Microbial Characterization, Factors Contributing to Contamination, and Household Use of Cistern Water, U.S. Virgin Islands

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ABSTRACT: Households in the United States Virgin Islands (USVI) heavily rely on roof-harvested rainwater stored in cisterns for their daily activities. However, there are insufficient data on cistern water microbiological and physicochemical characteristics to inform appropriate cistern water management. Cistern and kitchen tap water samples were collected from 399 geographically representative households across St. Croix, St. Thomas, and St. John and an administered survey captured household site and cistern characteristics and water use behaviors. Water samples were analyzed for Escherichia coli by culture, and a subset of cistern water samples (N = 47) were analyzed for Salmonella, Naegleria fowleri, pathogenic Leptospira, Cryptosporidium, Giardia, and human-specific fecal contamination using real-time polymerase chain reaction (PCR). Associations between E. coli cistern contamination and cistern and site characteristics were evaluated to better understand possible mechanisms of contamination. E. coli was detected in 80% of cistern water samples and in 58% of kitchen tap samples. For the subset of samples tested by PCR, at least one of the pathogens was detected in 66% of cisterns. Our results suggest that covering overflow pipes with screens, decreasing animal presence at the household, and preventing animals or insects from entering the cisterns can decrease the likelihood of E. coli contamination in USVI cistern water.

KEYWORDS: U.S. Virgin Islands, cistern, water quality, fecal contamination, enteric pathogens

1. INTRODUCTION

Globally, collection and storage of roof-harvested rainwater (RHRW) is a common practice to capture rainwater for potable and nonpotable use. RHRW can be used due to general water scarcity issues, such as long periods of drought or lack of freshwater resources, lack of desalination or freshwater treatment plants and piped networks, intermittent water supply issues, or simply as a sustainable water use practice. RHRW is used more frequently in certain parts of the world, such as sub-Saharan Africa, Australia, and other island settings with sufficient rainfall and roof catchment systems to collect volumes of water. On islands, freshwater sources are primarily limited to rainfall or ground water (if available), but islands often struggle with ground water contamination and are predicted to increasingly struggle with water scarcity due to climate change. Although sustainable desalination technologies to produce freshwater have increased, there are capital and logistical constraints for smaller islands to implement large-scale plants. Therefore, RHRW remains a robust and sustainable freshwater source in many settings. However, because catchment systems and cisterns are open to the environment and vulnerable to damage from extreme weather events such as hurricanes, RHRW is susceptible to microbial contamination.

There are an estimated 40,648 homes across the Virgin Islands of the United States (USVI), of which 95% collect and store water in cisterns. Less than 32% of USVI households have access to piped water from the local water utility, and households with access may also collect RHRW in cisterns for household use. While trucked water is available for filling cisterns, it is expensive and energy-intensive. In USVI, cisterns are large-volume water storage structures; a gutter system funnels the rainwater from the roof into the cistern. Most USVI cisterns are cement structures located beneath the home.

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Some households have above-ground polypropylene cisterns detached from the household, but these above ground systems retain the same rooftop catchment and gutter conveyance system that extends to the cistern. Because the U.S. Environmental Protection Agency (USEPA) and local USVI authorities do not regulate private water sources (e.g., ground water wells and cisterns), there are no water quality requirements for cistern water, placing the onus on USVI residents to protect and treat their own water supply.

Natural disasters may exacerbate water supply and security issues in USVI by causing extensive damage to infrastructure, including RHRW catchment and storage systems. In 2017, Hurricanes Irma and Maria passed through the Caribbean, causing localized flooding and extensive damage to residential buildings, schools, hospitals, and other critical facilities across the islands. Damage to municipal infrastructure resulted in a limited supply of potable water and required residents to solely rely on cistern water for drinking and other uses, if available. Despite the availability of guidance about cistern use after an emergency, only 12.5% of households that had access to cistern water after the storms heard public messaging about cistern cleaning and water treatment. Cases of melioidosis and leptospirosis in the weeks and months following the storms highlighted the impact of the hurricanes on infrastructure and water supply.

Considering the reliance on cistern water across USVI, the inherent vulnerability of catchment systems and cisterns to microbial contamination, a comprehensive study was conducted to ascertain cistern water microbial contamination, household water use habits, and associated site and cistern characteristics to inform recommendations about cistern water protection and routine cistern management. Previous studies conducted in USVI between 1987 and 1997 found that cisterns were contaminated with total and fecal coliforms, zoonotic pathogens such as Salmonella and Cryptosporidium, and environmental pathogens such as Pseudomonas aeruginosa and Legionella. These studies demonstrated the vulnerability of USVI cisterns to fecal and microbial contamination, but they were limited in size and scope and did not investigate factors contributing to contamination. Further, no known data exist on other environmental pathogens, such as Leptospira and Naegleria fowleri, despite documented cases in USVI in recent years.

