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SCIENTIFIC NOTE

A CROSS-SECTIONAL HOUSEHOLD SURVEY IN THE US VIRGIN ISLANDS (2019) REVEALS CISTERNS AS CHALLENGING PERIDOMESTIC *AEDES AEGYPTI* HABITATS

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ABSTRACT. Most residences in the United States Virgin Islands (USVI) rely on household rainwater-catchment systems and subterranean cisterns for long-term water storage that may provide suitable habitats for mosquitoes of public health relevance. We conducted a household cistern survey ($n = 164$) on the islands of St. Croix, St. John, and St. Thomas in 2019. The survey revealed that 45.7% (95% CI: 38.3–53.4%) of cisterns contained mosquitoes (adult and/or immature mosquitoes). *Aedes aegypti*, a vector of chikungunya, dengue, and Zika viruses in the USVI, was found in 27.4% (95% CI: 21.2–34.7%) of cisterns and accounted for 83.3% of the total mosquitoes identified in the study. The odds of detecting mosquitoes in a cistern were 5.45 times higher at locations where the residents reported that they had observed adult mosquitoes coming out of their cisterns (95% CI: 2.25–14.21), suggesting that vector control personnel should consider resident complaints about mosquitoes in their cistern as valid and likely reliable self-assessments. Resident mosquito management practices in cisterns did not correspond with decreased odds of mosquito detection. We conclude that cisterns in the USVI commonly provide habitat for immature and adult *Ae. aegypti*, which may decrease the effectiveness of area-wide mosquito control strategies. Additional studies are necessary to evaluate the importance of these cisterns as they relate to mosquito production and arbovirus transmission risk, and to assess physical and chemical control methods.

KEY WORDS Management practices, mosquito control, St. Croix, St. Thomas, St. John, rainwater-catchment systems

The United States Virgin Islands (USVI) is an American territory in the Caribbean Sea comprising 3 main islands: St. Croix, St. John, and St. Thomas. In 2020, the population on the islands was 87,146 with 42,261 (47.1%) persons on St. Thomas, 41,004 (48.5%) on St. Croix, and 3,881 (4.5%) on St. John (US Census Bureau, 2020). *Aedes aegypti* (L.), a well-established species on the islands, is the responsible vector for multiple anthroponotic arboviral outbreaks that have occurred in the USVI within the past decade (dengue virus: 2010, 2012; chikungunya virus: 2014–15; Zika virus: 2016–17) (Rosenberg et al. 2018). There is a paucity of recent studies detailing the distribution, abundance, and habitat associations of Culicidae within the islands, and little is known about the prevalence of *Ae. aegypti* or other mosquito species in peridomestic rainwater-catchment systems and retention structures such as household cisterns (Kenney et al. 2017, Seger et al. 2019, Rogers and Cruz-Rivera 2021).

Within the USVI, cisterns are often subterranean and, in most instances, are built directly underneath a residence with potential water storage volumes of more than 50,000 liters on average (Fig. 1). These rainwater-catchment systems are necessary for USVI households because the islands do not have universally available municipal water supplies, permanent freshwater sources are scarce, and rainwater is only episodically obtainable. Because of their importance for water security, household rainwater-catchment systems and large-volume storage tanks (i.e., cisterns) are required for most newly constructed or modified buildings (29 US Virgin Island Code [V.I.C.] § 308). Additional codes require that cisterns "shall be maintained in a watertight, clean, and mosquito proof condition at all times" (19 V.I.C. § 1401, 29 V.I.C. § 308, 29 V.I.C. § 333). More than 80% of the USVI population uses rainwater-catchment systems for activities such as bathing, washing dishes, and drinking; cisterns are often a source of drinking water during times of water scarcity, especially following natural disasters such as hurricanes (Marinova-Petkova et al. 2019). In this cross-sectional study, we sampled residential cisterns within the USVI to evaluate their role as a habitat for mosquitoes of public health concern. We examined the influence of water quality characteristics, cistern physical characteristics, and household

