that the government had done enough to let them know the safety of their water supply after the disaster. A similar fraction of the residents (43%) thought that the local and federal governments had done enough to provide them with safe sources of water (Figure 2). Satisfaction regarding government management of water after the disaster did not differ by income level. Results of χ^2 tests indicate that there were no significant differences between the two income groups in regard to the above questions (Figure 2).

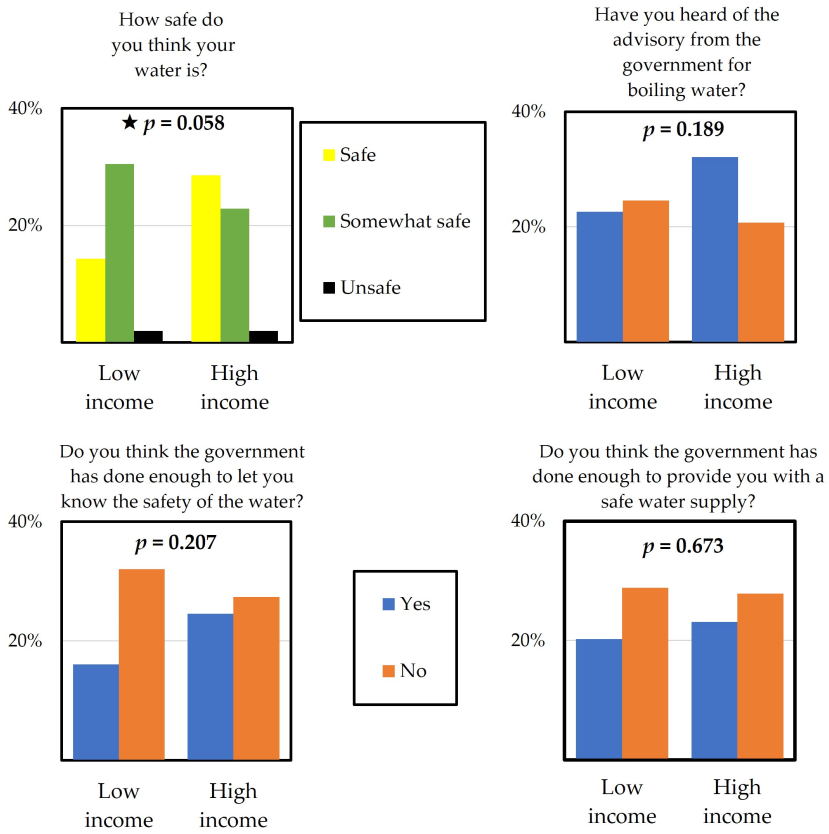

Figure 2. Survey responses to questions of perceived water quality and government role, grouped by income level (low and high). The statistical comparison of responses by the two income groups is indicated for each survey question. The ★ *p*-value indicates statistical significance defined in this study.

3.2. *Quantitative Microbial Risk Assessment*

The Monte Carlo simulation of *Legionella* concentrations in rain cisterns using the distribution of total viable heterotrophic bacteria (Figure 3a) and the fraction of *Legionella* bacteria among total bacterial community (Figure 3b) in cisterns showed that the *Legionella* concentration was distributed over a large range, with a median value of 8.8×10^3 CFU/L (Figure 3c). The daily risk of infection from aerosol inhalation during showering with rain cistern water was estimated by randomly sampling (10,000 iterations) for the parameters from Table 1 and simulated *Legionella* concentration in rain cisterns (under curve of Figure 3c). The daily risk of infection varied and had a median value of 3.5×10^{-6} for a cold shower and approximately 100-fold higher for a warm shower (Figure 4). For both scenarios, outliers approached a risk value of 1. The median annual risk based on theory of independence was estimated as 1.3×10^{-3} for cold showers and 2.5×10^{-2} for hot showers.

