UNIVERSITY OF CALIFORNIA SAN DIEGO
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# Risk factors and surveillance for arboviruses and their vectors in Guatemala and Puerto Rico

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in
Public Health (Epidemiology)
by
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The Dissertation of Zachary Joseph Madewell is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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2020

# EPIGRAPH

Mosquito or Man?

*Dr. Rubert Boyce*

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# LIST OF ACRONYMS

| | |
|---|---|
| AFI | Acute febrile illnesses |
| AOR | Adjusted odds ratio |
| CDC | Centers for Disease Control and Prevention |
| CHIKV | Chikungunya virus |
| CI | Confidence interval |
| DENV | Dengue virus |
| ELISA | Enzyme-linked immunosorbent assay |
| HAP | Household air pollution |
| IQR | Interquartile range |
| MSPAS | Ministerio de Salud Pública y Asistencia Social |
| OR | Odds ratio |
| PCA | Principal components analysis |
| PPV | Positive predictive value |
| PRDH | Puerto Rico Department of Health |
| RT-PCR | Reverse transcription polymerase chain reaction |
| SAGO | Sentinel autocidal gravid ovitraps |
| SD | Standard deviation |
| SE | Standard error |
| SES | Socioeconomic status |
| UI | Uncertainty interval |
| UVG | Universidad del Valle de Guatemala |
| VICo | *Vigilancia Integrada Colaborativa* |
| WNV | West Nile virus |
| ZIKV | Zika virus |

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Chapter 3, in full, is a reprint of the material as it appears in *PLOS Neglected Tropical Diseases*: Madewell ZJ**,** Hemme RR, Adams L, Barrera R, Waterman SH, Johansson MA. Madewell ZJ, Hemme RR, Adams L, Barrera R, Waterman SH, Johansson MA. Comparing vector and human surveillance strategies to detect arbovirus transmission: A simulation study for Zika virus detection in Puerto Rico.

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Chapter 4, in full, is a reprint of the material as it appears in *BMC Public Health*: Madewell ZJ, Sosa SM, Brouwer KC, Juárez JG, Romero C, Lenhart A, Cordón-Rosales C. Associations between household environmental factors and immature mosquito abundance in Quetzaltenango, Guatemala. *BMC Public Health.* 2019;19(1):1729. The dissertation author was the primary author of this material.

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Sosa S, Romero C, Ortíz L, **Madewell ZJ**, Juárez JG, Lenhart A, Cordón-Rosales C. Enhanced *Aedes* mosquito surveillance using ovitraps in rural and urban communities, Coatepeque, Guatemala (2017-2018).

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# ABSTRACT OF THE DISSERTATION

Risk factors and surveillance for arboviruses and their vectors in Guatemala and Puerto Rico

by

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Doctor of Philosophy in Public Health (Epidemiology)

University of California San Diego, 2020
San Diego State University, 2020

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**Background:** *Aedes aegypti*-borne diseases, including dengue, chikungunya, and Zika viruses, are increasingly important public health problems. Detecting and monitoring the transmission of arboviruses is critical for prevention and control activities. This dissertation research aimed to identify environmental, geospatial, and sociodemographic risk factors for arboviruses and their vectors in Guatemala, and compare vector and human surveillance strategies to detect local arbovirus transmission in Puerto Rico—both sites of recent large arbovirus outbreaks.

**Methods:** This dissertation includes three studies examining arbovirus and vector risk factors and surveillance strategies. Study one was a retrospective case-control study using data from a prospective public health surveillance system in hospitals and clinics in Guatemala and logistic regression to examine whether arboviral infections were associated with indicators of household air pollution. In study two, we used data from human and mosquito surveillance systems in Puerto Rico to develop a simulation model to compare these surveillance systems for detecting and monitoring Zika virus activity. Study three included data from two cross-sectional household surveys in Guatemala and used generalized linear and generalized additive models to assess whether household environmental and geospatial factors were associated with immature mosquito abundance.

**Results:** For study one, arboviral infections were inversely associated with cooking with firewood in the main house, on an open hearth, and ≥5 times per week. In study two, both vector and human surveillance strategies effectively identified transmission in simulated high Zika virus transmission scenarios. In simulated low incidence scenarios, vector surveillance had higher sensitivity than human surveillance and that sensitivity increased with more traps and tests. In study three, proximity to paved roads and other houses/structures was predicted to be associated with greater immature mosquito abundance. Households with low and high household infrastructure had fewer larvae/pupae than households in the middle range.

**Conclusion:** Better understanding of factors defining geographical distribution of arboviral vectors may allow for improved targeting of vector surveillance, prevention, and control measures in areas considered at higher risk for arbovirus transmission. Virological surveillance in mosquitoes may improve sensitivity for arbovirus detection compared to human surveillance, but resource availability is an important factor when considering the most effective approach.

# CHAPTER 1: INTRODUCTION

## OVERVIEW

Arthropod-transmitted viruses (arboviruses) pose important public health challenges and provide significant health threats in the Americas. There are over 500 recognized arboviruses worldwide, of which 150 are known to cause human disease (1). The arboviruses recognized today may be less than 1% of the total worldwide, as most arboviruses are zoonotic infections with vertebrate hosts other than humans as their primary reservoir (1). The most prevalent arboviral diseases globally are dengue (96 million cases per year), chikungunya (693,000 cases per year), Zika (500,000 cases per year), yellow fever (130,000 cases per year), Japanese encephalitis (42,500 cases per year), and West Nile (2,588 cases per year) (2, 3). Other arboviral diseases that pose medical and economic consequences are Eastern equine encephalitis, St. Louis encephalitis, La Crosse Encephalitis, Rift Valley fever, Spondweni, Mayaro, Usutu, O'nyong nyong, and Sindbis (4, 5). These viruses are both emerging in new areas and reemerging in areas where they had previously been eliminated (2, 6, 7).

Dengue (DENV), chikungunya (CHIKV), and Zika viruses (ZIKV) share several characteristics regarding epidemiology, transmission cycles, and clinical expressions. These viruses have attracted significant attention in recent years due to their increasing incidence, expanding geographic range, ability to cause disease, and consequent public health burden (7). DENV, CHIKV, and ZIKV are mainly transmitted by *Aedes* mosquitoes, particularly *Ae. aegypti* or *Ae. albopictus.* The clinical signs associated with infection from these arboviruses are often inapparent or mild, but may include serious complications, such as hemorrhage, chronic arthritis, congenital abnormalities, and others. Nearly 3.6 billion people in the world live in tropical and subtropical regions, which are endemic for *Ae. aegypti* or *Ae. albopictus* mosquitoes (8). Climate change, urbanization, migration, human behavior, and ecosystem alterations are among the myriad factors influencing the geographic spread of *Aedes spp.* and their associated viruses (7, 9, 10). However, other than for DENV, yellow fever, and Japanese encephalitis viruses, there are no commercially available vaccines for these viruses. The DENV vaccine has been licensed in more than 20 countries including the U.S., but the vaccine has not been incorporated into any national program to date because of difficult logistical requirement for prevaccination (11). Therefore, vector control and human behavior modification, to maintain mosquitoes below the density threshold required for local transmission, are the most widely used preventive strategies (12, 13). The expanding human population and greatly expanded international travel, trade, and tourism now pose daunting challenges to the control of these vectors and their viruses.

This dissertation research examines environmental, geospatial, and sociodemographic risk factors for pathogenic arboviruses and their vectors and compares vector and clinical patient-based surveillance strategies to detect local arbovirus transmission. These studies were completed in Guatemala and Puerto Rico—sites of recent large arbovirus outbreaks, and these results may be helpful in vector control and prevention interventions in those locales.

## CONCEPTUAL FRAMEWORK

These three manuscripts are based on an "eco-bio-social" conceptual model as proposed by the Ecosystem and Human Health Program of Canada's International Development Research Centre (IDRC) for research on factors responsible for the expansion and transmission of DENV in Asia (14-16). Briefly, the ecological components of *Aedes*-borne arbovirus infections include climate, natural, and geographical setting (Figure 1.1). Biological factors are vector transmission dynamics and host susceptibility. Social factors include public services, urbanization, SES, and community knowledge, attitudes and practices (KAP). Understanding the relative importance of each of these factors and their interactions can guide public health interventions or vector control practices, which are the emphases of this dissertation (17).

This dissertation research encompasses the epidemiological triad of agents, hosts, and environment with a centralized vector component (Figure 1.2). The specific agents are DENV, CHIKV, and ZIKV. The vectors are *Ae. aegypti* and *Ae. albopictus.* The environment includes extrinsic risk factors (e.g., climate, geography, urbanization, sociodemographic factors, population density, community preventive measures, housing types, waste management, and others) (18). Hosts are predominantly humans. Other host-related factors include age, sex, immune status, ethnicity, nutritional status, genetic predisposition, and previous infection. The dynamic interplay of hosts, vectors, agents, and environment contribute to the geographic spread of arboviruses. Developing appropriate and effective public health measures to control arboviruses requires that public health practitioners understand the biological, cultural, and social interrelationships among the environment, host, pathogens, and vectors. In this dissertation, I address these contextual interrelationships within three specific studies.

## Dengue virus

DENV is a single-stranded, lipid-enveloped, non-segmented RNA *Flavivirus* of the *Flaviviridae* family (19). Over half of viruses in the genus *Flavivirus* cause human diseases, including yellow fever, West Nile, Japanese encephalitis, and St. Louis encephalitis viruses. The first record of dengue fever was in a Chinese disease encyclopedia from the Chin Dynasty (265 to 420 A.D.), and the first major epidemics were reported in 1779 and 1780 in Africa, Asia, and North America (18). Dengue infections are caused by four serotypes (DENV 1-4) (19). A fifth variant, DENV-5, was isolated in October, 2013 (20).

DENV is the most prevalent and rapidly spreading of the arboviruses, with transmission occurring in 128 countries, thereby creating risk for almost 4 billion people (21-23). There are 390 million DENV infections (95% credible interval: 284–528 million) worldwide annually, including 96 million (95% credible interval: 67–136 million) clinical cases, 500,000 dengue hemorrhagic fever (DHF) cases, and 22,000 deaths, mostly among children <5 years of age (24). The incubation period for DENV is 3-14 days (7). DHF signs and symptoms include restlessness, thirst, abdominal pain, hemorrhage in gingiva and skin, frequent vomiting, and melena (25). One to twenty percent of DHF cases without proper treatment lead to death (24), whereas with treatment, the DHF case-fatality rate is <1% (21). Half of all DENV infections are asymptomatic, but all four DENV serotypes may cause febrile illness, sudden-onset skin rash, myalgia, headache, and vomiting (21, 26, 27). DENV infection may also cause dengue shock syndrome (DSS). DSS is defined as DHF plus excessive capillary permeability, leading to petechiae, ecchymosis, rapid heart rate, and weak pulse pressure (25). Death may ensue within 12-24 hours unless properly treated (25).

Infection from one DENV serotype confers lifelong immunity for that serotype, but only limited protection for other serotypes and for only a few months after infection (25). The risk of DHF may increase up to 15-500 times following a secondary infection with a different serotype (28). However, DHF/DSS is typically observed in only a small percentage of secondary DENV infections and is rare in primary DENV infections (29). There is evidence of cross-immunity between DENV and other *Flaviviruses* including ZIKV (30, 31) and yellow fever virus (32), although these immunologic patterns are poorly understood. There is also evidence, however, that prior DENV infection increased placental damage, fetal growth restriction, and fetal resorption in ZIKV-infected pregnant mice (33).

### Chikungunya virus

First identified in Tanzania in 1952, CHIKV is an enveloped plus-strand RNA *Alphavirus* (34, 35). The word ‘chikungunya’ in the Kimakonde language means “that which bends up,” which may have derived from the hunched posture among cases with severe joint and arthritic pain (36). CHIKV is of the *Togaviridae* family, which includes O'nyong'nyong, Mayaro, Ross River, and Semliki viruses (35). There are four lineages of CHIKV (West African, Asian, East/Central/South African, Indian Ocean), each with distinct genotypes (37). Outbreaks in 2004 in Africa and Asia were followed by outbreaks in the Americas in 2015 (38). Local transmission of CHIKV was described in the U.S. in 2014 and has been identified in nearly 40 countries (39). In contrast to DENV and ZIKV, most people infected with CHIKV are symptomatic (40). The incubation period for CHIKV is 1-12 days (41). Symptoms include fever, headache, conjunctivitis, nausea, rash, arthralgia, polyarthritis, and maculopapular rash (35, 40). Symptoms may last months to years, especially in those >35 years (42). Other complications include prolonged arthritis, meningoencephalitis, nephritis, retinitis, uveitis, myelitis, cranial nerve palsies, and acute encephalopathy (40).

## Zika virus

ZIKV is a single-stranded enveloped RNA *Flavivirus* of the *Flaviviridae* family. First identified in Uganda in 1947, ZIKV expanded into the South Pacific and Americas with 48 countries reporting active ZIKV by 2017 and 86 by 2019 (7, 43, 44). There are two ZIKV lineages (African and Asian) and three genotypes (West African, East African, and Asian), but only one serotype (45). Like other arboviruses, ZIKV infection confers lifelong immunity to reinfection (46). The incubation period for ZIKV is 3-14 days and symptoms last for 2-7 days (47). Although 60-80% of infected individuals are asymptomatic (48), ZIKV may cause fever, rash, conjunctivitis, arthralgia, myalgia, headache, dysesthesia, retro-orbital pain, asthenia, and arthritis (46, 49). ZIKV infection has also been linked to Guillain-Barré syndrome (47).

For ZIKV, other horizontal (e.g., sexual, blood transfusions) and vertical (mother-to-fetus) transmissions are also reported (7, 50). Sexual transmission of ZIKV has been reported up to 44 days after onset of symptoms, and the viral RNA may remain in semen for 180 days after symptom onset (51, 52). An estimated 5-15% of women infected with ZIKV during pregnancy give birth to infants with congenital abnormalities (47). Congenital Zika syndrome encompasses a range of disorders including microcephaly, encephalitis, craniofacial disproportion, cerebral palsy, hearing loss, brainstem dysfunctions, spasticity, joint deformities, clubfoot, and developmental and inflammatory ocular diseases (44, 46, 49, 53). The wide range of maladies associated with maternal and/or neonatal ZIKV infections are still being identified, and some of the long-term health consequences are still unknown.

## Transmission cycles

There are two cycles for DENV, CHIKV, and ZIKV amplification in nature: an enzootic sylvatic cycle between forest-dwelling *Aedes spp.* and primates (e.g., rhesus monkeys, capuchin monkeys and common marmosets); and an urban human cycle between *Aedes spp.* and humans (Figure 1.3) (25, 54, 55). Arboviruses have the potential to spillover from enzootic or sylvatic cycles to a cycle in which humans are the predominant hosts when humans encroach on new environments and become more exposed to enzootic vectors (56). Sylvatic cycles have been demonstrated in Asia and Africa (57). There is a documented enzootic cycle for Yellow Fever virus in the Americas, but not for DENV, CHIKV, or ZIKV (43).

At least 79 species of animals have been identified as capable of being naturally or experimentally infected with ZIKV, with 63 found to be infected naturally (43). Most are mammals, particularly primates, but birds, reptiles, and amphibians may also be infected (43). Only five monkey species, however, may have viremia levels high enough for transmission (43). Six-thousand primates and 10,000 mosquitoes are enough to maintain a ZIKV sylvatic cycle (58). Climate change could affect the sylvatic cycle, increasing contact between primates and mosquitoes (57).

## Host

Human susceptibility and clinical manifestations of arboviruses depend on viral strain, host age, sex, immune status, ethnicity, nutritional status, genetic predisposition, and previous infection (59, 60). Viral serotype, genotype, sequence of arbovirus infection, and interval between infections may also influence disease severity (61). Fewer *Aedes spp.* mosquitoes are required to cause local arbovirus transmission in immunologically naïve communities where herd immunity is low or non-existent and ambient temperature is high (13). There is no licensed remedy for DENV, CHIKV, or ZIKV (21, 62). Care is palliative, based primarily on rehydration (21, 62, 63).

*Ae. aegypti* and *Ae. albopictus* bite all people, but certain traits make some individuals more attractive than others. Mosquitoes are attracted to people with type O blood over types A, B, or AB (64). People with type O blood are bitten twice as frequently as people with type A (32). Mosquitoes may locate their targets by smelling carbon dioxide, lactic acid, uric acid, and ammonia, so people who exercise, have higher body temperatures, and release abundant $CO_2$ are more attractive to mosquitoes (65, 66). Pregnant women are especially attractive to mosquitoes, perhaps because they exhale 21% more $CO_2$ and are on average 0.7 °C warmer around their abdomens than non-pregnant women (67). Colonization of skin with certain bacteria makes some people more susceptible to mosquito bites (68).

Other characteristics that make some people more attractive include deodorants or perfumes, and beer drinking (32, 69). However, 85% of what makes humans more attractive to mosquitoes is intrinsic to hosts (blood type, metabolic pathways, body odor, $CO_2$ levels, and other factors) (32).

## Vectors

*Ae. aegypti* are small, dark mosquitoes with white markings on their legs (34). They have four life stages: egg (2 days), larva (7-9 days), pupa (2-3 days), and adult (70). The life cycle takes approximately 8-10 days, depending on temperature, food availability, and larval density (71). Males may mate frequently throughout their lifetimes, but only one dose of sperm is needed by females in order to produce numerous batches of eggs (32). Females generally lay 100 eggs at a time and only need a very small amount of water in which to lay eggs (71). The eggs are durable, capable of remaining viable after freezing and surviving in desiccated settings for more than a year (4, 72). Photoperiodically induced egg diapause contributes to the establishment and spread of *Aedes spp.* in long winters and temperate latitudes (73). Male adults survive 2-3 weeks in the field, whereas female adults may survive 4-5 weeks (25). Even in highly endemic areas, however, weather and other environmental factors may prevent *Ae. aegypti* from living long enough to transmit arboviruses or to reach senescence (74, 75).

*Ae. aegypti* is highly anthropophilic, although it may feed on non-human primates and occasionally on domestic animals (76). *Ae. aegypti* may ingest 3-4 µL of blood, which is twice its body weight. Viruses replicate in *Ae. aegypti*'s midgut before traveling to the salivary glands for further replication (77). Viruses in *Aedes spp.* saliva may be transmitted to humans by mosquito bites from infected females. Male mosquitoes do not bite humans. In contrast to many other biting insects, *Ae. aegypti* mostly bite during the day, with greatest activity in the hours after sunrise and before sunset (78). *Ae. aegypti* females seek blood meals within 24-36 hours after mating (25). Females may take multiple blood meals to complete the gonotrophic cycle—the reproductive cycle that begins with blood meals and ends with laying eggs (17). *Ae. aegypti* are highly productive in urban environments and have a strong preference for human blood, whereas *Ae. albopictus* prefer rural, suburban, and urban settings and are more indiscriminate, feeding on both humans and animals (25). *Ae. aegypti* prefer to rest inside houses on walls, in closets, or underneath furniture, whereas *Ae. albopictus* generally rest outdoors. *Ae. mediovittatus* is another competent arbovirus vector found in the Caribbean (79). Other *Aedes spp.* may spread arboviruses in Africa, Asia, and the Pacific (61, 80). For example, ZIKV has been identified in 16 different *Aedes* species (43).

Vectors may become infected after feeding on a viremic human. DENV, CHIKV, or ZIKV viremia in humans may range from a few days to over a week after infection, particularly in pregnant women with ZIKV (25, 37, 81). Vertical transmission of arboviruses has also been reported for *Ae. aegypti* (4, 43, 82, 83). Once infected with DENV, CHIKV, or ZIKV, *Ae. aegypti* and *Ae. albopictus* remain infected their entire lives (74). *Ae. aegypti* and *Ae. albopictus* are vectors with high competence for DENV, CHIKV, and ZIKV (25). The extrinsic incubation period (EIP), or the period of time following an infectious blood meal until the mosquito is able to transmit an arbovirus, is 8-12 days. Secondary noninfectious blood meals shorten the EIP of ZIKV and increase *Ae. aegypti* competence for DENV, CHIKV, and ZIKV (84). *Ae. aegypti* are capable of transmitting more than one arbovirus during a single feeding episode (85). We incorporate some of these mosquito and arbovirus characteristics (e.g., mosquito biting rate, probability of human-to-mosquito transmissibility) to develop a model of virological surveillance in mosquitoes.

### Breeding habits

Once a forest-dwelling zoophilic mosquito, *Ae. aegypti* adapted into an anthropophilic, highly domesticated species, preferring urban areas in and around households (4, 86). *Ae. aegypti* spend the majority of their lives in the houses where they emerged, flying an average of 40-80 m during the course of their lifetimes (87). Oviposition sites are selected based on their physical, chemical, and biological characteristics, such as container type, depth, water quality, and sun exposure (88, 89). Ideal larval habitats for *Ae. aegypti* are dark-colored containers filled with stagnant water and organic material in shaded areas around houses (78, 88, 90). The black autocidal gravid ovitraps selected for use in this dissertation field research mimic these ideal breeding habitats by attracting female mosquitoes with water and hay (91). Productive container types include flowerpots, tires, vases, buckets, cans, rain gutters, fountains, bottles, and birdbaths (78, 88, 90). Single containers may be extremely productive; one septic tank in Puerto Rico produced over 1,500 *Ae. aegypti* per day (92). In this research, we enumerated the number of mosquito larvae and pupae in indoor and outdoor household containers, which are surrogate indicators of adult populations (93, 94). Natural habitats are uncommon breeding sites for *Ae. aegypti*, but include tree holes, fronds, and coconut shells (25). *Ae. albopictus* prefer rural, suburban, or urban areas and can live in a broader temperature range and cooler temperatures than *Ae. aegypti* (95). Upon arrival to new areas, the invasive *Ae. aegypti* and *Ae. albopictus* have demonstrated an ability to replace resident mosquito populations via competitive exclusion (96).

Urbanization, unplanned growth, and concomitant deforestation create environments suitable for *Ae. aegypti* (8, 54, 61, 97, 98). In 2014, 54% of people lived in urban areas worldwide, which is expected to increase to 66% by 2050 (99). Greater human population densities provide more feeding opportunities for *Ae. aegypti* (97). Urban environments may be more favorable for *Ae. aegypti* due to the absence of natural vegetation, competition, predation, and presence of storm water drains, septic tanks, wells, and vacant lots (60, 89, 97, 100). To that end, we focused our studies on the urban environment. Deforestation causes changes in sunlight exposure and water pH, which may influence environmental nutrients and aid mosquito survival (2). Urbanized areas may experience warmer temperatures than surrounding areas, which may accelerate vector development (2). Other environmental changes such as flood protection, irrigation, dam creation, and green spaces may also increase vector breeding habitats and extend transmission seasons (2, 7).

