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Ekwomadu Uchechukwu Kingsley

Date: 4/22/2019

Spatial Analysis of the Environmental and Demographic Drivers of Dengue, Chikungunya and Zika  
in Natal, Brazil.

By

Ekwomadu Uchechukwu Kingsley MPharm.

Master of Public Health

Global Environmental Health

Gonzalo M. Vasquez-Prokopec, PhD, MSc.

Committee Chair

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## Abstract

### **Spatial Analysis of the Environmental and Demographic Drivers of Dengue, Chikungunya and Zika in Natal, Brazil.**

By Ekwomadu Uchechukwu Kingsley

**Background:** Dengue, Chikungunya and Zika are vector borne diseases of major public health concern with common vectors *Aedes aegypti* and *Aedes albopictus*. Prevention and vector control have been a major issue, especially in resource scarce settings. Understanding similarities in spatial pattern can provide invaluable information in decision making for stake holders efforts in management of scarce resources used in vector control and public health preparedness.

**Objective:** To identify similarities in spatial patterns of Dengue, Chikungunya and Zika in the city of Natal, Brazil and how these patterns are associated with demographic and environmental factors.

**Methods:** Spatial analytical methods such as overlay analysis, and Local Identification of Spatial Autocorrelation (LISA) were used to investigate similarities in spatial patterns of disease spread as well as relationships between areas of spread and demographic and environmental factors. Chi-square test of independence was used to test and establish relationships between clustering of disease cases and *Aedes* mosquito population using *Aedes* mosquito eggs as a proxy.

**Results:** Presence of a relationship between high population density and high number of cases was observed from overlay analysis. Identification of clusters also showed similarities in areas where cases of Dengue, Chikungunya and Zika clustered. Statically significant association was established between significant cluster of high number of *Aedes* mosquito eggs and hotspot areas of all three diseases.

**Discussion:** The results of this study provides positive indications of the possibility of predicting either Dengue, Chikungunya and Zika in areas where at least one is present and the other two are not. It also shows that conditions that promotes the spread of one can likely promote the spread, outbreak or emergence of the other two.

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## Table of Contents

|                             |     |
|-----------------------------|-----|
| Abstract .....              | i   |
| Acknowledgments.....        | iii |
| Table of Contents.....      | iv  |
| INTRODUCTION.....           | 1   |
| MATERIALS AND METHODS ..... | 13  |
| RESULTS .....               | 17  |
| DISCUSSION.....             | 22  |
| REFERENCES .....            | 24  |
| FIGURE.....                 | 28  |

## **INTRODUCTION**

Globally, vector borne diseases are a major public health concern, especially in resource lacking societies and accounting for significant mortality and morbidity rates. According to the World Health Organization “the burden of vector borne disease is highest in tropical and subtropical regions of the world and poor and/or marginalized populations are affected the most”.

A vector is a living organism capable of transmitting an infectious pathogen from one living human to another or from an animal to a living human. Infectious diseases which are transmitted from animals to humans are also known as zoonotic diseases. Vectors can also be classified as biological vectors and mechanical vectors. Biological vectors (such as mosquitoes) are capable of ingesting infectious pathogens, which multiply within their bodies as part of their life cycle before transmitting it to humans. While mechanical vectors (such as flies), can pick up infectious agents without ingesting it and transmit it to humans when they come into physical contact with them.

Most vectors are blood feeding arthropods, such as mosquitoes, ticks, triatomine bug, and fleas.

Others include aquatic snails and lice. Examples of infectious diseases transmitted by these vectors includes; Malaria, Zika, Dengue, Chikungunya, Leishmaniasis, Chagas disease and Schistosomiasis.

Globally, vector borne diseases makes up more than 17% of all infectious diseases, and are responsible for more than seven hundred thousand deaths every year. Dengue, which is one of three diseases focused on in this project, is one of the most important infectious diseases with more than 3.9 billion people within 128 countries being at risk of infection with the dengue virus and an estimated 96 million cases annually [1].

Some infectious diseases, like Malaria and Schistosomiasis, have effective treatments. While others such as Dengue, Zika and Chagas have no effective treatment, with current treatment approaches focused on management of clinical symptoms. Some challenges facing treatment includes; lack of



effective medications, asymptomatic characteristics of diseases and high probability of re-infection due to high prevalence and persistence of the disease vector. Vector control measures have the most widely used approach in mitigating the burden of most infectious diseases all over the world. But this approach is also riddled with various difficulties such as logistical cost, unintended adverse health impacts of chemicals used, and development of resistance by vectors to widely used vector control chemical agents [2].

Scientific observations have shown that the dynamics of vector distribution are not fixed but subject to change due to the influences of many factors, most of which are environmental. The reason why these factors affect the distribution of vectors can be attributed in some part to the biological design of arthropods, which are ectothermic in nature, in other words cold-blooded [3] and human behavioral change, which in turn affects human vector interaction [4].

Major environmental factors affecting global vector distribution includes; climate change, urbanization, land use and vegetation cover. It is difficult to quantify the direct impact of climate change on vector distribution due to lack of rigorous analytical methods.

Urbanization has been implicated as a major factor affecting the spread of vectors and the infectious diseases they carry. The world's population increased from 1 billion at the start of the 20<sup>th</sup> century to 6 billion by the end of the same century and it's predicted to grow by another 10 billion by 2050 [5]. In the next 25 years Africa's and Asia's urban population centers are expected to double, while a 50% increase is expected in Latin America and Caribbean urban centers [6]. This rapid urbanization trend presents a serious public health problem, especially in countries where infrastructural growth can't keep pace with urbanization. A huge number of people living in close proximity (as seen in urban centers) without necessary infrastructures such as; piped water and properly built sewer systems can create an ideal environment for the breeding of vectors and transmission of infectious diseases.

With increasing population, demand for natural resources and expansion of cities and urban centers, and agricultural needs, human activities will be forced to encroach into areas not previously habited by people. This will lead to increased deforestation, which affects land cover. Changes in land cover and land use can cause a disturbance or breakdown of the habitat of various infectious pathogen hosts, and lead to a distortion of vector-host-parasite relationship [4].

This project focuses on three important vector borne diseases; Dengue (which has been around for a longer time and poses a serious health threat to millions of people around the world), Zika and Chikungunya. These three diseases have multiple commonalities that have motivated this study. For example, the vectors *Aedes aegypti* and *Aedes albopictus* and high burden in the geographical area of Latin America amongst others.

The *Aedes* genus is made up of over 950 mosquito species, many of which act as vectors for the transmission of various serious pathogens [7]. They were originally found in tropical and temperate regions of the world, but over time they have spread to every part of the world except for the Antarctica. Human movement and activity and to some extent climatic change have been the primary reason for the spread of these mosquito species [8].

The most notable species are the *Aedes aegypti* and *Aedes albopictus* which are primarily responsible for the transmission of various debilitating illnesses such as; Dengue, Zika, Yellow fever, Chikungunya and Malaria, just to mention a few. These illnesses have had a huge health burden on a significant percentage of the world's population, making the *Aedes* specie a target of various public health efforts.

The adult *Aedes* mosquito has a distinct appearance compared to mosquitoes of other species. Characteristic features include; a narrow black body with light and dark scale patterns on the thorax and abdomen. The adult female can be differentiated from the males by the shape of their abdomen, and also their maxillary palps are shorter than their proboscis. Unlike other mosquito

species the *Aedes* mosquito are day biting mosquitoes and are active during the day, with peak biting periods of early mornings and evenings just before it gets dark [9].

The life cycle of the *Aedes* mosquito consists of egg, larva, pupa and adult. The females deposits black eggs which have thin oval shapes and are very small. A basic light microscope is sometimes needed to properly visualize these eggs. Eggs are usually laid on moist surface close to the water line in place where small water pools accumulate. Household objects, such as clay pots, buckets, used tires left outside can accumulate water and create perfect breeding sites for these mosquitoes. The flight radius of the female adult mosquito is usually within 100 meters of where eggs are laid [10] [11]. It usually takes 7 days to several weeks after eggs are laid for the adult mosquito to hatch. Mosquito eggs can also endure long periods of desiccation.

Before going into the complex pattern of the similarities between Dengue, Chikungunya and Zika, that motivated the need for this project, it will be useful to provide a fairly broad explanation of these three diseases in order to create a good foundation in understanding the aim of this study.

## **Dengue**

Dengue fever is endemic in tropical and subtropical regions of the world (Latin America, Southeast Asia, Africa and the Pacific Islands) and infection is more prevalent in urban environments [12]. This is most likely due to the peri-domestic characteristics of the *Aedes aegypti* mosquito (primary vector). It can also be transmitted by the *Aedes albopictus* mosquito. These two mosquito species will be discussed in more details later. However, with many parts of the world decreasing in their rural set up and moving towards more urban city style, there has been increase in mobility of people which has enabled the circulation of the dengue virus and hence increased number of epidemics. Cases are usually high when the mosquito population (*Aedes specie*) is increased, mostly during increased rainfall. This is because of the optimal conditions created, that supports mosquito breeding. Also the exponential increase in dengue rates that have been witnessed between 1960

and 2010 have been attributed to a combination of urbanization, global warming, population growth and increased international travel [11].

