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Emerging arboviruses, old patterns? Observations on dengue, Chikungunya, and Zika in a dengue-endemic tropical state

By

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Master of Public Health

Epidemiology

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By

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B.S., Western Carolina University, 2016

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An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2018

Abstract

Emerging arboviruses, old patterns? Observations on dengue, Chikungunya, and Zika in a dengue-endemic tropical state

By Marissa Lorelle Taylor

Globalization and urbanization have contributed to the emergence of arboviruses such as Chikungunya and Zika. In areas with endemic arboviruses, existing data may be able to predict novel arbovirus patterns because of the shared mosquito vector, *Aedes aegypti*. In Mexico, a study of the Yucatán state's capital, Mérida, recently found dengue hot spots to recur as hot spots for Chikungunya and Zika. Analyses presented here are a follow-up to those findings. Statewide passive surveillance has been conducted for dengue since 2008, Chikungunya since 2015, and Zika since 2016. Epidemic curves were created from all confirmed and probable cases. Index cases of DENV-3, Chikungunya, and Zika were identified, and residence and travel history were described for early cases of Chikungunya and Zika. Data include the case's reported residence, and these addresses have been geocoded for around 80% of observations. Where spatial data were available, points were mapped to determine which were within the state's boundary and within urban centers. For all viruses and years, 52,257 of 56,152 (93%) points fell within the state's boundary. Of those, 90% were urban and 10% rural. Age characteristics were described by year and virus for all cases in the state boundary. For dengue, serotype presence was mapped by number of cases identified per municipality. Municipalities were also used to describe annual incidence for each virus (dengue, Chikungunya, and Zika). To address incomplete years of data (due to invasion or incomplete geocoding), incidence was also described monthly by urban center. To define local and global clustering and identify hot spots, the local Getis-Ord G* and global weighted k-function tests were performed for urban center centroid points for case counts and cumulative incidence for each virus. Of the 127 urban centers, 40 (31%) were identified as hot spots. Cities around Mérida were common hot spots across viruses. Spatial analyses should be utilized for their ability to identify recurring problem areas. As arboviruses continue to emerge, robust surveillance and analysis tools are critical.

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Acknowledgements

I would like to thank Dr. Gonzalo Vazquez-Prokopec for serving as my thesis advisor and for his mentorship throughout my time at Rollins. I would also like to thank Dr. Pablo Manrique-Saide, who hosted me in the summer of 2017. Working in the Yucatán state greatly helped me understand the details and context of my thesis. I am incredibly grateful to the Yucatán state, for my experience there and for the privilege of conducting these analyses. I would also like to share my gratitude to Emory University's GIS librarian, Megan Slemons, who helped me immensely with the initial difficulties of mapping large amounts of data. Finally, I would like to thank my support system- including my colleagues at the Centers for Disease Control's Integrated Vector Management Team, mentors and supervisors along the way, as well as my family and friends.

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1. Introduction

1.1 Background

Pathogens borne by mosquitoes pose a significant global health burden. The *Aedes aegypti* mosquito is of major concern for its ability to vector Yellow Fever, dengue, Chikungunya, and Zika viruses. On a regional scale, Zika and Chikungunya are following the same path of emergence as dengue virus (1). Zika and Chikungunya both emerged from Africa, then caused outbreaks in Asia before successfully expanding through the Americas (1). This trend is of concern because of dengue virus's recent acceleration in frequency and severity of cases in the Americas (2). Because Chikungunya and Zika viruses are spread by the same mosquitoes as dengue, these viruses may also become endemic in the same places. *Aedes aegypti* mosquitoes prefer living alongside humans in tropical environments, with urbanization and globalization contributing to the success of the species (3).

Aedes aegypti population levels are variable on fine-scales, with significant differences between houses within the same neighborhood (4). This variability has been attributed to differences in maintenance of potential larval habitats, such as water storage, combined with *Ae. aegypti*'s short flight distance (4). For this reason, control efforts should be precisely targeted to high burden areas (4). Spatial analyses can assist in this effort to direct control by defining hot spots of mosquito presence, or of reported cases. Passive disease surveillance, which collects case residences, can aid in the effort to target control. As pathogens continue to emerge in areas with existing endemic diseases, further study of overlapping diseases is increasingly important. Robust surveillance systems, including spatial analyses of collected data, are critical in the precise evaluation of disease threats.

1.2 Pathogens of interest

Dengue fever is caused by four viruses in the *Flaviviridae* family which share approximately 65% of their genomes (5). These closely related viruses are referred to as serotypes, known as DENV-1, 2, 3, and 4 (5). Dengue serotypes co-circulate in subtropical and tropical regions of the world (5), with half of the world's population living at risk (3). In the Yucatán state, DENV-1 and DENV-2 are the dominant serotypes, DENV-4 is also present, and DENV-3 was recently identified. Out of the 390 million dengue virus infections per year, 77% are estimated to be asymptomatic (6). Where infection does cause symptoms, illness can range from mild and self-limiting to severe, with a case fatality rate of 2.5% for severe cases (7). Dengue's common name, "breakbone fever" is due to its causing severe muscle and joint pain (7). Because of dengue's serotypes, individuals can become infected up to four times, although risk for severe dengue is highest in the first or second infection (7). The world's first dengue vaccine, Dengvaxia, was approved for prevention of dengue in people 9-45 years of age living in endemic areas (8). These requirements are informed by the possibility that safety and efficacy are of concern in seronegative individuals (9). In Yucatán, Mexico, seroprevalence of dengue in the target age group is estimated at 82.3% (8). A modeling study based on historic data which synthesized multiple scenarios estimated a 74% reduction in dengue cases over a 20-year period as a best-case scenario, highlighting that effective vaccination alone would not eliminate disease in the state (10).

With cost-effective and widespread vaccine use still in the future, dengue control efforts typically rely on controlling the vector, *Ae. aegypti*. Unfortunately, vector control has been limited by application of inappropriate techniques, as well as insufficient resources (3, 11, 12). Because *Ae. aegypti* has adapted to live alongside humans, dengue is especially

successful in urban tropical areas (3). Urbanization and globalization have made dengue the most important vector-borne disease to infect humans (3). A systematic literature review of dengue in Mexico published in 2014 identified the following gaps in epidemiological knowledge: local serotype distribution, genotype evolution, age-stratified incidence and prevalence, hospitalization rates, underreporting rates, and primary/secondary infections (13).

Chikungunya virus recently emerged as an arboviral threat in the Americas. Chikungunya is an *Alphavirus* belonging to the *Togaviridae* family, unlike dengue and Zika, which belong to the *Flavivirus* genus and *Flaviviridae* family (14). The name Chikungunya, meaning "that which bends up" comes from Tanzanian Makonde (15). The first epidemic was described in Tanzania, and the name describes the characteristic joint pain of disease (15). Although previous evidence for introduction to the Americas by travelers existed, the first occurrence of autochthonous transmission was in St. Martin and Martinique in December of 2013 (16). Chikungunya reached Mexico in late 2014, causing an outbreak in the southern state of Chiapas (14). The study in Chiapas described *Ae. aegypti* as the principle vector of the virus in Southern Mexico and did not describe the role of *Ae. albopictus* (14). A 2015 study in the Yucatán state successfully isolated Chikungunya from febrile humans and mosquitoes, revealing Asian lineage and a close relation to other strains isolated in the Americas (17).

Zika Virus, originally discovered in Uganda in 1947, became a pandemic when it reached the Americas (18). Autochthonous Zika transmission in the Americas was first reported in early 2015 in Brazil; however, most countries in Latin America and the Caribbean described autochthonous transmission within 1 year (19). *Ae. aegypti* is the principal Zika

virus vector in the region, although other *Aedes* mosquitoes, including *Ae. albopictus* have been described as competent vectors (19). Zika virus is significant because of its association with severe outcomes, including Guillain-Barre Syndrome (GBS) and congenital malformations including microcephaly (18). The microcephaly outbreak in Brazil associated with Zika was declared a public health emergency of international concern by the WHO in February 2016 (20). Although Yucatán surveillance data begins January of 2016, there is evidence of Zika virus in the peninsula since at least July of 2015 (21). As of late August 2017, autochthonous Zika transmission had been reported in 27 of Mexico's 32 states (22). Across all states, Zika has been confirmed in 5,667 pregnant women and 15 cases of congenital Zika syndrome have been reported (including 1 stillborn) (22). No deaths have been reported among cases (22). Geographic expansion of Zika and its subsequent outbreak can be seen as a troubling new pattern of arboviral disease emergence (18).

1.3 Surveillance & analysis

In Mexico, passive surveillance for human cases has been conducted for dengue fever since 2008, with Chikungunya and Zika viruses added in 2015 and 2016. Where spatial data are available, space-time analyses can be applied to re-construct key features of epidemics, which could include propagation following the index case, and arrival of the outbreak to a major city (23). A study which applied contact tracing was able to demonstrate the probability of positive transmission occurring within the main city (Cairns) or in a satellite town based on place of residence and its distance from Cairns (23). In the Yucatán state, roughly half of the population lives in the capital city, Mérida. A study of dengue, Chikungunya, and Zika overlap in Mérida found areas with historical dengue presence

recurred as hot spots during the subsequent Chikungunya and Zika invasions (24). Even without recreating specific networks, Mérida will have clear influence on statewide arbovirus transmission patterns. Further, studying spatial and demographic patterns of dengue is likely a helpful tool for understanding and predicting Chikungunya and Zika viruses. The recently emerged arboviruses share the principle mosquito vector, *Ae. aegypti*, with dengue, as well as a dependence on urbanization and globalization (1). Invasive arboviruses (Chikungunya and Zika) are hypothesized to follow similar spatial patterns as dengue (prominent serotypes- DENV-1 & DENV-2) because their transmission depends on *Ae. aegypti* distribution as well as human factors such as population density and human movement (1, 24).

2. Methods

2.1 Ethical considerations

Protocols for processing and analyzing data were approved by Emory University's ethics committee under protocol ID: IRB00088659. The protocol was also approved by the Ethics and Research Committee from the O´Horan General Hospital from the state Ministry of Health, Register No. CEI-0-34-1-14. All cases were anonymized before being analyzed.

2.2 Study Site

Beginning with the first suspected Yellow Fever outbreak in 1648, the state of Yucatán in southern Mexico has battled viruses transmitted by *Aedes aegypti* (25). In 2015, Mexico's population was 127 million, with an annual growth rate of 1.3% (26, 27). The population has risen from 109 million in 2015 and 94 million in 1995 (26). The Yucatán state has a population of 1.96 million (28), with 973,000 residents living in Mérida's urban agglomeration (29) (Figure 1). Mérida has a tropical climate, with mean monthly temperatures from 1991 to 2015 ranging from 23.6 ˚C in January to 29.3 ˚C in May (30). Although most dengue cases occur between September and December, cases are reported throughout the year, and recent data suggest that *Ae. aegypti* produces year-round in Mexico (13). Rapid population growth as well as urbanization, trade and tourism combined with a warm climate provide ideal conditions for sustained dengue virus transmission (13).

2.3 Data Sources & Management

Available data have been collected by passive surveillance conducted by the Yucatán State ministry of health and provided by the federal government of Mexico (Dirección General de Epidemiología, SINAVE: Sistema Nacional de Vigilancia Epidemiológica).

Information is available for individuals who presented to a clinic or hospital with signs or symptoms typical of arbovirus infection. Based on signs and symptoms or a diagnostic test (IgM, IgG, PCR, or NS1), individuals were assigned one of the following case status values: confirmed, probable, or discarded. Confirmed cases are those with a positive virus or serology result, while probable cases are diagnosed based on signs and symptoms. During identified outbreaks, percentage of suspected cases receiving diagnostic testing generally decreases. As such, relying only on laboratory confirmed cases will obscure patterns of transmission.

