I dedicate this work to my family, specially to my supporting husband and daughter for their unconditional support, love and patience through all these years.
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Abstract of Dissertation Presented to the Graduate School
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EPIDEMIOLOGY AND TRANSMISSION DYNAMICS OF ARBOVIRUS IN LATIN AMERICA

By

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Major: Epidemiology

Arthropod-borne viruses (arboviruses) are considered global public health priorities. Arboviruses are considered the cause of some of the most important emerging infectious diseases currently threatening the globe. Approximately three billion people worldwide live in areas infested with Aedes mosquitoes and are at risk for arbovirus infections like dengue virus, chikungunya virus and Zika virus. Dengue virus has been circulating since the 1980’s in Latin America and its incidence has increased significantly worldwide and in this region. Aedes aegypti is the main vector for most of the arbovirus transmitted in Latin America.

In the last three years two arbovirus had emerge in the Western Hemisphere causing important outbreaks. In 2013, Chikungunya virus emerged in the Caribbean and spread rapidly through the Caribbean, Central and South America with 1.8 million cases reported. Similarly in 2015 Zika Virus was isolated in Brazil and since then it spread in areas infested with Aedes mosquitos in Latin America and the Caribbean and also with authoctonous transmission in continental United States.

The aim of this dissertation is to provide new estimates about the epidemiology and transmission dynamics of Arbovirus in Latin America. In the first paper, the estimates of dengue seroprevalence in three settings in Yucatan, Mexico are presented to describe the baseline conditions of these populations and potential risk factors associated with dengue transmission. The second paper has the first results of the baseline dengue seroprevalence and first year follow-up of a community based cohort in Yucatan, Mexico; and the third paper
characterize the epidemiology and transmissibility of Zika virus in Colombia using surveillance data defining the epidemiological features of local ZIKV outbreaks in Colombia and estimating the transmission parameters and potential risk factors associated with Zika transmission.

This study offers a unique perspective on the epidemiology of arbovirus in Latin America given the timeframe where for the first time three arbovirus are co-circulating in this region. Due to the robust datasets available, this dissertation is one of the first studies describing the epidemiology and transmission dynamics of the three viruses in the Western Hemisphere.
CHAPTER 1
INTRODUCTION

Arthropod-borne viral infections, or arboviral infections, are common causes of disabling fever syndromes worldwide, but their cumulative impact on global disease burden has not been fully assessed. In their acute stages, arboviral infections cause a broad spectrum of disease, ranging from asymptomatic infection to severe disease. They can also progress to much more complex secondary conditions, or sequelae, such as encephalitis or hemorrhagic diathesis, which result in long-term physical and cognitive impairment or in early death\[1\].

More than 100 arboviruses are known to cause disease in humans. A significant subset, including members of the Flaviviridae, Bunyaviridae, and Togaviridae families, are transmitted widely in different areas of the world. Arboviruses are also considered to be emerging pathogens based on their geographic spread and their increasing impact on susceptible human populations \[1–3\]. This dissertation will provide new evidence about the epidemiology and transmissibility of arbovirus transmitted by Aedes mosquitoes from two countries in Latin America.

1.1 Epidemiology of Arbovirus

1.1.1 Epidemiology of Dengue Virus

Dengue virus (DENV) is the most rapidly spreading arbovirus worldwide \[4\]. It is transmitted predominantly by Aedes mosquitoes, mainly Aedes aegypti and Aedes albopictus. In the last 50 years, its incidence has increased 30-fold globally with growing geographic expansion to new countries and from urban to rural settings\[5\]. Currently more than 40% of the world population is at risk of DENV infection, and approximately 390 million DENV infections are estimated globally each year, of which 96 million have clinical manifestations of the virus\[4\]. The burden of the disease is higher in countries of South-East Asia and the Western Pacific regions, however, a dramatic increase of cases has been reported in the Latin America and the Caribbean during the last decade\[6\]. DENV has four closely related serotypes (DENV1, DENV2, DENV3, and DENV4)\[7\]. The infection with any serotype confers
life-long protective immunity directed against the serotype of infection but does not confer long-term protection against infection by other serotypes[8, 9]. Each of the four serotypes can be responsible for dengue epidemics and can also be associated with severe dengue disease depending on the sequence and time between infections, among other factors[10]. The clinical spectrum of dengue goes from asymptomatic infections to life-threatening severe disease; the proportion of asymptomatic infections ranges from 50% to 90%[11, 12]. DENV infection does not have specific treatment or clinical predictors to prevent severe disease[7].

Several factors are involved in the increasing incidence of dengue in the Americas. The main factors are the unsustainable and ineffective vector control strategies that target the vector in all stages of development and it still remains as the only measure for dengue prevention[2, 13, 14]. Also the Population growth, unplanned urbanization with poor sanitary conditions, lack of the public health infrastructure, and decreased access to health care has also contributed to the increase of disease burden. Globalization of the economy, international travel, and climatic changes might also explain the disease expansion[6].

The estimates of the transmission parameters for dengue fever vary considerably between studies[15–19] and the reasons of the variability on the parameters are not completely understood. It could be explained by the history of dengue transmission in the population, the quality of the epidemiological surveillance, and the climatological conditions that can affect vector densities and the macro factors already mentioned. Methods for estimating the reproductive number (R) use data on the intrinsic growth rate of the epidemic[16, 17]; on the relation between the reproductive number and the final epidemic size[15]; and age-stratified seroprevalence surveys[18, 20, 21]. The estimations of the basic reproductive number (R₀) for DENV epidemics range from 2 to 6 [15–19, 22–24]. Giving the complexity of dengue transmission, dengue prevention requires innovative interventions e.g. vaccines, effective vector control and genetically modified mosquitoes to provide more effective and sustainable strategies, especially in growing and complex urban environments[25, 26].
Currently five DENV vaccine candidates are in clinical stages of development[27]. Two of them are in advanced stages and only one vaccine has completed two Phase III trials (Dengvaxia) and it is being approved for introduction in some countries [28–30]. This vaccine has an estimated efficacy of 64.7% and 56.5% in the Latin America and South East Asia trials, respectively. The vaccine efficacy in the pooled analysis of both trials was found to be significantly higher in participants with pre-existing dengue neutralizing antibodies compared to those who were seronegative. The serotype-specific vaccine efficacy was heterogeneous in both trials, and the vaccine efficacy against hospitalization for dengue in South East Asia was 67.2% and in Latin America 80.3% [28, 29, 31].

In April 2016, the World Health Organization (WHO) Strategic Advisory Group of Experts on Immunization (SAGE) recommended to the dengue endemic countries to consider the introduction of Dengvaxia only in transmission settings with high endemicity, measured by seroprevalence surveys of approximately 70% or greater in the age group targeted for vaccination (≥ 9 year olds). The vaccine was not recommended when dengue seroprevalence is below 50% [32]. Based on the WHO recommendations and the particularities of this vaccine, field studies are needed to understand better the transmission dynamics of dengue and the full burden of disease. Also dengue seroprevalence surveys are a requirement to introduce the vaccine in endemic countries so this project will be key to plan vaccine introduction in different dengue transmission settings and to evaluate effectiveness of different interventions like vaccine effectiveness, vector control activities or the combination of both.

1.1.2 Epidemiology of Zika Virus

Zika virus (ZIKV) is flavivirus in the same genus as dengue virus and yellow fever virus. ZIKV was first isolated in the Zika Forest of Uganda in 1947 and primarily transmitted by Aedes mosquitoes[33]. Although ZIKV has circulated in Africa and Asia since the 1950s, little is known about its transmission dynamics[34]. Recent outbreaks in Yap Island in the Federated States of Micronesia (2007), French Polynesia (2013), and other Pacific islands, including Cook Islands, Easter Island (Chile), and New Caledonia (2014), indicate that ZIKV has spread
beyond its former geographic range [35–38]. In April 2015 ZIKV was isolated in the Northeast of Brazil[39] and since then it spread rapidly through Latin America and the Caribbean.

ZIKV Infection typically causes a self-limited dengue-like illness characterized by exanthema, low-grade fever, conjunctivitis, and arthralgia[40]. While illness is believed to be mild or asymptomatic in approximately 80% of the infections [41], an increase in rates of Guillain-Barré syndrome (GBS) has been observed during ZIKV outbreaks[42–44]. Furthermore, in October 2015, the Brazilian Ministry of Health reported a dramatic increase in cases of microcephaly in Northeast Brazil where ZIKV had been circulating [45]. On the basis of the possible link between ZIKV, GBS and microcephaly, the World Health Organization declared a public health emergency on February 1, 2016[46, 47].

As of August 2016, around 500,000 Zika virus disease (ZVD) cases have been estimated in Brazil, and autochthonous circulation has been observed in 45 countries in the Americas region including United States with local transmission in two counties in the State of Florida[48]. ZIKV is spread primarily through Aedes mosquitoes, especially. Ae. aegypti. The capacity of Ae. albopictus to transmit ZIKV is still unknown but is believed to be lower than Ae. aegypti [49, 50]. Also sexual [51–54], perinatal transmission of ZIKV[55–58] and the potential for transmission by transfusion is being described [43].

Most of the $R_0$ estimations for ZIKV have been estimated from ZIKV outbreak in French Polynesia in 2013 [59]. The ZIKV basic reproductive number ($R_0$) was estimated around of 2.33 (95% CI [2.15-2.51]) [59–61]. The $R_0$ for the Latin American outbreak is being estimated from Brazil. The $R_0$ estimates for ZIKV in Rio de Janeiro ($R_0=3.9$, 95% CI: [3.1 - 5.3]) [62].

The countries with ZIKV transmission focused their responses on vector control during the outbreak and advice to delay pregnancy, followed by an extended recommendation to all affected countries by WHO in June 2016[63]. Countries in the Americas reporting the highest incidence of ZIKV disease are also countries with historically endemic DENV transmission and recent CHIKV outbreaks. Currently, other countries with ZIKV outbreaks besides Brazil have
reported cases of microcephaly and other birth defects associated with ZIKV infection during pregnancy (Zika Congenital Syndrome) [64].

The magnitude and public health impact of this ZIKV outbreak is due to the spread in areas infested by *Ae. aegypti*, the full population was susceptible and ineffective vector control activities. Some factors can contribute to underestimation of the disease burden of ZIKV infection e.g. lack of adequate infrastructure in laboratory perform ZIKV detection, ZIKV in most of the cases is very mild or asymptomatic infections, and patients may not seek medical care[65]. As the transmission in the Americas is so recent very few is known about the epidemiology and transmission parameters to understand better the dynamics of ZIKV and how its transmission could be affected by the co-circulation with other arboviruses.

### 1.2 Transmission Dynamics of Arbovirus

Transmission of an arbovirus in a population is a function of local environment, the natural history of infection and the susceptibility of the population to infection. The suitability of the local environment for arbovirus transmission and the impact of the natural history of the disease are usually captured by the basic reproductive number (*$R_0$*), defined as the number of secondary infections expected from a single case in a population with no preexisting immunity. *$R_0$* and the transmissibility of vector-borne diseases is associated with strong spatial heterogeneity, driven by variability in vector abundance and characteristics of the exposed populations[3].

The combined impact of these factors and susceptibility are captured by the reproductive number (*R*), which is related to *$R_0$* by the equation *$R = R_0 \times S$*, where *S* is proportion of the population susceptible to a particular arbovirus. This value, combined with the serial interval (the time separating two consecutive infections in a chain of transmission) is important to understand how a pathogen will spread in a population. The size of an outbreak after an introduction will depend on *R* (*$R_0$* in a naive population), with small, self-limiting outbreaks becoming more likely as *R* approaches one, and increasing epidemics with larger Rs. Hence, ZIKV can successfully spread to a new region if *R* >1, which requires, among other factors,
sufficient density of the vector population[3]. The force of infection ($\lambda$) is a measure that is used to estimate the intensity of transmission (transmission hazard) in a given setting and evaluates the rate of acquisition of infection among susceptible individuals [18, 21, 66].

These parameters could be estimated from surveillance data when data from field studies are not available. For DENV as is being circulating for long time at least non-serotypes specific serosurveys are needed to estimate $R$ and $\lambda$ for monotypic infections. To estimate $R_0$ and force of infection for multitypic infections longitudinal field studies and data collection are needed.

Estimate these transmission parameters for CHIKV and ZIKV is less complex because it is just one serotype and as most of the viral infections we will assume that after an infection there is life-long immunity against the virus.

1.3 Field Studies to Understand Transmission Dynamics of Arbovirus

Seroprevalence surveys are useful to estimate the baseline hazard of DENV infection and basic reproductive number in a specific population, to establish the most recent serotypes circulating, and to determine whether people have been exposed to one or more serotypes of dengue [67–69]. This information allows to identify high risk populations for dengue infection where targeted interventions could be implemented and according to the WHO-SAGE recommendations will be needed to plan the introduction of the dengue vaccine. Additionally, testing for specific antibodies (monotypic vs. multitypic immunity) is considered essential to understand the transmission dynamics of dengue in an specific population [18, 70, 71]

Prospective cohort studies are key to estimate the risk of infection by dengue virus. Currently, there are several ongoing longitudinal studies in dengue endemic areas in South East Asia and Latin America that are collecting information considering all of the clinical spectrum of dengue infections[72–79]. Most of these studies include enhanced surveillance because it improves the detection of dengue infections and cases in the acute phase that will allow the virological confirmation of the infections using RT-PCR to identify the infecting serotype improving the regular virological surveillance that is done by the local public health laboratories[76, 80]. The incidence of severe cases among dengue infections are key to
determine possible risk factors that could be associated with the severity of dengue disease including viral, social, cultural, environmental and health care-related factors[74, 75, 81].

To better understand dengue transmission and estimate the burden of disease in endemic regions, several long-term prospective epidemiological studies have been established in South East Asia and Latin America [19, 72, 80, 82–85]. Additional research is needed to address some questions that are critical to future vaccine evaluation: estimation of disease burden in countries where dengue is not well characterized, explore the dengue serotype-specific transmission and dengue severity, determine unique viral and host factors that can contribute to differences in dengue risk, and assess the potential impact of a vaccine, vector control intervention or the combination of both [86].

The aim of this dissertation is to better understand the epidemiology and the transmission dynamics of arbovirus in different Latin American settings. The first two papers aimed to assess the baseline epidemiology to identify the dengue transmission dynamics in three different urban settings in Yucatan, Mexico. We design a field study that includes a seroprevalence survey in the general population and a school-based, prospective cohort study set in urban environments with different social, demographic, and economic profiles, and different dengue transmission patterns based on epidemiological surveillance data. An initial household survey will be used to assess risk factors, risk behavior, and clinical symptoms, accompanied by serological testing on all members of the family. The third paper is an analysis of Zika Virus outbreak and estimation of transmission parameters in two settings in Colombia using epidemiological surveillance data.
CHAPTER 2
METHODS FOR THE BASELINE FIELD STUDIES IN YUCATAN, MEXICO

2.1 Study Components

Understanding the full burden of dengue infections and other arbovirus dynamics, including asymptomatic infections, and the degree of underreporting on different transmission settings have important public health implications in transmission dynamics. This study will also characterize the disease spectrum and the epidemiological parameters associated with dengue transmission that may be influenced by interventions.

This study is designed to collect prospective baseline data in Yucatan, Mexico to measure: 1) age-specific seroprevalence, 2) age-specific attack rates of dengue determined by serological confirmation, 3) prevalence of asymptomatic infections, and 4) proportion of underreported asymptomatic and clinical dengue cases (including severe dengue cases).

