The influence of spatial patterns and dengue serotype on dengue fever severity in Mexico

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BY

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ABSTRACT

Low socioeconomic status (SES), high temperature, and increasing rainfall patterns are associated with an increase in the number of dengue case counts. However, the effect of climatic variables on individual dengue virus (DENV) serotypes have not been explored in prior literature. Furthermore, there is a knowledge gap about the extent to which serotype count affects the rate of severe dengue in Mexico. A principal components analysis was used to determine the poverty indices across Mexico. Conditional autoregressive Bayesian models were used to determine the effect of poverty and climatic variables on the rate of serotype distribution and severe dengue in Mexico. A unit increase in poverty increased the rate of DENV-1, DENV-2, DENV-3, and DENV-4 by 8.4%, 5%, 16%, and 13.8% respectively. An increase in one case attributable to DENV-1, DENV-2, DENV-3, and DENV-4 was independently associated with an increase in the rate of severe dengue by 0.02%, 0.1%, 0.03%, and 5.8% respectively. Hotspots of all DENV serotypes and severe dengue were associated mostly with coastal regions in Mexico. The Southeast region experienced higher humidity, a higher poverty index, and a lower average altitude. The association of these climatic parameters with severe dengue puts states like Oaxaca at increased risk of a higher number of severe dengue cases.

Pregnancy increases a woman's risk of severe dengue. To the best of our knowledge, the moderation effect of dengue serotype among pregnant women has not been studied in Mexico. This study explores how pregnancy interacts with dengue serotype from 2012 to 2020 in Mexico. Information from 2,469 notifying health units in Mexican municipalities was used for the analysis. Multiple logistic regression with interaction effects was chosen as the final model and sensitivity analysis was done to assess potential exposure misclassification of pregnancy status. Pregnant women were found to have higher odds of severe dengue [1.50 (95% CI: 1.41, 1.59)]. The odds of dengue severity varied for pregnant women with DENV-1 [1.45, (95% CI: 1.21, 1.74)], DENV-2 [1.33, (95% CI:1.18, 1.53)] and DENV-4 [3.78, (95% CI: 1.14, 12.59)]. While the odds of severe dengue were generally higher for pregnant women compared with non-pregnant women with DENV-1 and DENV-2, the odds of disease severity were much higher for those infected with the DENV-4 serotype. Future studies on genetic diversification and phylogeny may potentially elucidate this serotype-specific effect among pregnant women in Mexico.

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CHAPTER 1: INTRODUCTION

1.1 SPECIFIC AIMS

Within the last five decades, there has been a resurgence of diseases like dengue fever (DF) in Mexico [1]. Primary infection with a DF serotype confers immunity through the production of serotype-specific neutralizing antibodies [2]. However, the disease mechanism of secondary infections involves non-neutralizing antibodies. DF has 4 serotypes: DENV-1 to 4 (DENV-1, 2, 3, and 4), and in recent times, DF outbreaks typically involve a combination of more than one serotype [3, 4]. Hence, each DF serotype can elicit cross-reactive non-neutralizing and disease-enhancing antibody responses [2]. An individual infected with a heterologous DF serotype (a serotype other than the first serotype the person was infected with) has a higher risk of developing dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [5]. DHF is a severe form of DF with its critically defining stage characterized by signs of circulatory failure or hemorrhagic manifestations [6], and DSS occurs in patients with DHF who develop circulatory collapse [7]. Among DF patients, compared to those without DSS, cases with DSS are 50 times more likely to result in mortality [8]. Changes in the incidence and severity of DF are temporally associated with new circulating DF serotypes in a region [9]. The persistence of these serotypes in a region may result in hyperendemicity. However, hyperendemicity and simultaneous circulation of several serotypes may not be sufficient to result in a DHF epidemic [9]. It is suggested that the presence of a virulent strain is critical to the development of a clinically severe outcome. Therefore, the hyperendemicity and virulence of a DF strain may act synergistically to cause a DHF epidemic. DENV-2 is significantly associated with DSS [8]. Hence, a hyperendemic region, with a high incidence or prevalence of a virulent serotype like DENV-2, might require increased monitoring to improve emergency preparedness for DF epidemics.

Predictors of DF include extrinsic factors like changes in climate and weather patterns. Factors associated with climate change and host-virus interactions affect serotype dynamics and predict the rate of DF infection [10]. Serotype dynamics have previously been explored in Peru, with findings suggesting the introduction of different serotypes at different time points, and detection of a positive autocorrelation (degree of similarity across time) between DF case counts at a lag of approximately 70 weeks [11]. In their study, Stoddard et al (2014) found that, while analysis of climatic variables correlated weakly when measured yearly, analysis by trimester (three monthly intervals) revealed that the climatic variables correlated more strongly, albeit distributed over long lags [11]. Variations in DF transmission intensity occur across countries and regions in Latin America. These variations in patterns may be evident across different Mexican municipalities, which may differ in terms of landscape connectivity [12], weather patterns, and mosquito control programs [11], all of which may affect the incidence and severity of DF in a region. In addition to environmental factors largely implicated in the spread of DF [13, 14], it has been suggested that improved transportation may also contribute to the effective dispersal of more virulent DF strains which displace indigenous strains in the Americas [9]. while a higher socioeconomic status associated with the purchase of items like air conditioner, may help reduce human-vector interactions, thereby decreasing the risk of DF [15]. Investigation of variation of serotype pattern across municipalities in Mexico, accounting for these extrinsic factors may therefore reveal variations in DF severity.

Certain intrinsic factors may also influence the severity of DF, and these include host-virus interactions and comorbidities. For instance, pregnancy has been found to increase the risk of severe dengue [16-18]. Similarly, comorbidities like diabetes and hypertension have been associated with an increased risk for a severe clinical presentation of DF [19]. There is, however, a gap in literature portraying the severity of DF in pregnancy and how its occurrence in individuals with other

comorbidities is modified by the DF serotype. Hence, an objective of this study will be to determine whether the severity of DF is associated with the interaction between pregnancy and/or other comorbidities and DF serotype, in Mexico. The association between pregnancy and specific serotypes will guide policies related specifically to DF prevention among pregnant women or individuals with comorbidities in Mexico.

This dissertation had three specific aims. **Aim 1:** To explore the effect of climatic parameters and socioeconomic factors on the distribution of dengue serotype across Mexico. **Aim 2:** To determine how climatic parameters and socioeconomic factors predict the risk of dengue fever severity in Mexico. **Aim 3:** To determine the role of pregnancy and serotype on DF severity.

1.2 PUBLIC HEALTH SIGNIFICANCE

DF is the fastest spreading vector-borne viral disease worldwide, with endemicity in over 100 countries [20, 21]. Particularly, within the past five decades, factors like urbanization, globalization, and international mobility have aided in the emergence of arboviral disease epidemics in new areas, of which DF is the most common [22, 23]. DF occurs as a result of a flavivirus infection that is mostly spread in urban and suburban areas of tropical and sub-tropical regions [20]. About 40% of the world's population is at risk of being infected with DF [20]. Transmission of DF occurs through the *Aedes species* (*spp.)* mosquito. In Mexico, *Aedes aegypti* and *Aedes Albopictus*, of subgenus *Stegomyia*,[22] are ubiquitous [24, 25] and have been implicated in DF transmission [26, 27]. The *Aedes* mosquito has a life cycle between 8-10 days, consisting of an aquatic and terrestrial phase [2]. Of the two causative *Aedes spp*., *Ae. aegypti* is more implicated in dengue transmission [22]. *Ae*. *aegypti* has a preference for feeding on human beings during the daytime, around domestic environments, typically feeding on multiple people within short intervals [22]. Hence, overcrowding and man-made larval habitats, further

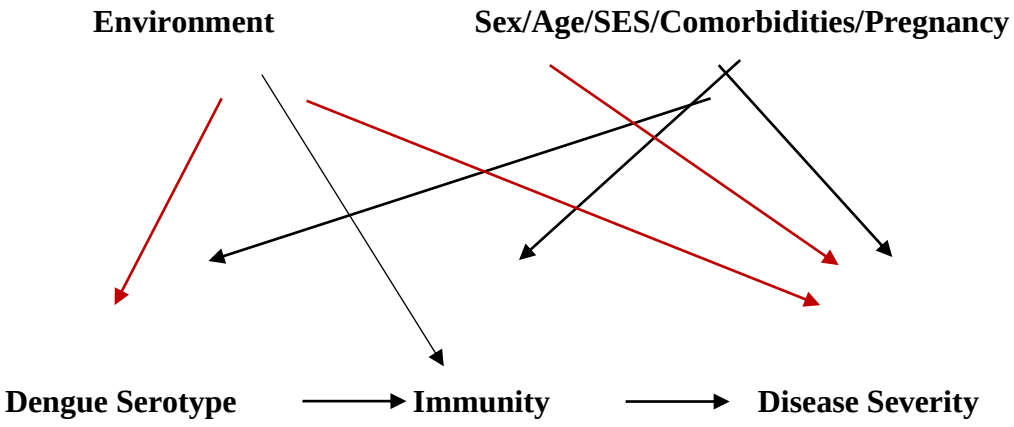
compound the spread of DF, particularly in tropical low-income countries [28]. Although less efficient than *Ae. aegypti*, the *Ae. albopictus* has also been implicated in the spread of DF in new locations since it easily adapts to new environments [2]. Because the *albopictus spp.* tends to be hardy in more temperate climates [29], the spread of DF is predicted to increase as temperatures fluctuate due to climate change [30, 31].