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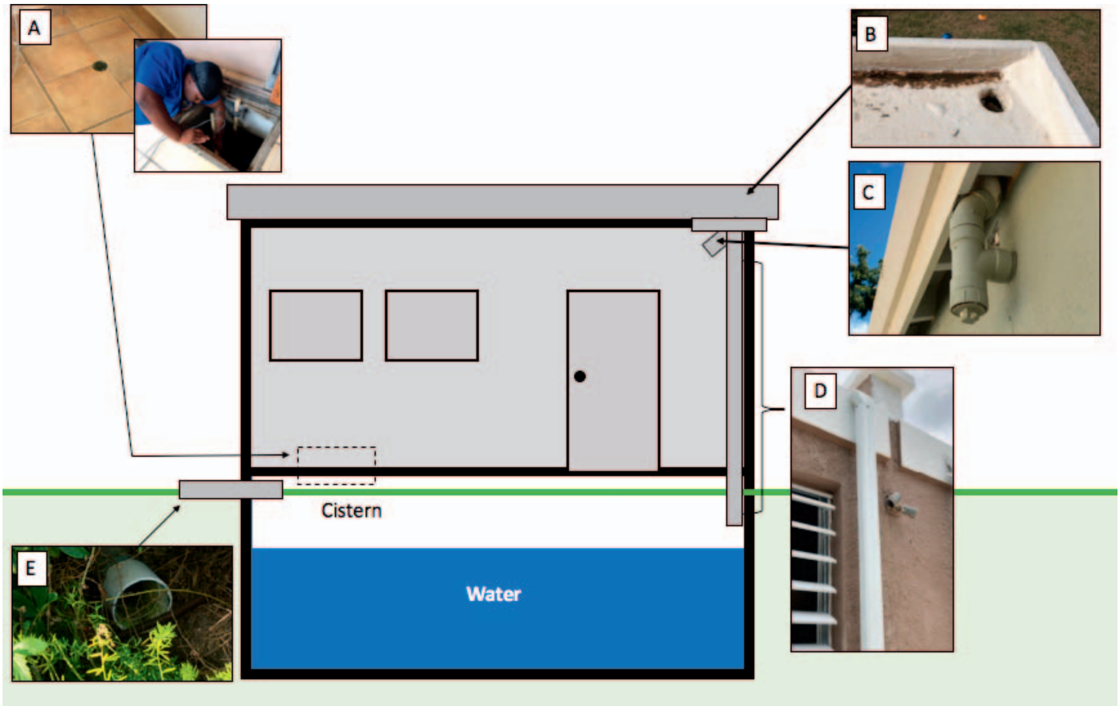

Fig. 1. Rainwater-catchment system schematic: This figure exemplifies common features of a rainwater-catchment system in the United States Virgin Islands. Physical access into the cistern is typically through a panel (A) above the water-containing vault. Rainwater is directed by gravity through an intake spout opening on the roof (B) where a "clean out" pipe may be available (C). Rainfall enters the cistern through an intake spout (D) that may be external (as demonstrated here) or internal (typically housed within the concrete blocks of an external house wall; demonstrated in C). When the cistern is full, excess water can leave the vault through overflow pipes (E) to prevent flooding within the residence.

practices as risk factors for mosquito presence in cisterns.

This study was conducted as part of a larger USVI Department of Health (VIDOH) and Centers for Disease Control and Prevention (CDC) project titled "Determining Risk Factors and Mitigation Strategies for Household Cisterns in the U. S. Virgin Islands" that received a nonresearch determination after review by the CDC's eClearance process (Project ID: 0900f3eb8190a63c); the study protocol was also approved by the VIDOH and Western Carolina University based on the provisions of 45 CFR 46.102(l)(2).

Mosquito surveys were conducted from July 15 through August 9, 2019. Mosquito survey teams consisted of an interviewer and at least 2 additional members to obtain water and mosquito samples and to assess the physical characteristics of cisterns. Cisterns were examined to determine type (i.e., above- or belowground) and construction material (e.g., concrete, plastic, fiberglass), calculate an estimate of size and volume, and determine the presence or absence of screens capable of preventing mosquito entry on intake spouts and overflow pipes.

Cistern access covers (Fig. 1A) were manually removed while the opening was simultaneously aspirated using an Improved Prokopack Aspirator (John W. Hock Company, Gainesville, FL) attached to an 8-ft (2.4-m) extension pole (Vazquez-Prokopec et al. 2009). The aspirator was immediately placed inside the cistern and an approximately 10-ft (3.0-m)-diam area was aspirated along the ceiling of the cistern and accessible wall areas as water levels within the cistern allowed. The cistern water was visually inspected for the presence of mosquito larvae or pupae using a flashlight. Any visible immature mosquitoes were immediately collected, if accessible, using an 8 × 4-inch (20 × 10-cm) meshed fishnet attached to a 12-ft (3.6-m) extendable pole. The net was then swept through the center of the water column directly underneath the cistern opening, at the edges of the water at any accessible walls, and at the bottom of the cistern in a steady sweeping manner to sample immature mosquitoes not directly visible. The presence of any adult and immature mosquitoes that were visualized but not collected was also documented. In some instances, investigators were able to access the cistern but were unable to reach the bottom of the cistern with the sweep nets and did not observe or aspirate adult mosquitoes; these attempts ($n = 5$) were not included in data analyses. Mosquitoes were microscopically identified using morphological characters described in Belkin et al. (1970) and Darsie and Ward (2005).