The first study is a baseline seroprevalence survey that includes a random sample of the population in the three different settings to estimate the previous exposure of these populations to dengue virus.

Second, a school-based prospective cohort study beginning enrollment once the seroprevalence survey is completed. The aim of this second study is to characterize the local epidemiology of dengue including age-specific attack rates, proportion of asymptomatic infections, and the estimated underreporting levels in the study area. The activities planned for the cohort include active surveillance, febrile detection, and an annual follow-up visit to all participants. These activities will last for the duration of the study. (Fig. 2-1). During the dengue season other studies will be added to the active follow-up. Cluster studies for dengue transmission and case-control studies of severe dengue will be helpful to better understand the disease spectrum of dengue in Yucatan, Mexico.

2.2 Study Sites and Organization

The state of Yucatan is located in the southeast peninsula bordering the Gulf of Mexico and the Caribbean Sea. Merida is the largest urban center in the region and capital city
Figure 2-1. Overview of the baseline dengue studies in Yucatan
with 814,000 habitants (2013). The weather is warm and humid, and the rainy season falls
between June and October; the mean annual temperature is 25.9°C (19.5 to 33.6) and annual
precipitation is 1050 (mm). Progreso is the main seaport in the state, 32 kms., away from
Merida, with 54,000 habitants (Fig. 2-2). This town has similar weather conditions and is the
weekend and holiday resort (July-August) for the people living in Merida, as well as a tourist
resort at the national and international levels. Ticul is a town 82 kms., south from Merida with
34,000 habitants whose main economic activity is dedicated to the production of shoes [87].

The cities of Merida, Progreso and Ticul were selected as three different epidemiological
settings for dengue transmission due to the distinct intensity of dengue outbreaks in the
past and the co-circulation of more than two serotypes in recent years. Incidence rates were
calculated for each city of suspected and confirmed cases from 1979 to 2013. These three
cities also have different dengue incidence rates, allowing us to explore possible differences in
dengue transmission scenarios[88].

This study is being conducted by the Regional Research Center "Hideyo Noguchi" of the
Universidad Autonoma de Yucatan, in Merida, the capital of the state of Yucatan in Mexico
with the participation of the Ministry of Health of Yucatan and the State Reference Laboratory.
Other research institutions involved in the project are the University of Florida, University of
Washington, and the Fred Hutchinson Cancer Research Center, creating an interdisciplinary
research network.

This study was approved by the Institutional Review Board at Universidad Autonoma de
Yucatan. Written informed consent and assent forms will be signed during enrollment and
before samples are obtained. Written informed consent will be obtained from participants older
than 18 years, and from parents of participants younger than 18 years old. The analysis using
de-identified data was approved at Fred Hutchinson Cancer Research Center, Seattle, WA.

2.3 Seroprevalence Survey

In this part of the study, we aim to characterize the baseline age-specific prevalence of
antibody-mediated immunity to dengue through a cross-sectional serological survey in the three
Figure 2-2. Map of the Yucatán and geographical locations of the study sites
selected cities. Serum samples will be collected from a random sample of children and adults aged 2 to 65 years old who live in the study areas. These data will provide information about the history of dengue transmission in each city.

The sampling scheme for the seroprevalence survey includes two different strategies. The first is a school-based survey with a random selection of schools in the three sites, followed by a random selection of children (5 to 19 years old) in the different educational levels (primary, secondary and high school). The adult population will be selected at random from the population attending the health centers in the three cities (one health center per city).

Inclusion criteria include adults (≥ 18 years old) who were not suffering from an acute febrile disease, and went to the healthcare center for an annual physical examination or were under clinical control, and therefore required a blood sample for their consultation. Individuals were randomly recruited until the target sample size is reached. Informed consent was requested and signed by adults (≥ 18 years old) and from parents of minors (<18 years old). Blood sample was taken in the school once the informed consent forms were signed from the parents. Results were delivered personally to the parents of minors while those from the adult population were delivered by telephone.

The age-group specific prevalence of pre-existing antibody-mediated immunity to any of the dengue viruses is defined as the proportion of the sampled individuals in the age group whose serologic specimen is positive to any serotype using Panbio Dengue IgG Indirect ELISA (titer ≥ 0.12). Confidence intervals (95%) for these prevalence estimates will be estimated [67–69].

2.4 Cohort Study

2.4.1 Selection of Risk Areas

This part of the study involves a school-based, prospective cohort in the low-to-middle-income districts of Merida, Progreso and Ticul. The primary aim of this study is to characterize the local epidemiology and transmission dynamics of dengue in Yucatan. To assess initially the risk of transmission by city three considerations were adopted: 1) Proportion of epidemiological
weeks with reported dengue cases compared to epidemiological weeks with no reported cases;
2) Duration of the dengue epidemic waves as a proxy of persistent dengue transmission;
3) Intensity of transmission measured as the incidence rate during the given period. The
school-based study is defined by a random selection of five extensive geographic areas with
different dengue transmission risks (high, medium or low).

The data obtained from the epidemiological surveillance system identified those areas
where dengue has been historically higher, persistent and where the transmission risk is
defined as high, medium and low. The sample includes low-risk areas in Merida and Progreso,
medium-risk areas in Merida and Ticul, and a high-risk area in Merida.

Each area of transmission includes several primary public schools, from which the cohort
of children from first to third grade will be randomly selected. Primary public schools will be
selected randomly and invited to participate in this study based on their criteria of risk (high,
medium and low).

2.4.2 Recruitment and Enrollment

Children will be enrolled at the beginning of the academic year from first through third
grade and are eligible to remain in the study until they graduate from sixth grade. During each
subsequent year, children 9-12 years old will be recruited from their primary and middle schools
in the areas of study. The only exclusion criteria is intent to move outside of the study area
during the months following enrollment. The students entering elementary school and those in
second and third grade will be selected and included in the cohort study with written consent
granted by their parents. Each group of children who are enrolled and their respective families
will be followed-up during their education track for the period of the study.

The strategy of creating and maintaining a cohort of individuals and their families
participating in long-term project requires ongoing effort. It highlights the importance of
sharing interim results, advances in the project, and understanding the community-level
protective measures against dengue epidemics. Therefore, each cluster will have a team
of community specialists consisting of local physicians, nurses, laboratory technicians,
microbiologists, and anthropologists who will gather information and organize activities directed to sensitize and educate cohort members and their families. They will participate in the enrollment of new first grade students and their families during the entire study period.

2.4.3 Follow-up Visits

Evaluations of the study population including baseline demographic information, biological and blood samples will be obtained once every year. Blood samples will be taken during the surveillance period each year (January - July). The study participants and their families will be followed from January 2015 through December 2020 at 12-month intervals for serological evidence of dengue infection after the dengue season every year. Participants will be considered lost to follow-up after a full year has passed since their previous blood draw, despite repeated attempts to locate the participant, or if there is a verifiable reason for dropping them from the study (e.g., direct request from the participant, movement from the study area, or death).

The follow-up period proposed allows us to measure the infection rates in different age groups as well as the proportion of asymptomatic cases or under-reporting of febrile cases if an outbreak should occur. The prospective follow-up of the primary school cohort and their families will provide information for the estimation of the age group hazards of infection for those represented in this sample.

We will investigate the effects of infection history and heterotypic antibody-mediated immune responses on the risk of severe illness once infected.

2.4.3.1 Suspected case definition

Participants from the cohort are classified as suspected dengue cases if they satisfy the following criteria: (1) are over 24 months in age; (2) have an oral temperature \( \geq 37.2^\circ C \) and onset of symptoms within the last 72 hours; and (3) present at least one clinical manifestation listed in the World Health Organization (WHO) dengue guidelines. Once the subject is laboratory confirmed as a dengue case, the family household will be studied clinically and serologically to determine dengue transmission in the household, including the detection of asymptomatic cases in all members \( \geq 2 \) years.
2.4.3.2 Identification of dengue infections and cases

The identification of symptomatic and asymptomatic dengue cases requires establishing different strategies to increase dengue case detection. Since not every febrile or clinical dengue case seeks health care, we want to identify more dengue cases through three strategies: enhanced surveillance, geographical related cases or clusters, and severe dengue or case control studies.

2.4.3.3 Enhanced surveillance

This strategy aims to identify dengue febrile cases in the school-based cohort through absentee surveillance at school and its surrounding area, and also from the traditional surveillance system.

This activity includes the detection of febrile illness in study participants using school-based surveillance in the selected areas by weekly visits to investigate school absenteeism and continuous active surveillance for febrile cases through mobile application and family use of a dengue alert number (1-800).

Cohort participants will be followed closely for all febrile illnesses, and children and their relatives who also have fever will be screened clinically and by laboratory testing, for signs and symptoms of dengue. Subjects with acute febrile dengue-like illness will be tested and identified by the field group working in the community; the absentee surveillance system that will be implemented in each school and/or by the traditional surveillance system will provide data on suspected cases occurring in the selected areas. The surveillance system will be used for the identification of suspected or probable dengue cases that are clinically and serologically studied to confirm dengue infection. During school vacation, children will be visited or contacted weekly at their homes.

Symptomatic dengue infection is classified as symptomatic non-hospitalized or symptomatic hospitalized, based on admission into the hospital as decided by the treating physician. Hospitalized symptomatic cases will be further defined as either dengue or severe dengue using the 2009 guidelines from the World Health Organization [5].
Acute-phase serum samples are tested with NS1, anti-DENV IgM and IgG antibodies by IgM capture enzyme-linked immunosorbent assay (ELISA). Febrile episodes will be classified as dengue infections based on NS1 and IgM paired serology. Infections identified with a four-fold increase titers IgM or IgG titer will be counted as seroconversions in all incidence calculations.

Inapparent (subclinical) dengue infection will be defined as a positive IgM antibody against any DENV serotype in the sera, obtained during the surveillance months without a febrile illness or school-absence identified during active surveillance in the time period, or a seroconversion of the cohort participant in the yearly follow-up samples. Clinical definitions of serologically or virologically confirmed dengue infection will be based on evidence of acute dengue infection.

2.4.3.4 Investigation of geographic associated cases (clusters)

During a dengue outbreak or high transmission season, cases will be detected through the standard surveillance system that identifies clusters of 5 to 10 confirmed and probable cases of dengue in a radius of 500 meters during the previous 2 weeks. Priority selection of cases to be studied will be performed in the areas under follow-up, and one cluster will be randomly selected every week. Once dengue cases and their families are identified, we will select a control family (without dengue like symptoms) paired by sex and age group of the index case in the surrounding households. We will interview and take the blood samples for the identification of asymptomatic and clinical cases in the household perimeter not captured by the surveillance system. This approach is justified by evidence that demonstrates that dengue cases are geographically clustered during the initial days of infection and around the index cases.

The outbreak investigations of clusters of dengue cases will help us determine whether the number of cases surrounding a confirmed case varies according to the transmission intensity defined by the emergence of a cluster of geographically associated cases, as well as potential individual and household level factors that may affect transmission dynamics.
2.4.3.5 Case-control study

One of the most important outcomes of dengue is severe dengue and associated mortality. To measure its impact, we propose a case-control study for severe dengue cases that will be paired to a control (mild-dengue case) based on age group, sex, hospital, and place of residence. The aim of this study is to measure the number of cases surrounding severe cases to distinguish a potentially different or more intensive transmission pattern. Patients of all ages will be eligible for recruitment when admitted to a reference hospital in and outside the selected areas with a clinical diagnosis of severe dengue according to the 2009 WHO guidelines[5].

Patients will be examined daily during hospitalization by a dedicated team of physicians with experience in dengue diagnosis and treatment. Signs and symptoms of severe dengue (hemorrhage, capillary permeability, shock) along with other relevant clinical and laboratory information will be extracted from the clinical records and will be prospectively recorded using standardized case record forms.

Family members of both cases and controls will be recruited and blood samples will be taken to measure and compare their dengue immunological or serological (IgM and IgG) profile. A household control (matched by age and sex) and their family members will also be selected with the purpose of comparing the family immunity status or clinical profile and to identify if recent and more intensive intra-household transmission of dengue acts as a risk factor for dengue severity. This particular issue will be important to estimate the indirect effect of vaccination once a dengue vaccine is available.

2.5 Data Collection and Storage

Data from this study will be collected and entered into an electronic database that can be accessed through a web-based system. The data will be captured using a mobile platform using mobile devices such as tablets and smartphones. This information will be synchronized when there is access to the Internet and will be continuously updated. The application will allow us to make predesigned and customized consultations in real time. This application includes
basic demographic data (age, sex, education level, occupation, etc.), with a specific section regarding clinical history of dengue (clinical signs and symptoms, dates of occurrence, access or demand of health services, hospitalization, etc.). Data from the behavioral, febrile and absentee questionnaires will also be included in the application. The behavioral questionnaire will explore movements between households, vicinity, neighborhood, locality and outside the cities. The febrile questionnaire (regular house to house visits) includes data regarding febrile periods, symptoms, dates, duration, severity, movements outside the area, demand of health services, contacts and blood sample results (serology) including all family members. The absentee questionnaire (active school-base surveillance) will be used weekly at each school to identify children who were absent in the previous week and reasons for their absence will be recorded. Blood sample results will be recorded from school children and family members.

2.6 Statistical Analysis

The age-stratified specific prevalence of pre-existing antibody-mediated immunity to any of the dengue viruses is defined as the proportion of the sampled individuals in the age group whose serologic specimen is positive to any serotype using Panbio Dengue IgG Indirect ELISA[67–69]. We plan to estimate confidence intervals (95%) for these prevalence estimates that will be based on the standard deviation from the Binomial distribution, assuming independent, randomly selected observations. It is also possible to estimate the baseline hazard of infection as well as the basic reproductive number using information from this age-stratified serological surveys [18, 20, 71].

The prospective cohort will estimate baseline seroprevalence for dengue virus and age specific dengue incidence rates and incidence rate ratios. We will investigate the effects of infection history on the risk of severe illness once infected [9, 80, 89]. We will also estimate the level of association between risk factors and the risk of severe illness given infection (i.e., pathogenicity). Risk factors for pathogenicity will include age, gender, city, as well as the individuals infection and immunologic history [11].
To analyze the information derived from the cluster investigations we will employ methods previously implemented by our group to estimate the effects of individual and neighborhood-level predictors of the transmission potential of an infection [90]. For the outbreak data collected from this study, we will use statistical methods that fit a chain-binomial model for the transmission of infection. The basic model will estimate two transmission-related parameters, the probability of within-household transmission and the probability of infection due to exposure (via a mosquito vector) to an infectious individual who is not a member of the same household (community-to-person) [91]. This partitioning of infection risk by the source of exposure will help describe and quantify the spatial and temporal heterogeneity of the risk of dengue transmission. The probability of within-household transmission will be quantified by the household secondary attack rate (SAR), which is defined as the average probability that an infected individual with dengue will transmit the virus to another household member during his/her infectious period (intrinsic + extrinsic infectious periods). In contrast, the probability of community-to-person transmission will be quantified as the community-to-person probability of infection (CPI). The CPI will be defined as the cumulative probability that over the course of the intensive follow-up period for a cluster of dengue cases, a susceptible individual will become infected due to exposure (via a mosquito) to a community-based source. This modeling approach permits the estimation of the effects of risk factors on both the infectiousness and susceptibility to infection. Since dengue cases tend to cluster in time and space, the SAR is expected to provide a substantial amount of information about the transmission of this virus. Special consideration will be taken to estimate the roles of infection and immunologic histories on susceptibility to infection, as well as on infectiousness.

