Dengue Fever in Ribeirão Preto, Brazil, 2003-2012: Patterns of Disease and Understanding of Prevention

by

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DEDICATION

To my Father, John, and to my God-Father, Cid (*in memorium*): the two people who most inspired me to seek the world's truths through the methods of science.

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ABSTRACT

Dengue fever is the most important arboviral disease of modernity and is a disease characterized by a large spectrum of clinical manifestations of varying intensities. It is caused by four serologically distinct viruses transmitted by mosquitoes of the genus *Aedes*. Over 2.3 million cases were reported in the Americas during 2013. More than half of the world's population is at risk of infection by the virus and the vectors' distributions are increasing. Current prevention strategies involve personal protection and disruption of vector breeding sites as no vaccine is available. Accordingly, it is important to better understand the underlying risk factors of dengue prevention if transmission risk is to be reduced.

To assess some of the underlying risk factors of dengue, three studies were conducted using data from Ribeirão Preto, Brazil. The first (Chapter II) characterized patterns of dengue surveillance data that included all reported cases of dengue in the city during 2003-2012. Epidemiologic patterns were analyzed in relation to a variety of individual- and population-level risk factors. The second study (Chapter III) employed a generalized linear model with a negative binomial distribution to assess the potential links between changes in weather variables and dengue incidence during this same time period. Finally, a large survey was conducted in 2012 aimed at characterizing population-level knowledge of dengue.

Annual dengue incidence varied considerably from <1 to ~500 cases per 10,000 inhabitants. Dengue incidence did not vary by sex, but was inversely associated with educational levels. Most cases of dengue visited clinics within 3 days of the onset of symptoms. Analyses suggested that roughly one-third of the city's 650,000 people had been infected with dengue during the study period. Results from the weather study indicated that increases in minimum temperature and precipitation were associated with dengue cases lagged by 6 and 8 weeks, respectively. Results from the survey indicated that residents had general knowledge about various aspects of dengue, but that individuals with lower income and socioeconomic status had lower levels of knowledge while individuals with previous infections had higher levels.

Taken together, these three studies provide additional information that may help explain the drivers of dengue transmission and disease risk in Ribeirão Preto, Brazil. Ultimately, it appears that a large proportion of the city may be primed for more severe disease in the near future. Also, upon validation, the dengue-weather model may help city officials to predict and better prepare for future outbreaks. Finally, survey findings indicated the necessity of investing in targeted campaigns to improve dengue knowledge amongst those at highest risk of disease. This combination of these epidemiological approaches provides a low-cost basis for concurrent assessment of multiple aspects of the etiology of dengue, which may be of particular interest in resource-limited locations.

CHAPTER I

Introduction.

1.1. History of Dengue.

The modern name of the disease, dengue, is believed to come from either Spanish or Swahili—the Spanish origin meaning “fastidious” or “careful”, and the second from the phrase “Ka dinga pepo”: a disease caused by an evil spirit (1). Phylogenetic research suggests that the different viruses that cause dengue fever arose independently— suggesting both Asian and African origins of the virus(2–7). First descriptions of dengue-like illness can be traced as far back as 3rd century China—this disease was referred to as “water-poison”, suggesting an association with the disease vector and its breeding site(7–9). In the 17th century, the disease was described with wide geographic range, likely having been spread by the global slave trade, and may have even reached pandemic proportions in the 18th century (1,7,10,11). In fact, Dr. Benjamin Rush described clinical manifestations of a disease in line with those of dengue in Philadelphia in 1780(11,12).

Despite the disease’s presence in the Americas for several centuries, dengue fever was actually eliminated in the mid-twentieth century as a side-result of an international campaign to eradicate yellow fever, transmission of which shares the same mosquito vectors. The vectors were mostly eliminated from the Americas, but campaigns ceased in the1970’s and *Aedes* mosquitos recolonized a large portion of the Americas, as seen in figure 1.1 (13,14).

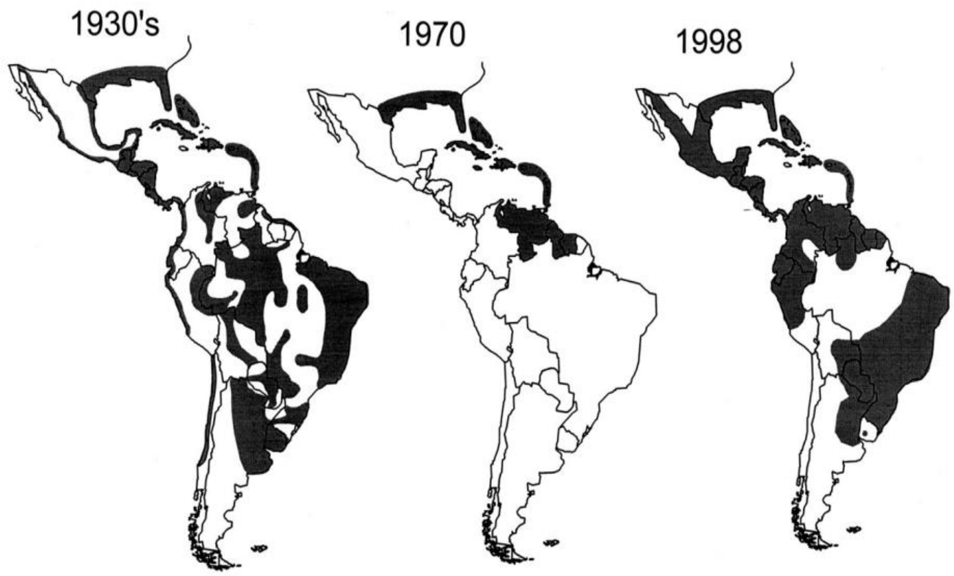

Figure 1.1. Distribution of *Ae. aegypti in the Americas, 1930-1998.* (14)*.*

Today *Ae. aegypti* and *Ae. albopictus* are present throughout much of the inhabited regions of the Americas and studies suggest that the range of the vector will likely continue to expand(15,16). Given that vector presence is one of the principal risk factors for dengue, the expanding range of dengue virus-competent *Aedes* spp. suggests that more people will be at risk of contracting the disease in the near future, further reinforcing dengue’s status as a priority of public health.

1.2. Epidemiology and Transmission.

It is estimated that over 2.5 billion people are at risk of contracting dengue worldwide, with an estimated 390 annual infections (range 284-528 million infections)—approximately 96 million of which manifest with clinical significance (24.6%) (17). It has actually been reported that as high as 87% of infections can actually be clinically irrelevant or inapparent (18,19). As can be seen in Figure 1.2, most dengue infections occur in tropical and subtropical areas, as cold winter temperatures restrict the distribution of dengue's vectors, and temperature and rainfall affect these mosquitoes' breeding patterns and life cycles (20,21).

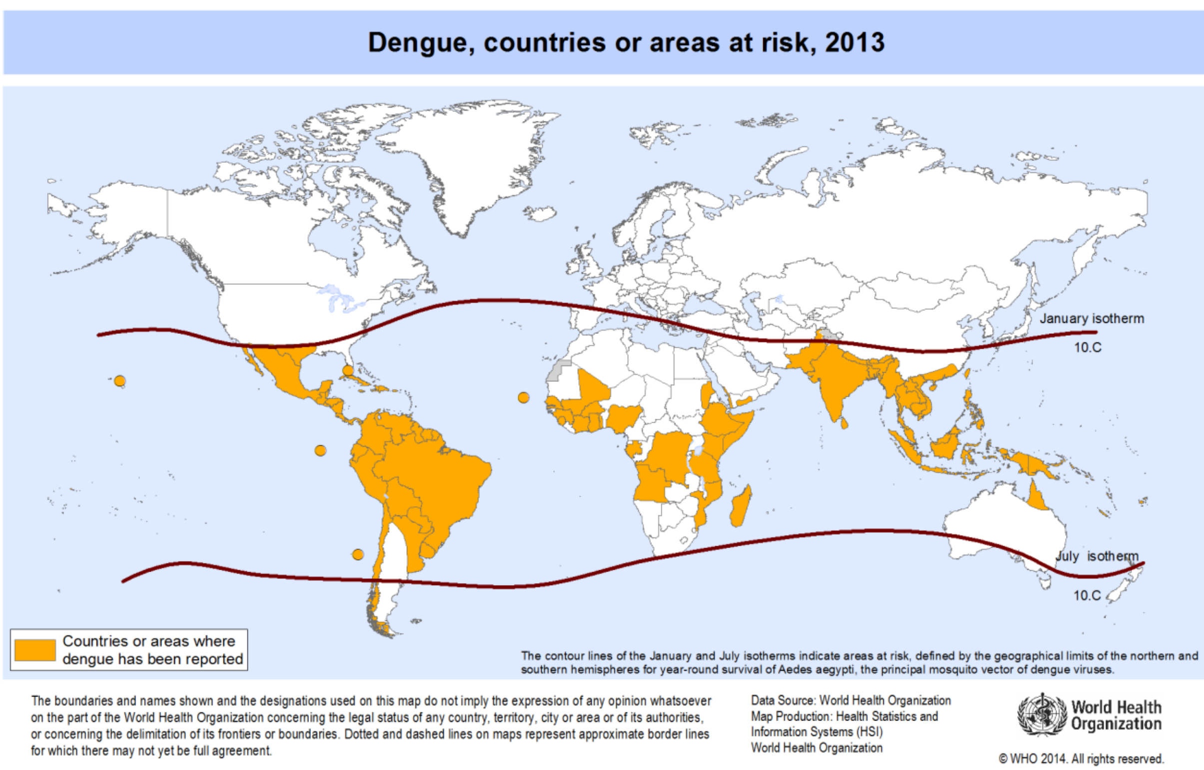

Figure 1.2. Distribution of Dengue—Countries or Areas at Risk(22).

An over 30-fold increase in dengue incidence has been seen in the past 50 years, and dengue even seems to be expanding into rural settings recently; human movement has been implicated as a driver of the expansion of the geographic range of the disease into previously unaffected areas (19,23–25). Dengue is known to follow seasonal and cyclical patterns, which related to a variety of demographic, immunological, and environmental factors(19,23,26–31).

Dengue fever is primarily caused by four serologically distinct viruses entitled Dengue-I, Dengue-II, Dengue-III, and Dengue-IV (DENV 1-4). These viruses are members of the family *Flaviviridae*, which includes other well-known disease-causing viruses like Yellow Fever, St. Louis encephalitis, Chikungunya, West Nile, and Hepatitis C. These are positive-sense, single-stranded RNA viruses that range from 9.6 to 12.3 kilobases in length, coding various structural and non-structural proteins (32).

Dengue virus transmission occurs in one of two distinct, mostly non-overlapping cycles—the common or “Urban” cycle, as well as a much less common “Sylvatic” cycle (Figure 1.3).

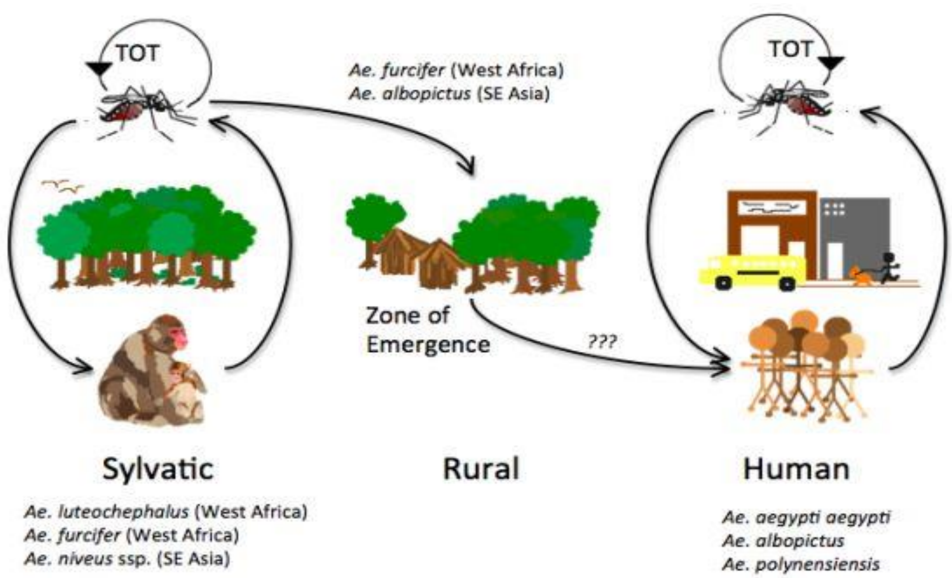

Figure 1.3. Urban (Human) and Sylvatic Dengue Transmission Cycles (7).

In the urban cycle, the virus is transmitted by infected female *Ae. aegypti*, *Ae. albopictus*, and *Ae. polynesiensis* to human hosts, as well as from female mosquitoes to their progeny through transovarial transmission (33–40). The sylvatic cycle consists primarily of transmission of dengue virus between non-human primates and arboreal vectors such *Ae. taylori*, *Ae. niveus*, *Ae. furcifer, Ae. luteocephalus,* and *Ae. africanus,* among others*.* This cycle is mostly observed in Africa and Southeast Asia and rarely affects humans (7,41,42). There is, however, the possibility that "spill-over" from the sylvatic cycle may occur, leading to new urban epidemics (43). The viruses circulating in each of these two cycles are genetically and environmentally distinct, indicating that there is truly a lack of overlap between them. In fact, it has recently been claimed that a new genetically distinct viral serotype, "Dengue 5", has been detected in the sylvatic cycle in Thailand, however there is currently no published primary research that addresses whether this is truly a new dengue serotype (44).

As mentioned above, mosquitoes of the genus *Aedes* are the sole known vectors of dengue fever. *Aedes aegypti* is generally considered to be the principal vector of the disease worldwide, with *Ae. albopictus* and *Ae. polynesiensis* serving as secondary vectors (7,43). It appears that *Ae. albopictus* may have been the original vector of dengue virus, yet it seems that *Ae.egypti* is actually better suited to sustain large-scale and long-term dengue transmission. This may be because *Ae. albopictus* is a peri-urban mosquito that oviposits in both natural settings, such as tree holes, and human-made ones (45–48). This mosquito will seek a blood meal from a variety of vertebrates, including humans. *Aedes aegypti*, on the other hand, feed exclusively on humans and oviposit in human-created containers including tires, flower pots, swimming pools, gutters, water collection tanks, jars, drains, as well as almost any other object that can accumulate water, with the types of containers used for oviposition varying according to season. *Aedes aegypti* primarily feed during the day and will often seek a blood meal from multiple human hosts (49,50). Upon combining oviposition and feeding preferences, *Ae. aegypti* behaviors put it in close and constant contact with human hosts, thus facilitating viral transmission.

Certain biological characteristics of the mosquitos' life cycle have made it particularly difficult to eradicate in modern times. For example, it is difficult to eliminate the juvenile stages of the mosquito since *Ae. aegypti* eggs are resistant to environmental changes, such as desiccation, for up to one year. The eggs hatch when a certain moisture threshold is achieved, such as after water-accumulating events such as rain (51). The larvae molt four times before becoming pupae, a stage that can last from one day to a few weeks depending on environmental conditions. Adult *Ae. aegypti* usually live an average of 11 days in urban environments, and females will lay an average of 63 eggs at each oviposition (51). Also, Adult female mosquitoes have been shown to be more active upon infection with dengue virus, further contributing to the spread of the infection (52). Finally, it is of interest to note that both *Ae. aegypti* and *Ae.albopictus* are able to successfully oviposit in brackish waters as well, further increasing the potential for greater vector abundance and, consequently, virus transmission (53).

Difficulty in reducing dengue incidence and mortality from dengue stems from the general inability to control and/or eliminate mosquito vectors. The current strategy for controlling the disease involves disrupting vector ecology, mostly by overturning containers that serve as water-filled oviposition sites, pouring insecticide into these oviposition sites, and peridomestic insecticide spraying (54,55). This has produced limited success, but has nonetheless helped mitigate the disease’s potential impact. A mathematical modeling study has suggested that had Singapore not employed such a control policy in the 2004-2005 dengue epidemic there, at least a 10-fold increase in incident cases could have occurred (54). Other "ecological" prophylaxis options may include the release of sterilized male mosquitoes, use of genetically modified vectors, and/or genetically modified endosymbionts (56–58).

Dengue fever is characterized by a large spectrum of different clinical manifestations of varying intensities. It has been well documented that three-quarters of infections produce little or no recognizable symptoms (59,60). Furthermore, many of the clinical manifestations of dengue closely resemble those of other febrile illnesses, most likely leading to underdiagnoses of true disease incidence worldwide (17,55,61). Classical symptoms of dengue include: fevers, myalgia, arthralgia, cephalalgia, weakness, general malaise, vomiting, rashes, exanthema, diarrhea, retro-orbital pains, and/or swelling of glands. There is also a range of covert symptoms diagnosable via laboratory exams, including thrombocytopenia, leukopenia, and increased hematocrit counts. In more severe cases of the disease described below, hemorrhagic-like symptoms may also occur, including: petechiae, bleeding of the gums, epistaxis, menorrhagia, hematuria, and even blood in the stool of those infected. In extreme cases, dengue infection can lead to severe neurological symptoms, shock, and even potentially death (55,62,63).

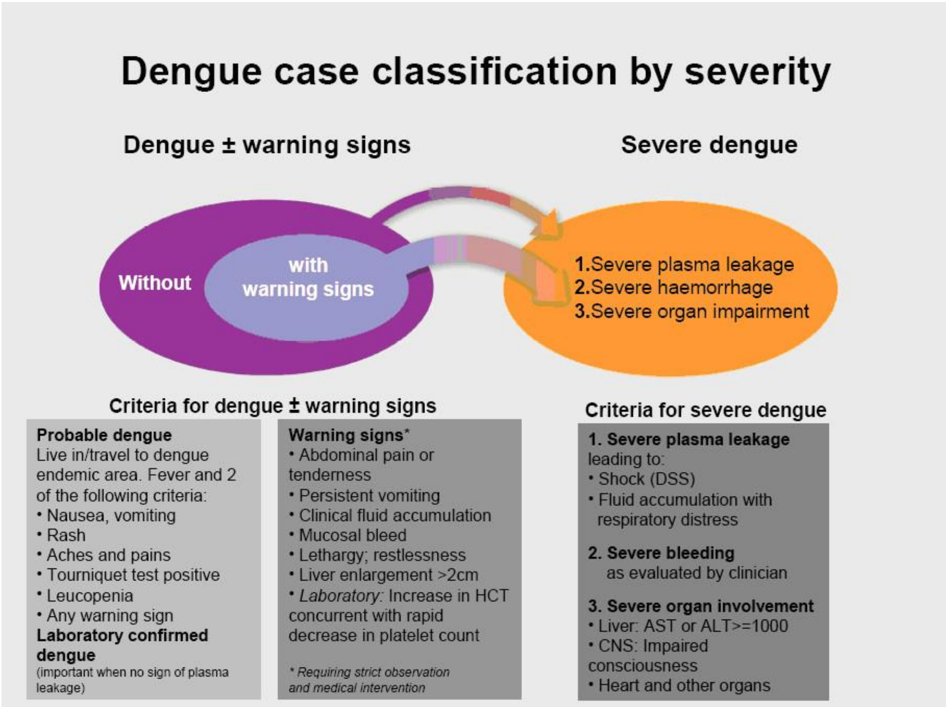

Figure 1.4. Dengue Case Classification by Severity: World Health Organization, 2009 Criteria. ALT = alanine aminotransferase; AST = aspartate aminotransferase; CNS = central nervous system; DSS =dengue shock syndrome; HCT = hematocrit (61).