The first infection with either of the DF serotypes results in asymptomatic disease or mild symptoms like retroorbital pain, fever, headache, flu-like illness, nausea, rash, muscle ache, vomiting, and swollen lymph nodes [20]. A second infection with any of the other serotypes usually results in a severe clinical manifestation like DHF, respiratory distress, and organ impairment [20]. In 2013, the global years of life lost due to premature mortality attributable to DF was about 576,900 and the years lived with disability attributable to DF was approximately 566,000 [32]. DF contributes to 1.14 million disability-adjusted life-years (DALYs) when considering both fatal and non-fatal outcomes [32]. Proper case management, may, however, reduce the mortality rate from about 20% to 1% [20]. Since multiple *Aedes*-transmitted diseases have similar epidemiology, specific tests are necessary to make a diagnosis of DF [22].

Viral titers have also been linked with DF epidemics. After a first epidemic, subsequent epidemics result in fewer cases due to herd immunity and as the immuned population ages, younger individuals become progressively susceptible [33]. This may explain the variance in the most susceptible age groups across the globe. As epidemics continue over time, the age-specific seroprevalence is expected to change, with small epidemics involving the younger generation. Higher titers result in more acute and shorter epidemics; these tend to be more intense, with more individuals involved and higher immunity levels than lower titers [33]. When interacting with other environmental factors, viral titers may be modified to result in better or less favorable outcomes. For instance, while

low-titer viral introduction into a DF-naïve population (a population that has not yet been exposed to DF) during wintertime might not produce an epidemic, a single introduction into that population could result in an epidemic at another time of the year. With a high titer introduction, there is a reduction in the magnitude of the effect of seasonal variables [33]. The lag (temporal autocorrelation) between monthly cases of DF goes back at least four months, with a lack of substantive correlation between current cases and environmental factors like weather, temperature, and rainfall [33]. The sequence of weather patterns has also been shown to influence DF occurrence; a sequence of the dry season followed by excessive rainfall may increase the risk of an outbreak [34]. The rainfall lag is at approximately 7 weeks and the minimum temperature lag associated with DF outbreaks is on average 12 weeks [34]. These findings suggest that epidemics may take several months to fully develop. Figure 1 shows a directed acyclic graph (DAG) demonstrating all the factors associated with DF transmission and progression to disease severity.

Figure 1: A Directed Acyclic Graph, showing the key drivers of DF transmission.



Specific variables associated with DF severity.

Regional differences and mobility: Due to the temporal and spatial variation in DF infectivity, the prediction, and prevention of DF have been considered to be a moving target [35], with a need for continued surveillance both globally and locally. Key drivers of DF transmission vary across different contexts [11, 35, 36] and show complex nonlinear relationships [37]. Like most other variables associated with DF, demographic variables also show variability across regions. In parts of the Americas like Brazil, DHF occurs mostly in adults while DHF predominantly occurs in children in Southeast Asia [38, 39]. The shift in the age distribution of the predominance of DF in the Brazilian adult population began to occur in 2007, years after DENV -1,-2 and -3 had, respectively, been introduced in Brazil [39].

While climatic factors play a role in driving DF epidemics, human mobility patterns, circulating serotypes [40], and serological factors act synergistically to determine the viable geographical range of disease transmission and determine the number of DF cases during an epidemic [41, 42].

Serotype: Investigating the DF-specific serotype involved in infections is important due to the risk of developing antibody-dependent enhancement (ADE) after secondary infection with a heterologous DF serotype. ADE occurs when sub-neutralizing antibodies enhance viral entry and heighten viral production [43]. The occurrence of ADE leads to the persistence of complex patterns that result in the relative abundance of serotypes, making it possible for the different serotypes to co-exist [44]. While cross-reactive immunological responses of closely related strains tend to induce competition between strains within the pathogen population, in the case of DF, cross-reactive antibodies enhance the severity of subsequent infection with another strain [44]. Hence, pre-existing immunity to DF is a risk factor for the development of severe disease (DHF and DSS) and enhancement from the cross-reaction between

different strains permits the coexistence of strains [44]. Both primary (an initial infection) and secondary infections (subsequent infections) with the DENV-2 serotype have been associated with more severe infections [45], and the severity of infection may be particularly so after it follows a DENV-1 infection [44].

Transmission of DF in a region may vary by serotype across time [11, 46]. Similarly, DF transmission may result in the co-circulation of DF serotypes. The mix of serotypes found in certain regions may be a result of redistribution during the year. DF might be lost during colder seasons in northern regions and reintroduced during warmer seasons from southern locations [33]. Due to a successful campaign organized by health authorities between 1947 and 1960 to eradicate *Ae. aegypti*, and mosquitoes were declared eradicated in Mexico in 1963 [9]. This was, however, short-lived with its reappearance two years later. DENV-1 epidemics occurred mainly on the east coast of Mexico between 1979 and 1980, after which DENV-4 was implicated in a major epidemic in the Yucatan peninsula in 1984 [9]. Between 1984 and 1985, DENV-1, 2, and 4 were reported across Mexico. In 1995, a surge in DF cases occurred, during which DENV-3 was first isolated in Mexico [9]. While epidemics have been seasonal, the proportion of DHF and DSS to all DF cases has progressively increased over the years [9]. Prior studies have alluded to seasonal patterns of DF transmission, but more recent studies have suggested inter-epidemic periods of DF transmission [47]. The inter-annual transmission variability of DF serves as an indicator of other important drivers of DF transmission [48]. Hence, while the transmission may be partly due to the reintroduction of the virus from neighboring countries, the persistence of DF in Mexico may also be due to an interaction between environmental, host-related factors and the continual circulation of the indigenous serotypes within the country [49]. Imported DF serotypes may increase the likelihood of an epidemic spread, but this is not always the case [49-51].

Due to co-circulation with multiple serotypes in endemic regions, a past DF epidemic may influence the innate reproduction number (R_o) of a serotype for which herd immunity has been reached [33]. The invasion of a strain *i* into a population with a primary infection strain j depends on the equation R_{oi} $[1/R_{oj} + ø\ (1- 1/R_{oj}) > 1]$ $i \neq j$. Where R_{oi} = the basic reproductive ratio of strain *i* and ø is the degree of enhancement (if $ø > 1$) or neutralization ($ø < 1$) [44].

Comorbidities: Comorbidities like diabetes, asthma, cardiac disorders, and hypertension have been found to increase the risk of severe clinical presentation of DF [52, 53]. Thrombocytopenia, a marker of severity of DF is worsened in patients with diabetes [53]. Hence, diabetes invariably predisposes DF-infected patients to DHF and DSS. Other variables associated with an increased risk of thrombocytopenia in dengue patients are hypoalbuminemia, hypertriglyceridemia, and older age [53]. When diabetic individuals have other comorbid conditions, the risk of DHF and DSS increases further [54]. For instance, compared with non-diabetics and non-hypertensives, individuals with diabetes and hypertension have been found to have a higher risk of DHF in a DENV-2 epidemic [19]. Hypertension, therefore, modifies the effect of diabetes on DF severity [19], and independently, individuals with hypertension have higher odds of developing DHF [55]. Limited studies on the effect of DF on the cardiovascular system have also elucidated that DF may affect the heart, further resulting in complications like bradycardia, cardiac arrhythmias, and death [56]. With the limited literature on the moderation effect of DF serotypes on DF severity among diabetes and hypertension patients, there's a need to explore the interaction effect between DF serotypes and comorbidities on DF severity.

Pregnancy: While most studies have focused on pregnancy outcomes associated with DF, a few have explored how pregnancy predicts the risk of severe dengue. One such study found that among women infected with dengue, 46.5% of pregnant women developed severe dengue, compared to 22.5% of non-pregnant women who developed severe dengue [16]. Other studies have reported that the link between

pregnancy and the severity of DF depends on the trimester of the pregnancy. Research involving DF serotypes has implicated DENV-1,2 in perinatal symptoms like fever, petechiae, and hepatomegaly [57]. To the best of our knowledge, there is still limited research exploring the association between DF-specific serotypes, pregnancy, and dengue severity. Since DF serotype has been shown to predict DHF, this study aims to bridge the gap in research by investigating if pregnancy moderates the effect of DF serotype in pregnancy.

Weather: While a lower-than-average rainfall intensity is an indicator of increased risk of DF, temperature has been shown to have a positive association with DF incidence [58-60]. Warmer temperatures increase the survival and development rates of the *Aedes* mosquitoes [61, 62] and may shorten the extrinsic incubation period (EIP; the time between the ingestion of a virus-laden blood meal and the time the mosquito becomes infectious and gonotrophic development rates) required for viral transmission [33, 58, 63, 64]. However, DF vectors are better at transmission when temperatures are moderate and humidity is high, with varying competency at mean and diurnal temperatures [35, 65]. Under the same environmental conditions, the four DF serotypes may be unequally distributed [49].

1.3 LIMITATIONS AND GAPS IN PREVIOUS RESEARCH

Limitations from prior studies involving DF serotypes include the evaluation of environmental parameters associated with DF serotypes in non-endemic regions where outbreaks are only triggered by the importation of new DF serotypes, with the limited effect of immune status from the population's limited exposure [49]. Since most DF cases are not laboratory-confirmed and lack information on DF serotype [42, 66, 67], several studies have had to use data based on clinical diagnoses in their analysis. Due to the mild clinical presentation of DENV-4, some studies have excluded it in their analysis due to smaller sample sizes [67]. Other important covariates that have been omitted in some studies include socioeconomic and demographic factors like population density [68]. Several studies have also reported

gaps since prior studies have not factored in data on vector control activities, other interventions, and policy changes [34].