Available geographic data for the Yucatán state include the political border of the state, the road and rail transportation network, and municipality divisions, which are the first political subdivisions of the state. There are also data for all 127 places designated by Mexico as cities (more than 2,500 inhabitants), which will be referred to as urban centers (Figure 2). For all municipalities and urban centers, census information is available on total population as well as three-year age categories.

Geographic information for cases corresponds to the physical address provided at the time of the doctor visit/hospitalization. Approximately 80% of observations are geocoded, meaning the patient's address is assigned global positioning system (GPS) coordinates, which can be processed with GIS software. Where available, the precision of the coordinates is included (address, zip code, municipality, etc.). Dengue data are available from January 2008 through December 2016, with coordinates available through July 2016. Chikungunya and Zika virus data are available starting in May 2015 and January 2016, respectively, with coordinates available through December 2016. Spatial data are available for dengue (n= 45,526), Chikungunya (n= 2,501), and Zika (n= 8,125). To make the best

use of available data, separate datasets were created for observations with and without spatial data. For both datasets, all observations with a case status of “discarded” were excluded, leaving confirmed and probable cases for dengue, Chikungunya, and Zika.

To create a statewide epidemic curve, all cases with a Yucatán residence were included, regardless of availability of spatial data (GPS coordinates). A line listing of symptom onset date and diagnosis was created for the entire nine-year period. An annual epidemic curve was constructed by aggregating data by virus and year and displaying data as stacked columns. Additionally, week number and year variables were calculated based on the date of symptom onset. Week and year were used to calculate a cumulative week (assuming 52.149 weeks/year), creating a continuous weekly observation for the entire nine-year period, equaling a total of 470 weeks. Data were aggregated by virus and cumulative week and displayed as stacked columns.

For all confirmed and probable cases date of symptom onset, city and municipality or residence are available. In some instances, cases are asked to provide recent travel history. To describe invasion for each novel arbovirus (DENV-3, Chikungunya, and Zika), index and early cases were identified. To identify the DENV-3 index case, all dengue cases were sorted by isolated serotype. Only one PCR positive DENV-3 case was identified; city and municipality residence were available, but travel history was not collected. To describe Chikungunya and Zika index cases, date of symptom onset was sorted for the first years of available data (2015 and 2016, respectively). Identified Chikungunya index cases (n=2) and the Zika index case had city and municipality of residence available, and travel history was collected, but no travel was reported. Because travel information was not available for

Chikungunya and Zika index cases, travel history (as well as residence) was described for the first 20 cases of each virus.

Age distribution

Because age distribution was described as a gap in epidemiologic knowledge for Mexico, statistics on age distribution for each year and disease have been calculated. These calculations were only performed on confirmed or probable cases with coordinates inside the Yucatán state. All observations have either birth date and date of symptom onset available, or an existing age in years integer variable. Age was not able to be calculated for Chikungunya in 2015, as birth date was not available in the database. In the place of a calculated continuous variable, the existing age integer variable was used to calculate mean and standard deviation. The calculated age variable was used to gather basic statistics for each disease and year, including counts, minimum and maximum values, mean, and standard deviation.

Serotype distribution

Serotype PCR results were available for select (n= 2,191) dengue cases from 2008-2015. Dengue serotype distribution maps were created for each of the four serotypes from all observations where PCR successfully identified a serotype. Maps were created to summarize distribution of serotypes by municipality, including number of reported cases of each serotype.

Municipality level incidence

To compare incidence of dengue over time and between municipalities, annual incidence was calculated for each municipality for all years of data. Confirmed and probable cases

were assigned municipalities based on their spatial locations in order to sum cases by municipality and year. Incidence was calculated by dividing these values by the municipality's population. These values were analyzed to determine the minimum, 25th percentile, median, 75th percentile, and maximum values of annual incidence at the municipality level. This calculation allowed for one classification to be applied to all municipalities and years, enabling visual comparison of maps. Spatial joining of cases to municipalities was repeated for Chikungunya and Zika data. In order to compare incidence of the invasive arboviruses with dengue incidence, the Chikungunya and Zika maps are displayed with the quartile values derived from dengue incidence statistics.

Urban incidence & spatial statistical analyses

To describe and compare arbovirus incidence in urban centers, each urban center was assigned the cases falling within its political boundary. For each virus, total number of cases were summed for each city or town, and crude monthly incidence was calculated by dividing sum of cases by population. Dengue data are available for 103 months, Chikungunya data for 20 months, and Zika data for 12 months. In order to display the urban centers and perform advanced spatial analyses, centroids (geometric centers) were calculated for each urban center.

2.4 Data analysis

Point pattern analysis of urban center centroids were used to perform local Getis-Ord G* tests (31). The local G* test uses geographic information (in this instance, centroid points) and their associated values (number of cases or incidence) to find statistically significant "hot spots", also referred to as members of clusters. The local G* test was applied to urban

center centroids for case counts as well as cumulative incidence for each virus, totaling six G* tests. G* calculates local clustering by comparing observed data with the assumption of spatial randomness. For each urban center, local clustering was calculated each 1 kilometer up to 50 kilometers and assigned a z-score value. According to the sample size of 127, the threshold for statistical significance at a p-value <.05 is a z-score of 3.2889 (Bonferroni correction). For each urban center with a significant z-score, maximum z-score values and distance at maximum z-score value were mapped. Maps were produced for case counts and cumulative incidence for each virus.

The Global Ripley's Weighted K-function test was performed for each virus for case counts as well as cumulative incidence, with the urban center centroids as the spatial reference for each town or city. Ripley's K was used to determine the pattern of clustering (or dispersion) with a 50-kilometer search window in 1-kilometer increments. The K-function is calculated by comparing the observed data to the assumption of complete spatial randomness, which was calculated with 1,000 Poisson distribution permutations. Ripley's K results are displayed as a plot comparing a random spatial pattern and its calculated confidence envelope to the observed data, based on Monte Carlo simulations.

3. Results

Epidemic curve & index cases

The yearly epidemic curve for dengue, Chikungunya, and Zika (Figure 3) provides a pattern of large outbreaks occurring approximately every 2-3 years: in 2009, 2011-12, and 2015. The first year with dengue case information (2008) has the fewest cases, at 1,126. Cases increased by more than 4.5 times in 2009, with a 5,274 case dengue outbreak. Following outbreak years, case counts decline; however, the second-lowest number of dengue cases in one year still reaches 3 times the 2008 level (at 3,441 in 2014). Chikungunya emerged in 2015, with cases only representing 10.7% of the year's arbovirus cases. Zika data begin in 2016, with 14,739 cases in its first year of records. Zika represented 72.4% of the year's arbovirus cases, with dengue representing 21.2% of cases and Chikungunya the remaining 6.4%.

The cumulative weekly epidemic curve (Figure 4) provides more detailed information on epidemic peaks. The maximum number of incident cases in one week in 2008 (weeks 1-53) was 52. In 2009, the maximum increased 10 times to 521 incident cases in one week. The 2009 dengue outbreak had the smallest incident cases per week peak, compared to 876, 653, and 1,524 per week during the 2011-12 and 2015 dengue outbreaks. The 2016 Zika outbreak's maximum weekly incidence reached 1,592 cases. The index case of Zika occurred in 2016 in cumulative week 419; peak weekly incidence occurred 40 weeks later, in cumulative week 459. The index cases of Chikungunya (n=2) occurred in 2015 in cumulative week 384. Peak weekly incidence occurred 20 weeks later, in week 404.

Each of the four described index cases occurred among residents of Mérida urban center and municipality (Table 1). Cases occurring soon after the index case (up to the first 20

cases) reached seven municipalities for Chikungunya and seven municipalities for Zika, of which two (Mérida and Valladolid) were shared among both (Table 2). Of the first 20 cases of Zika and Chikungunya, 18 of the 40 (45%) were among residents of the Mérida municipality, followed by 6 of the 40 (15%) in Valladolid municipality. Travel history in the last two weeks was collected for 12 of 20 (60%) early Chikungunya cases, of which three (25%) reported travel. One-month travel history was collected for two of those three cases, of which one reported travel. Travel among early Chikungunya cases was within Mexico, to two municipalities in the state of Chiapas, and to one municipality within the Yucatán state (Table 3). Travel history in the last three weeks was collected for all 20 early Zika cases. Three of the 20 (15%) reported the following travel: within Mexico to Chiapas (Palenque municipality) and Quintana Roo states, as well as to Cuba and the United States of America (Table 3). Travel to Chiapas was reported by three of the six early cases who provided travel history.

Age distribution

Statistics of mean and standard deviation were calculated for each virus and each year based on birth date and symptom onset (Table 4). Chikungunya in 2015 had the youngest mean age, at 21.7; in 2016 the virus had the oldest mean age, at 31.8.

Spatial information

Dengue records from 2008-2016 contained 81,736 observations, of which 23,365 (28.6%) were discarded (not confirmed or probable cases); 70,283 (86.0%) observations had GPS information available, of which 45,314 (64.5%) were confirmed or probable cases with

coordinates inside the state. 40,197 (88.7%) were classified as urban (located within an urban center), leaving 5,117 (11.2%) defined as rural.

Chikungunya records from 2015-2016 contained 3,139 observations, of which 226 (7.2%) were discarded (not confirmed or probable cases). All observations had GPS information available, of which 1,677 (53.4%) were confirmed or probable cases with coordinates inside the state. 1,463 (87.2%) were classified as urban (located within an urban center), leaving 214 (12.8%) defined as rural.

Zika records from 2016 included 14,942 observations, of which 215 (1.4%) were discarded (not confirmed or probable cases); 10,900 (73.0%) observations had GPS information available, of which 5,266 (48.3%) were confirmed or probable cases with coordinates inside the state. Of those, 5,121 (97.2%) were classified as urban (located within an urban center), leaving 145 (2.8%) defined as rural. Data breakdowns for each year are shown in Table 5.

Serotype distribution

Serotype test information was available for 2,191 dengue cases between 2008-2015. Of the tested samples, 1,960 (90%) had a positive result, with 1,027 DENV-2, 878 DENV-1, 54 DENV-4, and 1 DENV-3. 231 (10%) observations indicated a negative result for serotype PCR testing. Coordinate information was available for 1,815 (83%) of the 2,191 results. Observations falling outside the state's border (14 observations) were removed. Mapped positive results have the following distribution: 949 DENV-2 (52.7%), 797 DENV-1 (44.3%), 54 DENV-4 (3.0%), and 1 DENV-3 (<0.1%) (Figure 6). DENV-1 was identified in 81 municipalities, DENV-2 in 74, DENV-3 in 1, and DENV-4 in 15 (Figure 5).

Municipality level incidence

Incidence quartiles were calculated based on yearly dengue incidence values from 2008-2016 for all municipalities with observations above zero. The minimum municipality incidence above zero was 0.3 per 10,000, the 25th percentile: 4.8 per 10,000, the median: 11.9 per 10,000, the 75th percentile: 28.3 per 10,000, and the maximum: 290.3 per 10,000 (Figure 7). To map Chikungunya and Zika, the same interquartile cut points were applied (Figure 8); however, the minimum was 4.6 per 10,000 and the maximum was 91.4 per 10,000.