The diagnosis of dengue is done using medical assessment of patient symptomatology (clinical diagnosis), various laboratory tests, or a combination of the two. The symptoms and severity of dengue infection vary widely, due to factors such as age, sex, viral serotype/ genotype, previous infection, day of infection, among other factors (64–68). In fact, more severe disease has been reported among women than men in several studies, and it lower incidence among women than men has also been reported as well; it has been suggested that women are less likely to be exposed to dengue as they spend more time at home compared to men(24,69,70). Also, It has been shown that children, in general, are less likely to experience clinically relevant manifestations, but are at higher risk of severe outcomes when they actually are symptomatic (19,71–73).

Patients may be considered to have suspected dengue according to varying local criteria, including certain symptoms and a positive "tourniquet test" that assesses capillary fragility, although it is of questionable clinical utility in the diagnostic process (55,74). Given symptom variability and the problems related to the adequacy of the tourniquet test, diagnosing patients solely using clinical manifestations is not considered adequate (55). Laboratory tests may consist of testing for the covert symptoms of dengue described earlier, as well as for markers of infection by dengue virus. These laboratory tests may search for viral proteins (such as the NS-1 protein), patient anti-dengue immunoglobulin (IgA, IgM, and IgG for immediate, short-term and long-term indicators of infection, respectively) via techniques such as Polymerase Chain Reaction (PCR) or of ELISA (Enzyme-Linked ImmunoSorbent Assay), and even viral isolation (55,75–81). It should be noted that the classification of dengue symptoms has recently changed. Previously, the WHO categorized individuals as having classical dengue, dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) in growing order of severity. The WHO revised their classification criteria in 2009 and now symptomatic infections are classified as being either dengue (with or without complications) or severe dengue (Figure 1.4). This reclassification scheme has led to an increased number of patients who are considered to have more severe disease (82).

As previously mentioned, there is no currently licensed vaccine to protect against dengue fever. This is related to the fact that previous infection with one serotype does not provide immunity to other serotypes, instead apparently leading to more severe disease in secondary infection through a process known as Antibody Dependent Enhancement (ADE). It is hypothesized that antibodies developed from an infection with a particular dengue virus strain complex with the new virus strain, allowing the virus to infect more host cells. Mathematical modeling studies have shown that monovalent vaccines could be particularly dangerous in areas with circulation of more than one serotype, as the antibodies developed from the vaccine could interact with the circulating serotypes, leading to more severe infections (83). The same type of problem could arise with a polyvalent vaccine, as antibodies formed against one or more strains in the vaccine could enhance the infectivity of the other strains in the vaccine or the environment. Currently, there are over ten dengue virus vaccine candidates in various trial phases. One of the most promising vaccine candidates, Sanofi Pasteur‘s ChimeriVax, uses a chimerical virus to achieve protection. Using a Yellow Fever 17D vaccine virus as a backbone, the constructed dengue vaccine virus has been modified to have express pre-membrane and envelope proteins of all four dengue serotypes. Overall vaccine safety, as well as elevated short- and long-term seroconversion rates, were seen in Phase I and II trials (84–91). Other vaccine candidates include Inviragen's attenuated Primary Dog Kidney Vaccine, the National Institutes of Allergy and Infectious Diseases (NIAID) vaccine, the Instituto Butantã's live attenuated tetravalent vaccine, and Merck's recombinant subunit vaccine (92–94). A study conducted in Indonesia reported that 94% of parents would vaccinate their children against dengue if such a vaccine were available (95).

Given the lack of any commercially available vaccine-base prophylaxis, and the limited impact of environmental-level prophylaxis, clinical care has become the primary focus of dengue control. In most cases, rehydration therapy, rest, and use of acetaminophen to control febrile events and pains are recommended. Most other nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, are not recommended as they may lead to increased hemorrhaging. In more severe cases, clinical interventions may include procedures to counteract effects of shock, namely through the use of intravenous administration of colloids, crystalloids, or even blood transfusions when necessary(55,61).

The wide range of mosquito vectors, the potentially severe clinical outcomes associated with infection, and lack of prophylaxis converge to produce a large burden cause by dengue worldwide. A study by Halstead et al., found that the mean cost per case for clinically relevant dengue was US$ 514 for those cases that only required ambulatory care, and $1,304 per case that required hospitalization using data on eight countries in Asia and the Americas. It has also been estimated that clinical dengue costs greater than $1.9 billion per year in the Americas, suggesting that dengue burden actually exceeded those from other viral diseases such as human papilloma virus (HPV) or rotavirus (96,97). Researchers have also found that dengue is responsible for a loss of anywhere from 9-65.8 Disability Adjusted Life-Years (DALYs) per 100,000 inhabitants in dengue-endemic countries (98–102). It should be noted that the literature on the social and economic costs of dengue is fairly sparse, including for endemic countries such as Brazil (103,104).

1.3. Dengue in Brazil.

It can be argued that no country has a greater social and economic burden stemming from the impact of dengue than Brazil. Brazil has approximately 190 million inhabitants, and is undergoing transition from an underdeveloped to developed nation (105).Approximately 87% of the population is urban (105) and *Ae. aegypti* is found throughout most of the country, putting most of the nation's population at risk of infection from one or more of the circulating strains of dengue (106). Between 2003 and 2012, over 5,000,000 cases of dengue were reported to the Brazilian national surveillance system (Table 1.1). Considerable variation was seen between then ten years, with the largest amount of reported cases in 2009 and 2010. As previously mentioned, dengue follows cyclical patterns, therefore interruptions in cyclical patterns may indicate shifts in environmental factors associated vector presence, host susceptibility, and even expansion of viral diversity and range(19,25,27,28,30).

Table 1.1. Cases of Reported Dengue in Brazil.

Year	**2003**	**2004**	**2005**	**2006**	**2007**
Cases	341,902	203,789	346,550	559,954	585,769
Year	**2008**	**2009**	**2010**	**2011**	**2012**
Cases	591,254	1,011,647	764,032	565,510	203,789

Source: (107).

Up to 560 DALYs per million inhabitants are lost to dengue each year in certain areas of the Brazil (99), resulting in the national healthcare system dedicating over US$1 billion annually towards dengue control(108). Brazil has a well-developed and reasonably well-maintained national public health-care system—the SUS: *Sistema Único da Saude*. According to the Brazilian constitution that was drafted in 1988, all Brazilian citizens have access to the SUS with absolutely no utilization costs. The SUS is responsible for patient care as well as for preventive public health measures(109). Despite having a well-planned and relatively effective health-care system, Brazil has not been able to mitigate the incidence of dengue, as evidenced by outbreaks that are occurring in major urban centers throughout the country (110–114).

1.4. Dengue in Ribeirão Preto.

Ribeirão Preto is a city of approximately 650,000 inhabitants located in the northern region of São Paulo State, Brazil, (47°W, 21°S) occupying an area larger than 650,000 km^2 (115). A wealthy city by Brazilian standards, Ribeirão Preto has a Human Development Index (HDI) of 0.800 and a robust economy based primarily on agriculture and related activities (115,116). Despite its strong economy, the city has endured several dengue outbreaks since 1990 (114), with all four dengue serotypes known to have circulated in the city(117). Table 1.2 shows the number of reported cases of dengue in the city from 2003 to 2012(118). Similarly to the numbers reported in table 1.1 for Brazil as a whole, wide variation in reported cases were seen in Ribeirão Preto. Some of this variation can be explained by shifts in viral serotypes throughout the years(117), which is consistent with the cyclical nature of the disease (19,23,26–31). However, it is not known what role these other factors that contribute to this cyclical nature have played in the history of dengue in Ribeirão Preto.

Table 1.2. Cases of Reported Dengue in Ribeirão Preto.

Year	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Cases	2,864	988	2,611	12,186	7,363	6,369	4,484	34,986	46,707	3,903

Source:(118).

These data come from a public health surveillance database—known as *SINAN*— managed by the Municipal Secretariat of Health (Secretaria da Saúde de Ribeirão Preto) as mandated per the Brazilian Ministry of Health (Ministério da Saúde). Patients who were deemed to have a suspected dengue infection as per the opinion of a qualified healthcare professional had information collected according to a standardized dengue notification form—*Ficha de Notificação de Dengue*—and uploaded onto the previously described digital database. These data were used in part of the analyses presented in this dissertation.

Much dengue research has been conducted on dengue in Ribeirão Preto, however most of the work has focused on clinical and microbiological aspects of the disease, with only three recent known studies directly assessing epidemiological characteristics. Even then, only one known study directly examined the characteristics of the population affected by dengue: Hino et al (2003) looked at 10 years of routine surveillance data from 1994-2003 and characterized those years in terms of overall number of cases, cases by sex, and annual incidence rates (119). Although this study was important in furthering the understanding of the impact of dengue in Ribeirão Preto, several factors shown to potentially be important in other dengue studies such as education, race, and age were left unassessed(24,120–123). As previously, considerable variations occurred in the number of reported dengue cases. This included several large outbreaks, particularly in the later years, which may be due in part due to introduction and re-introduction of the different viral serotypes(118). Accordingly, it was determined that further analyses and deeper detailing of the dengue outbreaks were required.

1.5. Relationship between Weather and Dengue.

The etiology of dengue is complex, with many aspects influencing the overall numbers of dengue infections. Among such factors are those that are intrinsically linked to the disease vector, affecting vector oviposition patterns, growth and development, and activity; these, in turn, will affect vector ubiquity and behavior, thus directly affecting viral transmission and disease incidence. Weather has been implicated in impacting mosquito ubiquity and dengue disease in several ways. First, precipitation has been implicated as a determinant of dengue (124–128), which is likely related how water abundance and/or humidity are necessary for vector development (129,130). Furthermore, temperature has been found to be associated with increased dengue(131,132). Studies have shown that higher temperatures lead to the shortening of the extrinsic incubation of the dengue virus, which may lead explain this phenomenon (19,133–135). Several studies aimed at assessing the impact of weather on dengue have been conducted in Brazil, however no such study has ever been conducted in Ribeirão Preto. Accordingly, it was determined that it such analyses was needed for the city of Ribeirão Preto to further the understanding of the local determinants of dengue.

1.6. Knowledge, Attitudes, and Practices and Dengue.

Dengue transmission is intrinsically linked to vector ubiquity and vector ubiquity, in turn, is influenced by creation of oviposition sites, which are often driven by human behaviors (136–139). Consequently, it is important to understand host factors that drive creation of these oviposition sites, namely people's knowledge, attitudes, and practices (KAPs). Several such studies have been conducted in Brazil and elsewhere, exhibiting a wide range of sampling strategies and survey methods (140–142) (143–146). Findings have included disparate levels of Knowledge, Attitudes, and Practices in culturally and geographically-similar areas (144,147). Furthermore, it was found that KAPs levels were greater amongst individuals who previously had dengue fever than among those who did not while, conversely, it was seen that KAPs levels did not vary by socioeconomic levels(141,148–151). However, no such studies have ever been conducted in the city of Ribeirão Preto, where findings from such a study could potentially provide helpful insight to the Municipal Secretariat of Health. Accordingly, a large KAPs study was conducted in Ribeirão Preto with assistance of employees of the Municipal Secretariat of Health.

1.7. Purpose of Dissertation.

The surveillance data show that the burden of dengue in Ribeirão Preto has been widespread and elevated over many years. As a result, the city spends considerable resources on dengue prevention across several different public health agencies. Accordingly, analyses were developed to assess various aspects of recent dengue outbreaks in the city of Ribeirão Preto between the years of 2003 and 2012, with the purpose of better understanding the epidemiological, social and ecological drivers underlying such high and widespread incidence, thereby hopefully aiding the Municipal Secretariat of Health in its mission to combat this disease. As can be seen from Figure 1.4, various components of transmission, infection and disease must be considered when assessing patterns, and eventually developing approaches to control and prevention.

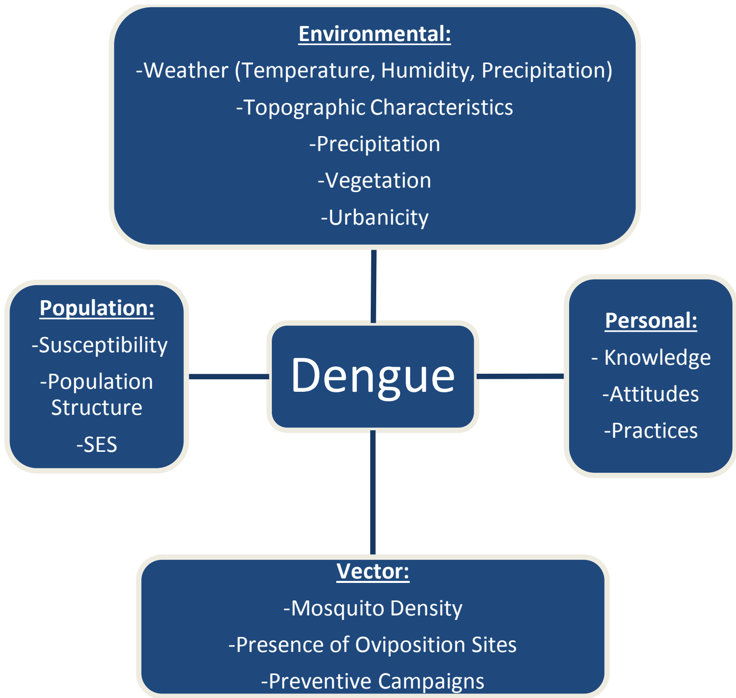

Figure 1.5. Theoretical Framework of Risk Factors for Dengue.
Adapted from:(152–154).

A variety of kinds and sources of data were used to assess these risk factors—including population information through services such as censuses, disease surveillance data, mosquito abundance data (e.g. Breteaux indices), individual-based data (surveys, focus groups, etc), as well as meteorological & geographical information. In using such diverse data, analyses in this dissertation aim to better understand aspects of this multifaceted problem, and eventually to provide useful insights for the Municipal Secretariat of Health preventive efforts. Three main data sources were used - the dengue surveillance database, local climatological data, and data from our own a Knowledge, Attitudes, and Practices survey. Unfortunately, other databases of interest were not available for this project, thus limiting analysis involving geographic and vector-related risk factors. This project consisted of three primary Aims, each focusing on a different component of epidemiological interest:

Aim 1. *Characterize and analyze the basic dengue disease patterns in the city of Ribeirão Preto*: Using 10 years of surveillance data from the SINAN database, we assessed epidemiological patterns of dengue, with the purpose of understanding the determinants of risk to the population that has been and will be affected by dengue (Chapter 2).

Aim 2: *Evaluate the potential relationships between various climatological variables and dengue outbreaks in Ribeirão Preto*: By combining the SINAN dataset with climatological data from the same period (2003-2012) available through CIIAGRO (*Centro Integrado de Informações Agrometeorológicas*—Integrated Agro-Meterological Information Center of the State Government of São Paulo), we analyzed the relationships between various climatological variables and observed temporal patterns of dengue in the city (Chapter 3).

Aim 3: *Assess the Knowledge, Attitudes, and Practices (KAP) of the residents of Ribeirão Preto to better understand personal risk factors and prevention in relation to the patterns of disease*. We conducted a large KAP survey in 2012 in collaboration with the Municipal Secretary of Health to assess personal behaviors and understanding of city residents associated with dengue, which ultimately may help improve health-related communication and dengue prevention campaigns that target inhabitants of Ribeirão Preto (Chapter 4).

In summary, findings from Chapter 2 will be used to better understand the population structure and potential population-level risk factors for dengue, providing in depth analyses of the disease that has been lacking for recent years. Chapter 3 will provide the first analyses of potential relationships between weather-related risk factors and dengue, while chapter 4 will address the absence of previous assessments of personal-level risk factors such as disease knowledge, attitudes, and practices in Ribeirão Preto. In the final section of this dissertation (Chapter 5), the findings across the three Aims are interpreted and synthesized in order to develop a more comprehensive understanding of what factors increase dengue risk. By addressing the above mentioned shortages in the current dengue knowledge for Ribeirão Preto, I hope to pave the way for future dengue studies, as well as to provide findings that can be used by the Municipal Secretariat of Health in its future dengue prevention efforts.

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Chapter II

Dengue in Ribeirão Preto, 2003-2012: Understanding Population Structure and Dengue Susceptibility.

2.1. Abstract.

Dengue fever is the most important arboviral disease of modernity. Dengue virus, the pathogen responsible for the disease, is transmitted by the bite of an infected female *Aedes* mosquito. There are four serotypically distinct viruses and infection by any one only provides temporary immunity to the other serotypes, thus allowing for multiple infections. Factors like age, gender, viral serotype/ genotype, and previous infection affect potential disease severity. It has been shown that the majority of infections actually produce little or no symptoms, leading to under-diagnosis of the true infection levels. Accordingly, it is imperative to understand population structure and serotype susceptibility. As such, we conducted a study in Ribeirão Preto, Brazil, assessing such factors between 2003-2012 using data from routine dengue surveillance.

Data from this study came from routine dengue surveillance in Ribeirão Preto from January 1st, 2003 through December 31st, 2012, and from the 2010 Brazilian National Census. All reported cases, confirmed through either laboratory-based serological testing or clinical-epidemiological criteria, were included for analyses which included assessing incidence, characteristics of cases compared to city's population, assessing time taken between onset of symptoms and clinical visits, as well as estimating number of susceptibles for each serotype.