Table 1. United States Virgin Islands residential cisterns containing mosquitoes (2019).

Location (n = cisterns sampled)	Percent positive[1] (95% CI)	Percent containing immature mosquitoes (95% CI)	Percent containing adults (95% CI)	Percent *Aedes aegypti* (n = count)[2]
Overall (164)	45.7 (38.3–53.4)	22.6 (16.2–29.2)	40.2 (32.7–47.7)	83.3 (222)
St. Croix (71)	32.4 (22.7–43.9)	19.7 (10.4–29.0)	25.3 (12.5–32.7)	78.0 (50)
St. Thomas (74)	52.7 (41.5–64.7)	21.6 (12.2–31.0)	48.6 (37.2–60.0)	82.4 (142)
St. John (19)	68.4 (46.0–84.6)	31.6 (10.7–52.5)	57.9 (35.7–80.1)	96.7 (30)

[1] Positive: resting adult or immature mosquitoes observed and/or collected from cisterns.
[2] A total of 268 mosquitoes were sampled across all 3 islands; 83.3% were *Ae. aegypti*.

For water quality measurements, water samples were obtained from the cisterns directly below the cistern access panel. The water temperature was recorded from the retrieved sample immediately. A Hach (Loveland, CO) Pocket Pro™+ Tester (Prod. No. 9532800) was used to measure pH, conductivity, total dissolved solids, and temperature. Turbidity was measured using the Hach 2100Q Portable Turbidimeter (Cat. No. 2100Q01); free and total chlorine residuals were determined using the Hach Pocket Chlorimeter II (Cat. No. 58700-00).

For statistical methods, descriptive statistical measures were calculated with R (R Core Team 2018) using the RStudio integrated development environment (version 1.1.463); all statistical tests were performed with an a priori alpha level of 0.05. For each predictor variable, we analyzed the probability of mosquito presence using a single category of "all forms" (i.e., adult and immature mosquitoes, collected or visualized) and individually, as either adult or immature mosquitoes; the results were similar in all cases. Binary logistic regression was used to assess associations between continuous variables and the presence or absence of mosquitoes. Fisher's Exact test was used to assess relationships between binary categorical variables and the presence or absence of mosquitoes. A chi-square test was used to determine whether the proportion of cisterns containing mosquitoes differed among the islands.

A total of 164 cisterns were successfully sampled, and the corresponding water quality analyses and resident interviews were obtained for each; 45.7% of the cisterns had adult and/or immature mosquitoes. The proportion of cisterns containing mosquitoes differed by island ($\chi^2 = 10.48$, df = 2, $P < 0.01$), and we were 1.3 to 2.3 times (by island) more likely to detect adult mosquitoes versus immature mosquitoes (Table 1). Immature mosquitoes were observed and/or collected in 22.6% of sampled cisterns, while adults were observed and/or collected in 40.2% of sampled cisterns. In the cisterns where larvae were collected, the median number of larvae obtained by sweeping was 3 (range 1–37); pupae were rarely encountered (<2% of cisterns). In cisterns where adults were collected, the median value was 1 (range 1–13). Of the 268 mosquitoes obtained from sampling the cisterns, 83.3% were *Ae. aegypti*. Additional species (*Ae. mediovittatus* (Coq.), *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, and *Psorophora johnstonii* (Grabham) [a single aspirated adult]) were less commonly collected from the cisterns than *Ae. aegypti*. This indicates that subterranean cisterns could serve as reclusive resting habitats for adult *Ae. aegypti*, providing harborage near human hosts.

The odds of detecting mosquitoes in cisterns where residents reported previously observing mosquitoes "come out of the cistern" was 5.45 times higher than in cisterns where residents had not observed mosquitoes (Fisher's Exact test, 95% CI: 2.25–14.21, $P < 0.001$). This suggests that vector control personnel should consider resident complaints about mosquitoes in their cistern as valid and likely reliable self-assessments. Residents who reported that they treated their water for any reason were more likely to have mosquitoes detected during our sampling efforts (Fisher's Exact test: odds ratio = 2.66, 95% CI: 1.08–7.09, $P = 0.02$). Although most (76.2%) residents stated that they treated their cistern water, only 20.7% said they treated the water specifically for mosquitoes. Of those who treated their cisterns specifically for mosquitoes, there was no observed relationship (protective or associative) with the odds of detecting mosquitoes. Answers to other survey questions did not predict the presence or absence of mosquitoes in cisterns (data and analyses available upon request).