Urban incidence

Mean monthly dengue incidence was highest in Pisté, Cuncunul, Maxcanú, Muxupip, and Ticul (Figure 9). For Chikungunya, incidence was highest in Cantamayec, Santa Elena, Chankom, Yobaín, and Yaxcabá (Figure 10). For Zika, incidence was highest in Acanceh, Pisté, Valladolid, Cuncunul, and Tinum (Figure 11). Four of the five highest incidence urban centers are in three adjacent municipalities: Tinum (Tinum and Pisté), Cuncunul, and Valladolid.

Spatial statistical analyses (G and Ripley's K)*

Of the 127 urban centers, 40 (31%) had a significant G* z-score (Gi* > 3.2889; $P < 0.05$) for either dengue, Chikungunya, or Zika case counts or incidence. The highest significant G* z-scores for dengue cases (Figure 12), Chikungunya cases (Figure 14), and Zika incidence (Figure 17) all occur in Mérida and its surrounding urban agglomeration. Significant urban centers for dengue incidence (Figure 13) were Mérida and Caucel (a Mérida suburb), Tinum, and Pisté. Significant urban centers for Chikungunya incidence

(Figure 15) were all in the southern part of the state, reaching Peto, Tekax, Chapab, Sotuta, and Yaxcabá municipalities. The highest significant urban centers for Zika cases (Figure 16) were Acanceh and Pisté. Timucuy and Leona Vicario, near Acanceh, were also significant. Eight cities near Pisté also had significant z-scores.

Of the 40,197 dengue cases within cities, 25,558 were within the 14 cities identified as hot spots. These hot spot cities (11% of all cities) represent 63.6% of all urban dengue cases. Of the 1,463 Chikungunya cases within cities, 648 were within the 11 cities identified as hot spots. These hot spot cities (9% of all cities) represent 44.3% of all urban Chikungunya cases. Of the 5,121 Zika cases within cities, 622 were within the 12 cities identified as hot spots. These hot spot cities (9% of all cities) represent 12.1% of all urban Zika cases.

Ripley's weighted K-function was also calculated for urban center case counts and cumulative incidence for dengue, Chikungunya, and Zika. Global weighted K-function plots for cases of dengue, Chikungunya, and Zika all look similar to each other. The lower confidence envelopes are below the expectation of randomness, indicating no statistically significant clustering or dispersion (Figures 18, 20, 22). Global weighted K-function plots for incidence of dengue, Chikungunya, and Zika also look similar to each other, showing no statistically significant clustering or dispersion (Figures 19, 21, 23).

4. Discussion

4.1 Limitations

This study has several limitations. First, because the data have been collected from passive surveillance, only symptomatic cases are included. Considering dengue's asymptomatic rate of nearly 80%, the data analyzed here represent only a small portion of actual cases (6). Further, because of reductions in diagnostic testing during outbreaks, confirmed cases are limited, and probable cases have been included in analyses. Diagnosis of probable cases is particularly difficult in the scenario of overlapping pathogens which also share symptoms. Further, emerging arboviruses may experience a delay in detection, be more difficult to diagnose, and have limited availability for diagnostic testing. Diagnostic tests are more likely to be available in wealthier areas and urban centers, which may create a bias in the data. Another limitation is the reliance on case residences for spatial analysis, which previous studies have shown may not reflect where infection occurred (23, 32). Spatial data requires geocoding to link an address to its geographic location. Geocoding errors for 2016 Chikungunya data may be prevalent based on a low proportion of cases for which spatial data fell within the state's border.

4.2 Conclusions / Implications

While the presence of mosquito-borne diseases is not unusual for Mexico's Yucatán state, troubling new patterns of disease emergence and epidemiological changes in previously described diseases present cause for concern. Traditionally, dengue has been observed across regions to cycle in outbreaks separated by multiple years (33, 34). This pattern has been previously described in the Yucatán (35), and can be observed in the presented epidemic curves. However, as novel arboviruses invade, epidemiological shifts are likely

to occur. Even dengue can be considered a novel arbovirus, as serotypes expand geographically, or mutations contribute to virulence. For example, the first occurrence of severe dengue (hemorrhagic fever- DHF and shock syndrome- DSS) in the Americas (Cuba, 1981) was associated with a virulent strain of DENV-2 which came from Southeast Asia (35, 36). In the Pacific, DENV-3 was associated with five outbreaks across the region after being reintroduced in 2012 (34). The ability of DENV-3 to become the Pacific's dominant serotype has been attributed to low immunity after its 18-year absence from the region (34).

In 1983 when a DENV-4 outbreak in the Yucatán caused nine hospitalizations and four deaths, a study warned of a significant shift in the spectrum of dengue illness in Mexico (36). When DENV-3 was first isolated in Mexico in 1995, the potential for outbreaks and severe infections (secondary infections and DHF or DSS) was described (37). Though this work was published 23 years ago, the authors highlighted what are presently regarded as key factors in dengue's emergence as a threat to global public health: the roles of urbanization, globalization, international trade, and human movement (37). Further, tourism, which is described as essential to Mexico's economy was described as a method for dengue to migrate (37).

Mexico's National Institute of Anthropology and History (INAH) maintains several prominent historical sites, including the Chichén Itzá archaeological zone, with two million annual visitors (38). The Yucatán state also claims many cenotes, limestone sinkholes, which attract many tourists. Visitation to these sites is enabled through the region's bus network, with connectivity to hubs such as Cancún, Mérida, and Valladolid. These cities and their suburbs as well as cities and towns with connectivity to major highways were

found to also be significant for arbovirus transmission. The urban centers with highest mean monthly dengue incidence were a Valladolid suburb with connectivity to Mérida, a large town along the highway which connects Yucatán and Campeche states, a Mérida suburb with a cenote, and a city close to the Uxmal archaeological site with connectivity to Campeche. This pattern of high incidence repeats for Zika, with Valladolid, Mérida and Valladolid suburbs, towns neighboring archaeological sites. While two of Chikungunya's five highest incidence urban centers fit the same pattern, three (Cantameyec, Yobaín, Yaxcabá) of the highest incidence urban centers were rural centers with small populations.

Described patterns of Chikungunya in Yucatán may serve as a case study for the unpredictable nature of invasive arboviruses. Despite reaching the capital city of a two-million person naïve population, Chikungunya did not displace dengue and cause a major statewide outbreak. Just one year later, Zika virus caused a major outbreak. Zika is known to have been present in the state before 2016, and a possible explanation for dengue dominating arbovirus cases in 2015 is competition between Chikungunya and Zika limiting either from surmounting a major outbreak until dengue's typical cyclic outbreak had subsided. Other explanations for Chikungunya's relatively few cases include insufficient diagnostic testing or a strain with low infectivity to humans. Considering half of the early cases with available travel information traveled to Chiapas, where Chikungunya was first identified in Mexico, some additional cases are likely tied to these introductions and should be considered associated with importation.

Observations made from this analysis are the result of passive epidemiologic surveillance, which is a critical part of health systems capacity. Surveillance can be used to describe patterns of endemic disease, predict novel invasions, respond to outbreaks, and target

disease control measures as well as estimate their efficacy. For example, the Yucatán state used its surveillance data to direct vector control during the 2016 Zika outbreak (24). Studies which seek to predict the success of novel measures, such as those related to dengue vaccine efficacy, rely on historical data for information such as seroprevalence to make inferences (8, 9, 39). A challenge to successful surveillance, as described in the Pacific region, is the reliance on presentation of syndromes. Syndromic surveillance for co-circulating dengue, Chikungunya, and Zika is complicated by other diseases occurring at the same time with similar clinical presentations. A potentially effective solution to this challenge is increased diagnostic testing. In Mexico, diagnostic testing for dengue already uses Real-Time PCR or NS1 testing (40). Additions to standard PCR protocols, such as described serotype-specific fluorescent probes (41) should be considered for their ability to reduce the cost to efficiently collect dengue serotype information.

The factors which have allowed dengue to establish itself as an endemic disease in Mexico will also serve as contributors to the emergence of novel arboviruses. The capacity of health systems to survey for and respond to threats is increasingly critical considering the trends in urbanization, globalization, and trade and travel (42). Predictions surrounding currently emerging arboviruses have described these same factors for emergence- urbanization, aircraft transportation, and mosquito adaptation to the urban environment (43). The study highlighted Yellow Fever, Rift Valley Fever, O'nyong'nyong virus, and Spondweni virus as likely to follow the emergence path of dengue, Chikungunya, and Zika (43).

The findings of the applied spatial analyses (local Getis-Ord G* test and local weighted k-function) are important to understanding pathogen co-circulation and arbovirus transmission dynamics. The local Getis-Ord G* test detected significant clustering, while

the local weighted k-function did not. This suggests that global tests should not be relied on, because while no statement about clustering can be made state-wide, there are significant clusters present. Spatial analyses should be utilized to understand how endemic pathogens behave, and to elucidate differences in transmission dynamics between endemic and novel pathogens. Although the details of yet-to-occur outbreaks may remain difficult to predict, the patterns shared among their predecessors should be considered as certainties.

4.3 Future directions

Following this study, additional analyses should attempt to further explain transmission dynamics. Risk factors within cities that contribute to clustering should be elucidated so cities with these characteristics may be prioritized for disease control. These characteristics may be shared across regions with arboviral diseases, which would be useful for predicting transmission. In particular, total population and population density may be predictive of hot spots because of *Ae. aegypti*'s success in urban settings. Additional studies of human movement, especially between cities within the state could also provide additional information into pathogen migration within the state.

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Tables

Table 1: Date of symptom onset & residence for invasive index Cases

Invasive Arbovirus	Symptom Onset	Urban Center	Municipality
Dengue Serotype 3	9/8/2013	Mérida	Mérida
Chikungunya	5/3/2015*	Mérida	Mérida
Zika	1/3/2016	Mérida	Mérida
	* two cases		

Table 2: Municipality of residence of first 20 Chikungunya and Zika cases

	Chikungunya		Zika		Sum	
Municipality	Cases	%	Cases	%	Cases	%
Chemax	--	--	1	**5**	1	**2.5**
Cuzamá	--	--	1	**5**	1	**2.5**
Espita	2	**10**	--	--	2	**5**
Kanasín	--	--	1	**5**	1	**2.5**
Kaua	--	--	1	**5**	1	**2.5**
Mérida	5	**25**	13	**65**	18	**45**
Oxkutzcab	--	--	1	**5**	1	**2.5**
Progreso	1	**5**	--	--	1	**2.5**
Tinum	3	**15**	--	--	3	**7.5**
Tizmín	4	**20**	--	--	4	**10**
Umán	1	**5**	--	--	1	**2.5**
Valladolid	4	**20**	2	**10**	6	**15**
	20	--	20	--	40	--

Table 3. Travel history among first 20 Chikungunya and Zika cases where collected (32 out of 40) and reported (6 out of 32).