The matched case-control sub-study will be analyzed using a standard conditional logistic regression approach, with age, sex, and location (hospital where the patient was seen) included as independent predictors. All other important risk factors will be determined a priori and included in the regression model. Inclusion of information regarding immune profile, characteristics, and recent transmission history of case and control households as risk factors
for severe dengue may require the adaptation of existing statistical methods [92]. We will develop and validate the performance of these methods prior to conducting such analysis. A preliminary approach for dealing with this issue will be to aggregate the individual-level information about immunity and transmission history at the household-level, thereby losing some information but allowing us to use standard analytic methods for matched case-control data. The hospital-based, matched case-control sub-study will provide information about risk factors for severe dengue, including a characterization of the immune profiles of the members of the cases and controls households, as well as the history of transmission in these families.
CHAPTER 3
DENGUE SEROPREVALENCE SURVEY IN THREE URBAN SETTINGS IN YUCATAN, MEXICO

3.1 Background

Dengue has become the most rapidly spreading vector-borne viral disease transmitted throughout the Americas. Brazil, Colombia and Mexico accounting for the largest burden of disease in the region [6, 93]. While the cases reported are a small proportion of the total burden of disease occurring in vulnerable communities, there is scant information regarding the extent of transmission in cities and suburban areas in endemic countries like Mexico. The estimation of the real burden of dengue disease requires strong epidemiological surveillance methods and modeling techniques [94–96].

To identify the real burden of dengue, it is important to acknowledge multiple challenges: 1) Only a small fraction of symptomatic cases are diagnosed and reported through the surveillance system; 2) Data describing the proportion of asymptomatic and mild infections is limited, and hospital records only partially report the incidence of severe dengue cases [97]; 3) Differential diagnosis between dengue and other endemic infections (leptospirosis [98], rickettiosis [99] and the recently introduced chikungunya virus[100] and Zika virus[101] is often difficult in the absence of adequate serological and virological tests; 4) Surveillance data can have reporting biases in settings where different endemic pathogens are commonly co-circulating [102, 103].

To fill the gaps of the epidemiological surveillance systems, dengue seroprevalence surveys are needed to characterize the spectrum and dynamics of dengue infections in selected population groups in endemic areas. Dengue virus has been circulating in Mexico since the late seventies. Despite an increase in the reported number of dengue and severe dengue cases[104], seroprevalence surveys are very limited and will be helpful to understand the changes in the epidemiology of the disease over the last three decades. This is particularly important because larger and more frequent dengue outbreaks with an increasing proportion of severe cases have been reported to the epidemiological surveillance system throughout the country. Also co-circulation of the four serotypes of dengue virus is more
frequently detected [105]. The state of Yucatan in Mexico emerges as a region with a long history of dengue transmission. Dengue was first reported in the Yucatan in 1979, followed by large dengue outbreaks and annual co-circulation of at least two serotypes during the last two decades[104]. Cross-sectional dengue serosurveys provide the baseline information of dengue and it is key to assess the effectiveness of different interventions available like innovative vector control interventions and vaccines, among others [27, 106–110]

3.2 Aims

Estimate the baseline dengue seroprevalence in three different transmission settings in Yucatan, Mexico. This study will provide information that will be key to assess the effectiveness of different interventions available like innovative vector control interventions and vaccines among others.

3.3 Methods

3.3.1 Sampling

The survey sample was estimated based on 50% prevalence with 3% error and 95% confidence interval (95% CI) that gave a sample size of 1,307 individuals. We increased the sample size to 1,700 in order to have half of the samples from the children population (<18 years old) and distributed according to the population size in the three settings, with a higher proportion of samples from Merida, followed by Progreso and Ticul.

The sampling scheme included two different approaches. The first one was a school-based survey with a random selection of schools in the three cities followed by a random selection of children (5 to 18 years old) in the selected elementary, middle and high schools of Merida, Ticul and Progreso: eight schools were selected from Merida (five were elementary schools), six schools from Progreso (four were elementary schools) and five from Ticul (three were elementary schools) (Fig. 3-1). Prior agreements and an explanation of the study aims were made with the local ministries of Health and Education of Yucatan. We requested the lists of students by grade to obtain ages of the participants and their information. We did a stratified randomization by grade and school to have a proportional sample in each age group. We
excluded participants whose siblings were previously randomized and have provided signed informed consent. In the city of Merida, we confined the study to schools in the central metropolitan area. Blood samples were obtained in the school after the parents signed the informed consent.

Figure 3-1. Location of the selected schools for the dengue seroprevalence survey in Yucatan, Mexico. A) Location of the schools in Merida; B) Location of the schools in Progreso; and C) Location of the schools in Ticul.

The adults (≥ 18 years old) were randomly selected and stratified by age from people attending public primary health care centers in Merida, Progreso and Ticul. We included individuals who did not have signs of acute febrile illness, who were requesting a health certificate or were under clinical follow-up for a non-communicable disease. Blood samples were obtained after the adults signed the informed consent. The results were delivered personally to the parents of the children participants in the schools. For the adult population the results were reported by telephone or home visits directly to the participants.

3.3.2 Study Procedures

Participants were asked to provide a 5 mL venous blood sample and to complete a brief questionnaire with basic demographic information. Blood samples were collected in anticoagulant-free tubes by trained and certified health personnel, centrifuged within 1.3 hours of collection, and transported to the state laboratory. Samples were stored at -70±5°C until the serological testing was done. Serum samples were obtained from individuals and tested against dengue infection in state public health laboratory (LESPRE) of the Ministry of Health.
Prior exposure to dengue and age-specific serostatus were determined using Panbio IgG indirect ELISA. Prior exposure to dengue and age-specific seroprevalences were determined using the standard cut-off points to define positive (≥ 12 Panbio units) from negative samples (<9 Panbio units) and indeterminate for those in between.

3.3.3 Ethical Review

This study was approved by the institutional review boards at Fred Hutchinson Cancer Research Center and the General Hospital Agustín O’Horan of Yucatan. Written consent was obtained from all adult participants (≥ 18 years old) after providing them with a detailed explanation of the study and procedures. Parents/guardians of all child participants (<18 years old) were asked to provide written consent on their behalf.

3.3.4 Statistical Analysis

Descriptive analysis of the demographic variables and a logistic regression model were done using R (version 3.2.2). We grouped age in ≤ 8 years old, 9-14, 15-19, 20-49 and ≥ 50. We estimated the age specific dengue seroprevalence and fit a logistic regression model of the variables independently associated with dengue seropositivity as the outcome variable. The independent variables considered for the model were age, gender, place of residence, previous history of dengue and previous dengue confirmation. The age-stratified specific prevalence of pre-existing antibody-mediated immunity to any of the dengue viruses is defined as the proportion of the sampled individuals in the age group whose serologic specimen was positive to any serotype using Panbio Dengue IgG Indirect ELISA[67–69]. We plan to estimate confidence intervals (95%) for these prevalence estimates that will be based on the standard deviation from the Binomial distribution, assuming independent, randomly selected observations [18, 20, 71, 111].

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3.4 Results

3.4.1 Description of the Population of Study

A total of 1,731 serum samples were obtained for this study. The samples were obtained from January through June 2014 in the cities of Merida, Progreso and Ticul. Most of the samples (43%) were collected in Merida (748), 27% in Progreso (472) and 30% in Ticul (511). Most of the samples were obtained from healthcare centers (56%), 24% from elementary schools and 20% from middle and high schools. After processing the samples 3.6% (63/1,731) had indeterminate results for the IgG ELISA and were excluded from this analysis. We analyzed 1667 blood samples from people from the three cities: Merida (700), Progreso (469) and Ticul (498). The samples were located geographically widely through all the neighborhoods in the selected cities (Fig. 3-2). While some clustering of cases appeared in Merida and Progreso, this may result from oversampling in one or two schools.

![Figure 3-2. Location of the households of the participants in dengue seroprevalence survey in Yucatan, Mexico. A) Location of the participants in Merida; B) Location of the participants in Progreso; and C) Location of the participants in Ticul. The red dots show dengue seropositive individuals and green the seronegatives.](image)

The mean age in the overall population was 25.8 years (SD: 18.04). The mean age of the individuals did not have significant differences between three cities (p=0.172). The population was balanced by age groups with the exception of the 9 to 14 and 15 to 19 year olds groups in Progreso that contributed with different proportions compared to the other cities in this study (Table 3-1). Most of the population of this study (94%) was born in the
Yucatan. This proportion is significantly higher in Ticul (98%), followed by Progreso (93.8%) and Merida (89.1%). Ticul is a rural town so it is possible that the mobility of its population is more within the Yucatan state compared with the other cities. A few proportion of the participants (5%) mentioned having previous history of dengue. This proportion was found higher in Merida (8.6%), followed by Progreso (3.4%) and Ticul (2%). We also found a small proportion of people recalled having previous laboratory confirmation of dengue. Just 2.8% had previous laboratory confirmation for dengue and most of the participants confirmed were from Merida (3.9%). Another interesting finding was that the proportion of laboratory confirmation increased with age. A possible explanation for these two last variables could be that the accessibility to healthcare in Merida that is the capital of Yucatan is higher compared with the other cities like Progreso and Ticul.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Merida</th>
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<th>Ticul</th>
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<tr>
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<td>487 (97.8%)</td>
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<td>490 (98.4%)</td>
<td>1620 (97.2%)</td>
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3.4.2 Dengue Seroprevalence

The overall estimated dengue seroprevalence in Yucatan was 73.6% (95% CI 71.4% - 75.7%) showing that most of the population had already been exposed to dengue infection.
The dengue seroprevalence in the Yucatan increased with age in all three study settings (Fig. 3-3). The lowest overall seroprevalence was 51.4% (95%CI 45%-57.9%) in the group ≤ 8 year olds and the highest for the population ≥ 50 years old (83.4% (95%CI 77%-88.2%) (Figure 5A). The seroprevalence in Merida was 68.6% (95%CI 65%-72%), in Progreso 68.7% (95%CI 64.2% - 72.8%) and in Ticul 85.3% (95%CI 81.9% - 88.3%). The estimated dengue seroprevalence in Merida went from 43.6% (95%CI 33.7%-53.8%) in the group ≤ 8 year olds to 79.8% (95%CI 69.9%-87.6%) in the population ≥ 50 years old (Figure 5B). In Progreso the seroprevalence went from 45.1% (95%CI 33.2%-57.3%) in the younger age group to 71.4% (95%CI 56.7%-83.4%) in the population ≥ 50 years old (Figure 5C). Ticul had the highest dengue seroprevalence in all age groups compared with the other two cities. In Ticul the seroprevalence went from 69.6% (95%CI 57.3%-80.1%) in the ≤ 8 years old to 95.9% (95%CI 88.5%-99.1%) in the population ≥ 50 years old (Figure 5D).

We fitted a simple logistic regression model for dengue seropositivity. We estimated the odds ratio for each of the variables collected in the survey (age, sex, city, born in Yucatan, and prior dengue clinical or laboratory diagnosis). The analysis showed that the odds of being seropositive also increase with age. The reference group for age was the ≤ 8 years old group. The 9-14 years group has 2.43 (95%CI 1.69-3.50) times the odds of being seropositive compared to the reference group (Table 3-2). The ≥ 50 years group has 4.74 (95%CI 3.08-7.46) times the odds of being seropositive compared to the reference group. Females had 1.27 (95%CI 1.02-1.59) times the odds of being seropositive compared to males. The participants from Ticul had 2.67 (95%CI 1.99-3.60) times the odds of being seropositive compared to the participants from Merida. The variables being born in the Yucatan, having history of previous infections with dengue and having a previous confirmation of dengue were not associated with seropositivity (Table 3-2).

3.5 Discussion

This is the most recent cross-sectional dengue serosurvey conducted in three cities in the state of Yucatan, Mexico. Our study estimated an overall dengue seroprevalence of
Figure 3-3. Dengue seroprevalence to Indirect IgG by age group and city, 2014
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<th>Total (N=1667)</th>
<th>Seropositives (N=1227)</th>
<th>Seronegatives (N=440)</th>
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<td>≤ 8</td>
<td>241 (14.5%)</td>
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<td>1.69-3.50</td>
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<td>285 (17%)</td>
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<td>116 (6.9%)</td>
<td>77 (6.3%)</td>
<td>39 (8.9%)</td>
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<td>Ref</td>
</tr>
<tr>
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<td>1150 (93.7%)</td>
<td>401 (91.1%)</td>
<td>1.45</td>
<td>0.96-2.16</td>
</tr>
<tr>
<td><strong>History of previous dengue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1581 (94.9%)</td>
<td>1163 (94.8%)</td>
<td>418 (95.2%)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>86 (5.1%)</td>
<td>64 (5.2%)</td>
<td>21 (4.8%)</td>
<td>1.09</td>
<td>0.67-1.86</td>
</tr>
<tr>
<td><strong>Previous confirmation of dengue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1620 (97.2%)</td>
<td>1190 (97.0%)</td>
<td>428 (97.3%)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>47 (2.8%)</td>
<td>37 (3.0%)</td>
<td>12 (2.7%)</td>
<td>1.25</td>
<td>0.64-2.70</td>
</tr>
</tbody>
</table>
73.6%, similar to other dengue serosurveys done in Mexico and the Yucatan [15]. In 1985, the prevalence of anti-dengue antibodies in the urban population of Yucatan was 72.5% [112]. Another study in a cohort of school children from 8 to 14 years old (1987-1988) including urban and rural localities of Merida reported an overall dengue seroprevalence of 56.3% in children living in urban areas compared to 63.7% in children living in rural areas [113]. More recent studies have found an overall seroprevalence of 59.9% and 81.5% in 1996 and 2006 respectively [114].

The cases reported to the surveillance system indicated that both women (51%) and men (49%) were similarly exposed to dengue [113], which is a different finding compared to our study where women were more likely to be seropositive compared to men (OR:1.14). The age specific seroprevalence was as low as 51.5% in children ≤ 8 years old and increases with age up to 83.4% in the age group of ≥ 50 years old. The seroprevalence in Merida was 68.6%, Progreso 68.7% and Ticul 85.3%. Ticul had the highest seroprevalence in all age groups.

This serosurvey of three different urban areas in Yucatan highlights the heterogeneous level of exposure to dengue virus these settings. The seroprevalence estimated in our study was similar to estimates from Venezuela and Brazil among other endemic countries from Latin America but lower compared with the estimates from Nicaragua[76, 103, 115, 116]. In our study the overall dengue seroprevalence in children and adolescents 9-14 years old was 72%. This is a relevant finding giving the World Health Organization (WHO) recommendations for introduction of the first dengue vaccine that is also licensed in Mexico (CYD-TDV-Denvaxia) [117]. This vaccine was licensed targeting the population from 9 to 45 years old. The WHO recommends introducing the dengue vaccine CYD-TDV only in geographic settings where epidemiological data suggest a high burden of disease in the populations to be targeted for vaccination. This high burden can be objectively measured by dengue seroprevalence surveys. The seroprevalence should be >70% in the age group that is going to be targeted for vaccination in order to maximize public health impact and cost-effectiveness[117, 118] This dengue serosurvey provides data from school children and the adult population attending public health services provided
by the Ministry of Health and may represent the lower and medium socioeconomic levels, nevertheless, the distribution of the sample in the three cities demonstrated that exposure to dengue infection is widespread. Data from the early 80’s show that exposure to specific serotypes in the past has also been intense and that the exposure to dengue serotypes 1 and 2 has been predominant in the last two decades [15, 112]. The regression model results showed that the main risk factor associated with dengue seropositivity in Yucatan were age, sex and city. The variables being born in the Yucatan, having history of previous infections with dengue and having a previous confirmation of dengue were not associated with seropositivity in this study.