Ever since the introduction of Dengue, regions infested with the *Aedes aegypti* have suffered their share of epidemics. In the Americas, dengue was first introduced within 1600 to 1946 [13].

There is also high risk of recurrent epidemics in endemic areas, especially when an increased number of individuals get infected within a common area and within a short period of time. This risk is even higher when there is a significant lack of immunity to any of the virus in the same area. In endemic countries, like Mexico, Brazil and many other Latin American countries immunity to either one or all of these viruses is common.

In Latin America Dengue has adopted a pattern of cyclical peaks, with high and low incidence periods. This cycle tends to happen every 3 to 4 years with a reduced incidence level of dengue followed immediately by a 2 to 3 years' period of high incidence levels. In the last decade incidence peaks have had an upward trend [14].

Dengue is thought to have originated in monkeys and moved to humans in Africa and Southeast Asia over 100 years ago. Today, according to the Center for Disease Control and Prevention (CDC) "about 2.5 billion people (up to 40% of global population) are living in areas where there is a risk of dengue transmission". And the World Health Organization estimates that "50 to 100 million infections occur every year, with about 22,000 deaths with majority being in children".

Brazil and many Latin American countries had been free of *Aedes aegypti* (main dengue vector) until 1976 due to a successful vector eradication program aimed at preventing yellow fever, which is also transmitted by *Aedes aegypti*. This successful effort was coordinated by the Pan American Health Organization. However, efforts were not made to maintain this success. This led to re-

infestation of the *Aedes aegypti* mosquito and subsequent reemergence of dengue virus in 1986 [15].

Dengue fever is a mosquito-borne illness, characterized by sudden onset of elevated body temperature (fever), vomiting, joint and muscle pain and a characteristic skin rash. The clinical signs of Dengue fever usually begin three to fourteen days after infection occurs. Patients who are infected usually recover within two to seven days. However, in a small percentage of cases the disease progresses more, leading to severe clinical outcomes such as; hemorrhagic fever, low levels of blood platelets, blood plasma and life threatening low blood pressure levels in a medical condition known as Dengue Shock Syndrome.

The transmission of Dengue is largely dependent on the *Aedes* mosquito species (*Aedes aegypti* and *Aedes albopictus*). However, transmission is much less dependent on the *Aedes albopictus* specie. The *Aedes* mosquito is very well adapted to the urban environment [15]. This vector will be discussed in more details later.

The pathogenic agent of the Dengue fever is the Dengue virus (DENV), which is spread mainly by the *Aedes aegypti* mosquito [15]. The dengue virus is a single positive-stranded RNA virus belonging to the family *Flaviridae* and genus *Flavivirus* [16]. So far, there are 5 identified types of this virus, usually referred to as; DENV-1, DENV-2, DENV-3, DENV-4 and DENV-5 [17]. Each of these serotype have the potential to cause Dengue fever. A patient who has been infected with any of the serotype acquires lifelong immunity to reinfection of that same serotype [18] and provides no immunity to any other serotype. However, it is important to state that infection with a serotype after a being previously infected with different serotype can lead to dangerous clinical complications in the manifestation of the disease.

Dengue fever can be diagnosed using microbiological laboratory testing, such as virus culture, polymerase-chain reaction (PCR) or serologic assays. However, many times in endemic areas,

laboratory diagnostic methods are not always readily available and physicians may have to rely on a combination of travel history and clinical symptoms for preliminary diagnosis [15].

There are currently no vaccines and antiviral medication available for the prevention and treatment of Dengue. The only method used for prevention is vector control which has been regarded by many as expensive and ineffective and poses a lot of logistical challenges in implementation, especially in an urban setting [19].

Vector control approaches commonly used includes; eliminating breeding sites for vectors, spraying of insecticide (organophosphate and pyrethroids), ultra-low volume insecticide application, larviciding and preventing bites by use of mosquito bed nets, insect repellent etc.

Some of the challenges with currently practiced measures as stated earlier includes; the health impact of human exposure to insecticides, logistics and cost of residual spraying (especially in urban areas), human behavior in areas with poor infrastructure and socio economic status, environmental factors such as temperature and rainfall that affect the dynamics of vector spread. All these, have posed big challenges in scaling up vector control interventions.

### **Chikungunya**

Chikungunya fever is a viral disease caused by the Chikungunya virus (CHIKV). As stated earlier, this virus is also transmitted primarily by the *Aedes aegypti* and *Aedes albopictus* mosquitos.

Chikungunya has been around for a much shorter time than Dengue. According to the World Health Organization “the disease was first described during an outbreak in southern Tanzania in 1952, and also got its name from the local Kimakonde dialect meaning “to become contorted”; a description of the stooped posture taken by patients with severe muscle pain (a common clinical symptom of this disease)”.

Since then, confirmed and suspected cases of Chikungunya have been reported in Asia, other African countries, the Indian subcontinent, the Americas and Europe. According to the Pan American Health Organization, “since 2004 continuous outbreaks have been occurring in Asia and Africa, infecting over 2 million people with attack rates as high as 68% in some areas” and “in 2013 confirmation was received on the first autochthonous transmission of Chikungunya in the Americas, before that, people from the Americas who have travelled to Asia and Africa have become infected with Chikungunya”.

The major risk factor for contacting the virus is proximity of households or other living areas to breeding sites of the *Aedes* mosquito. The *Aedes albopictus* mosquito is more responsible for exposure in Europe, due to their ability to persist in temperate climate and their recent invasion in the European continent [20]. Importation or migration from high prevalent areas is also responsible for some cases seen in Europe.

The incubation period of the virus before onset of symptoms usually takes 2 – 12 days. Clinical symptoms includes mainly; sudden fever, generalized body pain, joint pain and in some cases rash, headache and swelling of the joints. In most patient symptoms resolves within a week, while in immune-compromised patients (the elderly, children, pregnant women, hypertensive and diabetics) the disease could lead to more severe outcomes.

The Chikungunya Virus (CHIKV) belongs to the genus *alphavirus* and the family *togaviridae* [21]. It is a positive single-stranded RNA virus sensitive to temperatures above 58 degrees Celsius. The virus is closely related to many other alphaviruses, most of which are known to cause arthritis [23]. Three genotypes of the virus have been described, which includes; West African, East Central, and Asian which are all named based on their geographical distribution [24].

Patients are usually misdiagnosed in areas where Dengue and Zika are also prevalent. However, one distinguishing symptom for Chikungunya is the severe joint pain, which is not usually present

in these other diseases. More sophisticated methods such as enzyme-linked immunosorbent assays (ELISA) and reverse transcriptase-polymerase chain reaction (RT-PCR) can be used to reach more accurate diagnosis.

There are no commercially available medications for treatment or vaccines for prevention of Chikungunya. Current treatment approaches are focused on management of symptoms, for example using Non-Steroidal Anti Inflammatory drugs for the pain and swelling, fluids for hydration and Anti-Pyretic for the fever [24]. Vector control through the use of insecticides, larvicides and elimination of breeding places are the current prevention approaches being practiced.

### **Zika**

Zika fever disease is an infection caused by the Zika virus (ZIKV), which is also primarily transmitted by the *Aedes aegypti* and *Aedes albopictus* mosquitoes [25]. This disease is named after the Zika forest in Uganda [26].

Zika was known to be restricted to the African and Asian continents in the 1950s with spread to the Americas being more recent (in the 2000s) [27]. Even though the disease was discovered in Uganda, human cases were first reported in Nigeria in 1954 [26].

Since its discovery, there have been a few outbreaks of Zika in Asia and Africa. However, the first major outbreak was reported in 2007 in the Yap islands of the Federated States of Micronesia. This outbreak had 108 confirmed cases using Polymerase Chain Reaction (PCR) and serology [28]. This outbreak was also the first reported outbreak of Zika fever outside of Asia and Africa [29].

In August of 2014 medical doctors in Natal, Brazil started investigating an outbreak of a peculiar illness. Patients presented with fever, joint pain, and conjunctivitis amongst others. The illness also spread quickly to three other states. This was initially thought to be dengue, given that such clinical



symptoms were also seen in dengue cases. However, dengue was later ruled out and Zika fever was confirmed to be the disease using RT-PCR [30].

Despite all these, the emergence of Zika virus in Latin America didn't get much attention until early 2015 when there was a widespread epidemic of Zika fever, which started to spread northwards and more importantly, pregnant mothers were giving birth to babies with microcephaly; a serious congenital defect due to the virus' ability to cross the placenta and harm the fetus [31] [32] and rapid onset of muscle weakness due to damage of the peripheral nervous system, in what is referred to as Guillain-Barre syndrome [23].

These events led to the World Health organization declaring the outbreak as a Public Health Emergency of international concern [33] [34].

Scientific researchers mostly agree that Zika virus was brought to Brazil and Latin America by infected travelers from French Polynesia. This hypothesis has been supported by phylogenetic analysis of the virus in the first cases in Brazil, which strongly indicated that the circulating strain is much similar to the Asian strain rather than the strains found in Africa [35] [36]. Other research papers have also suggested the process of Zika spread from Africa and Asia to Oceania and to the Americas as resembling that of Dengue and Chikungunya [35].