Incidence rates varied from 0.1 cases per 10,000 inhabitants in 2004 to 491 cases per 10,000 inhabitants in 2010. Overall, there were little-to-no differences in incidence by gender. Mean age of infection did not show an overall pattern, however it was seen that incidence rates were usually higher among adults than children. Dengue incidence was varied slightly by Census racial categories and overall inversely associated with education. A multivariate logistic model showed that sociodemographic differences exist between epidemic and non-epidemic years, particularly in regards to

race and education. We also saw that nearly 80% of patients visited medical centers within 5 days of the onset of infection. Finally, it was estimated that nearly 1/3rd of city inhabitants may have been exposed to dengue.

This study provides evidence of a high incidence of infection of dengue among people in the city of Ribeirão Preto and that this is the case among various aspects of the population. Most cases were among working-age adults, meaning dengue may hamper overall family income and the Ribeirão Preto's economy. More studies are required to better assess differences in racial/ethnic differences in dengue. Given the amount of potential infections during this period, it was seen that a large number of the population of Ribeirão Preto may have been primed for a more severe, secondary dengue infection in the future which needs to be accounted for by the local healthcare system.

2.2. Introduction.

Dengue fever is arguably the most important arboviral disease of modernity. The Pan-American Health Organization (PAHO) reports that in 2013 alone there were over 2.3 million cases of dengue in the Americas, with approximately 1.5 million of those cases occurring in Brazil alone (1). Dengue virus, the pathogen responsible for dengue disease, is transmitted by the bite of an infected female *Aedes* mosquito, mostly by *Ae. aegypti* in the Americas. There are four serotypically distinct viruses (DENV I-IV) in the primary/urban cycle of transmission, and infection by any one virus only provides fleeting immunity to the other serotypes, thus allowing for multiple infections (2–4).

Disease associated with dengue virus is generally characterized by a multitude of symptoms including: fevers, myalgia, arthralgia, cephalalgia, weakness, general malaise, vomiting, rashes, exanthema, diarrhea, retro-orbital pains, and hemorrhages. In the most severe of cases, infection by dengue virus may even lead to shock and, potentially, death (5,6). Severity of disease can also be affected by multiple individual factors such as age, viral serotype/ genotype, previous infection, time since infection, and possibly race/ethnicity (7–14). Finally, it has actually been documented that more than 75% of infections produce little or no recognizable symptoms (15,16).

Given the differences in severity in disease based on personal factors, several studies have been conducted to assess population structure and its effects on dengue outcomes. For example, it has been seen that the proportion of individuals affected may actually not vary by race or socioeconomic studies, as has been seen in other studies conducted in Latin America (17–20). Also, age of individual at the time of infection appears to be decreasing in dengue endemic areas, suggesting that older-age individuals have already been exposed to the circulating viruses in the region (21). Furthermore, it has also been seen that men may be disproportionately affected by dengue than women (19,22,23). As sociodemographic variables may be associated with dengue outbreaks, it is also important to understand exactly how these factors are related to disease in the interest of public health (24).

Another factor associated with disease severity is day of infection; that is, the amount of time between viral infection and the particular day of manifested disease affects clinical aspects of the disease. Accordingly, the ability for the different serological tests to properly diagnose dengue infections is affected by the time of symptom onset (5,6) and it can be argued that inhabitants of historically endemic cities may recognize potential infection and seek medical care quickly. Thus, infection patterns in relation to the population structure of both the at-risk individuals in a dengue endemic setting, as well as those who become infectious is important for understanding transmission dynamics and, thus, preparing public health prevention and response. Accordingly, in-depth analysis of a multi-year, extensive, surveillance database containing all reported dengue cases in the dengue-endemic city of Ribeirão Preto, Brazil, was conducted to assess the characteristics of those affected by dengue between 2003-2010.

2.3. Methods.

In this study, we assessed the demographic patterns of dengue cases in the city of Ribeirão Preto, Brazil (21.2° S, 47.8° W), a metropolis in the southeastern part of the country that has suffered from severe outbreaks of dengue virus transmission occurring several times during the past few decades.

2.3.1. Dengue Surveillance Data.

Surveillance data project came from a public health surveillance database, known as SINAN, managed by the Municipal Secretariat of Health of Ribeirão Preto (*Secretaria Municipal da Saúde de Ribeirão Preto*) as mandated per the Brazilian Ministry of Health (*Ministério da Saúde*). Data included all reported cases of dengue in Ribeirão Preto between January 1st, 2003 and December 31st, 2012, which were digitized and made available by the Secretariat. Patients suspected of having dengue, as per the opinion of a qualified healthcare professional, had information collected on them according to Municipal Secretariat of Health's standard guidelines at the aforementioned public health-centers, some with referrals from private-sector medical facilities. Information was collected according to a standardized dengue notification form (*Ficha de Notificação de Dengue*) and uploaded onto a digital database belonging to the local public health system. Information collected included basic bio-sociodemographic information, clinical manifestations (both common and more severe/hemorrhagic symptoms), hospital visit information, previous laboratory-confirmed dengue infection, laboratory test results, patient outcome (recovery/ death), as well as any other possibly pertinent information. The notification form content changed during the period of this study, and, consequently, information on most symptoms was no longer available after 2006. Accordingly, the variables used for these analyses consisted only of those available for the entire study period.

Only dengue-positive patients (patients who were deemed to have dengue infection, either through laboratory-based tests (ELISA, PCR, NS-1 viral protein detection, Viral Isolation), or through clinical-epidemiological criteria during large outbreaks when the laboratory was unable to process all samples) were selected for this study. Clinical-epidemiological diagnosis involved a qualified physician's assessment of dengue-like symptoms (including absence of certain symptoms that would indicate potential non-dengue infections such as sore throats), as well as criteria such as living in an area with current high transmission in the midst of a large outbreak. Identifiable information was removed from the database prior to analysis.

2.3.2. Population Data.

Population data from the 2000 and 2010 national censuses conducted by the Brazilian Institute of Geography and Statistics (*IBGE*) for Ribeirão Preto were used to assess the population structure of cases and to estimate the age-specific incidence (25). Population numbers for 2000 and 2010, as well as population estimates from IBGE for the years of 2011-2013, were combined to calculate the estimated population for each year in the study. Findings are reported using the Brazilian Census' definitions of race, with only one possible classification possible per individual:

- *Amarelo*—"Yellow"; this is the IBGE's terminology to refer to Brazilians of Asian descent.
- *Branco*—"White"; this is the IBGE's terminology to refer to Brazilians primarily of European descent.
- *Pardo*—"Brown"; this is the IBGE's terminology to refer to Brazilians of mixed descent, but who are not classified as either *Preto* or another IBGE racial group.
- *Preto*—"Black"; this is the IBGE's terminology to refer to Brazilians of primarily African descent.
- *Indígena*—"Indigenous"; this IBGE's terminology to refer to Brazilians of primarily Native Brazilian descent.

2.3.3. Laboratory Serotype Data.

Laboratory-based, viral serotype data from Ribeirão Preto assessed using viral isolation techniques or RT-PCR techniques (26) for the study period (with the exception of 2004, when there were very few notified cases of dengue) were used. Table 2.1 shows the overall distribution of the different serotypes of dengue detected among samples of dengue-positive patients in Ribeirão Preto.

Table 2.1: Number of Dengue Serotypes by Year from Patient Samples Tested at the *Instituto Adolfo Lutz* Laboratory. The number in parenthesis represents the percentage of samples that contained a particular serotype in a particular year. Source (26).

	DEN1	DEN2	DEN3	DEN4	Total
2003	0	0	5 (100%)	0	5
2004	0	0	0	0	0
2005	0	0	5 (100%)	0	5
2006	0	0	9 (100%)	0	9
2007	1 (3.1%)	0	31 (96.9%)	0	32
2008	1 (33.3%)	1 (33.3%)	1 (33.3%)	0	3
2009	22 (12.8%)	9 (5.2%)	141(82.0%)	0	172
2010	60 (76.9%)	8 (10.3%)	10 (12.8%)	0	78
2011	215 (100%)	0	0	0	215
2012	21 (39.6%)	1 (1.9%)	1 (1.9%)	30 (56.6%)	53

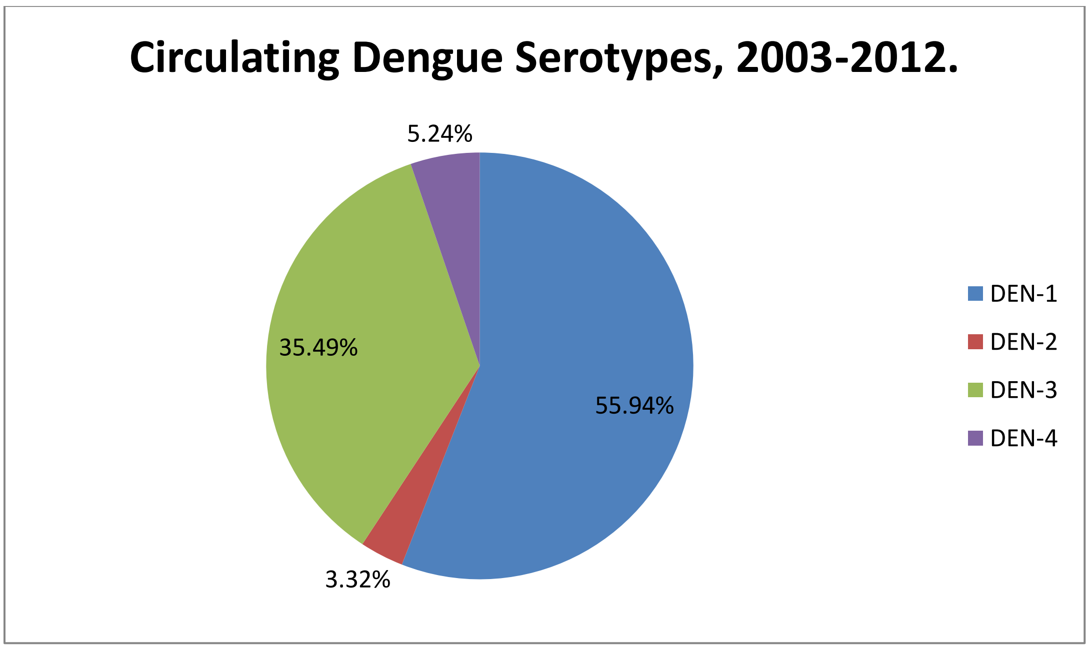

Figure 2.1 Percentage of the Four Circulating Serotypes of Dengue Virus Detected as Part of Routine Surveillance in Ribeirão Preto, Brazil, during the Period of 2003-2012. Source: (26).

2.3.4. Analytical Methods

Data from the surveillance database were cleaned and dengue-positive cases were selected for analysis. Variables assessed included: age, gender, race/ethnicity, day of reporting, day of first symptoms, date of birth, schooling level, diagnostic criteria (clinical-epidemiological versus laboratory-based testing), whether patients were hospitalized, and final outcome (e.g. recovery or death). Age was calculated by subtracting the patient's date of birth from the day of reporting. This was deemed necessary as the age variable that was already present in the dataset was known to have large inaccuracies stemming from clinical professionals/ data-entry individuals' errors and/or misreporting. Analyses were conducted using SAS statistical software, version 9.3(27). Tests conducted included binomial tests for proportions, chi-square tests for categorical variables, T-tests for differences in means, and multiple logistic regression. The logistic regression was employed to assess how sociodemographic characteristics varied between epidemic and non-epidemic years, using these sociodemographic characteristics as dependent variables. For the purposes of the analyses, epidemic years are defined as the years with over 100 reported cases of dengue per 10,000 inhabitants in Ribeirão Preto, which coincides with the highest quartile of cases/year All sociodemographic variables used in other analyses were included; age was included as a categorical variable (pediatric/ adult) in keeping with the analyses conducted in 2.4.3.

2.3.5. Ethical Approval.

This project used secondary analysis of government surveillance data that received ethical approval from the Municipal Secretariat of Health of Ribeirão Preto and had no individual identifying information. Data used in this study involved de-identified, surveillance data approved as part of a larger project approved by the Ethics Committee at the Medical School of Ribeirão Preto of the University of São Paulo; accordingly further Institutional Review Board approval was not needed.

2.4. Results.

2.4.1. Annual Incidence.

To estimate the total population for each year, we assumed that the population growth rate of Ribeirão Preto grew at an exponential rate. Population estimates for 2000, 2010, and the 2011-2013 were used to interpolate the city's population for 2003-2009. Using these annual population estimates and reported disease count data, overall annual incidence rate of dengue per 10,000 city residents for each year was estimated (Table 2.2) (28).

Table 2.2 Reported Cases and Estimated Annual Incidence of Dengue per Year in Ribeirão Preto, Brazil, 2003-2012.

Year	**2003**	**2004**	**2005**	**2006**	**2007**
No. of Cases	789	46	616	6,140	2,726
Annual Incidence per 10,000	15.21	0.85	11.39	111.92	47.59
Year	**2008**	**2009**	**2010**	**2011**	**2012**
No. of Cases	1,059	1,641	29,710	23,837	289
Annual Incidence per 10,000	18.15	27.62	491.33	389.28	4.66

Source:(28).

Table 2.2 shows that there was variation in the magnitude of disease outbreaks during the study period, varying from a low incidence rate of <1 case of dengue per 10,000 persons in 2004, to a high of nearly 500 cases per 10,000 in 2010.

2.4.2. Incidence by Gender.

Dengue incidence was assessed by a variety of sociodemographic factors present in the SINAN database. First, we characterized reported dengue cases by gender. A binomial test was conducted to compare the gender distribution among cases over the 10-year to that of the 2010 census estimate, which showed that 52.01% of the population of Ribeirão Preto was female. It was seen that 53.93% of reported cases were female, differing in a statistically significant way from the census ($p<0.\ 01$), suggesting that women affected in greater proportions than what they represent in the city of Ribeirão Preto, albeit only slightly given the very large sample size. Gender-specific incidence rates per 10,000 for all years based on the city's yearly population estimates were calculated as seen Table 2.3. Incidence rates were calculated as the number of reported cases by gender divided by the total estimated population by gender for each year.

Table 2.3. Gender Specific Incidence Rates & Female-Male Incidence Rate Ratio per 10,000 Inhabitants, 2003-2012.

Year	**2003**	**2004**	**2005**	**2006**	**2007**
Female	15.49	1.03	12.01	118.35	47.90
Male	14.91	0.65	10.72	104.96	47.25
IRR	1.04	1.58	1.12	1.13	1.01
IRR 95% CI	1.01, 1.07	1.05, 2.34	1.06, 1.18	1.11, 1.15	1.00, 1.02
Year	**2008**	**2009**	**2010**	**2011**	**2012**
Female	19.21	28.48	518.45	393.40	5.12
Male	17.00	26.69	461.94	384.81	4.17
IRR	1.13	1.07	1.12	1.02	1.23
IRR 95% CI	1.08, 1.18	1.04, 1.09	1.11, 1.13	1.02, 1.03	1.10, 1.36

In all years, women had higher dengue incidence rates than men. Incidence rate ratios varied from 1.01 in 2007 to 1.58 in 2004. In many years the 95% confidence intervals approximated or even included the null value (1.0). The year of 2004 had the widest range, reflecting the small sample size of less than 50 cases per year. This, in turn, may have affected the gender-distribution and, thus, led to the single highest incidence rate ratio in the study period. As such, the incidence rate ratios indicate that women and men are affected almost equally by dengue in Ribeirão Preto, with women being marginally affected more than men, which is consistent with the previously mentioned binomial test. As such, we conclude that dengue patterns do not differ in a relevant way in Ribeirão Preto during the study period.

2.4.3. Incidence by Age.

Mean age of infection by year was also assessed, as were the annual incidence rates among children versus adults. Individuals whose age was either less than one year or greater than 95 years were excluded, as these represented a small proportion of the population and likely would have been calculated using data entered incorrectly into the surveillance dataset. Age was assessed, as shifting towards older mean ages would indicate arrival of new serotypes as the older population would likely be susceptible to the new serotype. The average age and 95% confidence interval of reported infection during 2003 through 2012 was calculated (Figure 2.2).

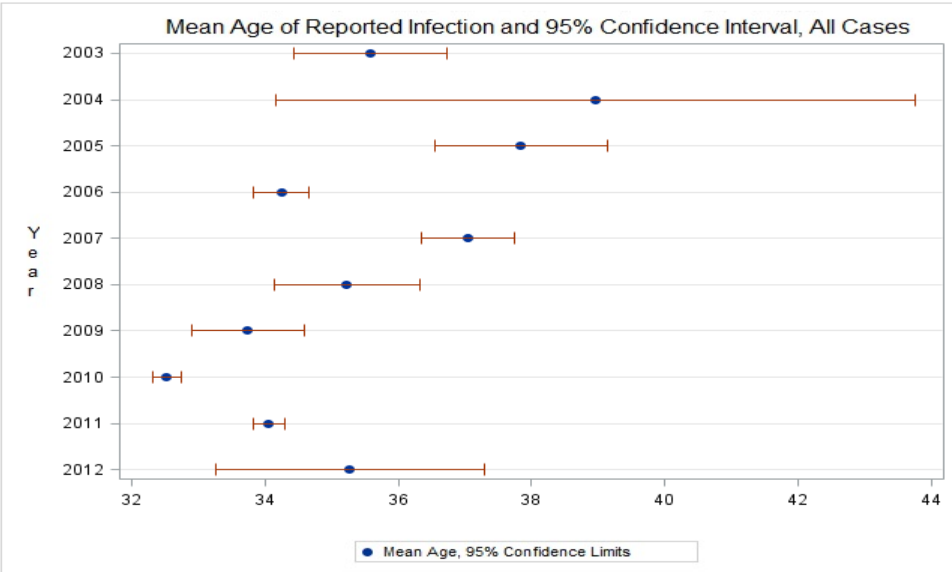

Figure 2.2. Age (Mean and 95% CI) of Dengue Cases by Year among Residents of Ribeirão Preto, 2003-2012.

Figure 2.2 shows that there is inter-annual variation in the age of infection, but no systematic pattern. Several means and confidence intervals overlap, while mean age generally trended toward decreasing between 2003 and 2010 and then increased again in 2011.

Mean age of infection and separate incidence rates for adults (age 20 years or older) and for pediatric cases (age 19 years or less) were analyzed to more easily compare our findings with census categories. Figures 2.3 and 2.4 present the mean age of infection among the two groups and Table 2.5 shows the dengue incidence rates by year for adults and children, as well as the incidence rate ratio comparing adults and children.