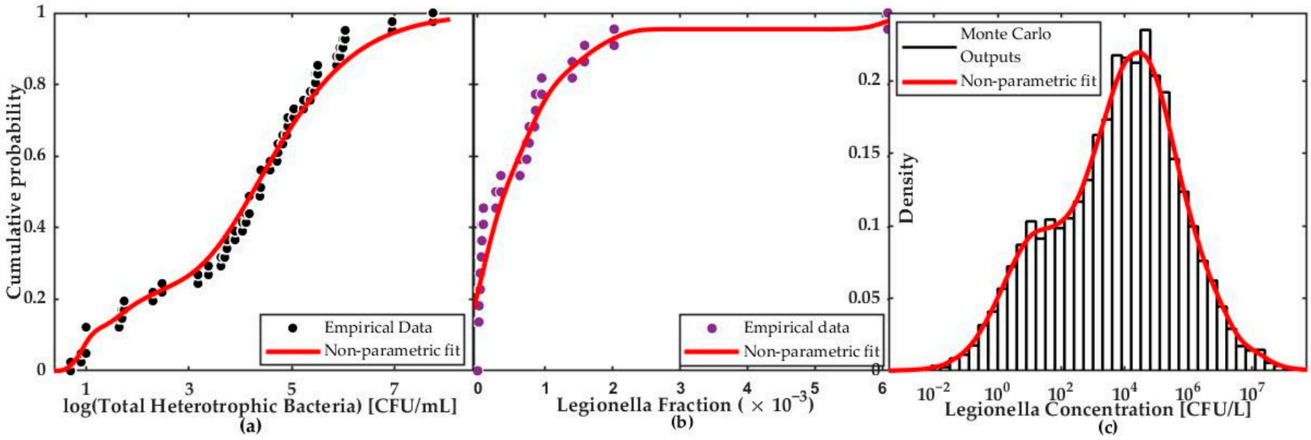

Figure 3. Non-parametric cumulative distribution fits of total heterotrophic bacterial data in cisterns (**a**), the fraction of *Legionella* bacteria among total microbial community in cisterns (**b**), and Monte Carlo simulation outputs of *Legionella* concentration in cisterns of St. Thomas, Virgin Islands (**c**). Empirical data for (**a**) and (**b**) were collected by Crabtree et al. (1999) [19] and Jiang et al. (2020) [9], respectively.

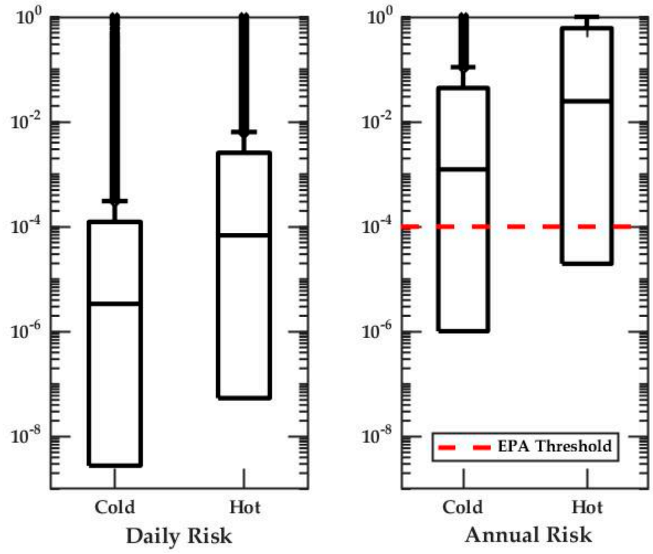

Figure 4. Boxplots of daily and annual infection risk based on aerosol inhalation of *Legionella*-contaminated cistern water during cold and hot shower scenarios. The median is shown as the mark inside the box, 25th and 75th percentile values are the bottom and top edge of the box, respectively.

The results of the sensitivity analysis (Figure 5) indicated that for both scenarios, the concentration of *Legionella* in the cistern was the most influential model parameter (C_{Leg}) (ρ = 0.99) regardless of the shower water temperature. This was further broken down to fraction of *Legionella* among the total microbial community ($\%_{Leg}$, ρ = 0.60) in the cisterns and total heterotrophic bacterial counts (C_{bac}, ρ =0.65). Spearman rank coefficients indicated that shower duration (t) and aerosol mass deposited in the alveolar-bronchiolar region (M_{AB}) were not sensitive input parameters in influencing the model output (ρ is close to 0).