Population based serosurveys are useful to identify those age groups more at risk and which ones would be the best target for interventions[80, 108, 119, 120]. Similar studies should be pursued elsewhere in Mexico and other endemic countries to better understand the transmission dynamics of dengue and to help evaluate the effectiveness of interventions like vector control and dengue vaccines in the near future. Dengue serosurveys have also become key for modeling potential impacts of the different interventions already available and in development [110, 121–126]. Previous dengue serosurveys were done in Mexico in the early 1980’s but these data are very limited to certain population groups or localities nevertheless all of them demonstrated high dengue exposure in different time periods[127–130].

One of the main limitations of this study is the lack of dengue serotype specific seroprevalence that is key to better understand the intensity of transmission, and establish whether the population has been exposed to one or more serotypes as well as the level of exposure in the different age groups. Another limitation is that it is not a true random sample of the population giving the sampling strategy to enroll adults in the healthcare centers instead of the community random sample. Also a degree of recall bias is present when interviewing people about past experiences, such as having had dengue Dengue epidemics have increased significantly worldwide [4, 7, 131] The global estimates of dengue distribution and disease burden remain imprecise in most of the endemic areas[132, 133] The real burden of the
Dengue is unknown since most of the dengue infections are asymptomatic, misdiagnosed or not reported[134]. Some of the main issues in dengue surveillance include the lack of standardized reporting procedures, variable diagnostic laboratory capacity in traditional surveillance systems, along with the absence of reporting from the private health sector, which in our study population represent an important proportion of all health care providers[135]. Seroprevalence surveys are invaluable identifying the burden of both symptomatic and asymptomatic infections and quantifying infection prevalence and incidence in different epidemiological settings[119, 122, 129, 136, 137].

As established by the Mexican Dengue Expert Group it is essential to develop an evidence-based proactive strategy that provides evidence to decision makers[135]. These data should include results from clinical trials, epidemiological studies and better burden of disease estimates in order to create a sustainable immunization program with all the resources required for the adoption of the new vaccine and its further evaluation[135]. This particular study contributes to provide evidence regarding the serological status of potential target populations in a highly endemic region of the country.
CHAPTER 4
EPIDEMIOLOGY OF DENGUE AND OTHER ARBOVIRUS IN A SCHOOL-BASED COHORT IN YUCATAN, MEXICO: BASELINE AND FIRST YEAR FOLLOW-UP

4.1 Background

Dengue is the most rapidly spreading mosquito-borne virus worldwide. In the last 50 years, its incidence has increased 30-fold with growing geographic expansion to new countries and from urban to rural settings [5]. Currently more than 40% of the world population is at risk of infection, and approximately 390 million infections are estimated globally each year, of which 96 million have clinical manifestations of the virus [4]. Currently there is no specific treatment for DENV infection or clinical predictors to prevent severe disease [7].

Dengue Virus (DENV) has four closely related serotypes, each of the four serotypes can be responsible for dengue epidemics. Each of the four serotypes can also be associated with severe dengue disease depending on the sequence and time between infections, among other factors [8–10]. The clinical spectrum of dengue ranges from asymptomatic infections to life-threatening severe disease; approximately 50% to 90% of dengue infections are asymptomatic [11, 12].

Measuring the burden of disease attributable to dengue infection is needed to understand the potential impact of dengue control interventions. Therefore it is very important to strengthen and increase surveillance to improve our knowledge of how many cases are actually occurring in the community. An accurate risk analysis and allocation of resources for dengue control depends on timely disease surveillance [138].

Surveillance systems based on the monitoring and notification of symptomatic cases have low sensitivity and rarely detect low or sporadic transmission [139, 140]. The proportion of asymptomatic infections vary widely within the same populations, geographical areas, and over different epidemiological periods [141]. Underreporting of dengue cases to national surveillance systems hinders accurate local, regional and global calculations of disease burden [142]. It is needed to strengthen passive surveillance systems by incorporating active surveillance methods (e.g., house-to-house visits, school absenteeism or self-identification of fever episodes) and
improve the recognition of inapparent infections and under reporting of unspecified febrile dengue infections.

Vector control strategies still remain as the only measure of dengue prevention [13, 14]. These traditional approaches that target the vector in all stages of development have proven to be unsustainable and ineffective at preventing dengue transmission [2]. Dengue prevention requires innovation e.g. vaccines, effective vector control and genetically modified mosquitoes to provide more effective and sustainable strategies for dengue control, especially in growing and complex urban environments [25, 26].

Currently five vaccine candidates are now in clinical stages of development. Two vaccines are in Phase II trials, and two are in Phase I testing [27]. Only one vaccine has completed two Phase III trials and it is being licenced for introduction in some countries [28–30]. This vaccine has an estimated efficacy of 64.7% and 56.5% in the Latin America and South East Asia trials, respectively. The vaccine efficacy in the pooled analysis of both trials was found to be significantly higher in participants with pre-existing dengue neutralizing antibodies compared to those who were seronegative. The serotype-specific vaccine efficacy was heterogeneous in both trials, and the vaccine efficacy against hospitalization for dengue in South East Asia was 67.2% and in Latin America 80.3% [28, 29, 31].

The availability of a licensed vaccine poses different challenges to current dengue control programs since it is not expected to universally cover all susceptible and at-risk populations or even provide complete coverage in target groups. Vaccine introduction will therefore be a gradual process. In each of these populations there are several questions that need to be addressed regarding the clinical spectrum and transmission risks where the vaccine may have a potential benefit [143].

4.2 Aims

The aim of this study is to characterize the current local epidemiology of arboviral infections estimating age-specific attack rates, the proportion of asymptomatic infections in
the study area, incidence rate ratios and assess the covariates associated with infections in a prospective cohort study in three transmission settings in Yucatan, Mexico.

4.3 Methods

The description of the study sites, enrollment activities and the study procedures were described in detail in the second chapter of this dissertation.

4.3.1 Case Definitions

4.3.1.1 Dengue case definition

Dengue cases were classified according to the 2009 WHO Case Classification [5].

4.3.1.2 Dengue without warning signs

Dengue without warning signs is defined by acute fever (≥ 38.5°C) and two or more of the following symptoms: headache, myalgia, arthralgia, retroorbital pain, rash, hemorrhagic manifestations or leukopenia [5].

4.3.1.3 Dengue with warning Signs:

Dengue fever with any of the following warning signs: abdominal pain, persistent vomiting, fluid accumulation, lethargy, mucosal bleeding, fluid accumulation, liver enlargement, or increasing hematocrit with decreasing platelets [5].

4.3.1.4 Severe dengue:

Dengue fever with any of the following: severe bleeding, severe plasma leakage leading to shock or fluid accumulation with respiratory distress, or organ failure or involvement as evidenced by liver ALT or AST ≥ 1,000, impaired consciousness, failure of the heart or other organs [5].

4.3.1.5 Laboratory criteria for confirmation for dengue

**Acute dengue case** A symptomatic participant who tested positive for dengue evidenced by: 1) detection of DENV RNA by RT-PCR, 2) viral isolation of DENV, 3) seroconversion as determined by a DENV-specific immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA) using paired acute and convalescent sera, and/or 4) a ≥ 4-fold rise in total antibody titer between acute and convalescent sera as measured by Inhibition ELISA [144–146].
Inapparent dengue infection A participant whose paired annual serum samples demonstrated seroconversion as \( \geq 4 \)-fold increase in antibody titer as determined by Inhibition ELISA and who did not experience a symptomatic dengue infection during the time of follow-up [144–147].

Primary and secondary dengue infection A dengue infection is classified as a primary DENV infection if seroconversion was observed and was considered a secondary DENV infection if a \( \geq 4 \)-fold increase in antibody titer was observed in paired consecutive annual samples, as determined by Inhibition ELISA. If a participant’s serum contained anti-DENV antibody at enrollment or the participant experienced a previous DENV infection during the cohort, prior to a documented subsequent DENV infection, this was also considered a secondary DENV infection [148]. First and second DENV infections are identified by counting the number of the infections documented in a participant who entered the cohort dengue-naïve, using a \( \geq 4 \)-fold rise in titer by Inhibition ELISA in paired annual samples to identify inapparent infections or diagnostic assays in acute samples for symptomatic cases [145, 147, 148].

4.3.1.6 Dengue prior exposure

Dengue-naïve. A participant who did not have detectable anti-DENV antibody at enrollment as evidenced by Inhibition ELISA and has not experience a DENV infection during the cohort study.

Non-dengue-naïve. A participant who had anti-DENV antibody at enrollment as evidenced by Inhibition ELISA[145, 147, 148].

4.3.2 Chikungunya case definition

4.3.2.1 Acute chikungunya infection

A participant with abrupt onset of fever \( \geq 38.5^\circ \) accompanied by joint pain, muscle pain, headache, nausea, fatigue and rash. The joint pain is often very debilitating, but usually lasts for a few days or may be prolonged to weeks [149].
4.3.2.2 Laboratory criteria for confirmation for chikungunya

A symptomatic participant who tested positive for chikungunya evidenced by: 1) detection of CHIKV RNA by RT-PCR, 2) viral isolation of CHIKV, 3) enzyme-linked immunosorbent assays (ELISA) confirming the presence of IgM and IgG anti-chikungunya antibodies [149].

4.3.3 Zika case definition

4.3.3.1 Zika virus disease

Patient with rash usually pruritic and maculopapular with two or more of the following signs or symptoms: fever, usually ≥ 38.0°C, conjunctivitis (non-purulent/hyperemic), arthralgia or myalgia [150, 151].

4.3.3.2 Laboratory criteria for Zika confirmation

Participant who meets the criteria for a suspected case AND has laboratory confirmation of recent Zika virus infection by 1)RNA or Zika virus antigen in any specimen (serum, urine, saliva, tissue or whole blood); 2) Positive Zika IgM antibodies AND Plaque reduction neutralization (PRNT90) for Zika virus (titers ≥ 20) and four or more times greater than the titers for other flaviviruses; AND exclusion of other flavivirus [152].

4.3.4 Ethics Statement

This study was conducted as a collaboration between the Ministry of Health of Yucatan and the Center for Inference and Dynamics Infectious Diseases. This study was approved by the Institutional Review Boards at Fred Hutchinson Cancer Research Center and the General Hospital Agustín O’Horan of Yucatan. Written consent was obtained from all adult participants (≥ 18 years old) after providing them with a detailed explanation of the study and procedures. Parents/guardians of all child participants (≤ 18 years old) were asked to provide written consent on their behalf.

4.3.5 Statistical Analysis

The age-specific baseline seroprevalence for dengue virus was estimated for the entire cohort. For the first year of follow-up, the follow-up time was estimated as the time between enrollment and the end of the reported study period (August 2016), or withdraw from the
study. For those who were lost of follow-up, person-years were calculated as the time between enrollment and the last contact with study personnel, plus one-half the time between the last contact and the date recorded as lost to follow-up.

The analysis of dengue infections was limited to those participants who completed the year and contributed a blood sample at the beginning and the end of the year. In order to provide conservative estimates of dengue infection incidence, since the exact timing of the dengue infection could not always be ascertained, persons who experienced a dengue infection in the first year of follow-up contributed person-time for that entire year. For the analysis of dengue infections, the age of the participant was defined as the age when their annual sample was collected. The crude incidence per 1,000 person-years was 1,000 times the number of dengue infections divided by the number of person-years in the new dataset (\(= 1,000 \times \text{infections}/(\text{sum(days)}/365.25)\)) [103, 144, 145].

The incidence rate ratio was also estimated [153–155]. The incidence rate was estimated for each group (non-naïve and dengue naïve). Then the incidence rates in dengue non-naïve and naïve people, respectively, are:

\[
\hat{r}_0 = \frac{m_0}{T_0} \quad \text{in the non-naïve (control group)},
\]
\[
\hat{r}_1 = \frac{m_1}{T_1} \quad \text{in the naïve (exposed group)}. \tag{4-2}
\]

Then, the incidence rate ratio is

\[
\text{IRR} = \frac{m_1}{m_0} \cdot \frac{T_0}{T_1},
\]

which can take any value in [0, \(\infty\)). To calculate a confidence interval for the true IRR, we first use a log transformation:

\[
\ln \hat{\text{IRR}} = \ln \left( \frac{m_1}{T_1} \right) - \ln \left( \frac{m_0}{T_0} \right). \tag{4-4}
\]

Since outcomes in the two groups are independent,
\[
\text{Var} \left( \ln IRR \right) = \text{Var} \left( \ln \frac{m_1}{T_1} \right) + \text{Var} \left( \ln \frac{m_0}{T_0} \right) . \tag{4-5}
\]

By the delta method, we get the first-order approximate variances

\[
\text{Var} \left( \ln \frac{m_i}{T_i} \right) \approx \left( \frac{T_i}{m_i} \right)^2 \text{Var} \left( \ln \frac{m_i}{T_i} \right) \tag{4-6}
\]

Using an exponential model for the times to events, i.e., Poisson process, we get

\[
\text{Var} \left( \ln \frac{m_i}{T_i} \right) = \frac{m_i}{T_i^2} . \tag{4-7}
\]

Substituting this back into equation 4–6, we get

\[
\text{Var} \left( \ln \frac{m_i}{T_i} \right) = \left( \frac{T_i}{m_i} \right)^2 \left( \frac{m_i}{T_i^2} \right) = \frac{1}{m_i} . \tag{4-8}
\]

for \( i = 0,1 \). Therefore, the estimated variance of \( \ln IRR \) is approximately

\[
\frac{1}{m_0} + \frac{1}{m_1} \tag{4-9}
\]

and an approximate 95% confidence interval for the true \( \ln IRR \) is

\[
\ln \frac{m_1 T_0}{T_1 m_0} \pm 1.96 \sqrt{\frac{1}{m_0} + \frac{1}{m_1}} \tag{4-10}
\]

The corresponding 95% confidence limits for \( IRR \) are

\[
\frac{m_1 T_0}{T_1 m_0} \exp \left( \pm 1.96 \sqrt{\frac{1}{m_0} + \frac{1}{m_1}} \right) \tag{4-11}
\]

The hazard ratios (HRs) and 95% confidence intervals (95% CIs) for dengue infections were estimated using Cox proportional hazards models and adjusting for potential confounders [155, 156]. One advantage of the survival model is that it is valid with right censoring (lost-of follow-up). Let \( T_0 \) and \( T_1 \) be the total person-time contributed before analysis time \( t \) in the control group and the exposed group, respectively. We define the hazard function at time \( t \) for subject \( i \) with \( p \) covariates (explanatory variables) \( x_i = (x_{i1}, \ldots, x_{ip}) \) as
\[ \lambda(t|X_i) = \lambda_0(t) \exp(\beta_1 X_{i1} + \ldots + \beta_p X_{ip}) \]

4.4 Results

4.4.1 Baseline Characteristics of the Participants

A total of 767 families corresponding to 3,400 participants were enrolled from January to June, 2015 from the three sites in Yucatan. The majority of the families and participants were from Merida (2,021 (59.4%)), followed by Ticul (738 (21.7%)) and Progreso (641 (18.9%)). Among the participants 1,869 (54.97%) were females, 1,463 (43.03%) were 14 years old or younger, and 2,970 (87.35%) were born in the state of Yucatan (Table. 4-1). These participants contributed with 3430.87 person-years for the first year of follow-up. The mean participation time is 1.01 years (368.31 days) per participant (range 0.04- 1.94).