Natural disasters such as earthquakes or hurricanes may also drive the spread of arboviruses by displacing populations, contaminating water, generating aquatic habitats in damaged properties, forcing people to live close together in confined spaces, and interfering with healthcare delivery and vector control programs (54, 101-103).

## Climate

Numerous ecological and environmental factors affect the ability of vectors to transmit arboviruses (vector competence) and vector abundance including rainfall, humidity, wind speed, photoperiod, and elevation (16, 104-107). Barometric pressure and atmospheric oxygen may also be influential (108). Weather and climate factors influence rates of virus incubation, fecundity, development, survival, and biting behavior (54, 107). A cyclical pattern of DENV transmission is observed following the monsoon season (109). *Aedes spp.* prefer tropical and subtropical zones with temperatures between 16-35 °C (105). *Aedes*-borne arboviruses are rarely reported above 2,000 m (106), although *Ae. aegypti* has been found in elevations as high as 2,300 m in Colombia (110).

Geographic expansion of arboviruses is believed to occur in response to climate change (2, 10). Higher temperatures drive the spread of arboviruses by creating more suitable environments for *Aedes spp.*, decreasing the extrinsic incubation period for *Ae. aegypti*, increasing the rate of larval development and biting rate, and lengthening the transmission season in areas where *Aedes spp.* are already locally established (25, 54, 104, 111). Increased ambient temperature accelerates the *Ae. aegypti* life cycle resulting in smaller mosquitoes which require more frequent blood meals (17). Climate change may also influence human behaviors, such as water storage practices, creating domestic breeding sites for arbovirus vectors (54, 107). Canopy coverage and water turbidity, depth, and substrate also impact breeding preferences of *Aedes* (97). *Ae. albopictus* may exist in more temperate climates than *Ae. aegypti*, extending the potential range of arboviruses.

Climate change is expected to increase global temperatures by 2-4.5 °C by 2100, potentially exposing an additional two billion people to arboviruses including higher latitude U.S. states (60, 112). It is believed that >60% of the world’s population will be at risk for DENV by 2080 (113). Limiting climate change to 1.5 °C above preindustrial levels could prevent 3.3 million DENV cases per year in the Americas compared to a no-policy scenario that warms the earth by 3.7 °C (114).

## Vector control

Effective prevention programs involve massive coordinated efforts, demanding large resource use and rigorous community engagement including persistent public education and human behavior modifications (107, 115, 116). Successful vector control programs involve collaboration among the public health sector, education, urban planning and housing, as well as nongovernmental organizations and the private sector (25, 115). *Ae. aegypti* control programs include habitat reduction, waste management, improved housing, municipal water infrastructure, covered domestic water containers (e.g., tanks, flowerpots, vases) and reservoirs, construction site management, landfill and recycle program management, and tire disposal management (25). Integrated Vector Management (IVM), promoted by WHO, aims at improving cost-effectiveness, ecological soundness, and sustainability of vector control through collaboration with health sector programs and communities (117). IVM recommends a combination of chemical and non-chemical vector control methods, including destruction of breeding sites, mobilization of communities, strategic insecticide application, and entomological and epidemiological monitoring.

Vector control strategies target different stages of the mosquito life cycle and may be biological, chemical, or environmental (21). Immature mosquito control strategies include larval source reduction campaigns, and cleaning or treating water-containing containers with bleach, insecticides, copepods, larvivorous fish, or entomopathogenic fungi (116). Adult mosquito control strategies target different mosquito behaviors such as mating (e.g., release of insects with dominant lethality), sugar feeding (e.g., toxic sugar traps), blood feeding (e.g., personal protection such as repellents, protective clothing, and bed nets), resting (e.g., indoor residual spraying), or egg laying (e.g., lethal ovitraps) (116). Smoke produced from burning biomass fuels may also have a repellent effect on mosquitoes (118). This dissertation research evaluates the association of arboviral infections with routine household cooking using biomass fuels (firewood). Other vector management efforts include mass deployment of gravid ovitraps, release of genetically modified male mosquitoes that express a dominant lethal gene, or release of *Ae. aegypti* transfected with the endosymbiont bacterium *Wolbachia* (91, 119-122). *Ae. aegypti* have developed resistance to commonly used insecticides, including pyrethroids (123). Insecticide resistance varies greatly between sites and may be induced through genetic, metabolic, environmental, or biological factors.

### Surveillance

Data from arboviral surveillance programs are used for early detection of virus circulation, tracking the spread of arboviruses, identifying outcomes associated with arbovirus infection, and categorizing transmission routes (124, 125). Rapid detection may reduce human morbidity and mortality resulting from arbovirus infection by connecting patients to healthcare services in a timely manner and implementing mosquito control interventions (126). Vector surveillance programs (e.g., larval, pupal, and adult surveys and oviposition traps) are useful for determining vector locations, vector abundance, the impact of interventions, and insecticide resistance (25, 115). A previous assessment of human arbovirus surveillance strategies for U.S. counties where no known arbovirus transmission had occurred found that testing people seeking medical care with signs of Zika virus was a more effective strategy than testing blood donors or pregnant women (127). An alternative strategy to testing humans is to test the mosquitoes directly for viruses. In this study, we used data from human and mosquito surveillances during the ZIKV epidemic of 2016 in Caguas, Puerto Rico, to compare these two strategies for detecting and monitoring ZIKV activity.

### Socioeconomic status

Social, political, and economic changes impact community sanitation, infrastructure, water access, vector control, and human migration, all of which influence *Aedes* populations (9, 60, 101). *Ae. aegypti* have limited dispersion capabilities, so arbovirus introduction to new areas is largely attributed to increased international commerce and mass global transport via air, land, and sea (7, 54, 60, 128, 129). Travelers carry infections to regions with established vector presence and susceptible populations (7, 130). Civil unrest or armed conflicts may spur human migration, introducing pathogens into susceptible areas (101).

Poverty creates ideal conditions for vector proliferation, such as limited access to water infrastructure, garbage disposal services, street drainage, sewage systems, and yard services (54, 107). Irregular supply of water or insufficient plumbing may increase the presence of uncovered containers around the household, providing greater breeding habitats (16, 60, 105). Lower SES neighborhoods may lack mosquito control infrastructure and use fewer prevention measures such as repellents, air-conditioning, and insect screens on windows (60). Individuals of lower SES are more likely to have outdoor jobs, thus increasing the probability of contact with mosquitoes (131). Other risk factors among lower SES populations are associated with higher vector abundance; these include household characteristics (number of people in the household, makeshift housing construction, proximity to abandoned properties, shaded patios, nearby gardens), sanitation (pit latrines, pigs on property), and individual characteristics (low family income, higher unemployment, lower educational attainment, older age, male sex) (128, 132-140). Because low SES is associated with *Ae. aegypti* abundance and arboviruses (132, 135), it is important to evaluate the social and environmental constructs of SES that are found in lower middle income countries, including cooking with firewood, proximity to roads and highways, housing density, and household infrastructure.

### Knowledge, attitudes, and practices

Community KAP regarding arboviruses may affect implementation of vector control or prevention measures, and these may be influenced by media coverage, public interest, and the magnitude and intensity of public health responses (54, 128, 141-143). Misunderstandings of arboviruses or fear of health outcomes such as congenital malformations may affect the probability of infected individuals seeking care. Publicized geographic risks for some areas may affect tourism and travel, particularly for couples considering pregnancy in the case of ZIKV (54). Vector control efforts may be complicated by public resistance to the use of pesticides or the release of genetically-modified mosquitoes (144, 145). Understanding that ZIKV may be sexually transmitted is important for prevention of horizontal infection by using condoms (142, 143). Education campaigns (e.g., mass media, school involvement, distributions of printed materials) may reduce *Ae. aegypti* populations in the community by informing members how to eliminate standing water, cover containers, and use proper personal protection measures (115).

### Diagnosis

IgM and IgG immunoglobulin isotypes appear in response to arbovirus infection and may be detected with Enzyme-Linked Immunosorbent Assays (ELISA) or Plaque Reduction Neutralization Tests (PRNT) (146). IgM antibodies are typically detectable 3-5 days after onset of illness and up to 12 weeks or longer following DENV, CHIKV, or ZIKV infection (147, 148). For example, ZIKV IgM may be detected 12-19 months after illness onset (149). IgG antibodies are detectable at low levels by the end of the first week following infection and may persist for years (150-152). Following a secondary DENV infection, IgG isotypes will precede IgM (153). DENV, CHIKV, and ZIKV viral genomes are also detected by nucleic acid sequence-based identification or reverse transcription polymerase chain reaction (RT-PCR), which was used in this dissertation (25, 146).

### Vaccines

There is a prototype vaccine for DENV, Dengvaxia® (CYD-TDV), which is a three-dose live recombinant tetravalent vaccine, developed by Sanofi Pasteur, licensed and commercially available in some countries (11). WHO recommends the vaccine only be used in people who previously had a DENV infection, however, as the vaccine may increase the risk of developing severe dengue in people who had not been previously infected with DENV. Therefore, WHO recommends serological testing for past DENV infection (e.g., ELISA IgG) prior to vaccination. Dengvaxia® was approved temporarily by the U.S. FDA for use in the U.S. territories of American Samoa, Guam, Puerto Rico and the US Virgin Islands (11). Other DENV vaccines are currently in Phase I and II clinical trials (154). CHIKV or ZIKV vaccines have not yet been approved for clinical use, but several are also in Phase I and II clinical trials, including a DNA vaccine, purified inactivated vaccine, live attenuated vaccine, mRNA vaccine, and several viral vector based vaccines (155, 156).

## STUDY SETTING

This dissertation research focused on the Central American/Caribbean region, where DENV, CHIKV, and ZIKV are endemic (157-159). Specifically, the research was conducted in Guatemala and Puerto Rico, geographic areas endemic for many arboviruses. Puerto Rico is home to *Ae. aegypti* and Guatemala has both *Ae. aegypti* and *Ae. albopictus*. Both countries host all four DENV serotypes, which significantly increases risk for development of severe dengue (157). The ZIKV strains in Guatemala and Puerto Rico are all within the Asian genotype and are closely related to strains from Brazil (2015) and French Polynesia (2013) (160). Understanding mosquito and arbovirus activity in the Central American/Caribbean region is critically important for limiting the spread of these viruses in mainland United States and elsewhere.

### Guatemala

With 16.6 million people, Guatemala has the largest population in Central America and the largest indigenous population in Latin America (161, 162). Indigenous peoples, including Mayan, Xinca, and Garifuna, represent 40.0% of the national population (162). The largest Mayan groups are the K'iche' (11.3% of the total population), Q'eqchi' (7.6%), and Kaqchikel (7.4%) (162). The remainder of the population is Ladino or non-indigenous (162). The country spans 108,890 km$^2$, lies between the Pacific Ocean and Caribbean, and borders Mexico, Belize, Honduras, and El Salvador (163). The country is divided administratively into 22 departments and 340 municipalities.

Guatemala represents real life manifestation of the milieu of biological, environmental, social, and behavioral factors that sustain the spread of arboviruses. These include political, economic and social changes, rapid urbanization and deforestation, human migration, socioeconomic disparities, and limited public health infrastructure (163-165). Only ¾ of people aged ≥15 in Guatemala are literate, which is the lowest literacy rate in Central America (166).

Guatemala is a lower-middle-income country with both high under-5 mortality (2,900 per 100,000 live births) and high adult mortality (21,200 and 11,200 per 100,000 people aged 15 to 60 for men and women, respectively) (167, 168). The life expectancy at birth for males and females is 69.8 and 73.9 years, respectively (162). Infectious diseases constitute a major cause of death and disability in Guatemala, accounting for 27.9% of all disability-adjusted life-years (DALYs) lost and 25% of all deaths in 2016 (169). Guatemala also has a high total fertility rate (3.6 births per woman) and rapidly growing population (170). Its annual population growth rate of 2.5% is over twice the average rate of other Latin American countries (1.1%) (170).

The GDP per capita is $7,700 USD per year and the number of people in poverty (measured at the upper middle-income class line; $5.5 USD per person per day in 2011), increased from 51% in 2006 to 59.3% in 2014 (163). Of those living in poverty, 52% are indigenous (163). The Guatemalan government spends $473 USD per capita annually on health (both healthcare and public health) (23, 24), and many of these funds are used to control arbovirus outbreaks (25). Public health expenditure is 2.6% of the total GDP, and approximately one-fifth of the population lack access to health services (171, 172).

Guatemala has weather and climate favorable for vector proliferation (86). Guatemala has also been severely affected by man-made and natural disasters including civil war, earthquakes, mudslides, volcanic activity, and hurricanes that affect vector habitat (165, 173, 174). Natural disasters disproportionately affect low income households in Guatemala, which have limited ability to manage these problems (165).

Many households in Guatemala lack running water and sanitation infrastructure, so individuals store water in containers at home and use latrines, providing breeding sites for mosquitoes (175-177). Other arbovirus risk factors include adobe, mud, or stick walls, incomplete plastering, open water containers for animals, poor waste disposal programs, and uncovered windows (176, 178-180).

Barriers to implementation of arbovirus preventive measures in Guatemala include the high costs of mosquito prevention, low perception of arbovirus risk, poor knowledge of prevention methods, and disbelief that ZIKV is sexually transmitted (175, 181, 182). Rural, indigenous populations are less likely to seek and receive health care due to distance from health care facilities, lack of available transportation, language barriers, mistrust in the providers, discrimination, and reliance on traditional healers (183, 184). One study of 599 residents of 29 communities in 11 municipalities of Guatemala demonstrated that most participants had not heard of microcephaly or Guillain-Barré syndrome (182). Cultural factors and stigmatizations may also prevent women from visiting family planning offices, accessing birth control technologies, or purchasing condoms (165, 172, 175).

DENV was first detected in Guatemala in 1978 (185). Large DENV outbreaks occurred recently in Guatemala with nearly 20,000 cases in both 2014 and 2015 (186) and 15,000 cases in 2019 as of August 12 (187). In 2014 and 2015, there were 27,000 and 30,000 cases of CHIKV, respectively (186). ZIKV was first detected in Guatemala in November 2015. From 2015-2017, there were approximately 4,000 suspected ZIKV cases and 1,000 confirmed cases, including 140 confirmed congenital syndromes associated with ZIKV infection (159). Although CHIKV and ZIKV clinical cases in Guatemala in 2019 have not risen to levels of previous years, the vectors are in place, and the viruses are circulating, with 184 and 202 confirmed cases of CHIKV and ZIKV, respectively, as of August 12 (187). Although DENV, CHIKV, and ZIKV are included in the list of notifiable diseases in Guatemala, case identification is limited by non-specificity of clinical signs and lack of diagnostic facilities. The above estimates underreport the true burden of disease as robust population-based data are lacking. Ministerio de Salud Pública y Asistencia Social in Guatemala maintains a passive arboviral surveillance program that includes arbovirus case information from hospitals and clinics (186). To our knowledge, there have been no formal evaluations of arbovirus surveillance systems in Guatemala.

The response of the Ministerio de Salud Pública y Asistencia Social in Guatemala to arboviruses has included: community-based vector control; application of larvicides; population-based education and communication campaigns; elimination of breeding sites; and epidemiological, entomological and laboratory surveillance (182, 188). These control methods have been unevenly applied across the country.

**Puerto Rico**

Puerto Rico has a population of 3.2 million and a land mass of 3,515 square miles. It is the third most densely populated state or territory in the United States and fourth largest island in the Greater Antilles (189). Like Guatemala, there are a number of environmental, political, economic, and social factors that render Puerto Rico a likely habitat for arbovirus vectors, including climate, population growth, internal migration, unplanned urban areas, poverty, poor sanitary conditions, and limited mosquito prevention measures (60). Approximately 12.4% of Puerto Ricans are unemployed and 45% live in poverty (<$34 per day), over twice as high as any U.S. state (189). Communities with higher poverty and population densities have more stagnant water and reduced access to mosquito control resources (190).

Puerto Rico has a tropical climate with an average annual temperature of 25° C. The dry season is December to April, and the rainy season is May to November (60). Dry, boreal winters in Puerto Rico have lower numbers of circulating *Ae. aegypti*, but enough to sustain arbovirus transmission because people in Puerto Rico frequently use household water containers (191, 192). In Puerto Rico, the influence of weather on vector density is high in areas of high population density and low median household income (193).

Puerto Rico has had cyclical 2-3 year DENV epidemics of different serotypes since the 1970s (194). Outbreaks have increased in severity, beginning with DENV-3 (1977) and followed by DENV-1 (1978), DENV-4 (1981), DENV-2 (1984), and DENV-3 (1997) (194). A DENV-4 outbreak in 1986 marked the emergence of epidemic DHF, leading to high incidence of DHF and DSS in subsequent years (195). The most recent DENV epidemics in Puerto Rico were in 2010, 2012, and 2013, and included 21,298, 12,877, and 18,164 reported cases, respectively (121, 196). There are 3,400 to 7,000 DENV cases in non-epidemic years in Puerto Rico, with most occurring during the rainy season from August to November (196). DENV has been reported in Puerto Rico every month since 1986 (92).

Local transmission of CHIKV was reported in Puerto Rico in May, 2014, and led to 28,327 cases during the first year of transmission in all 78 municipalities (197). There have not been epidemics of DENV or CHIKV in Puerto Rico since 2013 and 2014, respectively.

Local transmission of ZIKV in the United States was reported in December, 2015, in Puerto Rico (198). ZIKV soon became a major epidemic with over 40,000 cases by the end of 2017, including over 4,000 pregnant women, more than in any other U.S. state or territory (199). During this period, there were 424 hospitalizations, 5 deaths, 53 Zika-related cases of Guillain-Barré syndrome, and 49 reported birth defects, including microcephaly, neural tube defects, and ophthalmic diseases (199, 200). Like many DENV epidemics in Puerto Rico, there was an early peak for the ZIKV epidemic, which may have been partly attributed to the occurrence of a super El Niño one year earlier (201, 202). El Niño may have increased mosquito biting rates and shortened the extrinsic incubation period (43). El Niño was followed by a cool and wet La Niña from July-September, 2016, which may have facilitated the end of the ZIKV epidemic from December, 2016, to April, 2017 (121). DENV, CHIKV, and ZIKV have since been detected in *Ae. aegypti* around houses that had human cases and in mosquito surveillance sites (121, 203, 204).

KAP studies in Puerto Rico demonstrated that people believe DENV transmission may be prevented by improved public green space and building maintenance, improved water infrastructure, fumigation activities, comprehensive education programs about arboviruses, and waste and recyclable collection services (205, 206). KAP data also indicated misconceptions about DENV, lack of perceived importance of DENV, and denial of personal responsibilities to reduce DENV (60, 205, 206). Efforts to control sexual transmission of ZIKV are inhibited by sexuality-related stigmatization, poor quality sex education, limited access to contraception, low levels of reproductive services, and low condom use rates (207). Contraception access is limited by incomplete insurance coverage, lack of trained providers, and high costs (207).

Vector control in Puerto Rico is managed by the Puerto Rico Department of Health with technical support from the CDC's Dengue Branch (with its laboratory in Puerto Rico), and includes source elimination, outdoor fumigation, larvicidal measures, educational campaigns, and epidemiological surveillance (208).

## DISSERTATION RESEARCH

The rapid emergences of DENV, CHIKV, and ZIKV underscore the reality that new pathogens causing dangerous epidemics may emerge at any time. The introduction of West Nile virus in North America and its rapid spread throughout the western hemisphere provides a vivid example of the ability of an emerging arbovirus to cause a significant public health impact in a new environment. The presence of competent vectors and ecological similarities between Central America, the Caribbean, and North America indicate that infections in Central America or the Caribbean could pose similar threats to the southern tier of the United States. To date, *Ae. aegypti* has been frequently, albeit sporadically, reported in California, Texas, Florida, Louisiana, Mississippi, Georgia, and Arizona (209). The potential range of *Ae. aegypti* in the United States is enormous, however, encompassing the entire U.S. southern tier (210).

The first manuscript of this dissertation explores the interactions among social and ecological factors by evaluating the associations of arboviral infections in human hosts with routine household cooking with firewood. There is a growing worldwide movement to reduce levels of household air pollution by switching from biomass fuels to cleaner forms of energy. There is some evidence, however, that smoke from biomass fuels may be an effective mosquito repellent (211-215). It is therefore important to determine whether the transition to cleaner fuels may increase the risk of mosquito-borne diseases, which would call for additional mosquito prevention measures. This study is based in rural Guatemala, where arbovirus vectors flourish and where firewood is the most common cooking fuel.

The second manuscript compares vector and host-based surveillance strategies to detect ZIKV, in Puerto Rico. Virological surveillance in mosquitoes involved trapping gravid *Ae. aegypti* females and testing them for ZIKV. Human surveillance included identifying cases with two or more ZIKV symptoms seeking care in an emergency department. Many ecological, biological, and social factors affect these mosquito and human surveillance systems as to their efficacy in identifying positive mosquito pools or human cases. Vectors must become infected by biting an infected human during the window of human infectiousness and then be captured. An infected human must be symptomatic, seek care, and be viremic at the time of testing. In both systems, the agent must then be identified with a specific laboratory assay. Using simulation models for human and mosquito surveillance strategies, we evaluate different transmission scenarios with varying ZIKV infection rates. Results from this study may be used to guide surveillance efforts given different ZIKV transmission contexts. Sensitive surveillance systems may allow more timely vector control interventions and may provide information on the efficacy of vector control activities in reducing disease outcomes.

The third manuscript explores relationships among the environment and arbovirus vectors by evaluating household and geospatial predictors of immature mosquito abundance in urban and rural areas of Guatemala. In this study, we determined the association between household environmental factors and the presence of mosquito larvae and pupae in household containers. We also determined whether proximity to other houses and roads was predictive of immature mosquito abundance. The results of this study may be useful for targeted surveillance and vector control interventions in areas where arboviruses are endemic.

The ecological, biological and social influences of arboviral vectors and their diseases are extraordinarily complex and dynamic over time. These studies are but snapshots immediately subject to changing environmental and social conditions. However, these three studies may offer new insights for public health practice in tropical settings that include more focused consideration of the interactions among host, agent, and environmental factors for arboviruses and their vectors.