The objectives of this study were to investigate USVI household cistern water microbial contamination and the mechanistic drivers of contamination from (i) a survey of cistern and household characteristics, as well as cistern water use and treatment practices; (ii) a characterization of bulk cistern and kitchen tap water physicochemical water parameters and fecal contamination as indicated by culturable Escherichia coli and the molecular human-specific fecal marker (Bacteroides HF183); (iii) a characterization of cistern pathogen contamination through molecular testing for select waterborne pathogens (Salmonella, Cryptosporidium, Giardia, Naegleria fowleri, and pathogenic Leptospira); (iv) an assessment of associations between cistern microbial contamination and cistern and site characteristics; and (v) a multivariate model to identify associations between E. coli contamination and cistern and site characteristics to identify recommendations for the prevention of cistern water contamination.

2. MATERIALS AND METHODS

2.1. Study Design and Data Collection. Between July 15 and August 16, 2019, the study enrolled 399 households across the three main islands comprising the USVI territory. The study was powered to investigate the prevalence of E. coli in cisterns among households across USVI with the following populations: St. Croix = 50,601; St. Thomas = 51,634; and St. John = 4170 people. Sampling units were households per island, not individuals. Additional details on sample size estimates can be found in Text S1. This study was deemed as a nonresearch public health evaluation under 45 CFR 46.102(I) by the U.S. Centers for Disease Control and Prevention (CDC) and USVI Department of Health Institutional Review Board (IRB) committees.

Statistical analyses were conducted at the household level (not individual), and to account for potential geographically related differences in water quality, all multivariate statistical analyses were clustered by islands. Households were recruited through two processes to obtain a geographically representative sample set across the three islands (N = 399 total enrolled households). One enrollment process included asking households previously enrolled in a leptospirosis seroprevalence survey (N = 114 households). Briefly, after excluding census blocks with 0 population and those comprised of tourist housing, selected blocks were chosen at random. The other enrollment process consisted of randomized census block enrollment using 2010 United States Census Bureau (i.e., census blocks) data. Households were randomly selected from census blocks that were not visited during the leptospirosis seroprevalence study (N = 285 households). Up to 8 households per census block were enrolled before moving to the next census block to decrease any clustering biases. Households were enrolled by starting at the center of a census block and visiting every 3rd house to inform residents of the study and recruit. A coin toss determined a left or right direction when enumerators reached the end of a block. An eligible household invited to participate was defined as a USVI residence that was (1) inhabited at the time of the survey and (2) had an accessible cistern. Eligible residences included single-family homes and multiunit apartment or condominium buildings, regardless of whether the home was owned by the surveyed resident, provided the cistern was accessible. All households were assigned a unique, anonymous household ID.

Field teams for survey administration and water collection were comprised of USVI Department of Health (USVI DOH), Love City Strong (a nonprofit based in St. John, USVI), and CDC staff. Information was collected about the number, ages, and health statuses of household members. Respondents were asked which water sources (i.e., cistern, bottled, and municipal) were used as the primary or supplementary sources for a variety of household activities and what types of water treatment methods or technologies they employed. Data were collected about the components of the catchment system (e.g., roof and gutters) and their condition as well as the cistern and its condition (e.g., cistern dimensions and material, water volume, damage, screens and overflows, and presence of foreign objects). Information was also collected about the surrounding household environment (e.g., animal sightings, location of septic systems, and vegetation/terrain) around catchment and cistern access points. Whenever possible, cistern and site characteristics and measurements (e.g., cistern dimensions, presence of overflow screens, etc.) were directly
observed and/or measured by the field teams. Survey responses and information were collected using Epi Info (CDC, Version 7) on handheld tablets.

2.2. Water Sample Collection. Grab water samples (300 mL) from each household's cistern and kitchen tap were collected for microbiological and physicochemical water quality testing. Cistern water was collected directly from the cistern opening hatch using a retractable rope and a 500 mL polypropylene beaker. The beaker was disinfected between each use by spraying with 70% ethanol and drying with lint-free paper towels. An aliquot for testing fecal indicator bacteria (FIB), total coliforms and *E. coli*, was poured from the beaker into a 300 mL Whirl-Pak bag containing sodium thiosulfate to neutralize any chemical disinfectant present. The remaining beaker volume was used for on-site measurement of temperature, pH, and electrical conductivity using a Hach Pocket Pro + Multi 2 Tester (Hach, Loveland, CO). Total and free chlorine residuals were measured using a Hach DR300 Pocket Colorimeter regardless of whether a household reported treating with bleach. Turbidity was measured using a Hach 2100Q Portable Turbidimeter. Grab samples of tap water were collected directly from kitchen faucets for the same testing as grab cistern samples. Before sample collection, the outside of the tap was disinfected with an alcohol wipe and then flushed for 30 s. Households serviced by the Virgin Islands Water and Power Authority (WAPA) can switch between using WAPA water or cistern water, and households using WAPA water at the time of the visit were recorded. All water quality measurements were recorded on the chain of custody forms and on the survey forms to allow for cross-checking double entries. A field blank was collected by one sampling team each day by filling a Whirl-Pak bag with an aliquot of sterile water. A subset of households (*N* = 47) agreed to collection of large-volume cistern water samples by dead-end ultrafiltration (DEUF) for pathogen and fecal indicator molecular analyses. One hundred liters of water were pumped directly from the cistern and concentrated by ultrafiltration on-site using commercially available dialysis filters (REXCEED-25S, Asahi Kasei) as described previously.9,30

Samples collected for microbiological testing on St. Croix were placed in a cooler over ice packs and transported to the USVI DOH laboratory on the day of collection. Samples collected on St. Thomas and St. John were held overnight at 4 °C and flown to St. Croix the following morning in insulated coolers containing ice packs. All samples were received at the USVI DOH laboratory for processing of ultrafilters and quantification of culturable FIB within 24 h of collection. Households received their culturable FIB results from the USVI DOH within 1 week. Households that also provided large-volume samples received molecular testing results within 6 months.

