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Environmental and Socioeconomic factors associated with Dengue, and the mosquito vector Aedes aegypti

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Environmental and Socioeconomic factors associated with Dengue, and the mosquito vector *Aedes aegypti*

Dissertation

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Chapter 1: Literature Review

Introduction

Severe mosquito-borne diseases, including chikungunya, dengue, yellow fever, and Zika, have re-emerged; their re-emergence has created global concerns about the causes of their appearance, threats to health, and prevention and control of these diseases (Paixão et al., 2018). There are many vector-borne diseases such as West Nile and malaria that have also attracted interest in recent years due to the increase of incidence, geographical expansion, and threats to human health. More than 50% of the viruses in the genus *Flavivirus*, family Flaviviridae, can cause disease; viruses belonging to this genus include dengue, yellow fever, and Zika (Rigau-Pérez et al., 1998). Chikungunya is a member of the genus *Alphavirus*, family Togaviridae, (Escalante et al., 1998). These four viruses (chikungunya, dengue, yellow fever, and Zika) are transmitted by a common vector, the mosquito *Aedes aegypti* (Pless et al., 2017).

In recent decades, the incidence of mosquito-borne viral infections has grown dramatically. Since it was first reported in Saint Martin, chikungunya has spread to 45 countries causing >2.9 million cases and 296 deaths as of late July 2016 (Yactayo et al., 2016). In the past 50 years, dengue incidence has increased 30-fold with an estimated 96 million new cases annually (Bhatt et al., 2013; World Health Organization, 2012), and more than 2 billion people are at risk of infection (Halstead, 2008). Each year approximately 200,000 cases of yellow fever occur; 90% of those cases are reported in Africa (Barnett, 2007). Since the first major Zika virus outbreak occurred in 2007, Zika has continued to migrate eastward where between 500,000 and 1.5 million cases have occurred (Boeuf et al., 2016). The purpose of this literature review will be to summarize published findings about factors associated with these four vector-borne diseases and identify knowledge gaps of the following viruses transmitted by the *Aedes aegypti* mosquito: chikungunya, dengue, yellow fever, and Zika.

The vector

Aedes aegypti

Aedes mosquitoes are active during daylight hours, they are daytime feeders, and are highly adapted to urban living (Gubler & Clark 1995; Hawley, 1988; Morrison et al. 2008). These mosquitoes breed in and around houses, in natural or artificial water containers (Mammen et al., 2008). Female adults will lay eggs, a few at a time at intervals, in water tanks, flower vases, pot plant bases, discarded tires, buckets or other containers found around or inside homes (Christophers, 1960). *Aedes aegypti* (*Ae. aegypti*) is the main vector of the four viruses; it is considered one of the most efficient mosquito vectors for arboviruses and can be found in tropical, subtropical, and even temperate climates (Cunha & Trinta, 2017; Wilder-Smith et al., 2013). It is described as a small, dark mosquito with white lyre-shaped markings and banded legs (Becker et al., 2010).

A wide range of water-holding artificial containers located around homes is often exploited as sites for oviposition of eggs (Eisen & Moore, 2013; Focks & Alexander, 2006). Occasionally, *Ae. aegypti* will also use natural larval habitats, such as bromeliads and tree holes (Halstead, 2008). These mosquitoes are closely associated with human dwellings, and will take blood meals from the inhabitants of these homes (Kauffman & Kramer, 2017; Ngugi et al., 2017). Even when other hosts such as dogs, swine, rodents, bovines, and chickens are available, *Ae. aegypti* will prefer to feed on human blood (Scott et al., 2000). They often feed on multiple hosts during a single gonotrophic cycle thereby facilitating the transmission of human blood-borne pathogens with each blood meal (Harrington et al., 2014; Kauffman & Kramer, 2017).

In the life cycle of *Ae. aegypti*, the duration of the larval stages is 7-9 days at 25°C and that of the pupal stage is 2-3 days at the same temperature (Halstead, 2008). The average lifespan for females is about 8-15 days and 3-6 days for males. *Ae. aegypti* is killed rapidly at freezing temperatures; 62% of adults died when exposed for 1 hr. at 32°F (Knipling & Sullivan, 1957). In a study conducted in southwestern Georgia, most larvae died when the average ground temperature dropped to 48°F (Smith & Love, 1958). The lowest temperature that yellow fever infectiousness has been observed to develop in a mosquito is approximately 16.5 ºC (61.7 ºF) (Hindle, 1930). Conversely, temperatures greater than 35°C have been seen to negatively affect *Ae. aegypti* activity and survival (Biggerstaff et al., 2010; Christophers, 1960).

I. Subspecies of *Aedes aegypti*

There are two recognized subspecies of *Ae. aegypti* s.l. (Moore et al., 2013). The first subspecies is the ancestral form, *Ae. aegypti formosus*, a sylvan mosquito restricted to sub-Saharan Africa. The second subspecies, *Ae. aegypti aegypti*, is found globally in tropical and subtropical regions typically in association with humans. *Ae. aegypti* s.l. subspecies were discovered in the late 1950s in East Africa (McClelland, 2009). The intraspecific classification of *Ae. aegypti* was proposed by Mattingly (Mattingly, 1957).

The frequency of pale forms of *Ae. aegypti* were higher in populations in and around human dwellings than in adjacent forests. This correlation between color and behavior is what prompted Mattingly to revisit the biology and taxonomy of *Ae. aegypti* (Mattingly, 1967). Mattingly described *formosus* as a form restricted to sub-Saharan Africa and in West Africa; *formosus* would most frequently breed in natural containers such as tree holes, and would feed primarily on wild animals (Moore et al., 2013). The dark-scaled body parts of *formosus* are generally blacker than *Ae. aegypti aegypti*. *Ae. aegypti aegypti* is defined as either distinctly paler and browner in color (at least in the female); this species will breed in artificial containers provided by humans, will breed indoors, and has a preference for feeding on human blood (Mattingly, 1967).

a. Aedes albopictus

Aedes albopictus (*Ae. albopictus*), the Asian "tiger mosquito", is susceptible to and can transmit many arboviruses of public health importance (Shroyer, 1986; Mitchell, 1991). It ranks second only to *Ae. aegypti* in importance as a vector of dengue virus (Benedict et al., 2007). However, it is considered a competent vector of chikungunya, dengue (serotypes 1-4), yellow fever and West Nile (Moore, 1997). One of the most important differences between the North American populations of *Ae. aegypti* and *Ae. albopictus* is their latitudinal distribution (Estrada-Franco, Craig, & Pan American Health Organization, 1995). Populations of *Ae. aegypti* cannot tolerate very low temperatures and have been restricted to southern areas of the United states. In contrast, *Ae. albopictus* has evolved a photoperiod-induced egg diapause that allows them to colonize temperate and northern latitudes. North American strains of *Ae. albopictus* have showed egg cold hardiness; this enables the species to survive suboptimal winter temperatures (Hawley, 1988). The eggs of *Ae. albopictus* are the only life stage capable of coping with subzero temperatures (Kreß et al., 2017). Laboratory studies on colonies from Japan, Réunion, and the United States of America have demonstrated that populations of *Ae. albopictus* can breed and survive at a mean temperature above 10°C in Japan and Réunion (Delatte, Gimonneau, Triboire, & Fontenille, 2009; Kobayashi, Nihei, & Kurihara, 2002) and -5°C for populations in the United States of America (Mitchell, 1995).

While the distribution of *Ae. aegypti* and *Ae. albopictus* can overlap in urban areas, *Ae. albopictus* is more commonly found in suburban and rural areas where open, vegetated spaces are prevalent (Estrada-Franco et al., 1995). The larval breeding sites of *Ae. albopictus* are broad and can range from natural sites (e.g., bamboo stumps, bromeliads and tree holes) to artificial containers (e.g. water storage containers and old pieces of cars (Hawley, 1988). *Ae. albopictus* is mainly a daytime mosquito that prefers to bite early in the mornings and late afternoons (Paupy et al., 2009). This mosquito prefers to feed on mammals; however, the female may bite reptiles, birds, and amphibians as well. When given the choice between human and animal bait, *Ae. albopictus* preferentially chooses humans. This preference has been seen in host choice experiments using wild populations (Delatte et al. 2010) or analyzing blood meals from wild mosquitoes (Niebylski et al., 1994).

II. Introduction to California

In the fifteenth through seventeenth centuries, it was likely that *Ae. aegypti* migrated to the Americas in European slave ships; these slave ships provided a favorable environment thereby selecting traits that would increase the success of the domestic form of *Ae. aegypti* (Powell & Tabachnick, 2013). In California, it was not until 2013 when breeding populations of *Ae. aegypti* was first discovered; they were reported in Fresno, Madera, and San Mateo (Pless et al., 2017). Analyses suggest that these populations were introduced into California from South Central United States, particularly the region of Houston or New Orleans (Gloria-Soria et al., 2014).

Ae. albopictus was first discovered in August 1985 breeding in tire dumps in Harris County, Texas (Moore, 1997; Usinger, 1944; Estrada-Franco, Craig, & Pan American Health Organization, 1995). It may have entered the United States in shipments of used tires from northern Asia, where the species is widely distributed (Craven et al., 1988; Hawley et al., 1987; P. Reiter & Sprenger, 1987). This invasive mosquito originated from Asia; it is found in both temperate and tropical parts of the continent (Paupy et al., 2009). From its native range, it has spread to at least 28 other countries around the world (Benedict et al., 2007). In September 2011, *Ae. albopictus* was discovered in the City of El Monte, Los Angeles County, following a request from a citizen for vector control service (Zhong et al., 2013). It is uncertain how these mosquitoes were introduced; possible sources of infestation may include an introduction from other areas of the United States where *Ae. albopictus* is established, importation from Hawaii or abroad, or they are surviving mosquitos from past introductions.

III. Surveillance in California

Mosquito species, *Ae. aegypti* and *Ae. albopictus*, are found in nearly 200 cities within 12 central and southern California counties (California Department of Public Health, 2018). Last year (2017), *Ae.aegypti* was found in Merced and appears to have become established (Merced County Mosquito Abatement District, 2017b). Standard surveillance traps (New Jersey light, CO_2, and gravid) that are used in California may not capture *Ae. aegypti* and *Ae. albopictus*. Thus, particular devices have been developed specifically for the detection of eggs and adults of these mosquitoes; these devices are referred to as ovitraps and adult traps. These traps are placed throughout California. All data on *Aedes* collection efforts is reported and entered in the California Vector-borne Disease Surveillance System (CALSURV), a database for mosquito management in California.

Arboviral diseases

Dengue

I. Introduction

Dengue virus is an arthropod-borne virus of the genus *Flavivirus* that belongs to the family Flaviviridae (Martina et al., 2009). More than 100 countries are affected by dengue, including Europe and the United States (San Martín et al., 2010). Based on statistical modelling methods, 51 million dengue infections are estimated to occur each year (World Health Organization, 1999). Incidence rates of dengue are increasing mainly in tropical and subtropical regions of the world, and in the Americas (San Martín et al., 2010). Dengue is a disease that can be caused by four different dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) (Hasan et al., 2016). Each of the four serotypes have been individually responsible for dengue epidemics (Gibbons & Vaughn, 2002). Infection with one of four serotypes will not provide cross-protective immunity; thus, people that live in dengue-endemic areas can have four dengue infections during their lifetimes (Gubler & Clark, 1995). Dengue virus does not require an enzootic cycle in order to maintain the virus; it is preserved through a cycle between humans and the mosquito *Ae. aegypti* (Gubler & Clark, 1995; Marques, Guabiraba, Cisalpino, Teixeira, & Souza, 2014).

There is a wide array of clinical symptoms for dengue and as a result, it is often misdiagnosed for other tropical diseases or may go unrecognized (Amarasinghe et al., 2011). A dengue virus infection can range from asymptomatic illness to dengue fever (DF) to the severe illness of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) (World Health Organization, 2009). During the last decades, higher DF and DHF incidence rates have been observed (Brathwaite Dick et al., 2012). DHF/DSS was first documented in the 1950s in Southeast Asia (Patz et al., 1998). In the late 1970s, dengue and DHF/DSS re-emerged in the Americas. DF is a high-grade fever that can last from 3 days to a week (Narayanan et al., 2002). Severe headaches, lassitude, myalgia and painful joints, metallic taste, appetite loss, diarrhea, vomiting, and stomachaches are other reported manifestations (Hasan et al., 2016).

DHF is frequently seen during a secondary dengue infection. The hemorrhagic episodes in DHF are associated with vasculopathy, deficiency and dysfunction of platelets and defects in the blood coagulation pathways (Chiu et al., 2005). DSS is defined as DHF but is accompanied by narrow pulse pressure (<20mmHg), unstable pulse, restlessness, cold, clammy skin, and circumoral cyanosis (Hasan et al., 2016). Currently, no vaccine is available to treat dengue virus (Wilder-Smith et al., 2010). Creating a vaccine for dengue virus has become challenging as there are multiple dengue serotypes (Tolle, 2009). Supportive and symptomatic treatment has been key for treating symptoms of dengue virus such as intravenous hydration. The general diagnosis for dengue virus in many endemic countries has been clinical evidence of acute febrile illness with decreased platelet count (Wiwanitkit, 2010).

II. Environmental Factors

Environmental factors can play a vital role in the incidence of dengue. Factors such as rainfall or larval density have been identified as risk factors for this disease in many tropical countries (Gould, Mount, Scanlon, Ford, & Sullivan, 1970; Li, Lim, Han, & Fang, 1985). This positive association is most often seen in countries that are just above and below the equator (Kuno, 1995). Several studies have found that the greatest increase in density of *Ae. aegypti* occurred at the onset of a rainy season (Keating, 2001; Lu et al., 2009; Nagao et al., 2003). There have been reports of dengue virus epidemics however, in locations where rainfall or larval indices were unusually low (Biswas et al., 1993; Norman et al., 1991). It has been found that the virus that causes dengue can only be transmitted by *Ae. aegypti* mosquitoes when the ambient temperature is above 20°C (Delatte et al., 2009).

Climatic factors, high temperature and an increase in rainfall, have been found associated with a rise in cases of DF (Hu et al., 2012). High temperatures can accelerate the development of DF arboviruses therefore increasing the number of infectious mosquitoes (Patz et al., 1998). In southern Thailand, mean temperature, rainfall and relative humidity were key determinants for DHF in the areas that bordered the Andaman

Sea, while minimum temperature, rainy days and relative humidity were associated with DHF incidence on the Gulf of Thailand (Promprou et al., 2005).

Environmental conditions such as shop-houses, brick houses and houses with poor garbage disposal had a higher risk for contracting DHF in southern Thailand (Thammapalo et al., 2008). Shop-houses are buildings in Asia that are two or three stories high; the shop is located on the ground floor while the residence is situated above the shop. The majority of shop-houses are interconnected thereby allowing mosquitoes to travel freely between homes and from room to room. Brunkard et al. (2007) investigated risk factors for dengue virus transmission in Brownsville, Texas, and Matamoros, Tamaulipas, Mexico and found that risk factors that predicted past dengue infections were presence of larval habitat, absence of air-conditioning and street drainage. In El Salvador, people living in houses with infested discarded cans, infested discarded plastic containers, or discarded tire casings were more likely to have a recent infection of dengue (Hayes et al., 2003).

III. Socioeconomic Factors

Socioeconomic factors have also played a role in the incidence of dengue. Walker, Joy, Ellers-Kirk, & Ramberg (2011) found that housing age was a significant factor associated with the distribution of the dengue vector, *Ae. aegypti*. More mosquitoes were increasingly found around older homes. Houses that were located in poorer neighborhoods provided a better habitat for mosquitoes. These homes tended to be less maintained with an accumulation of objects in the yards and were close to vacant or abandoned properties. Older homes in low-income neighborhoods tended to have a higher egg count than houses of similar age in wealthier neighborhoods. In addition, homes in high-density, low-income neighborhoods tended to have higher egg counts than houses in high-density, higher-income neighborhoods. Brunkard et al. (2007) found that low income was the dominant risk factor for both recent and past dengue virus infections in the Texas-Mexico Border area.

In a vulnerability assessment to DF, poor neighborhoods with high proportions of young (i.e., <15 years) and illiterate residents, as well as a high percentage of individuals that were either unemployed or doing housework were the primary indicators of DF (Hagenlocher et al., 2013). Population density, age groups, and education levels have been identified as primary indicators in additional vulnerability assessments (de Mattos Almeida et al., 2007; Dickin et al., 2013). Spiegel et al. (2007) found that households where members were not economically active (student, homemaker, retired, or unemployed) had an increased chance of having a breeding site.

IV. Overview of Dengue Risk Factors

Known environmental risk factors of dengue have been rainfall, the number of rainy days, larval density, and high temperature. The environmental conditions of homes have also been found to significantly relate to cases of dengue. Shop-houses, houses made out of brick and those with poor garbage disposal were significant factors in contracting DHF. Homes that contain infested discarded cans, infested discarded plastic containers, or discarded tire casings are more at risk for dengue infection. The presence of larval habitat, absence of air conditioning and street drainage are also known risk factors. The known socioeconomic risk factors of dengue consist of housing age (older homes), homes in poor neighborhoods, low income, population density, age groups (<15 years), educations levels, and households where its members were not economically active.

Chikungunya

I. Introduction

Chikungunya is an arthropod-borne viral (arboviral) disease; the virus that causes chikungunya is a member of the genus *Alphavirus* (family Togaviridae) (Pialoux et al., 2007; Tolle, 2009). Chikungunya was first discovered in 1952 after an outbreak in southern Tanzania (Nasci, 2014). Although the chikungunya virus is not a newcomer among tropical viruses, it is still unknown by most people in the world including medical doctors (Powers, Brault, Tesh, & Weaver, 2000; Simon, Savini, & Parola, 2008). The first significant urban outbreaks of chikungunya fever were documented in Bangkok in the early 1960s (Nimmannitya et al., 1969) and in India from 1963-1973 (Padbidri & Gnaneswar, 1979). Minor outbreaks have occurred periodically over the 30 years, but no major outbreaks were recorded until 2004 in the coast of Kenya (Powers & Logue, 2007; Staples, Breiman, & Powers, 2009). According to the Pan American Health Organization, more than one million cases have been reported in the Americas in 2014 (OPS/OMS, 2016). In 2015, 666,311 cases were reported while 54% of those cases (359,728) were reported in Colombia (Oviedo-Pastrana et al., 2017).

Chikungunya has been identified in over 60 countries in Asia, Africa, Europe, and the Americas (World Health Organization, 2017a). The arrival of chikungunya virus in the Americas, however, has been fairly recent; its first infection was reported in December 2013 in the island of St. Martin (Leparc-Goffart et al., 2014). Chikungunya virus is maintained in an enzootic transmission cycle, which occurs between *Aedes* mosquitoes and animal reservoirs, with nonhuman primates being the presumed major reservoir host (Silva & Dermody, 2017). During epidemic periods, humans serve as chikungunya virus reservoirs (Mahendradas et al., 2013; Pialoux et al., 2007). Outside these periods, the main reservoirs are monkeys, rodents, birds, and other unidentified vertebrates. The primary vector of chikungunya is *Ae. aegypti* and it is occasionally transmitted by *Ae. albopictus* (Tolle, 2009).

As other viral diseases, a chikungunya viral infection may be asymptomatic or may produce a spectrum of clinical manifestations, ranging from milder forms to severe and disabling conditions. Symptoms of a virus infection include high fever, rigors, headaches, photophobia and a petechial rash or maculopapular rash (Schwartz & Albert, 2010). These clinical manifestations typically resolve within 7 days (Robinson, 1955). However, polyarthralgia may last as disabling and long-lasting joint pain (Brighton et al., 1983). There have been reports of peripartum mother-to-child infant transmission (Ramful et al., 2007), severe neurological involvement (Gérardin et al., 2008), and mortality (Beesoon et al., 2008; Josseran et al., 2006). An acute chikungunya infection may symptomatically resemble dengue fever; however, unlike dengue, a characteristic feature of chikungunya is recurring musculoskeletal disease primarily affecting the peripheral joints that can persist for months to years after acute infection (Couturier et al., 2012). There are no approved antiviral treatments currently available for a chikungunya virus infection (Thiboutot et al., 2010). Treatment for chikungunya consists of nonsteroidal anti-inflammatory drugs or steroids, bed rest, and fluids. Several methods can be used for diagnosis; serological tests such as enzyme-linked immunosorbent assays (ELISA) may confirm the presence of chikungunya antibodies (World Health Organization, 2017a).

II. Environmental Factors

In Phatthalung, Thailand, Nakkhara, Chongsuvivatwong, & Thammapalo (2013) found that having a garbage pile close to the house increased the risk of chikungunya infection. The presence of a garbage pile increased the risk of an asymptomatic infection 2.23 times and for a symptomatic infection, 3.97 times. Oviedo-Pastrana et al. (2017) compared two populations in the Colombian Caribbean that were severely affected by the chikungunya virus. Results showed that the lack of water supply and poor waste collection services could be determining factors in the proliferation of chikungunya virus. Mourya, Yadav, & Mishra (2004) investigated the effects of high temperatures during larval stages on the susceptibility of *Ae. aegypti* to chikungunya virus and found that there was a significant increase in the susceptibility of mosquitoes to chikungunya virus with increasing temperature. An increase in the susceptibility of mosquitoes to virus was noticed above 39°C. It was found that an increase of chikungunya cases occurred after 6 weeks of increasing cumulative rainfall with a variation of average daily temperatures (23.7−30.7°C) per week (Ditsuwan et al., 2011).