1.4 INNOVATION

This study helped to determine the spatial relationship between the environment and DF serotypes in Mexico. This was done with monthly aggregated confirmed data at the municipality level, using laboratory-confirmed diagnosis instead of clinical diagnosis. We also aimed to establish the relationship between climatic parameters, altitude, and DF serotypes in Mexico. Thirdly, we explored the interaction between pregnancy, and dengue serotypes and how they predict dengue severity.

1.5 BACKGROUND FOR SPECIFIC AIMS

1.5.1 Aim 1 and 2:

Aim 1. To explore the effect of climatic parameters and socioeconomic factors on the distribution of dengue serotype across Mexico. *Hypothesis:* Dengue Serotypes in Mexico are geographically clustered, which might be due to climatic parameters or/and socioeconomic variation.

Aim 2. To determine how climatic parameters and socioeconomic factors predict the risk of dengue fever severity in Mexico. *Hypothesis:* Some persistent dengue serotype and severity hotspots exist in Mexico and might be associated due to climatic parameters

Rationale:

An acute dengue fever (DF) infection may either be asymptomatic or present with symptoms like fever, rash, lymphadenopathy, body pains, and/or minor hemorrhage [69]. Minor symptoms may progress further into dengue hemorrhagic fever (DHF), which may lead to dengue shock syndrome (DSS) and/or death [70]. An individual with DHF presents with a temperature above 38 °C, signs associated with a

reduced platelet count (thrombocytopenia), a positive tourniquet test, and signs of plasma leakage, while DSS is a progression from DHF to circulatory failure [66]. DF is reported in 28 of 32 states in Mexico and the severity of the disease has increased over the past decades [71]. The increase in the number of DF cases has been partly attributed to the genetic diversification of DENV serotypes and the emergence of new genotypes across Latin America [72]. Pathogen genetic variation is further associated with infectious disease spread and the severity of diseases [73].

Immunological priming by a previous dengue virus (DENV) serotype is the most important risk factor for DF severity in Southeast Asia, and the circulation of the DENV-2 serotype has been associated with the severity of DF in the Americas [9]. Compared with the other three DENV serotypes, DENV-2 has been linked mostly with severe DF [74]. The pathogenesis of DF severity involves the interaction between the virus and the genetic background of the host, the specific viral serotype, and the immune response to a previous infection [75]. Serotypes with higher replication rates induce the production of antibodies at a faster rate and result in severer DF outcomes [74, 76].

In addition to viral-host factors, socioeconomic (SES) factors may further predict DF severity. Low SES independently increases the risk of DHF [77]. Prior studies have explored the effect of gross domestic product (GDP) in predicting DF distribution and there have been suggestions that more granular socio-economic indicators like access to education, information, and technological infrastructure are better predictive factors of DF distribution [78].

Different DF strains possess different virulence and consequently predict the severity of DF infection [72, 79]. Furthermore, the distribution of the mosquito vectors responsible for DF is influenced by environmental factors like temperature and altitude [80]. Hence, the investigation of the serotype-specific effect on DF severity needs to consider environmental factors that may modify the effect of DENV on DF severity.

To the best of our knowledge, no study has investigated the effect of environmental predictors on the number of DENV serotypes in Mexico. Similarly, the effect of DENV serotype, SES, and environmental parameters on DF severity in Mexico has not been investigated previously. We hypothesized that DENV serotypes in Mexico will be geographically clustered and that the rate of spread could be due to climatic parameters and/or socioeconomic variations. We also aimed to identify if persistent DF severity hotspots existed in Mexico and if the number of severe DF cases was associated with climatic parameters and the socioeconomic status of Mexican municipalities. Results from our study may be used to guide policies that help allocate public health resources to the most vulnerable municipalities in Mexico.

1.5.3 Aim 3:

To determine the role of pregnancy and serotype on DF severity.

Hypothesis: We hypothesize that the effect of DF serotype on DF severity will be moderated by hypertension, diabetes, in the general population, and pregnancy among the female population.

Rationale:

In Mexico, it is estimated that 139,000 symptomatic dengue fever (DF) cases occur yearly on average, with an estimated yearly cost of $170 million and an average annual disease burden of 65 disability-adjusted life years (DALYs) per million population [81]. The overlap between DF symptoms and the physiological alterations seen among women during pregnancy may make the identification of warning signs difficult [82]. However, for identified cases, pregnancy increases the risk of hospitalization and the development of severe dengue [82].

Maternal mortality rates (MMR) vary across and within regions, in Mexico [83]. MMR is associated with factors like pregnancy-related hypertension, obstetric hemorrhage, quality of health care

[84], and infections like DF [85]. Severe DF is associated with a high rate of fetal distress, intrauterine death, obstetric hemorrhage, preeclampsia and eclampsia, caesarian section deliveries, and death due to multiple organ failure days after delivery [85, 86]. Prior studies have also implicated DF in vertical transmission during late pregnancy, and implicated serotypes have been serotypes 1 and 2 (DENV -1 and DENV -2) [57]. DF-specific serotypes have been linked with severe outcomes of DF. Severer complications have mostly been associated with the DENV-2 serotype [87]. There is, however, a gap in literature portraying how the severity of DF in pregnancy is modified by the DF serotype in Mexico.

Other factors related to severe outcomes of DF are comorbidities. Adults with self-reported hypertension have 1.6 times the odds of developing dengue hemorrhagic fever (DHF) compared to non-hypertensives [55], while diabetes is associated with 2.75 times the odds of DHF [88]. Diabetes presents with a far worse prognosis in Mexico compared to high-income countries [89]. Approximately 20% of preventable deaths in Mexico are attributable to diabetes [90] and account for one-third of all mortality between the ages of 35 and 74 years [89]. Prior studies assert that the risk of dying among individuals hospitalized for dengue increases 11-fold when there are underlying comorbidities like diabetes [91]. A recent study done in Mexico, Brazil, and Colombia explored mortality associated with DF and found that comorbidities increase case fatality rates 3- 17-fold [92].

This study aimed to explore the moderation effects of DF serotype on pregnancy in causing severe DF. Findings from this will inform policies regarding the management of DF, particularly among pregnant women.

CHAPTER 2: LITERATURE REVIEW

2.1 Common Arboviruses in Mexico:

Mexico suffers from a burden of multiple mosquito-borne diseases, some of which present with similar epidemiology and symptomology. Particularly, in the last five decades, there has been an unprecedented rise in zika and yellow fever cases, and a resurgence of diseases like dengue and chikungunya [1]. The most common mosquito-borne diseases in Mexico are chikungunya, zika, and dengue [93]. Like chikungunya and zika, dengue is associated with the *Aedes aegypti* and *Aedes albopictus* mosquito species. These mosquitoes particularly survive well in tropical and subtropical regions. While dengue remains the most prevalent of the infections, co-infection with chikungunya and zika also tends to occur often [93].

Some studies have alluded to the fact that chikungunya, zika, and dengue tend to present more in females and people 15 years and older [93]. However, this may not be the case for all states or cities in Mexico; one study found that individuals 15 years and younger, were more susceptible to dengue infections and seroconversions, specifically in the state of Yucatan [94].

2.2 Dengue Epidemiology

Dengue is the fastest spreading vector-borne viral disease, with endemicity in over 100 countries [20, 21]. Particularly, within the past 5 decades, factors like urbanization, globalization, and international mobility have aided in the emergence of an arboviral disease epidemic in new areas, of which dengue is the most common [22, 23]. The interplay of vectors, human behaviors, and the environment characterize the epidemiological patterns seen in the transmission of dengue fever [95].

Dengue is a flavivirus that is mostly spread in urban and suburban areas of tropical and sub-tropical regions and about 40% of the world's population is at risk of being infected with dengue [20]. Transmission of dengue is by the *Aedes* spp mosquitoes, of subgenus *Stegomyia [22]*. Of the *Aedes* spp, *Ae. aegypti* is the commonest vector implicated in dengue transmission [22]. *Ae. aegypti* has a preference for feeding on human beings and does so during the daytime, around domestic environments, typically feeding on multiple people within short intervals [22]. Hence, overcrowding and man-made larval habitats, further compound the spread of the dengue virus particularly in tropical low-income countries [28]. Although less efficient than *Ae. aegypti*, the *Ae. albopictus* has also been implicated in the spread of dengue in new locations since it easily adapts to new environments [2].

Dengue virus has 4 serotypes: dengue 1 to 4 (DENV- 1, -2, -3, and - 4) and in recent times, dengue outbreaks typically involve more than one serotype [3]. When an individual gets infected with one serotype, that person gets long-term protection for that serotype but remains vulnerable to the other serotypes [9]. The first infection with either of the serotypes results in asymptomatic disease or mild symptoms like retro-ocular pain, fever, headache, flu-like illness, nausea, rash, muscle ache, vomiting, and swollen lymph nodes [20]. A second infection with any of the other serotypes usually results in a severe clinical manifestation like dengue hemorrhagic fever, respiratory distress, and organ impairment [20]. While primary infection confers immunity through the generation of serotype-specific neutralizing antibodies, the disease mechanism of secondary infections involves non-neutralizing antibodies; each dengue serotype can elicit cross-reactive non-neutralizing and disease-enhancing antibody responses [2]. These cross-reactive antibodies can gain access to host cells during a secondary infection with a different serotype [2] and this process is known as antibody-dependent enhancement (ADE).