Individuals infected with the Zika virus usually don't present with clinical symptoms and may be unaware that they are infected. The common clinical symptoms include; fever, rash, headache, joint pain, conjunctivitis and muscle pain [35]. Clinical symptoms usually manifest within 3 -14 days after infection and resolves within a week. Serious symptoms can arise in a few patients especially pregnant women. Such clinical symptoms include; microcephaly (fetus in pregnant women), Guillain-Barre syndrome, other congenital abnormalities [36].

The major concerns amongst physicians and other public health professionals with Zika virus is the severity of clinical complications and being able to be transmitted through sexual intercourse from men who may not even know they are infected to their partners [36]. Medical investigations have shown that the semen of infected men contains high amount of virus compared to blood or urine. Because of this, the Center for Disease Prevention and Control and the World Health Organization recommends that all men who travel to areas affected by the Zika virus should abstain from sex for 6 months before trying to conceive a child with their partner regardless of any absence of clinical symptoms [37][38]. There is currently no evidence that women can transmit the virus to men [39].

The Zika virus is a non-segmented single-stranded *flavivirus*, which makes it related to the dengue [40].

Differential diagnosis of Zika can be difficult due to a broad overlap of symptoms with Dengue and other arboviruses, especially in areas where other diseases with similar symptoms are endemic [41]. The use of RT-PCR is the most effective and accurate way to diagnose Zika. However, testing in ill patients needs to be done 1-3 days of symptoms onset if serum samples are used for testing, 1-5 days for saliva samples and up to 14 days for urine samples [42][43]. The short window for serum sample is due to the short viremia exhibited by the virus [44]. The Center for Disease Control and Prevention recommends that “pregnant women and infant be screened regularly if they are suspected to be exposed, regardless of the presence or absence of symptoms” [45].

Just like Dengue and Chikungunya, there is no commercially available medication or vaccine for Zika. Current treatment approaches are focused on supportive care via alleviating symptoms. Current prevention approaches are the same as Dengue and Chikungunya, giving they are spread by the same vector *Aedes mosquitoes* [42].

The approaches used in this project will provide more insight into the spatial distribution of Dengue, Chikungunya and Zika. This will provide information for adequate approaches in targeted

vector controls, especially in the hotspots areas, where all three diseases are endemic [47] [48]. More importantly, understanding similarities in environmental factors between the three diseases will help in informing proactive measures in preventing the emergence of any of the diseases (especially Zika and Chikungunya) in areas, where only one or two of the three diseases are endemic.

Other scientific papers such as Silver, J. et al. have also attempted to study the effects of environmental factors on dengue in Natal [46]. However, certain challenges still exist with a study like this, for example; challenges of clearly identifying where hotspots might exist, due to lack of clarity in the definition of what a hotspot is [48].

There for this thesis aims at looking at spatial patterns of Dengue, Zika and Chikungunya cases, taking into account environmental and demographic factors such as level of *Aedes* mosquito infestation, human population density and human population, and number of houses in order to look at the similarities in these patterns for all three diseases and how these environmental and demographic factors affect them.

## **MATERIALS AND METHODS**

### **Study Site**

Natal is the capital of the northeastern Brazilian State of Rio Grande do Norte (*shown in figure 1*) and home to over 800 thousand people according to 2010 census data. It has a population density of 4,808.20 persons per kilometer square [46].

This beautiful city also prides its self as a destination for tourists, with tourism being a lifeline of its economy, which also makes it significant in the spread of vector borne diseases and propagation of disease vectors.

The area has a typical tropical climate (according to Koppen climate classification system), with average high temperatures of 30 degrees Celsius and low temperatures of 23 degrees Celsius and relatively high humidity all year round.

In recent years natal has been a hotbed for Zika, Dengue and Chikungunya. Before the confirmation of the 2015-2016 outbreak of Zika, cases were first reported by physicians in Natal as early as 2014 [30].

### **Data Collection and Analysis**

Neighborhood shape files and geocoded epidemiological data of laboratory confirmed cases of DENV, CHIKV and ZIKV were obtained from the Ministry of Health of Rio Grande do Norte State, as part of a NSF-funded project. Sub district shapefiles of Natal was downloaded from ArcGIS online and Census data was downloaded from the Instituto Brasileiro de Geografia e Estatística online. Demographic information was also obtained from the census data such as; the number of houses per census tract, population per census tract and population density per census tract.

Data on the average number of eggs in each census tract was collected by placing ovitraps at different locations in each census tract mainly in areas of human dwelling. The eggs of this traps were collected weekly for the year 2016.

The census tract shape files were projected on ArcMap version 10.6 and spatially joined with geocoded epidemiological data of cases for each disease (DENV, CHIKV and ZIKV). This was used to quantify the cases per census tract.

### **Case Distribution**

ArcMap 10.6 was used to make choropleth maps of DENV, CHIKV and ZIKV case distribution per census tract to access the areas of the city with more cases and areas with less cases of all three diseases.

### **Overlay Analysis**

Overlay analysis was performed using ArcMap 10.6 to overlap the number of DENV, CHIKV and ZIKV cases per census tract represented in a dot density with the population density represented in graduated colors. This was done to assess the relationship between population density and level of disease occurrence per census tract and how they differ for all three diseases.

Other relationships investigated using the overlay analysis methods includes;

- a. 2016 Total number of *Aedes* mosquito eggs per ovitrap location with population density.
- b. 2016 total number of *Aedes* mosquito eggs per ovitrap location with cases distribution of ZIKV
- c. 2016 total number of *Aedes* mosquito eggs per ovitrap location with cases distribution of DENV

- d. 2016 total number of *Aedes* mosquito eggs per ovitrap location with cases distribution of CHIKV

### **Clustering**

The ArcMap version 10.6 software, was also used in conducting to a point pattern analysis, with the  $G^*(d)$  method to identify local indication of spatial association (LISA) of disease cases for DENV, CHIKV and ZIKV. The  $G^*(d)$  method evaluates the spatial location of the disease cases and compares it to multiple scenarios of Complete Spatial Randomness (CSR) to determine areas where clusters exist within a given radius. This radius (3000 meters) is predetermined by calculating one third of the shortest possible Euclidean distance from east to west. Areas with high number of cases next to each other can be considered as hotspots while areas of low number of cases next to each other can be considered as cold spots. The  $G^*(d)$  method was used in investigating clusters for Dengue, Chikungunya and Zika cases.

Clusters were also determined for high 2016 total number of eggs per ovitrap, using the same predetermined radius of 3000 meters. This was done to identify areas where the ovitraps had high number of total eggs collected.

The hotspots and cold spots are also categorized into different confidence intervals from (90%, 95%, 99%), depending on how significant the clusters are.

The cluster maps generated were used to investigate the differences in the case clusters between the three diseases.

These maps were also used to investigate the association between hotspots for Dengue, Chikungunya, Zika cases and areas with significant clusters of high number of total *Aedes* eggs collected. This was done by categorizing significant and non-significant clusters of eggs into binary values of 1's and 0's (where 1 = significant and 0 = non-significant clusters). The same was done for

hotspots of cases and non-hotspots of cases for each disease (Dengue, Chikungunya and Zika) (where 1 = hotspots and 0 = non-hotspots). Then the association between the clusters of *Aedes* mosquito eggs and each of the three diseases was tested using a Chi Square test of independence. This was done using the statistical analysis software SAS version 9.4.

## RESULTS

### Results from ArcMap analysis

#### Case Distribution

The distribution of DENV, CHIKV and ZIKV appears to follow a similar pattern in their occurrence. This corresponds to the fact that they share primarily the same disease vector *Aedes aegypti* and *Aedes Albopictus*. However, cases of dengue have a wider spread with majority of the census tracts having at least 1 case of Dengue (**Figure 3**). A similar pattern can also be observed with cases of Chikungunya (**Figure 4**). However, the same spread wasn't observed for Zika cases. Zika cases were more on the west as compared to the eastern area of the city (**Figure 5**).

#### Relationship between Case Distribution and Demographic Factors

The investigation into the relationship between the spatial distributions of cases of DENV, CHIKV, ZIKV and other demographic variables (Number of houses, population density) shows a relationship between population density and cases distribution. Higher number of cases (for all three diseases) occurring in areas of high human population density, as shown in (**Figures 6 – 8**).

There was no clear relationship between number of houses and cases distributions for (DENV, CHIKV and ZIKV), (**as shown in Figure 9-11**). Cases of all three disease are seen in census tracts with high number of houses; like in the north eastern and central areas of the city, while in the south western and some north eastern census tracts have less or no cases even though the number of houses are high.

#### Association between number of *Aedes* mosquito eggs and population density

Overlay analysis of total number of *Aedes* mosquito eggs in 2016 with population density (**Figure 12**), shows that ovitraps in areas of higher population density tend to have more eggs compared to



areas of lower population density. Interestingly, the ovitraps across the western part of the city had more eggs than the ovitraps in other area of the city.

### **Association between number of *Aedes* mosquito eggs and cases distribution.**

Overlay analysis of these two variables shows an association between the total number of eggs in 2016 and the distribution of cases. The higher number of eggs on the western area of the city as we observe (**Figures 13 -15**) happens to correspond with areas of high local clusters of disease cases for DENV, CHIKV and ZIKV as will be seen later.