2.3. Fecal Indicator Bacterial Quantification and DEUF Sample Concentration. Total coliforms and *E. coli* were enumerated from 100 mL samples using IDEXX Colilert-18 media and the Quanti-Tray/2000 most probable number (MPN) methodology (Westbrook, ME), according to Standard Methods for the Examination of Water and Wastewater Method 9223.31

The DEUF ultrafilters were backflushed with 500 mL of a 0.5% Tween 80/0.01% sodium polyphosphate/0.001% Antifoam Y-30 solution following methods described previously.29 The recovered sample concentrates were pelleted by centrifugation at 4000 × g for 15 min. The pellets were resuspended in 0.01 M PBS, preserved in an equal volume of UNEX lysis buffer (Microbiologics MR0501), and stored at 4 °C until shipment on ice to CDC in Atlanta, GA for molecular analyses. At CDC, preserved samples were subjected to nucleic acid extraction as previously described.32 An extraction blank consisting of nuclease-free water was included with each set of extracted samples (*N* = 12 samples).

2.4. Molecular Analyses of Pathogens and Fecal Indicators. Real-time polymerase chain reaction (PCR) with a hydrolysis probe was performed for the detection of *Salmonella* (ttg gene), *Cryptosporidium* (18S rRNA), *Giardia* (185 rDNA), *Naegleria fowleri* (ITS), pathogenic *Leptospira* (LipL32 gene), and a marker of human fecal contamination, *Bacteroides* HF183 (HF183) (Table S1).33–39 Although HF183 is not a pathogen, this human-specific fecal marker is useful in determining the prevalence of human fecal contamination that could contain enteric pathogens in cisterns (e.g., via sewage or septic intrusion). Five microliters of template DNA were analyzed in triplicate in 50 μL PCR reaction volumes for all assays except *Leptospira*, which used 20 μL reaction volumes. Primer and probe concentrations, thermal cycling temperatures, and applicable PCR facilitators were followed according to the assay citation. Standard curve information is described in Table S1 and Text S2. TaqMan exogenous internal positive control reagents (Applied Biosystems) were included to evaluate assay efficiency and the presence of PCR inhibition. Amplification was performed in a 7500 real-time PCR system using TaqMan Environmental Master Mix 2.0 (Applied Biosystems) for all assays except *Leptospira*, in which PerfeCTa qPCR ToughMix, Low ROX (Quanta Biosciences, Gaithersburg, MD) was used. Three no template controls were included with each instrument run. An analyte was scored as positive if amplification occurred at a quantification cycle threshold (Cq) below 45 for at least two replicates.

2.5. Data Analysis. Culturable total coliform and *E. coli* data were dichotomized as present in samples with concentrations ≥1 MPN/100 mL. Samples with concentrations <1 MPN/100 mL were assigned half the detection limit at 0.5 MPN/100 mL, and samples with concentrations >2419.6 MPN/100 mL (the upper limit of quantification) were assigned 2420 MPN/100 mL. Associations between *E. coli* presence and household demographic characteristics, cistern and site characteristics, ground animal sightings, and physicochemical water quality parameters (pH, temperature, turbidity, conductivity, and free and total chlorine residuals) were analyzed using chi-square tests, Kruskal–Wallis (Wilcox–Wallis rank sum) for multi-categories or Mann Whitney U tests for two categories, or Spearman rank correlation tests for continuous variables. Total coliform and *E. coli* concentrations and turbidity values were log_{10}-transformed for analyses. Statistical tests were considered significant for *p*-values <0.05.

To investigate a mechanistic model for cistern contamination, we used a linear mixed effect model with log_{10} *E. coli* concentration as the outcome variable for all models. Predictor variable selection was based on a priori knowledge, the published literature,40 and associations identified via bivariate analyses of household, physical, and water quality characteristic variables, including groupings. Missing data were addressed using multiple imputation chained equations with 50 iterations (mice package, R software version 4.0.2).41 Household island clustering was treated as a random intercept in the models. Potential cofounders controlled for in the models included water quality parameters that impact microbial presence or
disinfection effectiveness (e.g., free chlorine and turbidity) and household ownership as proxy for cistern maintenance. Grouped variables were selected into the comprehensive model when the variance inflation factor (VIF) < 5 and p-values < 0.10. Backward elimination was then conducted until an improved log-likelihood estimate was found, VIF < 5, and