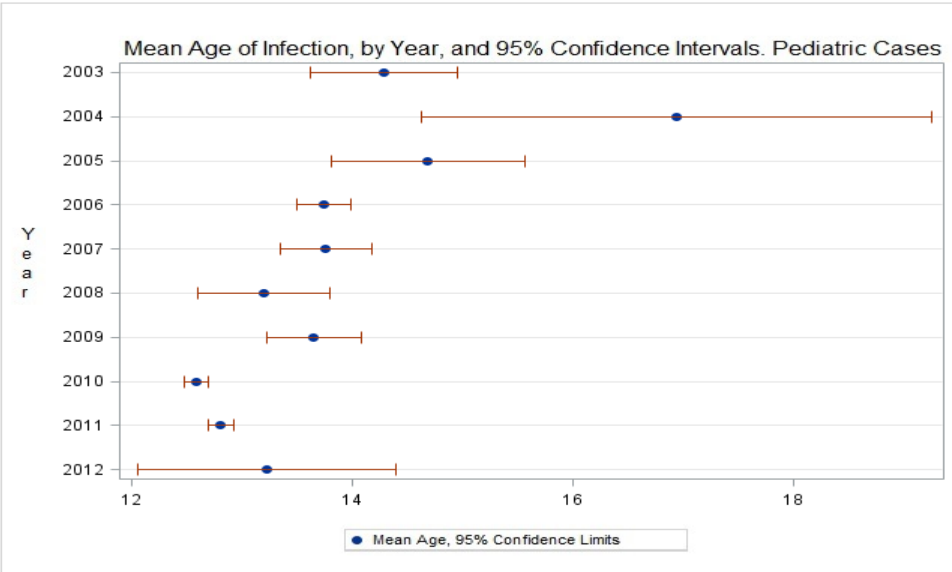

Figure 2.3. Age (Mean and 95% CI) of Dengue Pediatric Cases, by Year, for Children 19 Years Old or Younger in Ribeirão Preto, 2003-2012.

Among pediatric cases (Figure 2.3), there is also a trend toward generally decreasing age of dengue cases from 2003 to 2010, with the mean age once again increasing in 2011 and 2012.

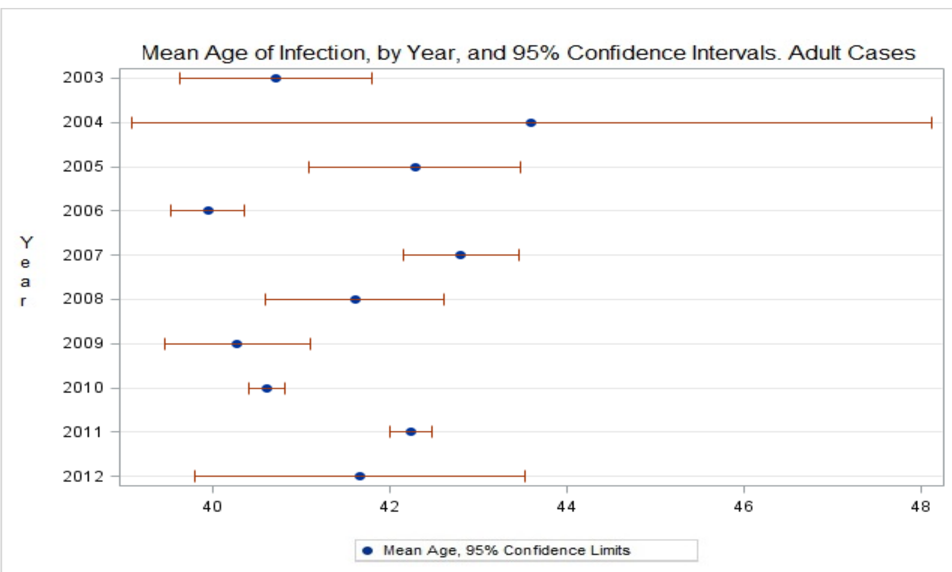

Figure 2.4. Age (Mean and 95% CI) of Dengue Cases, by Year, for Adults 20 years old or Older in Ribeirão Preto, 2003-2012.

Results in Figure 2.4 indicate that there was no simple pattern of change in the age distribution of dengue cases among adults (20 years or older) during the study period. Mean age fluctuated on an almost yearly basis, with a visible decreasing trend between 2007-2009, followed by increases again in 2010, and 2011.

Table 2.4. Incidence Rates per 10,000 for Adult and Pediatric Cases in Ribeirão Preto, 2003- 2012.

Year	**2003**	**2004**	**2005**	**2006**	**2007**
Adult IR	16.39	0.96	12.84	114.84	49.40
Pediatric IR	12.07	0.54	7.51	103.27	42.75
IRR	1.36	1.78	1.71	1.11	1.16
Year	**2008**	**2009**	**2010**	**2011**	**2012**
Adult IR	17.76	26.85	461.14	377.67	4.90
Pediatric IR	19.19	29.66	571.98	420.28	4.03
IRR	0.93	0.91	0.81	0.90	1.22

Incidence Rate per 10,000.

Results in Table 2.4 demonstrate that the incidence rate ratio (IRR) of Adult-to-Pediatric cases was >1 for most years, with the exception of 2008-2011. An overall decrease was seen in those years, however the IRR went up again above 1.0 in 2012, the same year that DEN-4 was first detected in Ribeirão Preto.

2.4.5. Findings by Race and Education.

Table 2.5 presents Distribution of dengue cases by the 2010 Brazilian Census defined racial/ethnic categories as well as educational levels. Distribution of dengue among different educational levels was also evaluated. Education level may be considered a proxy for socioeconomic status, which could be useful since no financial/salary measurements were available in the surveillance dataset. Data in the *SINAN* database were originally coded into 10 different categories. Different educational levels were collapsed to make them comparable to how the 2010 *IBGE*

Census educational data were recorded for all individuals aged 10 years or older in Ribeirão Preto. Categorization was conducted as such:

1. **Incomplete/ No Primary Education**: Less than a completed 8th grade education. This also includes those with no education and/or illiterate individuals
2. **Completed Elementary, No/Incomplete Secondary Education**: Completed 8th grade education, but less than a completed high school education.
3. **Completed Secondary Education, No/Incomplete Tertiary Education**: Completed High School Education.
4. **Completed Tertiary Education**: Completed University Education and/or Technical-degree higher than High School Education.
5. **Undetermined-Education not determined**: refused to respond or not entered.
6. **Does not apply**: Educational levels do not apply to these individuals.

Table 2.5. Distribution by Census Racial/Ethnic and Education Categories in SINAN and Census.

	Category	2010 Census (%)	Dengue Cases (%)	Dengue Cases (%)*
Race/ Ethnicity	**"Yellow"**	0.93	0.51	0.63
	"Black"	6.36	7.35	9.13
	"Indigenous"	0.09	0.14	0.18
	"Brown"	22.83	12.92	16.05
	"White"	69.76	59.57	74.01
	Refused/ None	0.02	19.51	0.00
Education	**Incomplete/ No Primary Education**	36.74	21.04	40.31
	Completed Elementary Education, No/ Incomplete Secondary Education	18.29	17.81	34.11
	Completed Secondary Education, No/Incomplete Tertiary Education	28.10	11.38	21.80
	Completed Tertiary Education	16.22	1.97	3.80
	Undetermined	0.64	45.04	0.00
	Did not apply (Only for SINAN)		4.76	0.00

*Among cases reporting race/ethnicity and/ or education levels, accordingly.

As a large portion of individuals either refused to report race or did not have it reported/entered into the *SINAN* system, it is difficult to accurately compare racial distributions in the surveillance data and the census. Chi-square tests were conducted to see if the racial-proportions found among cases differed from the expected racial/ethnic proportions in the 2010 census (tests not shown). This test was conducted in two forms: one including the “refused/ missing” category as well as one without that category (using just the reported racial groups). For both tests, it was seen that the proportions differ from the expect distribution according to the census, both with p-values <0.0001.

Among those who had educational levels assessed, it was seen that dengue is inversely associated with education, affecting those with lower educational levels in higher rates than those with higher education. This was seen both within the surveillance database and when comparing the surveillance database to the census. A large portion of cases in the *SINAN* database did not have educational levels available recorded, therefore inferences from this analysis are limited. However, a much lower proportion of dengue cases were seen among those with the highest level of education when compared to the city’s educational distribution according to the census using a Chi-Square test (p-value <0.0001).

2.4.6. Epidemic x Non-Epidemic Differences.

A logistic multiple regression model was conducted to understand how case demographics varied by epidemic and non-epidemic years. All demographic variables studies in this chapter were included in the model. Odds ratios comparing epidemic to non-epidemic years and their respective 95% Confidence Intervals from the logistic regression are presented in table 2.6, with significant odds ratios presented in Italics.

Table 2.6. Multiple Regression Comparing Sociodemographic Factors of Cases between Epidemic and Non-Epidemic Years.

Variable	**Category**	**Odds Ratio**	**95% CI**
Education[1]	**Primary**	*1.92*	*1.77, 2.08*
	Secondary	*0.28*	*0.25, 0.32*
	Tertiary	*0.42*	*0.36, 0.53*
Race[2]	**Black**	*0.71*	*0.64,0.79*
	Brown	*0.77*	*0.67, 0.87*
	Yellow	1.43	0.96, 2.13
	Indigenous	0.63	0.25, 1.60
Age[3]	**Adult**	*1.82*	*1.66. 1.99*
Gender[4]	**Female**	1.00	0.92, 1.08

References: 1—Less than Primary Education, 2—White, 3—Pediatric (<20), 4—Male. Observations used=25, 502.

Overall, it can be seen that education levels and racial/ethnic categories differed between epidemic and non-epidemic years. In particular, it was seen that the odds of being infected during an epidemic year versus a non-epidemic years was 1.92 comparing those with completed primary education to those with less than primary education, while lower odds were seen comparing highest education levels to less than primary education. It was also seen that lower odds of having dengue during an epidemic versus non-epidemic year was seen in most racial groups when compared to white inhabitants of Ribeirão Preto, with exception of comparing “yellow” and “white” inhabitants. The odds of having dengue in epidemic years compared to non-epidemic years showed an odds ratio of 1.82 comparing adults to children. No statistically significant differences were seen between genders between epidemic and non-epidemic years.

2.4.7. Time to Clinical Visit.

The amount of time between the appearance of first symptoms and health center visits of dengue cases was assessed. This is of clinical importance as it affects which diagnostic dengue laboratory test would be most effective in detecting potential disease. Time between first symptoms and clinical visits was classified as 0-4 days (individually), 5-10 days, and 10-15 days. Cases with more than 15 days between first symptoms and healthcare center visit were considered to be incorrectly input in the system and were therefore removed from the analyses, as were those with missing information. Table 2.13 details the time between the appearance of the first dengue-related symptoms and individual's visits to the clinic.

Table 2.7. Time between First Symptoms and Clinical Visit.

Days	Frequency	Percent
0	6992	10.87
1	16128	25.08
2	11344	17.64
3	9446	14.69
4	6648	10.34
5-10	12378	19.25
10-15	1380	2.15

Missing=2726.

It can be seen that 78.62% of individuals with dengue visit health clinics in the first 5 days of dengue-like symptoms, with 53.59% of cases visiting clinics in the first 3 days of the clinical manifestations of the disease. Further analyses were conducted to see if the amount of time between onset of symptoms and clinical visit varied by age group (broken down by quartile), race, gender, educational levels, and year of outbreak, but differences were only seen by year of infection (not shown). The two lowest mean amounts of days were in the 2010 and 2011 outbreak years, which reported mean 2.60

and 2.40 days respectively, while the highest amount of days seen in 2004 (5.76). The lows in 2010 and 2011 are consistent with those two being the two outbreak years, and the high in 2004 coincides with the lowest year in terms of cases in Ribeirão Preto. Chi-square testing indicated differences in days by years were indeed statistically significant ($p<0.0001$).

2.4.8. Serotype-Specific Estimates.

As previously mentioned, most primary dengue infections do not produce clinically relevant symptoms. In fact, studies have shown that upwards of 75% of infections are asymptomatic, leading to severe underdiagnoses of the disease in surveillance (29,30). Thus, in order to calculate what might be the true number of infections, we modified the SINAN data as follows. First, we assumed that the SINAN database only captured 25% of true infections, so multiplied the reported values by 4. Furthermore, we also assumed that the serological laboratory tests for specific dengue serotypes were both accurate and truly representative of the distribution of infections occurring during each year. Finally, we assumed that all individuals were susceptible to all serotypes at the start of the study and that all individuals added to Ribeirão Preto's estimated population were also susceptible to all four serotypes.

Because no viral serotype data were available in 2004, we assumed that the infections were caused by DEN-3, as it was the only detected circulating serotype in 2003, 2005, and 2006. As a result, new, estimated total infections were calculated for the study period (Table 2.8) and accumulated total of estimated serotype-specific infections over the study period in Figure 2.5.

Table 2.8. Estimated Actual Total Number of Incident Infections by Year of Study.

	DEN1	DEN2	DEN3	DEN4	Total
2003	0	0	2,430	0	2,430
2004	0	0	138	0	138
2005	0	0	1,887	0	1,887
2006	0	0	18,885	0	18,885
2007	256	0	7,922	0	8,178
2008	1,059	1,059	1,059	0	3,177
2009	630	258	4,036	0	4,923
2010	68,562	9,142	11,427	0	89,130
2011	71,511	0	0	0	71,511
2012	344	16	16	491	867
Total	142,360	10,474	47,800	491	201,126

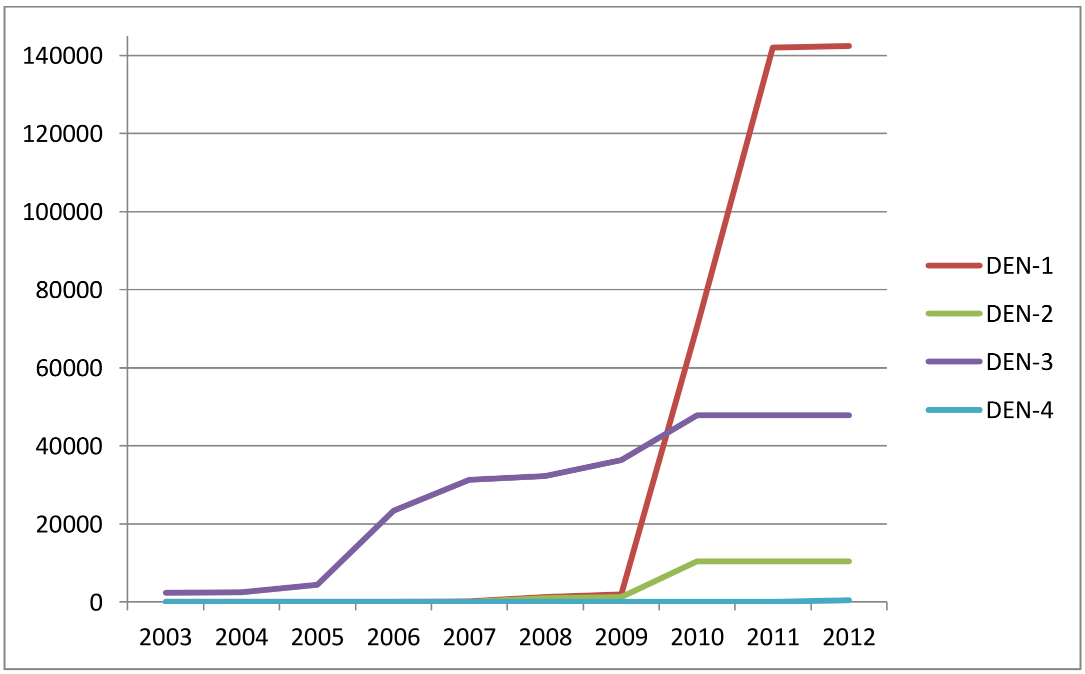

Figure 2.5. Estimated Accumulated Dengue Infections in Ribeirão Preto, 2003-2012.

Based on these estimates, DEN-1 and DEN-3 were the predominant circulating serotypes, and it can be concluded that many individuals in Ribeirão Preto have been exposed to at least one serotype. In fact, if each estimated infection were to be for a separate individual, it would mean that nearly 1/3rd of the population had been exposed to at least one dengue serotype during the study period. This, however, would lead to an over-estimation of infections as individuals affected by one serotype are still-susceptible to infections with other serotypes.

As such, we proceeded to estimate the number of non-primary infections during the study period. To estimate the number of non-primary infections, it was assumed that all severe cases represented secondary infections as more severe disease is often associated with subsequent infections (2–4). This is likely an overestimate of true cases of secondary infections as it is known that primary infections can also lead to severe clinical manifestations. Severe cases of dengue were defined in the dataset as any of the following:

1. Having been classified as having dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) as per the old disease criteria.
2. Having been classified as having severe dengue according to the revised WHO criteria.
3. Having been hospitalized in the course of dengue infection
4. Dying from dengue infection.

Deceased cases must either have died due to dengue or have been directly classified in one of the disease classification criteria as having severe dengue. If the above criteria did not apply, then cases were considered to have been "non-severe disease". Then, the proportion of primary: non-primary infections was applied to the estimates from table 2.8. The number of estimated primary and non-primary dengue infections can be seen in table 2.9, as can the estimate for the dengue infections among the entire city population.

2.9. Estimated Primary x Non-Primary Dengue Infections in SINAN Surveillance Database and the Population of Ribeirão Preto, 2003-2012.

	Primary Infection	**Non-Primary Infection**	**Population Primary Infection**	**Population Non-Primary Infection**
2003	754	56	2,262	168
2004	43	3	129	9
2005	586	43	1,758	129
2006	6,204	91	18,612	273
2007	2,708	18	8,124	54
2008	1,052	7	3,156	21
2009	1,626	15	4,878	45
2010	29,346	364	88,038	1,092
2011	23,283	554	69,849	1,662
2012	274	15	822	45
Total	65,876	1,166	197,628	3,498

It can be seen that approximately 98% of infections in Ribeirão Preto may have been primary infections during the study period. Overall, larger proportions of severe dengue cases occurred during the first three years of the study period, as well as during the last one, than among other years of the study. These years, however, represented years with lower overall numbers of cases. Chi-square analyses showed that the number of severe cases varied among years in a statistically significant way, $p<0.0001$. This resulted in a much higher estimate of severe dengue disease in Ribeirão Preto than previous estimates done by the Municipal Secretariat of Health (28). The above population estimate suggests that 31.9% of inhabitants of Ribeirão Preto have been exposed to at least one viral serotype. As such, the potential for severe manifestations of disease in the near future is increased (2–4).