Virus	Municipality	State	Country
Chikungunya	Tonala	Chiapas	Mexico
Chikungunya	Arriaga	Chiapas	Mexico
Chikungunya	Valladolid	Yucatán	Mexico
Zika	--	Havana	Cuba*
Zika	--	--	United States of America*
Zika	Palenque	Chiapas	Mexico
Zika	--	Quintana Roo	Mexico
			* Same case

Table 4. Age statistics by virus & year

	n	Mean	Standard Deviation
Zika 2016	5,217	26.7	14.9
Chikungunya 2016	203	31.8	15.3
Dengue 2016	659	26.5	17.7
Chikungunya 2015*	1,474	21.7	15.6
Dengue 2015	11,265	27.7	18.1
Dengue 2014	3,202	24.5	16.1
Dengue 2013	4,790	23.7	15.8
Dengue 2012	12,767	25	16
Dengue 2011	8,022	25.3	16.1
Dengue 2010	3,069	24.2	15.6
Dengue 2009	1,164	25.64	15.13
Dengue 2008	375	24.7	15.4
* no birth date available, used existing integer age variable			

Table 5. Description of available data

	Observations with coordinates	Cases with coordinates	Cases with coordinates within state	% in state	Urban cases	% urban	Rural cases	% rural
Dengue 2008	638	377	375	99.5	328	87.5	47	12.5
Dengue 2009	1,782	1,180	1,164	98.6	979	84.1	185	15.9
Dengue 2010	5,128	3,099	3,070	99.1	2,779	90.5	291	9.5
Dengue 2011	11,965	8,041	8,022	99.8	7,393	92.1	629	7.9
Dengue 2012	16,851	12,855	12,767	99.3	11,284	88.4	1,483	11.6
Dengue 2013	8,283	4,804	4,790	99.7	4,128	86.2	662	13.8
Dengue 2014	4,733	3,221	3,202	99.4	2,770	86.5	432	13.5

Dengue 2015	15,217	11,279	11,265	99.9	10,032	89.1	1,233	10.9
Chikunguny a 2015	1,607	1,607	1,474	91.7	1,264	85.8	210	14.2
Dengue 2016	953	670	659	98.4	504	76.5	155	23.5
Chikunguny a 2016	1,532	894	203	22.7	199	98.0	4	2.0
Zika 2016	10,899	8,125	5,266	64.8	5,121	97.2	145	2.8

Figures

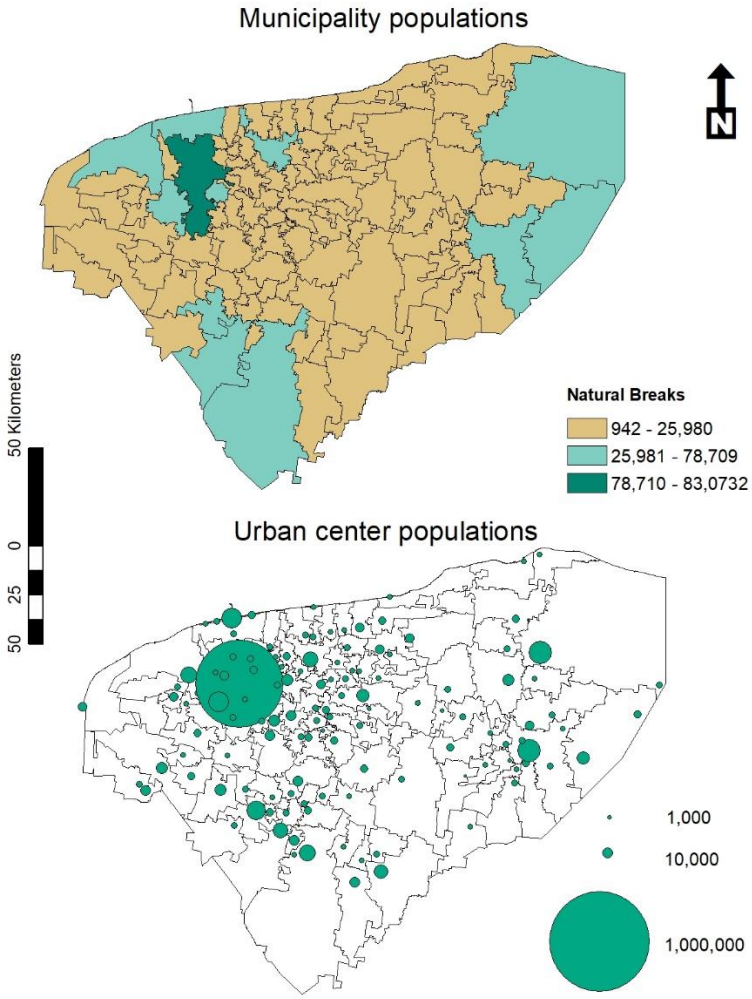

Figure 1. Populations of Yucatán state's municipalities and urban centers, based on 2010 census. The municipality legend uses natural breaks. The maximum municipality population is Mérida municipality, at 830,732. The maximum urban center population is Mérida at 777,615.

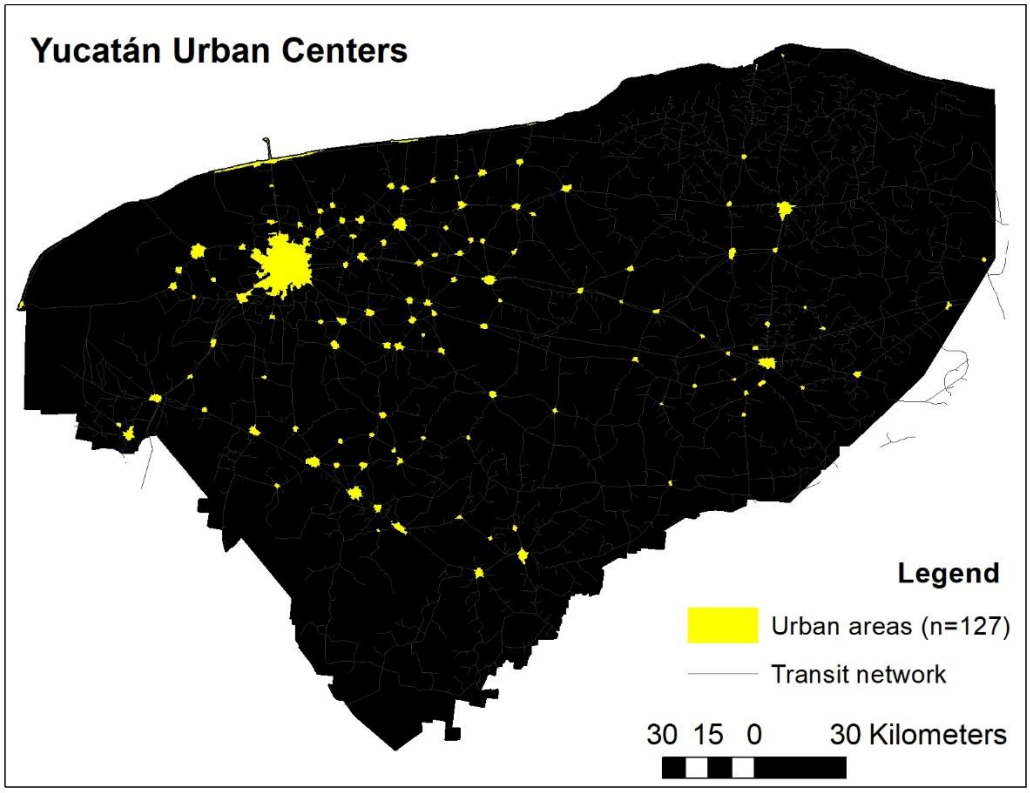

Figure 2. Geographic areas of Yucatán state's urban centers (based on Mexico's designation-population > 2,500) and state highway, road, and rail network. The largest geographic urban center is the capital city, Mérida.

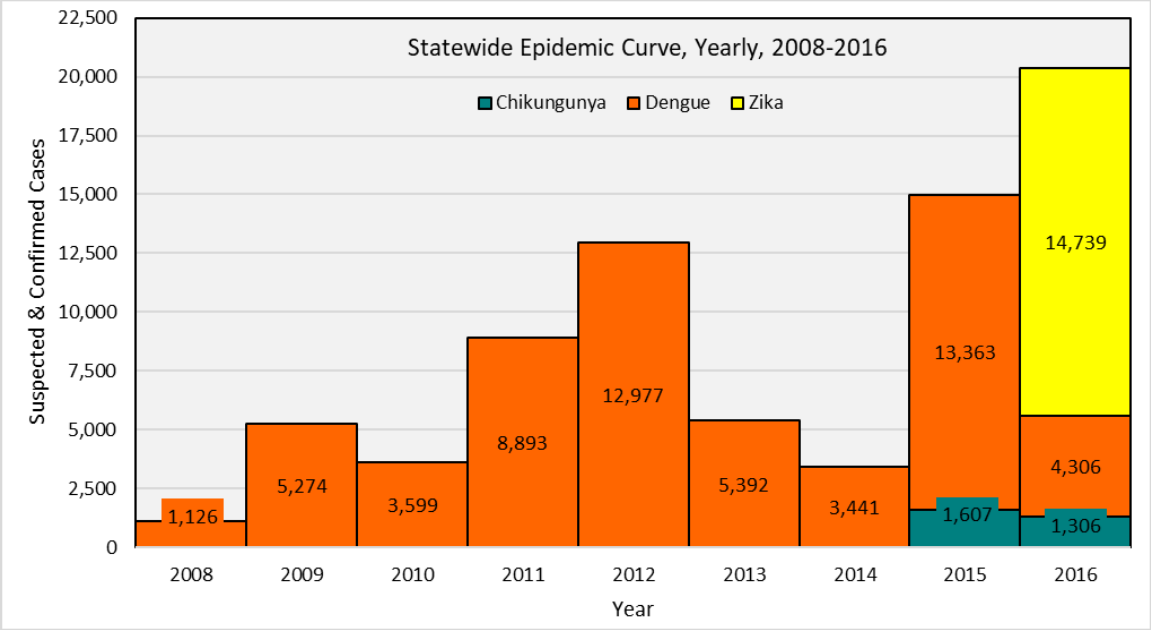

Figure 3. Statewide yearly epidemic curve for dengue, Chikungunya, and Zika from 2008-2016.

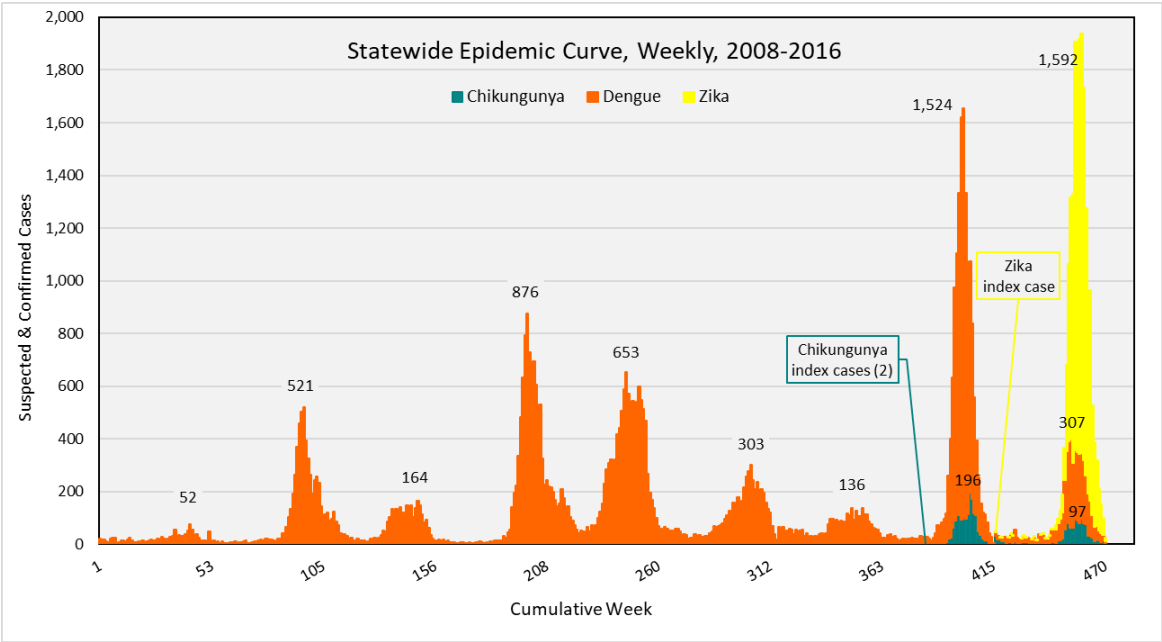

Figure 4. Statewide weekly epidemic curve for dengue, Chikungunya, and Zika from 2008-2016.