Figure 1. Distribution of mean log_{10} E. coli concentrations (MPN/100 mL) from cistern (top) and household tap water (bottom), overall and by USVI Island (STX = St. Croix, STT = St. Thomas, STJ = St. John). Samples with E. coli concentrations < 1 and > 2419.6 MPN/100 mL were assigned as 0.5 and 2420 MPN/100 mL, respectively, before log_{10} transformation. 1 log_{10} = 10^1 MPN/100 mL. Dashed lines indicate detection limits. Ninety-six total coliform samples (26%) and twelve E. coli samples (3%) exceeded the upper limit of quantification. Tap water samples from households serviced by WAPA (N = 19) were excluded from descriptive statistics.
remaining variables in the model were interpretable. Interaction terms between free chlorine and turbidity were assessed, and the height of overflow pipes from the ground and the slope of the ground were assessed due to their potential mechanistic association but neither proceeded into the final model. Average marginal effects were calculated using the mean of these unit-specific partial derivatives over the designated sample (margins package, R software). Pooled estimates were obtained based on linear mixed-effect model fit from original and imputed data for restricted maximum likelihood (REML) estimates (lme4 package, R software). Sensitivity analyses on free chlorine residual categorization was performed to determine an optimal REML fit.

3. RESULTS

3.1. Household Demographics. Of the 399 participating households, 78% (n = 305) were single-family homes and 86% (n = 337) of all homes were owned by the study respondents (Table S2). The mean household size was 2.69 persons, and 2% (n = 6) of households included a pregnant woman. Forty-seven percent (n = 178) of households included individuals with a chronic health condition defined as asthma, cancer, diabetes, or tobacco use, 7% (n = 24) had members that experienced gastrointestinal symptoms (e.g., nausea, stomach- ache, or diarrhea), and 12% (n = 41) had members that experienced respiratory symptoms (e.g., cough) in past 2 weeks.

3.2. Characteristics of Cistern and Tap Water. 3.2.1. Cistern Characteristics and Physicochemical Water Quality. Cistern characteristics and physicochemical water quality across all islands are described in Table S3. Physicochemical water quality summaries for tap water samples are presented in Table S4. The tap water results from 19 households were excluded from descriptive statistics because WAPA water was being used at the time of the visit. The mean turbidity levels of cistern and tap water were 1.4 NTU (±4.3 [SD]) and 1.2 NTU [1.8], respectively. Free and total chlorine residuals were detectable (levels ≥0.02 and <2.0 mg/L) in 48% (171/353) and 61% (215/352) of cisterns, respectively, and the median concentrations were 0.03 and 0.04 mg/L, respectively. Free and total chlorine residuals were detectable in 45% (158/355) and 60% (215/354) of tap water samples, respectively, and the median concentrations were 0.04 mg/L for each.

3.2.2. Total Coliforms and E. coli. Total coliforms and E. coli were detected in 95% (350/370) and 80% (296/370) of cistern water samples, respectively. Total coliforms and E. coli were detected in 83% (308/372) and 58% (217/372) of household tap water samples, respectively. Mean \( \log_{10} \) total coliform concentrations were 2.52 (±0.12 [SD]) and 1.72 (±0.25) \( \log_{10} \) MPN/100 mL for cistern and tap water, respectively (Figure S1). Mean E. coli \( \log_{10} \) MPN/100 mL were 1.19 [1.14] and 0.59 [0.99] for cistern and tap water, respectively (Figure 1).

For the bivariate analyses, increases in pH and free chlorine residuals were correlated with lower E. coli levels (p < 0.005), while increased turbidity was correlated with higher E. coli levels (p < 0.005) (Table S3). Cistern E. coli presence and concentration associations with cistern and site characteristics are presented in Table S5. Cisterns with unscreened overflow pipes were associated with a higher likelihood of E. coli presence (p = 0.002) and higher E. coli concentrations (mean difference = 0.38 \( \log_{10} \) MPN/100 mL, p = 0.003), compared with cisterns with screened overflow pipes. Households with ground animals reportedly seen daily or weekly were associated with increased likelihood E. coli presence (p = 0.002) and higher E. coli concentrations (mean difference = 0.89 \( \log_{10} \) MPN/100 mL, p = 0.02), compared with households reporting monthly or less frequent sightings of ground animals. The presence of objects in the cistern water was associated with an increased likelihood of E. coli detection (p = 0.036).

The multivariable model was built on a mechanistic evaluation of E. coli contamination in cistern water; therefore, nonstatistically significant variables remained in the model if they were hypothesized to be mechanistically associated with cistern contamination. Results of the linear mixed model using multiple imputation data, adjusting for island-specific random effects, are presented in Table 1. Household ownership was not significantly associated with E. coli concentration but remained in the model as a proxy for cistern maintenance, and galvanized