2.3 Direct and Indirect cost of Dengue Fever:

Between 2000 and 2007, the estimated aggregate cost attributable to dengue in the Americas was estimated at US $2.1 billion [3]. In 2019, there were 3.1 million DF cases, 20% higher than a previous high record in 2015 [3]. The economic impact of dengue includes family-level costs from food, transportation, and hospital bills, which may result in an average of 4.8 to 4.2 missed workdays [96]. Symptoms of DENV infection also tend to affect multiple family members at a time, with an average duration of illness being about 9.1 days and in 2001, dengue resulted in 427 DALYs lost per million in Thailand [96]. Globally, the years of life lost to premature mortality attributable to dengue was 576, 900 and the years lived with disability attributable to dengue was 566, 000 in 2013 [32]. Dengue contributed to 1.14 million disability-adjusted life-years (DALYs) in 2013 when considering both fatal and non-fatal outcomes [32]. Proper case management reduces the mortality rate from about 20% to 1% [20].

2.4 History of Dengue in the Southern Americas:

An increase in dengue hemorrhagic fever (DHF) over the years has been attributed to reasons like the trafficking of different DENV serotypes and strains which cause secondary infections, resulting in an immune-enhancement phenomenon. It is also hypothesized that improved transportation systems may be contributing to the effective dispersal of more virulent DENV strains which displace indigenous strains in the Americas [9]. Changes in the incidence and severity of dengue are temporally associated with new circulating DENV serotypes and genotypes in a region [9]. The oldest dengue serotype in the Americas is the DENV-2 and the youngest is the DENV-4 serotype [9]. This corresponds to their nucleotide diversity; while DENV-2 has the highest nucleotide diversity, DENV-4 has the lowest [9]. It is suggested that DENV -1, -2, and -3 were introduced into the Americas through airline traffic [3]. After the Pan

American Health Organization (PAHO) led a campaign to eradicate DF in the 1950s and 60s, some Caribbean Islands continued to house the DENV-2 and DENV-3 serotypes. The DENV-1 and DENV-4 serotypes reappeared in the 1970s after the PAHO campaign was over. Infection with either serotype typically resulted in dengue fever till the emergence of dengue hemorrhagic fever in 1981 in Cuba, across South America in 1989, and in Central America in 1994, which was concurrent with the reintroduction of DENV-3 [9]. Uruguay, continental Chile, and Canada are the only American countries out of 53 countries free of dengue fever [3].

In Mexico, *Ae. aegypti* mosquito was declared eradicated in 1963 but reappeared 2 years later [9]. In the late 1980s and early 1990s, DENV cases decreased but this reversed in 1995 when the DENV-3 was isolated for the first time and cases of DHF were confirmed. Overall, the reported proportion of DHF cases increased from about 0 before 1994 to 14-28% from 2002 to 2004 [9]. Dengue transmission typically has two different epidemiological patterns; it is endemic in the coastal states of the Gulf of Mexico and seasonal on the Pacific coast Yucatan peninsula [9]. There has also been the detection of low-level DENV transmission during inter-epidemic periods. The reintroduction of dengue in Mexico may therefore be through continual circulation within the country or from endemic viruses in neighboring countries.

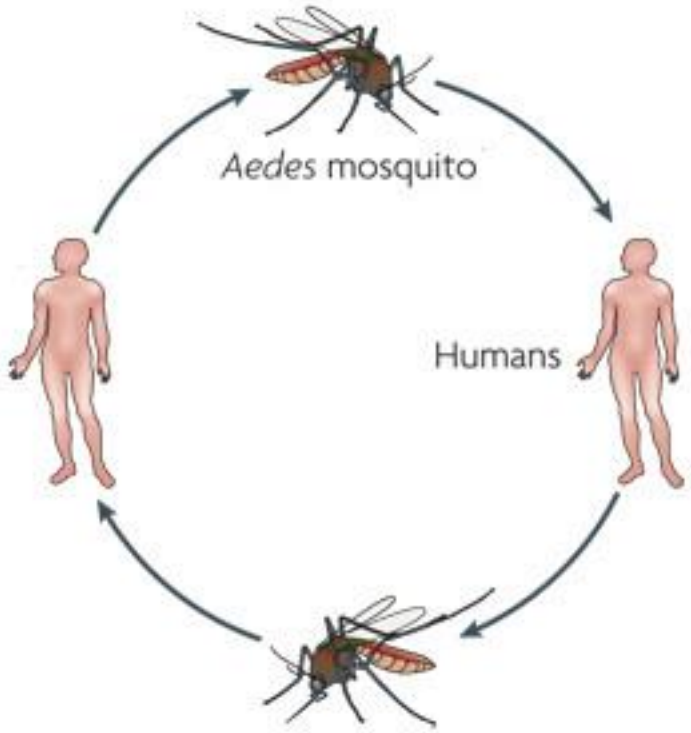
Phylogenetic analysis shows that the DENV-4 serotype was introduced at a one-time point in the Americas from a strain that circulated in the Pacific in the late 1970s and early 1980s [9]. After its introduction in 1977, the DENV-1 serotype is suggested to have emerged from different countries, after which independent lineages were formed [9]. Hence, it is hard to trace its origin. It is suggested that DENV-3 has an Asian origin but also has a weak phylogenetic association with the African strains. While several DENV-2 serotypes circulated in the Americas, these were progressively displaced by strains originating from Asia, along with the emergence of DHF. In some countries like Jamaica, the

introduction of a new DENV serotype was not accompanied by an outbreak of DHF in the country of introduction but in other countries [97, 98]. This suggests that a population's immune status and genetics might interact with the effect of the viral strain.

Hyperendemicity and simultaneous circulation of several serotypes are not sufficient to result in epidemic DHF. However, it is suggested that the presence of a virulent strain is critical to result in a clinically severe outcome [9]. Hyperendemicity and virulence of the DENV strain may act synergistically to cause a DHF epidemic. Hence, it is important to monitor the DENV serotypes to improve emergency preparedness for Dengue epidemics.

2.5 Life Cycle of *Aedes aegypti:*

Depending on its competency to be a vector, an *Ae. aegypti* mosquito, which is the most common causative organism of dengue fever, may feed on a viremic human, after which, the virus travels to the midgut to establish infection [99]. From the midgut, the virus may be disseminated to other tissues, including the salivary gland [99].



Adapted from Whitehead, S. S. *et al*. Prospects for a dengue virus vaccine. *Nature Reviews Microbiology* 5, 518–528 (2007).

2.6 Clinical Presentation:

The clinical presentation of dengue runs on a spectrum from asymptomatic to life-threatening clinical manifestations. Dengue fever is self-limiting and typically lasts for 5 to 7 days. The main difference between dengue fever (DF) and dengue hemorrhagic fever (DHF) is plasma leakage [2]. DHF has 4 grades, where grade 4 is the severest. The most critical phase of the disease is the end of the febrile illness and this is characterized by plasma leakage, hemoconcentration, and thrombocytopenia [2]. Laboratory tests usually reveal leukopenia and thrombocytopenia in all ages [100].

2.7 Primary and Secondary Dengue Infection:

Primary infection with dengue may be caused by DENV -1, -2, -3, or -4 serotype and is characterized by high titers of immunoglobulin M (IgM) in 3-5 days and immunoglobulin G (IgG) antibodies from day 6- day 10, after symptom onset [2]. While the IgM disappears in 2-3 months, IgG persists in the body for life, providing lifelong immunity against the infecting serotype, but not the other three serotypes [2].

Secondary dengue infection may be either caused by the same serotype as the first infection, or by any of the other three serotypes. Of the people who have a secondary infection, 2%-3% of them progress to DHF, which may further result in dengue shock syndrome (DSS) and death [2]. ADE, which occurs as a result of secondary infections, is associated with severer infections. However, not all severe infections are a result of secondary infection [2].

2.8 Complications of Dengue infection:

DHF is characterized by a fever that lasts from 2 to 7 days, the presence of a hemorrhagic manifestation or a positive tourniquet test, thrombocytopenia, and signs of plasma leakage as evidenced by hemoconcentration or pleural effusion [101]. Dengue shock syndrome is an extremely fatal presentation of dengue DHF and is characterized by additional circulatory symptoms like a weak and rapid pulse with a narrow pulse pressure or age-specific hypotension, cold and clammy skin, and restlessness [101]. Like Zika, DENV is associated with Guillain-Barre syndrome (GBS), which is a neurological disorder [102].

2.9 Diagnosis of Dengue Fever

Diagnosis of dengue is done either by identifying viral genomic RNA, antigens, or eliciting antibodies during an infection. The NS1 protein is an antigen that is released into the bloodstream from dengue-infected cells [2]. NS1, along with IgM and IgG may be detected simultaneously in a 3-in-1 ELISA-based serological test [2]. Serological tests such as IgM ELISA are not specific for an infecting flavivirus due to cross-reactions among flaviviruses [22]. Since multiple Aedes-transmitted diseases have similar epidemiology, specific tests are necessary to make a diagnosis of dengue. One such test is the antibody test which can distinguish alphaviruses from flaviviruses [22]. Reverse transcription-PCR and non-structural glycoprotein-1 (NS1) ELISA are the most reliable techniques for detecting acute-phase infections [22].

2.10 Ecological Factors Associated with Dengue Transmission:

2.10.1 Climate Variability:

Mexico is particularly susceptible to dengue because it experiences no winter throughout the year [14]. The inter-annual and intra-annual variability in dengue results from both intrinsic and extrinsic factors. These factors which include factors associated with climate change and host-virus interactions affect serotype dynamics that predict the rate of infection [10].