### **Cluster maps for DENV, CHIKV and ZIKV cases.**

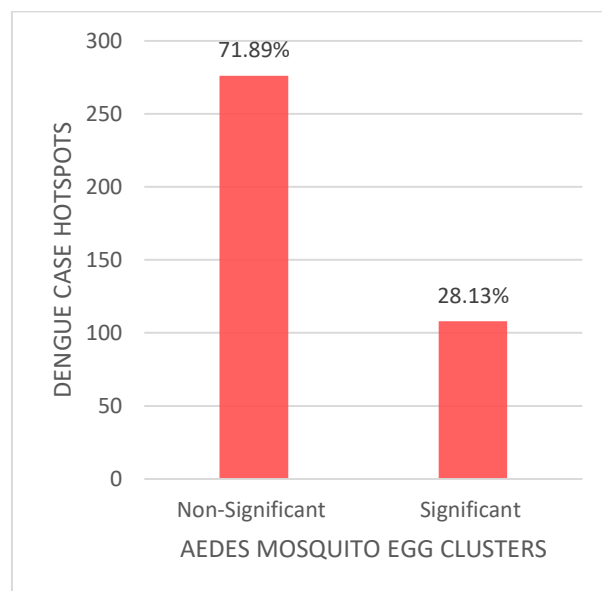
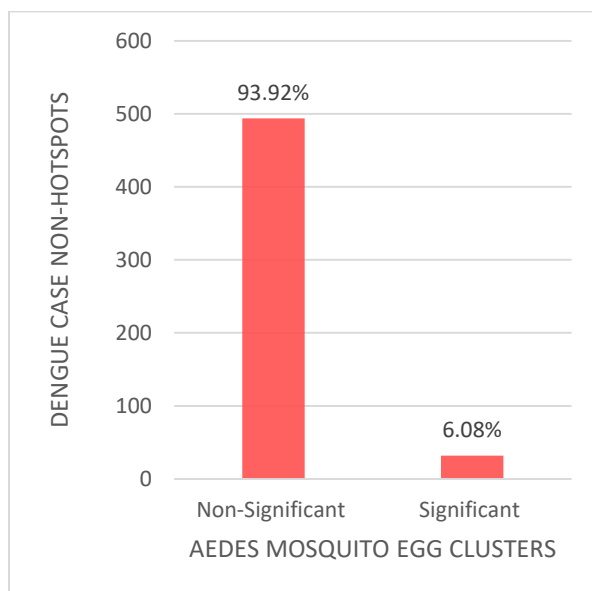
The cluster maps shows area of high disease cluster (hotspots) and areas of low disease clusters (cold spots) the red census tracts are areas of significant cluster and the blue census tracts are areas of no significant cluster. From the cluster of maps of cases (**16 - 18**) for the three diseases we observe that most of the hotspots are on the western area of the city and the most of the cold spots are on the eastern area of the city. The concentration of hotspots on the western area of the city corresponds to higher number of totals eggs seen in 2016 and it also corresponds to a large extent with areas of high population density.

### **Association between cluster of *Aedes* mosquito eggs and hotspots of disease cases.**

For this analysis we established an association between areas where significant cluster of *Aedes* mosquito eggs and areas of disease hotspots for all three disease. These associations were statistically significant according to the p-values of the Chi-square test of independence, as shown in the results below:

## Association of *Aedes* Mosquito Egg Clusters with Dengue Case Hotspots

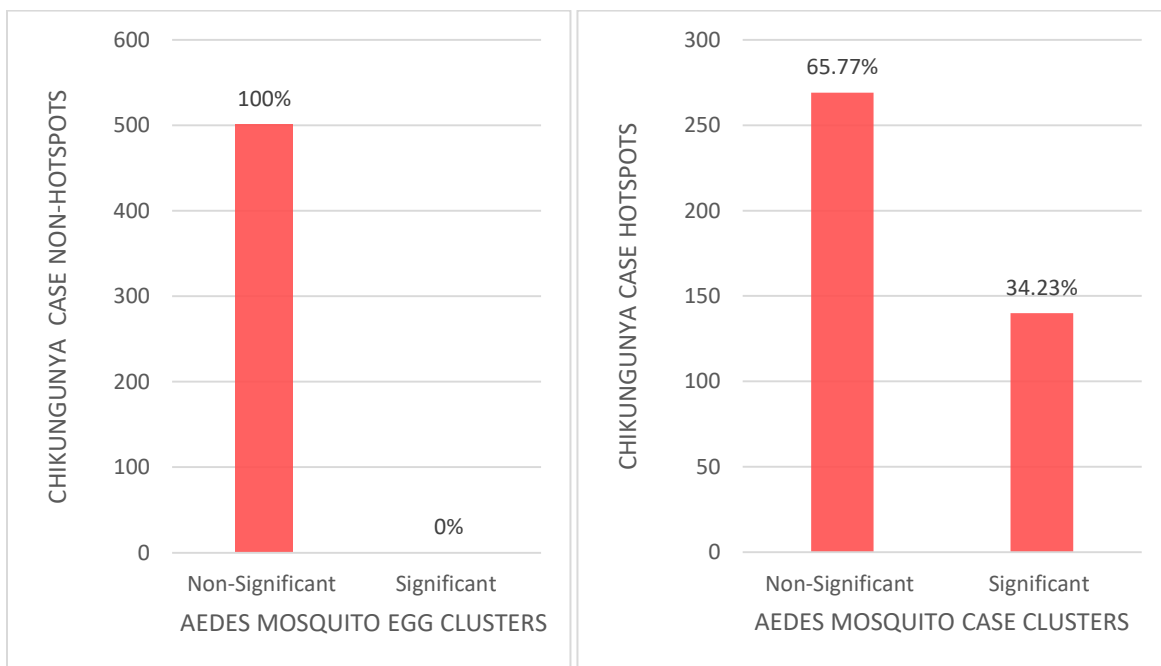
| Test Statistics | Value   | P-Value |
|-----------------|---------|---------|
| Chi-Square      | 82.8354 | <.0001  |



From the above graphs, in non-hotspot areas of Dengue cases, we have only 6.08% of significant clusters of *Aedes* mosquito eggs, while in areas with hotspots of Dengue cases, there are 28.13% of significant cluster of *Aedes* mosquito eggs.

## Association of *Aedes* Mosquito Egg Clusters with Chikungunya Case Hotspots

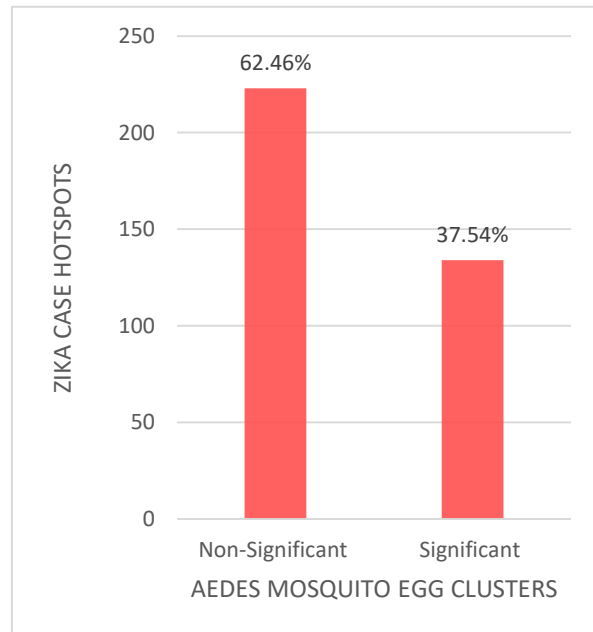
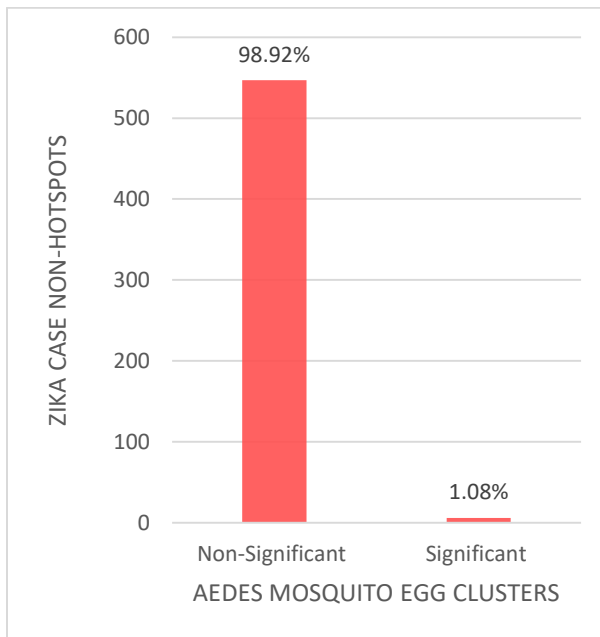
| Test Statistics   | Value           | P-Value          |
|-------------------|-----------------|------------------|
| <b>Chi-Square</b> | <b>202.6717</b> | <b>&lt;.0001</b> |



From the above graphs, in non-hotspot areas of Chikungunya cases, we have no of significant clusters of *Aedes* mosquito eggs, while in areas with hotspots of Chikungunya cases, there are 34.23% of significant cluster of *Aedes* mosquito eggs.