A total of 1,094 (32.2%) participants did not complete the first year of follow-up. A total of 320 (29.25%) of the participants were lost to follow-up, 210 (19.20%) moved out of the state of Yucatan and 564 (51.55%) asked for voluntary withdrawn from the study (Fig. 4-1).

Figure 4-1. Flowchart of participants in the dengue cohort, Yucatan, Mexico.
Table 4-1. Demographic characteristics of the cohort population enrolled in 2015 Yucatan, Mexico (N=3,400)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Merida (N= 2,021)</th>
<th>Progreso (N= 641)</th>
<th>Ticul (N= 738)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;8</td>
<td>593 (29.34%)</td>
<td>188 (29.33%)</td>
<td>198 (26.83%)</td>
<td>979 (28.79%)</td>
</tr>
<tr>
<td>9 -14</td>
<td>289 (14.30%)</td>
<td>86 (13.42%)</td>
<td>109 (14.77%)</td>
<td>484 (14.24%)</td>
</tr>
<tr>
<td>15 -19</td>
<td>85 (4.21%)</td>
<td>33 (5.15%)</td>
<td>40 (5.42%)</td>
<td>158 (4.65%)</td>
</tr>
<tr>
<td>20 -49</td>
<td>842 (41.66%)</td>
<td>277 (43.21%)</td>
<td>337 (45.66%)</td>
<td>1456 (42.82%)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>212 (10.49%)</td>
<td>57 (8.89%)</td>
<td>54 (7.32%)</td>
<td>323 (9.5%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>917 (45.37%)</td>
<td>286 (44.62%)</td>
<td>328 (44.44%)</td>
<td>1531 (45.03%)</td>
</tr>
<tr>
<td>Female</td>
<td>1104 (54.63%)</td>
<td>355 (55.38%)</td>
<td>410 (55.56%)</td>
<td>1869 (54.97%)</td>
</tr>
<tr>
<td>Number of families</td>
<td>463 (60.36%)</td>
<td>153 (19.95%)</td>
<td>151 (19.69%)</td>
<td>767 (100%)</td>
</tr>
<tr>
<td>Average number of people per family (Range)</td>
<td>4.36 (2.11)</td>
<td>4.18 (2.11)</td>
<td>4.88 (2.16)</td>
<td>4.43 (2.16)</td>
</tr>
<tr>
<td>Born in Yucatan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1775 (87.83%)</td>
<td>550 (85.80%)</td>
<td>645 (87.40%)</td>
<td>2970 (87.35%)</td>
</tr>
<tr>
<td>No</td>
<td>129 (6.38%)</td>
<td>52 (8.11%)</td>
<td>46 (6.23%)</td>
<td>227 (6.68%)</td>
</tr>
<tr>
<td>No information</td>
<td>117 (5.79%)</td>
<td>39 (6.08%)</td>
<td>47 (6.37%)</td>
<td>203 (5.97%)</td>
</tr>
<tr>
<td>Withdrawn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>778 (38.50%)</td>
<td>123 (19.19%)</td>
<td>195 (26.42%)</td>
<td>1096 (32.24%)</td>
</tr>
<tr>
<td>No</td>
<td>1243 (61.50%)</td>
<td>518 (80.81%)</td>
<td>543 (73.58%)</td>
<td>2304 (67.76%)</td>
</tr>
</tbody>
</table>
4.4.2 Dengue Baseline Seroprevalence

The baseline seroprevalence was estimated in 2,732 participants who authorized to take a blood sample at enrollment. The overall baseline dengue seroprevalence in the cohort was 73.39% (95% CI 71.7% - 75.0%) showing that most of the population had already been exposed to dengue infection (Fig. 4-2).

![Baseline dengue seroprevalence in the cohort of Yucatan, Mexico](image)

Figure 4-2. Age-specific baseline dengue seroprevalence in the cohort, Yucatan, Mexico.

The highest dengue seroprevalence was found in Ticul (81.59%), followed by Merida (73.99%) and Progreso (61.37%). The dengue seroprevalence increases with age and females in the cohort had higher seroprevalence estimates compared with males (Table 4-2).
### Table 4-2. Baseline exposure to dengue in the participants of the cohort enrolled in 2,015 in Yucatan, Mexico (n=2,732)

<table>
<thead>
<tr>
<th>Baseline exposure</th>
<th>Dengue-naïve (n= 708)</th>
<th>Dengue non-naïve (n=2005)</th>
<th>Indeterminate (n=19)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>City</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merida</td>
<td>404 (25.38%)</td>
<td>1178 (73.99%)</td>
<td>10 (0.63%)</td>
<td>1592</td>
</tr>
<tr>
<td>Progreso</td>
<td>195 (38.24%)</td>
<td>313 (61.37%)</td>
<td>2 (0.39%)</td>
<td>510</td>
</tr>
<tr>
<td>Ticul</td>
<td>109 (17.30%)</td>
<td>514 (81.59%)</td>
<td>7 (0.69%)</td>
<td>630</td>
</tr>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 8</td>
<td>418 (55.36%)</td>
<td>329 (43.57%)</td>
<td>8 (1.05%)</td>
<td>755</td>
</tr>
<tr>
<td>9 -14</td>
<td>150 (36.85%)</td>
<td>253 (62.16%)</td>
<td>4 (0.98%)</td>
<td>407</td>
</tr>
<tr>
<td>15 -19</td>
<td>30 (25.64)</td>
<td>86 (73.50%)</td>
<td>1 (0.85%)</td>
<td>117</td>
</tr>
<tr>
<td>20 -49</td>
<td>96 (7.67%)</td>
<td>1149 (91.84%)</td>
<td>6 (0.48%)</td>
<td>1251</td>
</tr>
<tr>
<td>≥ 50</td>
<td>14 (6.93%)</td>
<td>188 (93.07%)</td>
<td>0 (0%)</td>
<td>202</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>352 (30.39%)</td>
<td>972 (68.39%)</td>
<td>14 (1.21%)</td>
<td>1158</td>
</tr>
<tr>
<td>Female</td>
<td>356 (22.62%)</td>
<td>1213 (77.06%)</td>
<td>5 (0.32%)</td>
<td>1574</td>
</tr>
</tbody>
</table>
4.4.3 Suspected Symptomatic Arboviral Infections

Since the enrollment and baseline blood sample to the first year follow-up of the cohort, 199 suspected arbovirus infections cases or undifferentiated febrile illnesses were identified in the 3,400 population. These participants contributed with 3430.87 person-years for the first year of follow-up. The most common clinical diagnosis was undifferentiated fever 105 (52.76%), followed by 76 suspected dengue (38.19%), chikungunya 17 (8.54%) and one suspected case of Zika. (Table 4-3). The dengue incidence rate for suspected cases of 21.86 per 1,000 person-years.

All the consultations were in the outpatient clinic. The most common symptoms presented by the cohort population were: fever, headache, myalgia, arthralgia, rash and conjunctivitis. Not significant differences were found among all the arboviral clinical diagnosis (p=0.560) (Table 4-4). No severe symptoms were identified in the study population.

As the symptoms are unspecific among the arboviral infections, no incidence rates were estimated for probable dengue cases. The only incidence rate that could be estimated is the incidence rate of arbovirus suspected fever and it was 58.02 per 1,000 person years.

4.4.4 Confirmed Arboviral Symptomatic Infections

This analysis was done using as denominator the 199 participants with suspected arbovirus symptomatic infections or undifferentiated febrile illnesses were identified in the 3,400 cohort population. Among the symptomatic arboviral infections 12 (6.03%) were laboratory-confirmed as dengue-positive, 30 (15.08%) were confirmed as chikungunya-positive, 8 (4.02%) were confirmed as Zika-positive, and 149 (74.87%) were considered fever of unknown origen. These participants contributed with 3430.87 person-years for the first year of follow-up. The overall incidence rate of arboviral confirmed symptomatic infections was 14.57 per 1,000 person-years (95% CI: 10.82, 19.21). The incidence rate of confirmed symptomatic dengue was 3.49 cases per 1,000 person-years (95% CI: 1.87, 5.86). In the first year of the study, the majority of symptomatic cases were caused by the serotype DENV1 (52%); followed by serotype DENV2 (36%) and DENV4 (12%). DENV3 was not isolated during the study period.
<table>
<thead>
<tr>
<th>Probable symptomatic cases</th>
<th>Dengue</th>
<th>Zika</th>
<th>Chikungunya</th>
<th>Indeterminate</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue</td>
<td>7 (58.33%)</td>
<td>1 (12.5%)</td>
<td>11 (36.67%)</td>
<td>1 (100%)</td>
<td>56 (37.84%)</td>
<td>76 (38.19%)</td>
</tr>
<tr>
<td>Zika</td>
<td>1 (8.33%)</td>
<td>6 (75%)</td>
<td>0</td>
<td>0</td>
<td>10 (6.76%)</td>
<td>17 (8.54%)</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>1 (8.33%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.50%)</td>
</tr>
<tr>
<td>Undifferentiated fever</td>
<td>3 (25%)</td>
<td>1 (12.5%)</td>
<td>19 (63.33%)</td>
<td>0</td>
<td>82 (55.41%)</td>
<td>105 (55.41%)</td>
</tr>
</tbody>
</table>
Table 4-4. Symptoms of the febrile cases studied during the first year follow-up in the cohort of Yucatan, Mexico (N=199)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>189 (94.97%)</td>
<td>10 (5.03%)</td>
</tr>
<tr>
<td>Headache</td>
<td>158 (79.40%)</td>
<td>41 (20.60%)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>107 (53.77%)</td>
<td>92 (46.23%)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>140 (70.35%)</td>
<td>59 (29.65%)</td>
</tr>
<tr>
<td>Rash</td>
<td>102 (51.26%)</td>
<td>97 (48.74%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>13 (6.53%)</td>
<td>186 (93.47%)</td>
</tr>
<tr>
<td>Upper respiratory symptoms</td>
<td>11 (5.53%)</td>
<td>188 (94.47%)</td>
</tr>
</tbody>
</table>

No hospitalizations or deaths due to dengue symptomatic infection were reported during the first year of the cohort. The highest incidence of dengue was observed in the participants in the group from 15-19 years of age, females and in the participants from Merida (Table 4-5). The incidence rate of confirmed symptomatic chikungunya was 8.74 cases per 1,000 person-years (95% CI: 5.81, 12.30). The highest incidence of chikungunya was observed in the participants in the group 50 years old or more, females and in Ticul. (Table 4-5). The incidence rate of confirmed symptomatic Zika was 2.33 cases per 1,000 person-years (95% CI: 0.989, 4.529). The highest incidence rate for Zika was estimated also in Ticul (10.25 per 1,000 person-years). One case of co-infection of dengue and chikungunya was also identified. The majority of cases occurred from August to December 2015 that is historically the dengue season in Yucatan, Mexico.

4.4.5 Total Dengue Infections

The analysis of dengue infections was limited to 1,890 participants who completed the first year follow-up and contributed with a blood sample at the beginning (January 2015) and at the end of the first year of follow-up (June - October 2016). There were 1,037 (69.15%) participants from Merida, 379 (20.05%) from Progreso and 474 (25.08%) from Ticul. In total, the 1,890 participants contributed 2,271.14 person-years and 89 dengue infections were confirmed, for an incidence rate of 39.2 infections per 1,000 person-years (95% CI 31.66, 48.01) (Table 4-6). The overall ratio of symptomatic cases among dengue infections was 7.41 dengue infections per dengue symptomatic case. The highest incidence of dengue was observed in the
<table>
<thead>
<tr>
<th></th>
<th>Person-years at risk</th>
<th>Dengue IR (95%CI)</th>
<th>Chikungunya IR (95%CI)</th>
<th>Zika IR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>3430.87</td>
<td>3.45 (1.87, 5.86)</td>
<td>8.62 (5.81, 12.3)</td>
<td>2.3 (0.99, 4.53)</td>
</tr>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 8</td>
<td>1001.4</td>
<td>2.99 (0.602, 8.753)</td>
<td>9.99 (4.78-18.37)</td>
<td>3.99 (1.08, 10.23)</td>
</tr>
<tr>
<td>9 - 14</td>
<td>502.5</td>
<td>1.99 (0.03, 11.07)</td>
<td>7.96 (2.14, 20.38)</td>
<td>3.98 (0.45, 14.37)</td>
</tr>
<tr>
<td>15-19</td>
<td>145.3</td>
<td>6.88 (0.09, 38.29)</td>
<td>6.88 (0.09, 38.29)</td>
<td>0)</td>
</tr>
<tr>
<td>20-49</td>
<td>1490.1</td>
<td>4.69 (1.88-9.68)</td>
<td>7.38 (3.68, 13.21)</td>
<td>1.34 (0.15, 4.85)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>291.5</td>
<td>0</td>
<td>13.72 (3.69,35.13)</td>
<td>0)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1511.68</td>
<td>0</td>
<td>5.95 (5.81, 12.3)</td>
<td>2.29 (2.72, 11.30)</td>
</tr>
<tr>
<td>Female</td>
<td>1919.19</td>
<td>6.25 (3.23,10.92)</td>
<td>10.942 (6.78, 16.73)</td>
<td>2.61 (0.84, 6.08)</td>
</tr>
<tr>
<td>City</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merida</td>
<td>2164.26</td>
<td>4.16 (1.89, 7.89)</td>
<td>8.32 (4.93, 13.14)</td>
<td>0.92 (0.13, 3.34)</td>
</tr>
<tr>
<td>Progreso</td>
<td>601.76</td>
<td>3.32 (0.37, 12.01)</td>
<td>8.31 (2.68,19.29)</td>
<td>1.66 (0.02, 2.25)</td>
</tr>
<tr>
<td>Ticul</td>
<td>682.85</td>
<td>1.46 (0.02, 8.15)</td>
<td>10.25 (4.11, 21.12)</td>
<td>7.32 (2.36, 17.09)</td>
</tr>
</tbody>
</table>

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participants on the group from 15-19 years of age followed by the ≤ 8 year olds. The higher incidence rates were observed also Merida, females, naives and in the age group from 20-49 years-old with non symptomatic cases and the 9 to 14 years-old group with rates 6.66 cases per 100 infections.