III. Socioeconomic Factors

A study in Réunion Island identified factors responsible for a chikungunya epidemic that occurred in Réunion and Mayottte in 2005 (Setbon et al., 2008). Certain socioeconomic and demographic variables were strongly correlated with the distribution of cases. Generally, they observed that the wealthier were less affected on average than those who were more disadvantaged. The socio-demographic variables that were associated with the prevalence rate of disease were place of birth, level of education, age (ages 45-59), socio-occupational category and housing conditions ($p<0.05$). People living in a house (most often with a garden) had a much higher probability of having been infected than someone living in an apartment (OR=3.50; $p<0.001$). Individuals with the lowest incomes and who were manual workers (farm laborers) with little education and who were most often born on the island had the highest probability of being infected.

On the Indian Ocean island of Mayotte, Sissoko et al. (2008) investigated and identified risk factors for chikungunya virus infection among the primo-exposed population. Poor living conditions were strongly associated with a high risk for chikungunya virus infection. In particular makeshift housing and a low household asset index were associated with higher chikungunya virus seroprevalence rates. Minimal schooling was also associated with an increased risk of chikungunya virus infection. The short length of schooling (0-6 years of primary school) is a variable that is likely related to poverty. Moreover, on the island of Mayotte, Raude & Setbon (2009) investigated variables that predicted chikungunya infection. They found that people with lower material and educational resources had significantly higher chikungunya rates.

IV. Overview of Chikungunya Risk Factors

Risk factors associated with chikungunya are garbage piles near homes, lack of water supply, poor waste collection services, place of birth, level of education (minimal schooling), age (ages 45-59), socio-occupational category, housing conditions, low income, makeshift housing, low household asset index, and manual workers (farm laborers).

Yellow fever

I. Introduction

Yellow fever is caused by a prototype member of the genus *Flavivirus* (family Flaviviridae), and is of a single serotype (Monath, 2001; Monath & Vasconcelos, 2015). It was first isolated in West Africa in 1927 (Barrett & Higgs, 2007). According to the World Health Organization, 13 countries in the Americas are considered endemic for yellow fever (Pan American Health Organization, 2013; World Health Organization, 2014). *Ae. aegypti* was considered the only vector of yellow fever virus until the 1930s (Barrett & Higgs, 2007); however, it is now known that several other species of *Aedes* mosquitoes have spread the virus to the human population (Monath, 1994). Yellow fever has three transmission cycles: jungle, intermediate, and urban. The jungle cycle or sylvatic cycle involves the transmission of the virus between non-human primates (e.g. monkeys) and tree-hole breeding mosquitoes inhabiting the forest canopy (Barrett & Monath, 2003; Gardner & Ryman, 2010). Humans are infected incidentally when they enter the area (e.g., to work as foresters). In addition to the jungle cycle, an intermediate (savannah) cycle has been recognized in Africa. The intermediate yellow fever transmission cycle involves infected mosquitoes feeding on both monkey and human hosts (Gardner & Ryman, 2010). The urban cycle involves transmission of the yellow fever virus between humans by *Ae. aegypti* (Barrett & Monath, 2003).

The presentation of yellow fever ranges from fever, jaundice, hemorrhage, and renal failure (Barnett, 2007). There are three phases of yellow fever; the first phase is characterized by fever, malaise, generalized myalgia, nausea, vomiting, irritability, dizziness, and a general toxic appearance. There is an improvement in symptoms in the second phase; it includes a reduction in fever and in some infected individuals, they recover without developing jaundice. The third phase occurs in ≈15% of cases. This phase is characterized by the return of the fever, nausea, vomiting, jaundice, and bleeding diathesis. Shock and failure of multiple organs is typical during this stage. Infants and people of older age are associated with increased severity and lethality of infection with yellow fever virus. The case fatality rates for reported cases of yellow fever have been in the order of 15 to 50% (World Health Organization, 2000). Laboratory diagnosis of yellow fever is generally done by testing of serum to detect antibodies through ELISA (Monath, 2001).

There is a vaccine for yellow fever (Gubler, 2004). The yellow fever vaccine (YEL) has been used for more than 60 years (Khromava et al., 2005). It was once believed to be one of the safest vaccines (Khromava et al., 2005). Since 1996, that belief has changed, and more cases have been reported of YEL-associated viscerotropic disease (YEL-AVD) and YEL-associated neurotropic disease (YEL-AND) (Martin, Tsai, et al., 2001). Advanced age (65 years and older) has been identified as a risk factor for systemic adverse events associated with YEL (Lawrence et al., 2004; Martin, Weld, et al., 2001). There is also evidence that YEL-AVD and YEL-AND appear more frequently in people of advanced age ("Yellow Fever Vaccine-Associated Disease," 2004). YEL is recommend for people ages 9 months and older that are traveling to areas where yellow fever is endemic (Centers for Disease Control and Prevention, 2002). YEL is also recommended for military personnel that are on duty or are being deployed to an endemic area (Department of Defense (DoD), 1995)

In the 18th and 19th centuries, yellow fever epidemics have struck the United States repeatedly in small and large outbreaks (Patterson, 1992). After the discovery of the mosquito vector in 1900, measures were being made against *Ae. aegypti* breeding sites. This lead to the initiation of the *Ae. aegypti* eradication program by the Pan American Health Organization to prevent yellow fever outbreaks (Barrett & Higgs, 2007). This campaign was successful; they eliminated breeding sites and used insecticide spraying to kill adults. The last imported case of yellow fever in the United States was reported in 1924 (McFarland et al., 1997).

An ongoing outbreak of yellow fever currently exists in Brazil in the states of Rio de Janeiro, Minas Gerais, and São Paulo, including areas close to the city of São Paulo (Centers for Disease Control and Prevention, 2018). A number of unvaccinated travelers to Brazil have contracted yellow fever since early 2018. From July 2017 to February 2018, there have 464 confirmed cases of yellow fever reported in Brazil, including 154 deaths (World Health Organization, 2018). The list of areas where yellow fever vaccination is recommended for international travelers to Brazil has been expanded. Many will require vaccinations. People who have never been vaccinated against yellow fever are told to avoid traveling to areas of Brazil where yellow fever vaccination is recommended. Currently, there is a vaccine supply issue. Since the outbreak of yellow fever in Angola, there’s been a depletion of the vaccine that forced health officials to give out fractional doses in the related Democratic Republic of Congo outbreak (Ahuka-Mundeke et al., 2018). Before yellow fever manufacturers could fully recover, a large outbreak of yellow fever occurred in Brazil.

II. Environmental Factors

The extrinsic incubation period of yellow fever virus is dependent on temperature. The warmer the temperature, the shorter incubation period from the time the mosquito consumes the infective blood until the mosquito is able to transmit the virus by bite (Shope, 1991). Increasing warm temperatures in the United States would cause not only a wider distribution of *Ae. aegypti* and a faster generation time for mosquitoes but also the yellow fever virus would have a shorter extrinsic incubation period and would cycle much more rapidly in the mosquito (Shope, 1991). Hamrick et al. (2017) analyzed geographical and environmental factors and their relationship with yellow fever human cases in 14 YF-endemic countries. Altitude was associated with the distribution of yellow fever human cases in the Americas; altitudes between 318 and 784 meters had six times higher risk of yellow fever compared to countries at higher altitudes. Annual rainfall between 1,067 and 2,762 mm had four times higher odds of yellow fever. For temperature, 14.4°C and 20.0°C was the favorable temperature range for the presence of yellow fever. Finally, the study also found that for every one additional genus of non-human primate host present in the area, the odds of yellow fever occurrence doubled.

This suggests that primate diversity can be associated with environmental factors that favor the presence of yellow fever human cases.

III. Socioeconomic Factors

In 1853, New Orleans faced one of the worst epidemics of yellow fever; Pritchett & Tunali (1995) investigated this epidemic to identify the factors that made one susceptible to the yellow fever virus. The variables that were examined were age, gender, nativity (foreign born and U.S. born), years the person has lived in New Orleans, charity burial, and whether they died from the fever. Results showed that relative risk from yellow fever was lower for the aged and especially for children. Individuals born outside of New Orleans, either in other countries or in other states, were at greater relative risk of death from yellow fever. The French had the greatest risk, followed by the British, the Germans, the Irish and then native Americans born outside of New Orleans. It was found that the longer an individual resided in New Orleans, the greater the likelihood of acquired immunization; those who survived yellow fever acquired an immunity to the disease. Information on the form of payment of burial expenses was used as a proxy for poverty status at the time of death. Nearly 65% of burials that were made on charity, 84% were yellow fever victims; for private burials, only 20% were yellow fever victims. A likelihood ratio test indicated that the duration of residence and the form of burial payment were significant determinants of the cause of death.

IV. Overview of Yellow Fever Risk Factors

The known factors associated with yellow fever include high temperature, altitude, rainfall, presence of non-human primate hosts, poverty status, and nativity.

Zika

I. Introduction

Zika virus is a *Flavivirus*, in the family Flaviviridae (Petersen et al., 2016). It was discovered in 1947 when it was first isolated from a febrile rhesus macaque monkey in the Zika Forest of Uganda and later identified in *Aedes africanus* mosquitoes from the same forest (Dick et al., 1952). Initially, Zika was only endemic in Africa and Asia, but this pattern changed in 2007 when a major outbreak of Zika virus infection occurred in Yap State, Federated States of Micronesia (Hennessey et al., 2016). Since then, Zika virus infection has spread quickly with outbreaks occurring in French Polynesia, Cook Islands, Easter Island, New Caledonia, and most recently, the Americas (Plourde & Bloch, 2016). Zika virus is transmitted to humans mainly by mosquito species, *Ae. aegypti* (Marano et al., 2015).

Monkeys are presumed to be the reservoir hosts for Zika virus, although the primary species has not yet been identified (Hayes, 2009; McCrae & Kirya, 1982). Humans are considered the amplifying hosts for this virus; there is no evidence that animals other than humans and nonhuman primates serve as amplifying hosts (Lazear & Diamond, 2016). This suggests a mode of transmission similar to dengue virus, yellow fever virus, and chikungunya. There have been recent reports of perinatal, sexual, and transfusion transmission. For blood-borne infections, a Zika virus donor could potentially contaminate the blood supply; cases of Zika transmission through transfusions of donated blood have been reported in Brazil (Kleinman, 2015; Musso et al., 2014). There is evidence of sexual transmission of Zika virus (Foy et al., 2011; Hills et al., 2016); including, the detection of Zika virus RNA in semen (Atkinson et al., 2016; Musso, Roche, Robin, et al., 2015). The infectious virus has also been found in urine (Gourinat et al., 2015), saliva (Musso, Roche, Nhan, et al., 2015), and breastmilk (Besnard et al., 2014).

Infection is likely asymptomatic in ≅ 80% of cases (Duffy et al., 2009; Ioos et al., 2014). About 20% of individuals who are infected progress to a clinically apparent febrile illness (Duffy et al., 2009; Hayes, 2009). Symptoms associated with Zika include rash, fever, arthralgia, myalgia, fatigue, headache, and conjunctivitis. On average, these symptoms appear after 3 to 7 days of mosquito inoculation (Lazear & Diamond, 2016). The World Health Organization (WHO) concluded that Zika virus infection during pregnancy is a cause of congenital brain abnormalities, including microcephaly; and that Zika virus is a trigger of Guillain-Barré syndrome (World Health Organization, 2016). There is no specific antiviral treatment available tor Zika, and treatment generally consists of rest, fluids, and use of analgesics and antipyretics (Hennessey et al., 2016). Zika virus infection can be diagnosed through serological tests such as ELISA (Marano et al., 2015).

II. Environmental Factors

Very little is known about the factors affecting the transmission of Zika virus. It is known that weather and climate shape mosquito geographic distribution, lifespan, population abundance, and transmission potential (Ali et al., 2017). A recent study suggests Zika virus transmission can occur between 18°C and 34°C, with a peak of 29°C (Mordecai et al., 2017). In the state of Rio de Janiero, a study identified environmental drivers of the arbovirus, Zika (Fuller et al., 2017). Areas of high incidence were not just areas where a high mosquito density was present. Instead, the high level of urbanization and the limited access to municipal water were factors that greatly contributed to the Zika virus epidemics in the city of Rio de Janiero. This study also found that heavy rainfall preceded cases of Zika by three weeks; heavy rainfall was concluded as a predictor of potential outbreaks.

In countries where they experienced infections during the 2015-2016 outbreaks, a study investigated the effects of potential explanatory variables for the spread of Zika virus and assessed the risks of future outbreaks (Teng et al., 2017). Results indicated that higher temperature, vapor pressure, precipitation, higher water coverage and the occurrence of dengue virus were significantly associated with the occurrence of Zika virus infection. Findings also revealed that water-filled environments (vapor pressure >18hPa) with stable warm temperatures (22-28°C) would provide favorable conditions for mosquitoes to transmit the virus.

III. Socioeconomic Factors

Socioeconomic conditions such as poverty and traveling from Zika-affected areas have been identified as key variables that influence the potential for Zika outbreaks (National Center for Atmospheric Research, 2016). Cities such as Miami, Houston, and Orlando that had a large volume of air travelers also had a high number of *Ae. aegypti*. Poverty is associated with lower air-conditioning use; these conditions lead to open windows and doors which can raise the probability of human-vector contact (Monaghan et al., 2016). Zika is more frequently seen in women and in terms of age, it has been seen in all age groups (Paixão et al., 2016). Pregnant women are at risk for Zika; microcephaly is the most striking finding in the fetuses of affected mothers by Zika virus (Hajra et al., 2017; Jamali Moghadam et al., 2016; Li et al., 2017).

IV. Overview of Zika Risk Factors

The known factors associated with Zika virus are high temperature, high urbanization, little access to municipal water, precipitation, vapor pressure, high water coverage, occurrence of dengue virus, poverty, pregnant women, and travel. Zika cases are more frequently seen in women.

<u>Other Vector-borne diseases</u>

West Nile virus

I. Introduction

West Nile virus (WNV) is an arthropod-borne virus of the family Flaviviridae (Kleinschmidt-DeMasters & Beckham, 2015). It was first discovered in 1937 from an infected person in the West Nile district of Uganda (Petersen & Marfin, 2002). This virus is widely distributed in Africa, Europe, Australia, and Asia; since 1999, it has spread rapidly throughout the western hemisphere, including the United States of America, Canada, Mexico, the Caribbean, and into parts of Central and South America (Kramer et al., 2007). The majority of human WNV cases are as a result of mosquito bites (Shapshak et al., 2015). A small proportion of human cases develop from blood transfusions, organ transplants, and transmittance from mother to child during pregnancy, delivery, or through breastfeeding.

Mosquitoes belonging to the genus *Culex* are considered the primary carriers of WNV. In California, the mosquitoes which commonly carry WNV are *Culex pipiens, Culex quinquefasciatus,* and *Culex tarsalis* (Lindsey et al., 2010). The majority of human WNV cases are as a result of mosquito bites (Shapshak et al., 2015). WNV is not spread through casual contact such as touching or breathing in the virus. The virus is maintained primarily through a cycle between birds and mosquitoes. Most infections of WNV are symptomless (Campbell et al., 2002). People who are 50 years of age or older are at the greatest risk of developing a severe illness (Petersen & Marfin, 2002). At this time, there is no vaccine available for humans against WNV. (Seino et al., 2007). Recommended treatment for mild cases in humans has been taking over-the-counter pain relievers to reduce joint pains or fevers. With severe WNV cases, hospitalization may be necessary along with fluids that are given intravenously.

II. Environmental Factors

The importance of environmental factors and their influence of where human WNV cases occur has been investigated since WNV arrived to the United States (Gibbs et al., 2006). In southern California, a study included ten counties and examined factors associated with where WNV cases developed; the counties included were San Luis Obispo, Kern, San Bernardino, Santa Barbara, Ventura, Los Angeles, Orange, Riverside, San Diego, and Imperial County (Liu & Weng, 2012). The purpose of this study was to provide a more precise surveillance method by incorporating additional environmental factors not typically included in mosquito surveillance. The study found that climatic factors that contributed significantly to the transmission of WNV were summer mean temperature, annual mean deviation from the mean temperature, and land surface temperature. Elevation and slope of different regions were also significantly related to the spread of WNV. Landscape patterns and vegetation water content also had a significant influence in the distribution of the virus.

High temperature has been consistently associated with WNV outbreaks (Hoover & Barker, 2016). The rise in temperature can cause an increase in growth rates of vector populations, reduce the time between blood meals, increase transmission to birds, and reduce incubation time from infection to infectiousness in mosquitoes (Paz, 2015). Seasonal variation that results in mild winters has been associated with increased WNV activity and if followed by hot, dry summers can favor the transmission of infections (Epstein, 2001; Wimberly et al., 2014). In the United States from 2001 to 2005, a 5□C increase in mean maximum weekly temperature was associated with a 32-50% higher incidence of WNV infection (Soverow et al., 2009).

Mosquito species thrive in specific habitats. In Shelby County, Tennessee, *Cx. pipiens* and *Cx. quinquefasciatus* were positively associated with urban habitats (Savage et al., 2008). In contrast, the abundance of *Cx. pipiens* complex mosquitoes was lower in rural sites. A common principle in epidemiology states that above-average precipitation may lead to greater mosquito abundance and an increase for WNV outbreaks in humans (Landesman et al., 2007; Soverow et al., 2009). However, in urban areas a prolonged time of drought can result in increased abundance of mosquitoes that can intensify transmission of WNV (Hoover & Barker, 2016). The reason for this is the result of urban storm water management systems are not being regularly flushed by rainwater during droughts. Thus, they are supplied with water from landscape irrigation systems that is mixed with organic materials. This quickly becomes a favorable habitat for mosquitoes, especially *Cx. pipiens*. In particular, *Cx. pipiens* (one of the principal WNV vectors) prefers water sources with high organic material such as dairy ponds, catch basins, and septic tanks (Meyer & Durso, 1998). *Cx. tarsalis* instead favors open habitats such as swamps, marshes, rice fields, and open pastures (Horsfall, 1955).

III. Socioeconomic Factors

Some studies have found that economic variables and anthropogenic characteristics of the environment best explained and predicted WNV prevalence. Orange County, an urban region in southern California, has been considered a WNV hotspot since the year 2004. A study which examined human WNV cases from 2004-2008 revealed that lower-income areas (per capita income) had higher prevalence levels of WNV in vectors (Harrigan et al., 2010). An important variable that provides an explanation for years of high WNV prevalence was the density of neglected swimming pools (Reisen et al., 2008). During the period that this study was performed, Orange County experienced a large increase in home foreclosures and neglected swimming pools. As a result, these findings suggested that abandoned pools stimulated WNV amplification and provided a link between WNV propagation and lower income, populated areas (Harrigan et al., 2010). In addition, Bakersfield, Kern County experienced a 300% increase in notices of delinquency that produced large numbers of neglected swimming pools; this was associated with a 276% increase in human WNV cases (Reisen et al., 2008). There are three possible explanations as to why a link may exist between low-income areas and WNV propagation. The first explanation states that areas with dense populations predominately occur in flatland areas of low elevation. These areas are characterized with old infrastructure and outdated water runoff systems that can serve as breeding habitats for mosquitoes (Su et al., 2003).The second explanation is that lower-income communities are less likely to spend for upkeep of their property. Findings for this conclusion were drawn from the rise in number of neglected swimming pools. The last reason for the link between WNV and lower income areas would be education (Harrigan et al., 2010; Milligan et al., 2004). People with higher education show more involvement with mosquito control. For example, they request pest control services such as spraying. This study also suggests prevalence of WNV is related to the bird diversity in an area. Avian species are considered the primary hosts, and if there is a loss of diversity, an increase of WNV infections is seen among humans the alternative hosts.

An aerial survey conducted in the Bakersfield area of Kern County (Reisen et al., 2008) similarly found a substantial amount of swimming pools that had been neglected. Neglected pools contain large amounts of organic matter that result in green-colored water. Neglected swimming pools were frequently found among new housing areas and not just restricted to just old neighborhoods.

Considered as a hotspot of WNV activity, Orange County from 2004-2012 had a record total of 240 human WNV infections and 9 deaths (Liao et al., 2013). WNV is now considered endemic in this area; its particularly important to identify which predictors are significant. The strongest predictor in the Orange County study were street gutters followed by housing unit density, neglected swimming pools, mean per capita income, number of mosquito breeding sites and ditches, and housing average age. Variables that were found to be statistically insignificant were catch basins and flood control waterways, population density, the proportion of people who were over the age of 65, and the location of WNV positive mosquitoes as well as the area where infected dead birds were found.

A study in Chicago and Detroit assessed which natural and socioeconomic factors greatly influenced the transmission of WNV (Ruiz et al., 2007). A strong association was seen between the risk of WNV transmission and the age of housing in both locations. A possible explanation for this relationship would again be the storm water sewer system. The combination of standing water and organic matter can affect breeding of *Culex* mosquitoes. Vegetation found in the inner suburb areas of Chicago and Detroit was found to be significant for WNV transmission. Distinct features of these areas consisted of small yards with cemeteries, shrubs, hardwood trees, and grassy alleys. Results of this study provided insight to factors that are strong predictors for urban areas. However, additional socioeconomic factors in this study would have been helpful to understand WNV amplification.