## Association of *Aedes* Mosquito Egg Clusters with Zika Case Hotspots

| Test Statistics | Value    | P-Value |
|-----------------|----------|---------|
| Chi-Square      | 221.4173 | <.0001  |



From the above graphs, in non-hotspot areas of Zika cases, we have only 1.08% of significant clusters of *Aedes* mosquito eggs, while in areas with hotspots of Chikungunya cases, there are 37.54% of significant cluster of *Aedes* mosquito eggs.

The above results shows that the presence of significant clusters of *Aedes* mosquito eggs is more related to the presence of cases hotspots than case non-hotspots.

## DISCUSSION

The goal of this study was to analyze the spatial pattern of distribution while taking into account some environmental and demographic factors of Dengue, Chikungunya and Zika within an area where these three disease are known to be prevalent. By doing this, similarities in pattern as they relate to the aforementioned factors were established in order to show the possibility that one disease can be used as a predictor for the emergence of the other two. This knowledge would be useful in areas where either one or two out of the three diseases are prevalent to inform public health specialists and other stake holders of the potential risk of emergence of either of the three disease that is not already present. The project also highlight some factors that could come together to create the right environmental and demographic mix favorable for the emergence or outbreak of any these three diseases in area where they are not currently prevalent.

From the results we can see the conduciveness that can be provided by a densely populated area to the transmission of vector more diseases, especially for a peri-domestic day biting vector such as the *Aedes* mosquito. Densely populated area are usually characterized, by active human movement, activities and infrastructures. This makes transmission and spread of infectious disease relatively easy.

The higher total number of *Aedes* mosquito eggs experienced in areas with high human population density can be attributed to better chances of mosquitoes to find a blood meal, making it more capable of laying eggs. However, this does not explain some of the observed high number of *Aedes* mosquito eggs seen in areas of low human population density, which could also be as a result of autogeny that have been observed in some *Aedes aegypti* species as described by Ariani, Cristina V *et al.*

The higher number of *Aedes* mosquito eggs seen in areas of higher number of cases Dengue, Chikungunya and Zika, which are also areas of higher human population, provides good insight into

how a proper mix of environmental and demographic factors can create a dangerous platform for the spread of vector born disease.

The cluster maps of case counts of the Dengue, Chikungunya and Zika cases, shows similarity in areas where areas of hotspots and cold spots are found. The presence of more clusters for Dengue and Chikungunya compared to Zika is as result of higher number cases for the first two, which increases the likelihood of clusters. The clusters seen for each disease, supports the observation that most cases are in the west of the city compared to the east of the city.

Based on these results, efforts in directing vector control resources and other disease burden mitigating resource would have a larger impact if directed to census tracts along the western areas of the city. This is very important to consider especially in resource scarce situation. These patterns also provides useful insight in making public health preparedness plan in the event of an outbreak or implementing vaccination campaigns, if ever there is a commercially vaccine.

Despite best efforts to conduct a rigorous investigation and provide deep insight into the complex relationship between these three illnesses and with environmental and demographic factors, some limitations still exists in this study. It would have been very useful to consider the variations in level of vegetation cover across the entire city. This could have further helped to establish the relationship between urbanization and spread of *Aedes* spread infectious diseases. Also data on the temporal pattern of cases would have been useful in quantifying and comparing how rapidly these three disease spread over time. This will also help in conducting more rigorous risks assessment.

Further study would be needed to explore the relationship between Dengue, Chikungunya and Zika patterns without these limitations and also considering other important, environmental (example; temperature and rainfall) and demographic (example; water collection sites, trash disposal) factors that were not considered in this project.

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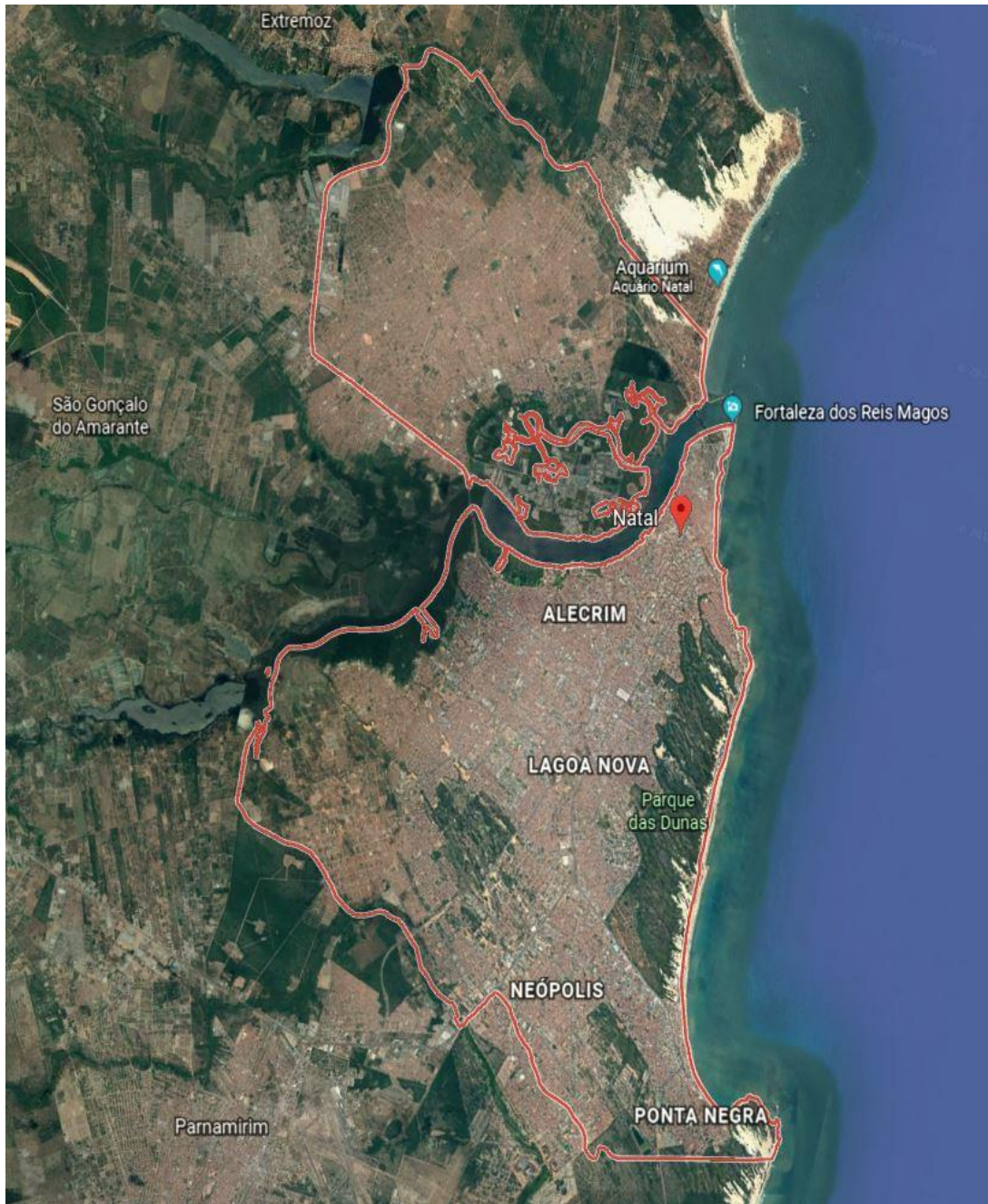
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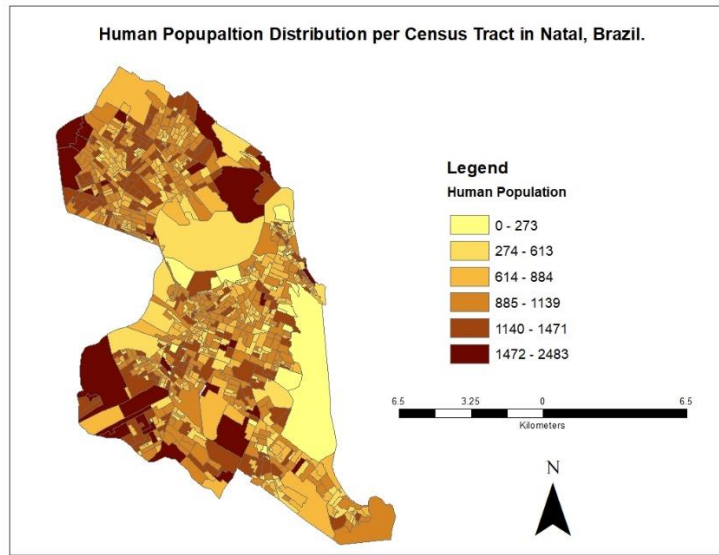
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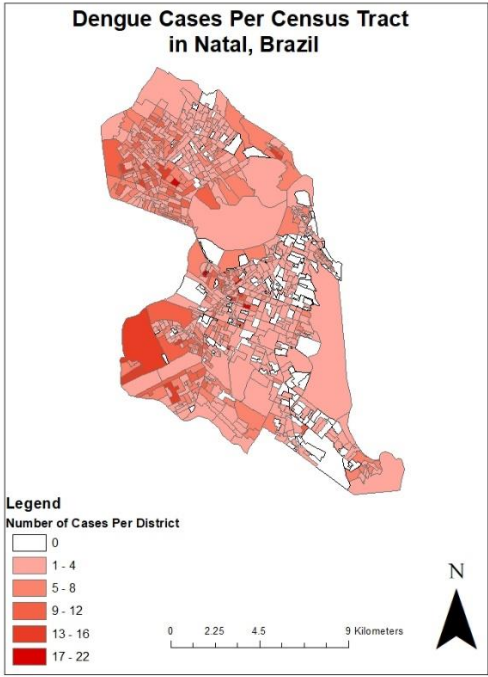
## Figures