Table 4-6. Incidence rates (IR) per 1,000 person-years of all dengue infections (N=1,890)

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Person-years at risk</th>
<th>Dengue infections</th>
<th>IR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>2271</td>
<td>89</td>
<td>33.9 (31.7, 48.0)</td>
</tr>
<tr>
<td>≤ 8</td>
<td>692.84</td>
<td>35</td>
<td>50.5 (35.7, 69.5)</td>
</tr>
<tr>
<td>9 -14</td>
<td>359.74</td>
<td>17</td>
<td>47.3 (28.5, 74.1)</td>
</tr>
<tr>
<td>15 -19</td>
<td>92.91</td>
<td>9</td>
<td>96.9 (47.2, 177.8)</td>
</tr>
<tr>
<td>20 -49</td>
<td>959.68</td>
<td>23</td>
<td>23.9 (15.6, 35.4)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>166.11</td>
<td>5</td>
<td>30.1 (11.0, 66.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>984.49</td>
<td>27</td>
<td>27.4 (18.4, 39.4)</td>
</tr>
<tr>
<td>Female</td>
<td>1286.79</td>
<td>62</td>
<td>48.2 (37.3, 61.4)</td>
</tr>
<tr>
<td>City</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merida</td>
<td>1248.14</td>
<td>51</td>
<td>40.9 (30.7, 53.3)</td>
</tr>
<tr>
<td>Progreso</td>
<td>481.76</td>
<td>27</td>
<td>56.1 (37.7, 80.4)</td>
</tr>
<tr>
<td>Ticul</td>
<td>540.38</td>
<td>11</td>
<td>20.4 (10.7, 35.4)</td>
</tr>
<tr>
<td>Prior exposure to dengue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>645.8</td>
<td>80</td>
<td>123.9 (98.9, 153.4)</td>
</tr>
<tr>
<td>Non-naive</td>
<td>1625.2</td>
<td>9</td>
<td>5.5 (2.7, 10.2)</td>
</tr>
</tbody>
</table>

4.4.6 Primary Dengue Infections in the Naïve Population

Among the 708 participants who entered the cohort as dengue-naïve, 478 dengue-naïve participants completed the first year follow-up and contributed with 490.31 person-years of time. Over the first year of follow-up 80 dengue-naïve participants experienced a dengue primary infection. The overall proportion of seroconversion was 16.74%. The incidence rate of primary dengue infections was 163.16 infections per 1,000 person-years (95% CI: 130.2, 202.1). The highest proportion of seroconversion in naïves was estimated in Merida (18.47%), followed by Progreso (17.48% and the lowest was in Ticul (10.47%) (Table 4-7).
Table 4-7. Seroconversion in the dengue-naïve at baseline (n=478)

<table>
<thead>
<tr>
<th>Seroconversion in naïves</th>
<th>Merida (n=249)</th>
<th>Progreso (n=143)</th>
<th>Tícul (n=86)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>46 (18.47%)</td>
<td>25 (17.48%)</td>
<td>9 (10.46%)</td>
<td>80 (16.74%)</td>
</tr>
<tr>
<td>No</td>
<td>203 (81.53%)</td>
<td>118 (82.52%)</td>
<td>77 (89.53%)</td>
<td>398 (83.26%)</td>
</tr>
</tbody>
</table>

4.4.7 Incidence Rate Ratio of Arbovirus Confirmed Symptomatic Cases and Dengue Infections

The incidence rate ratio (IRR) was also estimated and the 95% confidence interval using an exponential model for the times to event. The IRR assuming as exposed group the population that was naïve at baseline and the unexposed the non-naïves. The IRRs were estimated for dengue confirmed symptomatic cases, chikungunya confirmed symptomatic cases, Zika confirmed cases and overall dengue infections. The IRR for total dengue infections and Zika symptomatic cases were significant using the Logrank test (Table 4-8). More zika confirmed cases were detected in naïves and more chikungunya confirmed cases were detected in non-naïves.

Table 4-8. Incidence rate ratio for all arboviral infections in the first year of follow-up of the cohort in Yucatan, Mexico.

<table>
<thead>
<tr>
<th>Event</th>
<th>IRR (95%CI)</th>
<th>Logrank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue confirmed cases</td>
<td>1.4 (0.47, 4.14)</td>
<td>p=0.539</td>
</tr>
<tr>
<td>Dengue total infections</td>
<td>22.2 (11.13, 44.18)</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Chikungunya confirmed cases</td>
<td>0.5 (0.21,1.24)</td>
<td>p=0.114</td>
</tr>
<tr>
<td>Zika confirmed cases</td>
<td>3.7 (1.06, 13.26)</td>
<td>p=0.041</td>
</tr>
<tr>
<td>Any arboviral infection</td>
<td>0.9 (0.49, 1.61)</td>
<td>p=0.625</td>
</tr>
</tbody>
</table>

4.4.8 Survival Model for Dengue Infections

A Cox proportional hazard model was fitted for total dengue infections. The variables included in the model were: Age, dengue prior exposure, gender, city, overcrowding in the household (more than 5 people living in the same household), and one or more infections in the same household. In the univariate analysis, age as a continuous variable was significant as a protective factor for dengue infections (HR= 0.98, 95%CI: 0.96, 0.99). There was no significant association between total dengue infections and overcrowding in the household but the rest of the variables were significantly associated with the having a dengue infection.
The highest hazard ratios were estimated for baseline exposure to dengue (HR= 20.5, 95%CI: 10.30, 40.91) and having more people infected with dengue in the household (HR=57.9, 95%CI:37.21, 90.08). Those two variables were included in the final model as potential confounders. In the final model, having a dengue infection during the first year of follow-up was significantly associated with female gender, living in Ticul or Progreso, one or more infections in the household, and being dengue naïve at baseline. Age was not significantly associated with the outcome, it was confounded by prior immunity to dengue that increases with age. (Table 4-9).

Table 4-9. Hazard ratios for total dengue infections in the first year of follow-up of the cohort in Yucatan, Mexico.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 8</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>9 -14</td>
<td>1.06</td>
<td>0.59, 1.89</td>
</tr>
<tr>
<td>15 - 19</td>
<td>1.77</td>
<td>0.79, 3.96</td>
</tr>
<tr>
<td>20 - 49</td>
<td>1.27</td>
<td>0.72, 2.24</td>
</tr>
<tr>
<td>≥ 50</td>
<td>1.17</td>
<td>0.44, 3.09</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Females</td>
<td>1.65</td>
<td>1.04, 2.62</td>
</tr>
<tr>
<td>City</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merida</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Ticul</td>
<td>7.17</td>
<td>3.12, 16.49</td>
</tr>
<tr>
<td>Progreso</td>
<td>2.04</td>
<td>1.12, 3.76</td>
</tr>
<tr>
<td>Baseline status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dengue non-naïve</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Dengue naïve</td>
<td>15.35</td>
<td>7.19, 33.08</td>
</tr>
<tr>
<td>One or more infections household</td>
<td>22.31</td>
<td>14.01, 35.55</td>
</tr>
</tbody>
</table>

4.5 Discussion

Over the last five decades, dengue has emerged as a major health problem in the tropical regions worldwide including Latin America and the Caribbean [6, 7, 29, 146] and more recently chikungunya and Zika virus that emerged in the Americas causing significant epidemics in most of the countries infested with Aedes aegypti [157–160]. Mexico is considered one of
the endemic dengue countries in the region and since 2015 the three arbovirus have been co-circulating in many areas of the country [15, 104, 134].

This cohort enrolled 767 families and 3,400 participants from three cities in the state of Yucatan, Mexico. The majority of the families were from Merida (59.4%) the capital city of the state of Yucatan, followed by Ticul (21.7%) and Progreso (18.9%). The median age of the cohort was 24.8 years and around 46% of the participants are 14 years-old or younger. The ratio female: male is 1.2 similar to the ratio in the state of Yucatan [87]. Most of the participants were born in the state of Yucatan (87.35%), the average number of people per family is 4.43 with a range from 2 to 16. The largest families are in Ticul that is also the most rural setting of the study.

Most of the dengue cohorts in Latin America and South East Asia are pediatric cohorts [76, 84, 92, 103, 144, 145, 147, 148, 161]. The Yucatan cohort is unique given that it has participants from all age groups but the recruitment starts at the schools and it extends to the families. This cohort gives the opportunity to collect prospective data in all ages to understand better the full burden of dengue and other arbovirus that have emerged in the region.

The 3,400 participants contributed with 3,480 person-years during the first year of follow-up. The estimated baseline seroprevalence in the cohort was 73.39%. The city with the higher seroprevalence was also Ticul (81.9%), followed by Merida and Progreso. The seroprevalence increased with age as expected. These seroprevalence estimates are very similar to the seroprevalence estimated in the dengue seroprevalence survey in the previous chapter of this dissertation (73.6%).

A total of 199 suspected arboviral symptomatic infections were detected during the first year of follow-up. The most common symptom was fever (95%), followed by headache (79%) and myalgia (70.35%). These symptoms are similar among arboviral infections that is why the clinical diagnosis might not be accurate. It is needed to confirm the suspected cases to know which virus are circulating in endemic areas. The incidence rate of arboviral suspected cases was 58.2 per 1,000 person years. The dengue incidence rate for suspected cases of 21.86
per 1,000 person-years (95% CI 17.32, 27.25). This estimate falls in the confidence interval of incidence rates estimated in the Nicaraguan dengue cohort [145, 162, 163].

Among the symptomatic arbovirus cases just 6.60% were confirmed as dengue. The incidence rate of confirmed symptomatic dengue infections was 3.49 per 1,000 person-years. This proportion of confirmation and the incidence were lower than these estimates in other dengue cohorts [76, 80, 84, 92, 103, 144, 145, 147, 148, 161].

This can be explained that during this first year of follow-up chikungunya virus was introduced in Yucatan, Mexico and these arbovirus that are transmitted by Aedes aegypti might compete in the vector causing lower circulation of the other arbovirus in this case dengue [164–166]

The majority of the symptomatic cases were caused by DENV1, followed by DENV2 and DENV4. DENV3 was not isolated in the cohort samples. These results are consistent with the isolates from the national virology laboratory in Yucatan[88]. All dengue symptomatic cases were classified as dengue without warning signs according to the WHO 2009 case definition for dengue[5] The incidence rates of confirmed symptomatic Zika and confirmed chikungunya were 2.33 cases per 1,000 person-years and 8.74 cases per 1,000 person-years respectively. One case with co-infection of dengue and chikungunya was also detected as in some other endemic countries with co-circulation of multiple arbovirus [167].

From the original cohort a total of 1,094 (32.2%) participants did not complete the activities of the first year of follow-up but 2,304 are being followed from the original cohort. Most of the participants withdrew from the study at the time of taking the first year follow-up blood sample or they were considered lost of follow-up. The mobility on this population is very high so assessing potential mobility is important for the future of the cohort to be able to assess transmission of arbovirus as plan for this population.

The analysis of the dengue infections was done in 1,890 participants who had a baseline and follow-up blood sample. The incidence rate for dengue infections was 39.19 infections per 1,000 person-years. This incidence rate is lower compared with other cohorts around the

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world but it is showing that the dengue transmission was low during this first-year of follow-up [76, 80, 84, 92, 103, 144, 145, 147, 148, 161]. The highest incidence of dengue was observed in the participants on the group from 15-19 years of age followed by the ≤ 8 year-olds. The group from 15-19 years of age is just 4.65% of the cohort and just 25.64% of them were naïve at baseline. More participants from this age group have been enrolled in the last months so it is expected to have better denominators for the next years of follow-up.

Among 708 participants who were naïve at baseline, 478 (67.51%) completed the first year of follow-up. In the naïves were confirmed 80 dengue infections for an overall seroconversion rate of 16.74%. The incidence rate on primary infections was 123.88 per 1,000 person-years. This is very similar to estimates from other dengue prospective cohorts in endemic areas[144, 145]

The incidence rate ratio of confirmed overall dengue infections among naïves and non-naïve at baseline was 22.179 (95%CI 11.13, 44.18). In the Yucatan cohort the population that was dengue naïve had 22.17 times the incidence of dengue infections as those that were non-naïve at baseline. The incidence ratio of Zika confirmed cases was 3.7 (95%CI 1.1, 13.3). The other IRR estimated were not significant.

The hazard ratios estimated for dengue infections during the first year of follow-up were significant for female gender, living in Ticul or Progreso, one or more infections confirmed in the household, and being dengue naïve at baseline. In the final model after controlling for baseline dengue status age was not significantly associated with the outcome. To our knowledge, this is the first dengue cohort study that uses survival analysis as a tool to understand better the transmission dynamics of dengue and other arbovirus.

The limitations of this study include ascertainment of cases through enhanced passive surveillance. Thus, some dengue cases may not have been detected due to participants not seeking healthcare. Another limitation is that serum samples for cohort-wide serological testing are only available for participants once per year so it will be ideal to have more seroprevalence data points in time but logistically is difficult to manage. This study relies on Inhibition ELISA
to assess inapparent or asymptomatic infections. The gold standard to assess DENV infection is the plaque reduction neutralization test (PRNT) but it is very labor intensive and expensive so testing all the participants was not feasible.

In summary, this study reported baseline seroprevalence, incidence of dengue infections, dengue cases and other arboviral cases in a community-based cohort from three settings in Yucatan, Mexico using analytic methods. This is the first report of the results from this prospective cohort that is the only way to determine the incidence of arboviral infections to estimate the true rate of disease, which is usually underestimated by passive surveillance. These data will be used to estimate disease burden. The incidence estimates will be useful for policy makers and to evaluate interventions like vector control strategies and vaccines. Future analysis of household transmission, cluster analysis and effectiveness of interventions are planned.
CHAPTER 5
THE EPIDEMIOLOGY AND TRANSMISSIBILITY OF ZIKA VIRUS IN GIRARDOT AND SAN ANDRES ISLAND, COLOMBIA

5.1 Background

First isolated in the Zika Forest of Uganda in 1947, Zika virus (ZIKV) is an arbovirus primarily transmitted by *Aedes aegypti* mosquitoes [33]. Although ZIKV has circulated in Africa and Asia since the 1950s, little is known about its transmission dynamics [34]. Recent outbreaks in Yap Island in the Federated States of Micronesia (2007), French Polynesia (2013), and other Pacific islands, including Cook Islands, Easter Island, and New Caledonia (2014), indicate that ZIKV has spread beyond its former geographic range [35–38]. In April 2015 ZIKV was isolated in the Northeast of Brazil [39]. As of March 11, 2016, around 500,000 Zika virus disease (ZVD) cases have been estimated in Brazil, and autochthonous circulation has been observed in 31 countries in the Americas region. Further spread to countries within the geographical range of *Ae. aegypti* mosquitoes is considered likely [42].

ZIKV is a flavivirus in the same genus as dengue virus and yellow fever virus. Infection typically causes a self-limited dengue-like illness characterized by exanthema, low-grade fever, conjunctivitis, and arthralgia [40]. While illness is believed to be mild or asymptomatic in approximately 80% of the infections [41], an increase in rates of Guillain-Barré syndrome (GBS) has been observed during ZIKV outbreaks [42–44]. Furthermore, in October 2015, the Brazilian Ministry of Health reported a dramatic increase in cases of microcephaly in Northeast Brazil where ZIKV had been circulating [45]. On the basis of the possible link between ZIKV, GBS and microcephaly, the World Health Organization declared a public health emergency on February 1, 2016 [46, 47].

Zika virus was first detected in Colombia in mid-September 2015 in a municipality called Turbaco on the Caribbean coast. Turbaco is located approximately 20 minutes from Cartagena, a well known commercial and tourist hub (Figure 5-1). In October 2015, ZIKV spread through the central region of the country, appearing in areas with endemic dengue and ongoing circulation of chikungunya (CHIKV) since 2014. Through March 2016, Colombia has reported
over 50,000 cases of ZVD, making it the second-most affected country in this outbreak, after Brazil. There were 2,090 laboratory-confirmed cases with the rest being suspected cases or confirmed by clinical findings [157, 168]. Up to March 2016, 280 cases of neurological complications including Guillain-Barré syndrome (GBS) and three deaths possibly associated with ZVD have been reported in Colombia [169]. As of March 2016, there have been several suspected but no confirmed cases of ZIKV-associated microcephaly in Colombia [157, 168].