| Table 1. Multivariable Linear Mixed-Effect Model for USVI Cistern Survey and Water Quality Associations and Cistern Water \( \log_{10} \) E. coli Concentrations* |
|---------------------------------|----------|----------|----------|----------|
| household or cistern variable | AME \( b \) | SE \( c \) | z-score | p-value |
| ground frequency of animals | | | | |
| daily or weekly (ref) vs. | 0.81 | 0.36 | 2.3 | 0.02 | 0.11-1.5 |
| monthly, >monthly or none | | | | |
| cistern \( \log_{10} \) turbidity (NTU) | 0.63 | 0.15 | 4.2 | <0.001 | 0.34-0.93 |
| cistern FCR (mg/L)* | -1.03 | 0.34 | -3.0 | 0.003 | -1.7--- 0.36 |
| cistern objects* | | | | |
| man-made vs no objects (ref) | 0.21 | 0.16 | 1.3 | 0.18 | -0.10--- 0.53 |
| debris vs no objects | 0.30 | 0.24 | 1.3 | 0.21 | -0.17--- 0.77 |
| animals/insects vs no objects | 0.37 | 0.17 | 2.2 | 0.03 | 0.05-0.70 |
| cistern damage* | | | | |
| yes vs no (ref) | 0.25 | 0.15 | 1.6 | 0.10 | -0.05--- 0.54 |
| household ownership | | | | |
| rent vs own (ref) | 0.12 | 0.16 | 0.72 | 0.47 | -0.20--- 0.43 |
| overlap pipes screened* | | | | |
| yes vs no (ref) | -0.35 | 0.12 | -2.9 | 0.003 | -0.57--- 0.12 |
| galvanized roof metal* | | | | |
| yes vs no (ref) | 0.17 | 0.11 | 1.6 | 0.11 | -0.94--- 0.38 |

*The bold p-values are statistically significant. \( a \)Average marginal effect, also known as the average derivative. \( b \)Standard error. \( c \)Cistern free chlorine residual (FCR) values above or below the detection limit (<0.02 or >2.00 mg/L) of the analytical instrument were censored. Measurements from 0.02 to 2 mg/L were included in analyses. \( d \)Objects in cistern water directly observed by the field team, if possible.
metal roof material verses not galvanized metal was included in the model to control for roofing material. Daily or weekly sightings of ground animals around the household were associated with a 0.81 \log_{10} higher \( E. coli \) concentration in cistern water than households reporting monthly or less frequent sightings of these animals, and cisterns with observable animals or insects inside the cistern were associated with 0.37 \log_{10} higher \( E. coli \) concentration than cisterns without these entities. Cisterns with a screen covering the overflow pipe were associated with a 0.35 \log_{10} lower \( E. coli \) concentration than cisterns without screen coverings. A 1 mg/L increase in free chlorine residuals was associated with a 1.03 \log_{10} lower \( E. coli \) concentration, and a 1-log NTU increase in turbidity was associated with a 0.63 \log_{10} higher \( E. coli \) concentration. Cisterns with any observable damage such as cracks along joints, root intrusion, or covers not fitted properly were associated with a 0.25 \log_{10} higher \( E. coli \) concentration in cistern water compared to undamaged cisterns; the difference was not statistically significant. Cisterns containing man-made objects or debris were associated with a 0.21 and 0.30 \log_{10} higher \( E. coli \) concentration, respectively, but neither of these associations were statistically significant.

3.2.3. Molecular Detection of Pathogens and Human-Associated Fecal Contamination. Of the 47 households at which large-volume sampling was conducted, \( Cryptosporidium \) DNA was detected in 17 (36%) cistern water samples, \( Salmonella \) DNA in 15 (32%) samples, \( Naegleria fowleri \) DNA in 14 (30%) samples, \( Bacteroides \) HF183 DNA in 4 (9%) samples, and pathogenic \( Leptospira \) DNA was detected in 3 (6%) samples (Table S6). \( Giardia \) DNA was not detected in any cistern water sample. Of the 32 households that had at least one detection of a molecular marker, 17 (53%) had one positive detection, 10 (31%) had two detections, 4 (13%) had three detections, and one household (3%) had four detections. At least one zoonotic pathogen, defined as any positive detection for \( Salmonella \), \( Cryptosporidium \), \( Giardia \), and pathogenic \( Leptospira \), was detected in 26 (55%) of cisterns tested. All positive and negative controls and extraction blanks for the suite of qPCR assays performed as expected. No PCR inhibition was observed in any of the samples.

3.3. Water Use Habits and Treatment Types. Household water use habits were summarized by the primary water sources or supplementary water sources used (bottled, cistern, and local water utility) by activity (Table 2). Cistern water was reported as the primary water source for all 13 water use activities presented in the survey, except drinking. Bottled water was the primary water source for drinking (82%); up to 40% of respondents also used cistern water as a primary or supplementary source of drinking water. Most respondents reported using cistern water as the primary or supplementary source for other activities that could result in consumption of water, including making ice (56%), washing fruits and vegetables (84%), cooking (70%), or brushing teeth (88%). Cistern water was reported as the primary or supplementary source for activities that involved dermal or mucosal membrane contact, including bathing (94%), cleaning contact lenses (12%), nasal rinsing (24%), washing wounds or first aid purposes (67%), and filling a swimming pool (38%). Households that reported treating their cistern water (85%, \( n = 332 \)) indicated that the treatment was intended to kill or remove germs (60%, \( n = 198 \)), to kill mosquitoes (16%, \( n = 54 \)), to improve smell (14%, \( n = 47 \)), to remove harmful chemicals (11%, \( n = 37 \)), or to improve taste (7%, \( n = 24 \)).