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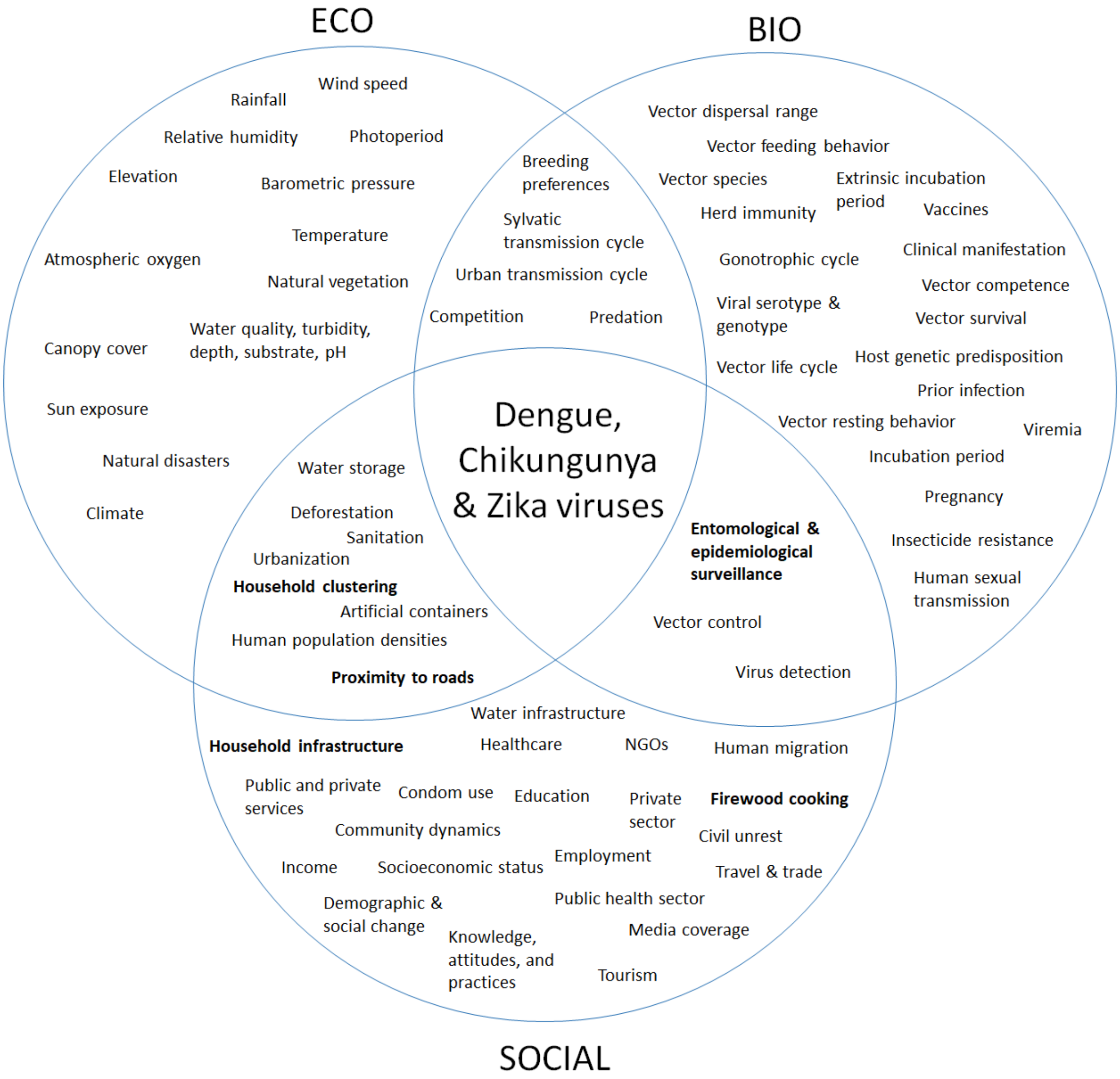

**Figure 1.1: Eco-Bio-Social Conceptual Framework.** Factors in bold were studied in this dissertation research.

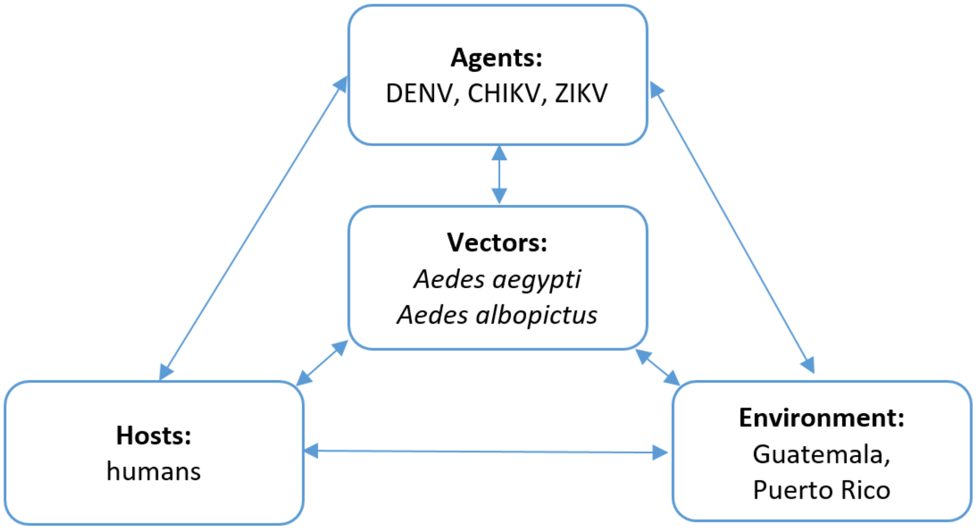

**Figure 1.2: Epidemiological Triad**

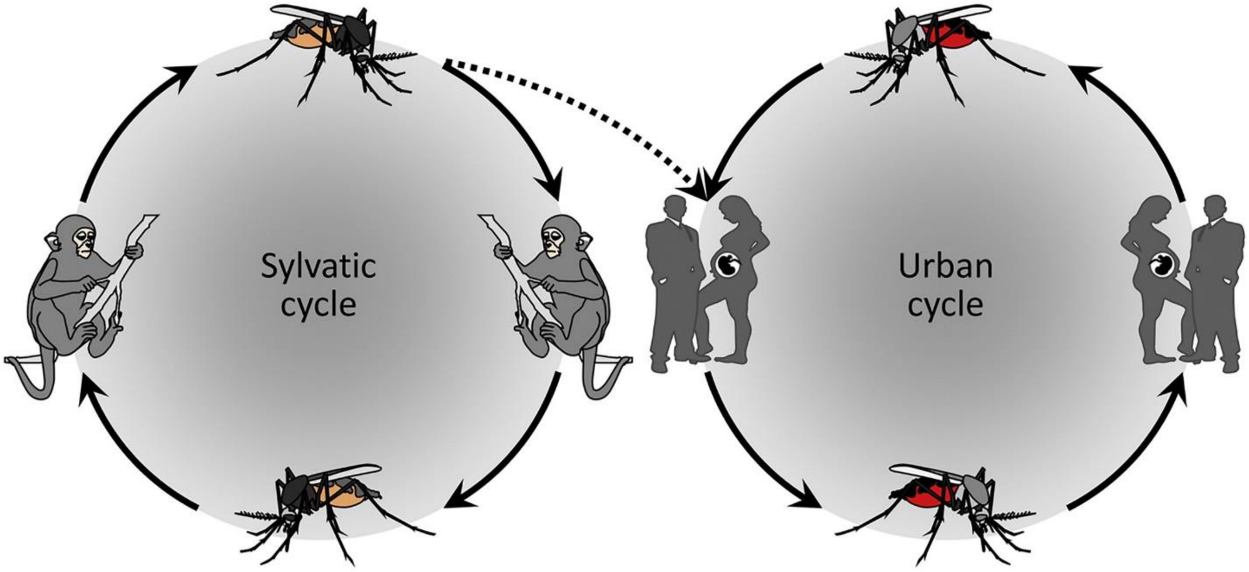

**Figure 1.3: Transmission cycles of Zika virus.** Source: Song BH, Yun SI, Woolley M, Lee YM. Zika virus: history, epidemiology, transmission, and clinical presentation. Journal of Neuroimmunology. 2017 Jul 15;308:50-64.

# CHAPTER 2: INVERSE ASSOCIATION BETWEEN DENGUE, CHIKUNGUNYA, AND ZIKA VIRUS INFECTION AND INDICATORS OF HOUSEHOLD AIR POLLUTION IN SANTA ROSA, GUATEMALA: A CASE-CONTROL STUDY

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## ABSTRACT

**Background:** Dengue, chikungunya, and Zika viruses are increasingly important public health problems. Burning vegetation, leaves, and other plant products have been shown to be effective mosquito repellents for their vector, *Aedes spp.*, but there has been scant research on whether firewood cooking smoke in households influences mosquito populations or mosquito-borne diseases. About 2.9 billion people worldwide use biomass fuel for household cooking and heating, resulting in an estimated 1.6 million deaths annually from household air pollution (HAP)-related diseases. Global health agencies now encourage households to transition from biomass to clean fuels, but it is unclear whether such interventions may actually increase risk for mosquito-borne diseases. This retrospective case-control study evaluated associations between arboviral infections and cooking with firewood in Santa Rosa, Guatemala.

**Method:** *Vigilancia Integrada Comunitaria* (VICo) was a prospective public health surveillance system for bacterial, parasitic, and viral causes of diarrheal, neurological, respiratory, and febrile illnesses in hospitals and clinics in the department of Santa Rosa, Guatemala. Enrolled VICo in-patients and out-patients during 2011-2018 were interviewed using standardized questionnaires on demographics and household characteristics. Blood and stool specimens were collected and tested to identify the etiologies presenting symptoms. Cases were defined as laboratory-positive for dengue, chikungunya, or Zika virus infections. Controls were laboratory-positive for bacterial and viral diarrheal illnesses (e.g., *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli*, rotavirus, norovirus, sapovirus, or astrovirus). Cooking with firewood, kitchen location, stove type, and firewood cooking frequency were the independent exposure variables. Logistic regression models were used to analyze unadjusted and adjusted associations between arboviral infections and exposures of interest.

**Result:** There were 311 arboviral cases and 1,239 diarrheal controls. Arboviral infections were inversely associated with cooking with firewood in the main house (AOR: 0.22; 95% CI: 0.08, 0.57), cooking with firewood on an open hearth (AOR: 0.50; 95% CI: 0.33, 0.78), and cooking with firewood ≥5 times per week (AOR: 0.54; 95% CI: 0.36, 0.81), adjusting for age, sex, ethnicity, socioeconomic status index,

number of people per household, community population density, community elevation, recruitment location, season, and admission year.

**Conclusion:** Several primary determinants of HAP exposure were inversely associated with arboviral infections. Additional studies are needed to understand whether interventions to reduce HAP might actually increase risk for mosquito-borne infectious diseases, which would warrant improved education and mosquito control efforts in conjunction with fuel interventions.

## INTRODUCTION

With the emergence and reemergence of dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV), arthropod-borne viruses (arboviruses) are increasingly important public health challenges (1-3). The first major DENV epidemics were reported in 1779 and 1780 in Africa, Asia, and North America (4). DENV is now the most prevalent and rapidly spreading of the arboviruses, with transmission occurring in 128 countries, thereby creating risk for almost 4 billion people (5-7). There are 390 million DENV infections (95% credible interval: 284–528 million) worldwide annually, including 96 million (95% CI: 67–136 million) clinical cases, 500,000 dengue hemorrhagic fever cases, and 22,000 deaths, mostly among children <5 years of age (8). CHIKV was first reported in the Americas in 2013, causing 1.8 million suspected cases from 2014-2017 in 44 countries and territories (3). CHIKV may also cause prolonged arthritis, meningoencephalitis, nephritis, retinitis, uveitis, myelitis, cranial nerve palsies, and acute encephalopathy (9). First identified in Uganda in 1947, ZIKV expanded into the South Pacific and Americas with 48 countries reporting active ZIKV by 2017 and 86 by 2019 (10-12). From 2015-2018, there were over 580,000 suspected and 220,000 confirmed ZIKV cases in the Americas (2). ZIKV has also been linked to congenital microcephaly, Guillain-Barré syndrome, craniofacial disproportion, cerebral palsy, spasticity, hearing loss, brainstem dysfunctions, joint deformities, and developmental and inflammatory ocular diseases (11, 13). This study focused on Guatemala, a country where arboviruses are endemic. Large arbovirus outbreaks have occurred in Guatemala with nearly 40,000 DENV cases from 2014-2015 (14), 57,000 CHIKV cases from 2014-2015 (14), and 4,000 suspected ZIKV cases and 1,000 confirmed cases from 2015-2017 (15).

The most common mode of DENV, CHIKV, and ZIKV transmission is via *Aedes (Ae.)* mosquitoes, particularly *Ae. aegypti* or *Ae. albopictus.* Climate change, urbanization, migration, increased air travel, human behaviors, and ecosystem modification are some factors driving the geographic spread of *Aedes* mosquitoes and their associated viruses (12, 16).

Low socio-economic status (SES) in many settings has been associated with increased risk for arboviral infection (17-19). Poverty creates ideal conditions for vector proliferation, such as limited access to water infrastructure, garbage disposal services, street drainage, sewage systems, and yard maintenance (20, 21). It is important to understand the associations between arbovirus transmission and environmental risk factors in order to apply appropriate control measures that may reduce transmission and eliminate arboviruses in endemic areas.

Smoke from burning biomass materials is the most widely used mosquito repellent in the rural tropics (22). Burning vegetation, leaves, and other plant products have been shown to be effective mosquito repellents (23-27), but there has been scant research on whether smoke from household firewood fires influences mosquito populations, mosquito bites, or mosquito-borne diseases. The few studies of associations between firewood smoke and mosquito abundance are inconsistent. Some studies report firewood smoke reduced *Anopheles* and *Culex* populations in the household resulting in fewer mosquito bites (28-30). Another study demonstrated inverse associations between firewood smoke and *Aedes* larvae (31). Other studies were unable to support firewood smoke as an effective mosquito repellent with respect to malaria infection, which is transmitted by *Anopheles* (32-34). The repellent effect is lost when occupants leave the home and its smoky environment, but smoke residue on skin may provide some repellency by masking human kairomones such as carbon dioxide (22). We are unaware of any studies assessing the impact of firewood smoke on *Aedes*-transmitted arboviruses. Mosquito repellent benefits from burning firewood are also likely outweighed by other serious health problems from inhaling biomass smoke (35).

About 2.9 billion people worldwide depend on biomass fuel, such as wood, charcoal, coal, animal dung, and crop residues, for their household cooking and heating (36). However, use of these fuel sources inside houses produces high levels of household air pollution (HAP), including particulate matter, methane, carbon monoxide, polyaromatic hydrocarbons, and volatile organic compounds, which may penetrate into organs and tissue (37). Exposure to HAP contributes to 1.6 million deaths annually from stroke, ischemic heart disease, chronic obstructive pulmonary disease, and lung cancer (35, 38). HAP exposure has also been linked with other cancers (e.g., cervical), pneumonia, decreased lung function, adverse pregnancy outcomes, asthma, and cognitive impairment (35, 39). Consequently, major global health investments are now made to accelerate the transition from biomass to clean fuels. For example, the Global Alliance for Clean Cookstoves is working to reduce the use of fuel burning stoves and to increase the number of improved cook stoves in Guatemala (40). Given the growing public health importance of arboviruses in the Americas, it is important to understand whether such interventions might have unintended consequences, such as increasing risks for mosquito-borne infectious diseases. This study focuses on firewood, which is the predominant energy source for cooking in Central America (41). To our knowledge, this is the first study to investigate associations between DENV, CHIKV, or ZIKV infection and household air pollution (HAP) or specific characteristics of firewood cooking in the household. This study evaluates the associations of cooking with firewood, the location in the house where someone cooks with firewood, the type of stove used to cook with firewood, and the times per week cooking at home with firewood, with arboviral infections in Santa Rosa, Guatemala, where firewood cooking is the most common cooking method (40).

## METHODS

### Study design

*Vigilancia Integrada Colaborativa* (VICo) was a prospective public health sentinel surveillance system for bacterial, parasitic, and viral causes of diarrheal, neurological, respiratory, and febrile illnesses in Guatemala. Hospital surveillance began in Cuilapa, Santa Rosa, in November 2007. Health center surveillance began in Nueva Santa Rosa Municipality in July 2007. We conducted a retrospective case-control study to examine associations between *Ae. aegypti*-transmitted arboviruses (DENV, CHIKV, or ZIKV infection) and HAP exposure. Additional details of VICo methodology are described elsewhere (42-46).

### Study setting

The Department of Santa Rosa, Guatemala, (14.16 ° N, 90.48 ° W) has a population of approximately 400,000 in an area of 2,295 km$^2$ and is semi-tropical (Figure 2.1) (47). Its altitude varies from sea level on the Pacific Coast to 1,945 m on top of the Tecuamburro volcano. The mean annual temperature is 23.5°C and mean annual precipitation is 1,412 mm (48). The population of the Department is 55% rural and 45% urban and is almost equally divided between women and men (49). Countrywide, 2.1 million households (59.7%) use wood fuel, including 1.3 million in rural and 0.8 million in urban areas (40, 49). In 2013, 97% of rural and 85% of urban residences used firewood for fuel in Santa Rosa (40).

The National Hospital of Cuilapa serves all 400,000 residents from Santa Rosa as well as referrals from municipalities of neighboring Departments of Jutiapa and Jalapa. VICo also included a health center in Nueva Santa Rosa municipality, located 30 km north of Cuilapa (43).

### Study population

Inclusion criteria for the study included residency in Santa Rosa, Jutiapa, and Jalapa Departments during the 30 days before presenting to the National Hospital of Cuilapa or health center in the Nueva Santa Rosa municipality. All ages and both males and females were included.

At the hospital and health center, surveillance staff searched the emergency room records, inpatient logs, and ward registers to identify patients admitted or presenting with signs or symptoms suggestive of acute febrile illnesses (AFI) or diarrhea. Patient and household information was obtained from clinical evaluations, medical records, laboratory, and standardized interviews using hand-held personal digital assistants. Following informed consent, all patients who met the case definitions and agreed to participate in the project were asked for information regarding demographics, clinical information, and risk factors, and the appropriate laboratory samples were collected to determine the etiology of their infections.

AFI was defined as self-reported fever that began within one week of the current illness, or documented oral or axillary fever of ≥38ºC at presentation or within 24 hours of admission to the hospital or health center. Patients with evidence of an obvious source of fever on physical examination (e.g., otitis media, septic arthritis, pyogenic soft tissue infection) determined by the examining healthcare provider were excluded.

Four ml of whole blood were collected from hospitalized patients with AFI, which were transported to the Universidad del Valle de Guatemala (UVG) laboratory. Plasma was separated and frozen at -70°C. Reverse transcription polymerase chain reaction (RT-PCR) (DENV, CHIKV, ZIKV) and enzyme-linked immunosorbent assay (ELISA) IgM (DENV) tests were done at the UVG laboratory to confirm arboviral infection. Patients with AFI enrolled in the study were not tested for diarrheal illnesses.

Cases were defined as those presenting AFI who tested positive for DENV, CHIKV, or ZIKV infection based on RT-PCR or ELISA tests from 2011 (when the questions regarding cooking with firewood were added to the survey) to 2018. Controls were confirmed bacterial and viral diarrheal illnesses during 2011-2018 (i.e., *Salmonella* spp., *Shigella* spp., *Campylobacter* sp., *Escherichia coli*, rotavirus, norovirus-1 and 2, sapovirus, and astrovirus). We only included diarrheal controls with confirmed bacterial or viral infections because diarrhea may be a symptom of an arbovirus infection (50). Stool culture was used to detect bacterial infections including *Salmonella* spp., *Shigella* spp., and *Campylobacter* sp. ELISA IgM was used to detect rotavirus. RT-PCR was used to detect norovirus-1 and 2, sapovirus, and astrovirus. Conventional PCR was used to detect *Escherichia coli*. Due to relatively low sensitivity of fecal smear microscopy (51-53) and weak associations with diarrheal illnesses (54), parasitic infections (e.g., *Cryptosporidium parvum, Giardia lamblia*) were excluded from analyses. Patients enrolled into the study with diarrheal symptoms were not tested for arboviruses.

### Ethics statement

The protocol for the VICo surveillance project received approval from the institutional review boards of UVG (Guatemala City, Guatemala), the Centers for Disease Control and Prevention (Atlanta, GA, USA), and the Guatemala Ministry of Public Health and Welfare. This study uses de-identified data from VICo and was determined to qualify for exemption from full Institutional Review Board review.

Patients were asked for verbal consent for eligibility screening. If they satisfied the case definitions, they were asked for written, informed consent for participation in the study. We obtained verbal assent from minors <18 years of age and written, informed consent from their parents or legal guardians.

### Exposure and covariate measures

The key HAP exposure classifications were: firewood is used as the main fuel for cooking in household, location in the house where firewood cooking is done, type of stove used to cook with firewood, and times per week cooking at home with firewood.

We used principal components analysis (PCA) to create a HAP exposure score from firewood cooking location in house, firewood stove type, and firewood cooking frequency based on all cases and controls. First, we assigned scores ranging from 0-3 based on HAP exposure levels: firewood cooking location (3: in the main house, 2: in a kitchen that is separated from the main house or in an informal structure without walls or roofs, 1: outside the house, 0: does not cook with firewood), type of stove used to cook with firewood (3: open hearth, 2: improved stove without chimney, 1: improved stove with chimney, 0: does not cook with firewood), and times per week cooking at home with firewood (3: ≥5, 2: 3-4, 1: 1-2, 0: does not cook with firewood). The first component included all three variables and accounted for 88% of the variability in the data, and these variables were then weighted against their eigenvector coefficients (55). The resultant score was categorized into quintiles of HAP exposure levels (very low, low, middle, high, very high).

Covariates included year of admission (2011-2018), season (dry, rainy), age (continuous), sex (female, male), ethnic group (Ladino, Xinca, other), recruitment location (hospital, health center), number of people in the house (continuous), community elevation (continuous), and community population density (continuous). The ‘Ladino’ people are Central Americans with a mix of indigenous and Spanish descent. The ‘dry’ season was from November-May, whereas ‘rainy’ was from June-October. Geographical information system software (ArcGIS Pro 2.2.4 software; ESRI, Redlands, CA) was used to calculate average elevation (meters) and average population density (numbers of people per hectare) per community. Population densities were obtained from WorldPop 2015 (56). Elevations were from the Consortium for Spatial Information (CGIAR-SRTM) (57). PCA was used to create a SES index from 14 variables based on all cases and controls and included: presence of a refrigerator, computer, radio, washing machine, clothes dryer, car, television, telephone/cellphone, microwave; number of rooms in house; family monthly income; electricity; roof material; and floor material (Table S.1). Missing data for SES variables were assigned the lower category. One component was developed and retained which accounted for 29% of the variability in the data. Other components explained little variability in the data. These variables were weighted against their eigenvector coefficients. The SES index was categorized into quintiles with scores ranging from 0 to 5 with a higher score indicating higher SES.

### Statistical Analysis

Exposures and covariates for cases and controls were first evaluated with descriptive statistics (means and standard deviations for continuous variables and frequency distributions for categorical variables). T-tests and Chi-square tests were then used to assess differences between cases and controls for continuous and categorical variables, respectively. The Chi-square test for trend (extended Mantel–Haenszel) was used to test linear trends in HAP scores between cases and controls.