2.10.2 Temperature and Rainfall:

An increase in weekly minimum temperature and rainfall is associated with an increase in DF cases [103]. Increasing temperatures may lead to a decrease in the mosquito's extrinsic incubation period (time taken for an organism to develop an intermediate host) [104]. Similarly, infection proportions of the DENV are influenced by extreme temperatures, as well as, daily temperature fluctuation [99, 105]; while

large fluctuations are associated with slower development and infectivity of mosquitoes, at a low mean temperature, these fluctuations may accelerate DENV transmission [106, 107]. The effect of temperature on dengue is nonlinear; above a temperature of 29 degrees Celsius, the odds ratio of dengue risk begins to decrease [108].

2.11 Vaccine:

Several types of vaccines have been explored in the attempt to control dengue. One such vaccine was the cell culture passage-based live-attenuated viruses (LAV) which were developed in Bangkok, Thailand. The vaccine development was stalled because it failed at eliciting a balanced immune response and was associated with adverse reactions primarily related to the DENV-3 vaccine strain. A few vaccine candidates have been able to reach the clinical trial phase. Notably, Dengvaxia, which was developed by Sanofi Pasteur, is the leading vaccine and has been approved in several countries, including Mexico, Brazil, El Salvador, and the Philippines. In 2015, Mexico licensed the first dengue vaccine, Dengvaxia (CYD-TDV), and was registered to be used in individuals who were 9-45 years, and living in endemic regions [20].

Vaccines formed with serotype-specific neutralizing antibodies have not been found to cause mortality at any concentration [109]. While WHO recommends that alternative dengue vaccine candidates should be developed to elicit neutralizing antibodies in the absence of cross-reactive enhancing antibodies, it might also be worth exploring more serotype-specific vaccines which may better target the predominant serotypes in various regions of the world.

2.12 Mitigating the spread of *Aedes* spp-transmitted diseases:

There have been previous successes in the control of *Ae. aegypti* when thorough control measures were put in place [110]. Prior successes have been attributed to control measures like larval source

reduction, indoor residual, and space spraying [11, 111]. Although effective, these measures were rare and transient. For instance, there was a drastic reduction in *Ae. aegypti* population between the 1950s and 1960s during a regional control program across the Caribbean, Central, and South America [110, 112]. After the successful control of *Ae. aegypti* in Singapore and Cuba during the 1970s and 1990s [112], the *Ae. aegypti* reinvaded these countries and is now reported to be in Africa [113]. Multiple factors have contributed to impeding the success of control programs. These include environmental factors specific to the increased rate of transmission in recent times, as well as, a lack of political will, scarce resources for effective interventions, ineffective control programs, and population factors like decreased herd immunity [112, 114].

Although insecticide-treated bed nets have been effective against the malaria-causing *Anopheles* mosquito, they have limited use in mitigating the spread of the *Aedes spp.* [22]. Proposed personal protection strategies include the use of insecticide/ non-insecticide screens at home, new technologies that may be applied during the daytime to prevent mosquito bites, and safe insecticide-treated clothing [22, 115, 116]. Other proposed control measures include genetic engineering technology [117], sterilization of male mosquitoes, and infection of the *Ae aegypti* with the bacteria *Wolbachia* [118].

CHAPTER 3: METHODS

3.1 AIM 1 & 2

3.1.1 Inclusion criteria:

DF is a nationally notifiable disease in Mexico. Upon request, Mexico's Ministry of Health (MoH) provided the dataset used for the analysis containing nine years (2012 to 2020) of clinical diagnosis and laboratory-confirmed DF infection with dengue virus (DENV) serotype classification, through Mexico's transparency platform, a portal which allows anyone to access or request government data. The MoH dataset contains non-identifiable health information collected from notifying health units from 2,469 Mexican municipalities. Classification of DENV serotypes is determined from polymerase chain reaction (PCR) results collected at the municipality level. Beginning in 2011, the RT-qPCR has been the mainstay for identifying DENV serotypes in Mexico [119]. Here, a dengue case is defined as an individual with laboratory-confirmed evidence of DF using the non-structural protein (NS1) of DENV or a positive immunoglobulin M (IgM) and a clinical diagnosis of DF.

3.1.2 Dependent variables:

DF is reported as non-serious dengue, serious dengue, and dengue with alarming signs. These three classifications are synonymous with DF, DHF, and DSS, respectively [66]. DF severity was further dichotomized as non-severe dengue and severe dengue, where severe dengue was classified as having serious dengue or dengue with alarming signs. This classification takes into account the World Health Organization's emphasis on the probability and potential for dengue with alarming signs to result in severe dengue [120]. Missing classification or diagnoses classified as 'other' were excluded from the analysis. The total numbers of all individuals with severe dengue were aggregated to get a total count for each Mexican state and quarter for each year.

3.1.3 Exposure variables

The MoH dataset contains data on PCR results at the individual level and was reported in the dataset as DENV-1, DENV-2, DENV-3, or DENV-4. The number of individuals with each DENV serotype was aggregated to get the count of DENV-1, DENV-2, DENV-3, and DENV-4 reported for each state per quarter. A five-category variable was created based on the geographic region. These regions were defined as Center, Center West, Northeast, Northwest, and Southeast (see Codebook). Monthly climate data was retrieved from the Climate Hazards Group InfraRed Precipitation with Station (CHIRPS) [121] and the national center for atmospheric research (NCAR). Mean estimates of climate data are calculated by extracting data at the municipality, by using post offices as centroids to extract these values. The climate data included reported data from January 2012 to December 2020 on the mean, maximum and minimum temperatures, and mean and maximum rainfall. Due to the potential of introducing systematic bias using maximum and minimum temperature ranges [122], the mean temperature was used to estimate the effect of temperature. Similarly, average humidity and rainfall were used as measures of humidity and rainfall. Monthly mean temperature, rainfall and humidity were averaged for each quarter of each year. Data about altitude was retrieved from the 'Advanced Spaceborne Thermal Emission and Reflection Radiometer Digital Elevation Model' (ASTER DEM) [123].

SES data included information about the percentage of individuals in a municipality without basic socioeconomic determinants of health. These included illiteracy, lack of health services, living in houses with dirt on the floor, lack of toilet, no water pipelines, no sewage, or absence of electricity. The national council for the evaluation of social development policy compiles data on these socioeconomic variables every 5 years and projects these percentages to other years [124]. A principal components analysis was performed to retain latent factors associated with all the SES variables and a poverty index was created. Information about the covariance structures and the principal components were retrieved

based on the amount of variation each component explained. Based on the eigenvalues, the first factor contributed 77.19% of the variances seen and was the only factor with an eigenvalue greater than 1. This was further visualized using a scree plot. The first factor was retained and an unrotated factor 1 was used to determine standardized coefficients that were then used to calculate a poverty index.

3.1.4 Data Cleaning and preprocessing

SAS 9.4 was used for data cleaning and recoding of variables. Aggregated individual-level data containing counts of DENV serotypes and region categories were merged with environmental and socioeconomic data. Separate datasets were created and aggregated at the quarterly, yearly, and complete (2012 to 2020) levels for descriptive analysis, regression models, and spatial analysis. The final sample size was 289,287. Of this, DENV-1, DENV-2, DENV-3, DENV-4, and severe dengue counts were 33667; 35713; 944; 519, and 84,047 respectively. Missing serotype data on 218,444 individuals were similar for severe (76%) and non-severe dengue (71%) (Table S1a). Similarly, missingness patterns across regions were comparable (Table S1b). However, missingness was drastically reduced in 2019 compared to the other years (Table S1b). Based on these observations, missingness was assumed to be at random (MAR) and a complete case analysis was used to test all study hypotheses.

3.1.5 Statistical Analysis

3.1.5.1 Individual-level data

For each serotype, an estimate of the mean temperature, rainfall, humidity, altitude, and poverty index associated with each subgroup was determined and an ANOVA was performed to assess statistically significant differences across serotypes for the environmental and SES parameters. Frequency distributions were obtained for demographic regions and years. Chi-square tests were used to

assess statistically significant differences across the DENV serotypes for each categorical variable. Independent sample t-tests were performed to assess the mean differences across dengue severity classification for environmental parameters and poverty. Chi-square tests were also used to assess if differences across regions, years, and DENV serotypes were statistically significant across disease severity classification.

3.1.5.2 Aggregated Data:

A time-series analysis was performed for five models, by exploring factors that predicted the rate of occurrence of DENV-1, DENV-2, DENV-3, DENV-4, and severe dengue. For each serotype count, the effect of SES, environmental factors, and quarterly effect, on the rate of occurrence was determined. The final model explored the influence of environmental factors, socioeconomic status, and the number of individuals with DENV-1, DENV-2, DENV-3, and DENV-4 on dengue severity. The models were assessed for overdispersion by determining if there were equal means and variances. The assumption of equal means and variances were satisfied; the scale parameter which represents the residual deviance divided by degrees of freedom was less than one for all models. Hence, Bayesian Poisson regression models were used for all outcome variables.

Conditional autoregressive Bayesian (CARBayes) approach was used to determine the spatial autocorrelation to be modeled by random effects that are assigned a conditional autoregressive prior distribution [125]. Complete cases were used for each model and a Markov Chain Monte Carlo (MCMC) was used to estimate each model parameter. The first 20,000 of the 220,000 sampled were used as burn-in. The credible interval was computed based on the highest density interval. A time variable in the unit of ‘years' was created from the quarter variable. The variable (t), therefore, took the values 0.25-year, 0.5-year, 0.75-year, one year, etc. To estimate the rate, the offset option was used in the model statement

and the log (t) was used as the offset option. Trace, autocorrelation, and density plots were used as diagnostics for each model. The deviation information criterion and Watanabe-Akaike information criterion (WAIC) of all models were compared and the models with minimum WAIC were chosen and interpreted (Table S2). For each model, convergence was assessed using Geweke diagnostic results. Convergence was achieved when Geweke was found to be between -2 and 2 [126]. R package CARBayes was used to fit the models.