2.5 Discussion.

This study suggests that there may be a relatively high incidence of infection of dengue among people in the city of Ribeirão Preto and that this is the case among various aspects of the population. Findings suggest no overall differences in gender, especially in the presence of other sociodemographic factors, suggesting that other sociodemographic factors may operate at a stronger level than gender. It was also seen that gender did not differ between epidemic and non-epidemic years, suggesting that other factors may operate at a stronger level than gender in Ribeirão Preto. However, complete data was available for less than 40% of the total cases in the dataset, so these findings must be interpreted with caution. It is thus recommended that preventive campaigns should not be solely tailored for differences in gender, but rather focused on other sociodemographic factors.

These findings also suggested that, overall, the average age of reported dengue infection is not decreasing, perhaps indicating a large, stable susceptible adult population. Results indicated that the majority of cases occurred in adults of working age. This means that economically active individuals lose work time, thereby negatively affecting overall family income and the municipal economy. It has been shown that nearly 560 Disability Adjusted Life Years (DALYs) per million inhabitants are lost to dengue each year in certain areas of Brazil (31) In addition, the country’s national healthcare system spends over US$1 billion per year in dengue control and prevention (32). It is likely that such impacts from dengue are also occurring in Ribeirão Preto, further cementing the need for dengue prevention as a public health priority for the city.

No major differences were seen between race/ethnicity and education in individual analyses, but differences were seen in the multiple logistic regression model. The multiple regression model indicated that differences were seen between racial and educational groups when comparing cases in epidemic years to cases in non-epidemic years. Overall, epidemic years are characterized by lesser odds of disease in non-white inhabitants of Ribeirão Preto, as well as with higher educational levels compared to the less-than-completed primary levels. There were much missing and incomplete data for both race/ethnicity and education. Furthermore, race/ethnicity, when recorded, was often determined by the attending health professional, and not by the dengue patient, suggesting that there may be misclassification of patient-perceived race/ethnicity. As race/ ethnicity are cultural constructs, it is possible that these measurements are actually proxies for deeper-level characteristics arising from historical and social factors that may also be related to dengue risk, as is likely the case for educational levels. Accordingly, it is imperative that complete and more detailed sociodemographic data be collected as part of routine surveillance in Ribeirão Preto if inferences are to be drawn for research and prevention purposes.

It was also seen that, over the entire study period, most reported cases visited clinics in the first few days after the onset of symptoms. Furthermore, it was seen that patients generally visited clinics earlier in outbreak years than non-outbreak years. It is known that the laboratory-based serological tests as the NS-1 test can be used to detect infection earlier than many other commonly used tests such as IgM-Elisa(5,33–37). Accordingly, these findings suggest the Municipal Secretariat of Health continue using the NS-1 test as the primary serological laboratory test.

Finally, we used information on infection sensitivity of the surveillance system to estimate the true number of strain-specific dengue infections per year over the study period. This was likely an underestimate of cases as the surveillance system likely missed a large proportion of dengue cases, most notably among those with higher incomes. Although dengue is a reportable disease, private clinics may not always follow through the process and report their cases to the Municipal Secretariat of Health of Ribeirão Preto. Furthermore, laboratory-based diagnostic tests do not always accurately capture dengue infection status. In fact, it has been shown that the NS-1 viral protein detection test often used in Brazilian exhibited low sensitivity for DEN-4 infections (38). Based on our findings, it can be said that a large population is susceptible to becoming infected with different dengue serotypes than they may have previously been infected with, thus creating the potential for more severe disease outcomes in the near future as secondary infections are associated with more severe disease(39). As a result, there may be more hospitalizations, longer hospital stays, and even an increase in dengue-related deaths. Such an increase in disease severity will, in turn, likely result in greater public health and economic burdens. Accordingly, it is imperative to invest in a more comprehensive dengue surveillance system that is better able to capture the true number of cases, and more detailed personal-level data (as previously suggested based on sociodemographic findings). Such a system will lead to enhanced understanding of current disease patterns and the potential impacts of dengue on people of the city of Ribeirão Preto. The findings in this study are consistent with the model presented in Chapter 1, showing that dengue risk is indeed associated with certain aspects related to population structure such as age and educational level. It was seen, however, that differences by gender were not present. Overall, it can be concluded that various intricacies underlie Ribeirão Preto’s dengue situation as it was seen that incidence varied by several factors and that further, in-depth studies should be conducted to continue assessing these factors.

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Chapter III

Relationship between Dengue Fever Incidence and Weather Variation in Ribeirão Preto, Brazil, 2003-2012.

3.1. Abstract.

Dengue fever is the most important arboviral disease of modernity. Dengue virus, the pathogen responsible for the disease, is transmitted by the bite of an infected female *Aedes* mosquito. A considerable body of research has been published regarding dengue prevalence, incidence, and vector ecology in relation to various climatological and meteorological factors. These studies have shown that multiple aspects of climate or weather affect *Aedes* mosquito life cycles and, consequently, risk of dengue. Although several such studies have been conducted in Brazil, none have ever assessed this aspect in Ribeirão Preto. Accordingly we conducted the first known weather-dengue assessment for the city.

Disease data from this study came from routine dengue surveillance in Ribeirão Preto from January 1st, 2003 through December 31st, 2012, and weekly weather measurements were obtained from the CIIAGRO meteorological database. Basic descriptors for the different weather variables were assessed. Generalized linear models with a negative binomial distribution with several lagged weather variables were assessed as potential predictors for dengue. Further assessment included outbreak/non-outbreak year as well as seasonality.

Weather patterns follow a distinct yearly seasonality in Ribeirão Preto as do dengue cases. Bivariate analyses found that outbreak years were characterized by higher maximum temperature and lower minimum temperatures, while precipitation did not differ between outbreak and non-outbreak years. Results from regression models indicated that increases in mean minimum temperature lagged by 6-weeks and precipitation lagged by 8 weeks were significantly associated with increased cases of dengue. Inclusion of outbreak/ non-outbreak years and seasonality improved model fit. An interaction term between precipitation and minimum temperature was shown to be negatively associated with dengue in this study. Maximum temperature and mean temperature were not as predictive as minimum temperature, suggesting that higher minimum temperature was the strongest driver of dengue among the weather variables assessed in this study Our findings are consistent with other findings the region and internationally. Ultimately, this model, upon validation and adjustment, may serve as a suitable predictive model for dengue in settings where computational and statistically-related limitations are present, as is often the case in dengue-endemic settings.

3.2. Introduction.

Dengue fever is arguably the most important arboviral disease of modernity. The Pan-American Health Organization (PAHO) reports that in 2013 alone there were over 2.3 million cases of dengue in the Americas, with approximately 1.5 million of those cases occurring in Brazil alone(1). Dengue virus, the pathogen responsible dengue disease, is transmitted by the bite of an infected female *Aedes* mosquito, mostly by *Ae. aegypti* in the Americas. There are four serotypically distinct viruses (DENV I-IV) in the primary/urban cycle of transmission, and infection by any one virus only provides fleeting immunity to the other serotypes, thus allowing for multiple infections (2–4). There is currently no commercial vaccine available and clinical management of the disease is mostly restricted to rehydration therapy and the use of acetaminophen for pain and fever reduction. Current prophylactic strategies are essentially limited to disrupting vector ecology, mostly by overturning containers that serve as water-filled oviposition sites, pouring insecticide into these oviposition sites, and peridomestic insecticide spraying. These preventive measures have had limited success in preventing the disease (5,6). Given the overall lack of effective preventive strategies, it could be useful to find methods that assist in disease prediction for optimal preparation of relevant health authorities. One such method that has demonstrated real-world predictive abilities involves the assessment between various meteorological factors, vector presence and dengue cases.

A considerable body of research has been published regarding dengue prevalence, incidence, and vector ecology in relation to various meteorological factors. These studies have shown that weather factors such temperature, rainfall, and humidity affect *Aedes* mosquito life cycles and, consequently, risk of dengue (7–12). In general, higher temperatures and greater precipitation are associated with more cases of dengue(13–20). It has been found that higher temperatures shorten the extrinsic incubation of the virus in the vector, which, in turn, may lead to greater transmission and greater number of cases(21–24). Lower temperatures are associated with decreased numbers of dengue cases as colder temperatures generally diminish the extrinsic incubation period (25,26). As such, minimum temperature may be positively associated with dengue cases; that is, the lower the minimum temperature, the fewer cases of dengue ,while higher minimum temperatures might be associated with increased dengue. Greater precipitation also has been associated with increased number of dengue cases(15,27). Eggs of *Ae. aegypti* are known to hatch only upon becoming covered in water or under conditions of increased humidity (28,29). Accordingly, human water-storage behaviors could provide for constant presence of water and, hence, larval development regardless of precipitation. Other meteorological factors that have shown a relationship with dengue include: both positive and negative relationships between sunlight and mosquito presence (30–32), and a negative relationship between wind velocity and dengue (17). Furthermore, sea surface temperature (33,34), and even larger-scale factors such as the El Niño Southern Oscillation have been implicated as affecting mosquito numbers and dengue cases(15,18,22,35). Consequently, it is essential to understand factors that may drive the spread of dengue to better prepare for dengue outbreaks and their impacts on populations and local health systems. One such tool that has shown real-world success in predicting dengue cases is the use of weather-based regression models (36–39). Such a regression model was employed in the present study to assess relationships between ten years of weather and dengue surveillance data in the city of Ribeirão Preto, Brazil.

3.3. Analytical Methods.

3.3.1. Study Site.

Weather and dengue surveillance data were obtained for the city of Ribeirão Preto (21.2° S, 47.8° W), a metropolis in the southeastern part of the Brazil that has suffered from quasi-yearly severe outbreaks of dengue virus transmission for the past few decades.

3.3.2. Data Sources.

Dengue case data came from a public health surveillance database, known as SINAN, which is managed by the Municipal Secretariat of Health of Ribeirão Preto (*Secretaria Municipal da Saúde de Ribeirão Preto*) as mandated per the Brazilian Ministry of Health (*Ministério da Saúde*). Data on all reported cases of dengue in Ribeirão Preto during the 10 years between January 1st, 2003 and January 2nd, 2013, were used in these analyses. Patients diagnosed as having dengue by a qualified healthcare professional had information collected according to Municipal Secretariat of Health's guidelines. Information was collected according to a standardized dengue notification form—*Ficha de Notificação de Dengue*—and uploaded onto a digital database belonging to the local public health system (SINAN/SINANET). Only dengue–positive individuals were used in our analysis, i.e. those patients who were deemed to have dengue infection, either through laboratory-based tests (ELISA, PCR, NS-1 viral protein detection, Viral Isolation), or through clinical-epidemiological criteria during large outbreaks when the laboratory was unable to process all samples. For this study, all potentially identifiable information was removed from the database prior to analysis and a count of dengue cases by day was generated. Daily case counts were generated and then combined into weekly measurements following the temporal patterns in the weather data reporting.

Weather data came from the Integrated Center for Agro-Meteorological Information, CIIAGRO (*Centro Integrado de Informações Agrometeorológicas*). CIIAGRO is a research group belonging to the State Government of São Paulo (*Governo Estadual de São Paulo*), and operates 146 meteorological stations scattered throughout the State of São Paulo that provide meteorological data and assistance primarily for agricultural activities (40). For this study, we used data collected by CIIAGRO's Ribeirão Preto station between September 12th, 2002, and January 2nd, 2013 (41). Weather data for several weeks prior to the start of the dengue surveillance data were included to allow for exploration of various weather data lag effects on dengue cases. Because of variable periods over which data were reported, measurements were combined into one week intervals, the smallest temporal unit possible for these analyses. Thus, analyses involved 537consecutive weeks of weather data during the 10-year study period.

Potential variables for analyses from the CIIAGRO dataset included: maximum temperature, minimum temperature, mean weekly maximum temperature, mean weekly minimum temperature, mean overall weekly temperature (all in ºC), days with recorded precipitation (count-based), as well as total weekly precipitation, monthly accumulated precipitation, and potential evapotranspiration (all in mm).

Total monthly precipitation was used to assist in calculating precipitation between dates when measurements were missing. Potential evapotranspiration was not considered for these analyses as it a meteorological variable primarily of agricultural concern as it measures the demand for water for vegetation and soil (42). Average maximum and average minimum temperatures were not considered as part of the regression as being means they would dampen the impact of extreme highs and low temperatures.

Although data were available during all weeks in the study (i.e. no missing data for an entire week), missing measurements for parts of 8 weeks (<1.5%) were encountered. To address this, we used only the partial data available for those weeks for the temperature-based variables. We were able to determine missing precipitation measurements using the reported monthly totals.

3.3.3. Univariate and Bivariate Analysis.

Univariate analyses were conducted to determine variable means, standard deviations, as well as other common descriptive statistics. Bivariate analysis consisted of independent sample t-tests when data exhibited normal distribution and the Wilcoxon-Mann-Whitney Test for non-parametric tests of differences in means.

3.3.4. Model Selection.

Generalized linear models with Poisson/Poisson-like distributions that incorporate autoregressive parameters as covariates have been shown to be useful in assessing weather-related time-series based data(43–45). In fact, this approach (and very similar approaches) has recently been used in assessing the relationship between dengue/ dengue-related factors and weather (27,36,46–50), as well as other infectious diseases including malaria (51–59), and several non-infectious health outcomes (60–65). This model has limitations when compared to traditional time-series data analysis, but similar models have been shown to be accurate in real-world scenarios (36,37,65). This issue will be discussed in further detail in section 3.5 (Discussion). As such, a generalized linear model approach assuming a Poisson-like distribution using the number of dengue cases as our dependent variable was used. Because the dependent variable, cases, was highly dispersed with a much larger variance than the mean, (i.e., there were several weeks, particularly weeks in the late winter and early fall, where zero cases of dengue were reported in Ribeirão Preto, and other periods with >1,000 cases per week) generalized linear models with a negative binomial distribution were used. These had the general form, using similar notation as presented by Simões et al, 2013(36):

$$cases \sim Negative\ binomial\ (\mu_{yt})$$

$$\log(\mu_t) = \beta_0 + AR(1) + \sum_{p=1}^{P} \beta_p X_{t-lp} + \sum_{i=1}^{P} \sum_{j>i} \beta_{ij} X_{i,t} - l_i X_{j,t-l_j}$$

Where: *μ*= mean cases, *AR(1)*= autoregressive term *β*= coefficient (β_0 is the intercept, which was excluded from the final model(36,50)), *X*= weather variable, t= week, l_p,p=1…p (lagged weeks), with the last term representing potential interactions to be assessed(36). As previously mentioned, inclusion of an auto-correlated covariate has been used in such models (36,44,46) and, as such, autocorrelation functions (ACF) and partial autocorrelation functions (PACF) assessment was conducted. It was determined that incorporating a first-order autoregressive term would be appropriate for this model (data not shown). This was done as such interactions have previously been demonstrated to be present in the association between numbers of *Aedes aegypti* and weather in Southeastern Brazil and, as such, was also assessed in this study(36). Interactions assessed included those between dengue cases, meteorological variables, and seasonality.

Model selection followed the procedures presented in Earnest et al, 2012's study meteorological risk factors on the number of dengue cases in Singapore, Gomes et al. 2012 study in Rio de Janeiro, and Simões et al 2013 study conducted in Governador Valadares, Brazil(36,46,50). Single predictor models were set-up to identify the optimum lag for each weather-based variable related to weekly reported dengue case count. Lags were restricted up to 6 weeks for non-precipitation temperature variables based on mosquito survival time post-hatching and up to 8 weeks for precipitation to accommodate effect of rain, as water/humidity is necessary for egg-hatching(28,29,36,66). The most significant variable identified in univariate analysis serves as the base for the model to which the next most significant variable was also added, and retained if significance was maintained. This procedure was continued until all variables in the model were significant, producing a composite model, with no more than two meteorological variables being added at once to avoid multi-collinearity (36,46). At that point, Model fit was assessed via Pearson Chi-Square and Aikake's Information Criteria: lower AICs were favored in model selection(36,50) To account for differences related to outbreak years, an indicator variable was included for outbreak years, based on the total reported cases of dengue per year in Ribeirão Preto (67) An outbreak year was defined as any year in which the reported number of cases would fall in the highest quartile during the study period (i.e.>5,287 cases per year), which also corresponds to years with annual dengue incidence rates of over 100 cases per 10,000 inhabitants as shown in table 3.1.

Table 3.1. Reported Cases of Dengue per Year in Ribeirão Preto, 2003-2012.

Year	**2003**	**2004**	**2005**	**2006**	**2007**
No. of Cases	789	46	616	6,140	2,726
Annual Incidence per 10,000	14.82	0.85	11.15	109.17	47.59
Year	**2008**	**2009**	**2010**	**2011**	**2012**
No. of Cases	1,059	1.641	29,710	23.837	289
Annual Incidence per 10,000	18.15	27.62	491.33	289.28	4.66

Source: (67)

The years of 2006, 2010, and 2011 were classified as outbreak years and the other years were considered as non-outbreak years. Also an indicator variable was created to account for seasonality consisted of high versus low transmission periods (January to May versus other months). Analyses were conducted using SAS statistical software ver. 9.3. (68). Production of univariate figures used Microsoft Excel 2010 (69).

3.3.5. Ethical Approval:

The data used in this project included completely de-identified, surveillance data approved as part of a larger project approved by the Ethics Committee at the Medical School of Ribeirão Preto of the University of São Paulo, accordingly further Institutional Review Board approval was not needed. No ethical approval was necessary for use of the CIIAGRO data as it does not involve human subjects.

3.4. Results.

3.4.1. Univariate and Bivariate Analysis.

By combining the meteorological measurements in the *CIIAGRO* database, 547 weeks of weather data were produced to compare with 521 weeks of dengue case counts Weather in Ribeirão Preto followed strong cyclical patterns, with hot, wet summers and drier winters. Table 3.2 presents a summary of basic descriptive statistics, and Figures 3.1-3.4 show the variation in these variables throughout the study period.

Table 3.2. Basic Descriptive Statistics of Meteorological Variables for Weekly Averages in Ribeirão Preto between 1 January, 2003 through 2 January, 2013.