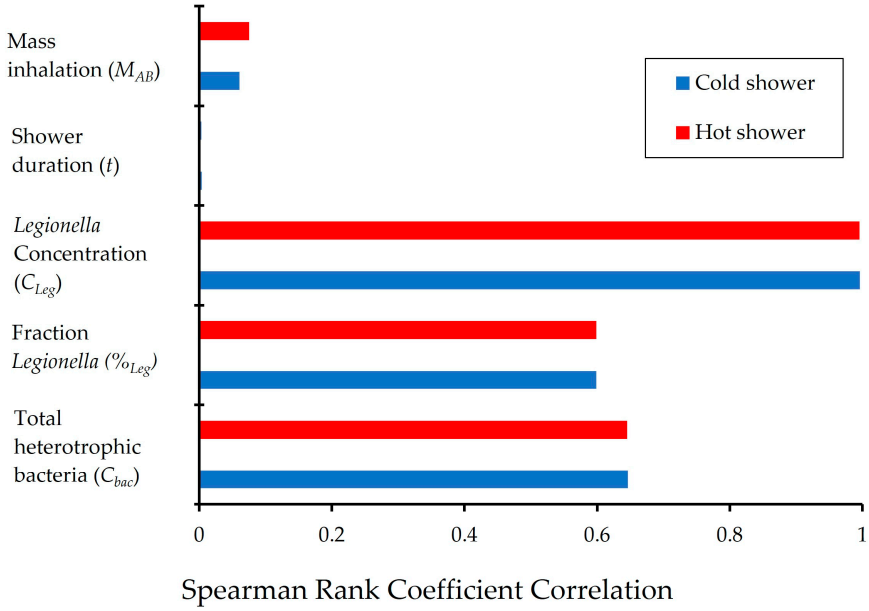

Figure 5. Sensitivity analysis for annual infection risk by scenarios and input parameters as defined in Table 1.

4. Discussion

4.1. Legionella Risk Post Hurricanes

Legionella risk in captured rainwater or recycled water has been discussed in the previous studies [2,20,31]. Yet not much is known of the impact of the hurricanes on rain cistern water quality and associated microbial infection risk. High occurrence of *Legionella* spp. discovered in rain cistern water on St. Thomas, VI, post hurricane season promoted the investigation of infection risk of this ubiquitous pathogen. The QMRA results indicated that *Legionella* risks post hurricanes were not significantly higher in comparison with two previous studies estimating the risks in captured rainwater (Table 2). The median annual risk of the current study is slightly higher than the value reported by Ahmed et al. (2010) [32] but is an order of magnitude lower than the risk estimated by Hamilton et al. (2017) [33]. At first glance, the comparative results suggest that hurricanes do not appear to increase the *Legionella* risk through shower water in the disaster-stricken region. However, discussions are warranted to better understand the contribution of the current study to the knowledge field and the limitation of the risk estimation.

Table 2. Comparison of simulated *Legionella* concentrations in rain cisterns and estimated annual health risk from showering using cistern water with literature values of similar studies.

	25th Percentile	Median	75th Percentile	Unit	Source
Legionella Concentration	3.0×10^{3}	9.0×10^{4}	1.3×10^{6}	Gene copies/L	[20]
	4.0×10^{3}	8.5×10^{4}	3.1×10^{6}	Gene copies/L	[20]
	1.6×10^{4}	2.5×10^{4}	1.0×10^{5}	Gene copies/L	[2]
	2.5×10^{2}	8.8×10^{3}	1.2×10^{5}	CFU/L	This paper
Annual Risk		1.0×10^{-2}		pppy (per person per year)	[33]
	1.1×10^{-3}		3.1×10^{-3}	pppy (per person per year)	[32]
	2.9×10^{-6}	4.5×10^{-3}	2.1×10^{-1}	pppy (per person per year)	This paper