3.1.6 Spatial Analysis

To determine spatial autocorrelation of the individual serotypes across Mexican municipalities, Moran's I was calculated. Moran's I for DENV-1, DENV-2, DENV-3, and DENV-4 were 0.016 ($p<0.001$), 0.03 ($p<0.001$), 0.03 ($p< 0.001$) and 0.01 ($p<0.001$). Hotspot analyses were performed using ArcGIS Desktop. The Getis-Ord Gi* was used to visualize variations in serotypes, poverty, and severe dengue in Mexico. The Getis-Ord Gi* statistic compares the local mean values (the count for a municipality and its neighboring municipality) to the global mean count (for all municipalities) [127]. It creates z-score, p-values, and confidence intervals for each municipality of interest [128], which represents the significance of the difference between the local and global means [127]. A significantly positive z-score (hotspot) represents clusters of high case count and a significantly negative hotspot represents clusters of low case counts unlikely to be a result of random spatial processes [127]. DENV serotypes and severe dengue were specified as input features. Hotspots were determined for all years combined and yearly hotspots were used to assess persistent severe dengue hotspots.

3.2 AIM 3

3.2.1 *Data Collection and Preprocessing*

The study was reviewed and approved as an 'exempt category' by the North Texas Regional Institutional Review Board and by the ethics and research committee of the Universidad Autónoma de Nuevo León. The total sample size was 94,832 women.

3.2.2 *Definition of Variables*

Figure 2 shows a directed acyclic graph (DAG) of factors associated with pregnancy and dengue severity. Analysis was restricted to women of their reproductive age and defined by an age range of 15 to 49 years [129]. A woman was identified as pregnant or not pregnant based on pregnancy status classification retrieved from the dataset. Region was categorized as Center, Center West, Northeast, Northwest, and Southeast. Classification of the region has been defined elsewhere [130]. Hypertension and Diabetes were binary variables with '1' indicating the presence of disease and '0' indicating the absence of disease. An individual with confirmed dengue had either DENV-1, DENV-2, DENV-3, or DENV-4 serotype. An individual with dengue was reported as having non-severe dengue, severe dengue, dengue without alarming signs, dengue with alarming signs, or others. Individuals with no dengue classification or 'other' classification were excluded from the analysis. The World Health Organization's revised 2009 classification of DF emphasizes the inclusion of warning signs as a diagnostic criterion for probable and potentially severe dengue [120]. However, this classification requires laboratory-confirmed results to prevent inflation of the number of severe dengue cases. Because our dataset contained both clinical and laboratory-confirmed diagnoses, severe dengue was defined as individuals with 'severe dengue or having dengue with alarming signs', while non-severe dengue was defined as individuals 'having non-severe dengue or dengue without alarming signs'.

3.2.3 *Analysis*

Differences between severe and non-severe dengue for each covariate were tested for statistical significance using chi-square and t-test scores for categorical and continuous variables, respectively. Statistical significance for the distribution of potential covariates was also computed by pregnancy status and region (Table S3, S4). Statistical significance was set to an alpha value of 0.05 for all computations. The pattern of the DENV serotype from 2012 to 2020 was visualized by DF classification. Bivariate analyses were performed to determine the relationship between each covariate and dengue severity and p-values were adjusted for False Discovery Rate (FDR).

3.2.4 *Model Selection*

Since hypertension increases the risk of diabetes and vice versa [131], a phi coefficient test was performed, before including both independent variables in the model. There was a weak positive correlation of 0.2769 between hypertension and diabetes. A multicollinearity test was performed and tolerance for hypertension (0.93) and diabetes (0.93) showed no multicollinearity between them. Similarly, there was no multicollinearity observed among the other covariates. Year and region were considered as potential random effects and were evaluated in a two-level hierarchical model. The Wald tests for random effects for the region (p= 0.049) and year (p=0.032) were both significant. A comparison between the random and fixed effects model using the Bayesian information criteria (BIC) revealed that the random-effects model performed better. Hence, both region and year were retained in the model as random effects. Based on the hierarchical model (1), a multiple logistic regression was performed with severe or non-severe DF as the outcome variable and pregnancy as the exposure variable.

$$y = X * _{\beta} + Z * u + \boldsymbol{\varepsilon} \quad \ldots\ldots (1)$$

Where X a matrix (N *p) with p predictor variables and Z is a matrix (N*q) for q random effects [132].

Two-way interactions were explored, and the interaction between pregnancy and serotype was retained. Other covariates in the model included diabetes, hypertension, and age. The final model was chosen by comparing AIC and BIC between models. The AUC for the predicted probabilities of the final model was 0.7156 (Figure S1).

3.2.5 *Sensitivity Analysis*

The dataset shows the results of individuals who were tested for pregnancy, diabetes, and hypertension. However, individuals without a test could potentially have been misclassified as 'negative'. To quantitatively assess this kind of systematic error, a sensitivity analysis is recommended [133]. Using a misclassification spreadsheet [134], results from the regression analysis were explored for exposure bias. As suggested for best practices [135], pairs of sensitivity and specificity were explored for exposure misclassification of pregnancy status (Table S5).

3.2.6 *Coding and Environment*

Data preprocessing, analysis, and generation of figures were done using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), R (version 4.1.2, The R Foundation, Vienna, Austria), and STATA/SE (Stata Corp LLC, College Station, TX, USA). All codes can be found in the supplementary file.

3.2.7 *Sample size determination*

A study conducted in Brazil found that 4 out of 707 (0.006) pregnant women and 19 out of 15,576 (0.001) non-pregnant had severe dengue [82]. To determine such an association with a power of 0.80, at an alpha of 0.05, a sample size of 4547 was needed. The sample size was determined using the G*Power 3.1.9.4 software. Our study was comprised of 94832 women of their reproductive age, of which 5431 were pregnant (5.31%) and 25018 (26.38 %) of all women had severe dengue.

CHAPTER 4: RESULTS

4.1 AIM 1 & 2:

The DENV serotype distribution across Mexico from 2012 to 2020 is shown in Table 1. Across the study period, the most prevalent serotype was DENV-2 and the least prevalent was DENV-4. While DENV-1 and DENV-2 were most prevalent in the center-west region, DENV-3 and DENV-4 were most prevalent in the Southeast region (Table 1). The chi-square statistic for the association between serotype and region was statistically significant ($\chi^2 = 2843$, $p < 0.0001$). Regional variations in serotype distribution varied by year. The Northeast region recorded the highest number of DENV-2 (Figure S2), with its peak count in 2020 (Figure 3). Serotype-specific differences across the years were statistically significant ($\chi^2 = 11943$, $p<0.001$). Higher mean rainfall was associated with DENV-1 and DENV-4 (Tables S2). DENV-3 and DENV-4 were both associated with higher humidity ranges and higher poverty indices. DENV-1 was associated with the highest altitude ranges while DENV-3 was associated with lower altitude ranges (Table S1). Differences observed across serotypes were all statistically significant (Table S1). Higher temperatures and poverty index were observed in the Southeast region compared to the other regions (Table S6). The Center region had the highest average rainfall and highest altitude, while the Northwest region was associated with the lowest altitude, and lowest humidity (Table S6). All regional differences were statistically significant (Table S6).

From 2012 to 2020, counts for all DENV serotypes and severe dengue rose around the third quarter and dropped to a one-year low usually around the first quarter of each year (Figure S3). The highest peak counts for DENV-1, DENV-2, DENV-3, DENV-4, and severe dengue were 4325, 6127, 126, 96, and 3442 respectively. The Southeast region had the highest proportion of severe dengue cases (Table 2). DENV-2 accounted for most of the severe dengue counts in all the regions (Figure S2). The highest proportion of severe dengue attributable to DENV-1, DENV-2, and DENV-3 occurred in 2019,

while the highest proportion of severe dengue cases among DENV-4 individuals occurred in 2015 (Figure S4). While the proportion of severe dengue decreased from 2012 to 2018, there was a spike in the number of severe cases in 2019 in the Center, Center west, and Southeast regions (Figure 4). The number of severe dengue cases did not vary substantially from 2017 to 2020 in the Northeast region, while cases began to rise in the Northwest region from 2017 (Figure 4). DENV-2 contributed to the highest proportion of severe dengue across the study period ($\chi^2 = 3596$, $p < 0.0001$) (Table 2).

A decrease in altitude was significantly associated with an increase in the rate of occurrence of DENV-1, DENV-2, and DENV-3 after controlling for other variables. However, the relationship between altitude and rate of DENV-4 occurrence was not statistically significant (Table S7-S10). A one-unit increase in the poverty index was associated with a percentage increase in the rate of occurrence [$(1-\exp^{(\text{median estimate})}) * 100$] of DENV-1 (8.4%), DENV-2 (5.0%), DENV-3 (16.0%), and DENV-4 (13.8%), after controlling for other variables. After controlling for other covariates, each degree Celsius increase in temperature was associated with a lower rate of all DENV serotypes while increasing humidity was associated with an increase in all DENV serotypes. After controlling for other variables, for each mm increase in rainfall, the rate of DENV-1 and DENV-2 decreased by 19.6% and 4.7% respectively (Tables S7 and S8).

All DENV serotypes had cold spots in the central region of Mexico (Figure 5). Except for DENV-3, cold spots associated with other serotypes extended into Southeastern states like Oaxaca. Hotspots were generally more associated with coastal regions. Hotspots for DENV-3 and DENV-4 were predominantly found in the Southeastern region. DENV-1 and DENV-2 hotspots were found in the center-west region (Figure 5). Hotspots in the northeastern region were associated with DENV-2, DENV-3, and DENV-4.