Logistic regression was used to analyze unadjusted (Model 1) and adjusted (Model 2) associations between arboviral infections and exposures of interest (cooks with firewood, firewood cooking frequency, kitchen location, stove type, and HAP score). In Model 2, confounders were identified *a priori* from the literature using directed acyclic graphs (58, 59): age (18, 19), sex (60), ethnic group (61), SES index (18, 62), admission year (63), season (63), number of people in household (64), population density (65), recruitment location, and elevation (66). We chose not to match on age, location, and year to prevent biases associated with matching in case-control study designs (58, 67). We considered linear, quadratic, and cubic forms of age and SES index. Since most of the cooking in Guatemala is done by women (40), we also looked to see whether there were interactions between sex and the exposures of interest on arboviral infections in Model 2. Tolerance values were used to assess collinearity among all independent variables. Hosmer-Lemeshow tests were used to assess goodness-of-fit of adjusted models. Odds ratios (OR) described the magnitude of associations between arboviral infection and exposures of interest. Statistical significance, defined as $p<0.05$, was evaluated through the Chi-square test. ORs, 95% confidence intervals, and p-values were reported. All analyses were conducted using SAS V.9.4 (SAS Institute, Inc., Cary, North Carolina).

## RESULTS

### Sample characteristics

Among the 311 arbovirus cases there were 219 DENV, 75 CHIKV, and 29 ZIKV infections (Table 2.1). Twelve (3.9%) had dual infections. Of arbovirus cases and diarrheal controls, 199 and 750 were recruited from the hospital, whereas 112 and 489 were recruited from the health center, respectively (Table 2.2).

The average age of patients was 21 years, 54% were male, and 76% were Ladino ethnicity (Table 2.2). Of cases and controls, 67% and 75% respectively cooked with firewood, 54% and 68% cooked with firewood at least five times per week, 32% and 53% cooked with firewood on an open hearth, and 2% and 7% cooked with firewood in the main house. Among all study participants who did not cook with firewood, 98% cooked with gas and 2% cooked with electricity.

### Arboviral infection and cooking with firewood

Unadjusted analyses demonstrated inverse associations between arboviral infections and cooking with firewood (OR: 0.66; 95% CI: 0.50, 0.86); cooking with firewood ≥5 times per week (OR: 0.59; 95% CI: 0.45, 0.78), in the main house (OR: 0.22; 95% CI: 0.09, 0.51), in an informal structure without walls/roofs (OR: 0.54; 95% CI: 0.33, 0.88), outside the house (OR: 0.54; 95% CI: 0.32, 0.92), and on an open hearth (OR: 0.44; 95% CI: 0.32, 0.60); and high HAP score (OR 0.40; 95% CI: 0.28, 0.57) and very high HAP score (OR 0.13; 95% CI: 0.04, 0.43) (Table 2.3). We found a significant linear trend in HAP score on arbovirus infection ($p<0.01$). Higher HAP exposure reflects lower odds of arbovirus infection.

Arboviral infections were no longer associated with overall cooking with firewood, cooking with firewood in an informal structure, and cooking with firewood outside, after adjusting for age, sex, ethnicity, SES index, number of people per household, community population density, community elevation, recruitment location, season, and admission year (p=0.11) (Table 2.3).

Even after adjustment, analyses showed associations between arboviral infections and the two highest level HAP exposure classifications (high HAP scores: AOR: 0.41; 95% CI: 0.25, 0.68; very high HAP scores: AOR: 0.12; 95% CI: 0.03, 0.44). Arboviral infections remained inversely associated with cooking with firewood ≥5 times per week (AOR: 0.54; 95% CI: 0.36, 0.81), cooking with firewood in the main house (AOR: 0.22; 95% CI: 0.08, 0.57), and cooking with firewood on an open hearth (AOR: 0.50; 95% CI: 0.33, 0.78), even after adjusting for relevant covariates (Table 2.3). Quadratic and cubic forms of age and SES index were not significant in all models (p-values≥0.34), but linear age and SES index were significant (p-values≤0.02) and therefore included. Hosmer-Lemeshow GOF tests demonstrated model fits were adequate (p-values≥0.24). Tolerance values for all independent variables were above 0.90, indicating no evidence of collinearity. Interaction terms between sex and exposures of interest were not significant (p-values≥0.13).

## DISCUSSION

Our analysis found fewer arboviral infections among patients exposed to higher levels of biomass smoke than among those who did not cook with firewood. We did not find associations between arboviral infections and lower levels of biomass smoke. These results were supported by analyses that treated HAP exposure as a composite score, which demonstrated inverse associations with the two highest HAP exposure levels.

The odds of cooking with firewood in the main house were less among patients with arboviral infections than among controls. Presumably, HAP exposure would be higher in households with kitchens in the main household rather than in a separate location (68). We did not find associations between arboviral infections and cooking with firewood outside or in a kitchen separate from the main house, implying that any arboviral-protective benefit from smoke exposure might be limited to confined household spaces. In Guatemala, approximately 90% of urban households and 70% of rural households conduct cooking activities inside the main house (40). Previous studies have demonstrated that firewood smoke was effective at reducing the number of *Anopheles spp.* found in households (28, 30). To our knowledge, only two studies have assessed kitchen location in relation to mosquitoes or mosquito-borne diseases. In Laos, cooking fires in the main living area or directly underneath houses were associated with fewer *Anopheles spp.* than fires in rooms separate from the house (30). In Ethiopia, individuals living in households that had a separate kitchen outside of the sleeping room were at greater risk for malaria (69). However, we are unaware of any studies examining the impact of cooking with firewood on arboviral infections. In Guatemala, cooking activities are carried out for approximately 13 and 14 hours per week in urban and rural areas, respectively (40). Additionally, women spend 4.6–6.8 hours in the kitchen per day (70). Cooking activities, which are mostly done in the daytime (40), likely have a differential effect on *Ae. aegypti*, which are primarily daytime feeders compared to *Anopheles spp.*, which are nocturnal but may include crepuscular feeders (71-73). *Ae. aegypti* preferentially rest indoors in dark places (e.g., on walls, in closets, or underneath furniture) and lay eggs in artificial containers around households (74), whereas *Anopheles spp.* rest indoors and outdoors, but prefer marshes, trees, swamps, fields, streams, and rivers as oviposition sites (75). *Ae. aegypti* are also more likely to bite indoors than outdoors (76), whereas *Anopheles spp.* mostly bite outdoors (77).

Cooking with firewood ≥5 times per week was less common among arbovirus cases than among controls, but there was no association with cooking with firewood <5 times per week. Smoke may therefore only act as a repellent during the cooking periods such as in the mornings, afternoons, and evenings when firewood smoke is produced (22). These findings are consistent with a study in rural Thailand that found inverse associations between firewood smoke and *Aedes* larvae abundance (31). Firewood cooking produces biomass smoke, which may influence mosquitoes by masking human odors such as carbon dioxide (22), interfering with mosquito chemoreceptors (78), or emitting organic compounds that serve as insecticides (22, 34). Alternatively, the heat from firewood cooking may reduce room humidity, creating an unfavorable environment for mosquitoes (28). Stove use monitoring could improve understanding of the relationships between cooking frequency and arboviral infections.

Arboviral infections were lower among patients who cooked on an open hearth, but there was no association observed with cooking with improved stoves. Chimney stoves reduce kitchen concentrations by approximately 90% and personal exposures in women by 61% (79), but may also inadvertently increase exposure to mosquitoes in the household (34, 80). Additional studies are needed to determine whether HAP interventions should be combined with mosquito prevention strategies (34). Insecticide-treated bed nets, window screens, protective clothing, and air conditioning are safe and effective arbovirus prevention measures (81-83).

The interaction between gender and firewood cooking on arboviral infections did not reach statistical significance. Cooking is mainly done by women in Guatemala (40), but it is conceivable that men are present in the household during cooking activities. It is also possible that we did not have adequate power to detect a gender-related interaction.

Three-quarters of cases and two-thirds of controls cooked with firewood, which is higher than the prevalence of firewood use in all of Guatemala (59.7%). This difference may be attributed to the high proportion of rural residences in Santa Rosa Department (58.1%), as well as the high prevalence of firewood use in Santa Rosa Department (rural households: 97%; urban households: 85%) (40).

This study has several limitations. First, there is likely unmeasured confounding in this study, such as whether participants used mosquito prevention measures (e.g., mosquito nets, fumigating), the number of open water-holding containers around patients' households, household sanitation, and arbovirus transmission site. It could be that cases were infected at work, school, or elsewhere away from the home where they were not exposed to firewood smoke. However, we do not expect other sources of smoke to be strong confounders, and our adjustment for urban versus rural residency should help reduce biases associated with arbovirus transmission sites. Second, this was a case-control study, so we are unable to make causal inferences about the relationship between arboviral infection and firewood cooking, only associations. Third, this study included patients from a hospital and health center and is thus not representative of all of Santa Rosa Department or Guatemala. Fourth, although we only included diarrheal controls with confirmed bacterial or viral infections in an attempt to ensure controls were not cases, most diarrheal controls were not tested for arboviruses. Non-differential misclassification of the outcome may dilute the magnitude of the odds ratios (biased towards the null). Fifth, we do not know when arbovirus transmission occurred in relation to wood smoke exposure, but our questionnaire reflects the patients' typical past exposures. Sixth, it is unknown whether hospital controls with diarrheal illnesses, like norovirus, influenced the susceptibility of the patients to arboviruses. Finally, it is unknown whether HAP exposure influences diarrheal controls' susceptibility to a diarrheal infection and therefore, selection into this study (Berkson's bias). However, one study in California demonstrated $PM_{10}$, COH, $NO_2$, and $O_3$ were not associated with gastroenteritis (84). An attempt was made to minimize this risk by limiting the controls to confirmed bacterial and viral diarrheal illnesses, and excluding respiratory infections and undiagnosed diarrheal illnesses.

Notwithstanding these limitations, this study included approximately four controls per case, which increased statistical precision. We assessed multiple measures of HAP exposure, including household kitchen location, firewood cooking frequency, and stove type and found associations that strengthened with increased HAP exposure. The interviewers collecting HAP exposure data were unaware that HAP might to be associated with arbovirus infections. Controls were recruited from the same catchment area as cases.

HAP exposure is a major risk factor for acute and chronic respiratory diseases. Particulate matter exposure risks include respiratory symptoms; acute and chronic decrement in pulmonary function; bronchial hyperactivity; acute phase reaction; respiratory infections, emergency department visits, and hospitalizations; asthma development; and premature mortality in people with chronic lung disease (35, 39). We found anecdotal evidence that suggests households that frequently cook with firewood may have fewer arboviral infections than households that do not cook with firewood. Rather than suggesting that biomass smoke be employed as a preventive measure, these findings suggest that arboviral surveillance studies should monitor levels and trends during efforts to reduce HAP exposures in order to help determine whether a causal relationship exists.  Given the public health importance of arboviruses in the Americas, it is important to understand whether interventions to reduce HAP might actually increase risks for mosquito-borne infectious diseases—especially during transmission season or outbreak periods, which would warrant expanded education and vector control efforts in conjunction with interventions to reduce HAP.

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**Figure 2.1. Cuilapa National Hospital and Nueva Santa Rosa Health Center, Santa Rosa Department, Guatemala.** Source: Santa Rosa department location map; by user Edgouno; licensed under CC BY 3.0 via Wikimedia Commons, https://commons.wikimedia.org/wiki/File:Quetzaltenango_department_location_map.svg.

**Table 2.1. Arbovirus frequency among cases; viral and bacterial infections among diarrheal controls, Santa Rosa, Guatemala, 2011-2018**

| | N (%) |
|---|---|
| *Cases (N=311)*[a] | |
| Dengue | 219 (70.4) |
| Chikungunya | 75 (24.1) |
| Zika | 29 (9.3) |
| *Controls (N=1,239)*[b] | |
| *Salmonella* spp. | 13 (1.1) |
| *Shigella* spp. | 144 (11.6) |
| *Campylobacter* sp. | 190 (15.3) |
| *Escherichia coli* | 326 (26.3) |
| Astrovirus | 49 (4.0) |
| Sapovirus | 49 (4.0) |
| Norovirus-1 | 48 (3.9) |
| Norovirus-2 | 388 (31.3) |
| Rotavirus | 290 (23.4) |

[a] Cases may have been diagnosed with multiple arboviruses (3.9%).

[b] Controls may have been diagnosed with multiple viral and/or bacterial infections among those listed (20.8%).

**Table 2.2. Cases with confirmed arbovirus infection (dengue, chikungunya, or Zika virus) and controls with confirmed diarrheal infections[a], Santa Rosa, Guatemala, 2011-2018**

| Characteristic | Cases<br>N = 311 | Controls<br>N = 1,239 | p-value[b] |
|---|---|---|---|
| *Categorical variables (n and %)* | | | |
| Cooks with firewood | | | **<0.01** |
| Yes | 207 (66.6) | 932 (75.2) | |
| No | 104 (33.4) | 307 (24.8) | |
| Times per week cooking at home with firewood | | | **<0.01** |
| ≥5 | 168 (54.0) | 837 (67.5) | |
| 3-4 | 14 (4.5) | 42 (3.4) | |
| 1-2 | 25 (8.1) | 53 (4.3) | |
| Does not cook with firewood | 104 (33.4) | 307 (24.8) | |
| Location in house where patient cooks with firewood | | | **<0.01** |
| In main house | 6 (1.9) | 82 (6.6) | |
| In a kitchen that is separated from main house | 158 (50.8) | 615 (49.6) | |
| In an informal structure without walls/roofs | 24 (7.7) | 137 (10.6) | |
| Outside the house | 19 (6.1) | 104 (8.4) | |
| Does not cook with firewood | 104 (33.5) | 307 (24.8) | |
| Type of stove used to cook with firewood | | | **<0.01** |
| Open hearth fire | 98 (31.5) | 656 (52.9) | |
| Improved stove without chimney | 66 (21.2) | 135 (10.9) | |
| Improved stove with chimney | 43 (13.8) | 141 (11.4) | |
| Does not cook with firewood | 104 (33.4) | 307 (24.8) | |
| HAP score[c] | | | **<0.01** |
| Very high | 3 (1.0) | 67 (5.4) | |
| High | 61 (19.6) | 449 (36.2) | |
| Middle | 76 (24.4) | 230 (18.6) | |
| Low | 67 (21.5) | 186 (15.0) | |
| Very low | 104 (33.5) | 307 (24.8) | |
| Sex | | | **0.03** |
| Female | 161 (51.8) | 559 (45.1) | |
| Male | 150 (48.2) | 680 (54.9) | |
| Ethnic group | | | **<0.01** |
| Ladino | 198 (63.7) | 985 (79.5) | |
| Xinca | 103 (33.1) | 207 (16.7) | |
| Other | 10 (3.2) | 47 (3.8) | |
| Recruitment location | | | 0.26 |
| Hospital | 750 (60.5) | 199 (64.0) | |
| Health center | 489 (39.5) | 112 (36.0) | |
| Season | | | 0.55 |
| Dry | 108 (34.7) | 408 (32.9) | |
| Rainy | 203 (65.3) | 831 (67.1) | |
| Admission year | | | **<0.01** |
| 2011-2012 | 5 (1.6) | 302 (24.4) | |
| 2013-2014 | 42 (13.5) | 359 (29.0) | |
| 2015-2016 | 247 (79.4) | 383 (30.9) | |
| 2017-2018 | 17 (5.5) | 195 (15.7) | |
| *Continuous variables (median and IQR)* | | | |
| Age | 22.6 (12.7-40.0) | 18.2 (10.2-32.4) | **<0.01** |
| Number of people per household | 5 (4-6) | 5 (4-6) | *0.56* |
| Socioeconomic status index[d] | 2.1 (1.5-2.7) | 1.7 (1.2-2.4) | **<0.01** |
| Number of people per hectare per community | 2.5 (2.0-2.8) | 2.6 (2.1-2.9) | 0.05 |
| Community elevation (m) | 1,043 (941-1,217) | 1,150 (1,098-1,232) | **<0.01** |

HAP: household air pollution, IQR: interquartile range

[a] *Salmonella* spp., *Shigella* spp., *Campylobacter* sp., *Escherichia coli*, rotavirus, norovirus-1 and 2, sapovirus, and astrovirus.

[b] Categorical variables: p-value from chi-square test; continuous variables: p-value from t-test

[c] HAP score was derived from principal components analysis and included: firewood cooking frequency, firewood cooking location, and stove type. The chi-square test for trend (extended Mantel–Haenszel) was used to test linear trends in HAP scores between cases and controls.

[d] Socioeconomic status index was derived from principal components analysis and included: a refrigerator, computer, radio, washing machine, dryer, car, television, phone, and microwave; number of rooms in house; income; electricity; roof and floor material. Score range: 0 to 5

**Table 2.3. Unadjusted and adjusted[a] associations between arboviral infection (dengue, chikungunya, or Zika virus) and indicators of household air pollution exposure, Santa Rosa, Guatemala, 2011-2018 (N = 311 cases and 1,239 controls[b])**

| Characteristic | OR (95% CI) | AOR[a] (95% CI) |
|---|---|---|
| Cooks with firewood | **0.66 (0.50, 0.86)** | 0.71 (0.47, 1.07) |
| Does not cook with firewood | REF | REF |
| Times per week cooking at home with firewood | | |
| ≥5 | **0.59 (0.45, 0.78)** | **0.54 (0.36, 0.81)** |
| 3-4 | 0.98 (0.52, 1.87) | 1.27 (0.56, 2.87) |
| 1-2 | 1.39 (0.82, 2.35) | 1.08 (0.57, 2.05) |
| Does not cook with firewood | REF | REF |
| Location where patient cooks with firewood | | |
| In main house | **0.22 (0.09, 0.51)** | **0.22 (0.08, 0.57)** |
| In a kitchen that is separated from main house | 0.76 (0.57, 1.01) | 0.73 (0.48, 1.09) |
| In an informal structure without walls/roofs | **0.54 (0.33, 0.88)** | 0.58 (0.31, 1.10) |
| Outside the house | **0.54 (0.32, 0.92)** | 0.60 (0.31, 1.15) |
| Does not cook with firewood | REF | REF |
| Type of stove used to cook firewood | | |
| Open hearth fire | **0.44 (0.32, 0.60)** | **0.50 (0.33, 0.78)** |
| Improved stove without chimney | 1.44 (1.00, 2.09) | 1.24 (0.72, 2.13) |
| Improved stove with chimney | 0.90 (0.60, 1.35) | 0.67 (0.40, 1.12) |
| Does not cook with firewood | REF | REF |
| HAP score[c] | | |
| Very high | **0.13 (0.04, 0.43)** | **0.12 (0.03, 0.44)** |
| High | **0.40 (0.28, 0.57)** | **0.41 (0.25, 0.68)** |
| Middle | 0.97 (0.69, 1.37) | 1.00 (0.61, 1.63) |
| Low | 1.06 (0.74, 1.52) | 0.76 (0.48, 1.20) |
| Does not cook with firewood | REF | REF |

CI: confidence interval; OR: odds ratio; AOR: adjusted odds ratio; HAP: household air pollution

[a] Adjusted for age, sex, ethnic group, admission year, season, number of people in household, recruitment location, community population density, community elevation, and socioeconomic status index. Socioeconomic status index was derived from principal components analysis and included: a refrigerator, computer, radio, washing machine, dryer, car, television, phone, and microwave; number of rooms in house; income; electricity; roof and floor material.

[b] Diarrheal illnesses included *Salmonella* spp., *Shigella* spp., *Campylobacter* sp., *Escherichia coli*, rotavirus, norovirus-1 and 2, sapovirus, and astrovirus.

[c] HAP score was derived from principal components analysis and included firewood cooking frequency, firewood cooking location, and stove type.

# CHAPTER 3: COMPARING VECTOR AND HUMAN SURVEILLANCE STRATEGIES TO DETECT ARBOVIRUS TRANSMISSION: A SIMULATION STUDY FOR ZIKA VIRUS DETECTION IN PUERTO RICO

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## ABSTRACT

**Background:** Detecting and monitoring the transmission of arboviruses such as Zika virus (ZIKV), dengue virus, and chikungunya virus is critical for prevention and control activities. Previous work has compared the ability of different human-focused surveillance strategies to detect ZIKV transmission in U.S. counties where no known transmission had occurred, but whether virological surveillance in mosquitoes could represent an effective surveillance system is unclear.

**Objectives:** We leveraged a unique set of data from human and virological surveillance in *Ae. aegypti* during the 2016 ZIKV epidemic in Caguas, Puerto Rico, to compare alternative strategies for detecting and monitoring ZIKV activity.

**Methods:** We developed a simulation model for mosquito and human surveillance strategies and simulated different transmission scenarios with varying infection rates and mosquito trap densities. We then calculated the expected weekly number of detected infections, the probability of detecting transmission, and the number of tests needed and compared the simulations with observed data from Caguas.

**Results:** In simulated high transmission scenarios (1 infection per 1,000 people per week), the models demonstrated that both approaches had estimated probabilities of detection of greater than 78%. In simulated low incidence scenarios, vector surveillance had higher sensitivity than human surveillance and sensitivity increased with more traps, more trapping effort, and testing. In contrast, the actual data from Caguas indicated that human virological surveillance was more sensitive than vector virological surveillance during periods of both high and low transmission.

**Conclusion:** In scenarios where human surveillance is not possible or when transmission intensity is very low, virological surveillance in *Ae. aegypti* may be able to detect and monitor ZIKV epidemic activity. However, surveillance for humans seeking care for Zika-like symptoms likely provides an equivalent or more sensitive indicator of transmission intensity in most circumstances.

## INTRODUCTION

First identified in Uganda in 1947, Zika virus (ZIKV) emerged in the Americas in 2015 (1, 2), with local transmission of ZIKV first reported in December, 2015, in Puerto Rico (3). The emergence of ZIKV highlighted several challenges for arbovirus surveillance. Many infections do not result in apparent illness (4) and acutely ill individuals do not always seek care or receive confirmatory testing (5, 6). Moreover, multiple surveillance strategies are possible (e.g. active and passive, vector-based and human); all have tradeoffs that may vary depending on the epidemiological situation. For example, a previous assessment of human-based surveillance systems to detect ZIKV transmission in U.S. counties found that despite low probabilities of care-seeking among ZIKV-infected individuals, testing symptomatic people seeking medical care was a more effective strategy than testing blood donors or all pregnant women (5). Testing pregnant women was found to be a less efficient strategy because it requires more tests, has a much higher false positive rate, and has a lower probability of detection than testing only patients with two or more Zika symptoms.

Effective prevention and control of arboviruses such as ZIKV, dengue virus, and chikungunya virus is dependent on timely and accurate detection of elevated viral activity in the population. Surveillance systems detect virus circulation, track geographical spread, monitor epidemic progression, detect adverse health outcomes associated with infection, and guide response efforts (7, 8).

Although previous work suggested that testing clinical patients with at least two ZIKV symptoms was a relatively effective strategy for detecting ZIKV transmission in humans, the probability of detection was <25% even under optimal circumstances and incidence of one infection per 10,000 people per week in a population of 100,000 (5). Moreover, none of the surveillance strategies evaluated could detect even 5% of all ZIKV infections.