In Suffolk County, New York, socioeconomic conditions were the key predictors of human WNV infections (Rochlin et al., 2011). The effects of urbanization and increased WNV activity were strongly associated. Such effects included fragmented natural areas, increased road density, and urban areas where there were high numbers of people with a college education. This finding suggested that middle class suburban neighborhoods or “inner suburbs” had the greatest activity and risk for WNV. The inner suburbs appear to provide favorable conditions for WNV transmission in contrast to wealthier neighborhoods. In this study, high income neighborhoods have more vegetation, less habitat fragmentation, and higher biological diversity. This high biological diversity resulted in less activity of human WNV infections as there was higher availability of preferred avian hosts. Natural wetland areas that have fragmented areas provide favorable habitat to mosquito larvae in contrast to manmade wetlands.

IV. Overview of West Nile virus Risk Factors

Factors that have been key predictors of WNV include temperature, elevation and slope, landscape and vegetation water content, urban habitat, low-income areas, and abandoned pools. Risk factors: street gutters, housing unit density, number of mosquito breeding sites and ditches, and age of housing have also been associated with WNV.

Malaria

I. Introduction

Malaria is caused by a parasite that belongs to the genus *Plasmodium*, family Plasmodiidae (Escalante et al., 1998). It is a major global health problem; in 100 countries, over 40% of the world’s population is at risk of malaria exposure (Tangpukdee et al., 2009). In 2016, 91 countries reported a total of 216 million cases of malaria, an increase of 5 million cases over the previous year (World Health Organization, 2017b). Although it is prevalent in several large areas of the world; its main impact is in sub-Saharan Africa where at least 90% of deaths from malaria occur (Greenwood & Mutabingwa, 2002). Malaria is caused by one of several protozoan parasites of the genus

Plasmodium: *P.vivax*, *P. malariae*, *P. ovale*, and *P. falciparum* (Jotte & Scott, 1993). *P. falciparum* and *P. vivax* are known to cause the most infections worldwide (Cullen & Arguin, 2014). *P. falciparum*, however, is the species that commonly causes severe and potentially fatal malaria. *P. vivax* and *P. ovale* have dormant liver stages; they can reactivate and cause malaria several months or years after the initial infection. For *P. malariae*, if left untreated or treated inadequately, can cause long-lasting infections and persist asymptomatically in the human host for several years or even a lifetime (World Health Organization, 2013).

Parasites are spread to people through the bites of infected female mosquitoes and only mosquitoes of the genus *Anopheles* transmit parasites causing human malaria (Service, 2012). There are 476 species of *Anopheles* but only 70 can transmit human malaria. The larvae of *Anopheles* can occur in a wide range of habitats, but most species prefer clean, unpolluted water (Centers for Disease Control and Prevention, 2015). Larvae have been found in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers, and small temporary rain pools. There are many species that prefer habitats with vegetation while some breed in open, sun-lit pools.

Common signs and symptoms of malaria are fever, headache, back pain, chills, increased sweating, myalgia, nausea, vomiting, diarrhea, and cough (Cullen & Arguin, 2014). Evidence of severe disease of malaria includes cerebral malaria, severe anemia, renal failure, pulmonary edema, hypoglycemia, shock, disseminated intravascular coagulation, acidosis, and hemoglobinuria (Jotte & Scott, 1993). The standard for diagnosing malaria has been examination of thick and thin blood smears, but there has been development of more sophisticated techniques. There is recommended artemisinin-based combination therapies (ACT) that can be given to treat uncomplicated *P.vivax*, *P. malariae*, *P. ovale*, and *P. falciparum* malaria (*Guidelines for the Treatment of Malaria*, 2015).

II. Environmental Factors

In Kapkangani, a rural part of Nandi District, western Kenya, the spatial distribution of malaria was analyzed and risk factors associated with malaria were identified (Brooker et al., 2004). Active case surveillance was undertaken in three schools located in Nandi District, Western Kenya. Areas characterized by low altitude were strongly associated with the risk of malaria and spatial clustering. Ernst, Adoka, Kowuor, Wilson, & John (2006) found that ecological factors: lower altitude, proximity to swamp, and proximity to forest were associated with increased malaria risk in Kipsamoite, Kenya.

III. Socioeconomic Factors

Koram, Bennett, Adiamah, & Greenwood (1995) conducted a study with Gambian children to investigate whether socioeconomic factors are important risk factors for malaria (Koram et al., 1995). The risk of malaria infection among Gambian children was associated with poor housing and low socioeconomic status. Children from 6 months to 3 years of age, pregnant women, nonimmune travelers, and migrant workers have also been found to be individuals at risk for malaria (Jotte & Scott, 1993). The risk of malaria has been found higher for children who were underweight and lived in households where medicine was not kept at home (Brooker et al., 2004).

IV. Overview of Malaria Risk Factors

Low altitude, proximity to swamp, and proximity to forest were environmental risk factors associated with malaria. Socioeconomic factors found associated with malaria are poor housing, low socioeconomic status, not keeping medicine at home, and the following individuals: children from 6 months to 3 years of age, underweight children, pregnant women, nonimmune travelers, and migrant workers.

Knowledge gaps

Despite intense study, much is still unknown about mosquito-borne diseases. Many unanswered questions remain about these four diseases associated with *Ae. aegypti* and *Ae. albopictus*: chikungunya, dengue, yellow fever, and Zika, while the threat of their outbreaks and expansion continues to increase. A major gap in our knowledge of these diseases is in the factors that are associated with human cases of infection. As our understanding of the relationship between factors (environmental and socioeconomic) and cases of mosquito-borne disease increases, methods of preventing outbreaks can improve. Few studies regarding risk factors for chikungunya, yellow fever, and Zika are available. Chikungunya and Zika are relatively newer diseases for much of the world, and yellow fever has begun a resurgence. More information was found on dengue; however, in order to fully understand the connection between risk factor and infection, it is still insufficient.

For yellow fever and Zika, very few studies were found regarding risk factors especially socioeconomic. In regard to socioeconomic studies of yellow fever, only one study was found. This study of yellow fever was reviewing an epidemic that occurred in New Orleans in 1853; moreover, an epidemic that occurred over 150 years ago. Conditions at the point of time are no longer comparable to the conditions we live in now. Therefore, it is imperative that we have more updated studies of what can influence mosquito-borne disease now. The current outbreak of yellow fever in Brazil is likely to result in more current studies of risk factors. After the recognition of Zika virus transmission in South America in 2015, a lot of research activity became focused on the causal relationship between Zika and devastating anomalies in newborn infants. For Zika, there were few studies available that examined socioeconomic factors and very little was found for environmental factors. Significant gaps remain in our knowledge about factors that contribute to outbreaks of Zika. Knowledge on this subject can enhance vector control strategies to better detect and prevent this important infection. Although many of these diseases currently impact regions outside of the United States, both mosquito vectors are established in the United States; having an understanding of risk factors associated with these diseases and other mosquito-borne diseases could help with prevention and management of these infectious diseases.

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Chapter 2: Environmental Factors Associated with Dengue in El Salvador

Introduction

Over the past 50 years, there has been a global re-emergence of vector-borne diseases. One of these arboviruses is dengue, which is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue is considered to be the most widespread arboviral disease in the world and causes an estimated 50-100 million illnesses annually (Gibbons and Vaughn 2002, WHO 2009), while the number infected with dengue is estimated to be greater than 300 million (Bhatt 2013). More than 50% of the world's population lives in dengue-endemic countries and as a result, has a high risk of infection (Gubler 2002, 2011). Dengue is attributed to four different dengue virus serotypes (DENV-1-4) (Hasan et al. 2016). Infection with one serotype will not provide cross-protection against the other types. Creating a vaccine for the dengue virus has been challenging due to the multiple dengue serotypes (Tolle 2009). Mosquito control is one of the primary methods to reduce dengue, but vector control can have limits due to factors such as insecticide resistance. Therefore, understanding environmental factors associated with the occurrence of vector-borne diseases could contribute to dengue prevention.

Environmental conditions such as climate, weather, and vegetation coverage affect the distribution and risk of many vector-borne diseases including dengue (Bouma and van der Kaay 1996, Anyamba et al. 2001, Gubler et al. 2001, Enscore et al. 2002, Cazelles et al. 2005). Temperature influences vector development rates, behavior, and range (Christophers 1960, Tun-Lin et al. 2000, Siraj et al. 2014), as well as viral replication within the mosquito (Watts et al. 1987, McMichael and Haines 1997). A positive relationship has generally been found between temperature and dengue cases (Bhatt 2013, Messina et al. 2019), while higher temperatures may not be optimal for mosquitoes or the virus (Colon-Gonzalez et al. 2013). Precipitation variability influences habitat prevalence for *Aedes* larvae and pupae (Morin et al. 2013). In the tropics, dengue cases can occur year-round but are more frequent in the rainy season than the dry season (Nakhapakorn and Tripathi 2005, Pham et al. 2011, Colon-Gonzalez et al. 2013). The abundance of *Aedes* larvae can also relate to incidence of dengue (Hayes et al. 2003). Other factors related to dengue cases are vegetation and forest coverage, in particular deforestation and urbanization (Nakhapakorn and Tripathi 2005, Van Benthem et al. 2005, Troyo et al. 2009, Pham et al. 2011, Swain et al. 2019).

In Central America, four to five million people annually are estimated to be infected with dengue (Hotez et al. 2014). Factors associated with dengue in Central America have been investigated (Troyo et al. 2009, San Martin et al. 2010, Zambrano et al. 2019), yet there are few studies of dengue in El Salvador (Hayes et al. 2003). El Salvador is a country of approximately 6 million and one of the most densely populated and deforested countries in the Western Hemisphere (PAHO 2017, World Bank 2019). Dengue continues to be a persistent public health threat. The country has a tropical climate (Armentera et al. 2016) which is ideal for the dengue vectors *Aedes aegypti* and *Aedes albopictus*. An eradication campaign for *Aedes aegypti* in the Western Hemisphere in the 1950s and 1960s eliminated this mosquito from El Salvador (Soper 1967, Gubler 1993, Hayes et al. 2003). Unfortunately, *Aedes aegypti* was reintroduced and outbreaks of dengue have occurred since 1980 (Gubler 1993), and the mosquito is now widespread in El Salvador (Joyce et al. 2018). During a dengue epidemic in 2000 in El Salvador, a study of risk factors at the household level found that most participants were aware that mosquitoes transmitted dengue, yet 36% of homes had mosquito larvae (Hayes et al. 2003). There is currently no systematic surveillance of mosquitoes or *Aedes aegypti* in El Salvador (MINSAL 2014). In addition, Chikunguya virus and Zika which are also transmitted by *Aedes aegypti* have been reported since 2014 and 2015 in El Salvador, respectively (Wilson and Chen 2002, Higgs and Vanlandingham 2015, Shutt et al. 2017).

This study is the first to evaluate multiple environmental variables and their association with dengue cases at the municipality (county) level to examine factors associated with dengue throughout the country of El Salvador. The objective of this study was to determine which environmental factors are associated with dengue in the years 2011-2013, prior to the introduction of Chikungunya and Zika viruses. We hypothesize that temperature, precipitation, and non-forested area are associated with dengue cases. Understanding these factors could contribute to focusing efforts to reduce dengue incidence in El Salvador.

Methods

Description of the study area

El Salvador is located in Central America within the Pacific slope (13°47'39.1" N, 88°53'47.5" W). The country has a territorial extension of 20,415 km^2 with an approximate population of 6.4 million (World Bank 2019); it is divided into 14 departments and 262 municipalities (Fig. 1). The climate of El Salvador is tropical, and the dry season extends from November to April while the rainy season lasts from May to October (Armenteras et al. 2016). In El Salvador like other countries of Central America, extreme maximum temperatures are increasing, with high temperatures being more pronounced in the dry season (Aguilar et al. 2005, Quesada-Hernandez et al. 2019). El Salvador is frequently influenced by tropical hurricanes and is highly vulnerable to floods and droughts (Aguilar et al. 2009). Much of the primary forests have been deforested by agriculture and livestock and almost all ecosystems are categorized as threatened or endangered according to IUCN criteria (Crespin and Simonetti 2015, 2016). However, the country currently has a forest coverage of ~37% remaining throughout the country (mainly secondary forests and forest plantations) and much of the territory is dominated by urban areas, agriculture, and livestock (MARN 2018) It is estimated that in El Salvador, 71% of the population is urban and 29% is rural, and the poverty rate is 29% (World Bank 2019). The health care system is composed of 30 public National Hospitals and nearly 600 additional community health units distributed throughout the country (DIGESTYC 2007).

Dengue cases

Confirmed dengue cases were obtained from 262 municipalities in El Salvador. The data were available as total confirmed dengue cases per year (2011-2013) for each municipality, from the Ministry of Health of El Salvador. A municipality in El Salvador is similar to a county in California. There was no distinction made of whether a dengue case was one of the four dengue serotypes (Dengue 1, 2, 3 or 4). Data for subsequent years (after 2013) were not included in the present study since Chikungunya was reported since 2014 and Zika since 2015. Confirmed dengue cases are reported to the national surveillance system from the diagnoses of patients who expressed acute febrile illness (fever over 40°C, ~104 F) and two or more of the following symptoms (headache, retroorbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leucopenia (WHO 2020), as well as by lab confirmation of a positive ELISA test for IgM dengue antibodies (MINSAL 2010). The confirmed dengue cases per municipality for 2011-2013 were used to calculate the mean number of dengue cases per year (2011-2013) and the incidence of dengue per 100,000 for each municipality for 2011, 2012, and 2013, and the mean yearly incidence for 2011-2013 combined.

Environmental and climate variables

The precipitation and temperature data were obtained from databases of the Ministry of Environment and Natural Resources of El Salvador (MARN). The data comes from 24 meteorological stations distributed throughout the country. Daily temperatures were reported and were used to calculate the means for the minimum, maximum, and mean temperature (ºC) for each municipality for 2011, 2012, and 2013, and for 2011-2013 combined. Daily precipitation values were used to determine the cumulative total precipitation (mm) for each year, and for the three years (2011-2013) combined. For each municipality, the temperature and precipitation values were assigned from the meteorological station which was closest in distance and most similar in elevation. To assign a meteorological station to a municipality, the geographic coordinates were found for the 262 municipalities (county seats) and for the 24 meteorological stations and used to calculate distances between each municipality and nearby weather stations. If two stations were equal distance to a municipality, the station with a more similar elevation was assigned. The environmental variable of % forest cover per municipality was estimated using data available for El Salvador, which comes from high-resolution global maps (Hansen et al. 2013). Images have a resolution of 30 m^2 and coverage of forest canopy ≥ 25% with trees over 5 meters high. Using this data, percent area with forest coverage and percent area non-forested (1-forested area) were determined for each municipality. A significant negative relationship has previously been reported between forest cover and dengue (Husnina et al. 2019) so we used non-forested area as a variable. Geographic and spatial data were processed using Geographic Information System ArcGIS 10.3 software.

Statistical analysis

Descriptive data were first produced to examine the total number of confirmed dengue cases per year, mean cases per year for 2011-2013, dengue incidence per 100,000 people per year for each municipality, and mean dengue incidence per year (2011-2013). Dengue incidence per year was calculated as dengue cases divided by the total population of the municipality, multiplied by 100,000. Subsequently, negative binomial regression models were run to examine the relationship between environmental variables and dengue cases.

Environmental variables included elevation, non-forested area, total annual precipitation 2011-2013, and mean annual temperature for 2011-2013. All variables were used for each municipality. Negative binomial regression is used when the dependent variable is a count variable with a Poisson-like distribution. A negative binomial regression is used rather than Poisson when the data are considered to be over dispersed. Before running each model, the multicollinearity of the variables in each model was assessed with the Variation Inflation Factor (VIF) statistic; all variables included in models had values <3. All analyses were performed using the software STATA 15.1. The population density (total population/area in the municipality) for each municipality was included as a control variable in all models. For all models, the incidence ratios (IRR) were determined. Incidence ratios estimate the rate of change in the dependent variable associated with each unit increase or decrease in the independent variable in the model. Variables with p-values <0.05 were considered significant.

Results

Incidence of Dengue in municipalities

The total number of confirmed dengue cases per year (2011-2013) ranged from 4,668 in 2011 to a high of 13,689 cases in 2012, with a total of 29,764 confirmed cases for the three years. The mean incidence of dengue cases per 100,000 per year (2011-2013) was mapped for each municipality (Fig. 2); the overall mean incidence per year for El Salvador for 2011 to 2013 was 135.40/100,000 people. The ten municipalities with the highest cumulative number of cases from 2011-2013 and their respective incidence rates (2011-2013) came from the highly urbanized municipalities of Santa Ana [Department (Dept): Santa Ana] (2117 cases; 287.53/100,000), San Salvador (Dept: San Salvador) (1,888 cases; incidence 199/100,000) and its suburbs, and from San Miguel (Dept: San Miguel) (2,523; 187/100,000) (Table 1). The municipalities with the ten highest incidence rates ranged from 300-600/100,000; eight of the ten municipalities had less than 10,000 inhabitants, with one exception being Ilopango, San Salvador (Table 1, Fig. 2).

Municipalities with the lowest number of cases were primarily in the departments of Chalatenango and Morazán, mountainous departments which border Honduras, and included, San Isidro Labrador, San Antonio de La Cruz, Potonico, San Francisco Lempa, Ojo de Agua, Las Flores (all Chalatenango), Arambola, Torola and Perquín (Morazán, all 0-2cases).

Climatic variables

The minimum, mean, and maximum temperatures for each year (2011-2013) and for the three years combined were determined for each municipality (Table 2). The average maximum temperatures varied from 21°C to 36°C (mountainous and coastal regions), the mean temperature ranged from 17 to 29 °C, while the minimum temperatures averaged from 12°C to 24°C (Table 2, Fig. 3). The annual mean cumulative precipitation 2011-2013 oscillated between 1,482-2,855 mm (Fig. 4). The greatest forest cover predominates in the volcanic zones (Central Graben region), and at high elevation in large mountain ranges in the central and northern part of the country, near Guatemala and Honduras (Fig. 1,5). The non-forested areas are located from approximately 0-800 meters in elevation and coincide with urban areas having higher population densities such as Santa Ana, San Salvador, and San Miguel (Figs. 1,5).

Negative binomial regressions

In the case of environmental variables, the model was significant ($X^2_5 = 101.26$, $p < 0.001$) and found a positive relationship between dengue cases and non-forested area (Table 3). For each percent increase in non-forested area, there was a 46% increase in dengue (Table 3). The cumulative precipitation was related to a very modest decrease in dengue cases (<1%). Municipalities with higher cumulative precipitation had lower dengue cases. Municipalities with higher mean temperatures had lower dengue cases

(Fig. 2-4). For each 1°C increase in the mean annual temperature, the number of dengue cases decreased by 14%. Elevation was not significant in the model ($p<0.05$).

Discussion

This study investigated the relationship between environmental factors and dengue cases from 2011 to 2013 in El Salvador, Central America. El Salvador is a densely populated country with a tropical climate and pronounced rainy and dry seasons. There were over 29,000 dengue cases during the three-year period, with an average yearly incidence rate of 135.40/100,000 inhabitants. Models to investigate environmental factors associated with dengue cases found that temperature, precipitation, and non-forested area, were significant predictors.

The mean incidence rate of dengue in El Salvador was similar to the incidence previously reported in El Salvador and in nearby Honduras, Central America. A previous study of El Salvador found that the city Aguilares, located ~40km from the capital San Salvador, had an infection rate of 180/100,000 near the peak of an epidemic (Hayes et al. 2003). In neighboring Honduras, a dengue incidence was reported of 479/100,000 in 2016-2019 in Cortez, a densely populated department (Zambrano et al. 2019). In the current study, the highest number of mean cases (2011-2013) were found in densely populated municipalities in the urban capital San Salvador and its surrounding municipalities Soyapongo, Santa Tecla, San Martín, and Ilopango, and in the cities of Santa Ana and San Miguel. The mean incidence per year (2011-2013) in these municipalities ranged from 195 to 287/100,000. The ten municipalities with the highest yearly incidence rates ranged from 300-600 cases /100,000 people and were in the west and center of the country. One municipality with among the highest mean cases per year and the highest incidence rates was Ilopongo, San Salvador (380/100,000).

When examining environmental variables, a positive relationship was found between dengue cases and non-forested area. Deforestation is widespread in El Salvador with an estimated ~37% percent of land having forest cover (MARN 2018). Other studies report a relationship between numbers of dengue cases and forest cover, which can be measured directly or indirectly through vegetation indices (Araujo et al. 2015, Martinez et al. 2018, Swain et al. 2019, Husnina et al. 2019). In Thailand, residences further from natural vegetation had a higher risk of dengue seroprevelance (Troyo et al. 2009). Unforested urban areas generate heat islands, characterized by high temperatures and limited vegetation, and these areas promote the transmission of dengue (Araujo et al. 2015). In El Salvador, the decrease in forest cover is associated with large extensions of urban areas and the extensive development of agriculture and livestock. The relationship identified in this study between dengue cases and non-forested areas may be due to the fact that much of the environment of El Salvador is highly anthropogenized.

Climatic variables including temperature and precipitation are important predictive variables of dengue. Tropical regions such as Central America have pronounced rainy and dry seasons. Cumulative annual precipitation in tropical areas can vary; in this study, El Salvador reported mean cumulative precipitation between 1,400-2,800 mm precipitation. In neighboring Honduras, higher numbers of dengue cases were reported in the rainy season than the dry season (Zambrano et al. 2012,2019), and a similar trend is reported in other countries with tropical climates (Pham et al. 2011, Colon-Gonzalez et al. 2013). Dengue can also be more frequent at the beginning and end of the rainy season (Nakhapakorn and Tripathi 2005). The present study in El Salvador was limited to yearly dengue cases (not weekly), yet it is likely that a similar pattern occurs with more dengue cases during the rainy season than the dry season (CDC 1983, MINSAL 2014). A significant relationship was found with precipitation, with a modest decrease in cases (<1%) with each mm of additional rainfall. The highest levels of cumulative annual precipitation fall in more forested regions at high elevation, areas which are less populated, which may explain the slight decrease in cases with precipitation (Fig. 2,3). Overall, the climate of El Salvador is tropical with both temperature and precipitation being highly suitable for mosquito development.