In this paper we describe local ZIKV outbreaks in Colombia in Girardot and San Andres Island between September 2015 and January 2016 for which detailed epidemiological data are available. We conduct an investigation to define the epidemiological features of these outbreaks and to estimate corresponding transmission parameters.

5.2 Aims

To describe local ZIKV outbreaks in Colombia in Girardot and San Andres Island between September 2015 and January 2016 for which detailed epidemiological data are available. We conducted an investigation to define the epidemiological features of these outbreaks and to estimate corresponding transmission parameters.

5.3 Methods

5.3.1 Settings

5.3.1.1 San Andres

San Andres is the largest island in a Colombian archipelago in the Caribbean sea located about 750 km north of mainland Colombia and 230 km east of Nicaragua (Figure 5-1). The island has an area of 27 km², a population of 54,513 inhabitants across 13,652 households, and a population density of 2,932 habitants per km² in 2010 [170, 171]. The average temperature is 27.3°C, and 80% of the total annual rainfall of 1,700 mm occurs during the heavy rainy season between October and December. The weather is humid subtropical with occasional tropical cyclones and hurricanes. The population in San Andres has two main ethnic groups: Afro-Colombians (17.5%) and Raizal (an ethnic group of mixed Afro-Caribbean and British descent) (39.2%) [171]. The most productive breeding sites of *Ae. aegypti* in San Andres
are unprotected water containers located in the households. San Andres has experienced low dengue transmission since 1983. Since 1995, the frequency of dengue outbreaks increased to every two to five years with a mean annual incidence of 43.6 cases per 100,000 inhabitants between 1999 and 2010 [170]. In 2014, CHIKV began circulating in San Andres, reaching an annual incidence of 365.1 cases per 100,000 inhabitants [172].

5.3.1.2 Girardot

The city of Girardot is located 134 km (2 hours drive) from the capital city of Bogota (Figure 5-1). Girardot has 102,225 inhabitants across approximately 23,000 households based on the most recent census from the National Statistics Department (NSD) [173], though the population triples during weekends and holidays. Girardot is 289 meters above sea level. The average temperature is 33.3°C, and the relative humidity is 66%. The mean annual precipitation is 1,220 mm with a rainy season extending from May through October [174]. The most productive breeding sites of *Ae. aegypti* in Girardot are unprotected private water containers, such as water storage tanks used in the households during the dry and rainy seasons, while public spaces provide more breeding sites during the rainy season [175]. Girardot has experienced hyperendemic transmission of dengue since 1990 with simultaneous circulation of all four serotypes; the mean annual incidence was 572.2 per 100,000 inhabitants between 1999 and 2010 [170]. In late 2014, CHIKV began circulating in Girardot reaching an annual incidence of 394 per 100,000 inhabitants in 2014 and 8,416 per 100,000 inhabitants in 2015.

5.3.2 Case Definition and Laboratory Analysis

We analyzed surveillance data from nine local health care sites in San Andres and twenty-two local health care sites in Girardot. Standardized case definitions used in both areas were defined by the Ministry of Health (MoH) and Colombian National Institute of Health (C-NIH) at the beginning of the ZIKV epidemic. A suspected ZVD case is defined as a person who lived or traveled in an area below 2,000 meters above sea level who presents with maculopapular exanthema, temperature higher than 37.2°C, and one or more of the following: non-purulent conjunctivitis, arthralgia, myalgia, headache, or malaise. A laboratory confirmed
Figure 5-1. Map of Colombia
case is a suspected case with a ZIKV positive reverse-transcriptase-polymerase-chain-reaction (RT-PCR) result as determined by the C-NIH virology reference laboratory. A clinically confirmed case is a suspected case that lived or traveled in an area with laboratory confirmed ZIKV circulation prior to onset of symptoms [176].

At the start of the outbreak, all suspected cases were reported based on the suspected case definition to the Colombian national surveillance system. Once laboratory confirmation from C-NIH was performed for cases in Girardot and San Andres, the new suspected ZIKV cases were laboratory confirmed if they fell into the risk groups defined by the C-NIH: newborns, age <1 year, age >65 years, and cases with co-morbidities [176]. After ZIKV circulation was confirmed in the regions, all suspected cases were reclassified as clinically confirmed. [177]

5.3.3 Data Collection

The data was collected using the C-NIH standard report form for Zika surveillance. The form includes socio-demographic variables. We analyzed a deidentified data set with the following variables: gender, age, pregnancy status, date of symptom onset, date the case visited the health care facility, date the case was reported to the national surveillance system, and case type (suspected, laboratory confirmed, clinically confirmed) [178, 179].

5.3.4 Statistical Analysis

We calculated overall and age/gender-specific attack rates using population census data from NSD [173]. Surveillance data were analyzed using R version 3.2.0 [180]. For descriptive results, categorical variables are presented as proportions and continuous variables by the median and interquartile range (IQR) or range. The impact of age and gender on attack rates was tested using log-linear models for case counts with age category, gender, and an interaction as independent variables with population size as an offset.

To estimate the basic reproductive number $R_0$ in each population, we used maximum likelihood methods to fit a chain-binomial model to daily incidence data [181]. $R_0$ is the median effective reproductive number during the growth phase of the epidemic, after
accounting for early under-reporting. (See Supplementary Online Materials for additional details on the model.) [36, 40, 182, 183]

5.4 Results

5.4.1 San Andres

In San Andres, we identified 928 reported ZVD cases (Table 5-1). Of these cases, 52 (5.6%) were laboratory confirmed by RT-PCR on acute phase samples collected within five days of symptom onset, and 876 (94.4%) cases were clinically confirmed.

Table 5-1. Characteristics of reported cases of Zika Virus Disease

<table>
<thead>
<tr>
<th>Region</th>
<th>San Andres</th>
<th>Girardot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cases</td>
<td>928</td>
<td>1936</td>
</tr>
<tr>
<td>Laboratory confirmed cases</td>
<td>52 (5.6%)</td>
<td>32 (1.7%)</td>
</tr>
<tr>
<td>Clinically confirmed cases</td>
<td>876 (94.4%)</td>
<td>1904 (98.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>589 (63.5%)</td>
<td>1138 (58.8%)</td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>31 (15, 47)</td>
<td>34 (24, 46)</td>
</tr>
<tr>
<td>Median time, days, to visit healthcare facility from symptom onset (IQR)</td>
<td>4 (1, 16)</td>
<td>1 (1, 2)</td>
</tr>
</tbody>
</table>

The dates of symptom onset among cases in San Andres ranged from September 6, 2015, to January 30, 2016 (Figure 5-2). Though the earliest case reported symptom onset on September 6, 2015, the local health care authorities did not receive laboratory confirmation of ZIKV until October 22, 2015. The number of cases peaked in epidemiological week 45 (November 8 to 14) and subsided in the last week of December. The median time between symptom onset and visiting a health care facility was 4 days (IQR 1 to 16). 79% of cases were reported to the national surveillance system on the same day they visited the health care facility. The median age of reported ZVD cases in San Andres was 31 years old (IQR 15 to 47; range 12 days to 82 years). 589 (63.5%) of the reported cases occurred in females. During this time period 70 dengue cases and 10 CHIKV cases were confirmed in San Andres.

The overall attack rate for ZVD reported by local surveillance was 12.13 per 1,000 San Andres residents. The gender-specific attack rates were 15.34 per 1,000 females and 8.91 per 1,000 males; the difference was significant adjusting for age (p<0.001). Cases occurred among all age groups, but the incidence of ZVD detected by local surveillance was highest among
Figure 5-2. Daily ZVD incidence for San Andres, Colombia

persons 20 to 49 years old (Figure 5-3); there was significant heterogeneity across the age
groups (p<0.001). Attack rates were higher in females across all age groups 10 years old and
above; there was a significant interaction between age and gender (p<0.001).

Figure 5-3. Age- and gender-specific ZVD attack rates for San Andres, Colombia

Thirty two pregnant women with ZVD were reported in San Andres and are being followed
according to national guidelines. By March 2016, fourteen of them had given birth with no
microcephaly reported. There were eight neurological syndromes reported in San Andres,
including GBS and meningoencephalitis attributed to ZIKV and among them one death was
reported. The incidence rate of neurological syndromes among ZVD cases in San Andres is 8.6 per 1,000 cases.

5.4.2 Girardot

In Girardot, we identified 1,936 reported ZVD cases (Table 5-1). Of these cases, 32 (1.7%) were laboratory confirmed by RT-PCR on acute phase samples collected within five days of symptom onset and 1,904 (98.3%) were clinically confirmed. During this time period were confirmed 67 dengue cases and 37 CHIKV cases in Girardot.

The date of symptom onset among cases in Girardot ranged from October 19, 2015, to January 22, 2016 (Figure 5-4). The first suspected case was reported on October 23, 2015, with laboratory confirmation obtained on January 27, 2016. The number of cases peaked in epidemiological week 48 (November 29 to December 5) and subsided in early January. The median time between symptom onset and visiting a health care facility was 1 day (IQR 1 to 2 days). 89% of cases were reported to the national surveillance system on the same day they visited the health care facility. The median age of confirmed ZVD cases was 34 years old (IQR 24 to 46; range 15 days to 92 years). 1138 (58.8%) of the cases occurred in females.

![Figure 5-4. Daily ZVD incidence for Girardot, Colombia](image)

The overall attack rate for confirmed ZVD detected by local surveillance was 18.43 per 1,000 Girardot residents. The gender-specific attack rates were 20.53 per 1,000 females and 16.07 per 1,000 males; the difference was significant adjusting for age (p<0.001). Cases
occurred among all age groups, but the incidence of ZVD detected by local surveillance was highest among persons 20 to 49 years old (Figure 5-5); there was significant heterogeneity across the age groups (p<0.001). Attack rates were higher in females in all age groups except in those 10 to 14 and 65 to 69 years old; there was no significant interaction between age and gender.

![Figure 5-5. Age- and gender-specific ZVD attack rates for Girardot, Colombia](image)

Sixteen pregnant women with ZVD were reported in Girardot and are being followed according to national guidelines. By March 2016, seven of them had given birth with no complications or microcephaly reported. Nine cases with GBS have been reported after an initial suspected ZIKV infection; laboratory-confirmation of ZIKV is pending. There were no deaths attributed to ZIKV. The incidence rate of neurological syndromes among ZVD cases in Girardot is 4.6 per 1,000 cases.

### 5.4.3 Estimations of the Basic Reproductive Number

The basic reproductive number ($R_0$) was estimated using daily incidence data. The model assumes a mean serial interval (time between successive cases in a chain of transmission) of 22 days, based on a mean incubation period in humans of 5 days, an extrinsic latent period (time from infection to infectiousness within the mosquito) of 10 days, and a mean infectious period in mosquitoes of 10 days. Under-reporting is assumed to be high (only 10% of cases reported) at the start of the outbreak and full reporting is assumed to be achieved in four weeks after
the outbreak begins to grow. The estimated $R_0$ for the Zika outbreak in San Andres was 1.41 (95% CI 1.15 to 1.74), and the $R_0$ in Girardot was 4.61 (95% CI 4.11 to 5.16) (Table 5-2 and Figure 5-6). Odds ratios for gender and age effects were obtained from the likelihood model, indicating increased odds of transmission among females and adults aged 20 to 49 years old in both San Andres and Girardot (Table 5-2).

The estimation procedure was also applied to daily incidence data from a published outbreak in Salvador, Brazil, that occurred between February 15, 2015, and June 25, 2015; 14,835 cases were reported with an overall attack rate of 5.5 cases per 1,000 Salvador residents [39]. The estimated $R_0$ of the Zika outbreak in Salvador, Brazil was 1.42 (95% CI 1.35 to 1.49). Sensitivity analyses are reported in the Supplementary Online Materials, including varying the incubation period in humans, the infectious period in humans, the infectious period in mosquitoes, the duration of under-reporting, and the level of under-reporting at the start of the outbreak.

Table 5-2. Estimates of $R_0$ gender-specific odds ratios for transmission, and age-specific odds ratios for transmission for ZVD in San Andres and Girardot, Colombia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level</th>
<th>San Andres</th>
<th>Girardot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Est.</td>
<td>95% CI</td>
</tr>
<tr>
<td>$R_0$</td>
<td></td>
<td>1.41</td>
<td>(1.15, 1.74)</td>
</tr>
<tr>
<td>OR$_{gender}$</td>
<td>Male</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.71</td>
<td>(1.50, 1.95)</td>
</tr>
<tr>
<td>OR$_{age}$</td>
<td>0-19</td>
<td>0.86</td>
<td>(0.74, 0.99)</td>
</tr>
<tr>
<td></td>
<td>$\geq$ 50</td>
<td>0.74</td>
<td>(0.63, 0.88)</td>
</tr>
</tbody>
</table>

5.5 Discussion

We report surveillance data on ZIKV outbreaks in two regions in Colombia between September 2015 and January 2016. The first region, San Andres, is a small, densely-populated island that is relatively isolated from continental Colombia. The second region, Girardot, is a typical moderately sized Colombian municipality. Both regions have endemic transmission of dengue and experienced recent outbreaks of CHIKV. We describe key epidemiological features of the Zika outbreaks and estimate the $R_0$ from daily incidence data.
Figure 5-6. Estimates of effective reproductive number. A) Estimates of effective $R$ (red) and model-fitted daily case numbers (green) for the outbreak of ZVD in San Andres, Colombia. The reporting ratio is assumed to increase linearly from 10% on and before September 30, 2015, to 100% in 4 weeks. Dashed curves (both red and green) are conservative 95% CIs. Histogram in grey shows the epidemic curve. The horizontal orange line indicates the reference value of 1. The two vertical orange lines indicate the time interval used for the estimation of $R_0$. B) As A) for Girardot, Colombia. The reporting ratio increases on October 19, 2015.
The overall attack rates for ZVD as detected by local surveillance were 12.13 cases per 1,000 residents of San Andres and 18.43 cases per 1,000 residents of Girardot. These attack rates are similar to those reported from Yap Island (14.3 per 1,000) [35] but higher than those reported in Salvador, Brazil (5.5 per 1,000) [39]. In both areas, significantly higher attack rates are observed among women, especially those of child-bearing age. The Colombian government issued an epidemiological alert on December 2015 to actively search for pregnant women with Zika-like symptoms in areas with active transmission [184, 185]. This effort may partially explain the findings, though differences in gender-specific attack rates persist when only cases occurring prior to December are considered [186–188]. Cases occurred in all age groups, but the most affected age group was 20 to 49 year of age, similar to previously published outbreaks in Yap Island, Federated States of Micronesia, and in Salvador, Brazil [35, 39]. As the population was fully susceptible to Zika transmission before the outbreaks, it is reasonable that all age groups would be affected.

Forty-eight pregnant women with ZVD were reported from San Andres and Girardot. These women are being followed according to national guidelines [184, 185] with no confirmed cases of microcephaly observed yet. Seventeen neurological syndromes, including GBS and ZIKV-associated meningoencephalitis, were identified, similar to reports from French Polynesia and Brazil [44, 189]. Laboratory-confirmation of these cases is challenging because neurological symptoms generally appear two weeks after acute symptoms [190] at which time ZIKV diagnosis by RT-PCR is not possible and serological tests are unreliable because of cross-reactivity with dengue [167, 191]. As ZIKV can be detected in urine longer than in blood [192], using urine samples to confirm ZIKV in GBS cases may be an alternative [193]. These challenges underscore the need for reliable diagnostic tests that can detect ZIKV after the viremic period.