An alternative to detecting arboviruses in humans is virological surveillance in mosquitoes, which involves capturing and testing host seeking female mosquitoes. For West Nile virus, which has a primary transmission cycle between infected *Culex* mosquitoes and birds, virological surveillance in mosquitoes can facilitate viral detection prior to human disease case identification (9, 10). However, transmission of

Zika, dengue, and chikungunya viruses occurs mainly between *Aedes* mosquitoes and human hosts. Even during outbreaks, detecting these viruses in *Ae. aegypti* is infrequent (11). In this context, the possible tradeoffs between vector and human virological surveillance are unclear. Here, we leveraged a unique set of data from human and vector surveillance during the 2016 ZIKV epidemic in Caguas, Puerto Rico to compare strategies for detecting and monitoring arbovirus activity.

## METHODS

### Study site

Caguas is located in the central mountain range of Puerto Rico, 32 km south of San Juan (18.23412° N, −66.0485° W). It has a population of approximately 130,000 people (12). The municipality has a tropical climate with a mean annual temperature of 25.1 °C and mean annual precipitation of 1,755 mm (13).

### Vector surveillance data

From October, 2016, to August, 2017, 360 sentinel autocidal gravid ovitraps (SAGO) were placed in eight clusters in Caguas encompassing approximately 80,000 residents (14, 15). Within each cluster, fixed SAGOs were placed in outdoor areas of randomly selected households located at least 100 m from the adjacent clusters with 109–131 m between each trap. If the homeowner did not give consent or was not home, traps were placed in a location adjacent to the randomly selected site. Each cluster had 38-53 traps placed among approximately 3,000 built structures for an overall trap density of approximately 0.14 traps per building or one trap per 70.5 buildings. Traps were monitored on a weekly basis by field technicians, mosquitoes were sexed, and identified as *Ae. aegypti* in the field (14). Female *Ae. aegypti* from individual SAGOs were pooled by week with 1-20 mosquitoes per pool and stored at −80°C until they were tested for viral RNA using the Trioplex Real-time RT-PCR assay (16). ZIKV may be detected in *Ae. aegypti* held in traps at ambient temperatures (17). We also simulated collections with higher and lower trap numbers.

### Human surveillance data

We collected human case data for Caguas from October 10, 2016–April 16, 2017, as reported by the Puerto Rico Department of Health (PRDH) from the passive arboviral disease surveillance system (18). All symptomatic cases were reported to PRDH as suspected arbovirus infections based on clinical suspicion, with subsequent confirmatory laboratory testing. We assessed the number of ZIKV cases detected by RT-PCR (confirmed cases) compared with the total number of cases submitted for testing.

### Simulation model

We simulated surveillance for ZIKV-infected *Ae. aegypti* and humans considering the surveillance systems implemented in Caguas during the ZIKV epidemic. We developed simulation models to estimate ZIKV transmission and detection in humans and mosquitoes at varying transmission levels. Four surveillance strategies were assessed: mosquito trapping systems with 180, 360, and 720 traps; and human surveillance. We started by simulating population sizes of humans and vectors, the incidence of infection in both populations, and surveillance for infection in each population. We then compared the total number of detections and probability of detection between the strategies. These processes are described in detail below with key parameters in Table 3.1.

#### *Population sizes*

We used a population of 100,000 people, slightly smaller than the total population of Caguas and larger than the population of the area covered by mosquito surveillance. The total number of mosquitoes, $N_M$, was estimated from the density of *Ae. aegypti* ($r$) and human population size. We assumed there were 1 to 3 adult female *Ae. aegypti* per person, which we assumed to be gamma distributed (19-21).

#### *Incidence of infection*

We simulated scenarios with human incidence ranging from one infection per 100,000 per week to one infection per 1,000 per week. In these simulations, we used the incidence in humans to calculate the number of infected mosquitoes using an average number of mosquitoes infected by an infectious person ($R_{0HM}$). $R_{0HM}$ was calculated as the product of the density of mosquitoes ($r$), mosquito biting rate ($b$), probability of human-to-mosquito transmission given an infectious bite ($p_{HM}$), and duration of infectiousness in humans ($d$): $R_{0HM} = rbp_{HM}d$ (19). We estimated ranges and uncertainty distributions for each of these variables based on previous research. We assumed $b$=0.63—0.76 bites per mosquito per day, which we approximated with a uniform distribution (22), and $p_{HM}$=0.5 (SD=0.1), which we approximated with a normal distribution (19, 20). The duration of infectiousness in humans ($d$) was assumed to be similar to dengue, with a mean of 7 days (SD=0.3) and approximated with a gamma distribution (19). The expected number of ZIKV-infected female *Ae. aegypti* per week was estimated as the number of infectious humans multiplied by $R_{0HM}$. The probability of a single female *Ae. aegypti* being infected when trapped was calculated as the expected number of ZIKV-infected female *Ae. aegypti* divided by the mosquito population size ($N_M$).

### *Surveillance strategies*

We modeled four different surveillance strategies. Three of the strategies used *Ae. aegypti* surveillance data at different trap densities: the number of traps equivalent to what was used in the study area of Caguas (360 traps), half as many traps (180 traps), and twice as many traps (720 traps). The fourth model used human surveillance for acutely ill patients with ZIKV symptoms. The four surveillance strategies were evaluated at different transmission densities, ranging from one to 1,000 human cases per 100,000 per week.

Human surveillance data was assumed to be similar to previously published estimates for identifying ZIKV cases from emergency departments in the continental United States (5). Briefly, we assumed that 20–40% of infections result in symptoms, 10–50% of people with symptomatic infections seek care, 5–50% of those patients seek care in an emergency department, and 82.4–83.3% of those patients have at least two of the following ZIKV symptoms: arthralgia, conjunctivitis, fever, headache, or rash. Human surveillance in Puerto Rico differs from this simulation in at least two ways: reporting was not limited to emergency departments and reporting of suspect cases relies on clinical suspicion, not a specific clinical definition. We used the emergency department assumption and stricter case definition to approximate patients seeking immediate care for acute illness with clinical suspicion of ZIKV. For reported suspect ZIKV patients, we assumed all testing was performed with RT-PCR with sensitivity of 80-95% (26) and specificity of 99-100% (16).

For vector surveillance, we started each simulation with a specific number of traps and simulated the total numbers of mosquitoes and pools collected from those traps based on the Caguas collection data. The probability of a pool containing a positive mosquito was calculated as the product of the average number of mosquitoes collected per pool and the probability of an individual mosquito being infected (described above). Mosquito testing was performed with RT-PCR. Two studies demonstrated sensitivities of 88.7% (25) and 96.1% (27) for RT-PCR detection of ZIKV in mosquitoes. Based on those findings and studies of RT-PCR for detecting WNV in mosquitoes (23, 24), we assumed the test has a sensitivity of 85%-100% and a specificity of 99.9-100%, which we approximated with uniform distributions. ZIKV RNA is stable enough to allow detection for up to 30 days (28). Because mosquitoes were collected and tested every 7 days, we assumed that all infected mosquitoes had detectable RNA (5).

### *Probability of detection*

We first calculated the numbers of expected true and false positives from the human and mosquito surveillance strategies. For humans, the expected number of true positives ($E(P)$) was the product of the prevalence of infection, the probability of being tested in the surveillance system, and the sensitivity of the human RT-PCR assay. The expected number of false positives was the product of the probability of being tested in the surveillance system for people not infected with ZIKV and one minus the specificity of the human RT-PCR assay. The expected number of true positive mosquito pools ($E(P)$)

was the product of the total number of pools, the probability of a pool containing an infected mosquito, and the sensitivity of the mosquito RT-PCR assay. Finally, the expected number of false positive pools was calculated as the product of the total number of pools, the probability of a pool not containing an infected mosquito, and one minus the specificity of the RT-PCR assay.

To estimate the probability of detection for each system we assumed that the number of true infections detected in mosquito pools or human patients was generated from a Poisson distribution with the means as the expected number of true positives as described above ($E(P)$). We therefore calculated the probability of detection, $p_{detect}$, as $p_{detect} = 1 - e^{-(E(P))}$.

*Estimating infections in human population*

We fitted Bayesian negative binomial regression models to the simulation data to estimate the relationship between the number of positive pools or clinical infections and the total number of human infections ($I_H$). We used linear models for both the mean parameter ($\mu$) and the dispersion parameter ($\phi$):

$I_H \sim \text{NegativeBinomial}(\mu, \phi)$

$\mu = \beta_0 + \beta_X X$

$\phi = \beta_0^{\phi} + \beta_X^{\phi} X,$

where $X$ was the number of positive pools or the number of detected clinical infections.

To assess the relationship between our simulations and the observed data from Caguas, we first fitted these models to the simulation data in order to estimate the relationship between the number of infected humans or mosquito pools observed by the surveillance system and the number of underlying human infections in the population (130,000 for the human surveillance and 80,000 for the mosquito surveillance). We then used the fitted model to estimate the number of infections in the two populations based on the observed numbers of human cases and positive pools from the 2016-2017 surveillance data.

*Software*

For each surveillance strategy, we ran 10,000 Monte Carlo simulations. All analyses were performed in R 3.5.2 statistical software (R Development Core Team, Vienna, Austria). We used Stan to implement the Bayesian models. We used three chains with a burn-in of 1,000 samples and a further 1,000 samples collected from each chain with clear convergence and no significant autocorrelation.

## RESULTS

We first analyzed the expected number of positive mosquito pools, human cases, and the probability of detecting infected vectors or humans for the four simulated surveillance strategies as described above. In a population of 100,000 and one infection per week, the expected number of positive mosquito pools or positive humans was essentially zero for all four systems (Figure 3.1 A and B) (Table 3.2). Therefore, the probability of detecting a positive mosquito pool or infected human was very low, but highest (6.9%; 95% UI: 1.6%, 15.7%) for the mosquito trapping system with 720 traps and lowest for human surveillance (1.5%; 95% UI: 0.3%, 4.7%) (Figure 3.1 C and D). When the incidence of infection increased to 10 infections per week, the probability of detection was 51% (95% UI: 15%, 82%) for the 720-trap system, 30% (95% UI: 8%, 57%) for the 360-trap system, 16% (95% UI: 4%, 35%) for the 180-trap system, and 14% (95% UI: 3%, 38%) for human surveillance. At the highest incidence rate of approximately 1,000 infections per week, all systems had a mean estimated probability of detection of greater than 78%.

For the mosquito surveillance systems, higher numbers of traps increased the probability of detection; however, more traps also led to more tests and increased resource needs (Figure 3.2). In contrast, for human surveillance of patients with ZIKV symptoms, the expected testing resources required were much lower and only increased slightly with higher incidence. For example, with 360 traps, an estimated 335 (95% UI: 275, 396) tests were required per week for vector surveillance. In contrast, approximately 10 (95% UI: 6, 14) tests per week were required for human surveillance with no ZIKV transmission and 12 (95% UI: 8, 17) tests per week with a high transmission scenario of 1,000 infections per week. Both systems had similar positive predictive values, with human surveillance being somewhat higher in low incidence settings (Figure S.1).

To identify the relationship between detected infections and incidence, we assessed the relationship between the simulated infection incidence and the observed number of positive pools or human cases in the same simulations (Figure 3.3). There was high variability across simulations and high uncertainty in the relationship between the number of observed positive pools or confirmed human cases and the number of underlying infections. For each positive mosquito pool, we estimated an additional 23.3 infections (95% Credible Interval (CI): 0.7, 171.1) (Figure 3.3A). For each confirmed human case detected, we estimated 49.0 additional infections in a population of 100,000 people (95% CI: 0, 334.1) (Figure 3.3B).

Finally, we compared these simulations to the data reported from Caguas from October 10, 2016, to April 16, 2017. Over this period, there were 127 confirmed human ZIKV cases out of 452 suspect cases detected through passive surveillance (28.1% positive) in the entire municipality of Caguas and 49 ZIKV positive pools of female *Ae. aegypti* out of 8,518 pools tested (0.6% positive) in the area of Caguas with vector surveillance (Figure 3.4A). We reran the simulations using the populations of all of Caguas (130,000) and the study area (80,000) for the human and vector surveillance systems, respectively, to estimate the relationship between the number of infections in populations of these specific sizes and the number of positive mosquito pools or confirmed human cases detected. The relationships were similar to those generated with a uniform population size of 100,000 people for each system; mean expected number of infected humans increasing by 47.6 (95% CI: 0, 341.9) and 19.7 (95% CI: 0.6, 167.4) infections per additional confirmed case or positive pool, respectively.

We used this model to estimate the incidence of infection on a weekly basis using the observed number of confirmed cases and positive pools and calculated the rate of infection given the distinct population sizes (Figure 3.4B). Although the 95% CIs overlapped for all weeks, the 50% CIs had minimal overlap during late 2016 when incidence in both systems was relatively high. Overall, estimates based on human surveillance were substantially higher in most weeks of 2016 and the incidence rate of the entire study period was 90 (95% CI: 50, 140) infections per 10,000 people based on mosquito pool data in the vector surveillance area versus 500 (95% CI: 340, 680) based on human surveillance data in all of Caguas.

## DISCUSSION

Population-based surveillance and detection of ZIKV is critical to guide timely public health and medical responses to emerging epidemics. Optimal surveillance strategies should be sensitive, specific, and cost-effective (29). *Aedes aegypti*-transmitted arbovirus surveillance systems have widely focused on testing the human population at risk. In a previous comparison of human-focused ZIKV surveillance approaches, a strategy focusing on patients seeking emergency department care was found to be more effective and efficient than routine testing of pregnant women or blood donors (5). Virological surveillance of *Aedes* transmitted arboviruses in *Ae. aegypti* provides an alternative approach to early detection of virus circulation and monitoring of transmission dynamics during outbreaks that does not depend on human surveillance.

In our simulation models for high transmission scenarios, both vector and human surveillance strategies effectively detected ZIKV. In low transmission scenarios, routine mosquito surveillance with high trap numbers had higher detection probabilities, although the probability of detection was still low (7%). This increased sensitivity also implies that for every confirmed human case there are more infected humans in the population than for every positive mosquito pool. That difference may result from the higher barriers to testing among humans in our model, which included developing two or more symptoms seeking care in an emergency department, and being viremic at the time of testing (5, 30). Identification of a positive mosquito pool is not influenced by the same contingencies.

In contrast to the simulations, more human cases (127) than positive pools (49) were observed in Caguas, suggesting that human surveillance may be more sensitive than virological surveillance of *Ae. aegypti*. Part of this difference is explained by the different population coverage (130,000 for human surveillance and 80,000 for vector surveillance). However, using the model to estimate the infection rate in both populations, we found that the difference was still clear; in almost every week the estimated incidence was higher using human surveillance data. The discrepancy may result from higher risk in the additional 50,000 people covered by human surveillance, but there is no obvious reason for this to be the case. Alternatively, the difference may result from mischaracterization of human surveillance in the simulation model. The model was limited to patients seeking care in an emergency department with two or more ZIKV symptoms, whereas the actual surveillance data likely includes individuals from other health facilities, those with only one ZIKV symptom, patients suspected of having infection for other reasons, and individuals who may have been infected outside of Caguas. These components commonly vary across surveillance systems and locations, and all of these could lead to underestimates of the sensitivity of human surveillance in the simulations.

Overall, the simulations suggested that virological surveillance of *Ae. aegypti* as performed in Caguas could potentially provide improved sensitivity to detect virus transmission, but the data from Caguas tell a different story. The observed data confirm that both systems were able to detect transmission in high and low incidence weeks, but they also indicated higher sensitivity for human surveillance. It is also important to consider these approaches in context. The Caguas study was conducted in high and low incidence periods after the peak of the outbreak in July 2016, when clinicians and the local population were already sensitized to ZIKV, which may have increased the number of observed cases. Passive surveillance for arboviral diseases in clinical patients is routine in Puerto Rico (31) and many other locations, so testing of humans may represent little or no additional resource burden. In contrast, mosquito surveillance is less widely implemented and thus may require even more resources, especially for laboratory testing. The resource investment of mosquito-based surveillance is directly related to the sensitivity, which is not necessarily true for human surveillance. In the absence of human surveillance systems or to identify local transmission risk by detecting infected mosquitoes, virological surveillance of *Ae. aegypti* may be an effective approach.

An important limitation to this study is that we did not collect data on attendant costs required for virological surveillance in mosquitoes and humans (e.g., traps, field work, laboratory supplies). These resource considerations are not trivial in terms of man hours and laboratory test costs. A second limitation was the considerable uncertainty around key model parameters. We accounted for uncertainty by sampling from parameter distributions, but those distributions were based on data from other locations and therefore do not necessarily reflect the reality of surveillance in Caguas. As described above, this was particularly evident when comparing the simulations to the observed data. We also used a very generic representation of trapping effort, including only the number of traps per person and collections based on the data from Caguas. We therefore ignored all components of spatiotemporal variability in vector populations, trapping effort, and incidence, despite their importance (32, 33).

The simulations suggested some potential gains in sensitivity for virological surveillance of *Ae. aegypti*, a finding substantiated by the detection of ZIKV in mosquitoes in Mexico without reported human cases (34). However, the increased resources needed are likely prohibitive in most circumstances. For example, during the study period there were 8,518 tests on mosquito pools in a subsection of Caguas compared to 452 tests on suspect human cases in all of Caguas. Some of the testing burden may be ameliorated by using "superpools" for mosquito testing (35), but the trapping itself also remains a challenge. Moreover, despite the intensive mosquito trapping and testing in Caguas and the small proportion of ZIKV-infected people who seek care, human surveillance was more sensitive than mosquito surveillance. In communities without health care facilities or in which people do not seek care, virological surveillance in mosquitoes have an important role. Virological surveillance in *Ae. aegypti* could also potentially help detect low levels of virus circulation, but only with extensive trapping and testing. In most situations where human surveillance systems already exist, such as in Caguas, human surveillance is likely to be more effective.

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Chapter 3, in full, is a reprint of the material as it appears in *PLOS Neglected Tropical Diseases*: Madewell ZJ, Hemme RR, Adams L, Barrera R, Waterman SH, Johansson MA. Comparing vector and human surveillance strategies to detect arbovirus transmission: A simulation study for Zika virus

detection in Puerto Rico. *PLOS Neglected Tropical Diseases*. 2019 Dec 26;13(12). The dissertation author was the primary author of this material.

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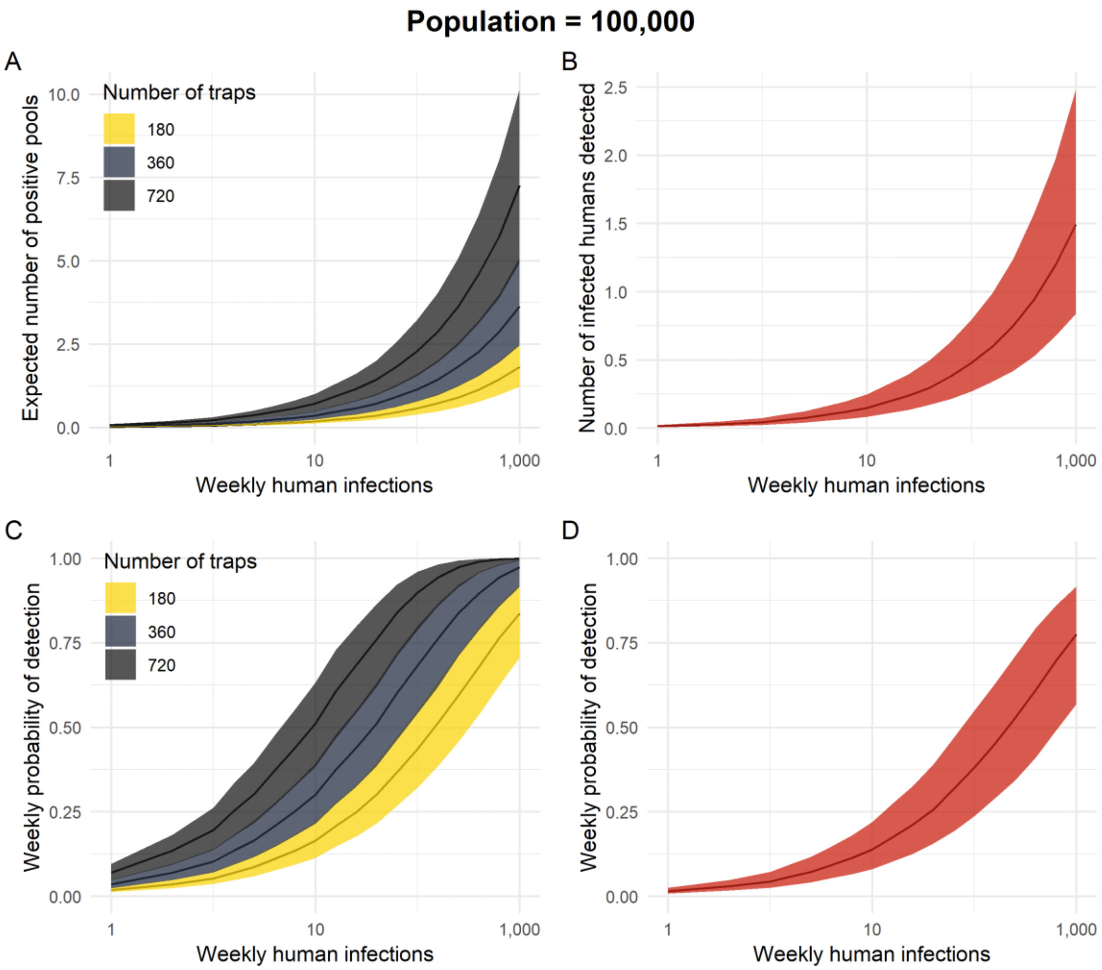

**Figure 3.1. The total number of Zika virus infections detected and probability of detecting local transmission of Zika virus by testing *Aedes aegypti* females collected from CDC sentinel autocidal gravid ovitraps (SAGO) and emergency department patients with two or more Zika virus symptoms.** Panel A shows the expected number of ZIKV-positive *Ae. aegypti* pools detected per week by testing pools of *Ae. aegypti* females trapped in the actual number of traps deployed in Caguas, Puerto Rico (360 SAGO traps: navy), half the number of traps as Caguas (180 SAGO traps: yellow), and twice the number of traps as Caguas (720 SAGO traps: black). Panel B shows the expected number of infected humans detected per week by testing patients in emergency departments showing two or more ZIKV symptoms (red). Panel C shows the weekly probability of detecting ZIKV by testing mosquito pools. Panel D shows the weekly probability of detecting ZIKV by testing symptomatic emergency department patients. The bands represent 50% uncertainty intervals.