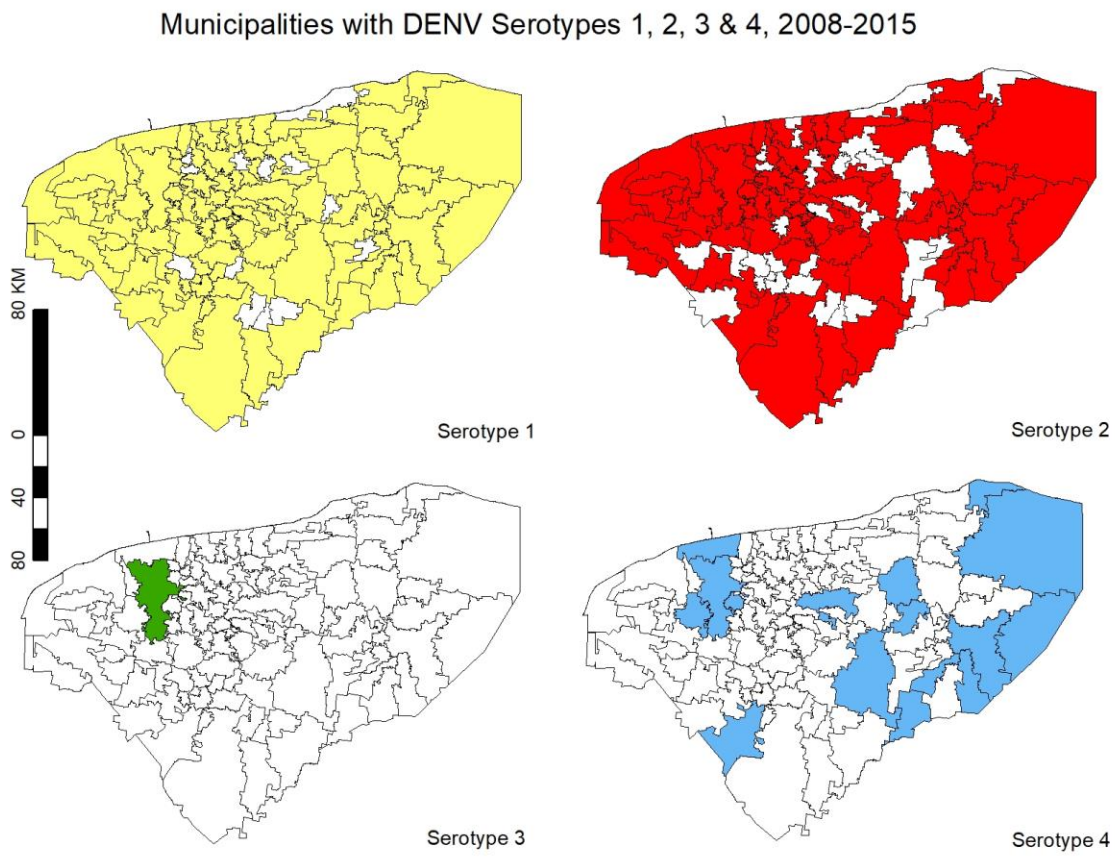

Figure 5. Municipalities with positive PCR result for DENV serotypes DENV-1, DENV-2, DENV-3, and DENV-4.

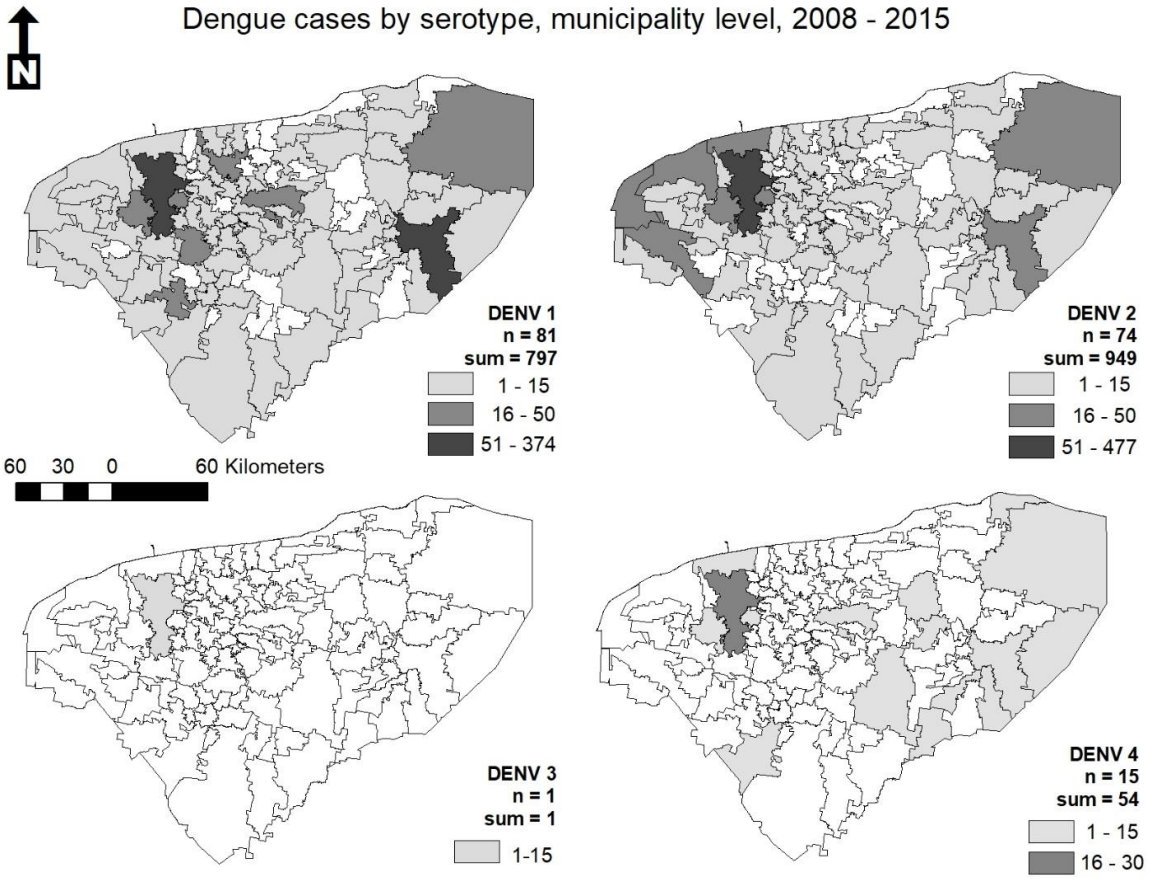

Figure 6. Number of municipalities and number of cases per municipality with positive PCR results for DENV serotypes DENV-1, DENV-2, DENV-3, and DENV-4 for 2008-2015.

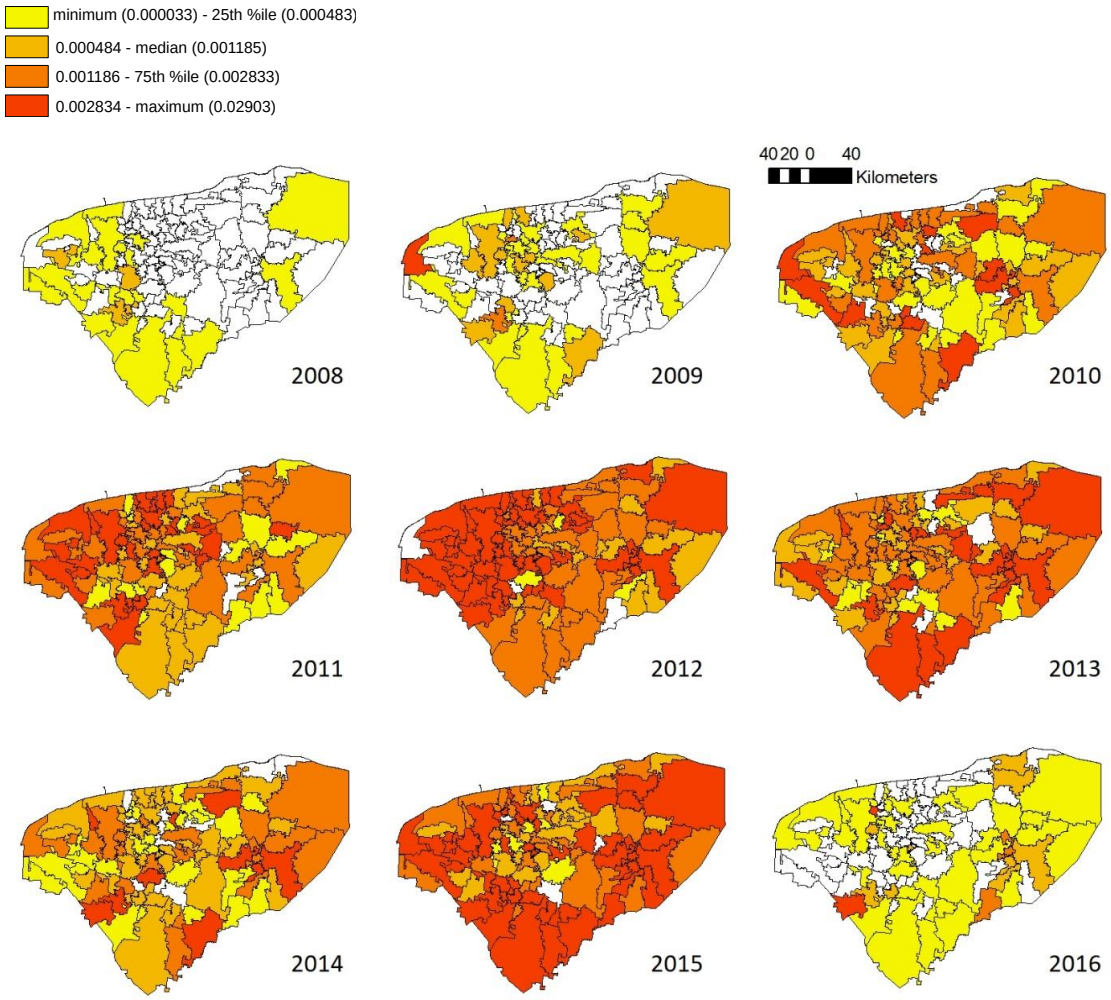

Figure 7. Annual incidence of dengue cases by municipality, 2008-2016. Annual dengue incidence for municipalities ranges from 3 per 100,000 residents to 3 per 100 residents, with median annual dengue incidence of 1 per 1,000 residents.