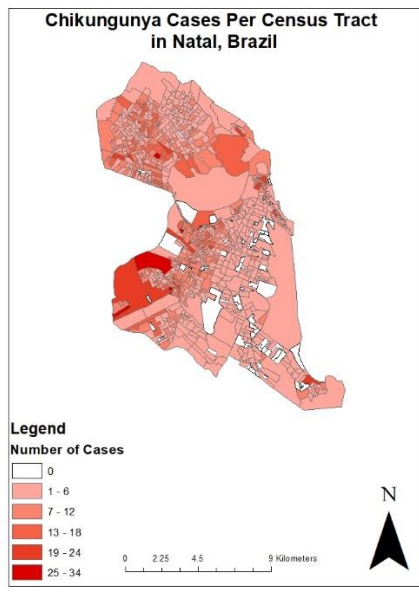
*Figure 1: Satellite image of Natal, Brazil (red boundary) from google earth.*



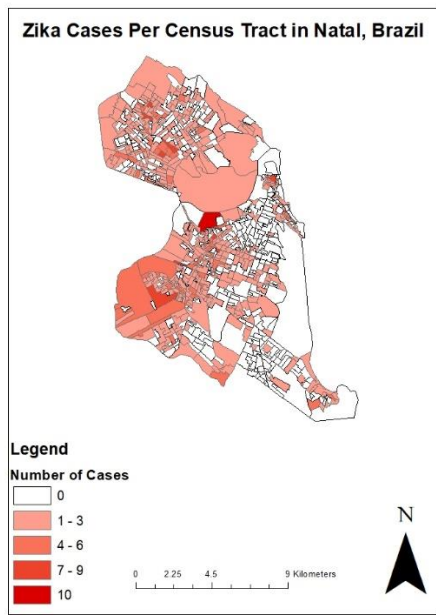
**Figure 2: Human Population per Census Tract in Natal, Brazil (2010 Census)**



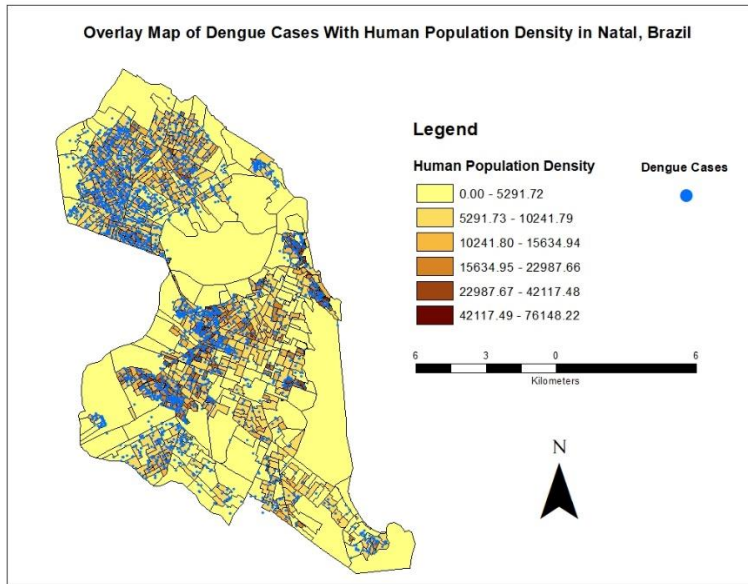
**Figure 3: Choropleth map for distribution of Dengue cases in Natal Brazil**



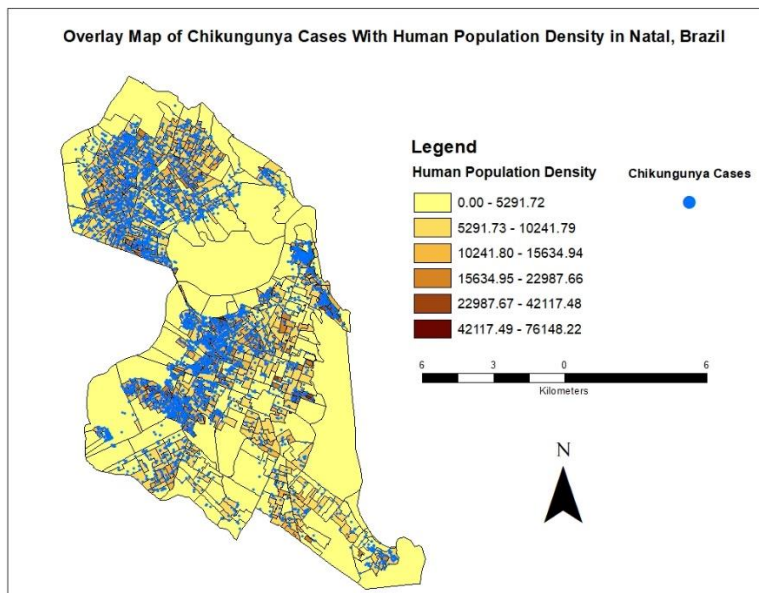
*Figure 4: Choropleth map for distribution of Chikungunya cases in Natal Brazil*



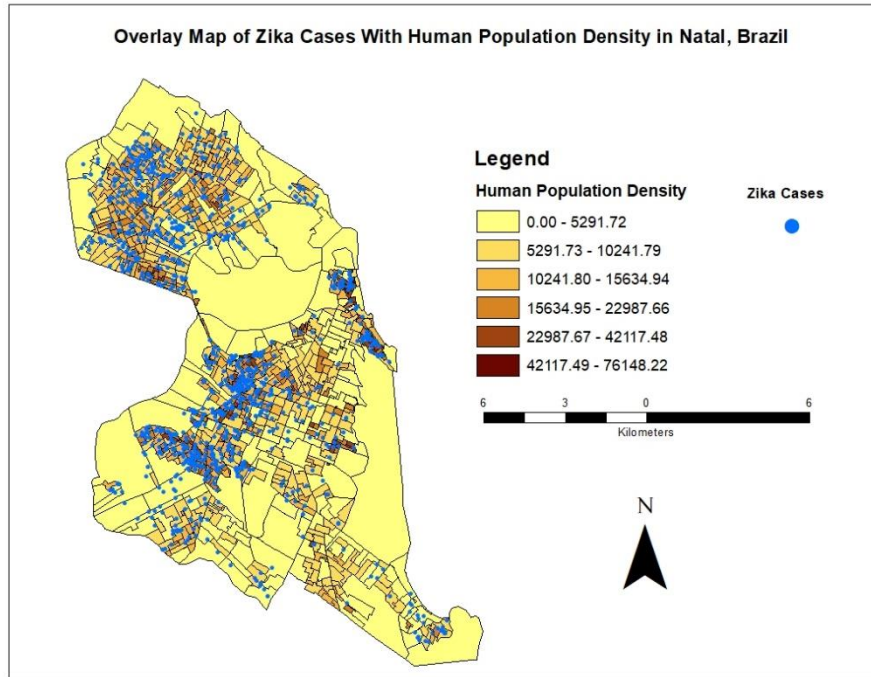
*Figure 5: Choropleth map for distribution of Zika cases in Natal Brazil*



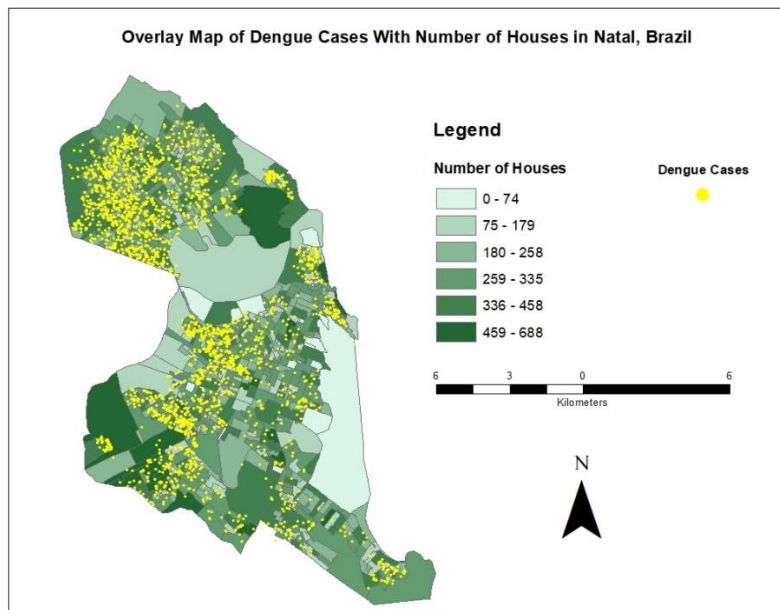
**Figure 6: Overlay map of Dengue cases with human population density**