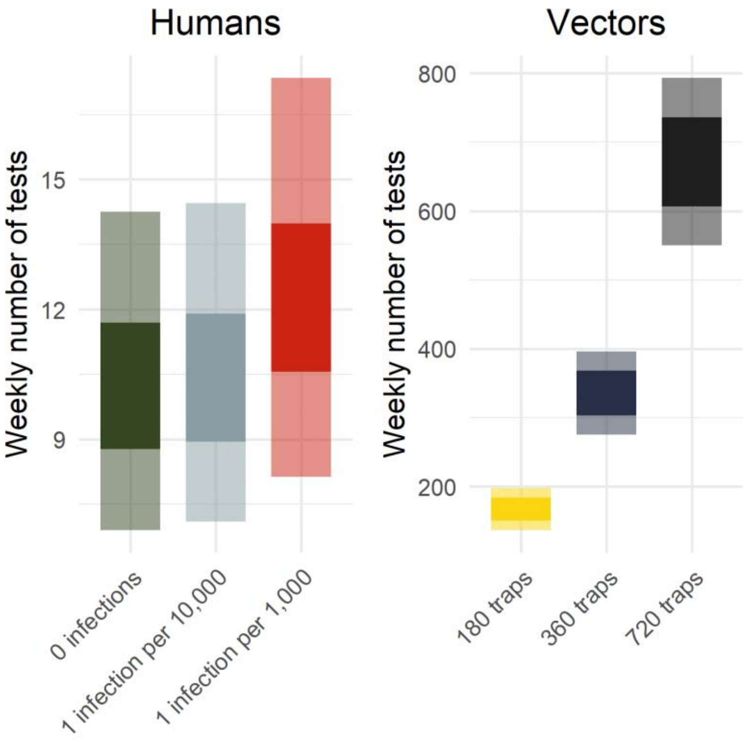

**Figure 3.2. Expected number of tests for vector and human surveillance strategies.** This figure shows the 50% uncertainty interval (UI, dark) and 95% UI (light) for the expected number of RT-PCR tests required per week for different surveillance strategies and transmission levels in a population of 100,000 people. The human surveillance strategy indicates the number of tests among emergency department patients with two or more ZIKV symptoms given transmission scenarios of 0 (green), 1 (grey), and 10 (red) infections per 10,000 people per week. The yellow, blue, and black bars are the number of tests required for pools of female *Ae. aegypti* for different numbers of traps in the same population.

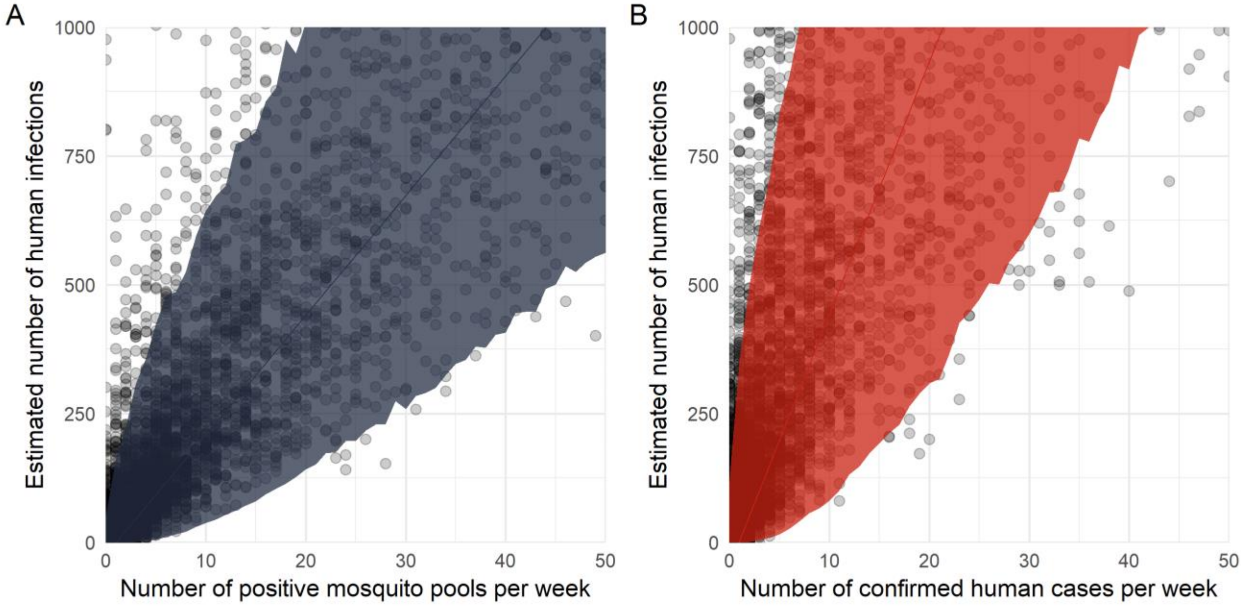

**Figure 3.3. Relationship between the number of simulated Zika virus positive mosquito pools and human infections, and estimated incidence rate of human infections.** Panel A shows the estimated incidence of human infections per week by the number of Zika virus positive female *Ae. aegypti* pools per week. Panel B is the estimated incidence of human infections per week by the number of infections in emergency department patients with two or more Zika virus symptoms per week. The lines are Bayesian negative binomial regression models fitted to predict the estimated incidence of human infections from the number of positive mosquito pools and the number of humans with two or more symptoms. The bands represent 95% credible intervals.

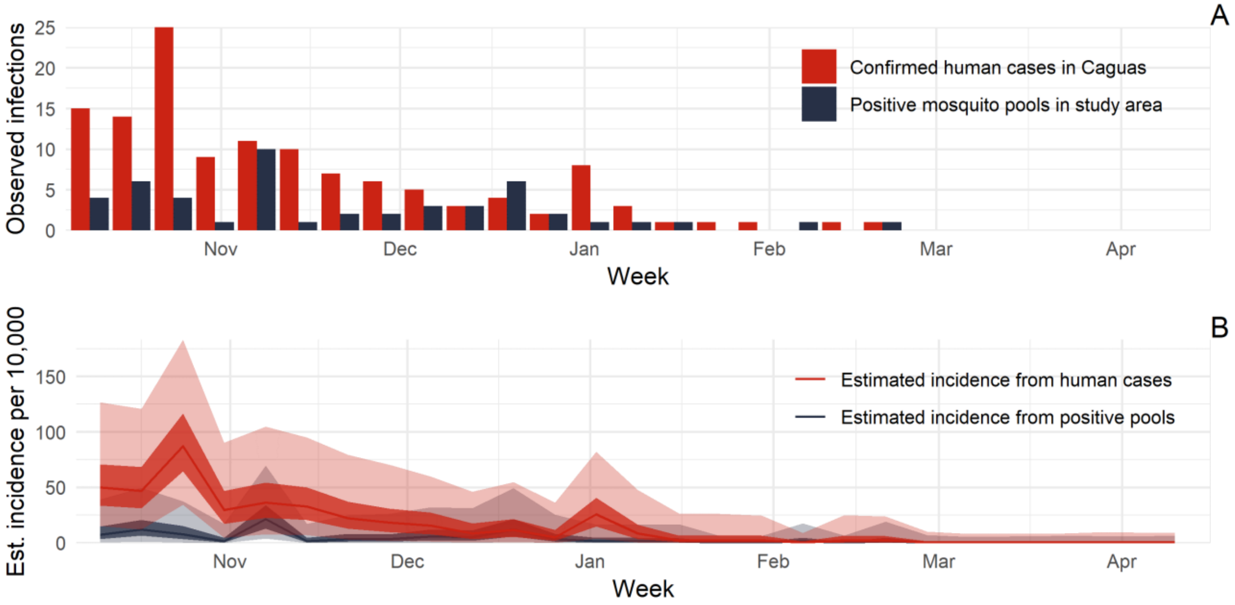

**Figure 3.4. Time series relationship between the actual number of positive mosquito pools and human cases in Caguas, Puerto Rico, and estimated incidence rate of human infections.** Panel A shows the actual number of ZIKV positive mosquito pools (navy bars) in the Caguas study area and confirmed human cases (red bars) in Caguas municipality from October 10, 2016, to April 16, 2017. In Panel B, we used the model to estimate the incidence rate of human infections from the number of positive mosquito pools (navy line) and the number of confirmed human cases (red line). The dark bands represent 50% credible intervals (CI), whereas light bands represent 95% CI for the estimated incidence of population infections.

**Table 3.1. Parameter assumptions for *Aedes aegypti* surveillance for Zika virus**

| Parameter | Estimate | Uncertainty distribution | Source |
|---|---|---|---|
| Duration of human infectiousness | 7 days (0.3 SD) | Gamma | (21) |
| Number of *Ae. aegypti* per person | 2 (1.5 SD) | Gamma | (19-21) |
| Mosquito biting rate | 0.63-0.76 bites per day | Uniform | (19, 22) |
| Probability of human-to-mosquito transmissibility | 0.5 (0.1 SD) | Normal | (19, 21) |
| Number of *Ae. aegypti* per pool | 5.17 | Poisson | Caguas data |
| Sensitivity of RT-PCR Assay | 85-100% | Uniform | (16, 24, 26, 27) |
| Specificity of RT-PCR Assay | 99.99-100% | Uniform | (16, 24, 26, 27) |

RT-PCR: reverse transcription polymerase chain reaction

**Table 3.2. The probability of detecting local transmission of Zika virus and the total number of Zika virus infections detected by testing *Aedes aegypti* pools collected from CDC sentinel autocidal gravid ovitraps and emergency department patients with two or more Zika virus symptoms**

| | Vector surveillance | | | |
|---|---|---|---|---|
| Strategy performance | 180 SAGO (95% UI) | 360 SAGO (95% UI) | 720 SAGO (95% UI) | Human surveillance |
| Weekly probability of detection | | | | |
| ZIKV incidence of 1 per 100,000 per week | 1.8% (0.4%, 4.2%) | 3.5% (0.8%, 8.2%) | 6.9% (1.6%, 15.7%) | 1.5% (0.3%, 4.7%) |
| ZIKV incidence of 1 per 10,000 per week | 16.4% (4.0%, 34.7%) | 30.1% (7.9%, 57.4%) | 51.2% (15.1%, 81.9%) | 14.1% (2.7%, 38.0%) |
| ZIKV incidence of 1 per 1,000 per week | 83.3% (33.6%, 98.6%) | 97.2% (56.0%, 99.9%) | 99.9% (80.6%, 100%) | 78.0% (24.2%, 99.2%) |
| Total number of ZIKV infections detected per week | | | | |
| ZIKV incidence of 1 per 100,000 per week | 0.02 (0.01, 0.04) | 0.04 (0.01, 0.09) | 0.07 (0.02, 0.17) | 0.02 (0.01, 0.05) |
| ZIKV incidence of 1 per 10,000 per week | 0.18 (0.04, 0.43) | 0.36 (0.08, 0.85) | 0.72 (0.16, 1.71) | 0.15 (0.03, 0.40) |
| ZIKV incidence of 1 per 1,000 per week | 1.79 (0.41, 4.26) | 3.58 (0.82, 8.52) | 7.17 (1.64, 17.07) | 1.51 (0.28, 4.79) |

UI, uncertainty interval; SAGO, sentinel autocidal gravid ovitraps

# CHAPTER 4: ASSOCIATIONS BETWEEN HOUSEHOLD ENVIRONMENTAL FACTORS AND IMMATURE MOSQUITO ABUNDANCE IN QUETZALTENANGO, GUATEMALA

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## Abstract

**Background:** *Aedes aegypti*-borne diseases are becoming major public health problems in tropical and sub-tropical regions. While socioeconomic status has been associated with larval mosquito abundance, the drivers or possible factors mediating this association, such as environmental factors, are yet to be identified. We examined possible associations between proximity to houses and roads and immature mosquito abundance, and assessed whether these factors and mosquito prevention measures mediated any association between household environmental factors and immature mosquito abundance.

**Methods:** We conducted two cross-sectional household container surveys in February-March and November-December, 2017, in urban and rural areas of Quetzaltenango, Guatemala. We used principal components analysis to identify factors from twelve variables to represent the household environment. One factor which included number of rooms in house, electricity, running water, garbage service, cable, television, telephone, latrine, well, and sewer system, was termed "environmental capital." Environmental capital scores ranged from 0-5.5. Risk factors analyzed included environmental capital, and distance from nearest house/structure, paved road, and highway. We used Poisson regression to determine associations between distance to nearest house/structure, roads, and highways, and measures of immature mosquito abundance (total larvae, total pupae, and positive containers). Using cubic spline generalized additive models, we assessed non-linear associations between environmental capital and immature mosquito abundance. We then examined whether fumigation, cleaning containers, and distance from the nearest house, road, and highway mediated the relationship between environmental capital and larvae and pupae abundance.

**Results:** We completed 508 household surveys in February-March, and we revisited 469 households in November-December. Proximity to paved roads and other houses/structures was positively associated with larvae and pupae abundance and mediated the associations between environmental capital and total numbers of larvae/pupae ($p \leq 0.01$). Distance to highways was not associated with larval/pupal abundance ($p \geq 0.48$). Households with the lowest and highest environmental capital had fewer larvae/pupae than households in the middle range ($p < 0.01$).

**Conclusions:** We found evidence that proximity to other houses and paved roads was associated with greater abundance of larvae and pupae.  Understanding risk factors such as these can allow for improved targeting of surveillance and vector control measures in areas considered at higher risk for arbovirus transmission.

## INTRODUCTION

Approximately 6.01 billion people currently live in areas suitable for *Aedes aegypti* disease transmission (1). *Ae. aegypti*-borne diseases, such as dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses, are found in tropical and subtropical zones with an abundance of these species, including Central America (2-4). Other than for yellow fever vaccine (5), no broadly licensed commercial vaccines are available for the principal *Ae. aegypti*-borne arboviruses, so vector control remains the primary strategy to limit their spread (6). Climate change, urbanization, migration, human behavior, and ecosystem modification are among the myriad factors influencing the geographic spread of *Ae. aegypti* and their associated viruses (1, 7, 8).

*Ae. aegypti* are highly productive in urban environments and have a strong preference for human blood (9). *Ae. aegypti* spend the majority of their lives in the houses where they emerged, flying an average of 40-80 m during the course of their lifetimes (10). Oviposition sites are selected based on their physical, chemical, and biological characteristics, such as container type, depth, water quality, and sun exposure (11, 12). Ideal larval habitats for *Ae. aegypti* are dark-colored containers filled with stagnant water and organic material in shaded areas around houses (11, 13, 14). Productive container types include flower pots, tires, vases, buckets, cans, rain gutters, fountains, bottles, and birdbaths (11, 13, 14). Greater human population densities provide more feeding opportunities for *Ae. aegypti* (15).

Studies of socioeconomic status (SES) impacts on *Ae. aegypti* abundance mostly report greater *Ae. aegypti* population densities in low SES areas (16-22). Most studies have only considered income, occupation, and education as the SES factors. Few studies have evaluated associations between household environmental measures as attributes of SES and mosquito abundance. The household environmental factors that can influence mosquito infestation are quite heterogeneous. These include piles of garbage (21), open wells (23, 24), storm sewers (25), and septic tanks (26). Less information is available on spatial risk factors, but proximity to vacant lots (27, 28), vegetation or green spaces (29), other houses/structures (30), and roads (31, 32), have been shown to be predictive of mosquito abundance. Household infrastructure may also influence the mosquito microenvironment (33-35). For example, the *premise condition index* has been shown to be an effective tool at classifying houses according to risk of having mosquito breeding sites (33-35). This index can be used to prioritize neighborhoods for vector control interventions.

For this study, we evaluated whether proximity to other houses/structures and roads, and household environmental factors were associated with immature mosquito abundance. A secondary objective was to determine how mosquito abatement interventions, including fumigation and cleaning possible larval habitat containers, influence immature mosquito abundance. It is particularly important to examine these relationships in Central America, which has been host to large outbreaks of arbovirus infection and where vector control resources are limited (36).

## METHODS

### Study Site

We selected two municipalities in the Guatemalan department of Quetzaltenango, Coatepeque and Génova (Figure 4.1), as study sites based on their high risk for arboviral disease transmission and high mosquito pupal index (>25% of houses with pupal infestations) (37). Coatepeque (14°42′00″N 91°52′00″O) and Génova (14°37′00″N 91°50′00″O) are located in the south-western region of the Republic of Guatemala and have a tropical climate. The mean annual temperatures for Coatepeque and Génova are 25.7°C and 26.2°C, respectively, the mean annual precipitations are 308 mm and 285 mm, and the mean elevations are 498 m and 350 m (38). This study included two communities in Coatepeque (La Unión, El Jardín) and six communities in Génova (30 de Junio, Robles, Nueva Italia, Génova, San Jose, Guadalupe) (Figure S.2). The communities in Coatepeque were selected based on the presence of *Vigilancia Integrada Comunitaria* (Integrated Community Surveillance), a prospective public health syndromic surveillance system for diarrheal, respiratory, and febrile illnesses of the Centro de Estudios en Salud/Universidad del Valle de Guatemala in collaboration with the Guatemalan Ministry of Health and the United States Centers for Disease Control and Prevention (CDC). In Génova, all of the communities reporting a high pupal index were included, with the exception of one community that posed a security risk for field personnel. Six sites in Génova were selected to achieve comparable population size to the two sites in Coatepeque. We remotely identified each probable house structure within each community using Google satellite imagery for 2016 in QGIS 2.2 (QGIS Development Team, 2019). The Ministerio de Salud Pública y Asistencia Social (MSPAS) provided detailed maps of each community in order to demonstrate community boundaries. All probable houses were identified and verified on-site to confirm classification of structures (39). Houses were then randomly selected in each village using a two-stage sampling procedure based on a geographic 100 x 100 m grid. We first randomly selected grids, enumerated households, and then used a random number generator to select one house within each grid. In both Coatepeque and Génova, selected houses accounted for 10% of the total community population (n=250 and n=258, respectively). If no one was at home during recruitment, if householders chose not to participate, or if the selected structure was not a house, we selected the nearest house to the right of the front door as a replacement.

### Container inspection and questionnaire

After obtaining informed consent from homeowners, we conducted cross-sectional surveys for container-inhabiting mosquitoes in February-March, 2017 (the local dry season) and November-December, 2017 (the local rainy season) in both Génova and Coatepeque. We conducted two surveys to capture immature mosquito abundance in Guatemala's two seasons. All containers $\geq$3L inside and outside the houses were inspected for any genera of mosquito larvae and pupae, and total numbers of mosquito larvae and pupae from all containers in each house and the containers with any mosquito larvae or pupae were recorded. Larvae and pupae were analyzed separately, as pupal counts are considered more representative of local adult mosquito populations (40, 41). We did not identify larval and pupal genus or species. We interviewed the heads of household or another adult residing in the house, and responses were transcribed onto Excel spreadsheets. Questions covered mosquito control measures, waste disposal, and socioeconomic indicators.

## Variables

We assessed household environment factors and distance from nearest house/structure, paved road, and main transportation corridor running through the city/village as risk factors for vector concentrations. We assumed the main transportation corridor was the nearest highway or the only paved road in villages that did not have highway access.

We used principal components factor analysis to identify factors based on 12 variables from the first household survey to represent household attributes of SES. These included: number of rooms in the house (1-4, >5), electricity (yes, no), running water (yes, no), a television (yes, no), a landline telephone (yes, no), a latrine (yes, no), cable television service (yes, no), a mobile phone (yes, no), trash disposal service (yes, no), a water well (yes, no), sewer system (yes, no), and a rainwater collection system (yes, no). The resultant compound factor, which we termed "environmental capital," included all of the variables except a mobile phone and rainwater collection system (Table S.2). Variables highly correlated with the factor were weighted against their eigenvector. This factor reflects some of the attributes of the Encuesta Nacional de Salud Materno Infantil (National Survey of Maternal and Child Health), which focuses on the health of children and adults in Guatemala (42). This household factor from the first survey explained 32% of the variability in the data and was used to represent environmental capital in the second survey as well. Higher environmental capital scores indicated higher SES and ranged from 0 to 5.5.

The measures of immature mosquito abundance were the total number larvae (continuous), total number of pupae (continuous), and positive containers (continuous). Categorical covariates included survey period (February-March vs. November-December), residence (urban vs. rural), self-reported cleaned (scrubbed, treated, or emptied standing water) containers (barrels, pots, tires, etc.) at least once in the last 6 months (yes, no), and self-reported homeowner or vector control authority fumigation inside/outside house at least once in the last 6 months (yes, no). Continuous covariates included the number of people in a household and the total number of containers $\geq$3L with water at the time of the visit per household (e.g., buckets, barrels, flower pots, etc.). ‘Urban’ residences were those in El Jardín, Coatepeque, whereas ‘rural’ residences were all other communities, as defined by the census (43).

### Spatial analysis

Coordinates of each house were entered into geographical information system software (ArcGIS Pro 2.2.4 software; ESRI, Redlands, CA) and overlaid on basemaps and satellite images from December 8, 2018, of Coatepeque and Génova (44). These maps were used to locate and visualize households and roads. We collected ground truth data through site visits during both survey periods. The distance between a house and its closest neighboring house or other structure (e.g., store, church) or road was ascertained by measuring the Euclidean distance between points taken from the front door of the house to the closest edge of lines representing roads (45-47). Within the sub-set of sampled houses in each community, we also attempted to detect spatial clusters of houses with larval infestations.

### Statistical analysis

Medians and interquartile ranges were reported for continuous variables (total number of larvae; total number of pupae; number of positive containers; number of containers ≥3 liters; number of people in household; distance to nearest paved road, highway, and house/structure; environmental capital). Frequency distributions were reported for categorical variables (cleaned containers, fumigation, urban/rural residence).

We used Poisson regression, which is used to model count data, to analyze unadjusted (Model 1) and adjusted (Model 2) associations between hypothesized risk factors (distance to nearest house/structure, paved road, highway), and immature mosquito abundance (number of larvae, pupae, and positive containers), with household as a repeated measure (two time points). We used generalized estimating equations to estimate the population-averaged effect and used compound symmetry as the covariance structure to account for correlations resulting from two measurements (February-March, November-December) of immature mosquito abundance on the same houses within each site. In Model

2, we used directed acyclic graphs (48, 49) to select each covariate for model inclusion based on *a priori* importance and evidence from the scientific literature of being potential confounders of associations between our exposures of interest and mosquito larvae and pupae abundance. The adjusted models included environmental capital (categorized by tertiles) (50, 51), survey period (52), urban/rural residence (53), the number of people per household (54), cleaned containers (55), fumigated inside/outside house (56), and the total number of containers ≥3 liters per household (50, 57). Tolerance values were used to assess potential collinearity between all independent variables (58). Due to the potential over-dispersion of larvae and pupae abundance, negative binomial regression models were fitted to evaluate the same associations as a sensitivity analysis (59).

We then used cubic spline generalized additive models to explore potential non-linear relationships between environmental capital and immature mosquito abundance (number of larvae, pupae, and positive containers) separately for both survey periods.