In this study, temperature was a significant predictor of dengue cases, although the relationship was negative between cases and increasing temperature. The relationship of temperature and dengue cases has been found to be positive or negative (Pham et al. 2011, Swain et al. 2019), and at times no relationship has been found between the two (Zambrano et al. 2019). The variation in the relationship of temperature and dengue cases in El Salvador may relate to the scale of the study. For example, a study in Thailand (Nakhapakorn and Tripathi 2005) reported an annual mean monthly temperature of 26 °C, and temperature was a negative predictor of cases; however, a mean monthly temperature of 42 °C was reported in April, followed by cooler temperatures in the rainy season, when more dengue cases occur. A similar pattern was shown by Swain et al. (2019). The present study of El Salvador is limited in that dengue cases were obtained at the yearly level for each municipality, rather than at a weekly or monthly level, so cases could not be related directly to mean monthly temperatures. Mean monthly temperatures in El Salvador follow the pattern described for other tropical countries; temperatures are higher at the beginning of the rainy season (April) and then decline during the rainy season (Fig. 6).

Another explanation for the negative relationship of temperature with dengue cases is that El Salvador's largest cities (most populated municipalities) and the cities with the highest dengue incidence are for the most part not in the eastern part of the country, where much higher temperatures occur (Fig. 2,3). In El Salvador, the mean annual temperatures for 2011-2013 in municipalities ranged from 17-29 °C. Municipalities with large populations such as San Salvador, Santa Ana, and San Miguel and high incidence rates had mean annual temperatures between 25-28°C, while municipalities with the highest mean annual temperatures (28-29 °C) were coastal or below ~600 m with smaller populations and lower dengue incidence. In addition, the eastern part of the country has the highest temperatures (Fig. 3), while incidence of dengue was lower in the eastern region of the country (Fig. 2). Some municipalities with high mean temperatures also have maximum temperatures which could approach or exceed the developmental thresholds for the mosquito vector (Almeida-Costa et al. 2010, Mohammed and Chadee 2011). As mentioned, there is no systematic mosquito surveillance in El Salvador, so it is not possible in this study to relate mosquito abundance to dengue cases.

In summary, this study is one of the first to describe dengue cases throughout the country of El Salvador. The objective herein was to describe the distribution of dengue cases prior to the first outbreak of Chikungunya and Zika viruses in 2014 and 2015 in El Salvador. We report a pattern of the highest number of dengue cases primarily in the capital San Salvador and its surrounding municipalities, as well as in two large cities in the west and east of the country, Santa Ana and San Miguel respectively. Other cities with the highest incidence occur in the western and central part of the country. The incidence of dengue cases is on the level of other epidemiological outbreaks. The distribution of reported confirmed cases is not likely a representation of where hospitals or clinics occur in the country, as clinics are widespread and dengue cases were reported countrywide, including in the smallest villages. The primary mosquito vector *Aedes aegypti* which transmits dengue is anthropogenic, and highly urbanized conditions promote the propagation of the insect, leading to exponential transmission of dengue in populated areas.

There are several limits to this study. One limit is that dengue cases were obtained at the yearly level, so we were not able to report the peak week or month of dengue cases for 2011 to 2013, and dengue cases could not be directly related to fluctuating monthly temperatures which occur throughout the year. Another limit is that the spatial scale of this study is at the municipality level for the entire country. Studies of dengue have examined factors associated with cases at the local, state, country, and even regional or global levels. Future analysis of dengue cases in El Salvador might benefit from a fine-scaled examination of cases in the cities with the highest incidence, as well as in the three large metropolitan areas of San Salvador, Santa Ana, and San Miguel which had high dengue number of cases. This could provide more detailed information about factors associated with dengue and aid in focusing health promotion and vector control efforts to reduce mosquito breeding sites. The tropical climate of El Salvador and high population density make it a region where dengue prevention will remain a pressing issue.

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FIG 1. Map of 262 municipalities in El Salvador, shaded by elevation (m). Darker shading indicates higher elevation. The capital city San Salvador is indicated by SS on the map.

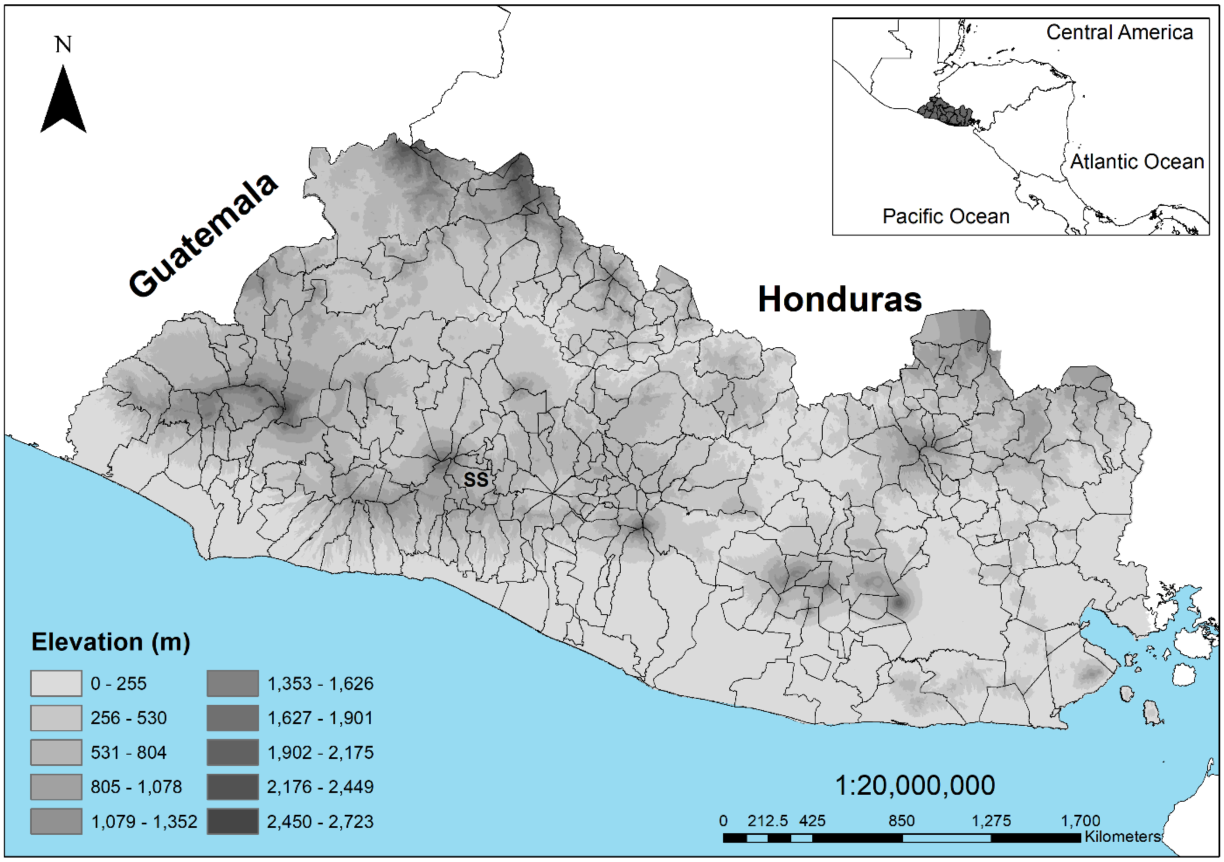

FIG 2. Mean yearly incidence per 100,000 of dengue cases in El Salvador (2011-2013).

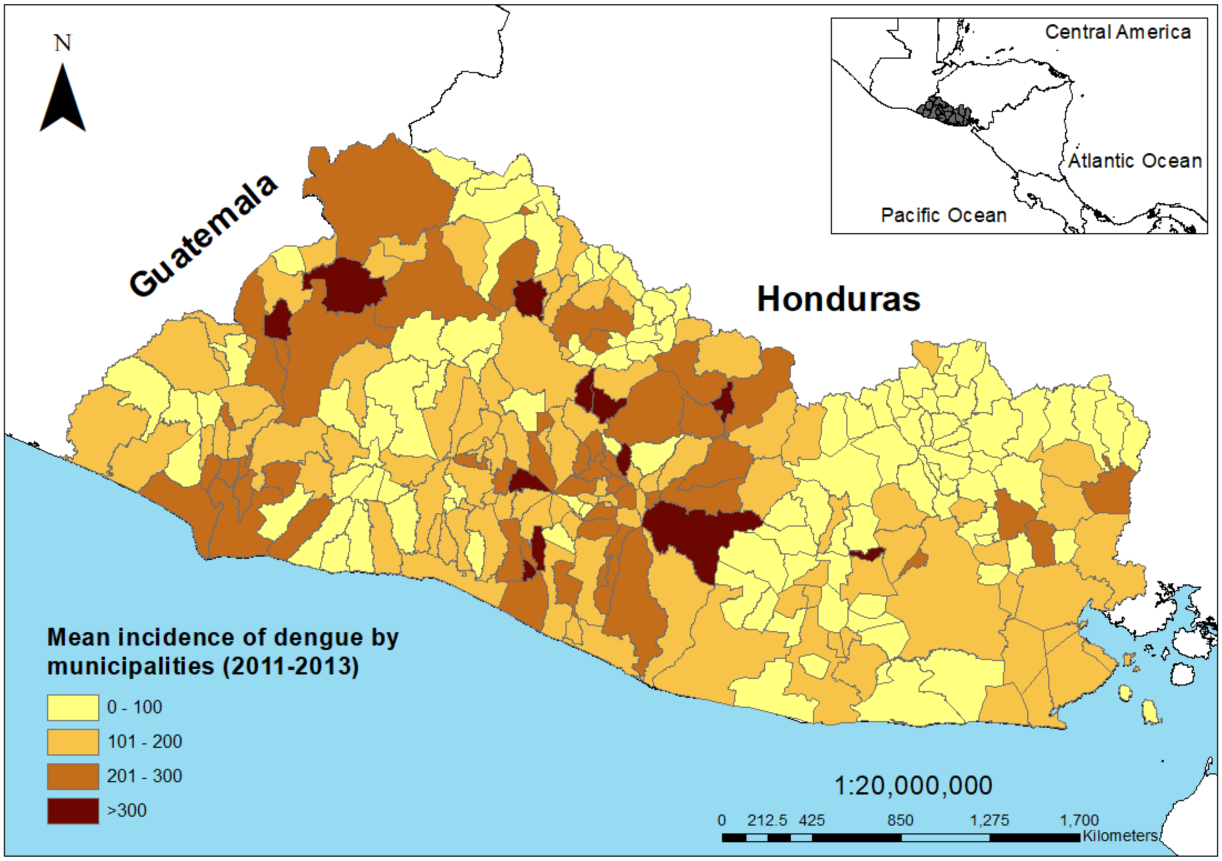

FIG. 3. The mean annual temperature (2011-2013) for each municipality of El Salvador.

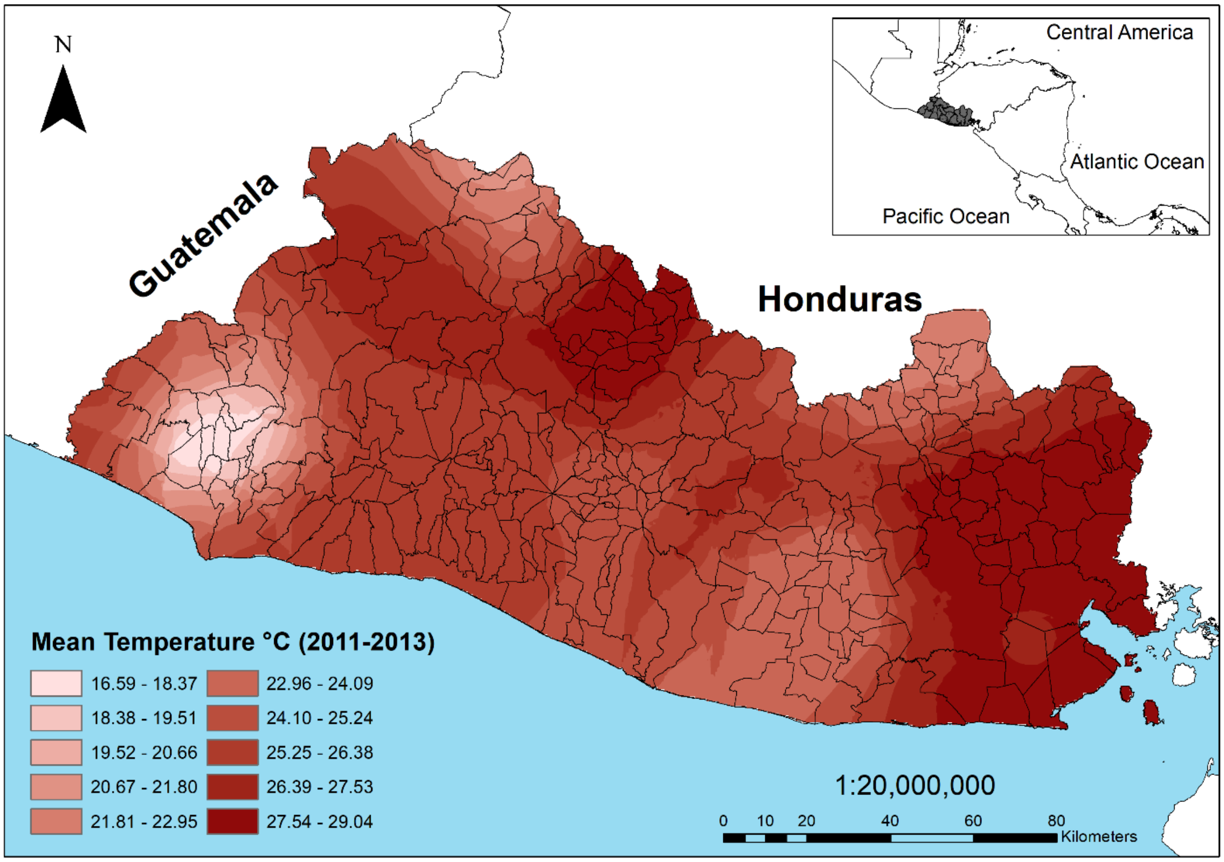

FIG. 4. The mean cumulative precipitation for 2011-2013 for each municipality in El Salvador.

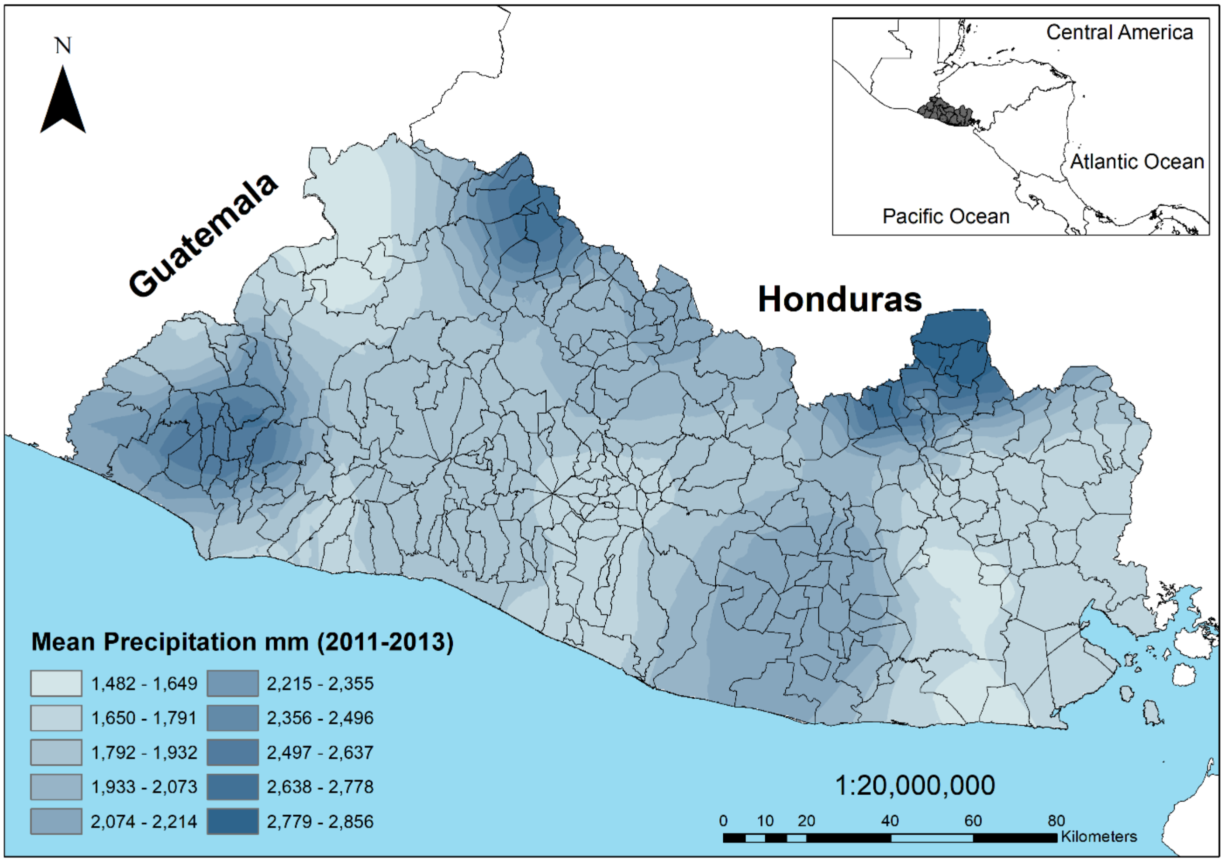

FIG. 5. The area with forest coverage for each municipality in El Salvador.

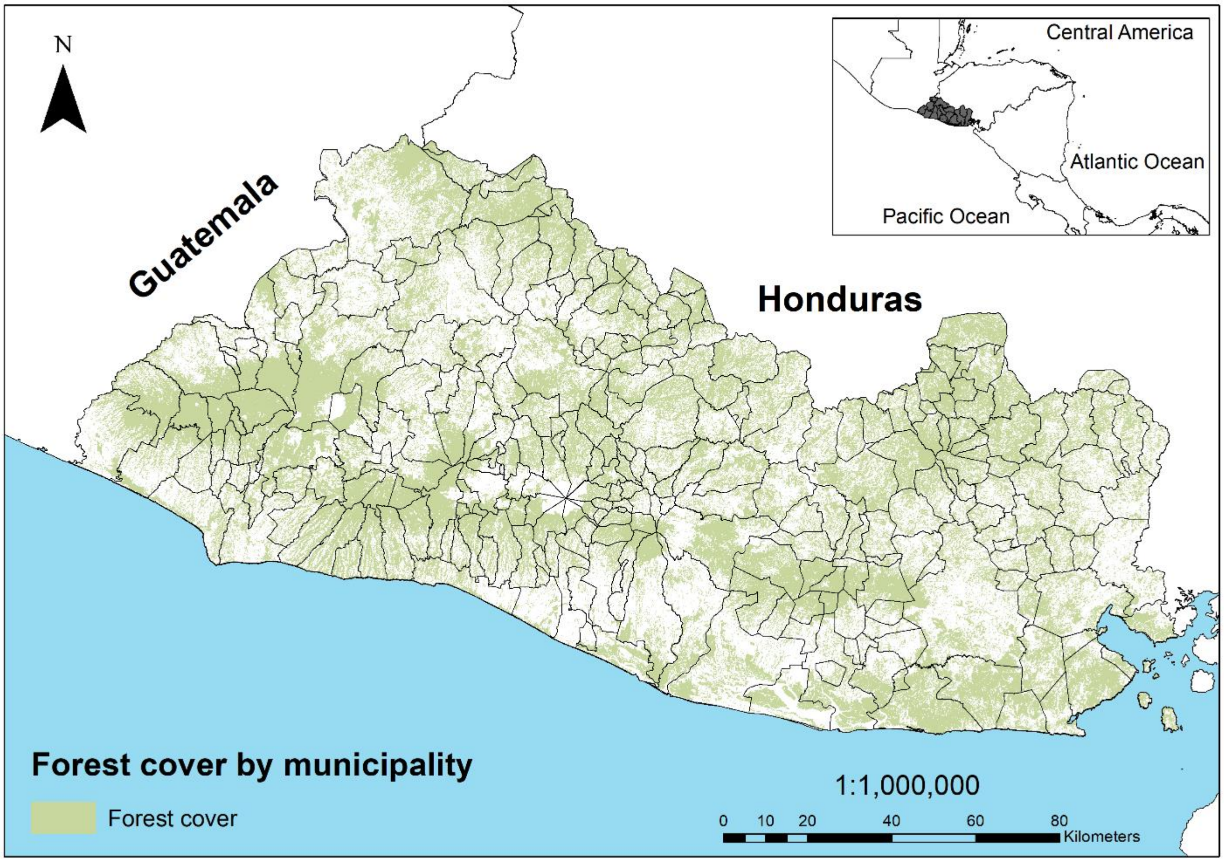

Fig.6. The mean monthy temperature for 2011-2013. The square represents the mean temperature, triangles are the mean maximum temperature, and inverted triangles indicate mean minimum temperature. Black color represents 2011 data, blue is 2012 data, and red indicates 2013.