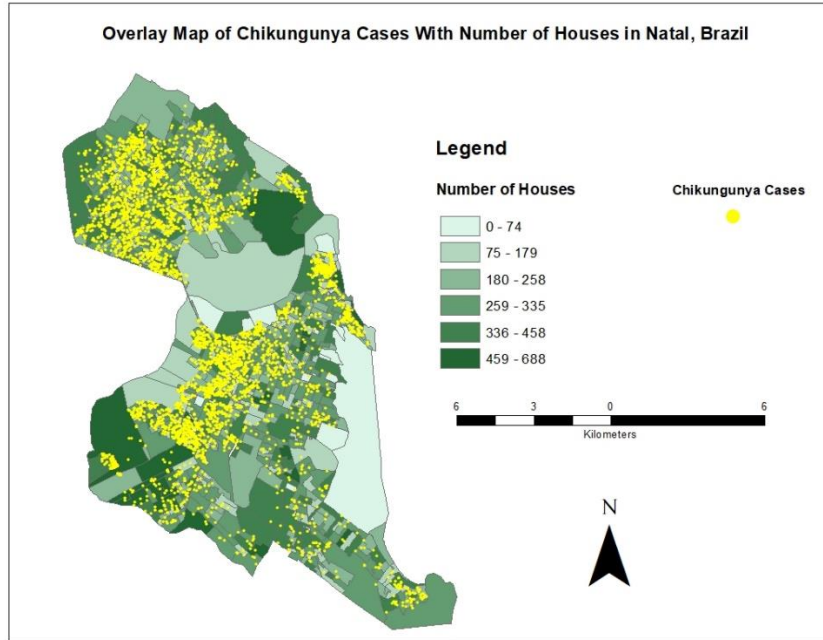
**Figure 7: Overlay map of Chikungunya cases with human population density**



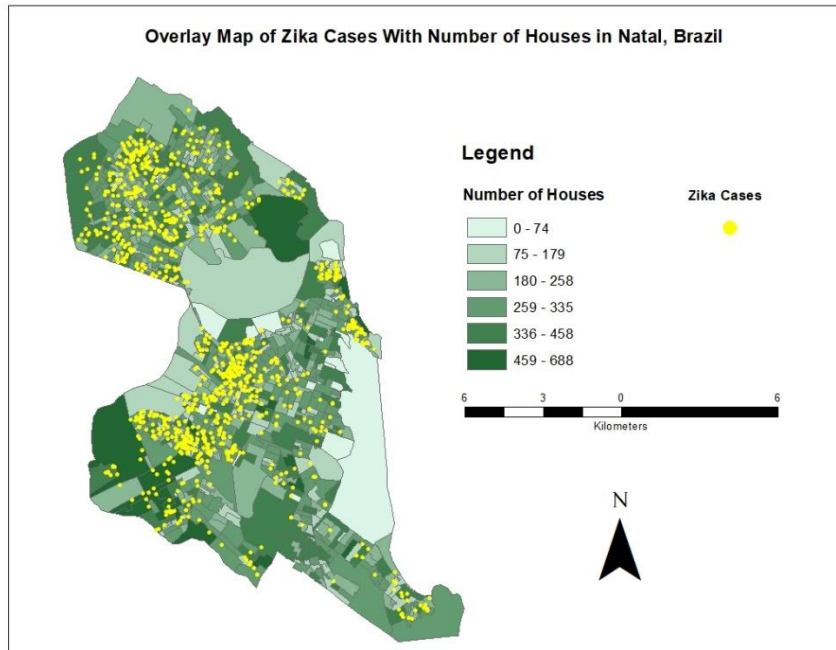
**Figure 8: Overlay map of Zika cases with human population density**