Finally, we assessed whether factors including fumigation, cleaned containers, and distance to nearest paved road, highway, and household/structure mediated the relationship between environmental capital and the total number of larvae, pupae, and positive containers. This analysis followed causal mediation analysis methods as previously described by VanderWeele (60). The mediation models were Poisson models to estimate the association between environmental capital and the distance to the nearest house/structure, paved road, and highway, and binomial models to estimate the association between environmental capital and cleaned containers and fumigation history, which are dichotomous variables. The outcome models were Poisson models that estimated the association between environmental capital and immature mosquito indicators (number of larvae, pupae, and positive containers), adjusting for the mediators. All hypothesized mediators were included in outcome models. The “mediation” package in R 3.5.2 statistical software (R Development Core Team, Vienna, Austria) was used for multilevel causal mediation analyses (61). We ran one thousand Monte Carlo simulations in this analysis for variance estimation. Estimates, standard errors, and the proportion mediated were reported. All analyses, other than mediation, were calculated using SAS V.9.4 (SAS Institute, Inc., Cary, North Carolina).

## RESULTS

### Household characteristics

In February-March, 508 household inspections were completed. In November-December, 469 of those households (92.3%) were revisited for a second survey (some houses were not revisited because the homeowner was unavailable). An additional 18 households that were eligible but unavailable during the first survey were included in the second survey. Of all houses, 72.7% were in rural areas (Table 4.1). There was a median of five people per household. The median distances to the nearest house/structure, paved road, and highway were 3.1 m, 13.9 m and 244.1 m for rural residences and 1 m, 4.9 m, and 144.3 m for urban residences, respectively. The median numbers of larvae, pupae, and positive containers were 8, 1, and 1 in rural residences and 20, 2, and 1 in urban residences, respectively.

### Geographical distances

Distance to the nearest paved road was inversely associated with the total number of larvae, pupae, and positive containers per house in Models 1 and 2 ($p \leq 0.01$) (Table 4.2). For every 10-meter increase in distance from the nearest paved road, the total number of larvae and positive containers decreased by a factor of 0.96 and the number of pupae decreased by a factor of 0.93, adjusting for environmental capital, urban/rural residence, the number of people per household, cleaned containers, fumigation history, and the total number of containers. Tolerance values were above 0.50, so there was no evidence of collinearity among any of the independent variables.

Distance to the nearest highway was not associated with the number of larvae, pupae, or positive containers per household in Models 1 and 2 ($p \geq 0.28$) (Table 4.2).

Distance from the nearest household/structure was inversely associated with the total number of larvae and pupae and number of positive containers per house in Models 1 and 2 ($p < 0.01$) (Table 4.2). For every 1-meter increase in distance from the nearest house/structure, the total number of larvae and positive containers decreased by a factor of 0.97 and the number of pupae decreased by a factor of 0.95, adjusting for relevant covariates. Results from negative binomial models were similar for distance to the nearest paved road, highway, and house/structure (Table S.3).

We did not verify measurements obtained using ArcGIS between houses and roads on the ground, but the ground resolution of the ArcGIS world imagery for our study sites is 0.46 m and objects in the map are within 5 m of their true location (62).

### Spatial clusters of larvae and pupae

High/Low clustering (Getis-Ord General G) analyses did not reveal spatially dependent clusters for immature mosquito abundance indicators (number of larvae, pupae, and positive containers) for either time point ($p \geq 0.40$).

### Environmental capital

Cubic splines demonstrated significant non-linear relationships between environmental capital and the number of larvae and pupae per house that were similar for both survey periods ($p<0.01$) (Figure 4.2). For both surveys, households with the lowest and highest environmental capital had significantly fewer larvae and pupae compared to those in the middle ($p<0.01$). Results for the number of positive containers were similar (Figure S.3).

Distance to the nearest paved road and house/structure were significant mediators of the relationship between environmental capital and the number of larvae and pupae ($p<0.01$) (Table 4.3). A one-unit increase in environmental capital was associated with a significant decrease in distance from the nearest paved road or house/structure, which in turn was associated with more larvae and pupae when environmental capital was held constant ($p<0.01$). Fumigated houses, cleaned containers, and distance to the nearest highway were not significant mediators of the association between environmental capital and the number of larvae and pupae. Results for the number of positive containers were similar (Table S.4).

## DISCUSSION

This study identified environmental factors and SES attributes that were associated with mosquito larvae and pupae abundance. Distance to the nearest paved road and house/structure were inversely associated with larvae and pupae abundance and were significant mediators of the relationship between environmental capital and the number of larvae and pupae per house. Cubic splines revealed that households of middle environmental capital had significantly more larvae and pupae than those with the lowest and highest environmental capital.

Our finding that households closer to paved roads had more larvae and pupae is consistent with previous studies from Kansas and Bermuda, which found greater numbers of adult mosquitoes and eggs closer to roads (31, 32). Proximity to paved roads may indicate greater population density, which would include more containers and greater availability of blood meals. The association remained significant after adjusting for the total number of containers ≥3L per household, which may suggest a greater presence of smaller containers like cups, cans, and bottles, in areas closer to roads (31). These containers are also conceivably productive larval habitats. This association was further supported by mediation analyses, which showed that distance to the nearest paved road was a significant mediator of the relationship between environmental capital and number of larvae and pupae. As environmental capital increased, distance to the nearest paved road decreased. Households closer to paved roads had significantly more larvae and pupae, holding environmental capital constant. It is conceivable that households with greater environmental capital, which are closer to roads, are more likely to own barrels and other large water storage containers, which may support larger mosquito populations if they are not properly managed. More mosquitoes in areas closer to paved roads may also increase the risk of the spread of arboviral infections, which was reported in a CHIKV study in Pakistan (63).

Distance to the nearest highway was not a significant predictor of larvae and pupae abundance. One study in Taiwan reported that the number of dengue fever cases corresponded inversely with distance from highways, further indicating that *Ae. aegypti* abundance may be associated with population density (64). Proximity to highways in our study was not necessarily suggestive of greater human population density, which may have greater influence on mosquito abundance (65, 66). These results may suggest that the immediate household environment contributes more to larvae and pupae abundance than more distant neighborhood factors (67-69). This is particularly important for *Ae. aegypti*, as immatures tend to be highly aggregated in space and time, rarely dispersing beyond 30-40 m of the household where they developed as larvae (67, 69).

Distance to the nearest house/structure was inversely associated with larvae and pupae abundance. Furthermore, mediation analyses revealed that households with higher environmental capital were closer to other houses/structures and had significantly more larvae and pupae. We are unaware of other studies assessing distance to the nearest structure as a mediator between SES and mosquito abundance. Previous studies of associations between distance to the nearest building and mosquito abundance are inconsistent. Some report greater *Anopheles* and *Aedes* abundance in houses/structures closer together (30, 70, 71), whereas others do not (31, 72). Urbanization and greater human population density lead to a greater number of artificial containers, which creates an abundance of potential habitats for mosquitoes, including tires, flowerpots, and cans (15). Urban environments may also be more favorable for *Ae. aegypti* due to the absence of natural vegetation, competition, and predation (12, 15, 73, 74). These results reinforce the premise that mosquito control requires community-wide efforts, as individual houses with disproportionately high numbers of mosquitoes may pose risks to their closest neighbors, and indeed the entire community (68).

Recent history of fumigation inside/outside of the house and containers that had been cleaned but could still serve as immature habitats for mosquitoes were not significant mediators between environmental capital and the number of larvae and pupae. Fumigating and cleaning containers with standing water are established mosquito control measures (55, 56, 75). Fumigation is only provided by MSPAS in Guatemala. It could be that our measure of environmental capital was not predictive of these preventive measures in these communities or that fumigation may not have been effective in these areas. Alternatively, our cross-sectional survey that asked whether participants performed these prevention measures in the last six months may have been insufficient to assess the efficacy of these interventions, which require repeated application.  Fumigation frequency and insecticide resistance should also be considered.

Households of middle environmental capital had significantly more larvae and pupae than households with the lowest and highest environmental capital for both surveys.  In this study, environmental capital included access to running water, improved sanitation, a sewer system, and trash disposal service, which are typically associated with reduced mosquito populations (23, 24, 52, 76-78). Greater environmental capital may also indicate higher values of other SES indicators, including income, occupation, and education, which are associated with greater mosquito prevention measures, such as removing containers with standing water (17-19, 21).  Conversely, low environmental capital was associated with greater distance to the nearest paved road, which was associated with fewer mosquitoes. It is conceivable that these distances exceeded the typical flight range for mosquitoes (79).  Moreover, houses with low environmental capital in this study had fewer barrels and other large water storage containers that were most productive for mosquitoes.

Our study did not characterize larval genus or species, but multiple species of *Aedes*, *Anopheles*, and *Culex* mosquitoes have been reported in Quetzaltenango department, where our study was conducted (80-83).  Specific species in Quetzaltenango include *Ae. aegypti* and *Ae. albopictus* (80, 81), which preferentially lay eggs in household containers (84); *An. hectoris, An. parapunctipennis,* and *An. xelajuensis,* which prefer marshes, trees, swamps, fields, streams, and rivers (85); and *Cx. corniger, Cx. peus,* and *Cx. quinquefasciatus*, whose breeding sites include storm sewers, cesspits, and polluted water (26, 86).  Given our container surveys occurred exclusively in households, we suspect that the majority of the immatures that we collected were either *Ae. aegypti* or *Ae. albopictus*.

Our study had several limitations.  First, we sampled communities based on high entomological indices and are thus these are not representative of all communities in Guatemala.  However, the households are representative of the local communities.  Second, cross-sectional surveys of mosquitoes are time sensitive (41) and our two surveys points were insufficient to fully capture the temporal variability of mosquito larvae and pupae, despite including both dry and rainy seasons.  Third, our survey assessments of whether participants fumigated inside/outside the house or cleaned their containers in the last six months were likely inadequate to assess the efficacy of these prevention strategies. Fourth, we did not include containers <3L on household premises such as discarded cups and cans, which could also serve as immature mosquito habitats.

The global human population is expected to peak around 9.6 billion by 2050, favoring the spread of vector-borne diseases (87, 88). With climate change, increasing temperatures, and more frequent flooding, the geographic range of *Ae. aegypti* and *Ae. albopictus* is increasing (1, 89). The findings reported here provide evidence that proximity to other houses/structures and paved roads was associated with more mosquito larvae and pupae in containers around households. Furthermore, households with higher environmental capital were closer to other houses/structures and paved roads, and had significantly greater larvae and pupae abundance. Finally, households with middle environmental capital had significantly more larvae and pupae than the lowest and highest tiers. In resource-limited vector control programs, findings such as these can be used to focus efforts on areas of greater population density closer to roads. The findings also highlight the importance of programs that take into account neighborhood-level risks and mitigation strategies when promoting the prevention of vector-borne diseases.

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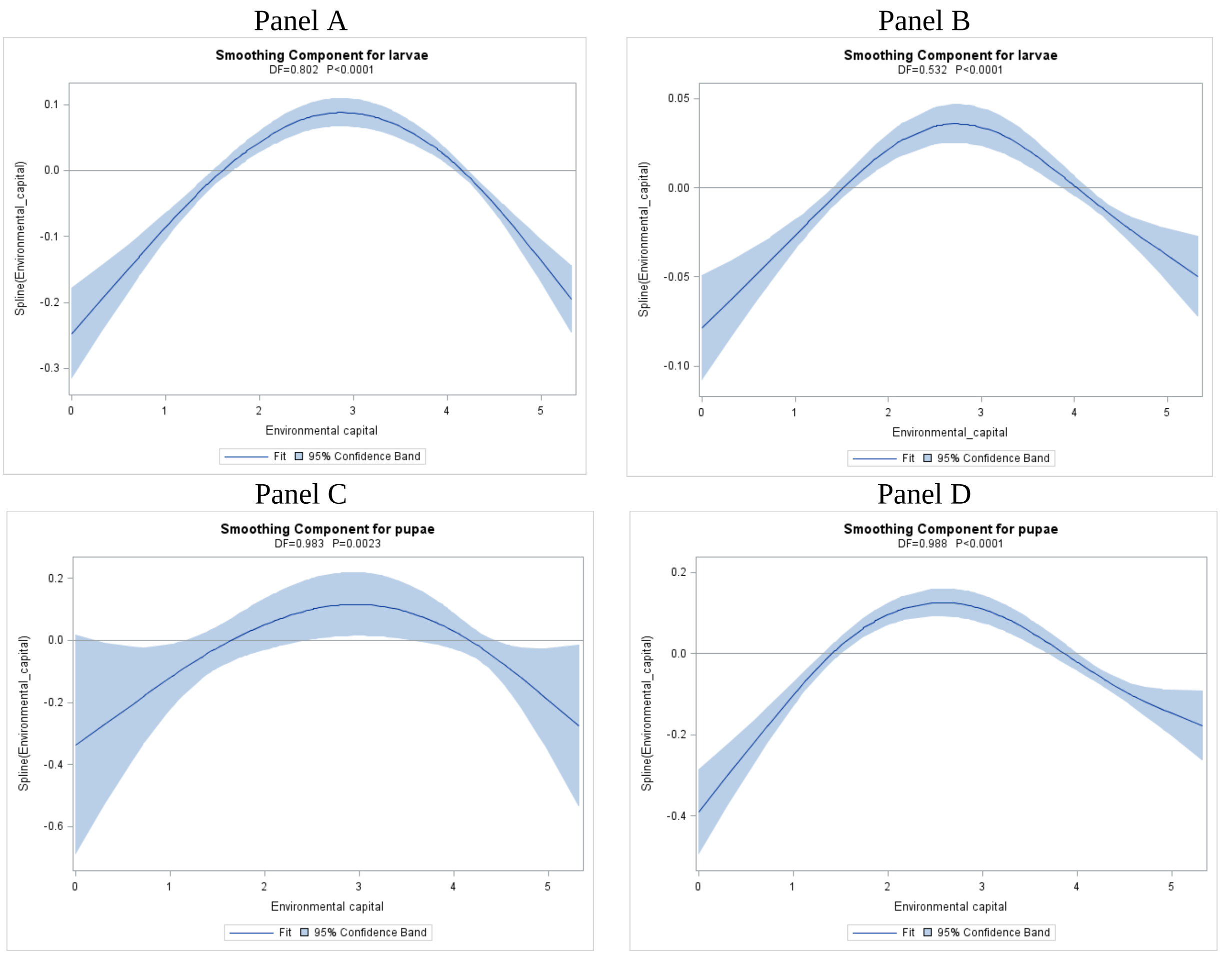
**Figure 4.1. Coatepeque and Génova, Quetzaltenango Department, Guatemala.** Source: Quetzaltenango department location map; by user Edouno; licensed under CC BY 3.0 via Wikimedia Commons, https://commons.wikimedia.org/wiki/File:Quetzaltenango_department_location_map.svg.



Panel A

Panel B

Panel C

Panel D

**Figure 4.2. Cubic splines of associations between environmental capital and total number of larvae and pupae per household, Coatepeque and Génova, Guatemala, 2017**. Panels A and B show results for larvae, whereas panels C and D show results for pupae. Panels A and C show results from the first survey in February-March, 2017, whereas Panels B and D show results from the second survey in November-December, 2017. The bands represent 95% confidence intervals.

**Table 4.1. Household characteristics and immature mosquito numbers, Coatepeque and Génova, Guatemala, 2017.** In February-March, 508 surveys were completed. In November-December, 469 of those households were revisited for a second survey (92.3%). At that time, an additional 18 households were surveyed.

| *Continuous variables* | Median (IQR) |
|---|---|
| Number of people living in household | 5 (4-6) |
| Total number of containers ≥3L per household | 4 (3-5) |
| Number of positive containers ≥3L per household | 1 (0-2) |
| Number of larvae in all containers ≥3L per household | 8 (0-50) |
| Number of pupae in all containers ≥3L per household | 1 (0-6) |
| Distance to nearest paved road (m) | 9.5 (3.3-28.1) |
| Distance to nearest highway (m) | 211.3 (57.4-404.3) |
| Distance to nearest building (m) | 2 (1.0-5.3) |
| Environmental capital[a] | 3.1 (1.8-4.1) |
| | |
| *Categorical variables* | % (SE) |
| Cleaned containers around house in previous 6 months | 53.8 (1.6) |
| Fumigated inside or outside house in previous 6 months | 30.1 (1.5) |
| Rural residence | 72.7 (1.4) |

IQR: interquartile range; SE: standard error

[a] Environmental capital was derived from principal components factor analysis and included: number of rooms in the household; presence of electricity, running water, a television, a landline telephone, cable, trash disposal, and sewer system; and absence of a water well and pit latrine. Score range: 0-5.5

**Table 4.2. Associations between geographical distances to roads/structures and immature mosquito abundance, Poisson regression, Coatepeque and Génova, Guatemala, 2017.**

| | Total number of larvae per household | | | Total number of pupae per household | | | Number of positive containers per household | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | β | SE | P-value | β | SE | P-value | β | SE | P-value |
| Distance from nearest paved road (10-m increase) | | | | | | | | | |
| Unadjusted | -0.04 | 0.01 | <0.01 | -0.06 | 0.03 | <0.01 | -0.05 | 0.01 | <0.01 |
| Adjusted[a] | -0.04 | 0.01 | <0.01 | -0.07 | 0.03 | <0.01 | -0.04 | 0.01 | <0.01 |
| Distance from nearest highway (100-m increase) | | | | | | | | | |
| Unadjusted | 0.01 | 0.01 | 0.50 | 0.01 | 0.01 | 0.28 | 0.01 | 0.01 | 0.52 |
| Adjusted[a] | 0.01 | 0.01 | 0.55 | 0.01 | 0.01 | 0.35 | 0.01 | 0.01 | 0.47 |
| Distance from nearest structure (1-m increase) | | | | | | | | | |
| Unadjusted | -0.03 | 0.01 | <0.01 | -0.04 | 0.02 | <0.01 | -0.04 | 0.01 | <0.01 |
| Adjusted[a] | -0.03 | 0.01 | <0.01 | -0.05 | 0.02 | 0.02 | -0.03 | 0.01 | <0.01 |

[a] Adjusted for environmental capital, survey period, urban/rural residence, the number of people in a household, cleaned containers, fumigated inside or outside the house, and the total number of containers. Environmental capital was derived from principal components factor analysis and included: number of rooms in the household; presence of electricity, running water, a television, a landline telephone, cable, trash disposal, and sewer system; and absence of a water well and pit latrine.

**Table 4.3. Mediation of distances to roads/structures and mosquito prevention measures on the association between environmental capital and immature mosquito abundance, Coatepeque and Génova, Guatemala, 2017**

| | Controlled direct effect | | Natural indirect effect | | Total effect | | |
|---|---|---|---|---|---|---|---|
| Characteristic | Estimate | 95% CI | Estimate | 95% CI | Estimate | 95% CI | Proportion mediated |
| Total number of larvae | | | | | | | |
| Fumigated house | 1.30* | 1.12, 1.48 | -0.14 | -0.37, 0.10 | 1.16* | 0.88, 1.45 | -0.12 |
| Cleaned containers | 1.24* | 1.06, 1.43 | -0.12 | -0.53, 0.28 | 1.12* | 0.65, 1.56 | -0.10 |
| Distance to paved road (m) | -0.26* | -0.46, -0.08 | 5.51* | 5.15, 5.88 | 5.25* | 4.95, 5.52 | 1.05 |
| Distance to highway (m) | 1.16* | 0.98, 1.34 | -0.03 | -0.05, 0.02 | 1.13* | 0.94, 1.35 | -0.03 |
| Distance to nearest structure (m) | -0.01* | -0.23, -0.19 | 3.22* | 2.80, 3.65 | 3.21* | 2.83, 3.59 | 1.00 |
| Total number of pupae | | | | | | | |
| Fumigated house | 0.01 | -0.08, 0.10 | -0.05 | -0.13, 0.03 | -0.03 | -0.16, 0.09 | 0.73 |
| Cleaned containers | -0.03 | -0.13, 0.06 | -0.02 | -0.08, 0.04 | -0.04 | -0.17, 0.06 | 0.33 |
| Distance to paved road (m) | -0.31* | -0.39, -0.23 | 1.53* | 1.39, 1.66 | 1.22* | 1.11, 1.31 | 1.25 |
| Distance to highway (m) | -0.03 | -0.13, 0.06 | -0.01 | -0.03, 0.02 | -0.04 | -0.15, 0.08 | 0.18 |
| Distance to nearest structure (m) | -0.31* | -0.42, -0.21 | 0.85* | 0.71, 1.01 | 0.55* | 0.41, 0.68 | 1.56 |

*p-value<0.05

# CHAPTER 5: DISCUSSION AND CONCLUSIONS

## OVERVIEW

This research intended to identify geospatial, environmental, and sociodemographic risk factors for pathogenic arboviruses and their vectors, and to evaluate surveillance strategies to detect local arbovirus transmission. The following aims were addressed by this research: Aim 1. To examine associations between measures of household air pollution and arboviral infections in Santa Rosa, Guatemala. Aim 2. To compare virological surveillance strategies in mosquitoes and humans to detect local Zika virus transmission in Caguas, Puerto Rico. Aim 3: to evaluate associations between proximity to other houses/structures and roads, and household environmental factors as attributes of SES, and immature mosquito abundance in Quetzaltenango, Guatemala. These studies identified several social and ecological factors that may be important in arbovirus transmission and described circumstances that might favor one surveillance strategy over the other. Results from this research may help focus arbovirus surveillance and vector control efforts in the context of specific sociodemographic attributes of local communities.

## SUMMARY OF FINDINGS

### *Arbovirus infections and indicators of household air pollution*

Two-thirds of arbovirus cases and three-quarters of diarrheal controls cooked with firewood. We found fewer arboviral infections among patients exposed to high levels of biomass smoke than those who did not cook with firewood. Arboviral infections were inversely associated with cooking with firewood in the main house (AOR: 0.22; 95% CI: 0.08-0.57), on an open hearth (AOR: 0.50; 95% CI: 0.33-0.78), and ≥5 times per week (AOR: 0.54; 95% CI: 0.36-0.81), after adjusting for relevant confounders. We did not find associations between arboviral infections and cooking with firewood outside, <5 times per week, or on improved stoves.

### *Comparing vector and human surveillance strategies to detect Zika virus*

In high Zika virus transmission scenarios (one infection per 1,000 people per week), all four surveillance systems evaluated (mosquito trapping systems with 180, 360, and 720 traps; and human surveillance) effectively detected transmission.  When the incidence of infection was one infection per 10,000 people per week, the probability of detection was highest for the 720-trap system, 360-trap system, 180-trap system, and human surveillance, in that order.  In low transmission scenarios (one infection per 100,000 people per week), the expected number of positive mosquito pools or positive humans was essentially zero for all four systems, but the probability of detecting a positive was highest for the mosquito trapping system with 720 traps and lowest for human surveillance.  The number of tests required for human surveillance was much lower than the mosquito surveillance systems.