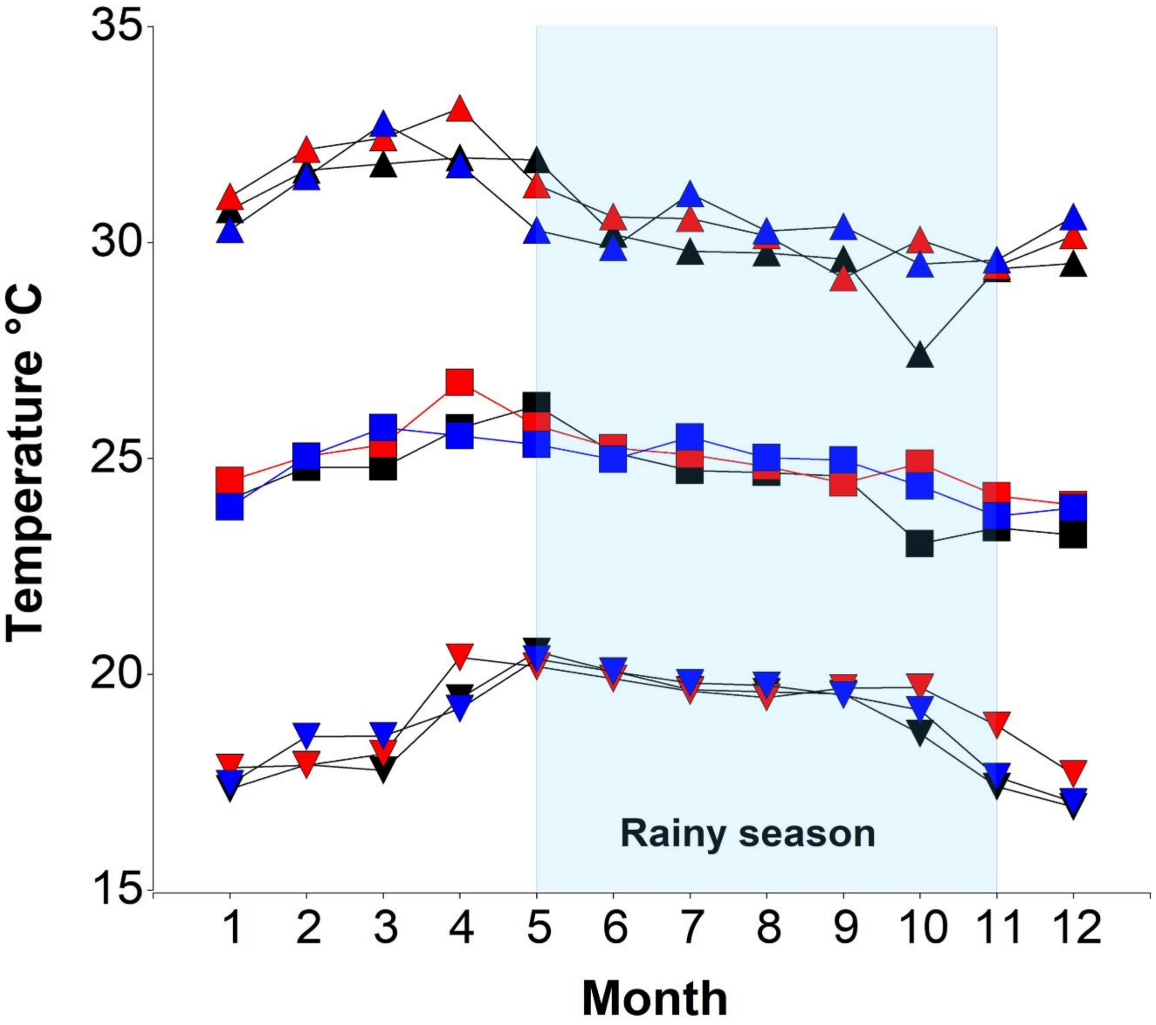

Table 1. Ten municipalities with the highest mean number of dengue cases per year (2011-2013), their associated population, and dengue incidence rate per 100,000**.**

Department	Municipality	Mean Cases 2011-2013	Population	Mean Rate 2011-2013
Santa Ana	Santa Ana	706	245,421	287.53
San Salvador	San Salvador	629	316,090	199.10
San Salvador	Soyapango	613	241,403	254.07
San Miguel	San Miguel	410	218,410	187.87
San Salvador	Ilopango	394	103,862	379.67
San Salvador	Mejicanos	305	140,751	216.46
La Libertad	Santa Tecla	229	121,908	188.12
Ahuachapan	Ahuachapan	187	110,511	169.21
San Salvador	San Martin	177	72,758	243.27
San Salvador	Tonacatepeque	176	90,896	193.63

Table 2. The minimum, mean, and maximum daily temperature (°C), the total precipitation per year (mm), and confirmed dengue cases for each year, 2011-2013.

Variable	Obs	2011	2012	2013	range
Min Temp °C	24	18.74 ± 3.26	18.94 ± 3.36	19.07 ± 3.34	11.79-23.57
Mean Temp °C	24	24.61 ± 3.96	24.83 ± 3.97	24.94 ± 3.91	16.59-29.04
Max Temp °C	24	30.48 ± 4.81	30.79 ± 4.61	30.79 ± 4.61	21.38-35.84
Precipitation (mm)	24	2,391.23	1,779.73	1,873.15	1482-2855
Total Dengue Cases	262	4668.00	13689.00	11407.00	29,764.00

Data is from 24 meteorological stations. Dengue cases come from 262 municipalities.

Table 3. Negative binomial regression for environmental variables associated with dengue cases 2011-2013 (X^2_5 = 101.26, p < 0.001, *n*=262).

Environmental Variables	IRR	Std. Err.	z	P>z	[95% Conf. Interval]	
Population density	1.001	0.000	4.93	0.000	1.000	1.001
Non-forested area	1.456	0.218	2.51	0.012	1.086	1.953
Elevation	0.999	0.000	-1.54	0.124	0.999	1.000
Precipitation	0.998	0.000	-4.74	0.000	0.998	0.999
Temperature	0.854	0.034	-3.74	0.000	0.787	0.928
Intercept	96266.99	166175.7	6.64	0.000	3260.958	283954

Chapter 3: Socioeconomic Factors Associated with Dengue in El Salvador

Introduction

Dengue is an arbovirus transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue is widespread and causes 50-100 million illnesses a year (Gibbons and Vaughn 2002, WHO 2009). Socioeconomic factors such as income, education, and urban conditions can contribute to dengue incidence. Known risk factors for dengue include low income (Brunkard et al. 2004), educations levels (Spiegel et al. 2007), and population density (Dickin et al. 2013, Swain et al. 2019). Poor garbage disposal or waste collection are commonly associated with dengue (Mourya et al. 2004, Thammapalo et al. 2008) as are homes that contained discarded containers or tire casings (Hayes et al. 2003). The incidence of dengue can increase due to peri-domestic water storage but has also been shown to increase when there is piped water (Colon-Gonzalez et al. 2013), suggesting the association with water storage may be complex. The absence of air conditioning and street drainage are also potential risk factors (Brunkard et al. 2007)**.** Understanding socioeconomic factors associated with dengue may help manage the mosquito vectors which transmit this disease.

In Central America, four to five million people annually are estimated to be infected with dengue (Hotez et al. 2014). Few studies of socioeconomic factors associated with dengue have been conducted in El Salvador (PAHO 2017, World Bank 2019). Dengue continues to be a persistent public health threat. The country has a tropical climate (Armentera et al. 2016) which is ideal for the dengue vectors *Aedes aegypti* and *Aedes albopictus*. Previous mosquito eradication campaigns for *Aedes* *aegypti* in the Western Hemisphere in the 1950s and 1960s eliminated this mosquito from El Salvador (Soper 1967, Gubler 1993, Hayes et al. 2003). However, the mosquito has since been reintroduced into El Salvador, and is now widespread (Joyce et al. 2018). A clinical study of risk factors in El Salvador at the household level found that most participants were aware that mosquitoes transmitted dengue, yet many homes still had mosquito larvae (Hayes et al. 2003). A larger scale national study of factors contributing to dengue in El Salvador would contribute to knowledge of which conditions are the strongest risk factors.

The objective of this study was to determine which socioeconomic factors are associated with dengue in the years 2011-2013, prior to the introduction of Chikungunya and Zika viruses. Socioeconomic variables that may be associated with dengue include education, housing infrastructure and population density. Understanding these factors could contribute to focusing efforts to reduce dengue incidence in El Salvador.

Methods

Description of the study area

A general description of El Salvador and the municipalities (counties) is provided in the previous environmental chapter. The 262 municipalities are outlines in Figure 1.

Dengue cases

Confirmed dengue cases were obtained from 262 municipalities in El Salvador. The data were available as total confirmed dengue cases per year (2011-2013) for each municipality, from the Ministry of Health of El Salvador, as described in the previous environmental chapter.

Socioeconomic variables

The socioeconomic variables were obtained from each municipality from the 2007 census of the Statistics and Census Bureau of El Salvador (DIGESTYC 2007), while poverty rate was obtained from the Fondo de Inversion Social para El Desarrollo Local (FISDL 2005). A total of 12 socioeconomic variables obtained for each municipality were included in these analyses (Table 1). Socioeconomic variables included poverty rate, literacy rate, and school attendance; yearly household income was not a variable in the census data, so poverty rate was used. Poverty rate came from another countrywide study at the municipality level in El Salvador; the source is called FISDL. Other variables included in the socioeconomic analysis included infrastructure related variables, specifically percent of households with lighting by electricity, percent of homes with sanitation service, percent of homes with municipal garbage service, and potable water. Four additional variables included were household construction materials used for flooring, which were cement foundation, cement bricks, ceramic tiles, and earthen floors. Population density was used as a control variable in all models, as described in the previous environmental chapter. These variables were used in the statistical analyses described below.

Statistical analysis

Descriptive data were first produced to examine the total number of confirmed dengue cases per year, mean cases per year for 2011-2013, dengue incidence per 100,000 people per year for each municipality, and mean dengue incidence per year (2011-2013) (see Ch. 2). Subsequently, negative binomial regression models were run to examine the relationship between socioeconomic variables and dengue cases.

Several negative binomial models were run for socioeconomic variables. The first model considered the factors of poverty rate, literacy, and school. The second model considered four infrastructure variables, municipal trash service, sanitary service, electricity for lighting, and potable water. A third negative binomial regression model was run to examine if any of four household flooring materials (cement foundation, cement brick, ceramic tile, or earth floor) as predictors of dengue cases. All variables were used for each municipality (county level) for the 262 municipalities in the country.

Negative binomial regression is used when the dependent variable is a count variable with a Poisson-like distribution. A negative binomial regression is used rather than Poisson when the data are considered over dispersed. Before running each model, the multicollinearity of the variables in each model was assessed with the Variation Inflation Factor (VIF) statistic; all variables included in models had values <3. All analyses were performed using the software STATA 15.1. The population density (total population/area in municipality) for each municipality was included as a control variable in all models. For the final three models, the incidence ratios (IRR) were determined. Incidence ratios estimate the rate of change in the dependent variable associated with each unit increase or decrease in the independent variable in the model. Variables with p-values <0.05 were considered significant.

Results

Incidence of Dengue in municipalities

The incidence of dengue was discussed in the environmental chapter and is illustrated in Figure 2.

Socioeconomic variables

The mean and range were determined for socioeconomic variables included. The mean percent literacy in the 262 municipalities was 84% (range 54-96%), school attendance was 85.3% (63.4-96.2%), and poverty rate was 52.02% (10.6-88.5%). For all municipalities, there was an average of 69.2% of households with potable water (3-99%), 88.6% of households had indoor plumbing (42-95%), households with electricity was 82.1% (30-99%), and 50% (0-96%) have municipal garbage service. The home flooring construction consisted of 18.12% of homes with cement foundations, 36.6% with cement brick flooring, 6.7% with ceramic tiles and 18.9% with earth floors.

Negative binomial regressions

The negative binomial regression for the socioeconomic variables poverty, literacy and school attendance on dengue cases was significant (X^2_3 = 214.56, $p < 0.001$) and all three variables were significant predictors ($P<0.05$) (Table 2). There was a negative relationship of dengue cases with poverty, literacy, and school attendance, with each percent increase in each variable there was approximately a 5% decrease in dengue cases (Table 2). A second negative binomial regression for infrastructure variables on dengue cases was also significant (X^2_4 =190.11, $p < 0.001$); there was a positive relationship of dengue cases with potable water, sanitary service and municipal waste service in homes ($P<0.001$) (Table 3). For each percent increase in sanitary service, there was a 3% increase in dengue cases. While potable water was a significant predictor, the associated incidence rate ratio for potable water was less than 1%. Finally, the percent of homes with municipal trash service was highly related to dengue cases, those with trash service had 8 times the rate of dengue compared to those without trash service. Materials used for home flooring were significantly related to dengue (X^2_4= 122.17, p < 0.001) (Table 4), and homes with cement brick floors were the strongest predictor (P<0.05), those with cement brick floors had 17 times the rate of dengue compared to those without cement brick floors.

Discussion

This study investigated the relationship between socioeconomic factors and dengue cases from 2011 to 2013 in El Salvador, Central America. El Salvador is a densely populated country with a tropical climate and pronounced rainy and dry seasons. There were over 29,000 dengue cases during the three-year period, with an average yearly incidence rate of 135.40/100,000 inhabitants. Models to investigate sociological factors associated with dengue cases found that municipal trash service, sanitary service, potable water, cement brick flooring in homes and population density were significant predictors.

The mean incidence rate of dengue in El Salvador was similar to the incidence previously reported in El Salvador and in nearby Honduras, Central America. In the current study, the highest number of mean cases (2011-2013) were found in densely populated municipalities in the urban capital San Salvador and its surrounding municipalities Soyapongo, Santa Tecla, San Martín, and Ilopango, and in the cities of Santa Ana and San Miguel. One municipality with among the highest mean cases per year and the highest incidence rates was Ilopongo, San Salvador (380/100,000). Urban areas with high population densities are the ideal environment for the transmission of dengue which occurs through a human-mosquito transmission cycle.

Built area and urbanization have a significant relationship with dengue cases in numerous locations including Thailand, Honduras, Mexico, and India (Nakhapakorn and Tripathi 2005, Zambrano et al. 2012, Colon-Gonzalez et al. 2013, Swain et al. 2019). There was a positive relationship between sanitary service, potable water, municipal trash service, and cement brick flooring in homes with dengue cases in El Salvador. While some studies find water storage in rural areas relates to increased dengue, others note that in cities with access to running water, occasional water stoppages also lead to water storage and increased dengue (Colon-Gonzalez et al. 2013). In the present study, potable water was a significant but marginal predictor of dengue cases. This may be because water storage in barrels and cisterns is common in both urban and rural areas of El Salvador, whether or not there is running water in homes. In rural areas, families store water for convenience, while in cities the storage of water is common to ensure water availability during occasional stoppages in delivery. Households with sanitary service (toilets and latrines) were a significant predictor of dengue cases. This may be due to additional water sources which provide potential above and below ground sites for mosquito breeding.

One objective was to describe the distribution of dengue cases prior to the first outbreak of Chikungunya and Zika viruses in 2014 and 2015 in El Salvador. We report a pattern of the highest number of dengue cases primarily in the capital San Salvador and its surrounding municipalities, as well as in two large cities in the west and east of the country, Santa Ana, and San Miguel respectively. Other cities with the highest incidence occur in the western and central part of the country. The primary mosquito vector *Aedes aegypti* which transmits dengue is anthropogenic, and highly urbanized conditions promote the propagation of the insect, leading to exponential transmission of dengue in populated areas.

There are several limits to this study. One limit is that dengue cases were obtained at the yearly level, so we were not able to report the peak week or month of dengue cases for 2011 to 2013, and dengue cases could not be directly related to fluctuating monthly temperatures which occur throughout the year. Another limit is that the spatial scale of this study is at the municipality level for the entire country. Studies of dengue have examined factors associated with cases at the local, state, country, and even regional or global levels. Future analysis of dengue cases in El Salvador might benefit from a fine-scaled examination of cases in the cities with the highest incidence, as well as in the three large metropolitan areas of San Salvador, Santa Ana, and San Miguel which had high dengue number of cases. This could provide more detailed information about factors associated with dengue and aid in focusing health promotion and vector control in areas with the highest incidence in order to reduce mosquito breeding sites. In addition, a national surveillance system for mosquitoes and mosquito-borne disease could contribute to reducing dengue cases. The tropical climate of El Salvador and high population density make it a region where dengue prevention will remain a pressing issue.

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FIG 1. Map of 262 municipalities in El Salvador, shaded by elevation (m). Darker shading indicates higher elevation. The capital city San Salvador is indicated by SS on the map.

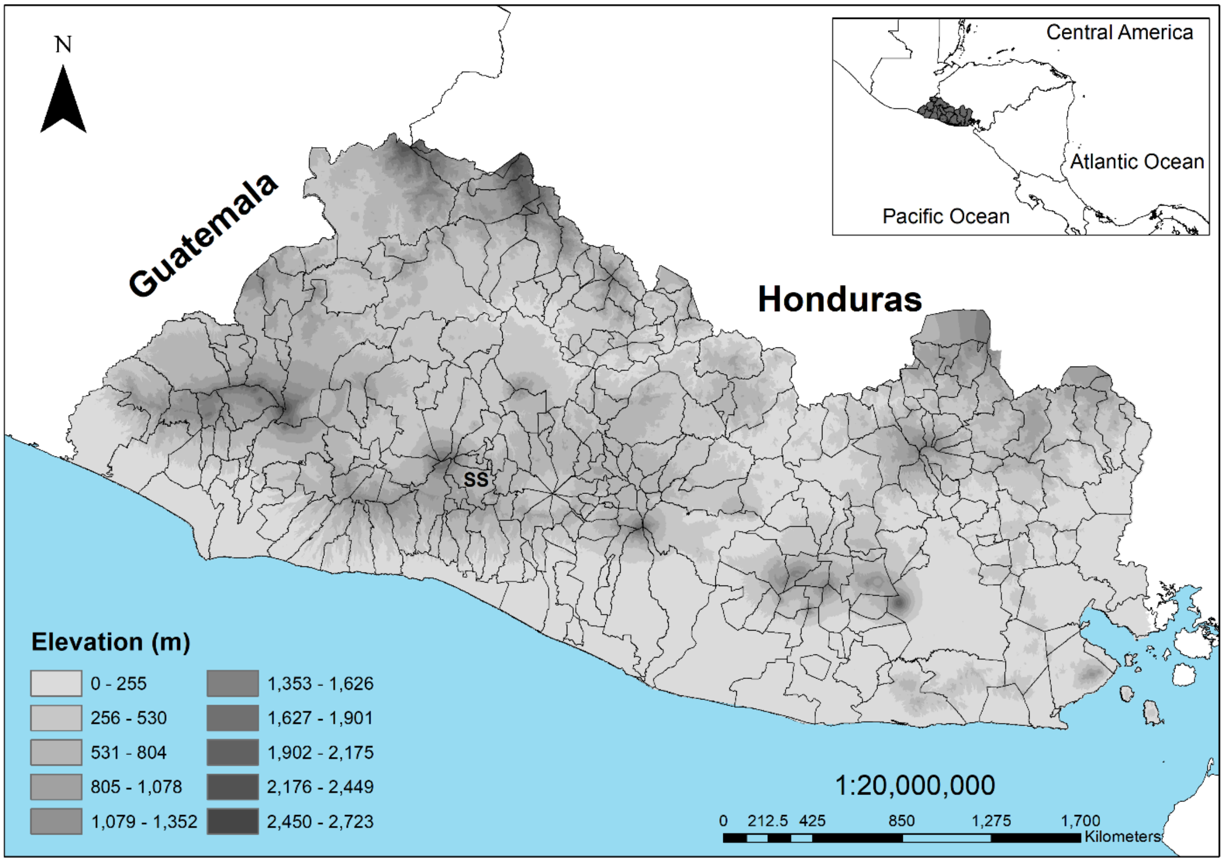

FIG 2. Mean yearly incidence per 100,000 of dengue cases in El Salvador (2011-2013).