Variable	Min	Max	Mean	SD
Absolute Maximum (ºC)	23.8	37.9	31.7	2.6
Absolute Minimum(ºC)	2.9	20.7	14.7	3.8
Average Temperature (ºC)	15.7	29.2	23.1	2.5
Precipitation (mm)	0.0	217.2	27.2	37.0

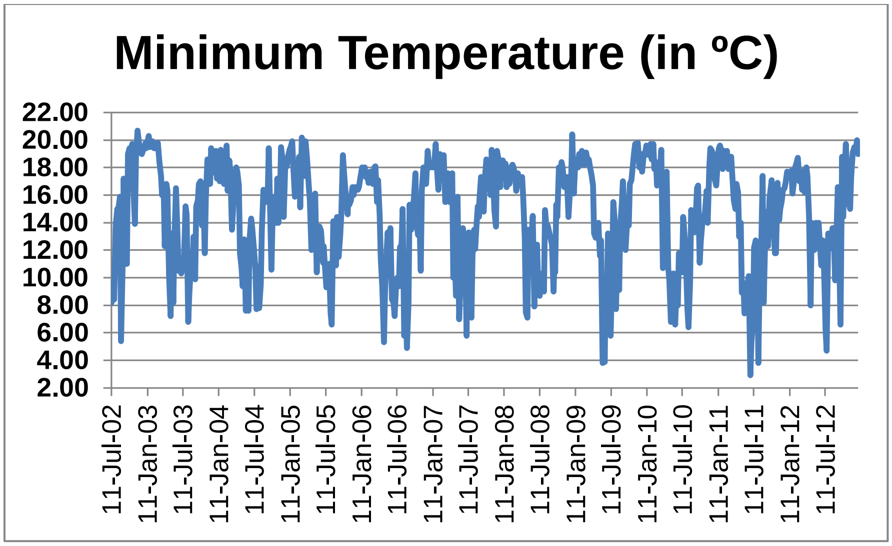

Figure 3.1. Absolute Weekly Minimum Temperature (ºC) in Ribeirão Preto, Brazil, 2002-2012.

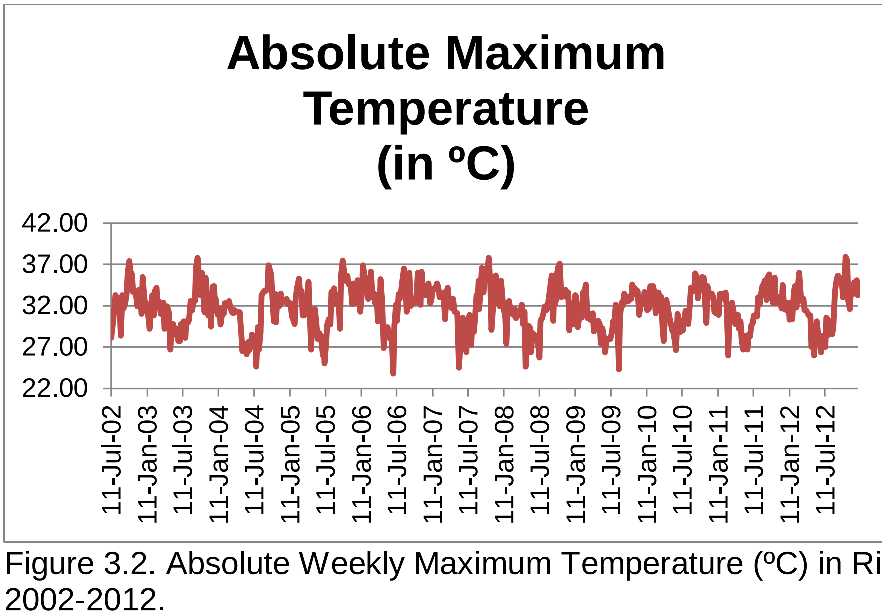

Figure 3.2. Absolute Weekly Maximum Temperature (ºC) in Ribeirão Preto, Brazil, 2002-2012.

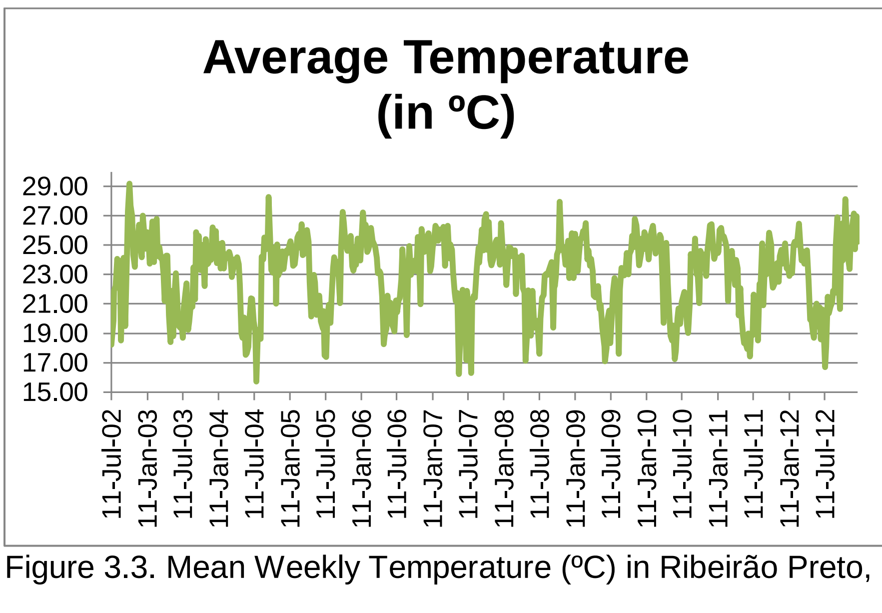

Figure 3.3. Mean Weekly Temperature (ºC) in Ribeirão Preto, Brazil, 2002-2012.

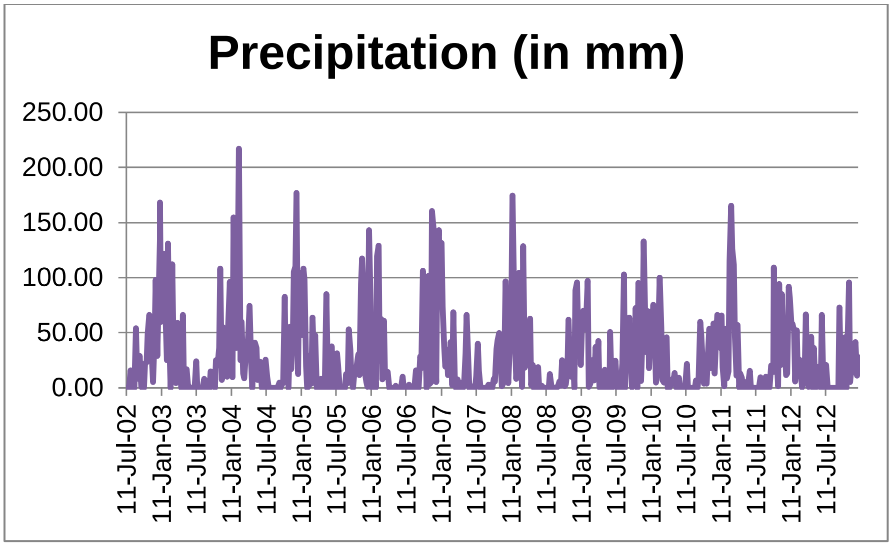

Figure 3.4. Absolute Weekly Precipitation (mm) in Ribeirão Preto, Brazil, 2002-2012.

Clear seasonality is evident in the temporal data in the figures above. In particular, temperatures are higher and precipitation is greater during December-March compared to June- September. Generally speaking, there appears to be little-to-no precipitation in the winter, and strong peaks of rain in the summer. Figures 3.5 3.2b shows the seasonal variation in weather variables by month and year in Ribeirão Preto.

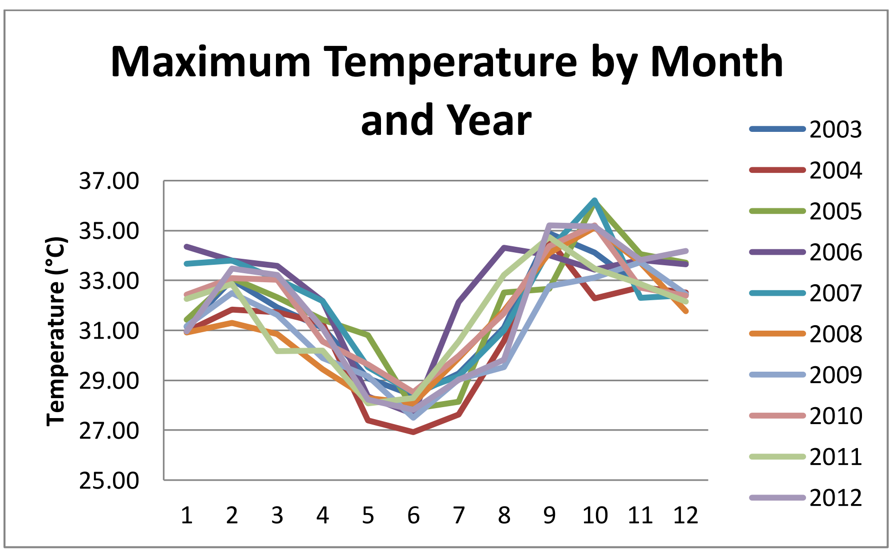

Figure 3.5. Maximum Temperature by Month and Year.

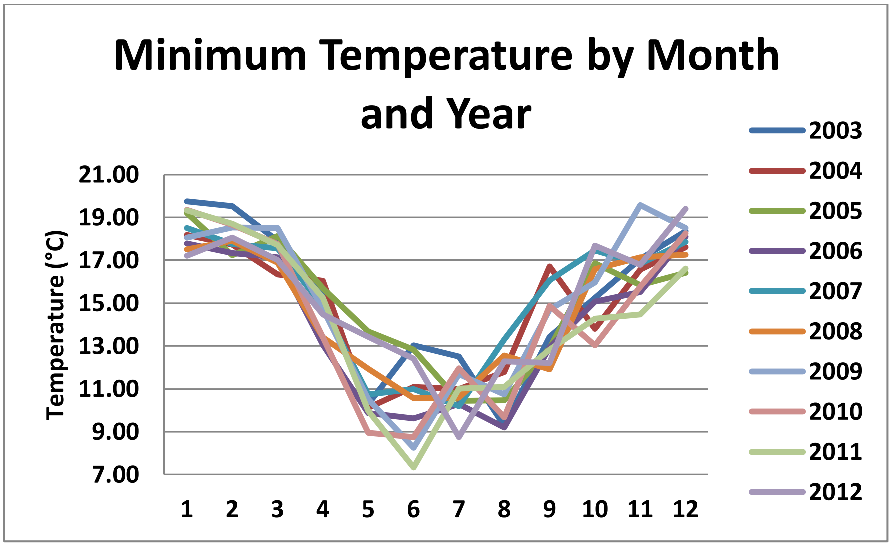

Figure 3.6. Minimum Temperature by Month and Year.

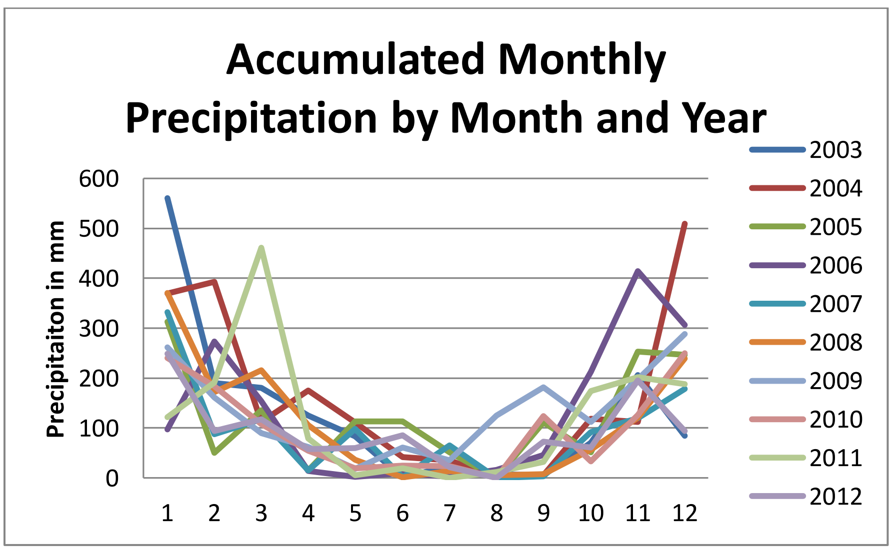

Figure 3.7. Accumulated Precipitation by Month and Year.

Figures 3.5-3.7 show clear within-year seasonality as well as variation between years. Overall high temperature generally peaks near the end of spring (November) and the lowest temperatures are generally in the winter months of June and July. Sharp peaks in December and January, and rapid drops soon after were observed, with little-no precipitation being reported in June and July. In fact, no precipitation whatsoever was reported for the month of July for the years of 2004, 2005, 2007, 2010, and 2012, and for June 2011. Figure 3.8 shows that most dengue cases occurred between February and May, representing the end of summer and start of fall, and less than 2% of reported cases occurred in between the months of July and November (winter and spring).

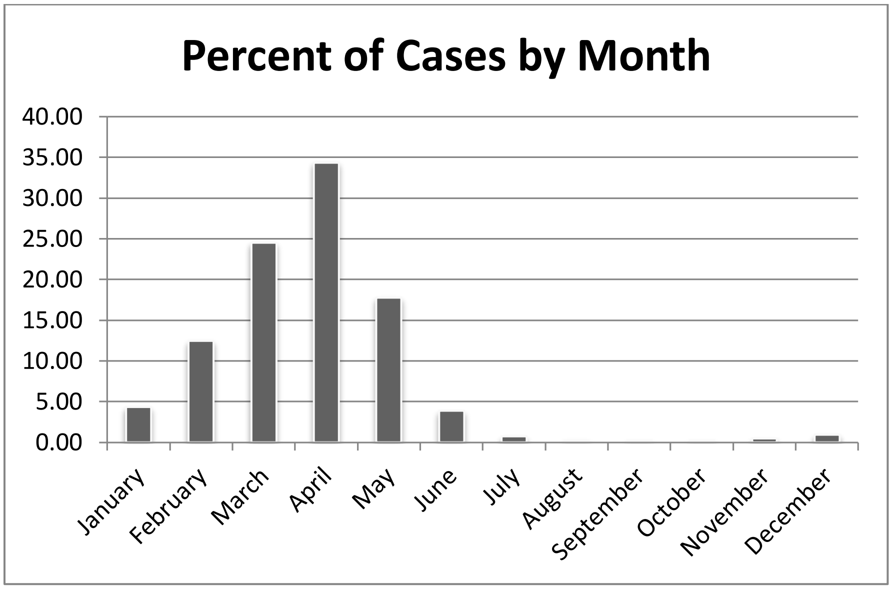

Figure 3.8. Percentage of Dengue Cases in Ribeirão Preto, 2003-2012, by month.

Table 3.3 and Table 3.4 show the relationship between weather variables by year, followed by the assessment of difference in means of the climatic variables grouped by non-outbreak and outbreak years, respectively.

Table 3.3. Mean Values of Meteorological Variables in Ribeirão Preto, 2003-2012

Year	2003	2004	2005	**2006***	2007	2008	2009	**2010***	**2011***	2012
Maximum (ºC)	31.6	30.9	32.0	**32.6**	32.2	31.2	31.1	**32.0**	**31.6**	31.8
Minimum (ºC)	15.1	14.8	15.0	**13.8**	15.0	14.6	15.1	**14.2**	**14.0**	15.0
Mean Temp. (ºC)	23.1	22.7	23.3	**23.2**	23.5	22.7	23.0	**23.0**	**22.6**	23.0
Precip. (mm)	29.9	37.4	26.6	**29.8**	20.6	27.1	30.3	**22.6**	**28.6**	21.3

*Bolded values represent outbreak years.

Table 3.4. Assessment of Difference in Mean Values of Meteorological Variables in Ribeirão Preto, 2003-2012.

	Non Outbreak	**Outbreak**	**T-Value**	**P-Value**
Years (N)	7	3		
Maximum (ºC)	31.5	32.0	-2.1[1]	**0.03**
Minimum (ºC)	14.9	14.0	2.4[1]	**0.02**
Avg. Temperature (ºC)	23.1	22.9	0.6[1]	0.56
			Z-Value	**P-Value[2]**
Precipitation (mm)	27.6	27.0	-0.72[2]	0.47

1.Satterthwaite Un-pooled Variance.2: Wilcoxon-Mann-Whitney Test.

Data in Table 3.3 were tested for differences between non-outbreak and outbreak years using a two-sample t-test for the temperature-related variables (Table 3.4). Because precipitation was not normally distributed, the non-parametric Wilcoxon-Mann-Whitney test was to test for differences. Statistically significant differences were seen in maximum and minimum temperatures between outbreak and non-outbreak years. In these years it was seen that the mean maximum temperature was greater during outbreak years while mean minimum temperature was lower by 0.9°C. Means of the measured average temperature and precipitation over the study period did not differ in a statistically significant way.

3.4.2. Multivariate Analysis.

Using the previously described methodology, variables found through single predictor models were selected for assembly into a larger composite model. As such, predictors selected for inclusion were: minimum temperature at lag 6 weeks and rain at 8 weeks. Maximum temperature was actually found to be the weakest of the predictor variables and mean temperature was not as strong of a predictor as minimum temperature. Furthermore, mean temperature was shown to be highly correlated with minimum temperature and, as such, was excluded from the final model to avoid introducing multicollinearity into the final, multivariate model. Table 3.5 presents the final selected model:

Table 3.5. Multivariate Negative-Binomial Regression Model Assessing Relationship between Dengue Cases and Weather Variables (incl. Weekly Lags) in Ribeirão Preto, 2002-2012.

Variable	Lag	Estimate	Std. Error	95% Confidence Interval	P-Value
Minimum Temperature	6	0.107	0.007	0.093, 0.121	<0.001
Precipitation	8	0.009	0.004	0.002, 0.016	0.01
AR(1) Term		0.002	0.000	0.001, 0.002	<0.001
Outbreak[1]		1.698	0.135	1.439, 1.968	<0.001
Seasonality[2]		1.667	0.181	1.310, 2.020	<0.001
Interaction Term[3]		-0.012	0.004	-0.020, 0.004	0.003

1: Non-Outbreak Years are reference. 2: Low-Transmission season is reference. 3: Seasonality x Precipitation.