**Figure 9: Overlay map of Dengue cases with number of houses**

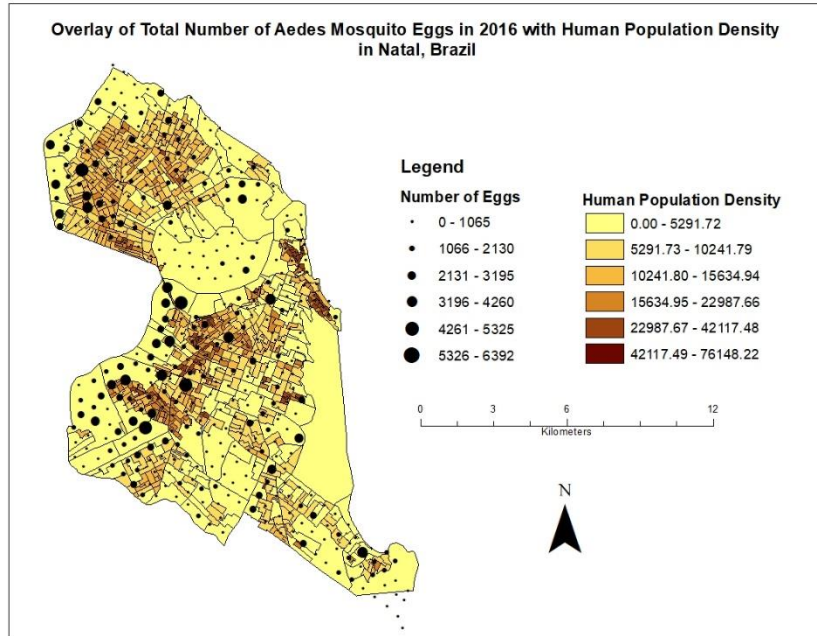


*Figure 10: Overlay map of Chikungunya cases with number of houses*

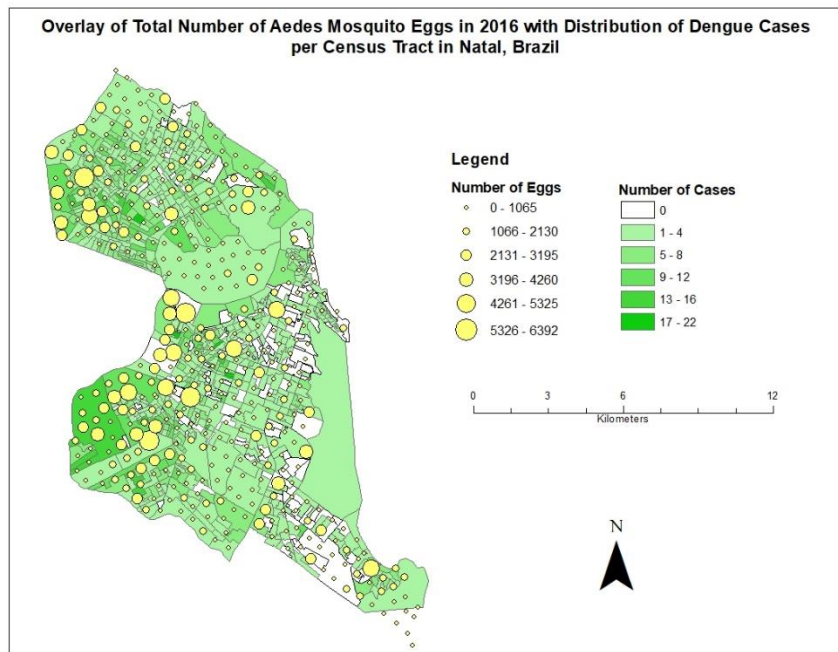


*Figure 11: Overlay map of Zika cases with number of houses*

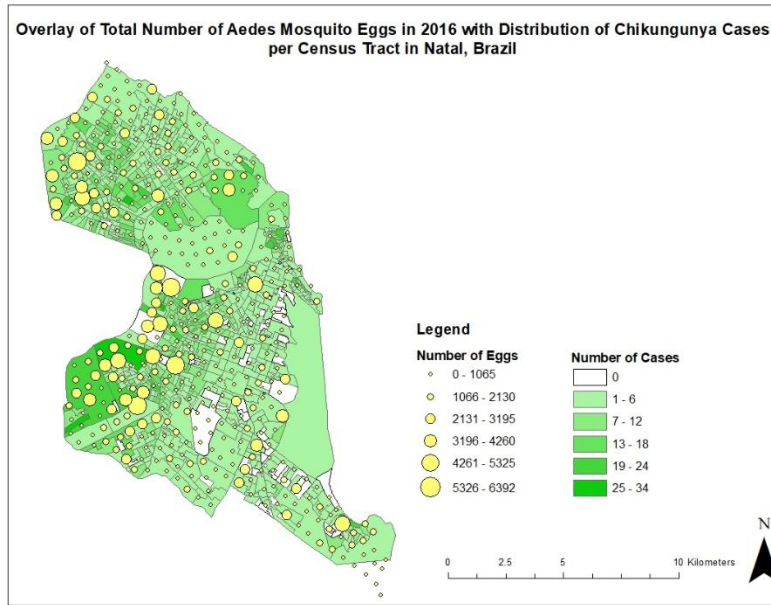




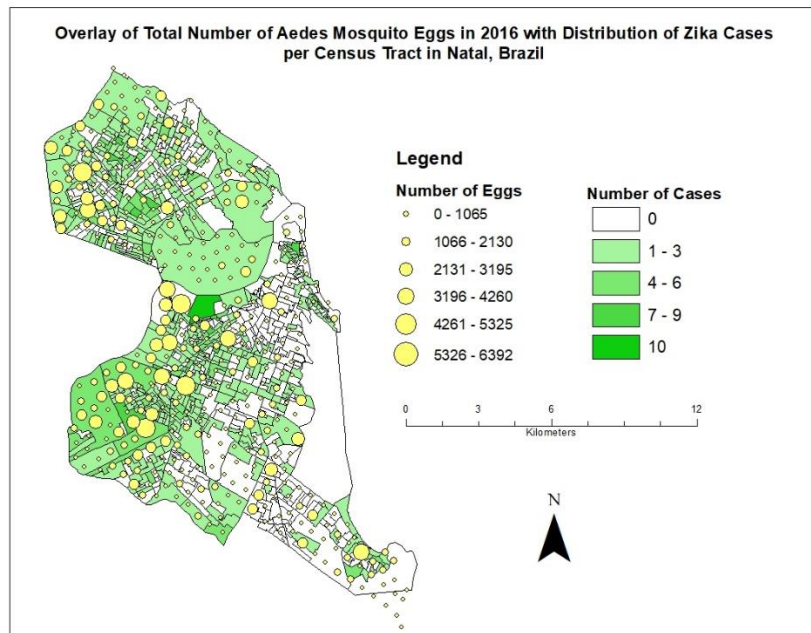
**Figure 12: Overlay map of total number of Aedes mosquito eggs in 2016 with human population density**



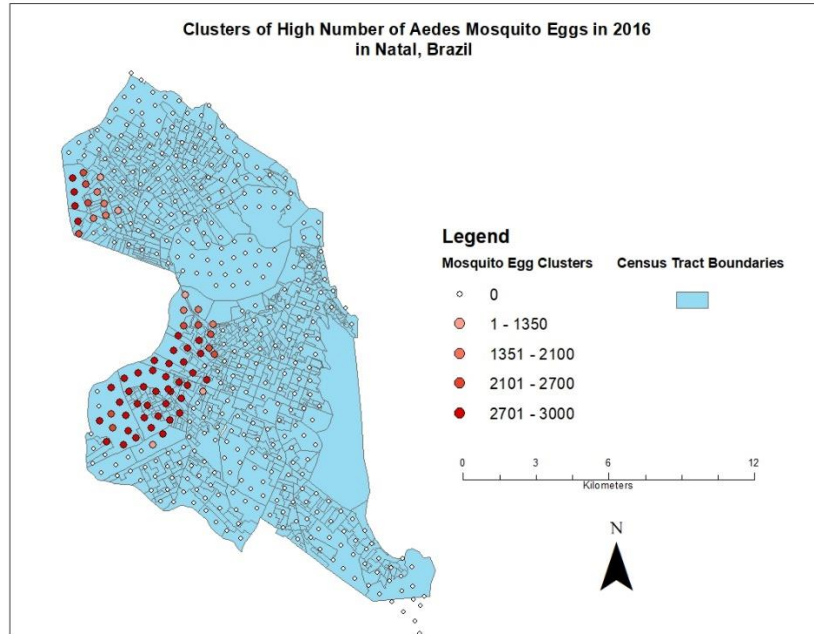
**Figure 13: Overlay map of total number of Aedes mosquito eggs in 2016 with distribution of Dengue cases**



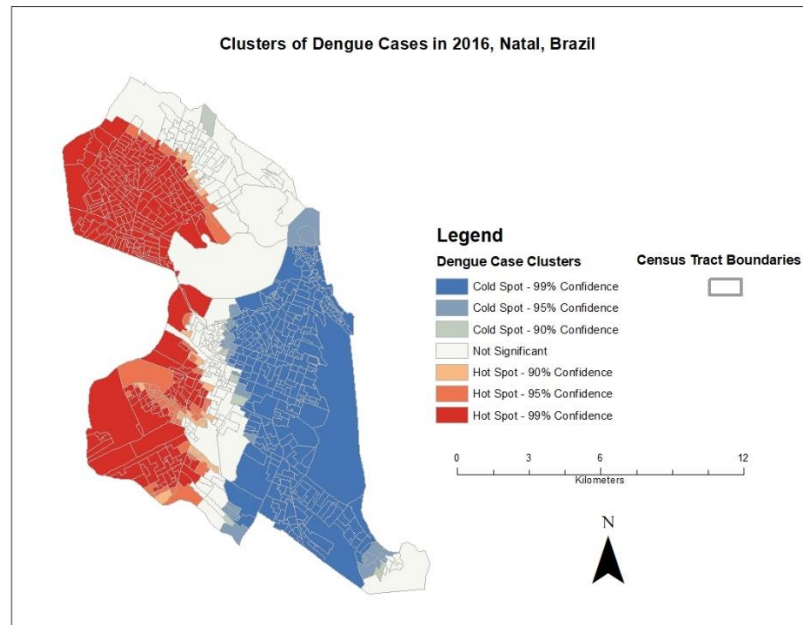
**Figure 14: Overlay map of total number of Aedes mosquito eggs in 2016 with distribution of Chikungunya cases**



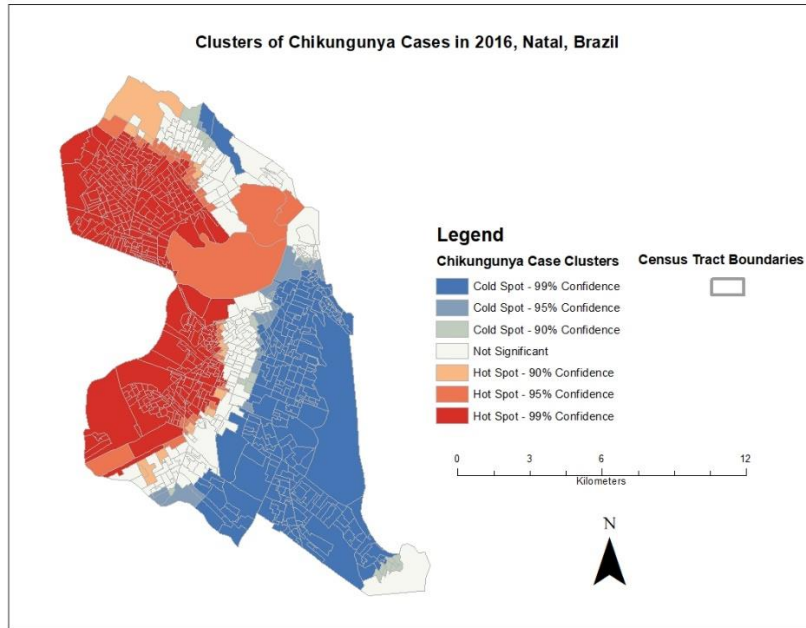
**Figure 15: Overlay map of total number of Aedes mosquito eggs in 2016 with distribution of Zika cases**



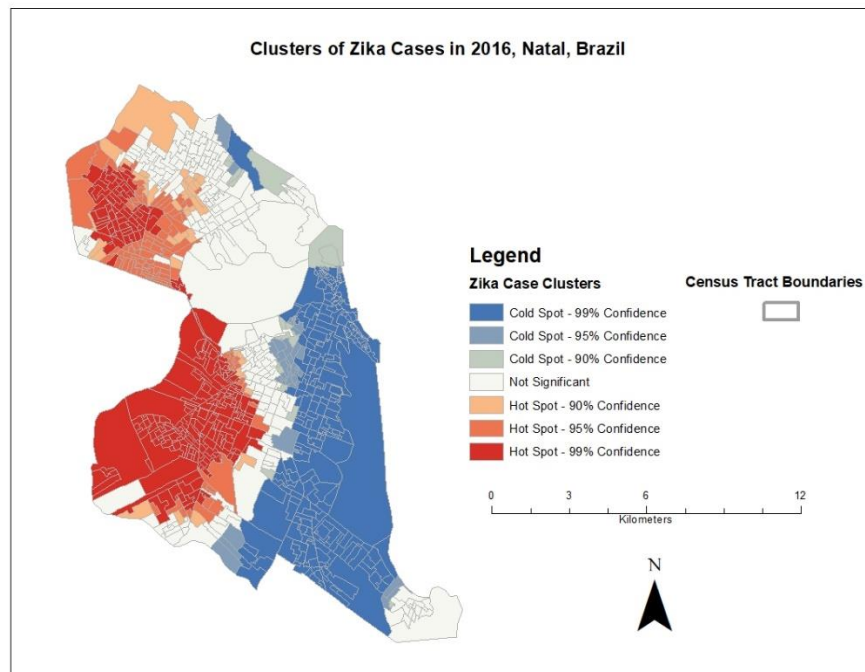
**Figure 16: Cluster map of Aedes Mosquito Eggs in 2016. Natal, Brazil**



**Figure 17: Cluster map of Dengue Cases in 2016. Natal, Brazil**



**Figure 18: Cluster map of Chikungunya cases in 2016. Natal, Brazil**



**Figure 19: Cluster map of Zika Cases in 2016. Natal, Brazil.**