### Table 2. Primary and Supplementary Water Sources Reportedly Used for Daily Household Activities, \( n \) Response (%)\(^a\)

| Water Use Habits | Bottled Water | Cistern | Local Water Utility\(^b\) |
|------------------|--------------|---------|--------------------------|
|                  | primary (%)  | supplemenary (%) | primary (%) | supplemenary (%) | primary (%) | supplemenary (%) |
| drinking         | 359          | 324 (82) | 35 (9)                  | 359          | 74 (21)    | 69 (19)            | 343          | 6 (2)     | 13 (4) |
| making ice       | 340          | 127 (37) | 17 (5)                  | 354          | 188 (53)   | 10 (3)             | 322          | 13 (4)    | 7 (2)  |
| washing fruits/vegetables | 348 | 62 (18) | 22 (6)                  | 388          | 308 (79)   | 19 (5)             | 343          | 28 (8)    | 8 (2)  |
| cooking          | 360          | 146 (41)| 30 (8)                  | 373          | 233 (63)   | 27 (7)             | 337          | 17 (5)    | 8 (2)  |
| washing dishes   | 339          | 8 (2)    | 11 (3)                  | 392          | 356 (91)   | 12 (3)             | 340          | 30 (9)    | 9 (3)  |
| washing kitchen surfaces | 335 | 2 (0.6)| 9 (3)                  | 391          | 358 (92)   | 10 (3)             | 336          | 30 (9)    | 12 (4) |
| bathing          | 335          | 7 (2)    | 3 (0.9)                 | 390          | 355 (91)   | 10 (3)             | 339          | 31 (9)    | 11 (3) |
| brushing teeth   | 338          | 41 (12)  | 19 (6)                  | 388          | 324 (84)   | 17 (4)             | 336          | 27 (8)    | 14 (4) |
| cleaning contact lens | 265 | 1 (0.4)| 5 (2)                  | 270          | 33 (12)    | 0 (0)              | 265          | 1 (0.4)   | 2 (0.8) |
| nasal rinsing    | 270          | 19 (7)   | 2 (0.7)                 | 271          | 64 (24)    | 1 (0.4)            | 265          | 3 (1)     | 1 (0.4) |
| first aid        | 328          | 52 (16)  | 9 (3)                   | 350          | 221 (63)   | 13 (4)             | 311          | 20 (6)    | 9 (3)  |
| medical device\(^c\) | 264 | 17 (6)  | 4 (2)                  | 273          | 46 (17)    | 1 (0.4)            | 260          | 1 (0.4)   | 2 (0.8) |
| filling pool     | 233          | 0 (0)    | 1 (0.4)                 | 240          | 85 (35)    | 5 (2)              | 231          | 8 (3)     | 2 (0.9) |

\(^a\)Denominator totals across a row may not always sum to 399 because respondents were not limited in selecting more than one option as their primary or supplementary water source for a specific activity. Shaded cells indicate the majority water source used, per activity. The fewer number of responses for use of local water utility water are reflective of the small portion of USVI residents that it serves (<32% of households [13,000/40,648]).\(^1\)\(^1\) A medical device was defined as a humidifier or continuous positive airway pressure (CPAP) machine.

\(^b\)A variety of methods and often more than one water treatment method were used. Among households that used cistern water for household activities, most respondents reported using
liquid, powder, or tablet forms of bleach (72%, n = 286). Of the households that used bleach, 62% (n = 178) stated that killing or removing germs was a reason for treatment. Reported bleach treatment occurred on average every 6.3 months, and the reported time since the last treatment at the time of the survey ranged from 0 to 48 months. For those who reported the amount of liquid bleach added (n = 179), a mean volume of 2.1 [4.8] liters (L) was poured directly into the cistern hatch, with a range of 0.06−45 L. If powdered bleach was used (n = 2), the mean amount added was 1.5 cups within a range of 1−2 cups. The primary liquid bleach concentration used by 87% (n = 191) of households was regular strength (~6%) and 17% (n = 6) of households reported mixing bleach using a pump, whereas 84% (n = 226) of households did not use a device to mix bleach. For those who reported the number of bleach tablets added (n = 13), the mean amount added was 1.5 tablets, ranging from 0.5 to 4 tablets. Other methods of reported water treatment included household-level treatments such as sediment filters (17%, n = 66), multistage filtration (8%, n = 33), and multistage filtration with ultraviolet (UV) treatment (6%, n = 22), as well as point-of-use treatments such as boiling (6%, n = 23) and carbon filter (12%, n = 48). Multistage filtration was defined as a household-level treatment system of >1 in-line filter, and multistage filtration with UV treatment was defined as a household-level treatment system of >1 in-line filter and observation of UV power box light turned on. Of the 85 households that provided information on their filters, there was a mean of 2 in-line filters (range 0−4) in a household with a mean pore size of 13 μm (median 5 μm; range 0.20−75 μm). The most predominant reasons for a household not having a multistage filtration treatment system were that respondents had not considered it or lacked knowledge (29%, n = 107), the associated costs were too high (18%, n = 66), or they believed that it was not needed (17%, n = 61).

3.4. Cistern and Site Characteristics. The majority of participating households had below-ground cisterns (80%, n = 308), and cisterns were primarily constructed of concrete (98%, n = 373); some above-ground systems were made of plastic or fiberglass (2%, n = 7) (Table S5). Approximately 62% (n = 239) of households had one cistern and 38% (n = 149) had partitions creating multiple cisterns in parallel. The median cistern capacity was 12,025 liters, containing a median of 6771 liters of water at the time of the study visit (Table S3). The mean cistern age was 35.5 years [SD 15].