Variables accounting for seasonality and outbreak/non-outbreak were added to the model as well, resulting in improved model fit. Furthermore, Pearson Chi-Square Goodness of Fit statistics and dispersion parameters (not-shown) increased, indicated the adequacy of the model and of the negative-binomial distribution. Inclusion of only the interaction term for precipitation and seasonality led to model adequacy improvement and statistically significant changes. All terms but the interaction term were positively associated with dengue, while outbreak years were the strongest predictor in terms of the magnitude of the coefficient. Maximum temperature was only found to be predictive at lag 0 and lag 6 (strongest at the former lag) and, even so, was less predictive than any other potential variable assessed in the model. Average temperature was less predictive than minimum temperature in all lags.

3.5. Discussion.

Various weather factors were found to be predictive of dengue, with minimum temperature and precipitation being the primary predictors of dengue in Ribeirão Preto between 2003-2012. As previously mentioned, maximum temperature was found to be predictive at lags 0 and 6 weeks, however in neither lag was it as predictive as minimum temperature, mean temperature, or precipitation. This may be related to the fact that Ribeirão Preto is a generally warm city, with a mean maximum temperature of 31.7° C, with the lowest maximum temperature recorded during the study period of 23.8°C, and that heat generally is positively associated with mosquito abundance (13–20). Despite this general association, it has also been demonstrated that temperatures exceeding 35°C are actually negatively associated with dengue fever as mosquito survival is diminished at these temperatures (22,70). In this study, less than 10% of weeks achieved or surpassed that temperature threshold at any point (data not shown). As such, generally high temperatures that rarely exceed the 35°C threshold may contribute to almost-constant ideal conditions for mosquito development and disease transmission for certain parts of the year.

Increases in average temperature and minimum temperature were both associated with increased dengue, with minimum temperature being more predictive than average temperature. Accordingly, minimum temperature was selected for inclusion the multivariate model at lag 6. In fact, both variables exhibited similar behaviors across the different lags; these findings suggest that much of the predictive aspects seen in the mean temperature variable is actually more due to the impact of the minimum temperatures than due to the impact of maximum temperature; in other words, increases in weekly minimum temperatures explain increases in dengue cases in Ribeirão Preto between 2003-2012. The six-week lag period indicates that these temperatures may be affecting early mosquito life, potentially even oviposition by the previous generation of mosquitoes given the mosquito's lifespan (36). This is in line with other findings in the region as a study by Simões et al. in another city of São Paulo state showed that low temperatures at 4 weeks were associated with mosquito survival and greater egg productivity in real-world/ non-laboratory controlled settings (36). Studies conducted in Singapore, Mexico, Taiwan, and Guadeloupe also found minimum temperature to be the most predictive of the meteorological factors (27,71–74). Finally, another study conducted in Barbados, Brazil, and Thailand showed that overall higher temperatures increased epidemic durations as well as led to quicker onsets of epidemics (75). Given these common findings, the onset of higher minimum temperatures should be considered to be a trigger for future dengue outbreaks.

Precipitation was also found to be a significant predictor of dengue in this study. Precipitation has actually been shown to be a strong predictor of vector numbers(8,10,76,77) as well as of dengue incidence(15,27). Several other studies in Brazil have actually found rain to be a significant predictor of dengue(8,10,11,76,78) thus further emphasizing associations between precipitation and dengue. The findings in this study suggest that precipitation, much like temperature, likely affects the earlier stages of mosquito development. Our findings of later lags support this as precipitation is required for eggs to hatch and, consequently, larval development(28,29). As dengue is only transmitted by adult mosquitoes to humans and precipitation directly affects hatching and larval development, the effects of precipitation on dengue would indeed be expected to be seen in later lags.

A statistically significant interaction effect was seen between precipitation and minimum temperature. Interaction between minimum temperature and humidity (which is necessary for precipitation) has previously been seen to be predictive of *Aedes aegypti* on mosquito abundance in São Paulo state(36). In Simões et al 2013 study, this interaction was seen to be positively associated with increased mosquito abundance and, consequently, it would be expected that it too would be associated with dengue. In our study it was seen that this interaction was negatively associated with dengue, albeit only marginally so. Excessive precipitation has been shown to wash *Aedes* eggs(29) out of oviposition sites , and the increased temperature and precipitation may be thus reflective of conditions associated with weather events related to greater precipitation such as storms.

Inclusion of the dummy term distinguishing between epidemic and non-epidemic years improved the model fit. Given the limited scope of our data and the limitations of our study methods, we believed this “outbreak/non-outbreak” variable and/or separate models would capture effects we could not directly measure with these data. This may be due to a variety of factors ranging from government environmental prophylactic campaigns to viral susceptibility of the human hosts: chapter 2 showed that multiple serotypes circulated in Ribeirão Preto during the city period and that many individuals may be susceptible to two or more dengue viral serotypes. Inclusion of a seasonality term also improved led to improved model fit, thereby further emphasizing the known seasonal patterns of dengue.

Overall, findings presented here corroborate other findings present in the dengue literature, especially findings from studies conducted in other locations close to Ribeirão Preto. First, no other known studies have assessed the relationship between weather variables and dengue in Ribeirão Preto. Our findings also contribute to the literature of other studies conducted in the region   (8,9,20,79–84). Studies in the region have found that higher temperatures are indeed related to higher mosquito numbers as well as dengue(20,83,85) Furthermore, only one other known study has looked at time-series related aspects of dengue in Ribeirão Preto (86), however it did not incorporate any weather variables in the analysis. Accordingly, our study adds to the existing body of literature for the São Paulo state region and for Brazil as a whole, while further demonstrating the need for more weather and time-series studies to be conducted in the area.

Methods for assessing the dengue-weather relationship range from simple correlation studies to complex ARIMAX models. For this study, we decided to use negative-binomial distributed generalized linear model. This decision was decided on the basis that it has been widely used in assessing the dengue-weather relationship that it has been shown to have predictive abilities, while having the advantage of providing a computationally simpler method that can be used by health agencies in developing nations. Several models using this distribution and the related Poisson-distribution have successfully been used to assess the dengue-weather relationships weather, including in real-world forecasting abilities (27,36,46–50). For example, a generalized linear model with negative-binomial distribution that also employed a first order autoregressive term from Brazil was not only successful in developing a model that explained impact of weather variables on mosquito abundance, but it was also successful in producing 19-week forecasts for mosquito abundance (36). A similar study conducted in Singapore used a multivariate regression with similar distribution (Poisson allowing for over dispersion) that assessed temperature and precipitation, and validated their findings against data from the year following their analysis. They found that their model was both highly accurate in predicting reported cases as well whether or not an outbreak would occur (37). Finally, findings from studies applying these methods have the advantage over more complex methods in that they can be used in settings that lack computational resources or where local health staff may not have the proper statistical training to employ more complex approaches.

The methodologies employed in this study present certain limitations. One such limitation is that our analysis is not a true time-series assessment of the relationship between various weather variables and dengue: this study used a generalized linear model with a negative binomial distribution that included an autoregressive term for the predictor, however that regressor only accounted for first order autocorrelations and it is likely that seasonal autocorrelation also occurred(87). Seasonal predictor terms were assessed to account for seasonal effects related to both weather and dengue cases, however these terms did not account for autocorrelation and likely only captured part of the seasonal effects. In addition, our models did not allow for assessment of multiple effects of a particular weather variable: only one lag per variable could be included in any particular model. Several time series approaches (such as spectral analysis and ARIMA) allow for the identification and even isolation of seasonal effect and thus permit identification of unusual factors that may explain (and even predict) unusual outcomes(86,88–91). Furthermore, as our models are not true time-series models, they do not permit for accurate forecasting of cases. Accordingly, using the estimated β-coefficients from this study to predict future dengue cases in Ribeirão Preto may lead to biased results without prior validation.

Other methods are present in the literature for analysis using time-series based data. For example, in other studies using negative-binomial/ Poisson-distribution GLMs, inclusion of Forrier terms and splines were often used to account for seasonality or limit autocorrelation effects(22,37,47,63,92,93), however these approaches may be considered inappropriate as they are unable to account for lack of stationarity in these types of data (22).Another approach used to look at potential weather-disease relationships includes the use of either generalized estimating equations (GEEs), Generalized Additive Models (GAMs), and recently, even, distributed non-linear lagged models (17,73,89,94–96). Finally, highly complex methods such as multivariate Autoregressive Integrated Moving Average Models (ARIMA) and Vector Autoreggressive Models that employ Granger Causality tests may also be used multiple time series analysis (88,89,97–100).

Another limitation is that these results are dependent on the accuracy of two separate databases. Dengue is often under-diagnosed due to factors related to factors such as diagnostic test accuracy and subclinical or clinically-irrelevant manifestations of infection. Also, there is potential of data entry issues that led to misclassification of dengue status in the database. However, we consider the SINAN database from Ribeirão Preto to be a well-maintained and accurate database, so we believe these issues may only play a limited role.

It is also possible that there may be accuracy issues involved with the weather database. It is possible that there may be calibration errors or that findings were incorrectly recoded if data were then entered by hand into the database. As our data came from an automated meteorological data collection station, we do not believe this is a likely problem. Another potential limitation with the weather data is that they came from one particular meteorological data collection station located in the southwestern part of the city (at 21º13'13"S, 47º51'10"W), which may not actually be applicable to the entire city of Ribeirão Preto. As mentioned in the methods section, there were some incomplete weather data, however they represented less 1.5% of the weeks used in the analyses. Another issue was there was a limited amount of variables were present in the data that could be used in the analyses: we did not have access to variables such as relative humidity and/or wind-speed, which have been shown to be predictive in other settings(36,42,94). There may also be several unmeasured confounders and effect measure modifiers masking the true relationship between meteorological variables and dengue such as vector abundance and locally estimated extrinsic and intrinsic incubations rates. In addition, weekly measurements as opposed to finer-level measurements may also affect the true relationships between dengue and weather: it is possible that within-day variation phenomena may affect the vector and its behavior, accordingly a larger-scale resolution would potentially miss such an effect.

Another limitation is that it is not possible to truly know what the outbreak variable is actually capturing. Outbreak years have been characterized in the literature by several weather and temporal aspects (75,101) and also may involve factors related to viral serotype, prevention campaigns, oviposition site creation through environmental modification and urbanization, individual behavior, and secular trends. Accordingly, we cannot run more sophisticated analyses to assess the specific relationship between the stratification variable and the other weather variables used in our study since we do not know how they would actually interact with other variables. This, in turn, would potentially allow us to understand deeper drivers that contribute towards dengue in Ribeirão Preto.

Nevertheless, this analysis showed strong relationships between certain weather predictors and weekly dengue cases. In particular, our study showed that weather-related predictors for dengue varied by summer/fall and winter/spring. This suggests that different drivers within the realm of weather are responsible for dengue numbers and, as such, more studies should be conducted on this subject. Secondly, this study used data that had greater temporal resolution than many other studies which used monthly assessments (50,73,75). This factor may be a strength because it allowed us to do more precise analysis than if we used a lower-resolution variable like months or years as weekly weather changes have been seen to affect *Aedes* presence and transmission of dengue(15,37), especially given the short mosquito life-span. Third, as previously mentioned, our study adds to the existing literature regarding climate-dengue relationships in the region(8,9,20,79–84).

Finally, these findings may assist the Municipal Secretariat of Health of Ribeirão Preto to better prepare for future outbreaks of dengue by providing potential case estimates. Ideally, our model will be tested against the 2013 DEN-4 outbreak in Ribeirão Preto when the data are available, as well as non-epidemic future years. The model will then be fine-tuned for more accurate dengue prediction. As previously mentioned, this study is not a true time-series analyses, as such the forecasting ability of the model may be limited. Yet, similar models have shown the ability to successfully predict future cases upon validation, with one particular model even correctly predicting whether or not an actual dengue outbreak would occur (36,37). Accordingly, we believe that fine-tuning our model could also provide helpful, real-world applications. In particular, we find that if we can maintain much of the simplicity found in our model and indeed fine-tune it, it will provide for a non-computationally heavy tool to aid in preparing for dengue outbreaks and combating the disease in Ribeirão Preto. Finally, we would like to test the models we developed against models using the same data, but with other methodologies including Generalized Estimating Equations (GEEs) and Generalized Additive Models (GAMs), as well as more traditional time-series analysis like Multivariate ARIMA and Vector Autoregressive Models. Findings from these analyses could also be used to fine-tune our findings and would allow us to compare and contrast the strengths and weaknesses of each model in a real-world setting.

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Chapter IV

People's Knowledge of Dengue in Ribeirão Preto, Brazil, 2012: Implications for Risk Reduction.

4.1 Abstract.

Dengue fever is the most important arboviral disease of modernity. Dengue virus, the pathogen responsible for the disease, is transmitted by the bite of an infected female *Aedes* mosquito. Mosquito ubiquity and behavior is intrinsically linked to the behavior of its human hosts, accordingly understanding such factors are imperative to understanding risk factors for dengue disease. To assess these factors, we conducted a large dengue Knowledge, Attitudes, and Practices (KAPs) study in Ribeirão Preto, Brazil.

A KAPs questionnaire composed of approximately 50 open- and closed-ended questions was developed. A small door-to-door pilot study was conducted, which was not representative of dengue cases reported to routine surveillance or of gender, age, and socioeconomic status, nevertheless indicated a larger study would be feasible. Thus, an expanded KAPs study was conducted in 26 supermarkets during four weekends in Ribeirão Preto in order to achieve a larger and more representative sample. Convenience sampling was used to recruit participants in each supermarket. Trained Vector Control Agents enrolled participants, administered the questionnaires and recorded participant responses. A knowledge score based on several questions was assembled, and univariate, bivariate and multivariate linear regression analyses were conducted.

Overall, 4,458 individuals were approached to participate, of which 2,031 eligible people (45.6% participation) were included in the study. The gender of study participants was not significantly different from non-participants or from the city's population. High response prevalence was seen for each survey question, with the lowest response rate of 90% among primary questions, and 77.5% among secondary questions. Overall, moderate levels of knowledge were apparent in the population, with 44.7% falling in the highest categories of knowledge levels.

Multivariate models indicated greater dengue knowledge scores among individuals who reported previously experiencing dengue disease, had more education, and who self-rated their dengue knowledge at higher levels. There were no differences in dengue knowledge scores in individuals who resided in households with children compared to households without children.

The study is the largest dengue knowledge study conducted in Brazil and used a novel methodology in such dengue KAPs studies to attempt to recruit a more representative sample. Although it is known that convenience sampling has limitations in surveys, this methodology allowed for inclusion of individuals that likely would not have otherwise participated in the survey. This study was the first known dengue study to also assess self-rated knowledge levels and was also the first study to assess if those who shared a household with children had higher dengue knowledge than those who did not, as concern for children's health could affect one's dengue knowledge. Self-rated knowledge scores were positively associated with dengue knowledge assessed using knowledge items, whereas having a child in the household was not. Findings from this study could provide information that will focus, and thus improve, public health campaigns conducted by local health authorities. In particular, future campaigns should focus on providing relevant information for different educational levels as opposed to focusing on the presence of children in the household to improve public dengue preventive campaigns in Ribeirão Preto.

4.2 Introduction.

Dengue fever is arguably the most important arboviral disease of modernity. The Pan-American Health Organization (PAHO) reports that in 2013 alone there were over 2.3 million cases of dengue in the Americas, with approximately 1.5 million of those cases occurring in Brazil alone(1). Dengue virus, the pathogen responsible for dengue disease, is transmitted by the bite of an infected female *Aedes* mosquito, mostly by *Ae. aegypti* in the Americas. There are four serotypically distinct viruses (DENV I-IV) in the primary/urban cycle of transmission, and infection by any one virus only provides fleeting immunity to the other serotypes, thus allowing for multiple infections (2–4). There is currently no commercial vaccine available and clinical management of the disease is mostly restricted to rehydration therapy and the use of acetaminophen for pain and fever reduction. Current prophylactic strategies are essentially limited to disrupting vector ecology, mostly by overturning containers that serve as water-filled oviposition sites, pouring insecticide into these oviposition sites, and peridomestic insecticide spraying (5,6). Given the limited preventive options, it is essential to understand various individual-level risk factors for the disease, especially how individuals' knowledge and, consequently maybe even future behaviors, may contribute to vector ubiquity and dengue transmission.

To assess such individual-level risk factors, one can employ a survey known as a Knowledge, Attitudes, and Practices (KAPs) survey, which enables researchers to acquire data specific to a population of interest with the intent of quantitative and or/qualitative analysis(7). This approach can be considered, in essence, an informational diagnostic tool for a community's KAPs regarding a particular health outcome including for dengue(8,9). *Knowledge* refers to remembering specific facts (usually related to biological/ biomedical aspects related to the outcome of interest for dengue and similar fields) or having the ability to explain concepts with knowledge acquired about that particular subject. The *Attitudes* component covers opinions, feelings, predispositions, and beliefs that are relatively constant towards an object, person, or situation. Finally, *Practice* involves the decision to execute an action. It should be noted that hypothetical questions are usually asked in the "practices" section, making it difficult to assess people's actual practices (7,8,10,11).

Various KAPs studies focusing on dengue have employed a range of sampling approaches and many types of KAPs assessment questionnaires. Studies range from small, focus-group based, open-ended questioning sessions in Puerto Rico(12) , to door-to-door household questionnaires in Vientiane, Laos(13), and even larger-scale random-digit dialing-based phone surveys assessing KAPs related to *Aedes*-borne diseases in Southeastern France(14). These studies, as well as several others, show that a diverse range of techniques can be used to assess KAPs according to characteristics and cultures of a particular population, resources available to researchers and/or government agencies, as well as other factors unique to a particular area. In turn, findings from these studies provide opportunities for effective interventions such as targeted public health campaigns aimed at curbing disease incidence. Relevant and specific research is needed to examine the correlates of behavior change and to understand the role of knowledge and attitudes in shaping behaviors (14,15). Accordingly, it is plausible that upon increasing individuals' knowledge, their attitudes and practices towards dengue may also improve as said individuals would potentially have greater understanding of how such attitudes and practices would affect dengue transmission and risk.