The serotype-specific effect on the number of individuals with severe dengue per year was statistically significant for all DENV serotypes (Table S11). For each count in DENV-1, DENV-2, DENV-3, and DENV-4, the rate of severe dengue increased by 0.02%, 0.1%, 0.03%, and 5.8% respectively, keeping other variables constant. Keeping other variables constant, an increase in altitude was associated with an increase in the rate of severe dengue while an increase in the poverty index was associated with an increase in the rate of severe dengue. An increase in temperature and rainfall both had negative effects on severe dengue while the humidity was significantly associated with an increase in dengue severity when controlling for other variables.

Hotspot analysis of the poverty index showed significant hotspots in parts of the Northwestern region and southeastern regions like Oaxaca (Figure S5). Cold spots for severe dengue extended from the central region to the southeastern region (Figure 6). Severe dengue hotspots were seen across municipalities in Chiapas, Campeche municipality, and some municipalities in Quintana Roo and Yucatan (Figure 4). States like Nayarit and Jalisco both had severe dengue hotpots when looking at data aggregated from 2012 to 2020. Although the hotspot trend for those with severe dengue was not persistent, hotpots occurred in coastal states and cold spots were specific to the central region when they occurred (Figure S6).

4.2 AIM 3:

Table 3 shows the distribution of the sample of women from 2012 to 2021. The average age was 29 years old, with most women living in the Southeast region (50.12%) of Mexico. The average age of pregnant women was 24 years and 29 years for non-pregnant women (Table S3) $t\ (_{7213.6}) = 57.40$ $p<0.0001$. Across regions, DENV-2 was the commonest serotype found among women with severe DF (Figure S7). Compared to the other four regions, pregnant women in the Northeast region had the highest

proportion of DENV-2 serotype (Figure S8). The chi-square statistic for the differences observed across various regions for dengue severity was statistically significant ($\chi^2 = 2799.85$, $p<.0001$). Although women with severe and non-severe dengue were within the same mean age, with median ages of 28 and 29 years respectively, the t-test for age ($t_{(59723)} = 3.60$, $p = 0.0003$) showed a significant difference between the severe and non-severe dengue sub-populations. Most individuals with severe dengue were not pregnant (Table 3). However, among pregnant women, 33.57% had severe dengue, compared to 25.23% of non-pregnant women (Table S3). The difference observed between pregnancy status for dengue classification was statistically significant ($\chi^2 = 187.12$, $p <.0001$).

Dengue severity has had both downward and upward trends from 2012 to 2020 (Figure S9). Until September 2019, DENV-1 remained the most prevalent serotype among women with non-severe DF (Figure S10). The proportion of DENV-2 increases around this period for non-severe DF and decreases for severe DF (Figure S11). However, DENV-2 remained the most predominant serotype from 2017 to 2020 among the severe dengue group. Among women with severe dengue, DENV-2 was the commonest variant, while DENV-1 was the commonest variant among those with non-severe dengue (Table 4). There was a similar distribution by pregnancy category; while most pregnant women had DENV-2, non-pregnant women mostly had the DENV-1 variant (Table S3). The proportion of hypertensives and diabetics was higher among women with severe dengue compared to those with non-severe dengue. IgM and IgG positive proportions were higher for those with severe dengue. These differences were statically significant (Table 4).

While the proportion of IgG-positive cases was higher among pregnant women, the proportion of IgM-positive cases was lower for pregnant women ($p<.0001$) (Table S3). DENV-2 was the commonest serotype in the Southeast region, while DENV-1 was the commonest serotype among women in the other

regions of Mexico (Table S4). The Southeast region had the highest proportion of severe dengue cases compared to other regions and this difference was statistically significant ($p<0.0001$) (Table S4).

Figure S12 shows variations in severe dengue prevalence from 2012 to 2020 across Mexican states and pregnancy status. Both pregnancy strata showed a similar pattern of spread of severe dengue, although the number of cases in non-pregnant women was higher. A look at the proportions between severe and non-severe dengue for each pregnancy strata shows variations across states (Figure 7). While most states recorded higher counts of non-severe dengue compared with severe dengue, Chiapas in the Southeast region, and Nayarit in the Center-West region had a higher prevalence of severe dengue for pregnant women. This was contrasted with non-pregnant women who had similar proportions across severity strata in both Chiapas and Nayarit.

Unadjusted odds of a pregnant woman experiencing severe dengue were 1.5 times the odds of that of non-pregnant women. When adjusted for in a multiple logistic regression model, serotype moderated the effect of pregnancy. Respectively, among individuals with DENV -1, DENV-2, and DENV-4, pregnant women had 1.45, 1.35, and 3.78 times the odds, of severe dengue, compared to non-pregnant women after adjusting for other variables. Compared to those living in the Southeast region, individuals living in the Center, Center West, Northeast, and Northwest regions, had 0.38, 0.31, 0.37, and 0.66 times the odds of severe dengue (Table 5). Based on the random effects from yearly variations, on average, severe DF cases were higher in 2014 and lower in 2017 and 2020, compared to non-severe dengue (Table S12). Similarly, severe DF cases were significantly higher in the Southeast and Center west regions. A one-unit increase in age was associated with 0.992 times lower odds of severe dengue after adjusting for other variables. All else equal, individuals diagnosed with diabetes had 2.6 times the odds, while those with hypertension had about 3.0 times the odds of severe dengue compared to those without diabetes and hypertension respectively. Figures S13 – S16 show a graphical representation of the interaction effects

between serotypes and pregnancy status, as well as post-hoc predictive probabilities of dengue severity. The variance seen among individuals with DENV-4 between pregnant and non-pregnant women is higher compared to the other serotypes. At a specificity of 99%, and sensitivity of 90% the adjusted OR for pregnancy was 1.63 (CI: 1.52,1.75) (Table S5) compared to the unadjusted OR of 1.49 (CI:1.41, 1.59).

CHAPTER 5: DISCUSSION

5.1 AIM 1 & 2

Severe dengue hotspots were associated with coastal regions and there were persistent cold spots in Center Mexico. Higher temperatures, increasing rainfall, and higher altitudes were associated with a decrease in the rate of severe DF while higher humidity and poverty were associated with an increase in the rate of severe dengue. States at lower altitudes, in humid regions with a high poverty index, are particularly at risk of severe DF.

Arid regions like the Baja California Sur have previously reported zero to few numbers of DF cases. Dengue hemorrhagic fever is now reported to have a cyclical trend in Baja California Sur [136]. Until 1985, there were no reported cases of DF in Baja California Sur [137]. However, from 2012 to 2014, the state saw a 652% rise in the number of reported DF cases, coinciding with the timeline of the first detection of DENV-2 [137]. The marked rise in cases was, however, attributed to floods and destructions from Hurricane Odile, which created a favorable condition for the breeding of mosquito vectors [137]. Changes in predominant serotypes and the presence of multiple serotypes in a region, at the same time, increase the possibilities of transmission and, consequently, increase the number of epidemics [137]. Our study shows an increase in the number of severe DF counts between 2014 and 2019. Severe dengue hotspots occur in the areas associated with DENV-1 and DENV-2 hotspots in the Center west region, and DENV-3 and DENV-4 hotspots in the Southeastern region.

The presence of DENV-3 and DENV-4 hotspots in the southeastern region might be attributable to the migration history of dengue serotypes. Historically, the southern states in Mexico have usually been the first and most affected by DF [9]. Hence, it has been hypothesized that there is a periodic introduction of dengue from Central America through the southern frontier [9, 138]. Findings of cold

spots in central Mexico are consistent with prior literature [139]. The center region of Mexico has higher altitude ranges compared to the other regions and prior literature suggests a reduced presence of *Aedes aegypti* above 1700m [80]. Further, increasing altitude was significantly associated with lower rates of all DENV serotypes and severe dengue.

Assessment of individual-level risk factors suggests that DENV-1, DENV-2, and DENV-4 increases the risk of severe dengue, and of these, DENV-2 increases an individual's risk higher than the other serotypes [140]. However, ecologically, our study suggests that a location's severe dengue rate may vary based on the presence or abundance of DENV-specific serotypes. An increase in DENV-4 count was associated with an increase in severe dengue rate much higher than the other serotypes. Being the youngest serotype in Mexico [9], this finding might suggest heterologous secondary infections with DENV-4 that result in worse outcomes, as primary infection with DENV typically results in less severe outcomes [141]. Future studies may further explore how primary or secondary infection with DENV-4 predicts severe dengue.

In our study, different climatic factors were observed to affect the rate of dengue attributable to the different serotypes. Increasing humidity was significantly associated with an increase in the rate of all four serotypes. While an increase in temperature was associated with a decrease in the rate of all serotypes, the negative effect of rainfall was only significant for DENV-1 and DENV-2. The effect of different climatic parameters on specific serotypes may further explain the serotype-specific hotspot patterns observed across Mexico. The proportion of DENV-2 was highest in the center-west region and this region had lower mean temperature ranges compared to the southeast region, where most DENV-4 serotypes were observed to occur. DENV-3 and DENV-4 were both associated with higher mean humidity compared with DENV-1 and DENV-2. Since southern and coastal Mexico tends to be more humid [142], this might explain the prevalence of DENV-4 in the region. Further research about the exact

mechanisms responsible for serotype diversity may further elucidate the reasons behind these findings. Particularly, possible mechanisms like transmission dynamics of the *Ae. aegypti* across time may help to predict future patterns and inform the current management of DF.