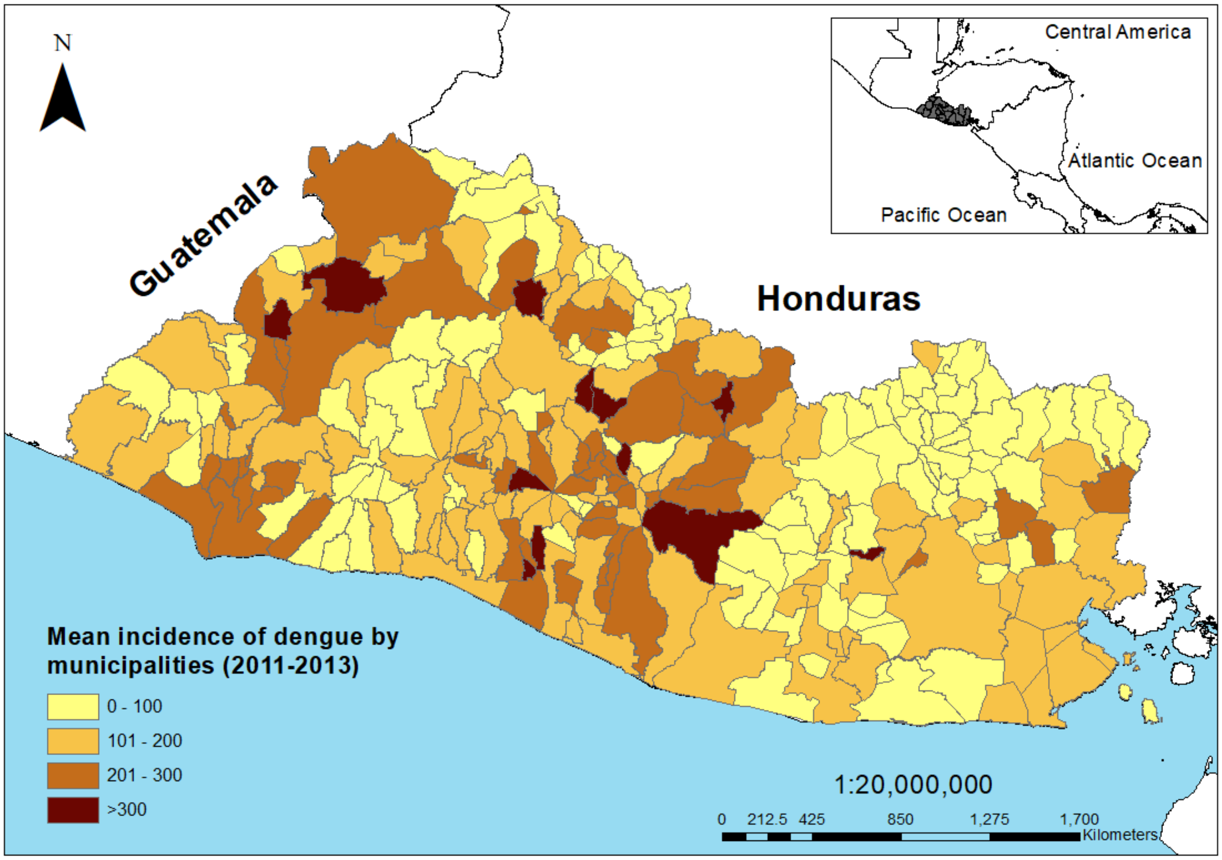

Table 1. El Salvador Census 2007 variables available at the municipality (county) level

Variable Name	Description of variable
Poverty rate*	Percent of population in poverty
School Attendance	Percent attending schooling in the formal system, age 7-14.
Literacy	A person who can read and write a paragraph is considered literate, regardless of whether or not they have attended a formal education center. Percent of population over 10 years old literate
Drinking water	Percent of homes with potable water, defined as having piped water in home or piped water on property.
Electricity	Percent of homes with electricity to provide lighting. Those not using electricity use another source such as gas or candles
Sanitary service	Percent of homes with toilet or latrine
Municipal garbage service	Percent with municipal trash service, not those who burn trash or dispose trash in the environment
Household flooring materials	Percent of homes with cement foundation, percent with cement brick floor, percent with ceramic, or percent with earthen floor

*Poverty rate variable comes from www.fisdl.gov.sv

Table 2. Negative binomial regression for socioeconomic variables associated with dengue cases 2011-2013 (X^2_3 = 214.56, p < 0.001, *n*=262).

Variables	IRR	Std. Err.	Z	P>Z	[95% Conf. Interval]	
Population density	1.0001	0.00007	1.76	0.079	0.999	1.0002
Literacy	0.946	0.013	-3.74	0.000	0.919	0.974
School attendance	0.952	0.018	-2.44	0.015	0.916	0.991
Poverty rate	0.948	0.005	-9.26	0.000	0.937	0.958
Intercept	178487.8	318089.2	6.79	0.000	5428.167	5868997

Table 3. Negative binomial regression for infrastructure variables associated with dengue cases 2011-2013 (X^2_4 =190.11, p < 0.001, *n*=262).

Variables	IRR	Std. Err.	Z	P>Z	[95% Conf. Interval]	
Population density	1.0001	0.00008	1.38	0.168	0.999	1.0002
Potable water	1.00004	0.00001	3.83	0.000	1.00002	1.00006
Electrical service	1.831	1.036	1.07	0.286	0.603	5.555
Sanitation service	1.031	0.007	4.47	0.000	1.017	1.044
Municipal trash service	8.615	3.846	4.82	0.000	3.591	20.671
Intercept	1.223	0.869	0.28	0.776	0.303	4.927

Table 4. Negative binomial regression for home flooring material variables associated with dengue cases 2011-2013 (X^2_4= 122.17, $p < 0.001$, n=262).

Variables	IRR	Std. Err.	Z	P>Z	[95% Conf. Interval]	
Population density	1.0004	0.0001	3.94	0.000	1.0002	1.0006
Cement foundation	3.723	6.021	0.81	0.416	0.156	88. 631
Cement brick	17.620	25.729	1.96	0.049	1.001	308.274
Ceramic tile	41.162	129.503	1.18	0.237	0.086	19611.79
Earthen floor	0.146	0.195	-1.44	0.151	0.011	2.009
Intercept	33.427	35.878	3.27	0.001	4.078	273.981

Chapter 4: Impact of Temperature on Oviposition of *Aedes aegypti*

Introduction

Climatic factors can strongly influence the life cycle of vectors such as mosquitoes. The mosquito, *Aedes aegypti*, is a day-biting mosquito which oviposits (lay eggs) in small water-holding containers found around homes, and transmits chikungunya, dengue, yellow fever, and Zika (Kweka et al., 2019). Reproduction and survival rates are affected by fluctuations in temperature (D. J. Gubler et al., 2001; Kovats et al., 2001). Oviposition behavior which is important for *Aedes aegypti* survival and dispersal can also be influenced by climatic factors (Dibo et al., 2008; Estallo et al., 2011, 2015; Romeo Aznar et al., 2013; Soares et al., 2015).

The most significant climatic factors for oviposition include rainfall and temperature (Estallo et al., 2011). Increased precipitation may increase vector abundance by expanding the size of existing larval habitats and creating new breeding sites (McMichael & World Health Organization, 2003). A positive correlation has been seen between the number of *Aedes aegypti* eggs collected in an ovitrap and rainfall (Almeida et al., 2013; Miyazaki et al., 2009). Moreover, a greater proliferation of *Aedes aegypti* eggs and larvae were found during periods of higher temperatures and greater rainfall (Vezzani et al., 2004). Although precipitation is a critical factor for mosquito survival and reproduction, it has also been found that *Aedes aegypti* oviposition activity can be continuous (but low) during dry winters (Estallo et al., 2011).

Establishment and oviposition are influenced by temperature for *Aedes aegypti*. Historically, *Aedes aegypti* has been able to establish in regions between the northern January and southern July 10°C isotherms (Jansen & Beebe, 2010). This mosquito is widely distributed in most tropical and subtropical areas; however, this does not reflect the maximum range of their potential distribution. In Paraiba, Brazil, optimal temperatures for development, longevity, and fecundity were between 22°C and 32°C (Beserra et al., 2006; Marinho et al., 2016). Under laboratory conditions in Trinidad, West Indies, a hatching rate of over 95% was seen after 48 hours at 24-25°C and 80% relative humidity, but the rate significantly declined as temperatures increased from 29°C to 35°C (Mohammed & Chadee, 2011). With higher temperatures *Aedes aegypti* can decrease egg-laying time and cause an increase in egg number (Costa et al., 2010). Yet, laboratory experiments have shown that *Aedes aegypti* larvae perished when the water temperature exceeded 34°C, and adults started to die when the air temperature exceeded 40°C (Christophers, 1960). The mosquito can also tolerate low temperatures to some extent. In Memphis, Tennessee, populations of *Aedes aegypti* have been known to persist in this area where minimum winter temperatures commonly fall below 0°C (Paul Reiter, 2001). The use of *Aedes* oviposition traps (i.e., ovitraps) and counts of pupae in breeding sites (i.e. pupal surveys) have been recommended as indicators of adult mosquito presence or abundance [Pan American Health Organization (PAHO),1994; Reiter & Nathan, 2001; Focks, 2003].

Aedes aegypti was first detected in California in the Central Valley counties of Fresno and Madera and the coastal county of San Mateo in 2013 (Gloria-Soria et al., 2014). From 2011-2015, *Aedes aegypti* was detected in 85 cities and census-designated places in 12 counties of California (Metzger et al., 2017; Porse et al., 2018). With the presence of *Aedes aegypti* comes the concern that mosquitoes will acquire infectious disease such as dengue and Zika from travel-related cases. Between 2015 and March 2018, 640 travel-related Zika infections were reported in California; 137 (21% of cases in the state) were in Los Angeles County (CDPH, 2018). In 2014, 141 cases of chikungunya fever and 133 cases of dengue were reported in California; these cases were of people who had recently traveled to dengue or chikungunya endemic areas (Porse et al., 2015). *Aedes aegypti* was detected in 2017 for the first time in Merced County in the city of Merced (Merced County Mosquito Abatement District, 2017a). In 2018, it was discovered in a second city (Los Banos) about 58 km from the city of Merced (Merced County Mosquito Abatement District, 2018), and more recently it was found it Winton and Atwater.

Surveillance of *Aedes aegypti* mosquito includes monitoring eggs, larvae and adults, and may use traps such as ovitraps, count larvae in containers, or use traps such as BG-Sentinel (BGS)or CDC-AGO adult traps (Metzger et al., 2017). Ovitrap egg traps are advantageous because they are inexpensive, easily deployed, and not invasive while container counts may require entering a homeowners property, and adult traps can be expensive (Centers for Disease Control and Prevention, n.d.).

Some mosquito indices include the house index (HI), container index (CI), and Breteau index (BI) (Romero-Vivas & Falconar, 2005) which are used to assess mosquito abundance. Indices can also be used to assess the risk of infectious diseases such as dengue or be used to assess if mosquito control is needed.

Understanding the oviposition of *Aedes aegypti* will contribute to containing and controlling the breeding of this vector within Merced County. The objective of this study is to examine the influence of environmental factors on oviposition of *Aedes aegypti.* An additional goal of this study was to determine the beginning of the seasonal activity of *Aedes aegypti* in Merced in order to target prevention effort in that time period. The results of this study will allow us to see what environmental factors influence oviposition of *Aedes aegypti* and to determine possible dates for this mosquito's peak activity. Previous studies suggest that highest abundance of *Aedes aegypti* eggs will be associated with high amounts of rainfall and high temperature. However, the Central Valley region of California has a hot summer with limited rainfall, so the dates with the highest abundance of the mosquito may correlate with high temperature alone. At this time, no endemic transmission of *Aedes aegypti* vector borne disease occurs in Merced County. The risk for transmission of *Aedes aegypti* vector borne disease is discussed.

Methods:

Aedes aegypti sampling

The sample location was Merced California, a city of approximately 80,000 in the Central Valley of California (Fig.1) (U.S. Census Bureau, 2019). The study site consisted of a neighborhood area in the north-east region of Merced (37.329275, -120.456259) where *Aedes aegypti* was first detected in the city. In this neighborhood, fifty-four residences were randomly selected as trapping sites. In each home's front yard, *Aedes aegypti* eggs were sampled using ovitraps. Ovitraps were black, plastic cups (750 ml) with the Merced County Mosquito Abatement District logo. Each cup contained a hay water mixture. According to the World Health Organization, hay infusions are an effective attractant to gravid *Aedes aegypti* female mosquitoes (Estallo et al., 2011). Each ovitrap contained a popsicle stick (1.5 cm x 15 cm) that had strips of seed germination paper (Nasco Company, Fort Akinson, WI) stapled to the stick to serve as an oviposition substrate and contained site numbers (Fig. 2). Traps were placed in the shade, as previous studies have found that many mosquito species prefer to oviposit in a less lighted or shaded areas (Madzlan et al., 2016).

Ovitraps were placed at field sites weekly for 12 months of the year, from September 27, 2017- September 27, 2018. Each week, ovitraps were inspected for the presence or absence of mosquito eggs. Egg sticks were first inspected for *Aedes aegypti* eggs in the field, and a rough estimate was made of the number of eggs found on each stick. Photos were taken of each stick for later egg determination. Egg sticks were bagged and transported to the lab where they were stored in a -20 freezer. Eggs were counted using a binocular microscope. Eggs were occasionally hatched in the lab and reared into adults to confirm that they were *Aedes aegypti*.

Each week the total number of eggs per trap was determined for each trap site. Egg counts were used for oviposition indices described below. The ovitrap index was calculated along with the egg density index. Both indices were calculated both for month and by week. These indices have been used successfully in previous studies (Lok et al., 1977; Morato et al., 2005; Romero-Vivas & Falconar, 2005; Soares et al., 2015).

Calculations for these indices are the following:

Ovitrap Index (OI) (Wong et al., 2011)

OI = (number of positive ovitraps ÷ number of ovitraps) *100

Egg Density Index (EDI) (Morato et al., 2005)

= total number of *Aedes aegypti* eggs found on popsicle sticks / number of positive ovitraps

Climate data

Daily values for mean temperature (°C), daily maximum and minimum temperature (°C), and precipitation (mm) from September 27, 2017- September 26, 2018 were obtained from the Oregon State University PRISM Climate Group website (PRISM Products Matrix, 2013). Mean, minimum, and maximum temperature and total rainfall were plotted to show the climatology of the city of Merced from September 27, 2017-September 26, 2018.

Trap site habitat characteristics

Each trap site was photographed to consider habitat characteristics which might influence mosquito abundance. We added a category to note if there was a combination of vegetation and overhead shade, and we also noted whether the trap site was within 3 feet or more from where a sidewalk meets a street (street gutter) which indicated water presence nearby. More habitat such as brush and more shade, and presence of street gutters/drainage might contribute to higher numbers of mosquitoes at a trap site.

Results

***Aedes* oviposition trends**

Traps at some sites were occasionally vandalized or disrupted, but 54 sites were regularly checked (Fig. 1). From January-April 2018, new traps were placed out every other week (rather than every week) and this led to 6 weeks without traps. Thus, traps were placed out during (41 weeks), with a total of 2,509 ovitraps were sampled in this study.

A total of 23,656 mosquito eggs were counted during the 12-month period. In each positive egg trap, from one to 368 mosquito eggs were found during a sample week. Very few eggs were detected in traps during cold winter months from December through April (Fig. 3); eggs were found in traps primarily from May through October while the largest number of eggs occurred the week of Aug. 23rd, 2018 (Table 1,2).

Ovitrap Index

A trap site was considered a positive premise if mosquito eggs were found in an ovitrap during any of the weekly visits during the month. From the 2,509 ovitraps, 736 traps were positive (~29%) for mosquito eggs over the course of the 1 year of trapping. The percent of positive traps per month ranged from approximately 1.2-67.2% positive per month (Table 1). From June to October, the ovitrap ranged from 43.9 – 67.3%. In July, August and September, traps ranged from 65.7 to 67.3% positive.

Egg Density Index

The mean egg density index (#eggs/#traps) varied between 1.0 and 44.6 eggs/ovitrap/month (Table 1). The lowest mean monthly egg density index occurred in the months of December through March when the index ranged from (1-3.3), and the highest mean monthly egg density index was from July to October and peaked in October at 44.6 eggs/index.

The mean number of eggs per month varied from a low of 0.01 in the month of March (low month) to a high of 26.03 in the August month (the highest month) (Table 1, Fig.3,4). The week with the highest number of eggs overall at a premise was August 23, 2018 (Table 2).

Climate data-temp and precipitation

Monthly trends

There was almost no precipitation from the months May to September (Fig. 4,5). Rainfall increased from October to April, the highest amount of precipitation 68.33 mm in the month of March. (Fig. 4,5). Interestingly, mosquitoes were present from May to October; these months had high temperatures and almost no rainfall.

For the mean monthly temperatures in Merced from 2017-2018, summer mean temperatures in months of June, July, August, and September were from 21.89 ˚C to 26.72 ˚C, (average around 23.76˚C) and winter mean temperatures December, January, and February ranged from 7.56 ˚C to 9.89 ˚C (average were near 9.20˚C). The maximum temperature was 36.56˚C in the month of July, and the minimum temperature -0.67˚C occurred in the month of December.

Trap Site Habitat Characteristics

Trap site habitat characteristics were examined. Nearly all sites (51) had vegetation and shade near the ovitrap, which makes an ideal site for oviposition. There was not much variation in home characteristics, since the study took place in a neighborhood with suburban homes of relatively the same age, all built in the 1990s.

Discussion

This study examined the oviposition of *Aedes aegypti* to quantify its abundance during 2017-2018, the first year it was detected in Merced in the Central Valley, California. More eggs were seen in warmer months (Fig. 7,8,9). In the month of May, the mean number of eggs per ovitrap began to increase due to warmer temperatures and remained high until October; the mean number of eggs per month was at its highest in the month of October and significantly decreased from November through April. There was almost no oviposition from December through April. This information is useful to understand the seasonality of this mosquito and will help management efforts target time frame of when mosquitoes are most abundant. Egg positivity is considered the most sensitive indicator to identifying the presence of *Aedes aegypti* (Morato et al., 2005). Gravid *Aedes aegypti* mosquitoes disperse their eggs over several sites with approximately 11-30 eggs per oviposition container (Apostol et al., 1994). Therefore, the 23,656 eggs collected in Merced, CA may represent 788.5 females (30 eggs per female).

In the months of June July, August, and September, and October, high levels of oviposition were associated with high temperatures. However, low levels of precipitation were recorded during these same months. Only highs levels of precipitation were found in the months of November, December, February, March, and April, when there were low temperatures and when oviposition did not occur. In these months there was a minimum of 20-70 mm of precipitation. In Costa Rica (Almeida et al., 2013), a high abundance of eggs was found due to high rainfall. The researchers believed the abundance of *Aedes aegypti* eggs was high during Costa Rica's rainy season because of the accumulation of water in natural and/or artificial reservoirs. This provides an increase in breeding sites for the hatching of eggs. The pattern of this study did not agree to what was found in this present study. In Mato Grosso, Brazil (Miyazaki et al., 2009), a positive relationship was also seen between the abundance of eggs and rainfall. They found that gutters would accumulate leaves and other debris that made it difficult for rainwater to drain. In Argentina, (Estallo et al., 2011) oviposition was strongly influenced by minimum temperature and increased rainfall. Increased temperatures in this present study were more strongly associated with oviposition. In Oran, a region in northwest Argentina, *Aedes aegypti* oviposition was strongly influenced by meteorological variables especially minimum temperatures (10°C) (Estallo et al., 2015); no eggs were found when temperatures dropped below 10°C in Oran. Moreover, there was a high number of eggs observed during the months of January, February and March because of rainfall that occurred 3 weeks prior. In the present study in Merced, high number of eggs occurred during the warmer months when there was limited precipitation. Water sources for oviposition during these months may have been from old fountains, pet bowls or excessive watering (Parker et al., 2019). These water sources which are potential mosquito oviposition sources in Merced neighborhoods would need to be shown to residents as sites where mosquito production occurs and can be reduced or eliminated.

In the city of Merced, mean values of the monthly ovitrap index (OI) ranged from 0.6% (in the month of March) to 65.7% (in the month of September). In Salvador, Bahia, a city found in Brazil, nine sentinel areas were assessed for *Aedes aegypti* infestations and both container and egg indices were estimated (Morato et al., 2005). Within this tropical climate, values of the mean monthly container index (same as OI) varied from

57.1% in one sentinel area (Lobato) to 75% in a second region Periperi). The CI values across the nine sentinel areas were overall much higher than the values observed in the study herein. In Salvador, Bahia the egg density index (EDI) varied between 35.6 (Lobato) and 106.2 eggs/ovitrap (Periperi). In the nine areas studied, the lowest mean monthly index occurred in the month of September and was highest in April. The mean EDI for all areas together was 60.0 eggs/ovitrap during each month studied. This study assumes that from the number of positive ovitraps and the number of eggs deposited throughout the study period that a constant replenishment of females was occurring. In Merced, the EDI ranged from 1.0 (in the months of December and March) to 44.6 eggs/ovitrap (October). Overall, our EDI values are lower; this could be due to the type of climate Brazil has which is tropical and humid and allows for year-round oviposition, while Merced is limited to oviposition in warmer summer months.

In countries where dengue is endemic, the ovitrap index and the egg density index would provide them information about *Aedes aegypti's* female reproductive activity in their environment. The indices monitor the most unfavorable times for the mosquito (dry seasons and/or lower temperatures). Thus, it allows us to identify areas where the vector is present and where control actions are needed in order to prevent the formation of more breeding grounds. When more breeding areas are formed, the density of *Aedes aegypti* rises and the occurrence of dengue increases as well. Climatic factors can also provide information in predicting transmission. For instance, a study found that a rise in the minimum temperature, rainfall and surface temperature of the sea was associated with an increase in dengue transmission levels in the coastal towns on the Gulf of Mexico (Hurtado-Díaz et al., 2007). Thus, they prepared a prediction model using time series, and proposed the development of an early warning system based on climate data for prevention and control of dengue epidemics.