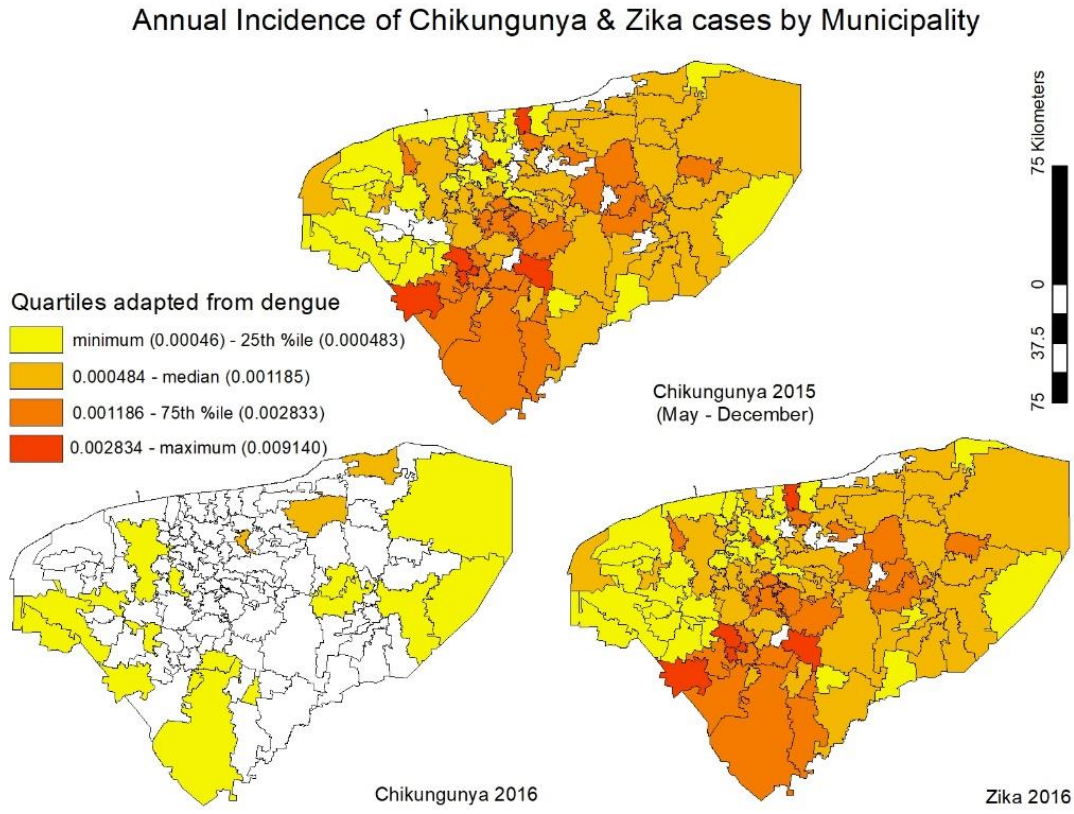

Figure 8. Annual incidence of Chikungunya and Zika cases by municipality, 2015-2016. Annual Chikungunya and Zika incidence for municipalities ranges from 4.6 per 10,000 residents to 9 per 1,000 residents. The median and 75th percentile classes from the dengue municipality map have been applied.

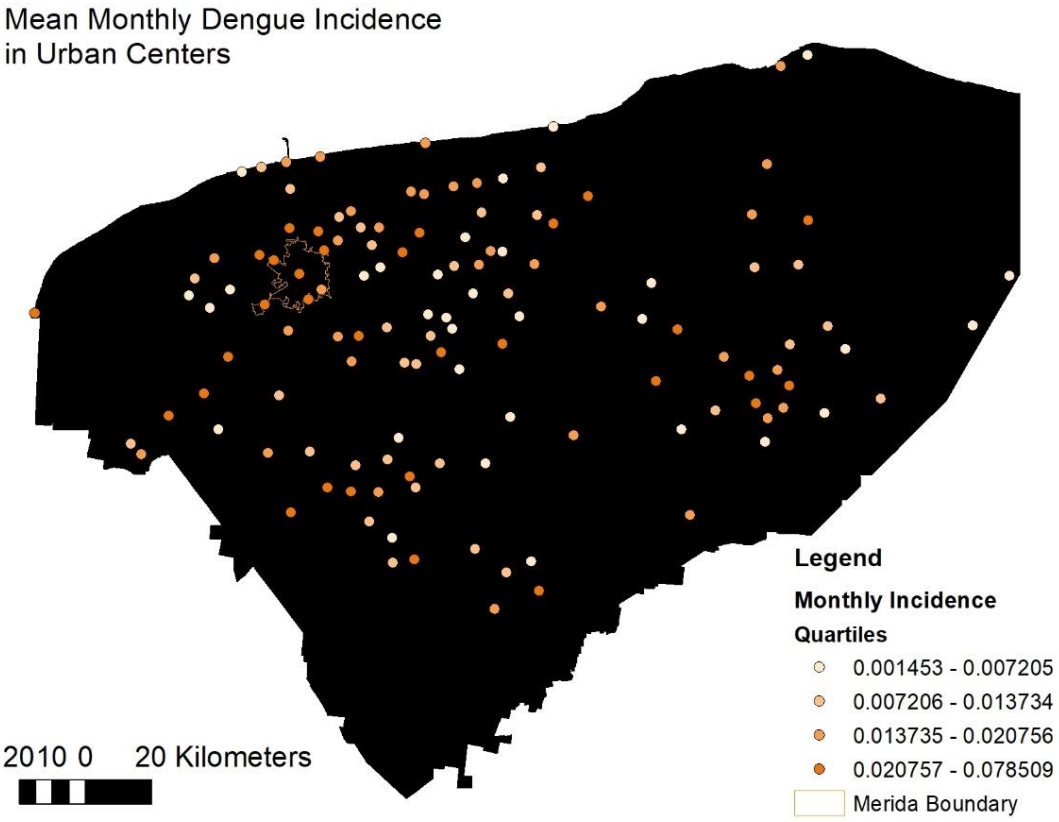

Figure 9. Mean monthly dengue incidence in urban centers from Jan 2008 - July 2016, with Mérida boundary. Monthly incidence across all cities for the whole time period ranges from 1.4 per 1,000 residents to 7.8 per 100 residents.

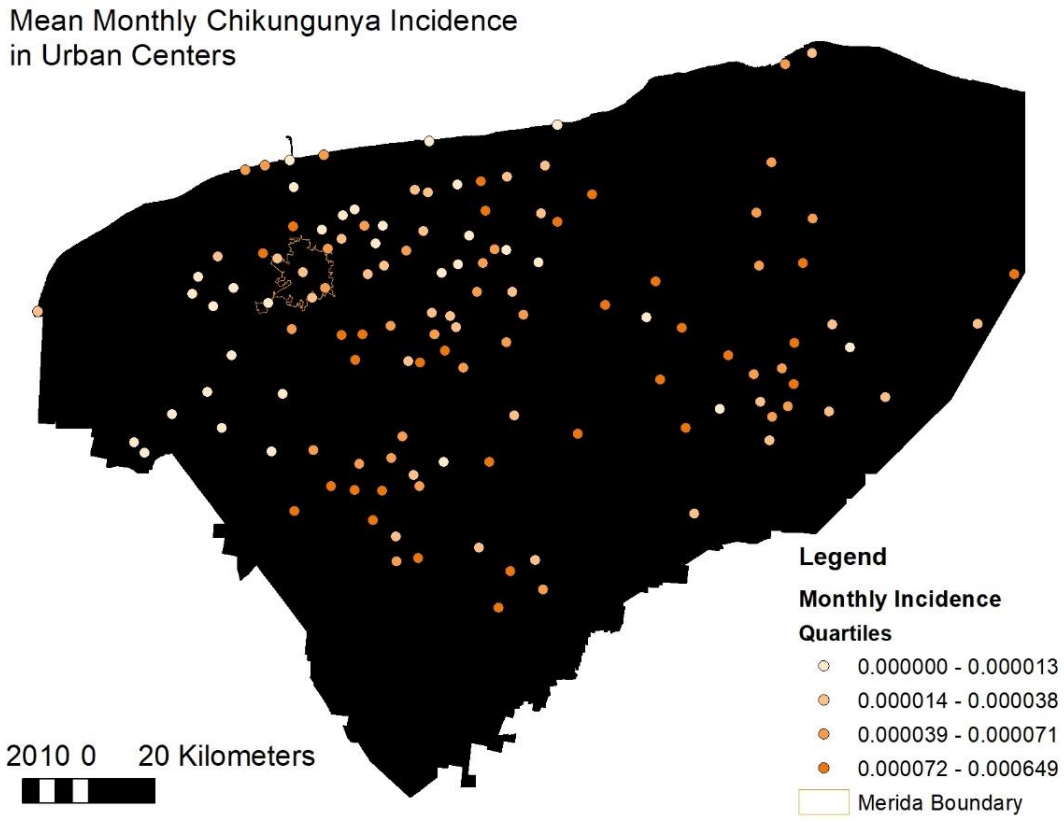

Figure 10. Mean monthly Chikungunya incidence in urban centers from May 2015 - December 2016, with Mérida boundary. Monthly incidence across all cities for the whole time period ranges from 0 to 6.5 per 10,000 residents.

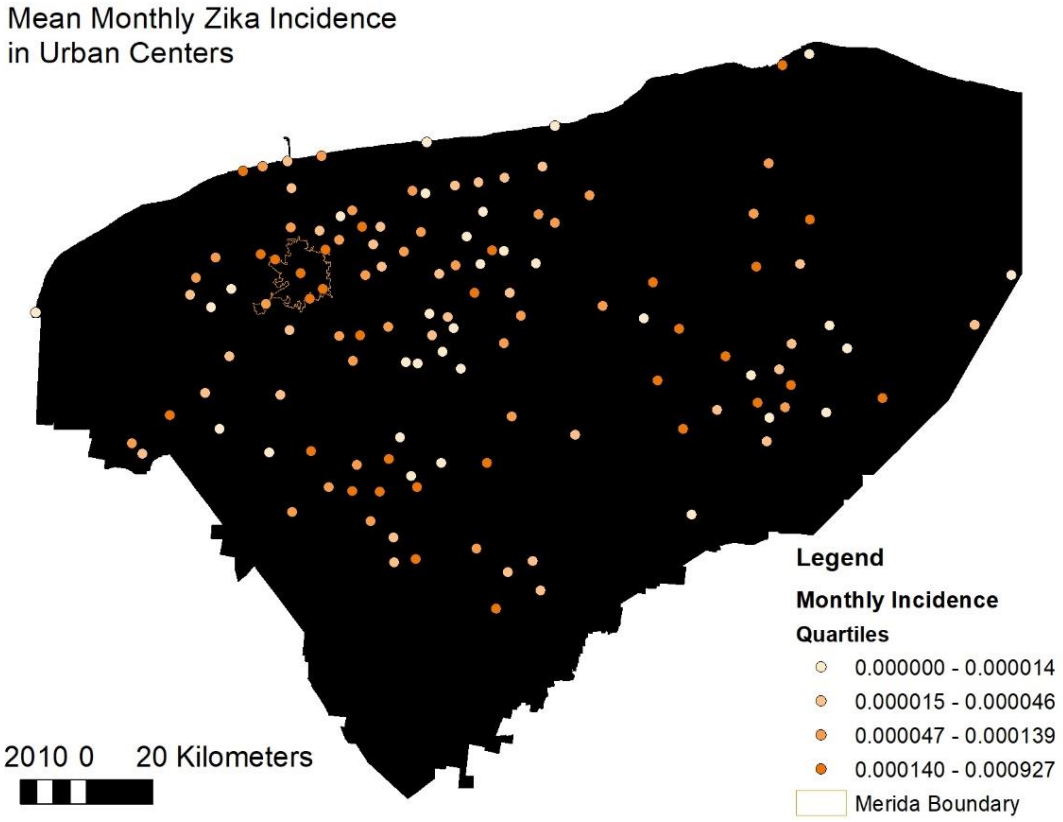

Figure 11. Mean monthly Zika incidence in urban centers from January 2016 - December 2016, with Mérida boundary. Monthly incidence across all cities for the whole time period ranges from 0 to 9.3 per 10,000 residents.