The *Legionella* concentrations in cistern water were estimated based on the fraction of *Legionella* spp. identified among the total bacterial community by NextGen sequencing of 16S rRNA gene [9]. The underlying assumption used in this study is that the fraction of *Legionella* spp. among the total microbial community in cistern water is the same regardless of assay methods (genome-based versus culture-based). Therefore, combining the $\%_{Leg}$ with C_{bac} (CFU/L) in cisterns could estimate the concentration of viable *Legionella* spp. in the water. However, a direct comparison between the sequencing-based approach (# *Legionella* spp. gene copies/# total gene copies) and the culture-based methods (# *Legionella* spp. CFU/# total CFU) has not been performed. No relationship between HPC counts and *Legionella* concentration or prevalence currently exists. Therefore, the assumption used here is an important limitation to the estimation of viable *Legionella* spp. in the water. Past research in estimating the risk of *Legionella* relied on PCR-based approaches to quantify *Legionella*-specific genes, which assumed the genetic fragments of *Legionella* equal to infectious *Legionella*. Therefore, a PCR-based approach is also not a perfect solution to identify infectious *Legionella*. Despite the limitations of 16S rRNA gene based NextGen sequencing, they are powerful data to understand the microbial composition in water. The HPC baseline data served to model a range of probabilistic values of *Legionella*. High HPC counts can result from cistern stagnation, lack of disinfection, or inadequate temperatures, which can also lead to *Legionella* growth [6]. The HPC values, therefore, reflect the condition of the cisterns, which bounds the range of *Legionella*. The HPC data were also used to correct for overestimation of infectious *Legionella* by the genetic method [2,20,34].

Significant progress has been made in recent years on improving the method for culture detection of *Legionella* in drinking water and the water distribution network [35–37]. The *Legionella* monitoring methods by ISO 11731 and the U.S. CDC also provide a reliable framework for culturing *Legionella* in drinking water [38,39]. However, these methods are still labor intensive and time consuming in comparison with genetic-based methods, which limit their implementation in a field study in the absence of a functional microbiology lab during the post-hurricane period. Nevertheless, culture-based methods should be considered whenever possible. Future studies should also carry out side-by-side investigations of culture-based vs. genetic-based *Legionella* detection in drinking water under various conditions. The outcomes of these comparisons will improve our understanding on the limitations of genetic-based methods and develop credible correlations between the two different monitoring approaches to improve future risk estimations.

A comparison of the range of *Legionella* concentrations from this study with those from other related studies is shown in Table 2. Hamilton et al. (2016) reported *Legionella* concentrations between 3×10^3 and 3.1×10^6 gene copies/L by qPCR based on 134 roof-top harvested rainwater samples collected in Southeast Queensland, Australia. Ahmed et al., (2014) [2] reported *Legionella* concentrations by qPCR to be between 1.6×10^4 and 1.0×10^5 copies/L, with a median concentration of 8.8×10^3 copies/L in 72 rainwater tank samples also from the same region in Australia. The simulated concentrations in this study ranged between 2.5×10^2 and 1.2×10^5 CFU/L, which are roughly one order of magnitude lower than qPCR results by Hamilton et al. (2016) [20]. The high end-values in this study are similar to the report of Ahmed et al. (2014) [2]. However, those previous studies reported concentrations as gene copies/L, whereas in this study the concentrations are CFU/L to represent viable counts. In drawing comparisons between the *Legionella* concentrations, it should be noted that qPCR results may overestimate the viable *Legionella*. Previous studies concluded that qPCR is useful for rapid detection and risk assessment, but often detect higher amounts than by culture methods, especially from water tanks which have been disinfected [34,35]. On the other hand, Hamilton et al., (2017) [33] noted in a study on seasonality and RHRW premise plumbing pathogens that culture-based methods can underestimate concentrations due to the presence of viable but nonculturable (VBNC) cells. These distinctions are important in interpreting risk results as conservative or liberal estimates. Nevertheless, in the absence of a better method to estimate the infectious *Legionella*, the genome-based approach in combination with the culture-based assessment of total HPC presents a useful method to estimate the viable *Legionella* for risk quantification.