The southeastern region was significantly associated with warmer temperatures, higher rainfall, humidity, a higher poverty index, and a lower altitude range compared to all regions, except for the Northwest. Of these environmental factors, humidity and altitude make the region suitable for severe dengue. Currently, there is scant literature that supports a definitive relationship between poverty and DF [143]. In this study, increasing poverty was associated with an increase in the number of all serotypes and severe dengue per year. However, there were few overlaps between poverty hotspots and DENV serotypes, as well as severe dengue hotspots. This may suggest that combining several determinants of severe dengue could potentially help to highlight states that require active surveillance. Of interest, are states like Oaxaca, which is in the southeastern region and had significant hotspots for the poverty index. Particularly, all four serotypes have been isolated in Oaxaca, with reported genetic recombination in the DENV-2 serotype [144].

This study has some limitations. Due to the mild presentation and self-management of most DF cases, DF tends to be under-reported [145] and sometimes misdiagnosed as other febrile illnesses [146]. To eliminate misclassification bias, lab-confirmed data was used in this study. Lack of information on heterotypic (infection with a subsequent DENV serotype other than one exposed to in a primary infection) DF infections and the order of infection made it impossible to explore the effects of cross-reaction between serotypes on DF severity. Lastly, although the serological diagnosis was made for diagnosed individuals, there were missing PCR results (75%), indicating that PCR tests are not done routinely. Removal of all missing PCR results resulted in a total sample of 71, 059 individuals with PCR results. Hence, although there was extensive missingness, we had enough samples to detect statistically

significant differences. Prospective studies may, however, focus on performing, monitoring, and documenting PCR results for further analysis. Furthermore, future spatiotemporal modeling or forecasting approaches may help to further track serotype distribution patterns and the risk factors associated with different DENV serotypes across Mexico.

Climatic factors predict the rate of occurrence of DENV serotypes differentially. Higher temperatures and increasing rainfalls predicted lower rates of severe DF, while humidity, lower altitude, higher poverty, and DENV serotypes were all positive predictors of dengue severity. Hotspots were associated more with coastal regions. States like Oaxaca in the southeast, with a high poverty index, should be monitored closely to help avoid complications associated with severe DF. Targeting such regions in DF control and management might help reduce complications associated with severe DF. Future research may focus on the effect of infection from a heterotypic DF serotype to determine if disease severity varies based on the order of serotype-specific infection.

5.2 AIM 3

The association between pregnancy and severe DF is moderated by the DENV serotype. Compared to other regions, the Southwest region had higher odds of severe DF. The effect of DENV-4 in pregnant women may indicate effects of genetic diversification and the emergence of new serotype-specific genotypes [147, 148] and this warrants further investigation. Dengue control programs and policies need to be expanded, using a multidisciplinary approach across Mexico.

Although a previous study found individuals with DENV-2 to have a lower risk of dengue hemorrhagic fever [67], the association between DENV-2 and a higher risk of severe dengue, particularly when compared to DENV-1 [149], is consistent with most literature [76, 149-151]. Similarly, DENV-2 and DENV-3 are more commonly associated with severe dengue compared to DENV-4 [149]. A recent

study in Brazil found that pregnant women had 1.92 times the odds of having DENV-4 serotype compared to non-pregnant women [82]. However, the authors concluded that the persistence of DENV-4 in a region and a higher number of cases in particular years may have explained the results found [82].

Pregnant women are generally more at risk and predisposed to certain clinal conditions [152]. The risk of hospitalization as well as DF severity tends to be higher among pregnant women compared to non-pregnant women [82]. A higher proportion of IgG-positive serology compared to IgM in pregnant women might point to a higher risk of severe DF among those with secondary infection. While a host's genetic background and immune status may influence disease presentation, it is suggested that certain viral structures may aid in replication in human target cells [153]. Differences among DENV serotypes may be attributed to genotype-specific (within serotypes) variances [67, 153]. Although the evidence does not support the transmission of antigenically aberrant strains, prior research suggests the displacement of DENV genotypes of less epidemiological significance by more virulent genotypes [153]. Genetic diversification and an emergence of a DENV-4 genotype-I have been found in a molecular analysis in Brazil and parts of tropical and subtropical America [147]. This may explain the moderation effect of DENV-4 serotype specifically among pregnant women compared to non-pregnant women. This is contrary to what is expected in the general population, where DENV-2 has been mostly associated with severer outcomes and DENV-4 has generally been associated with causing clinically mild diseases [67, 151]. It is also worth noting that although the effect of DENV-4 was statistically significant, the large confidence interval and smaller sample size may have influenced this finding. Hence, future prospective studies which may involve phylogenetic analysis or gene sequencing may further explore DENV-4 specific effects among pregnant women and the moderation effect of dengue serotypes in pregnancy.

The changing trend of increasing and decreasing cases of severe dengue may indicate a change in programs/policies associated with dengue fever eradication. The local health system mainly spearheads

the charge toward dengue prevention programs, with minimal effort from other sectors like water and sanitation [154]. When comparing regions, women in the Southeast regions had higher odds of severe dengue compared to those in other regions. Central regions on average had lower proportions of severe dengue. This may be explained by the fact that cities like Mexico City in the Center region are free of endemic mosquito-borne viral diseases [155]. With a subtropical climate and high elevation, there is a lower occurrence of *Aedes spp* in Central Mexico [155]. On the contrary, Pacific and Coastal regions tend to be at a higher risk of dengue [156]. For instance, states like Oaxaca in the pacific and southeastern region is one among the most affected states in Mexico, and the persistence of high dengue cases has been attributed to the presence of all four serotypes, a favorable climate, and socioeconomic level of the population [157].

Our study had several limitations. Firstly, only a confirmed diagnosis of pregnancy status was reported in the dataset. Hence, to address potential misclassification bias, a sensitivity analysis was performed to assess the misclassification of pregnancy status. The analysis showed that at a sensitivity and specificity higher than 80% and 97% respectively, findings from our study were conservative. Another limitation was missing data for all variables. Particularly, prevalence estimates for pregnant women in Zacatecas and Chihuahua were missing across the period of study. However, our sample size of 94832 women, satisfied the requirement for this study. Also, the cross-sectional nature of the study limits the inferential interpretation of results from the logistic regression model. The unavailability of information about vaccination status and behavioral factors that affect mosquito control also limits the study's ability to control for these confounders. However, the spatial trend analysis provides additional reasons for further exploration in future studies. Furthermore, since pregnant women are more likely than other women to visit the clinic for antenatal care and be hospitalized, the likelihood of being diagnosed with dengue might be higher than non-pregnant women. However, this assumes a high antenatal care

uptake and secondly, it also assumes that serological tests are performed routinely for pregnant women. Serological tests are performed upon clinical diagnosis of dengue. Since most dengue cases are asymptomatic and might go unnoticed, an acute presentation with a febrile illness among women is likely to present to the clinic regardless of pregnancy status. Lastly, restriction of the data to only women with serotype data means the likely exclusion of more people with non-severe dengue. However, the proportions of missingness among those with severe (76%) and non-severe (74%) dengue were similar. I higher proportion of missingness among non-pregnant women (75%) compared to pregnant women (60%), however, this supports the theory that more frequent access to healthcare may influence more diagnoses among pregnant women. Future studies may prospectively collect serotype data to ensure further limitation of potential bias.' has been added to the limitations.

Pregnancy increases a woman's risk of severe dengue. However, this may be modified by the DENV- specific serotype. Of note is the DENV-4 serotype, which is otherwise the least severe serotype in the general population. Across Mexican regions, the southwest region had the highest number of severe dengue cases. Particularly, perinatal care in states like Chiapas and Nayarit may warrant further surveillance. This may especially be important for individuals with comorbid conditions like hypertension and diabetes and under the age of 24 years. An intersectoral approach is still needed across Mexico, particularly in the Southeast region to address the risk of DF severity. Further research is needed to fully understand the moderation effect of dengue serotype in pregnancy.

5.3 Future Directions:

Future directions may include human-level variables that affect DF distribution dynamics. Theoretical approaches like ecosyndemics may be used. Ecosyndemics are disease interactions that occur

as a result of environmental changes due to human behavior [158]. This concept has been used in previous studies; one such study investigated the synergistic relationship between stress, vector-borne diseases, and sexually transmitted infections [158]. Another study used the ecosyndemic concept to explore the relationship between global warming and respiratory health [159]. It's been suggested that negative environmental changes may increase social disruption , exposure to infectious diseases ,and stress-induced immune suppression, which may reduce an individual's ability to respond to hazards [158].

Social factors:

Social factors that may influence regional mosquito control measures include how people from different cultural backgrounds may respond to mosquito control measures distinctly. Similarly, there may be variations in vector control in municipalities that are influenced by larger political and economic influences, which may further influence mosquito control in tourist areas. Future studies can, therefore, explore how social, cultural, political, and economic factors might influence differences in DF rates globally.

Migration patterns are associated with socio-economic changes, as well as the introduction of vectors into new regions. The rapid movement of people from rural to urban regions is often unmatched by the timely provision of adequate housing, sewage, and waste-management systems [160]. Future studies may further explore DENV serotype distributions, as well as severe dengue incidence across rural and urban regions.

Dengue, Pregnancy, and Comorbidities:

Studies and programs could address how pregnancies are monitored in various regions, as well as the progress of DF in pregnant women for each trimester to ensure that DF does not progress to severer

outcomes. Similarly, surveillance of the robustness of medical systems and dengue-related deaths that occur with comorbidities would provide insight into whether a stronger medical safety net prevents deaths linked to DF and its at-risk factors.

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