4. DISCUSSION

4.1. Cistern Contamination and Factors Contributing to Contamination. This evaluation of cistern water in USVI revealed the extent to which cisterns are vulnerable to microbiological contamination, as total coliforms and E. coli were detected in 95 and 80% of cistern water samples, respectively. The presence of these bacteria in cistern water demonstrated the open nature of RHRW catchment systems and cisterns to the surrounding environment. Globally, FIB contamination of RHRW cisterns has been recognized as a public health concern as it is commonly detected in RHRW cisterns worldwide, with reported E. coli prevalence ranging between 24 and 100%.

While USVI cisterns are not regulated as community drinking water systems, water containing total coliforms or E. coli does not meet U.S. drinking water quality standards. Detection of a human-specific fecal marker in cistern water samples suggests that the household septic system may pose a risk of cistern contamination through underground seepage. While the study design did not allow for determination of statistical associations between human fecal contamination and cistern characteristics, human-specific fecal markers are valuable tools for identifying septic seepage into ground water that can pose a health risk.

Molecular testing was limited to a subset of households; DNA from Cryptosporidium, Salmonella, Naegleria fowleri, pathogenic Leptospira, and a marker of human-specific fecal contamination were each detected in some cisterns. Salmonella and Cryptosporidium are common zoonotic gastrointestinal pathogens shed in the feces of many animals present in USVI and have often been found in RHRW in other countries. A 1976 outbreak of salmonellosis in Trinidad linked to RHRW highlights the potential risk of exposure to zoonotic pathogens through drinking and indirect consumption of contaminated cistern water. The presence of pathogenic Leptospira and N. fowleri in cistern water highlights different contamination sources and routes of exposure compared with zoonotic gastrointestinal pathogens. Leptospira is shed in the urine of animals and can cause disease in humans through ingestion or contact with contaminated water. N. fowleri is a ubiquitous environmental pathogen that can be found in warm freshwater bodies, sediment, and soil and can cause a rare but fatal illness when contaminated water enters the body through the nose during activities such as swimming or nasal rinsing. Illnesses caused by pathogenic Leptospira and N. fowleri have been documented in USVI in recent years. Detection rates of Salmonella and N. fowleri in USVI cisterns were higher than reported previously. Detection rates of Cryptosporidium fell within the ranges of previously reported prevalence, but HF183 was detected less frequently in USVI cisterns than in other studies. The concentrations of the detected microbes were lower than or on the low end of previously reported concentration ranges. Comparisons of detection rates for Leptospira were not possible because this was the first known detection of Leptospira in RHRW not associated with a case of leptospirosis.

Analyses showed an association between several cistern and household characteristics and cistern contamination. One potential route of cistern contamination is by channeling contaminants and environmental debris from the catchment system (roof and gutters) into the cistern during a rain event. Overhanging trees and vegetation can harbor animals, facilitating deposition of fecal matter onto a roof, either directly or via contaminated plant matter. Another potential route of cistern contamination is through openings to the cistern other than the catchment intake, such as overflow pipes, access hatches, or damage to the cistern. In USVI, frequent sightings of ground animals were associated with higher cistern E. coli concentrations. This finding is supported by the associations between observed animal presence in cisterns and un-screened overflows with higher cistern E. coli concentrations, suggesting that cistern water may become contaminated by animal feces directly when animals enter the cistern through openings such as an unscreened overflow. Furthermore, animal activity around a household may result in indirect fecal contamination of the cistern through animal feces in the soil surrounding the cistern, which may then wash into the cistern during a heavy rain event.

Taking measures to remove or better protect cistern access points may decrease fecal microbial contamination of cisterns.
Ensuring that rainwater catchment systems and overflows are properly screened and maintained would help prevent debris and small animals from entering a cistern. Closing off or sealing access points would prevent larger animals from entering a cistern, and keeping the roof clear of overhanging trees and vegetation would limit animal habitats within the catchment system. While not directly evaluated in this study, the use of first-flush diverters could help prevent debris and organic matter from entering a cistern at the onset a rain event. However, for these measures to be successful, USVI residents must have the resources to execute them. For example, educational resources would provide residents with knowledge of potential contamination routes and methods to secure and protect their cisterns.

4.2. Cistern Water Use and Treatment. Most USVI residents reported relying on RHRW collection for household use, likely in part due to limited distribution of desalinated seawater by the local water utility and the high cost of purchasing water to be delivered by tanker truck. Although bottled water was the most reported source for drinking, 40% of respondents reported some use of cistern water for drinking, and other household activities in which cistern water was used, such as food preparation or making ice, could also lead to consumption of cistern water. Cistern water was reportedly used for filling swimming pools, and swimming has been previously associated with infections and outbreaks caused by Cryptosporidium, Leptospira, N. fowleri, and fecal bacteria. Additionally, cistern water was reportedly used for nasal rinsing, which has been previously associated with exposure to N. fowleri.