Many cases of dengue have been reported in the country of Taiwan; approximately, 202-2,000 cases are reported yearly (Wu et al., 2009). In order to implement dengue control policies, it was important to understand the oviposition activity of its disease vectors. A study found that *Aedes aegypti* females were ovipositing throughout the entire year, significantly more females were collected indoors than outdoors, and that there were key areas where the distribution of *Aedes aegypti* was at its highest. This information allowed them to see that a productive *Aedes aegypti* control program could be implemented 14 weeks before the first case of dengue is detected. Through the use of ovitraps and indices, they concluded what measures are needed in order to protect the residents of Taiwan. Out of concern that dengue would be reintroduced in Galveston County, Texas, a study was performed to determine the density and distribution of *Aedes aegypti* (Moon & Micks, 1980). The relationship between the percentage of positive ovitraps and rainfall was also examined. It was found that in colder weather low numbers of adults and eggs were seen and high positive ovitraps rates were found associated with low rainfall. In addition, they found that the largest populations of *Aedes aegypti* were gathering in communities where there was more vegetation and trees present. With this information, they will now know when and where to target *Aedes aegypti* and prevent the introduction of dengue. In Recife, Northeast Brazil, they collected more than four million eggs that were laid in over a year (Regis et al., 2008). The egg density index ranged from 100 to 2,500 eggs. With this egg count information, they were able to identify where the vector was most concentrated and pinpointed areas that should be considered high priority for stepping up their control activities.

These studies are examples that will serve as models for the county of Merced. These studies will inform Merced County Mosquito Abatement on how to control and potentially eliminate populations of *Aedes aegypti*. Since there is no vaccine, it will be of great benefit for residents of Merced County to have *Aedes aegypti* eliminated. In addition, it will inform the abatement district where to concentrate their control efforts in high-risk areas to protect their residents and help promote its elimination.

A limitation within this study was that at times, traps were tampered with, tipped over, or removed; as a result, these traps were then excluded from the study. Another limitation within this study was that only 25-50 homes were examined. As an improvement, it would be recommended to expand the study area. I believe community outreach would be needed to accomplish this in order to get more people and neighborhoods involved. This outreach would also help the community be more aware of the importance of this research and how understanding what months and where oviposition occurs can help us eliminate mosquito breeding areas.

Another improvement of this study would be to determine how far a mosquito has traveled. This would be accomplished in a year by seeing how far a mosquito has traveled streetwise in a neighborhood. This would be possible to do when there’s a new detection in town. With a new introduction of a mosquito species, we can see how far on average they move, and if there is any clustering found surrounding homes. In addition, it would also help us understand if there are any anthropogenic barriers to their dispersal because of the environment they are found in. For example, habitat fragmentation due to roads or long, busy roads can impede dispersal (Hemme et al., 2010). In addition, house density, accessibility to water, and the amount of vegetation can also affect the distribution of *Aedes aegypti*. Ultimately, this information will help us to understand their behavior and to also improve our current control methods/programs.

Currently, *Aedes aegypti* mosquitoes are still being detected in Merced County and surrounding areas despite current control methods. Additional areas within the country have detections of *Aedes aegypti*, including Winton, Atwater and Los Banos (Merced County Mosquito Abatement District, 2018, 2020a, 2020b). No endemic dengue transmission has been detected; only tourist cases have been found. The indices indicate the presence and abundance of *Aedes aegypti*. The results of this study identified June, July, August, and September and October as the months with the highest mean of eggs per ovitrap. This result coincides that these months are also the months where temperatures were the warmest and there was almost no rainfall. The egg indices of the level observed in Merced are observed in other regions where dengue transmission occurs. Efforts to reduce this mosquito could focus on June to October, by having neighborhood residents remove standing water to reduce breeding mosquitoes, in combination with vector control efforts to reduce larvae and adult mosquitoes. Prevention efforts are important now before dengue or other vector borne illness associated with this species becomes endemic in the area.

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Table 1. Monthly ovitrap index and egg density index for *Aedes aegypti*

Month	Total # Eggs	#traps per Month (set/collected from)	Traps Positive/ Traps set	Ovitrap index (% positive traps)	Ovitrap index from traps collected (actual)	Egg density (eggs/positive traps)
October	3567	187 (157)	80/187	(80/187)*100=42.8	(80/157)*100 = 51.0	3567/80=44.6
November	693	270 (218)	49/270	(49/270)*100=18.1	(49/218)*100 =22.5	693/49=14.1
December	5	162 (106)	5/162	(5/162)*100= 3.1	(5/106)*100= 4.7	5/5=1.0
January	7	162 (94)	3/162	(3/162)*100= 1.9	(3/94)*100= 3.2	7/3=2.3
February	13	162 (91)	4/162	(4/162)*100= 2.5	(4/91)*100= 4.4	13/4=3.3
March	1	162 (83)	1/162	(1/162)*100= 0.6	(1/83)*100= 1.2	1/1=1.0
April	14	216 (206)	3/216	(3/216)*100= 1.4	(3/206)*100= 1.5	14/3=4.7
May	641	270 (263)	39/270	(39/270)*100= 14.4	(39/263)*100 = 14.8	641/39=16.4
June	2030	216 (214)	94/216	(94/216)*100= 43.5	(94/214)*100 = 43.9	2030/94=21.6
July	4919	216 (213)	140/216	(140/216)*100= 64.8	(140/213)*100= 65.7	4919/140=35.1
August	6820	270 (262)	176/270	(176/270)*100= 65.2	(176/262)*100= 67.2	6820/176=38.8
September	4946	216 (211)	142/216	(142/216)*100= 65.7	(142/211)*100= 67.3	4946/142=34.8

Ovitrap index is the total positive traps/traps set.

Table 2. *Aedes aegypti* weekly ovitrap index and egg trap index in Merced, California

Table 2. Weekly ovitrap index and egg density index for *Aedes aegypti*

Month	Eggs per week in positive traps	#traps per week (set/collected from)	Positive traps per week	Ovitrap index hypothetical (%traps positive)	Ovitrap index actual (%trap positive)	Eggs/ positive traps
4-Oct-17	58	25 (14)	6	(6/25)*100= 24.00	(6/14)*100= 42.86	58/6= 9.67
11-Oct-17	1748	54 (47)	29	(29/54)*100= 53.70	(29/47)*100= 61.70	1748/29= 60.28
18-Oct-17	888	54 (50)	25	(25/54)*100= 46.30	(25/50)=50.00	888/25= 35.52
25-Oct-17	873	54 (46)	20	(20/54)*100= 37.04	(20/46)*100= 43.48	873/20= 43.65
1-Nov-17	481	54 (45)	21	(21/54)*100= 38.89	(21/45)*100= 46.67	481/21= 22.90
8-Nov-17	42	54 (46)	6	(6/54)*100= 11.11	(6/46)*100= 13.04	42/6= 7.00
15-Nov-17	101	54 (44)	11	(11/54)*100= 20.37	(11/44)*100= 25.00	101/11= 9.18
21-Nov-17	23	54 (42)	5	(5/54)*100= 9.26	(5/42)*100= 11.90	23/5= 4.60
29-Nov-17	46	54 (41)	6	(6/54)*100= 11.11	(6/41)*100= 14.63	46/6= 7.67
6-Dec-17	1	54 (33)	1	(1/54)*100= 1.85	(1/33)*100= 3.03	1/1= 1.00
13-Dec-17	2	54 (36)	2	(2/54)*100= 3.70	(2/36)*100= 5.56	2/2= 1.00
20-Dec-17	2	54 (37)	2	(2/54)*100= 3.70	(2/37)*100= 5.41	2/2= 1.00
3-Jan-18	2	54 (34)	1	(1/54)*100= 1.85	(1/34)*100= 2.94	2/1= 2.00
25-Jan-18	5	54 (31)	2	(2/54)*100= 3.70	(2/31)*100= 6.45	5/2= 2.50
22-Feb-18	13	54 (30)	4	(4/53)*100= 7.41	(4/30)*100= 13.33	13/4= 3.25
8-Mar-18	1	54 (28)	1	(1/54)*100= 1.85	(1/28)*100= 3.57	1/1= 1.00
5-Apr-18	4	54 (51)	1	(1/54)*100= 1.85	(1/51)*100= 1.96	4/1= 4.00
26-Apr-18	10	54 (52)	2	(2/54)*100= 3.70	(2/52)*100= 3.85	10/2= 5.00
3-May-18	62	54 (53)	2	(2/54)*100= 3.70	(2/53)*100= 3.77	62/2= 31.00
10-May-18	158	54 (52)	5	(5/54)*100= 9.26	(5/52)*100= 9.62	158/5= 31.60
17-May-18	63	54 (53)	6	(6/54)*100= 11.11	(6/53)*100= 11.32	63/6= 10.50
24-May-18	120	54 (52)	10	(10/54)*100= 18.52	(10/52)*100= 19.23	120/10= 12.00
31-May-18	238	54 (53)	16	(16/54)*100= 29.63	(16/53)*100= 30.19	238/16= 14.88

7-Jun-18	311	54 (53)	19	(19/54)*100= 35.19	(19/53)*100= 35.85	311/19= 16.37
14-Jun-18	598	54 (53)	26	(26/54)*100= 48.15	(26/53)*100= 49.06	598/26= 23.00
21-Jun-18	532	54 (54)	26	(26/54)*100= 48.15	(26/54)*100= 48.15	532/26= 20.46
28-Jun-18	589	54 (54)	23	(23/54)*100= 42.59	(23/54)*100= 42.59	589/23= 25.61
3-Jul-18	643	54 (53)	30	(30/54)*100= 55.56	(30/53)*100= 56.60	643/30= 21.43
12-Jul-18	1083	54 (54)	39	(39/54)*100= 72.22	(39/54)*100= 72.22	1083/39= 27.77
19-Jul-18	1665	54 (54)	40	(40/54)*100= 74.07	(40/54)*100= 74.04	1665/40= 41.63
26-Jul-18	1528	54 (52)	31	(31/54)*100= 57.41	(31/52)*100= 59.62	1528/31= 49.29
2-Aug-18	1352	54 (52)	29	(29/54)*100= 53.70	(29/52)*100= 55.77	1352/29= 46.62
9-Aug-18	818	54 (51)	31	(31/54)*100= 57.41	(31/51)*100= 67.08	818/31= 26.39
16-Aug-18	1709	54 (51)	37	(37/54)*100= 68.52	(37/51)*100= 72.55	1709/37= 46.19
23-Aug-18	2037	54 (54)	39	(39/54)*100= 72.22	(39/54)*100= 72.22	2037/39= 52.23
30-Aug-18	904	54 (54)	40	(40/54)*100= 74.07	(40/54)*100= 74.07	904/40= 22.60
6-Sep-18	2014	54 (54)	42	(42/54)*100= 77.78	(42/54)*100= 77.78	2014/42= 47.95
13-Sep-18	1093	54 (52)	39	(39/54)*100= 72.22	(39/52)*100= 75.00	1093/39= 28.03
20-Sep-18	753	54 (51)	24	(24/54)*100= 44.44	(24/51)*100= 47.06	753/24= 31.38
27-Sep-18	1086	54 (54)	37	(37/54)*100= 68.52	(37/54)*100= 68.52	1086/37= 29.35

Figure 1. *Aedes aegypti* ovitrap sites in Merced during the study period.

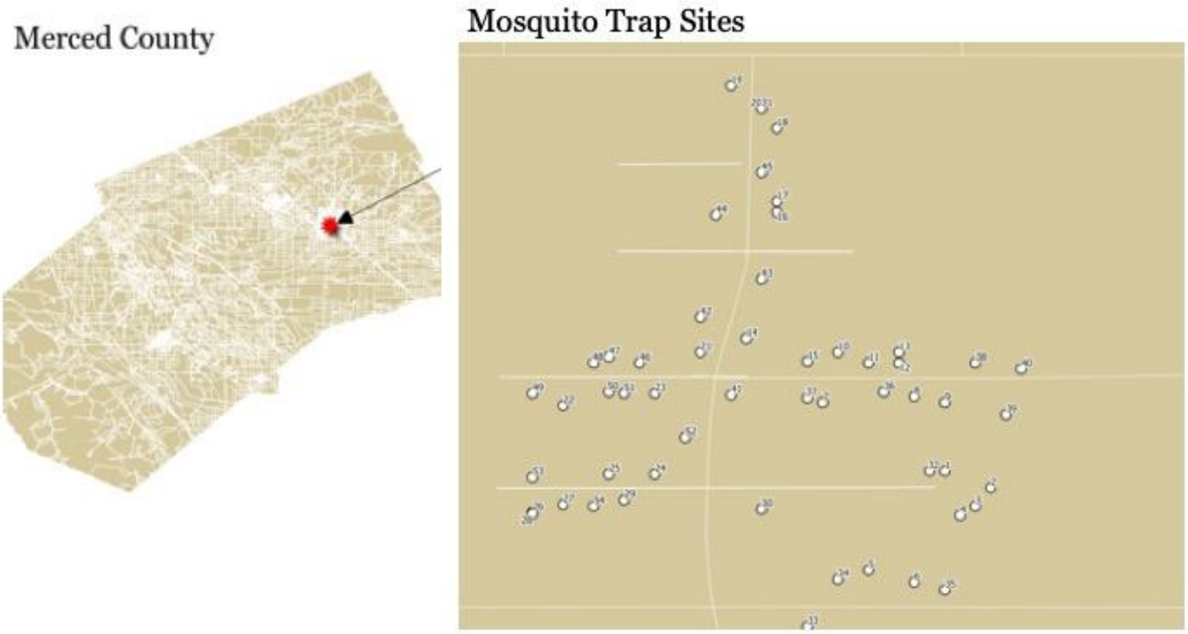


Figure 2. *Aedes aegypti* ovitrap with a popsicle stick

Figure 3. Mean eggs (+/- SE) per ovitrap for each month of sampling in Merced.

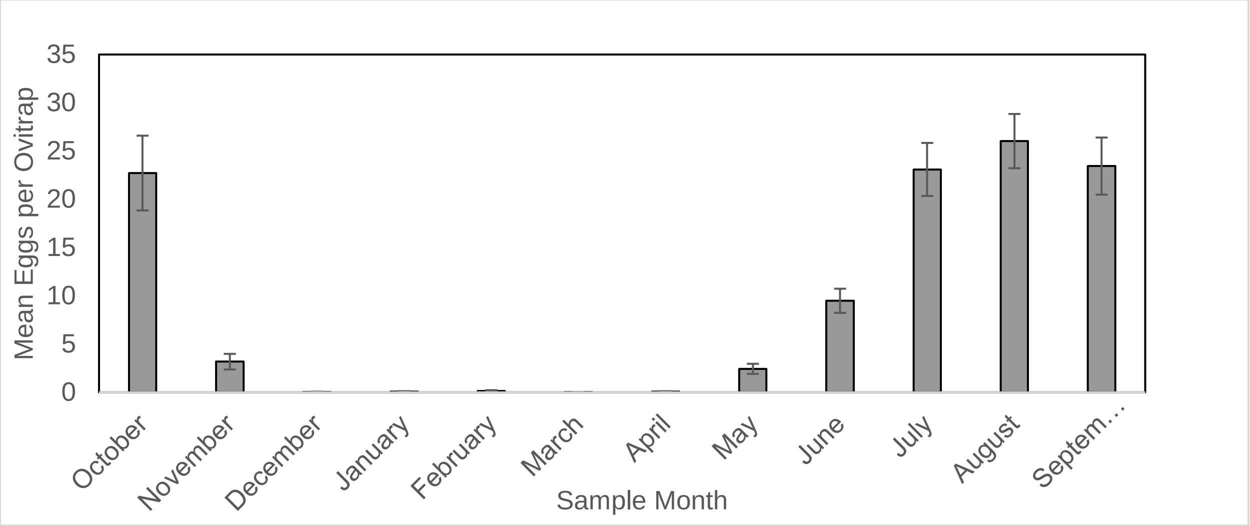


Figure 4. Actual Eggs (+/- SE) for each month of sampling in Merced (see table 1).

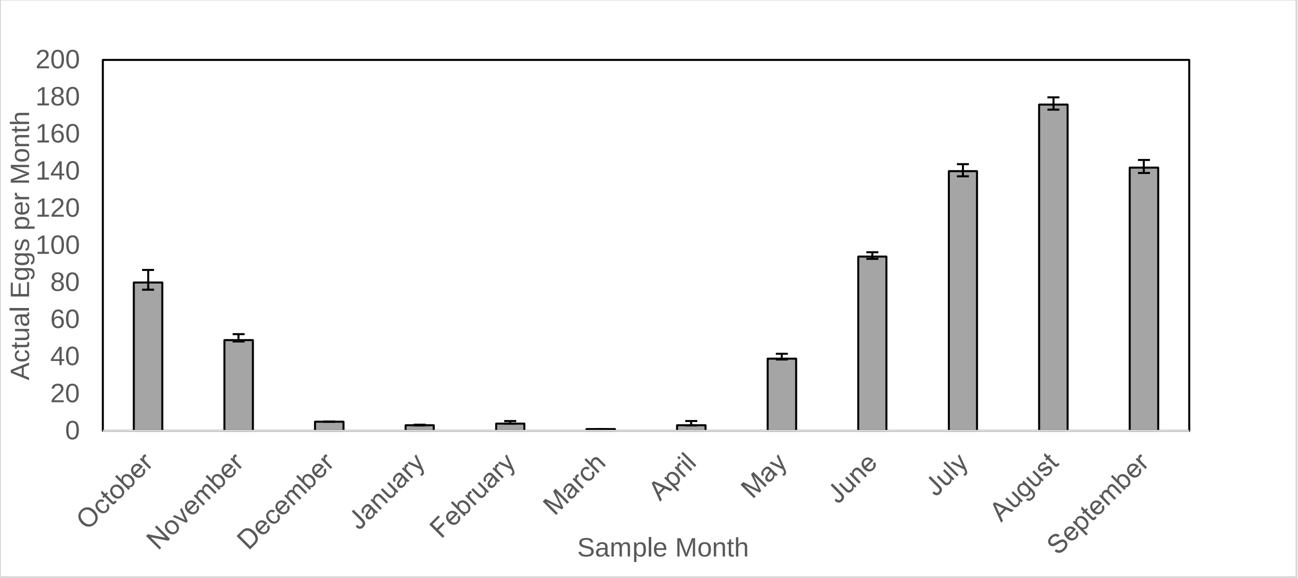


Figure 5. Monthly precipitation and temperature in Merced during the study period.

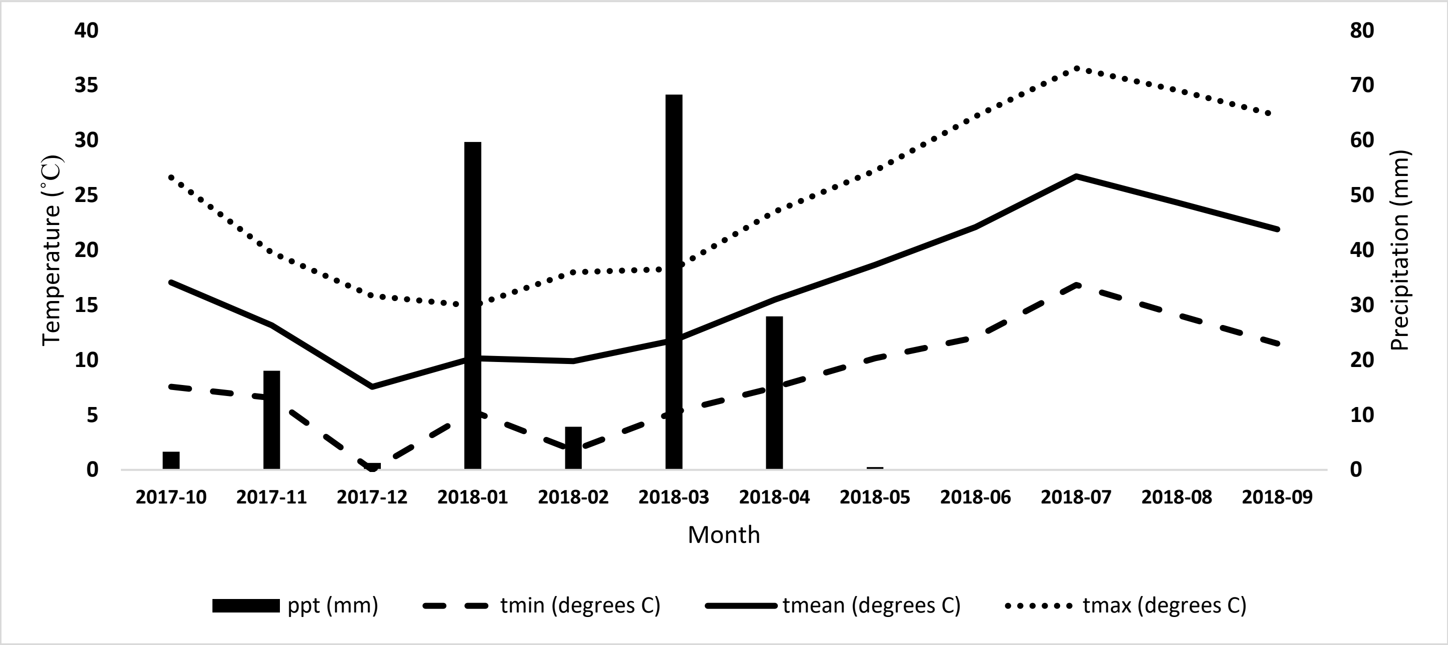


Figure 6. Weekly precipitation and temperature in Merced during the study period.