Comparison of the annual risks for combined warm and cold shower scenarios with those by Hamilton et al. (2016) [20] and Ahmed et al. (2014) [2] revealed a much wider range of estimated risks than those in the previous studies. This is due to a very large variability of the viable HPC detected in different cisterns. This variability could be attributed to the unevenness in cistern maintenance on the island. Alternative, the SMEWW 9215C method for HPC could also generate viable results because it uses a very small volume of water (0.1 mL) that could hit or miss of particle bound bacteria. The fractions of *Legionella* detected also varied among cisterns. Some of the cisterns may be impaired by the hurricane-induced storms as noted in the study by Jiang et al. (2020) [9]. During the sample collection effort, we covered as a broad of an area on the island as we could, but we did not have a pre-existing knowledge of income level of the households at the time of sample collection. Future study design should consider water quality assessment across different income levels. It should also be noted that both previous studies [2,20] equated qPCR genome copies with the viable CFU in the dose–response model, which may overestimate the risk of infectious *Legionella.*

Moreover, the uncertainty of the dose–response model is not only limited to ambiguity of the infectious *Legionella* concentration. Both this and previous studies adopted a dose–response model developed using guinea pigs rather than humans. Human infectivity requires clinical trials of exposing humans to *Legionella*, which are highly unlikely due to the ethical concerns. Research and data on *Legionella* infectivity in humans would be useful to further improve the risk assessment. Detailed epidemiology investigations of human exposure to contaminated water and health outcomes could be useful data to refit the human dose–response model.

The results of this study suggest investigations of HPC in cisterns can significantly further the understanding of the water quality and *Legionella* risk since HPC can reflect the condition of cisterns. The HPC is relatively simple to perform, but an identical HPC method should be used for comparison across seasonal and spatial samples. Moreover, identification of *Legionella* to the species level could improve our understanding of the pathogenic vs. non-pathogenic species in water. Additionally, our model does not account for additional *Legionella* that may be growing in the plumbing and showerhead due to biofilm release or the presence of amoeba [36]. There have also been reported differences in *Legionella* concentrations between the cistern and the in-home faucet due to fluctuations in water age and in uncertain chlorine residuals from chlorinated cisterns, causing pipes to act as *Legionella* reservoirs [31]. The growth in the plumbing and showerhead is especially important in stagnant water when the home is abandoned during a time of disaster. However, this situation was not applicable to this study. All households sampled during this study were occupied during the hurricane season because evacuation from an isolated island far from the mainland was more challenging.

It is unclear whether the hurricanes exacerbated the *Legionella* risk due to the lack of historical data on the cistern water quality for the Virgin Islands. The impact of the hurricanes on the water safety in the Virgin Islands may be reflected through the lack of access to chlorine or other disinfection methods after the disaster struck. Regardless of the source of the *Legionella*, the outcomes of this risk analysis suggest the need of a routine water quality monitoring and maintenance program to reduce the risk of *Legionella*. Since rain cisterns are considered private property, a public education program should be put in place to promote the self-monitoring and routine cleaning of the cisterns.

4.2. Risk Perception and Risk Management

Although the majority of islanders perceive their water to be safe or somewhat safe for household uses based on the on-site surveys, the QMRA results indicate otherwise. The median annual risk values for both warm and cold showers exceed the EPA recommended threshold of 10^{-4} pppy (per person per year). The perceived water safety may be related to water use patterns because over 90% residents answered that they used bottled water for drinking. Washing water is considered "less risky", and the aerosol transmission of pathogen through shower mist is not well known. We found that the perceived risk was divided by income levels; twice as many high-income participants deemed their water "safe", whereas most low-income participants answered only "somewhat safe." These low-income families may not have access to treatment methods such as chlorine, UV light, or filtration to disinfect their cistern water when electricity is compromised, which was the case during and after the hurricanes. They live in older, poorly maintained housing communities with aging water infrastructure and lack of economic resources to perform routine upkeep of the cisterns. They rely more on the cistern water that is free of charge, especially in times of crisis.