The basic reproductive number ($R_0$) was estimated in each area using daily incidence data. Our estimated $R_0$ for the Zika outbreak in San Andres was 1.41 (95% CI 1.15 to 1.74), and the $R_0$ for Girardot was 4.61 (95% CI 4.11 to 5.16). Applying the same methods with
previously published data, we estimated that the $R_0$ for Zika virus in Salvador, Brazil, was 1.42 (95% CI 1.35 to 1.49) [39]. We consider the estimate from San Andres to be the most reliable because it is a small, densely populated island, while Girardot has a higher risk of importation because the population fluctuates during weekends and holidays. The relative magnitudes of $R_0$ are consistent with the higher dengue transmission observed in Girardot versus San Andres. Estimates of $R_0$ in Zika are not widely available, though reports suggest an $R_0$ of 4.3 to 5.8 in Yap Island and $R_0$ of 1.8 to 2.0 in French Polynesia [194]. A recent manuscript considering the French Polynesian outbreak reported a range from 1.9 to 3.1 [59].

Relatively few cases were laboratory confirmed. The majority of cases were clinically confirmed, and the symptoms could be caused by other etiologies such as dengue. This report only includes symptomatic cases who attended a health care facility and were captured by the surveillance systems. ZIKV usually causes relatively mild illness lasting several days, and around 80% of infections are currently believed to be asymptomatic, so we are likely missing many mild or asymptomatic cases [41]. We also do not have a reliable estimate of under-reporting at these sites. Early under-reporting appeared to be especially apparent in the Girardot outbreak, and the sharp increase in cases observed may be due to increased public awareness of the disease. This phenomenon can result in an overestimate of $R_0$.

Well-designed studies can provide valuable insight. Phylogenetic analyses of circulating ZIKV strains will be critical for understanding whether mutations in the viral genome are associated with an increased severity of disease, as manifested by microcephaly and GBS in this outbreak. Household studies can allow for more accurate estimation of transmission dynamics and enhance understanding of asymptomatic infection. Studies are required to understand the interactions between ZIKV, dengue, CHIKV, and other co-circulating arboviruses and their impact on disease. It is also necessary to increase surveillance of neurological syndromes associated with ZVD, like GBS and encephalitis.

The evidence for a causal relationship between ZIKV and microcephaly is strengthening [195–197]. Recent evidence from the French Polynesian outbreak suggests an estimated number
of microcephaly cases associated with ZIKV is around one per 100 women infected in the first trimester[198]. Currently the Colombian Government is following a cohort of pregnant women that reported Zika-like symptoms anytime during their pregnancy. Those who are detected during the acute phase are being diagnosed with ZIKV RT-PCR. All women will be followed until the end of pregnancy, and the fetus will be evaluated during pregnancy and post-natally for twelve months. The prospective collection of data through this and other similar national cohorts will be essential for assessing causality, determining risk factors, and estimating rates of birth defects.

The results of this and other reports concludes that transmission of ZIKV may be widespread. Vector control has had limited success in controlling other arboviruses, such as dengue. A safe and efficacious vaccine, especially for women of child-bearing age, may be needed to reduce the disease burden.
Arboviral infections are considered public health problems worldwide but their cumulative impact on global disease burden has not been fully assessed. Approximately 40% of the world population is at risk of arboviral infections because they live in areas infested by Aedes mosquitoes, mainly Aedes aegypti and Aedes albopictus the vectors for dengue, chikungunya and Zika virus.

In their acute stages, arboviral infections cause a broad spectrum of disease, ranging from asymptomatic infection to severe disease. This wide spectrum of the disease makes difficult to assess the full burden and transmission parameters of these viral diseases using epidemiological surveillance data.

To better understand the epidemiology and transmission of arbovirus in Latin America, a field study was designed to collect the baseline dengue seroprevalence and a prospective school-based cohort to assess incidence and potential risk factors associated with transmission of these viruses in the population of Yucatan, Mexico. The state of Yucatan is considered a dengue-endemic state with heterogeneous local transmission among the different cities.

Initially the baseline field studies in Yucatan were design to understand better the transmission dynamics of dengue but chikungunya and Zika virus emerged in Mexico and in Latin America in 2015-2016 providing the opportunity to analyze the epidemiology and transmission dynamics of these virus too.

The first cases of Zika virus were confirmed in Latin America in 2015-2016 and Colombia was the second country in the region more affected by Zika virus after Brazil. The last chapter of this dissertation includes the analysis of epidemiological surveillance data from two Zika outbreaks in Colombia and the estimations of transmission parameters useful to estimate the impact of the outbreak and planning further interventions.
Epidemiological field studies are key to assess the baseline dengue transmission in endemic populations. The population based serosurveys are extremely useful to identify the age groups more at risk and which ones would be the best target for interventions.

The baseline seroprevalence survey was conducted in a random sample of the population from three urban settings in Yucatan, Mexico. These settings were classified based on epidemiological data as high, medium or low transmission. Merida de capital of Yucatan was classified initially as high risk, Ticul was classified as medium risk as Progreso as low risk.

In the survey the overall estimated dengue seroprevalence in Yucatan was 73.6% (95% CI 71.4% - 75.7%) showing that most of the population had already been exposed to dengue infection. We also observed that dengue seroprevalence in the Yucatan increased with age in all three study settings. The lowest overall seroprevalence was 51.4% in the group ≤ 8 year olds and the highest for the population ≥ 50 years old (83.4%) as expected given that the younger age groups have been exposed less to dengue compared with the oldest age groups.

The baseline seroprevalence in Merida was 68.6%, in Progreso 68.7% and in Ticul 85.3%. These seroprevalences were expected given that the state of Yucatan is a well-known dengue endemic area in Mexico. These findings were very interesting given that the initial risk classification using the epidemiological surveillance data was not completely accurate. Ticul ended up being the highest risk population, and Merida and Progreso were medium risk. It is important to take into account the size of the city and possible heterogeneities in dengue transmission in larger cities like Merida compared with smallest cities like Ticul.

In order to know the baseline exposure conditions to dengue and other flavivirus in endemic areas, field studies are required given the complexity of the transmission, the broad clinical spectrum of the disease (large proportion of asymptomatic infections), and the underreporting to the surveillance systems. The model results showed that the main risk factor associated with dengue seropositivity in Yucatan were age, sex and city. The variables being born in the Yucatan, having history of previous infections with dengue and having a previous confirmation of dengue were not associated with seropositivity in this study.
Prospective cohort studies are important to estimate the risk of infection by dengue virus. Currently, there are several ongoing cohort studies in dengue endemic areas in South East Asia and Latin America. The Yucatan cohort study is the second study from the baseline dengue studies. During the first year of follow-up dengue and other arbovirus incidence rates were estimated.

This cohort is unique given that it has participants from all age groups. The enrollment was initially at elementary schools and extended to the families. This cohort gives the opportunity to collect prospective incidence data to understand better the local transmission determinants and full burden of dengue and other arbovirus transmitted by Aedes mosquitos that have emerged recently in the Americas.

This cohort enrolled 767 families and 3,400 participants from three cities in the state of Yucatan, Mexico. The majority of the families were from Merida (59.4%) the capital city of the state of Yucatan, followed by Ticul (21.7%) and Progreso (18.9%). The mean age of the cohort 18.40 years and around 46% of the participants are 14 years-old or younger. Most of the participants were born in the state of Yucatan (87.35%), the average number of people per family is 4.43 with a range from 2 to 16. The largest families are in Ticul that is also the most rural setting of the study.

The estimated baseline seroprevalence in the cohort was 73.39%. The city with the higher seroprevalence was also Ticul (81.9%), followed by Merida and Progreso. The dengue seroprevalence in the cohort increased with age as observed in the initial dengue seroprevalence survey of the random sample of the population (73.6%). The match on the results of the two dengue seroprevalence is reassuring that the three settings are endemic areas and that Ticul has the highest risk for dengue transmission.

The most common symptom of the symptomatic cases was fever (95%), followed by headache (79%) and myalgia (70.35%). These symptoms are similar among arboviral infections that is why the clinical diagnosis and case reporting to the epidemiological surveillance system are not accurate as expected. It is needed to enhance epidemiological
and laboratory surveillance to be able to detect not just the viruses that are circulating but also
give information that could be useful for policy makers. The clinicians also need to know the
individual diagnosis to be able to prevent particular complications that are associated with each
arbovirus and suspect co-infections.

Among the symptomatic arbovirus suspected cases just 6.60% were confirmed as
dengue. The incidence rate of confirmed symptomatic dengue infections was 3.49 per
1,000 person-years. This proportion of confirmation and the incidence were lower than these
estimates in other dengue cohorts. One possible explanation is that there were more arbovirus
circulating in Yucatan, Mexico during this first-year of follow-up so these viruses might compete
and decrease dengue transmission. Currently we are also planning to review and update the
protocols for confirmation of acute cases given the current conditions of co-circulating viruses
in the area that could be leading to biases in the diagnosis.

The incidence rate for dengue infections was lower compared with other cohorts around
the world. It could be useful to support the fact that this first year of follow-up was a
low transmission season for dengue. The highest incidence of dengue was observed in the
participants on the group from 15-19 years of age followed by the ≤ 8 year olds. The group
from 15-19 years of age represents 4.65% of the cohort and just 25.64% of them were naïve
at baseline. During the second semester of 2016 were enrolled participants for middle and
high schools to have a representative number of participants of this particular age group
that is also the age group that could be potentially targeted for dengue vaccination. Among
the participants who were naïve at baseline, the overall seroconversion rate of 16.74%. The
seroconversion rate is the best estimate of force of infection given that those were primary
infections.

In the Yucatan cohort the population that was dengue naïve at baseline had 22.17
times the incidence of dengue infections as those that were non-naïve at baseline. Also
the population that was dengue naïve at baseline had 3.743 times the incidence of Zika
symptomatic infections as those that were non-naïve at baseline. These are very interesting
results given that being non-naïve for flavivirus infections could be potentially be protective for symptomatic Zika but this hypothesis would need more detailed immunological research. The other incidence rate ratios estimated were not significant.

The hazard ratios estimated for dengue infections during the first year of follow-up were significant for female gender, living in Ticul or Progreso, one or more infections confirmed in the household, and being dengue naïve at baseline. This is the first dengue cohort study that uses survival analysis as a tool to understand better the transmission dynamics of dengue and other arbovirus.

The main limitation of this first year follow-up results is that 32.2% of the participants during the first year of follow-up were lost of follow-up or withdrew from the study. There were not diferencial lost of follow-up and withdraws among naïves and non-naïves. Most of the participants withdrew from the study at the time of taking the yearly follow-up blood sample or because they moved out of the state of Yucatan. Currently new strategies to increase adherence to the cohort have been implemented.

The incidence rates of confirmed symptomatic Zika and confirmed chikungunya were 2.33 cases per 1,000 person-years and 8.74 cases per 1,000 person-years respectively. The state of Yucatan had a chikungunya outbreak in 2016 so that explains why this rates is higher than the dengue rates. Zika emerged also in Yucatan but it was introduced at the end of the traditional dengue transmission season (July-December). Few number of cases were reported and this could be consistent with the incidence rate estimated in the cohort study. This information is useful to design studies to evaluate effectiveness of public health interventions like vector control or Zika vaccine trials.

In contrast with Mexico, Colombia was the second most affected country by Zika virus during 2015 - 2016 outbreak in the Americas. Colombia has a good surveillance system compared with other countries in the region. We analyze epidemiological surveillance data from two local Zika virus outbreaks between September 2015 and January 2016. At the beginning of the Zika virus outbreak most of the transmission parameters were unknown as it was a
virus that recently emerged to the western hemisphere. In the last paper we described key epidemiological features of the Zika outbreaks and estimate the \( R_0 \) from daily incidence data from two settings: 1) San Andres that is a small, densely-populated island that is relatively isolated from continental Colombia; 2) Girardot, that is a typical moderately sized Colombian municipality. Both regions have endemic transmission of dengue and experienced recent outbreaks of CHIKV.

The overall attack rates for Zika virus disease and detected were 12.13 cases per 1,000 residents of San Andres and 18.43 cases per 1,000 residents of Girardot. In both areas, significantly higher attack rates are observed among women, especially those of child-bearing age. These higher attack rates could be explained by the effort of the Colombian government to capture all the pregnant women possibly infected with Zika virus to understand better the spectrum of congenital Zika syndrome. In both settings as the population was fully susceptible to Zika transmission before the outbreaks, all age groups were affected.

Forty-eight pregnant women with Zika virus disease were reported from San Andres and Girardot. These women were followed during pregnancy according to national guidelines and no confirmed cases of microcephaly were observed. Seventeen neurological syndromes, including GBS and Zika-associated meningoencephalitis, were identified. The laboratory-confirmation of Zika infections is challenging and in Zika infections with neurological symptoms could be more challenging because these symptoms generally appear two weeks after acute symptoms, at which time Zika virus diagnosis by RT-PCR is not possible and serological tests are unreliable because of the high cross-reactivity with dengue. As ZIKV can be detected in urine longer than in blood, using urine samples to confirm Zika in GBS cases should be an alternative. These challenges underscore the need for reliable diagnostic tests that can detect Zika after the viremic period.

The basic reproductive number (\( R_0 \)) for Zika was estimated in each area using daily incidence data. Our estimated \( R_0 \) for the Zika outbreak in San Andres was 1.41 (95% CI 1.15 to 1.74), and the \( R_0 \) for Girardot was 4.61 (95% CI 4.11 to 5.16).
We consider the estimate from San Andres to be the most reliable because it is a small, densely populated island, while Girardot has a higher risk of importation because the population fluctuates during weekends and holidays. The relative magnitudes of $R_0$ are consistent with the higher dengue transmission observed in Girardot versus San Andres. Estimates of $R_0$ in Zika in the Americas are not widely available, so these parameters have been used to model transmission dynamics of Zika in the Americas and to estimate probable areas where Zika vaccine trials should be run in order to detect cases during the trial. With the prospective cohort in Yucatan we expect to collect prospective data about Zika transmission and validate our estimations with the Colombian data.

In summary, it is a unique moment for arbovirus in Latin America given that three viruses that are transmitted by the same vector are co-circulating in most of the areas at risk. The Yucatan cohort will provide information to better understand the transmission dynamics of these viruses. Vector control had limited success in controlling these arboviruses transmitted by Aedes mosquitoes, such as dengue, Zika and chikungunya so new approaches to control mosquito densities are needed. Also safe and efficacious vaccines to prevent arboviral infections and symptomatic disease will be key to reduce transmission and burden of disease. In the case of Zika virus, the vaccine should target especially women of child-bearing age to prevent Zika congenital syndrome.
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BIOGRAPHICAL SKETCH

Diana P. Rojas Alvarez is a Medical Doctor from Colombia, specialist in tropical infectious diseases. Her research interest focuses on epidemiology and transmission dynamics of tropical infectious diseases in Latin America. Diana was the director of the national epidemiological surveillance system in the Colombian National Institute of Health for several years. She worked also as a researcher in the Universidad Industrial de Santander (Bucaramanga-Colombia) doing observational studies to measure impact of dengue infection in the Colombian population and phase 3 trials of a dengue vaccine. In 2013, she got awarded a Fulbright-Colciencias doctoral scholarship that allowed her to start her PhD training in Epidemiology at University of Florida. Diana is been involved in multiple projects since she started her program mainly in dengue, chikungunya and Zika virus transmission. She received her Ph.D. from the University of Florida in the summer 2017.