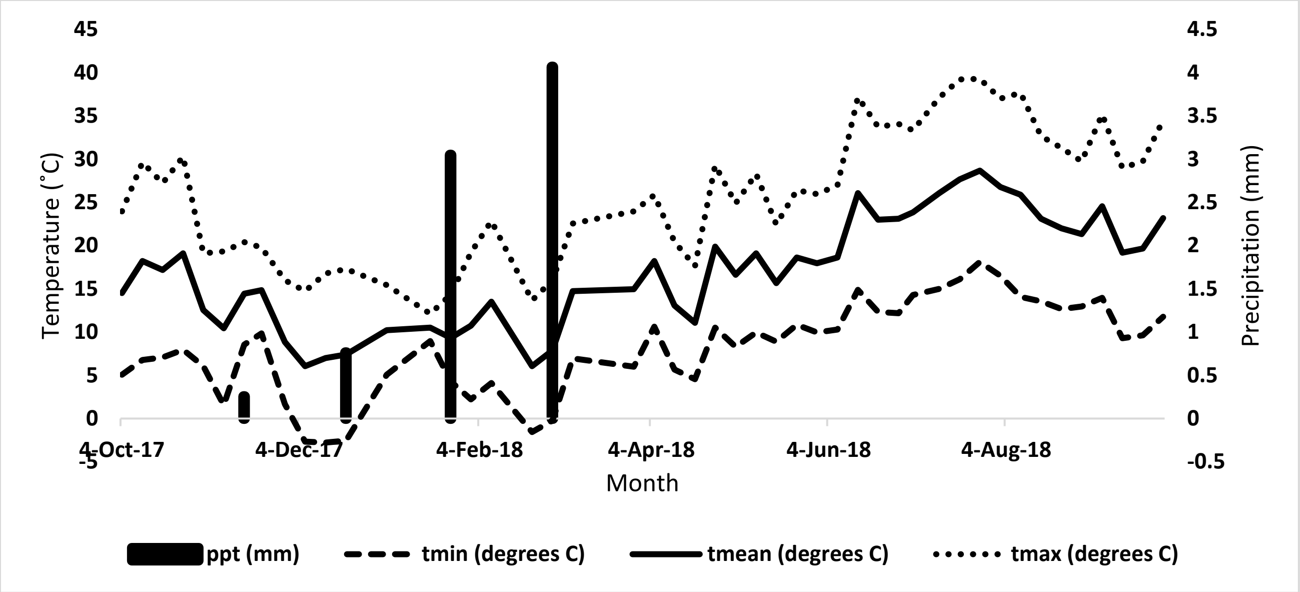
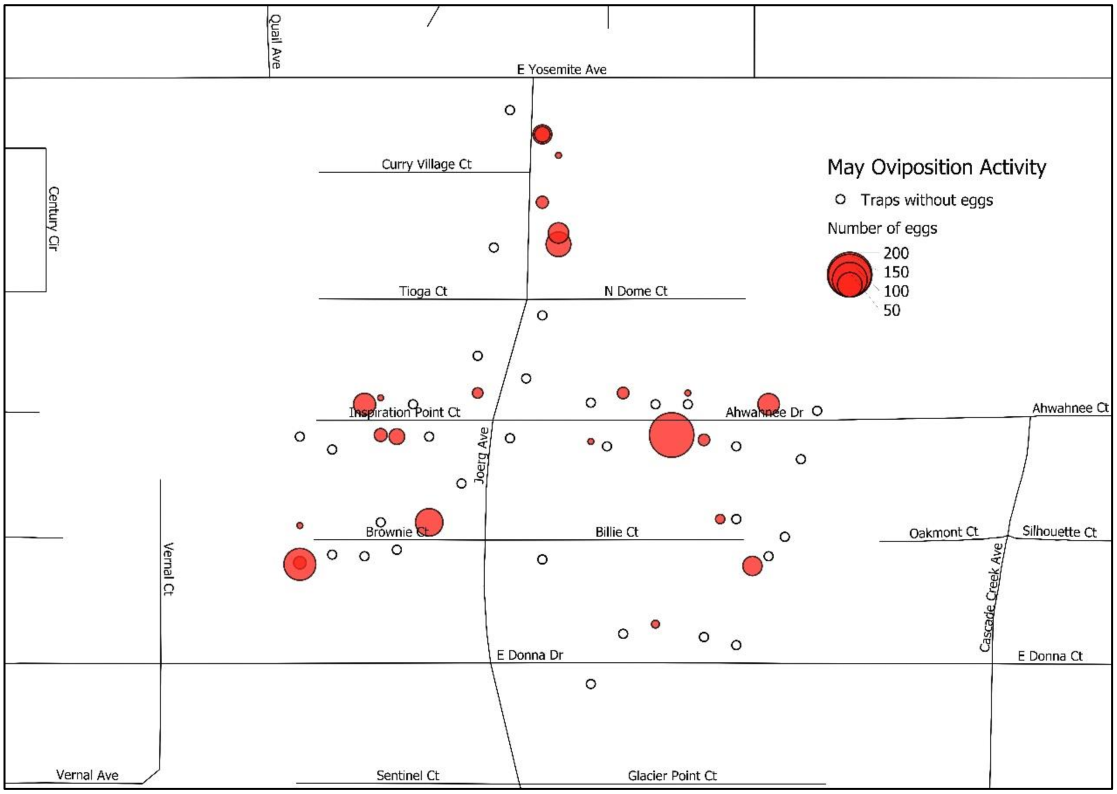


Figure 7. Maps of *Aedes aegypti* oviposition activity in May and June.



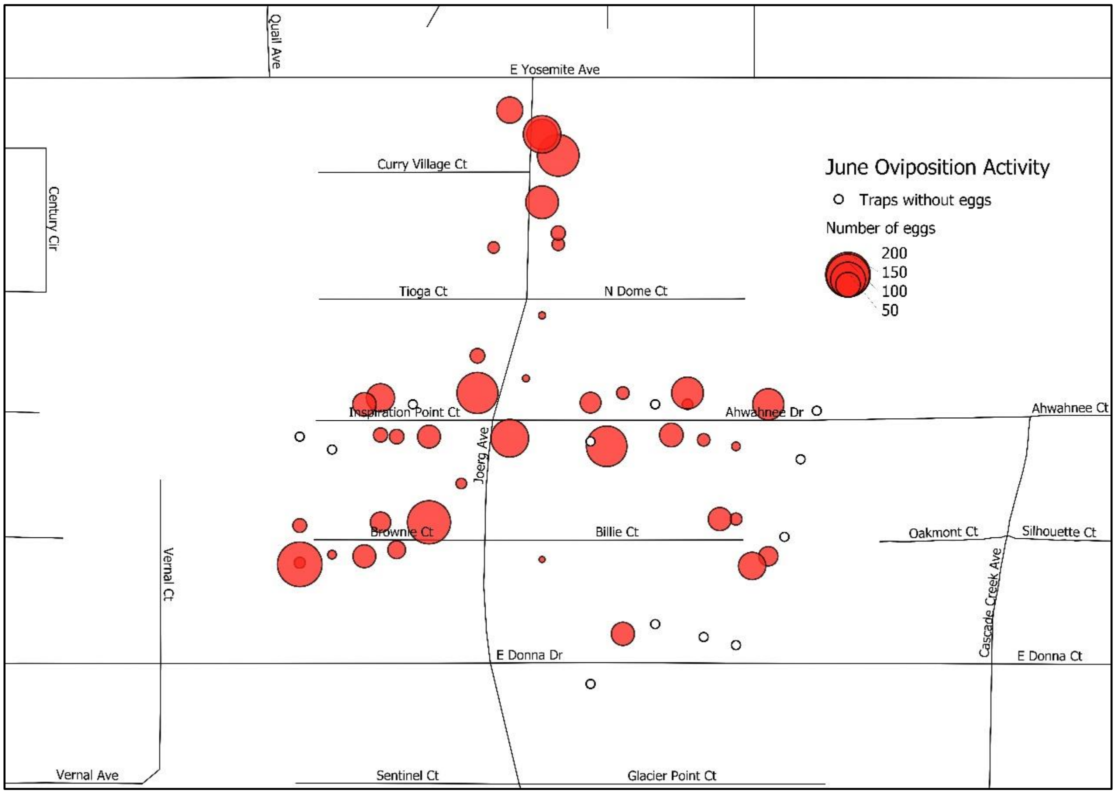
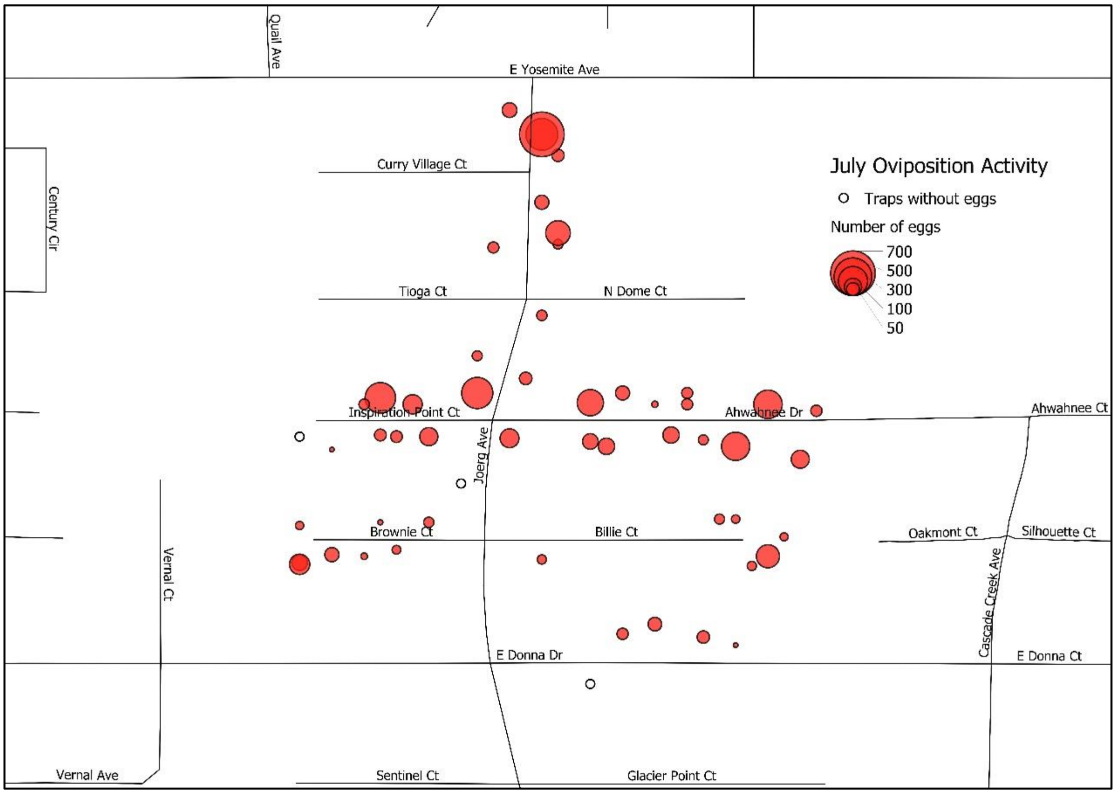


Figure 8. Maps of *Aedes aegypti* oviposition activity in July and August.



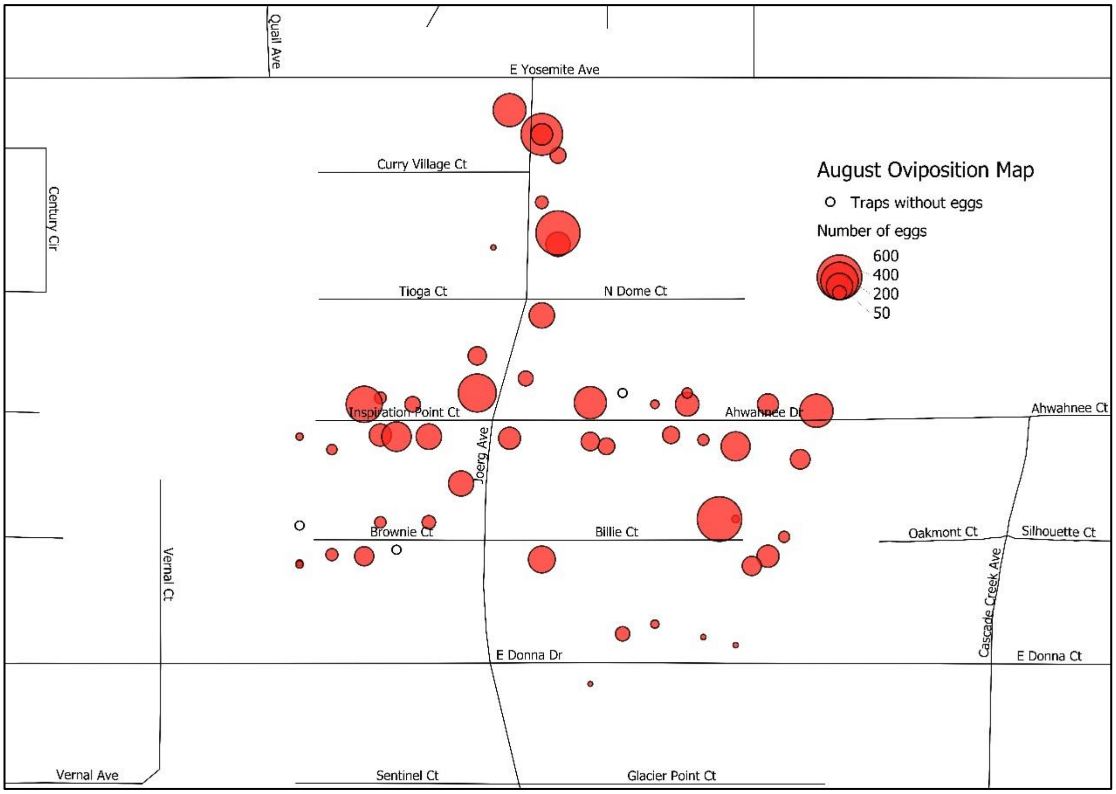
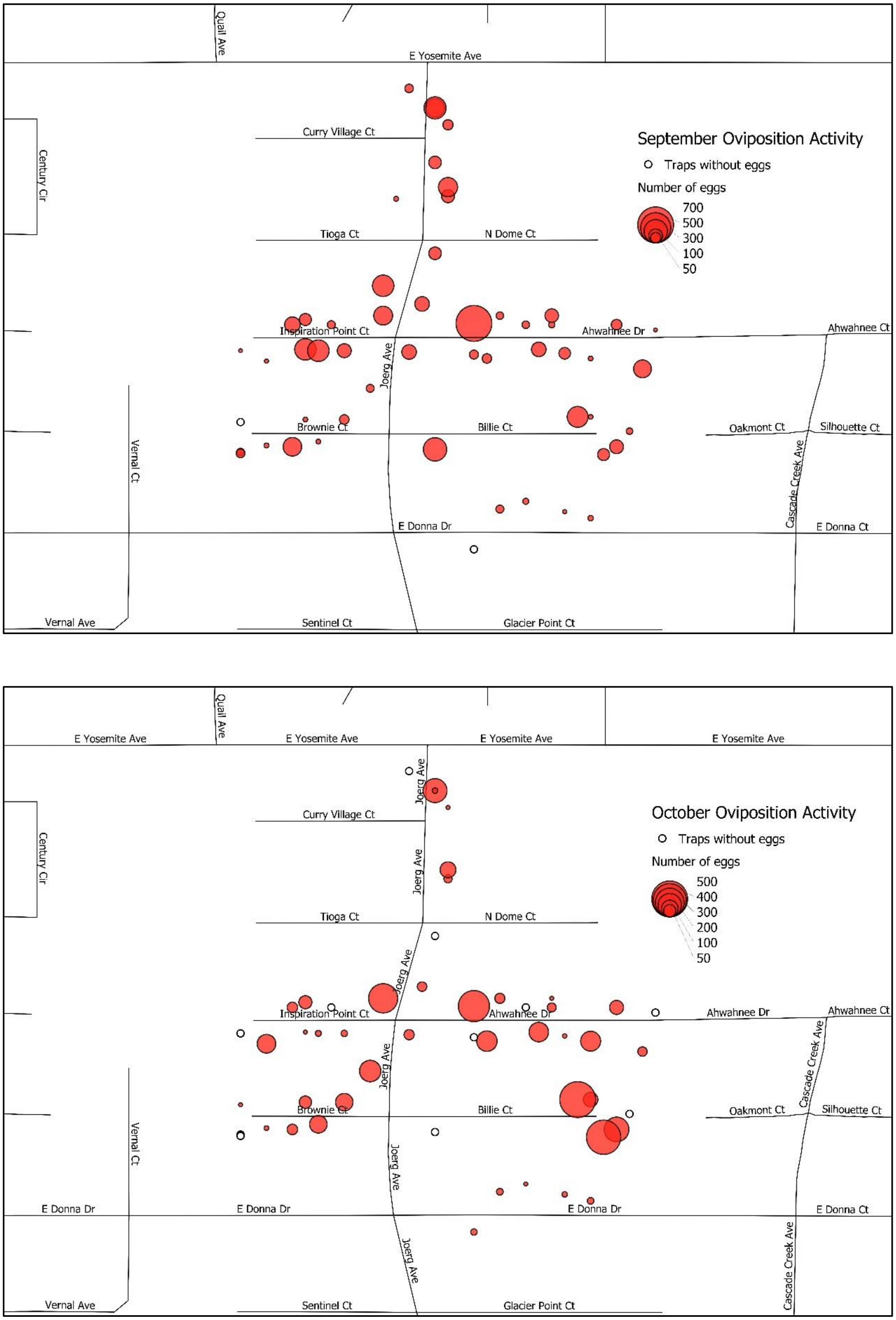


Figure 9. Maps of *Aedes aegypti* oviposition activity in September and October.



Chapter 5: Conclusions

Aedes aegypti is considered a successful vector species for transmitting dengue and other arboviral pathogens (Brady & Hay, 2020). Adult females are known to feed in the day and lay their eggs in different urban environments, stay in close proximity to humans and prefer to feed on them, have multiple feeds per gonotrophic cycle, and have desiccation-resistant eggs. It is for these reasons why we must have a better understanding of risk factors that are associated with the diseases that *Aedes aegypti* transmits.

The study of environmental factors and their association with dengue is important for countries such as El Salvador that have a very tropical environment. In studying the relationship between environmental factors and dengue cases, this study has shown that temperature, precipitation, and non-forested areas were the significant predictors. Most densely populated municipalities such as those in the urban capital San Salvador and the municipalities that surround it, have had the highest number of mean cases from 2011-2013. In El Salvador, deforestation is extensive. These non-forested areas are considered urbans areas with widespread agriculture and livestock; *Aedes aegypti* prefers to occupy human dwellings and are well adapted to urban environments which would explain the significant relationship between dengue cases and non-forested areas.

In tropical zones such as El Salvador, reproduction, and abundance of *Aedes aegypti* will depend on precipitation and temperature and therefore can influence the number of dengue cases. Within this study, a significant relationship was found between precipitation and dengue cases. The highest levels of cumulative annual precipitation were found in more forested areas at high elevations; these areas tend to be less populated. This could explain why a slight decrease in cases was found with precipitation (<1% decrease in cases with each mm of additional rainfall). A negative relationship was found between cases and increasing temperature. This relationship could be due to dengue cases being obtained at the yearly level for each municipality rather than at a weekly or monthly level. This prevented cases from being directly related to mean monthly temperatures. In additional explanation, is that El Salvador’s largest cities and the cities with the highest dengue incidence are located in the eastern part of the country where higher temperatures occur. The eastern part of El Salvador has the highest temperatures, but the incidence of dengue is lower in this part of the country. In El Salvador, non-forested areas and climatic variables, temperature and precipitation, are important predictors of dengue.

In addition to the study of environmental factors, socioeconomic factors should also be considered to better explain why diseases transmitted by *Aedes aegypti* such as dengue continue to persist. Guha-Sapir & Schimmer (2005) stated that the identification of social, economic, demographic and behavior factors involved in the transmission of infection diseases is key for the effectiveness of control measures. Within this study, poverty rate, literacy, school, municipal trash service, sanitary service, electricity for lighting, potable water, and household flooring materials were examined to determine the relationship between dengue cases and these variables. Dengue cases were significantly associated with variables which relate to urban areas, such as municipal trash service, sanitary service, potable water, cement brick flooring in home and population density. This is crucial information in helping to encourage health promotion which will reduce mosquito breeding sites that have been caused by these significant socioeconomical variables.

The patterns observed regarding dengue in Central America can help us here to prevent dengue in Merced, California. Understanding the oviposition of *Aedes aegypti* is highly important and will help to control the breeding of this mosquito in Merced County. It will lead us to understand the seasonality of this mosquito and target the time frame in which *Aedes aegypti* is most abundant. This study has found that the months of June, July, August, September, and October had the highest mean of eggs per ovitrap which may be as a result of warmer temperatures and almost no rainfall. High levels of precipitation were only found in the months of November, December, February, March, and April where no oviposition was found, and low temperatures occurred. Water sources in Merced neighborhoods during the months of high egg oviposition may have been from old fountains, pet bowls or excessive watering. It is important within these months to promote awareness in removing any potential breeding sites. The neighborhood where the mosquito was abundant had a relatively uniform socioeconomic condition of newer homes, but in the future in Merced, the mosquito may favor neighborhoods with different socioeconomic variables, and it will be important to consider this when managing the mosquito.

To summarize, these studies provide information which can be used to help control efforts of this mosquito which transmits dengue. This work is important as it aids in preventing the transmission of infectious disease. In combining the study of oviposition, environmental and socioeconomic variables, information will be useful in serving as an early warning tool for predicting peaks of activity by *Aedes aegypti*. It will not only be useful to the country of El Salvador but to even small counties such as Merced California.

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