Unscreened overflow pipes and intake spouts (Fig. 1) were commonly observed at residences (36% and 41%, respectively). There was no observed association with the presence of screens capable of preventing adult mosquito passage on cistern overflow pipes and intake spouts and the presence or absence of mosquitoes. Likewise, no significant relationship was detected between estimated cistern volume, estimated water volume, cistern type (above- or belowground), or cistern material (e.g., concrete, plastic) and the likelihood of detecting the presence of mosquitoes. Total and free chlorine residual, pH, total dissolved solids, conductivity, and turbidity were not significant predictors of the probability of detecting mosquitoes in cisterns. The odds of detecting mosquitoes in a cistern increased by 68% for each 1°C increase in temperature. However, a lack-of-fit test on the model with temperature as a predictor suggests that the fit of the model was poor ($\chi^2 = 213.7$, df = 160, $P = 0.003$).

Mosquito control strategies like area-wide ultra-low volume spraying and peridomestic source reduction may have reduced efficacy in the USVI if cisterns provide protected habitats for *Ae. aegypti* (Perich et al. 2000, Britch et al. 2018). Thus, additional studies are needed to determine if cisterns serve as mosquito harborage that decreases the effectiveness of common mosquito control strategies. It is beyond the scope of this study to infer the *Ae. aegypti* productivity in USVI cisterns. However, subterranean habitats (e.g., service manholes, wells, septic tanks) are well known to be suitable habitat and harborage for *Ae. aegypti* (Kay et al. 2000). Similarly, underground septic tanks are recognized as productive larval habitats harboring cryptic *Ae. aegypti* populations that may contribute to dengue endemicity in Puerto Rico (Barrera et al. 2008).

Sampling residential cisterns is inherently challenging, time-consuming, and potentially hazardous. Cisterns are a confined space and may be regulated as such in some countries (e.g., US Code of Federal Regulations: 29 CFR 1910.146). Cistern depths observed during our study commonly exceeded 10 ft (3 m) and ventilation was limited. Safety ladders or poles are rarely available within the cisterns. The openings do not have safety rails or other fall-prevention engineering. The access panels are mostly indoors and frequently in "tight quarters" such as a small closet. Thus, these challenges and concerns were considered during the sampling method development and during the survey. As a result, this study was not designed to quantify the total abundance of immature or resting adult mosquitoes within cisterns but focused on safely and swiftly determining the presence or absence of mosquitoes within cisterns. There are limitations to this approach. It is estimated that the sampling area for a typical cistern using this method is no more than 5% of the cistern water volume for an average cistern. Immature mosquito distribution within a cistern is not expected to be uniform, and opening the cistern access panel and using a handheld aspirator and flashlight to collect mosquito adults may have impacted the distribution of the larvae within the available sampling area.

This study occurred over an approximately 4-wk period during the summer of 2019. Precipitation in the region during the 3 months prior to the study was lower than normal, and on the island of St. Thomas these months had a cumulative 53% reduction in precipitation as compared to normal values (departures from normal [inches]: April: –1.71, May: –0.72, June: –1.95; St. Thomas Cyril E. King Airport Station [National Weather Service, National Oceanic and Atmospheric Administration]). Because this study was cross-sectional with a single sampling effort for each cistern, we are unable to consider how precipitation or other environmental factors influence mosquito presence in cisterns over time. In this study, pupae were rarely collected in cisterns ($<2\%$), suggesting that cistern productivity may be low at times. Furthermore, we did not sample rainwater-catchment intake spout clean out pipes (Fig. 1C) as part of this study. We have observed immature mosquitoes in these structures, and it is likely they may be flushed into the cistern during a rainfall event. Thus, we are unable to confidently state how the cistern water reservoirs or the rainwater-catchment intake spout clean out pipes contribute to overall adult mosquito abundance or productivity within a cistern. Additionally, overflow pipes and intake spouts are commonly unscreened (or have damaged screens or screens not suitable for preventing mosquito entry), so we cannot be certain if the *Ae. aegypti* aspirated from cisterns in this study originated in the cistern. Mosquitoes may be produced in other peridomestic habitats and seek resting harborage within cisterns. Taken together, our study suggests the clear and immediate need for additional field evaluations to determine the seasonal productivities of cisterns (including mosquito egress and ingress movement) and evaluate which physical and chemical control methods are appropriate.

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