The large discrepancy between the risks estimated based on the QMRA and the perception of adequate water quality suggests that the prevalence of *Legionella* in cistern water and its risks are not always apparent. Public education and routine monitoring programs are necessary for public health protection. HPC monitoring could be a simple solution for reducing the risk of *Legionella.*

Temperatures in the range of 20 °C (68 °F) to 45 °C (113 °F) favor the growth of *Legionella*. Therefore, finding *Legionella* in the cisterns on the Virgin Islands, where the temperature is around 24 °C year-round, is not surprising. *Legionella* can be inactivated when temperatures rise above 50 °C [37]. Heating water to 60 °C is effective at reducing *Legionella* in shower water. However, thermal inactivation is only effective on the heated portion of the water, while *Legionella* may still be present in the cold-water portion mixed to achieve a desired final shower water temperature. In fact, our results showed the warm shower risk was higher than the cold-water risk. This is because, as shown in Table 1, a hotter shower produces more aerosols per minute in the shower stall resulting in a higher concentration of aerosols within the shower stall. A shower temperature that is reasonably hot and much higher than room temperature causes a chimney effect in the shower stall, in which aerosols are carried upward with convective flow [25]. A higher aerosolization rate therefore results in more aerosols inhaled for the duration of the shower. The increase in aerosolization between warm- and cold-water showers is up to a factor of 100 and is directly proportional to the increase in the dose for each shower event. Although the high temperature provided by a conventional water heater is adequate for heat inactivation of the bacterium, it is not enough to reduce the risk. The amount of hot water needed from the water heater to mix with ambient temperature water is low since the ambient water temperature in the Virgin Islands is relatively warm due to the warm, tropical climate. The amount of inactivation for a reduction in the concentration of *Legionella* is small in comparison to the increase in aerosolization.

To better mitigate risk associated with RHRW household use, routine cleaning of cisterns and flushing of premise plumbing should be planned on a fixed schedule to reduce the opportunistic pathogens in shower water. In anticipation of an increase in *Legionella* prevalence in the rainwater by hurricane-induced storms, infrastructure damage (i.e., connection of underground cisterns with surface floodwater), and loss of power, stocking up on chlorine tablets before hurricane season and organizing quick transport and distribution of chlorine tablets immediately after the hurricanes to the disaster area could be helpful to reduce the waterborne and water-related illness. Other water treatment methods, such as UV and reverse osmosis membrane filtration, are less effective in the case of loss of power from electricity grids. Other types of fuel (no natural gas, gasoline is in significant shortage) are hard to access on the islands during and after the hurricanes.

Public education is an important tool to enhance the awareness of *Legionella* risks. Currently, there is no routine monitoring program, cistern water quality standard, nor uniformed recommendations for cistern management on the islands. Cisterns are considered private property; the governmental "interference" on the cistern water was not embraced by the local residents. There is a general mistrust of governmental agencies in advising of water quality and water use. Such mistrust and dissatisfaction among the public was reflected in our survey results, regardless of the household income levels. Public outreach programs using the research outcomes from objective QMRA could instill trust in the local residents about the governmental role in cistern water management. Development of transparent public policy with sufficient time for residents' input and buy-ins are necessary to improve the relationship between the government and the citizens. This trust is critical to improve cistern water quality through monitoring, routine cleaning, and addressing technological treatment requirements. Cistern water quality management is above and beyond the hurricane seasons.

5. Conclusions

A QMRA was carried out to estimate the *Legionella* risk through daily showering using untreated cisterns water on St. Thomas, Virgin Islands, following the 2017 hurricane season. The results showed that

- The estimated *Legionella* annual risks exceed the EPA guideline of 10^{-4} pppy.
- The model outputs are sensitive to the concentration of *Legionella* in individual cisterns, which was highly variable and uncertain due to unevenness in the cistern water management on the island.
- There is a disparity between perceived risk and QMRA estimated risk of cistern water, suggesting the *Legionella* risk associated with the shower water is not apparent to the local residents.
- Moreover, the perception of water safety is divided by income group. Most people in the high-income group considered their water "safe", while people in the low-income group only considered their water "somewhat safe".
- Both income groups believed that the government could have done more to help them understand the water quality and water safety at the time of natural disaster. A fact-based public education program should be developed to bring residents onboard to manage the cistern water quality collaboratively.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4441/13/7/889/s1, Table S1: Aerosol inhalation deposition rates in the alveolar-bronchiolar region of the lungs. UCI Survey: Household Water Resource Survey.

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