Eighty-five percent of households reported employing some form of cistern water treatment, and 60% stated that the reason for treatment was to kill or remove germs. However, the detection of total coliforms and E. coli in kitchen tap samples may suggest ineffective water treatment compared to perceived treatment efforts. While our study was not designed to evaluate treatment effectiveness, many of the treatment methods reported in USVI are not sufficient to remove or inactivate all viral, bacterial, or protozoan pathogens, some of which were detected in cistern water samples in this study. Some treatment methodologies may be effective at removing or inactivating certain microbe classes, but their effectiveness is dependent on many factors, some of which are beyond the awareness, expertise, or budget of household residents. Filtration options such as carbon or sediment filters do not achieve microbial removal from water. Bleach disinfection, which was by far the most prevalent treatment method among USVI households, can effectively kill bacteria and inactivate viruses but would not kill certain protozoa, such as Cryptosporidium. Moreover, effective bleach disinfection requires an adequate and sustained chlorine residual, which is difficult to achieve by pouring bleach directly into the cistern without knowing the volume of the water in the cistern, adequately mixing, accounting for chlorine demand in the water, and measuring chlorine residuals. Among households reporting using liquid bleach, the mean volume of liquid bleach added through the cistern hatch (4 L), lack of mixing, and mean frequency of treatment (6 months) would be insufficient to achieve and maintain a sufficient chlorine residual, considering the average size of cisterns. This is illustrated by the finding that a chlorine residual was only detected in 45% of cisterns for households that reported using bleach treatment.

Multistage filtration with UV disinfection is one of the most effective treatment options for USVI cisterns because the combined technologies of particulate filtration and UV disinfection treat all microbe classes. Multistage filtration/UV systems were the least common treatment technology reportedly used among surveyed households, likely due to the high cost, perceived lack of need, and the lack of awareness that the less expensive treatment options (e.g., liquid and tablet bleach) were not as effective. Education and messaging efforts to inform USVI residents about effective water treatment options would provide households with the knowledge needed to make informed decisions about water treatment methodologies and risks associated with cistern water use practices when existing treatment methods are not sufficient. If households do not have the ability to effectively treat and safely store all of their cistern water, they could consider filtering and disinfecting smaller volumes of water that will be ingested. This would ensure that they can reliably improve their drinking water, but it will not address the quality of water used for other purposes. Further investigation into the cost effectiveness of the available treatment options should be considered in addition to efforts to design novel, affordable treatment methods designed for RHRW systems and cisterns.

4.3. Limitations. This study was subject to several limitations. The self-reported survey data obtained during this study may be biased or reflect underreporting due to privacy issues. Additionally, this study was a cross-sectional evaluation of cistern characteristics and water quality. For a better understanding of water quality trends, routine sampling and testing would further clarify the relationship between cistern characteristics and microbial contamination. Many of the measured chlorine residuals were at or near the detection limit of the test (0.02 mg/L). This may have falsely indicated the presence of chlorine residuals and overestimated the number of samples with detectable chlorine, due to instrument imprecision, user error, or matrix interferences. This limitation did not impact statistical modeling because chlorine residuals were treated as continuous variables. Molecular results for pathogens and HF183 were constrained by several limitations. Molecular testing does not provide information about the viability or infectivity about the organisms detected. Additionally, the study was not powered to detect statistical associations between pathogen and human fecal marker molecular detection and water use behaviors or cistern characteristics due to the low detection rates in the subset of samples tested. HF183 has been found to be highly sensitive and specific for human fecal waste in the United States, although the sensitivity and specificity were not evaluated with local samples for this study. Finally, statistical associations between tap and cistern water quality were not assessed, as the study was not designed to evaluate premise plumbing contamination.

5. CONCLUSIONS

We present a microbial characterization of roof-harvested rainwater cisterns and associations between microbial quality and cistern and household site characteristics and physiochemical quality across the U.S. Virgin Islands. We also present data on how cistern water is treated and used across USVI. Our findings demonstrate the vulnerability of USVI cisterns to fecal and environmental microbial contamination and identified factors that likely contribute to the risk of contamination. These findings suggest that microbial contamination may be reduced by means of screening open access
points to the cistern, decreasing animal presence at the household, and sealing undesired access points to reduce introduction of animals and foreign objects into the cistern. Treatment of cistern water before use presents an opportunity for its safe use, but education and outreach efforts are warranted to provide USVI residents with the knowledge to select appropriate treatment methodologies.

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**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00389.

(Text S1) Sample size calculations; (Table S1) real-time PCR assays used for the pathogen and human fecal marker analytes and performance of standard curves; (Text S2) real-time PCR standard materials and quantification; (Table S2) demographic characteristics of participating USVI households (HH), N (%) or mean [SD], overall and by USVI island; (Table S3) cistern and physicochemical water quality measurement descriptive statistics and nonparametric correlation (r_s) with log_{10} E. coli concentrations; (Table S4) tap physicochemical water quality measurement descriptive statistics; (Figure S1) distribution of mean log_{10} total coliform concentrations (MPN/100 mL) from cistern (top) and household tap water (bottom), overall and by USVI island (STX = St. Croix, STT = St. Thomas, STJ = St. John); (Table S5) bivariate statistical associations between E. coli detection, mean-log_{10} concentration, and cistern and site characteristics; (Table S6) detections and mean concentration per 100 mL of zoonotic pathogens, a human fecal marker, and Naegleria fowleri, overall and by USVI Island (PDF)

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