### *Associations of household environment with immature mosquito abundance*

Immature mosquito abundance was inversely associated with distance to the nearest paved road and house/structure, but was not associated with distance to the nearest highway.  Households with the lowest and highest environmental capital had significantly fewer larvae and pupae compared to those in the middle range.  Distance to the nearest paved road and house/structure were significant mediators of the relationship between environmental capital and immature mosquito abundance, but fumigated houses, cleaned containers, and distance to the nearest highway were not.

## RESEARCH CONTRIBUTIONS AND IMPLICATIONS

This research evaluated human and mosquito surveillance systems for detecting and monitoring arbovirus activity and identified several environmental risk factors for arboviruses.  Early arbovirus detection and vector location and behavior information may guide response efforts.  There is also a paucity of literature on arboviruses and their vectors in Central America.  Understanding mosquito and arbovirus activity in Central America and the Caribbean is critical for guiding surveillance and vector control efforts in the United States and elsewhere.

To our knowledge, we presented the first study examining associations between *Ae. aegypti*-transmitted arboviruses and household cooking with firewood.  Nearly three billion people depend on biomass fuel for cooking and heating, but use of these fuels increases risk for developing stroke, heart disease, and cancer (1, 2).  Interventions to reduce HAP, such as improved stoves or ventilation are effective at reducing personal exposures (3), but there is anecdotal evidence that households that frequently cook with firewood may have fewer arboviral infections than households that do not cook with firewood.  Additional studies are needed to determine whether HAP interventions should be combined with safe mosquito prevention strategies, such as bed nets and window screens.

Interventional strategies are predicated on accurate and timely surveillance of viruses and vectors. We are unaware of other studies comparing tradeoffs between human and vector surveillance strategies to detect urban transmission of ZIKV, which involves predominantly human hosts.  Our results indicated value in both virological surveillance of *Ae. aegypti* and clinical patients, whereas the observed data from Caguas, Puerto Rico, showed human surveillance to be more sensitive.  In higher ZIKV transmission scenarios, both approaches effectively identified transmission.  In the absence of an organized human surveillance approach, virological surveillance of *Ae. aegypti* may have a role indicating local transmission intensity.  Sometimes decisions regarding surveillance strategies are based on anecdotal evidence.  This study may be valuable for epidemiologists and other public health personnel to consider when planning for ZIKV (and other *Ae. aegypti*-transmitted viruses) surveillance activities, as limited resources and budgets need to be directed to the most effective programs.  Rapid detection of ZIKV may trigger an immediate aggressive vector control program.

The underlying environmental and spatial risk factors for variation in mosquito infestation are not well understood.  Our study demonstrated that proximity to paved roads and proximity to houses were predictive of the number of larvae and pupae found in household containers and mediated associations between household environmental attributes of SES and immature mosquito abundance.  We also found that households with high and low environmental capital had lower immature mosquito abundance than those in the middle range.  These findings may be used to focus vector control on areas of greater household and population density closer to roads. The findings also emphasize the importance of programs that highlight neighborhood-level risks and mitigation strategies when promoting the prevention of vector-borne diseases.

These studies had strengths and limitations, which were detailed in each chapter.

## FUTURE DIRECTIONS

Results from our studies suggest that arboviral surveillance research should involve monitoring levels and trends in HAP exposures to help determine whether a causal relationship between cooking with firewood and arboviruses exists. If it is determined that firewood cooking smoke is indeed preventing arbovirus infections, then HAP interventions should be combined with safe and cost-effective mosquito prevention strategies. Furthermore, if arbovirus smoke is deemed an effective *Ae. aegypti* prevention measure, identification of the repellent components would be useful.

Although our surveillance study calculated the number of tests required for virological surveillance in vectors and humans, we did not evaluate the attendant costs of each system, which would be required for introduction of these methods. Furthermore, although our study examined different numbers of mosquito traps as virological surveillance systems, we did not account for the spatial heterogeneity of mosquito trap distribution and vector populations. Future studies could evaluate different trap densities for virological surveillance in *Ae. aegypti*, while accounting for spatial distribution patterns.

Although our study found associations between proximity to roads and houses and immature mosquito abundance, we did not assess mosquito-borne disease outcomes. Additional studies are needed to determine whether the associations identified with immature mosquitoes pertain to adult mosquitoes, and whether these associations have an impact on human disease prevalence. Our findings indicate that effective local mosquito control strategies might target closely spaced houses adjacent to roads. Future studies are needed to develop neighborhood-specific vector control strategies. Additional research is needed to examine impacts of environmental, household, and geospatial factors on the behaviors of specific mosquito genus and species.

As a prelude to any mosquito control program, it is imperative to document *Aedes spp*. populations at southern USA borders, and to assess control strategies to reduce or eliminate arbovirus vectors. Since the early 1960s, complete elimination of *Ae. aegypti* populations has not been achieved with any vector control approach, including larviciding, adulticiding, lethal genes, or mosquito traps (4). In response to recent arbovirus epidemics, the obligate intracellular bacterial endosymbiont *Wolbachia pipientis* has been used to limit the vector's ability to transmit viruses. *Wolbachia spp.* are not naturally occurring in *Ae. aegypti*, but *Ae. aegypti* may be transfected with *Wolbachia*. *Wolbachia* was originally identified in *Culex* mosquitoes in the 1920s and infects 65% of all insects (5, 6). *Wolbachia* is considered a biopesticide, not a genetically modified organism, and may be a useful nonchemical means of controlling *Ae. aegypti* in areas showing resistance to insecticides (7, 8). The EPA has stated the ecological risk posed by *Wolbachia* on non-target organisms is minimal (9, 10).

*Wolbachia* is transmitted vertically via eggs and may manipulate the biology of their hosts in many ways. *Wolbachia* may induce feminization of male mosquitoes during which they transform into females, cause shorter lifespans of *Ae. aegypti*, reduce the frequency of blood meals, delay larval development, reduce overall fitness, incite parthenogenesis in which females reproduce asexually, and cause reproductive abnormalities including sperm-egg cytoplasmic incompatibility (CI) (8, 11-13). CI occurs when *Wolbachia*-infected males mate with *Wolbachia*-free females, producing non-viable offspring (14, 15). Because females typically mate only once, all subsequent eggs will be sterile. Sterilization via males is known as the incompatible insect technique, which may drastically reduce overall vector numbers (16, 17). The proof of concept that *Wolbachia*-transfected mosquitoes can be an integral component of *Aedes spp.* elimination strategies in geographically unique settings has been described (15, 18-20). Confirmation of these findings for *Ae. aegypti* in other geographic settings is important to determine optimal mosquito control strategies, as well as the long-term measures to maintain the *Ae. aegypti*-free environment.

## CONCLUSIONS

Mosquitoes have been around for 190 million years (21). The pathogens they carry have killed a wide array of animals, including dinosaurs, and an estimated 52 billion people throughout the course of history (21). Mosquitoes remain the most dangerous animal to humans today, responsible for 830,000 deaths in 2015 (22). In comparison, humans, snakes, and sharks account for 580,000, 60,000, and 6 deaths per year, respectively (22). Approximately 6.01 billion people currently live in areas suitable for *Ae. aegypti* transmission and 6.33 billion people live in areas suitable for *Ae. albopictus* (23). The global human population is expected to peak around 9.7 billion by 2050 and 11.2 billion by 2100, favoring the spread of vector-borne diseases (24, 25). With climate change, increasing temperatures, and more frequent flooding, the geographic range of *Ae. aegypti* and *Ae. albopictus* is increasing (23, 26). As global warming increases, the mosquito's range will continue to expand north and south into areas that were free of mosquito-borne diseases, including higher altitudes. Despite major advances in science and medicine, both old (e.g., DENV) and new arboviruses (e.g., ZIKV) will continue to infiltrate susceptible human populations. We do not know what the next arbovirus epidemic will be, but other arboviruses including Mayaro and Spondweni viruses may be emerging viral threats (27, 28). Recent outbreaks of Yellow Fever virus in Brazil and Africa also may be a harbinger of what is to come in North America (29). Unless we identify a method of eliminating all 110 trillion mosquitoes on Earth (21), particularly *Aedes spp.*, *Anopheles spp.,* and *Culex spp.*, which may have unanticipated consequences, we will continue to struggle to contain arbovirus outbreaks as best as we can with our limited tools available. As there are currently no widely used commercially available vaccines for DENV, CHIKV, and ZIKV, robust surveillance programs and aggressive vector control efforts are the best methods of limiting their spread.

This dissertation identified several environmental risk factors for arbovirus vectors, including household infrastructure and crowding factors that may be useful for directing vector control efforts in limited resource settings. We also discussed tradeoffs between virological surveillance systems in humans and mosquitoes under different transmission scenarios, which may guide epidemic response efforts. Finally, we found anecdotal evidence that there may be fewer arbovirus infections in households that frequently cook with firewood than households that do not.

I have included 372 references, a small slice of the myriad publications on the enigmatic and disturbing associations between pathogenic viruses, vectors, and their unwitting human hosts.

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# APPENDIX

## SUPPLEMENTARY FIGURES

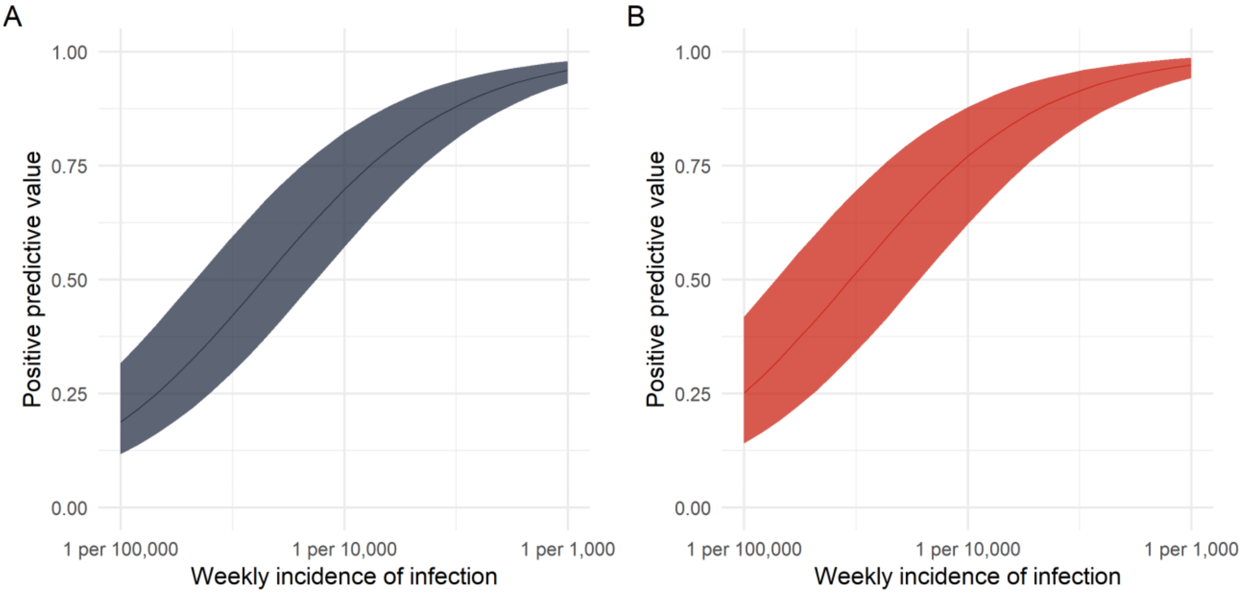

**Figure S.1. Positive predictive value of testing mosquito pools for Zika virus and emergency department patients with two or more Zika virus symptoms.** Panel A describes the positive predictive value (PPV) of a single positive Trioplex Real-time RT-PCR Assay test result on a pool of *Ae. aegypti* females. Panel B describes the PPV of a single positive RT-PCR test result on an emergency department patient. The bands represent 50% uncertainty intervals for the PPV of a positive test over a range of possible ZIKV incidences.

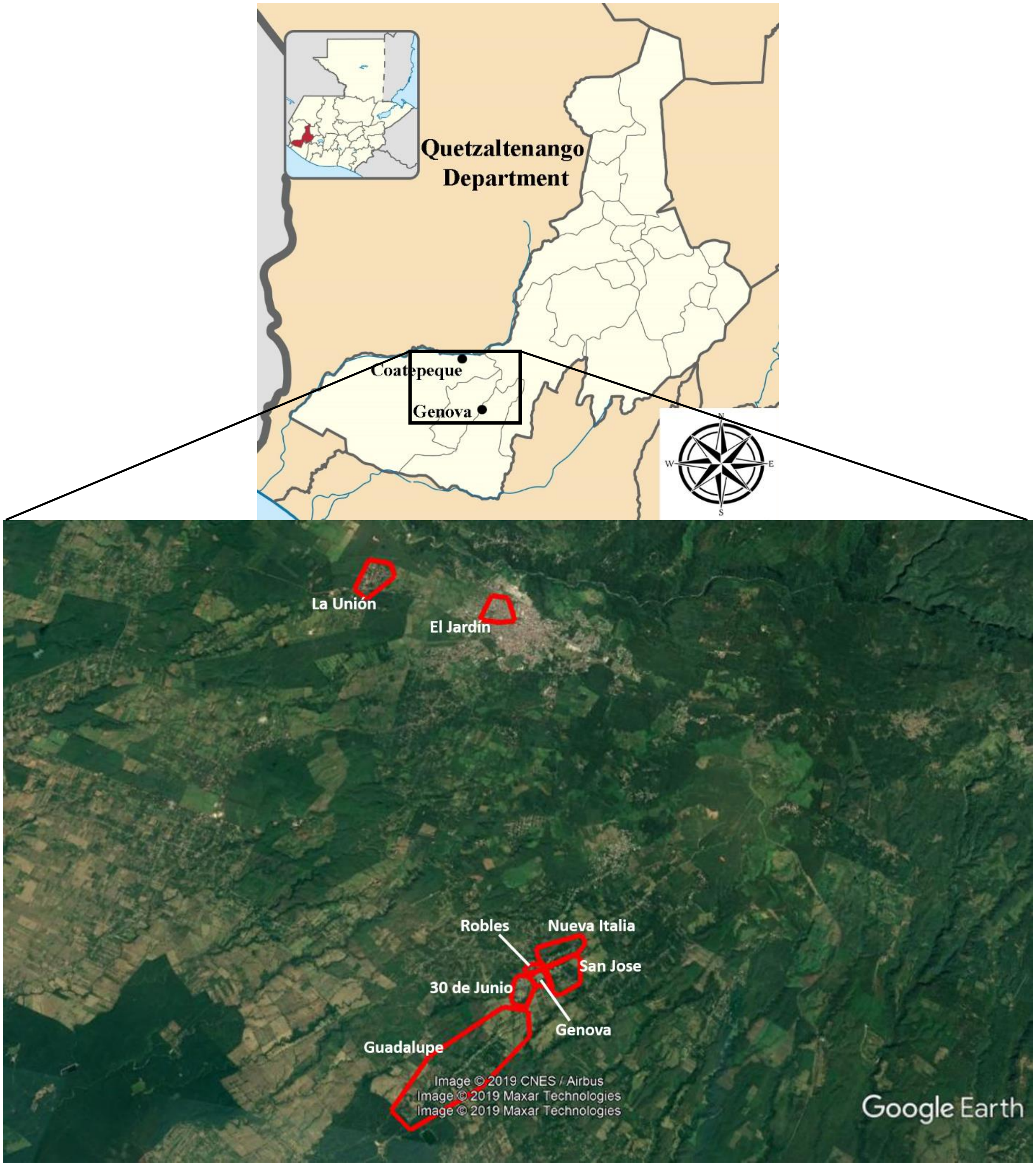

**Figure S.2. Aerial view of communities in Coatepeque and Génova, Guatemala, 2017.** The study sites are enclosed in red. Source: Quetzaltenango department location map; by user Edouno; licensed under CC BY 3.0 via Wikimedia Commons, https://commons.wikimedia.org/wiki/File:Quetzaltenango_department_location_map.svg.

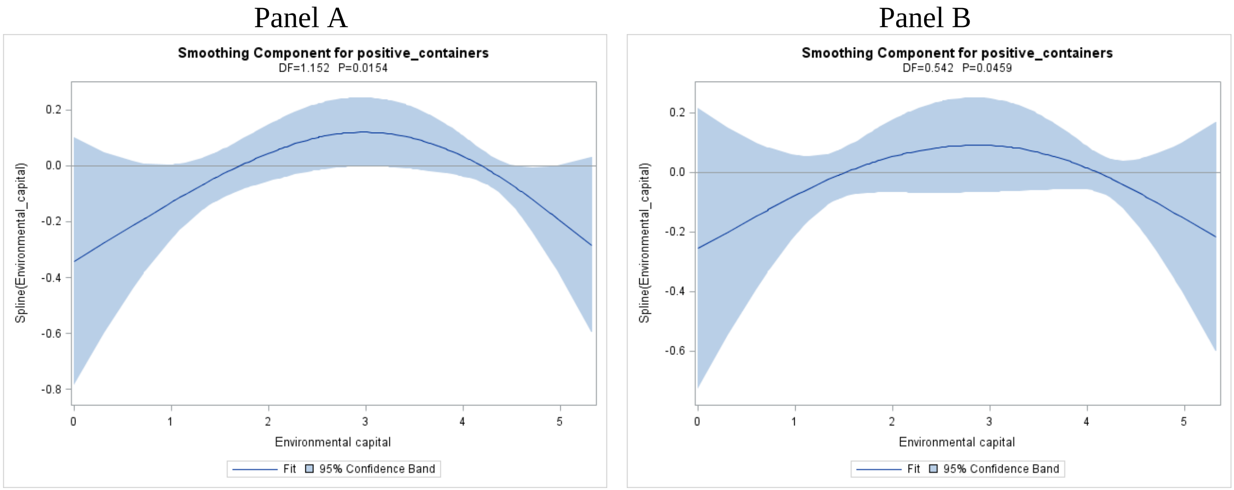

**Figure S.3. Cubic splines of associations between environmental capital and the number of containers with any mosquito larvae or pupae per household, Coatepeque and Génova, Guatemala, 2017**. Panel A shows results from the first survey in February-March, 2017. Panel B shows results from the second survey in November-December, 2017. The bands represent 95% confidence intervals.

# SUPPLEMENTARY TABLES

**Table S.1. Principal components analysis of socioeconomic and household air pollution variables, Santa Rosa, Guatemala (n=1,550)**

| Characteristic | Eigenvector |
|---|---|
| **Socioeconomic status index** | |
| Car | 0.31 |
| Computer | 0.32 |
| Microwave | 0.30 |
| Radio | 0.25 |
| Refrigerator | 0.38 |
| Telephone | 0.18 |
| Television | 0.31 |
| Washing machine | 0.29 |
| Dryer | 0.10 |
| Electricity | 0.24 |
| Number of rooms | 0.24 |
| Roof type | 0.13 |
| Floor type | 0.27 |
| Income | 0.30 |
| *Eigenvalue* | 3.63 |
| **Household air pollution score** | |
| Firewood cooking frequency | 0.59 |
| Firewood cooking location | 0.58 |
| Stove type | 0.56 |
| *Eigenvalue* | 2.64 |

**Table S.2. Principal components factor analysis of household environment variables, Coatepeque and Génova, Guatemala, 2017 (n=508)**

| Characteristic | Factor pattern |
|---|---|
| Electricity | 0.31 |
| Running water | 0.71 |
| Television | 0.43 |
| Landline telephone | 0.35 |
| No pit latrine | 0.41 |
| Cable television | 0.65 |
| Garbage service | 0.72 |
| No water well | 0.60 |
| Sewer system | 0.72 |
| Number of rooms in house | 0.43 |
| *Eigenvalue* | 3.16 |

**Table S.3. Associations between geographical distances to roads/structures and immature mosquito abundance, negative binomial regression, Coatepeque and Génova, Guatemala, 2017.**

| | Total number of larvae per household | | | Total number of pupae per household | | | Number of positive containers per household | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | β | SE | P-value | β | SE | P-value | β | SE | P-value |
| Distance from nearest paved road (10-m increase) | | | | | | | | | |
| Unadjusted | -0.04 | 0.01 | <0.01 | -0.06 | 0.02 | <0.01 | -0.04 | 0.01 | <0.01 |
| Adjusted[a] | -0.04 | 0.01 | <0.01 | -0.08 | 0.02 | <0.01 | -0.04 | 0.01 | <0.01 |
| Distance from nearest highway (100-m increase) | | | | | | | | | |
| Unadjusted | 0.01 | 0.01 | 0.49 | 0.01 | 0.02 | 0.27 | 0.02 | 0.01 | 0.41 |
| Adjusted[a] | -0.01 | 0.01 | 0.73 | 0.02 | 0.02 | 0.64 | 0.01 | 0.01 | 0.78 |
| Distance from nearest structure (1-m increase) | | | | | | | | | |
| Unadjusted | -0.02 | 0.01 | <0.01 | -0.06 | 0.01 | <0.01 | -0.04 | 0.01 | <0.01 |
| Adjusted[a] | -0.02 | 0.01 | <0.01 | -0.06 | 0.02 | <0.01 | -0.03 | 0.01 | <0.01 |

[a] Adjusted for environmental capital, survey period, urban/rural residence, the number of people in a household, cleaned containers, fumigated inside or outside the house, and the total number of containers. Environmental capital was derived from principal components factor analysis and included: number of rooms in the household; presence of electricity, running water, a television, a landline telephone, cable, trash disposal, and sewer system; and absence of a water well and pit latrine.

**Table S.4. Mediation of distances to roads/structures and mosquito prevention measures on the association between environmental capital and the number of containers with any mosquito larvae or pupae per household, Coatepeque and Génova, Guatemala, 2017**

| | Controlled direct effect | | Natural indirect effect | | Total effect | | |
|---|---|---|---|---|---|---|---|
| Characteristic | Estimate | 95% CI | Estimate | 95% CI | Estimate | 95% CI | Proportion mediated |
| Fumigated house | 0.08* | 0.05, 0.11 | -0.01 | -0.02, 0.01 | 0.08* | 0.04, 0.11 | -0.08 |
| Cleaned containers | 0.08* | 0.05, 0.11 | -0.01 | -0.02, 0.01 | 0.07* | 0.04, 0.11 | -0.04 |
| Distance to paved road (m) | 0.01 | -0.02, 0.04 | 0.22* | 0.16, 0.29 | 0.24* | 0.18, 0.28 | 0.94 |
| Distance to highway (m) | 0.08* | 0.04, 0.10 | -0.01 | -0.03, 0.01 | 0.07* | 0.04, 0.10 | -0.02 |
| Distance to nearest structure (m) | 0.03 | 0.03, -0.01 | 0.12* | 0.06, 0.18 | 0.15* | 0.10, 0.19 | 0.80 |

*p-value<0.05