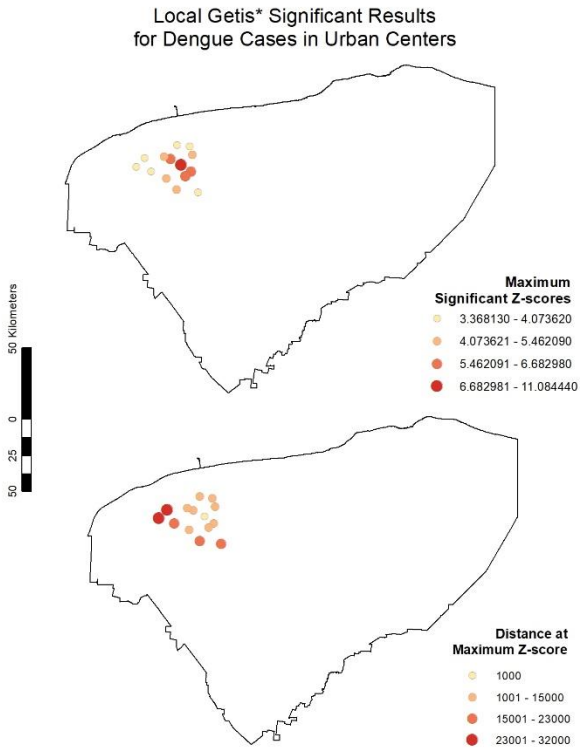

Figure 12. Urban centers identified as hot spots of dengue cases, with maximum z-scores and distances at maximum z-score for each city with a z-score larger than 3.2889.

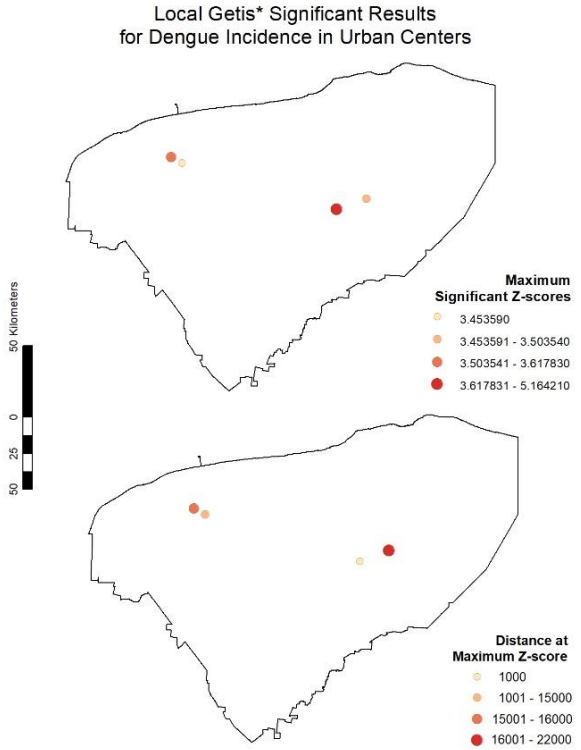

Figure 13. Urban centers identified as hot spots of dengue incidence, with maximum z-scores and distances at maximum z-score for each city with a z-score larger than 3.2889.

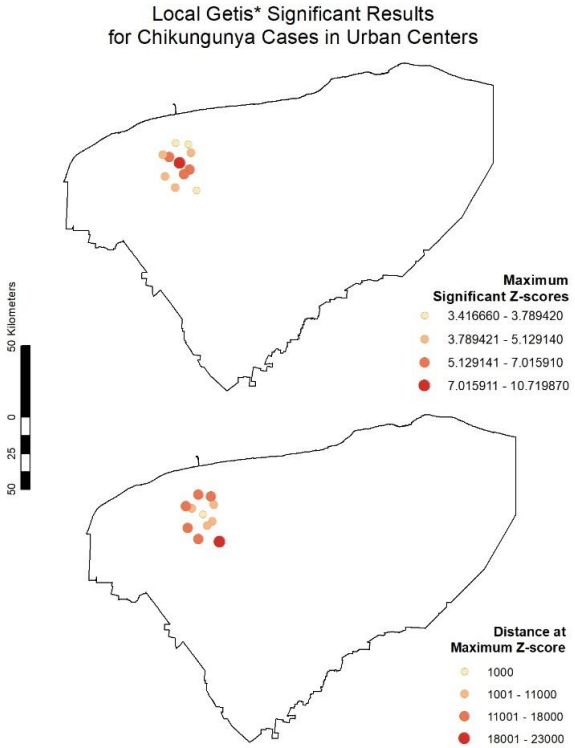

Figure 14. Urban centers identified as hot spots of Chikungunya cases, with maximum z-scores and distances at maximum z-score for each city with a z-score larger than 3.2889.

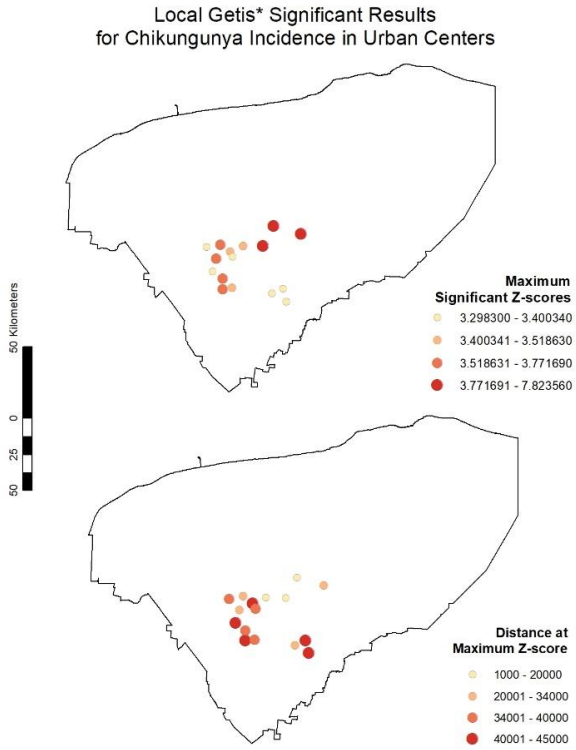

Figure 15. Urban centers identified as hot spots of Chikungunya incidence, with maximum z-scores and distances at maximum z-score for each city with a z-score larger than 3.2889.

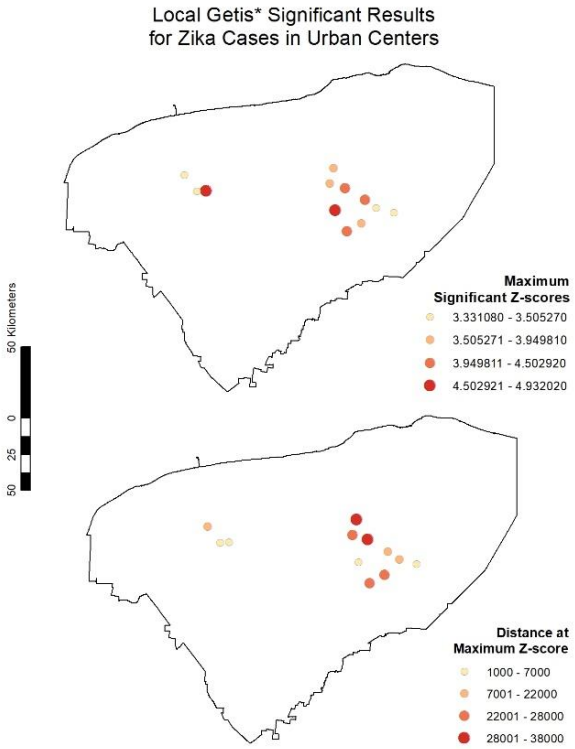

Figure 16. Urban centers identified as hot spots of Zika cases, with maximum z-scores and distances at maximum z-score for each city with a z-score larger than 3.2889.

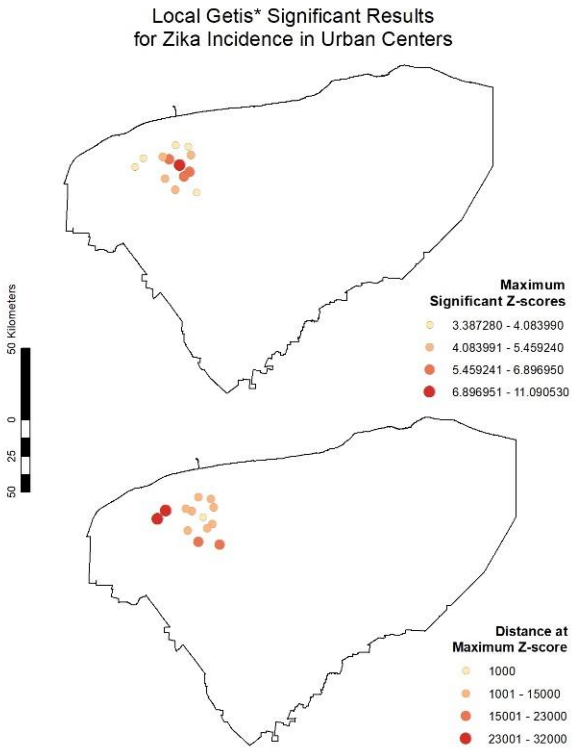

Figure 17. Urban centers identified as hot spots of Zika incidence, with maximum z-scores and distances at maximum z-score for each city with a z-score larger than 3.2889.

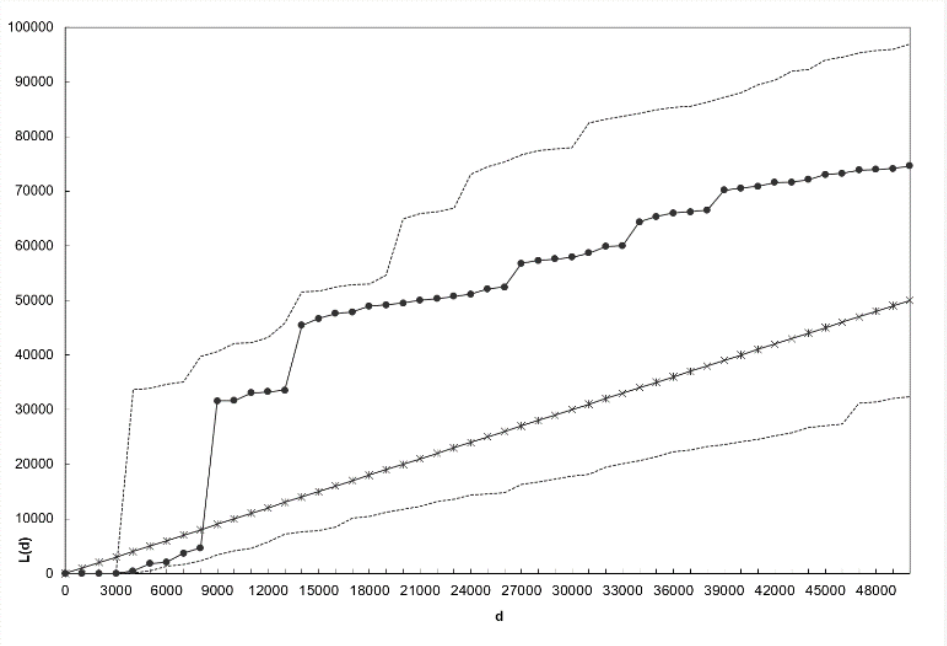

Figure 18. Dengue cases weighted k-function plot.
Plot indicates no statistically significant global clustering or dispersion pattern up to 50,00 meters

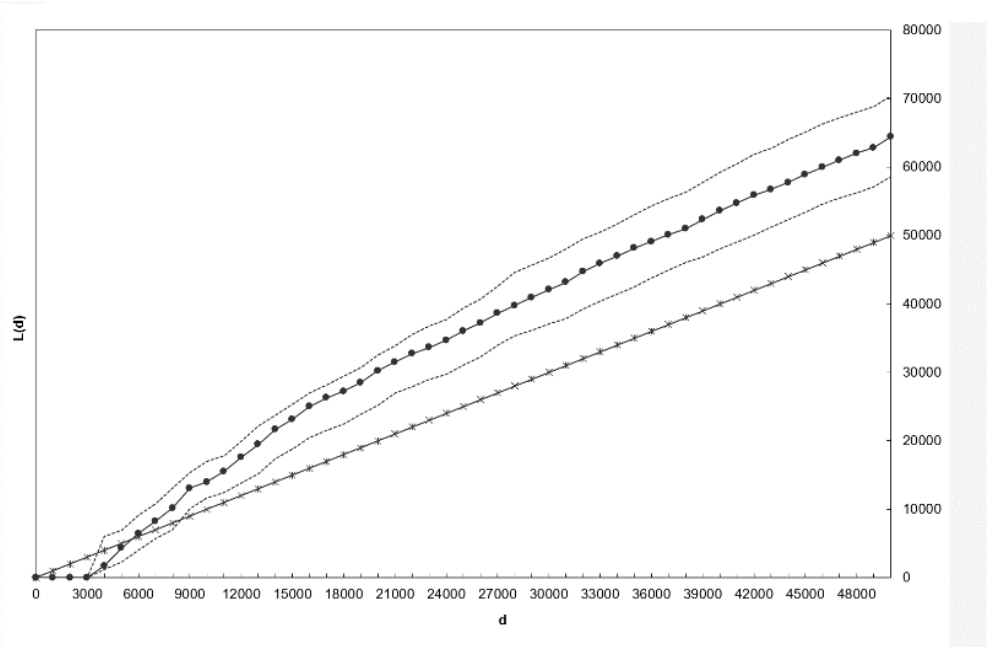

Figure 19. Dengue incidence weighted k-function plot.
Plot indicates no statistically significant global clustering or dispersion pattern up to 50,00 meters

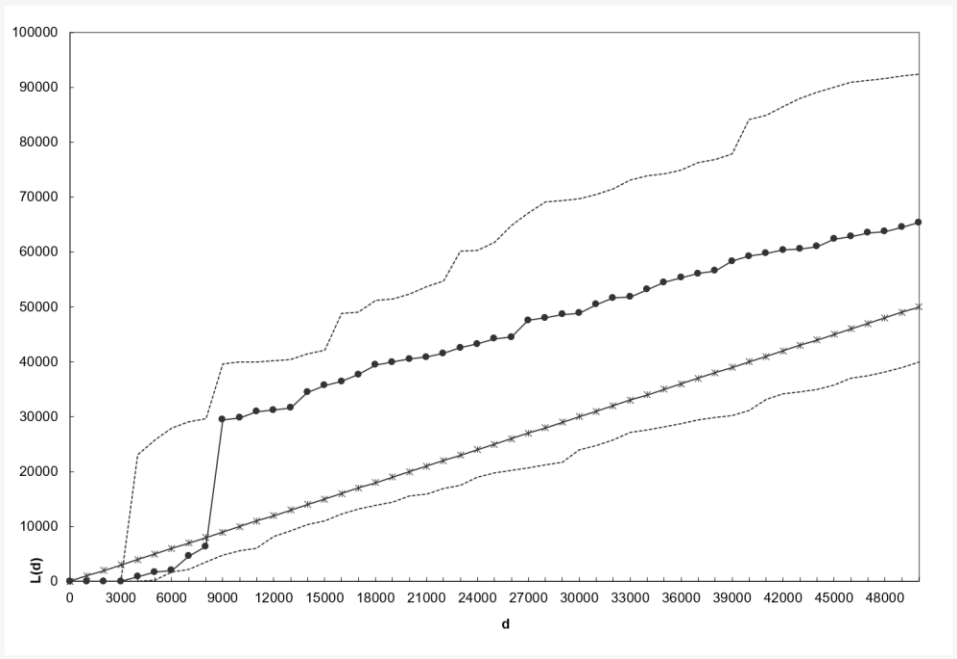

Figure 20. Chikungunya cases weighted k-function plot.
Plot indicates no statistically significant global clustering or dispersion pattern up to 50,00 meters

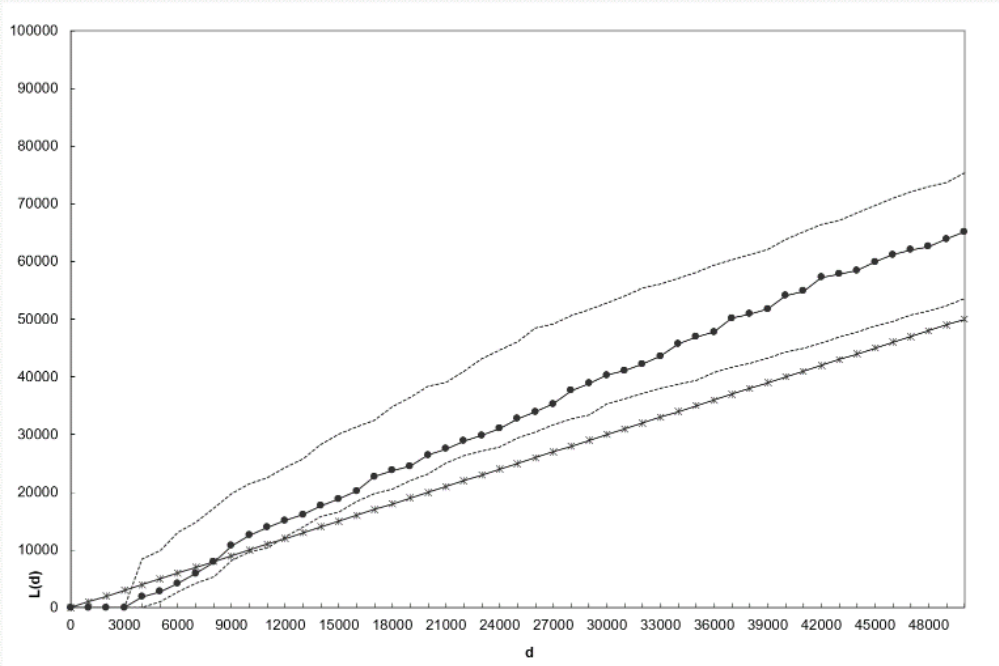

Figure 21. Chikungunya incidence weighted k-function plot.
Plot indicates no statistically significant global clustering or dispersion pattern up to 50,00 meters

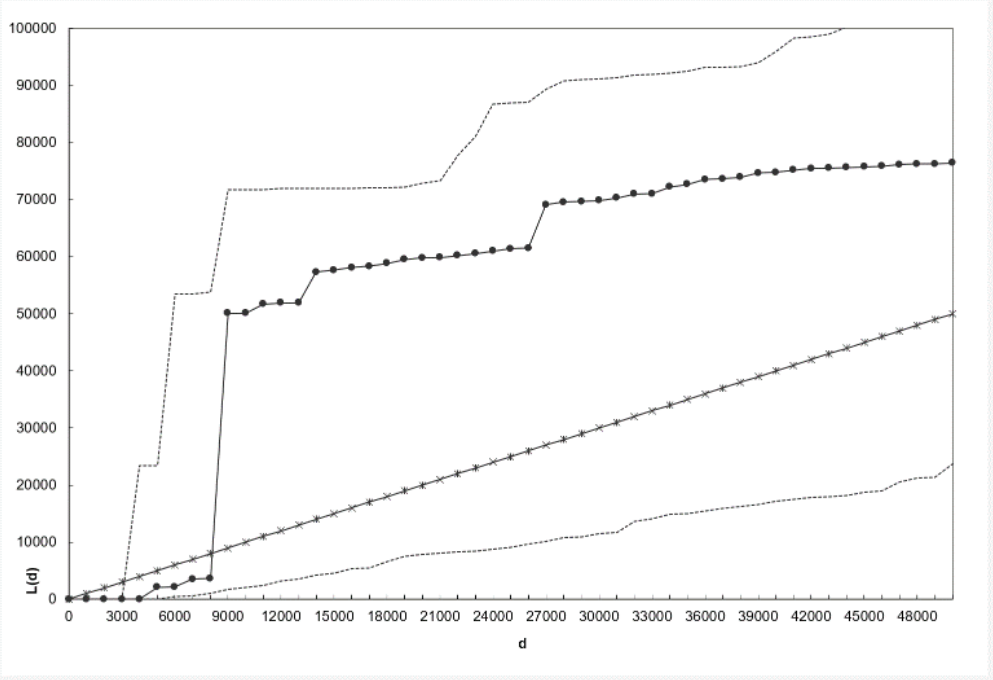

Figure 22. Zika cases weighted k-function plot.
Plot indicates no statistically significant global clustering or dispersion pattern up to 50,00 meters

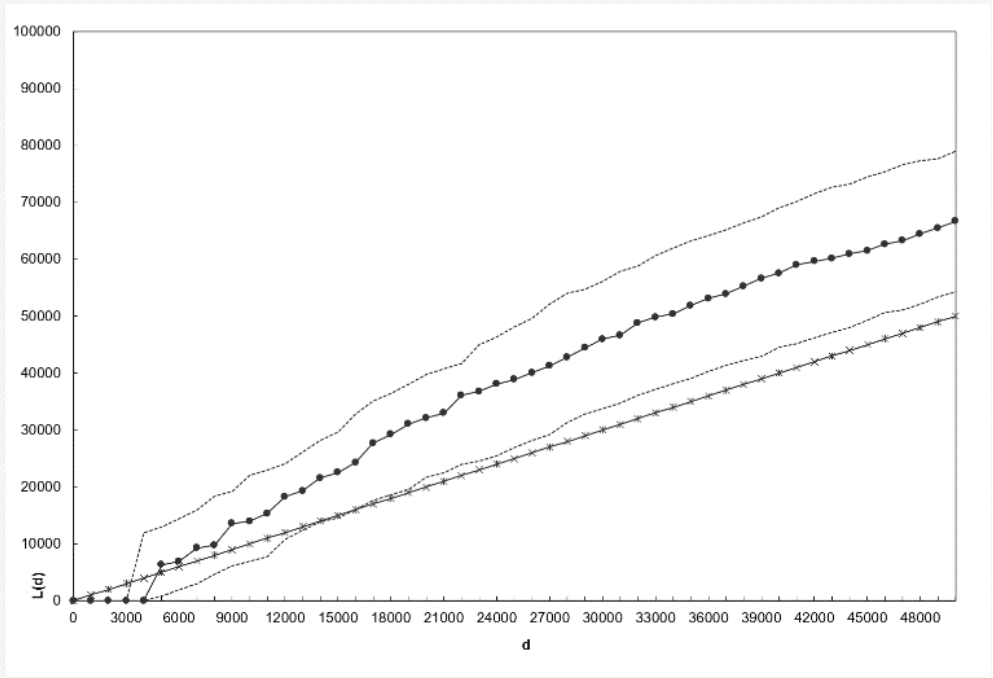

Figure 23. Zika incidence weighted k-function plot.
Plot indicates no statistically significant global clustering or dispersion pattern up to 50,00 meters