Several dengue KAPs and KAP-like studies have been conducted in Brazil (as described in further detail in the discussion section, yet no such studies have ever been undertaken in Ribeirão Preto, a metropolis where autochthonous dengue transmission was first detected in 1990(16). This city is located in Southeastern Brazil, (21.2° S, 47.8° W) and has a population of nearly 650,000(17). Ribeirão Preto has a Human Development Index (HDI) of 0.800 and a robust economy based primarily on agriculture and related activities(18,19). This city has suffered from quasi-yearly outbreaks of dengue, with over 50,000 cases of dengue reported between 2003-2013.Considerable effort and financial resources have been spent by local authorities in combatting dengue, yet outbreaks still persist. Local health authorities have tried to engage city residents in dengue prevention and control efforts, but much of Ribeirão Preto's population has little interaction with health agents due to factors such as gated communities being closed, and conflicting work schedules of health agents and residents. For these and other reasons, little is known regarding the local population's knowledge and behaviors in relation to dengue disease, transmission, and prevention. Accordingly, a large, city-wide, Knowledge, Attitudes, and Practices (KAPs) survey in Ribeirão Preto, Brazil, was conducted to assess people's understanding and behaviors regarding the causes and prevention of dengue. This questionnaire was conducted with future preventive campaigns in mind and, as such, focused on assessing knowledge levels. This decision was informed, in part, by the local research staff and the Municipal Secretariat of Health, who believed the knowledge base could be effectively improved upon to combat dengue in Ribeirão Preto.

The impact of dengue on Ribeirão Preto has resulted in the population becoming aware of the disease, and in trying to prevent dengue, residents may record high or very high mean knowledge scores (≥42 points, a measure discussed in depth further on).In fact, I hypothesize that more than 50% of participants will have a knowledge score within the High/Very range of 42-69. Such high levels of dengue knowledge have been reported elsewhere in dengue-endemic areas in Brazil(8,21,22) and, as such, high levels of knowledge are also expected to be found in Ribeirão Preto. Given that dengue has affected Ribeirão Preto ubiquitously, and that previous studies have reported that dengue knowledge does not vary by socioeconomic position (23–25), I hypothesize that mean knowledge scores will not vary by different levels of income as dengue has previously been . Furthermore, I hypothesize that knowledge scores will not vary by gender also due to the ubiquity and long-term presence of disease. Previous dengue infection may also lead to higher knowledge scores as individuals who previously had the disease may be more attuned to factors associated with dengue. This relationship between previous dengue infection and knowledge has been found in other locations(26). Accordingly, individuals with previous infection will have higher mean knowledge scores. A final potential factor that may be associated with higher dengue knowledge is the presence of children in the household of the interviewee. This is because of concern for the well-being of children will lead to higher dengue knowledge as well as engaging in more risk-reducing behavior. Rationale for this hypothesis came from clinicians in research staff and their experiences in conversing with parents and grandparents of pediatric cases in Ribeirão Preto, thus this was a novel exploratory question in the dengue KAPs literature.

4.3. Methods.

4.3.1. Creation of the KAPs Questionnaire.

After reviewing the current dengue KAPs literature, a structured questionnaire was designed to address the issues that seemed relevant to the cultural and ethnic characteristics of Ribeirão Preto residents as determined by local research staff. It was comprised of four general parts: basic sociodemographic information, dengue knowledge, attitudes towards dengue and dengue prevention, and people's practices aimed to reduce vector oviposition sites and adult vector abundance.

The questionnaire was developed in collaboration with the School of Medicine of Ribeirão Preto of the University of São Paulo (*Faculdade de Medicina de Ribeirão Preto* *da Universidade de São Paulo*), and with workers of the Municipal Secretariat of Health of Ribeirão Preto (*Secretaria Municipal da Saúde de Ribeirão Preto*), most notably several members of the Zoonotic Disease Control Division (*Divisão de Controle de Zoonoses*) and the Epidemiological Surveillance Division (*Vigilância Epidemiológica*). Questions were first created in English from epidemiological and entomological concepts using formal/technical terminology. Each question was then translated into Brazilian Portuguese and subsequently modified to a more familiar local vernacular. To ensure that the questions would be properly understood by people of various socio-economic and educational statuses members of the Zoonotic Disease Control Division of the Secretariat of Health (who routinely interact with the population of Ribeirão Preto and, thus, are more acquainted with how to effectively communicate the ideas in the study) provided considerable input into question development. Particular caution was taken in choosing language to assess practices, as often in KAPs studies, that section generally assesses theoretical or hypothetical, rather than actual, practices. Vector control agents were selected to conduct the survey as they are considered to be well-respected by the community and are accustomed to interacting with the local population.

Questions were derived from one of two sources: other dengue KAPs in the current literature and from brainstorming by the research team based on identified gaps for questions of interest. Questions were taken or adapted from other studies if they seemed both appropriate for and applicable to Ribeirão Preto. Every question was first pilot tested among workers of the Zoonotic Disease Control Division to assess their applicability in the field. This was done for both open-ended as well as closed-ended questions. The questionnaire, as a whole, was also administered to other Zoonotic Division agents and other health-field professions to explore potential issues that could arise in the field. This process was conducted for both the Pilot Study and the larger dengue KAPs study. Although we believe this method led to an applicable and well-rounded survey, there was no testing of internal reliability or validity. Many of the questions were derived from other questionnaires, most of which also lack information on reliability and validity.

4.3.2. Pilot Study Methods and Findings.

For the pilot study (November 2011), five agents of the Zoonotic Disease Control were trained by the research staff in how to conduct the survey and record the results. The questionnaire was administered in two neighborhoods near the Western District's Epidemiological Surveillance Division/ Zoonotic Disease Control's location, selecting 50 homes via simple random sampling. The pilot questionnaire consisted of nine sociodemographic questions, and 42 KAPs-related questions. Inclusion criteria for participation were: being age 18 years or older, being present when the house was approached, being a resident of the selected household, and consenting to participate in the pilot study. Exclusion criteria were failing any of the inclusion criteria or declaring an inability to complete the entire questionnaire after being informed of its estimated length. If the homeowner did not respond, the surveyor moved to the first house on the right. For people who responded but refused to participate, an effort was made to ask the reason for refusal. Household-based selection methods could potentially introduce several biases into the sample such as gender bias (as men are more likely to work outside the home than women in Ribeirão Preto(27)) or age bias (retirees more likely to be home), however this selection method was chosen due to practical reasons such as limited staff availability, both in terms of personnel and timing. No personally identifiable data were collected during the pilot study.

Of the 50 homes selected, 48 home-owners responded to our inquiries to participate in the survey. Of these, six (12.5%) refused to participate, with the predominant reason (5 of 6) being related to time constraints; the one remaining individual reported she was en route to the hospital to give birth, but actually asked us to return later to give her the survey. Results of this pilot study suggested that a larger KAPs study would be feasible, particularly given the high participation (84.0%) and general enthusiasm that was found. The pilot produced an unbalanced study population with a generally older population (mean age 47.5 years) that was also weighted heavily toward females (77.7%). This atypical demographic was likely due to the time of visits (weekdays, during work-hours). As this study was conducted in a relatively small, financially homogenous geographic area, it was seen that the entire pilot study population had similar educational levels and almost equal monthly incomes. As previously mentioned, Ribeirão Preto is a highly-developed city for Brazilian standards and actually boasts a considerable amount of individuals in middle and higher-income groups, and, as such, the pilot sample excluded these individuals. These findings were used to design a more representative sample in the large-scale KAPs study.

4.3.3. KAPs Study Methods.

Although the pilot study demonstrated feasibility, the sampling methodology did not achieve a representative sample of city residents. If the same household-based methodology were to be used in the larger study, we would expect under-sampling of middle-upper and higher SES-based people due to difficulties in obtaining permission to enter gated communities or apartment complexes, which is a common problem reported by the Zoonosis Control Division agents from their routine dengue prevention efforts. Furthermore, we realized that conducting the survey during work-hours on weekdays also would exclude much of the economically active population, as they would be at their place of employment instead their household, preventing participation in our survey. Thus, a new sampling methodology aimed at improving representativeness of our study population was developed (described below).

The KAPs questionnaire employed in the larger study consisted of 56 open- and closed-ended questions (Appendix A), the topics of which are summarized in Table 4.1.

Table 4.1 KAPs Questionnaire Categories: Description.

Knowledge	**Attitudes**	**Practices**
• Vector biology • Oviposition site recognition • Disease transmission • Recognition of clinical manifestations • Recognition of necessity of proper clinical care • Self-rated dengue knowledge (0-10 scale)	• SES perceptions of Dengue risk • Perceptions of dengue prevention responsibilities • Collective x individual • Attitudes regarding future availability of vaccines	• Domestic cleaning habits • Peri-domestic cleaning habits • Oviposition site creation behaviors • Response to clinical manifestations

Finally, the research staff decided against basing sample size on the power calculations suggested in some current KAPs literature(7), as a distribution of a particular biologically measurable effect such as biomarker levels among the population (such as prevalence of a particular aspect of dengue knowledge) was not being assessed. As such, sample size was determined by comparison with a similar KAPs study conducted in Santa Bárbara d’Oeste, a culturally-similar city ~200 km from

Ribeirão Preto, and adjusted for the population of Ribeirão Preto (28), based on a calculation assuming knowledge levels and potential refusals This resulted in a desired sample size of 1,786, which was later increased for practical purposes regarding questionnaire distribution. Assuming that 50.01% of adult residents met the criteria of dengue knowledge presented in hypothesis 1, and given Ribeirão Preto's population, such a survey would require a sample size of approximately 385 individuals, had it used truly random sampling (17). However, the convenience based sampling method did not allow for assessment of how representative the study sample truly was of Ribeirão Preto's adult population and, as such, a larger survey sample was used as it may lead to diminished amounts of bias(29).

To improve representativeness of the study, surveys were conducted in 26 markets and supermarkets distributed throughout Ribeirão Preto (and its southern satellite district of Bonfim Paulista), on four consecutive Saturdays in October and November 2012. Locations and dates were chosen so as to sample individuals representing the diversity of ages, socioeconomic statuses, and genders of city residents. The markets and supermarkets were chosen according to: geographic location within the city, ease of access both for people relying on public and on private transport, the market niche (financial / demographic), and the likely importance for consumers (e.g. was it the largest market in the neighborhood; the most likely to be frequented, etc). This selection process was undertaken with the input of several key members of the Zoonotic Disease Control and the Epidemiological Surveillance divisions. Permission to conduct our KAPs surveys in the locations of interest was obtained from either the owners or the employee in-charge for each market. The distribution of the supermarkets at which surveys were conducted throughout the city is shown in Figure 4.1.

Figure 4.1. Distribution of KAPs survey sites throughout the city of Ribeirão Preto, Brazil. Each red dot represents a location where surveys were administered in the larger KAPs study. City size= 650, 955 km2. Source: (Instituto Brasileiro de Geografia e Estatística, 2014).

Using population estimates for the five regions of the city (as determined by the Office of the Mayor—*Prefeitura Múnicipal de Ribeirão Preto*), sample sizes for each region were calculated, dividing them equally among the supermarkets located in each region. To facilitate this process, the proposed sample size was increased to 2,000 for a more even distribution of surveys among markets and agents, with the number of questionnaires distributed in each region and market weighted to the regional population and the number of markets in each region.

Approximately 30 agents, mostly from the Zoonotic Disease Control Division, went through a rigorous training process conducted by the research coordinator. Training included detailed explanations of how to conduct the questionnaire, as well as specific information relative to each question asked in the survey. For this convenience sample, agents were instructed to approach the first individual they could (regardless of apparent age, gender, SES status, etc.), to assess their interest in participating in the study. This convenience sampling was used to address budgetary constraints, vector agent well-being and safety, vector agent availability, and constraints imposed by those responsible for the research sites such as dates and times in which the study could be conducted. Potential impacts of this strategy were assessed by comparing available descriptors to those of the city census. One of the main limitations of convenience sampling is that generalizability of the results to the larger source population cannot be claimed via this method as it is unknown how those who opted to not participate differed from both the participating sample and the source population, as well as what kind of biases may be introduced via this sampling mechanism (30–33). Strengths and limitations of this method will also be addressed in greater detail in the discussion section.

Inclusion criteria for participation in the study included: 1) being a resident of the city of Ribeirão Preto or the suburb of Bonfim Paulista; 2) being 18 years of age or older; and 3) consenting to participate in the study, including filling out and signing a written consent form entitled the *TCLE: Termo de Consentimento Livre e Esclarecido*. Exclusion criteria included not fulfilling any of the inclusion criteria or having previously participated in this same study.

Administration of the KAPs surveys lasted approximately 15-20 minutes. Participants were not offered any compensation, financial or otherwise, for taking part in the study. Interviewers were asked to document the gender of those who refused participation.

4.3.4. Statistical Analysis Methods.

Two independent variables were used in the analyses: self-rated dengue knowledge (0-10 scale) and the constructed dengue knowledge score (0-69 scale). Bivariate analysis included use of independent t-tests for assessing differences in means, paired t-test comparing self-rated score and calculated knowledge score, and ANOVA tests to assess differences in categorical variables. Univariate and bivariate analysis were conducted for all participants who were eligible to respond to each particular question: response eligibility in the statistical analysis portion is defined as providing a response to a primary question that triggers the secondary question. Accordingly, those who answered “no” to a yes/no primary question were not eligible to respond to the secondary question. Participants were allowed to report that they “did not know” a response to particular question.

Linear regression was employed to evaluate relationships between knowledge scores and various socio-demographic factors, with statistical significance set at $p \leq 0.05$. Variables selected for the linear regression were selected among non-KAPs based questions, and then on findings in univariate and bivariate analyses,). Other factors, such as R^2 and parsimony were also considered when assembling potential models. Continuous predictors were transformed into categorical variables when deemed appropriate; for example, age was categorized into quartiles instead of being used as a continuous predictor as it permits assessment of potential target groups for future preventive campaigns. Income was also assessed in such a fashion (and continuously), as these groupings may represent more realistic societal structures. Dummy variables were created for categorical predictors. Data were manually entered into a Microsoft Access database using a custom form set-up to ensure proper and accurate data-entry.

Finally, linear regression was employed to explore the relation between knowledge scores and various socio-demographic factors, with statistical significance set at $p \leq 0.05$. Other factors, such as R^2 and parsimony were also considered when assembling potential models. Continuous predictors were transformed into categorical variables when deemed appropriate; for example, age was categorized into quartiles instead of being used as a continuous predictor as it was believed by the research staff that these groupings could reflect temporal trends experienced by different age generations better than a continuous age predictor. Income was also assessed in such a fashion (and continuously), as these groupings may represent more realistic societal structures. Dummy variables were created for categorical predictors. Data were manually entered into a Microsoft Access database using a custom form set-up to ensure proper and accurate data-entry. Statistical analysis was conducted using SAS statistical software, version 9.3(34,35).

4.3.5 Ethical Approval.

Institutional Review Board approval was obtained from the Medical School of Ribeirão Preto of the University of São Paulo Ethics Review Board as this study was deemed to cause no harm whatsoever to participants, did not involve the surveying of any minors, prisoner populations, or any other such groups requiring greater approval, as well as only completely de-identified data was used in the analyses. Further approval for the study was also given by the Municipal Secretariat of Health of Ribeirão Preto. No individually identifiable data were uploaded into the database and the questionnaires and their accompanying consent forms were kept in a secure location. Exemption from further review was obtained from the University of Michigan IRB [HUM00068660].

4.3.6. Measures.

As is common in the KAPs literature, a “score” was assembled to be used as a measurement of people’s dengue knowledge (23,26,36,37). Following the methods presented in these studies, questions were selected that directly pertained to dengue risk and assigned them numerical values as follows: each question could have a maximum of 3 points that could be awarded for answers that were either correct or evidenced protective/ risk-reducing behaviors. Incorrect answers could lead up to a 3 point deduction from the knowledge score. Other scores within the range were possible in certain questions as partial correctness was considered for certain questions. More detailed methods for this knowledge score calculation are present in Appendix B. Unanswered questions were assigned a score of 0, meaning they neither increased nor decreased final knowledge score. Secondary questions—those that required a particular response in another question to even be asked of the participant—were combined with primary questions and contributed as one part of the score, as also shown in Appendix B; in other words, the primary and secondary questions together could only contribute a maximum of 3 points and a minimum of -3 points.

Knowledge question values were tallied and it was seen that the maximum possible score was 33 points and a minimum possible of -27 points. The differences in maximum and minimum total points are because not all questions could result in negative points, whereas all of them did provide the potential for positive points. Given that this score is supposed to represent people's calculated knowledge, we added 27 points to all scores so that the new theoretical maximum score was 69 points and the new minimum was 0 points—signifying no dengue knowledge & no risk-reducing behavior whatsoever. For the purposes of the analysis in this section, we also categorized the levels of knowledge as shown in Table 4.2, by breaking the data into five approximately equal categories selected *a priori* as per the decision of the research staff:

Table 4.2. Knowledge Score Categories.

Level of Knowledge	**Knowledge Score**
None-Very Low	0-14
Low	15-28
Adequate	29-41
High	42-55
Very High	56-69

The other variables used in the analyses were also derived from the questionnaire in Appendix A. Many of these came from sociodemographic questions present in the first portion of the survey. Self-rated knowledge levels were determined simply by asking participants to rate on a 0-10 scale their own level of knowledge. This was determined to be the most culturally-relevant approach by the research team to investigate how people self-perceived their knowledge of dengue as this was considered to be a scale that would be easily interpreted by the local populous, as per the opinion of the research staff. Self-rated score was assessed to explore how inhabitants of Ribeirão Preto perceived their own knowledge levels versus several knowledge aspects deemed to be important by the Secretariat and the research staff for effective preventive efforts as well provide insights as to how open people may be to obtaining new information: key for future preventive efforts. Income could be reported as either an actual income amount (such as R$4,500 per month) or in monthly “minimum salaries” (*salarios mínimos mensais*) which is a standard measurement in Brazil used by the government to determine minimum wage. At the time of this survey, one minimum monthly salary was equivalent to US$306.76 per person per month(38). Participants were also asked if they were ever previously diagnosed with dengue, which is reflected in the “previous dengue” variable; this is a self-reported variable and not necessarily a reflection of any particular laboratory-based serological test.

4.4. Results.

4.4.1. Overall findings and Basic Descriptors.

A total of 4,458 individuals were approached in 26 supermarkets spread throughout Ribeirão Preto and Bonfim Paulista during the study period. Of those people, 2,039 (45.7%) consented to participate. Eight (0.4%) of the interviewed individuals were subsequently deemed ineligible to participate according to the inclusion criteria, and were removed from analysis. Of the 2,031 eligible participants, 14 (0.7%) choose to not complete the entire questionnaire for a variety of reasons, and so only completed responses for these participants were considered in the analysis.

Questions were divided into primary and secondary categories to assess completeness of responses. Primary questions were defined as those that could be asked without dependence on previous responses, hence for which all participants were asked to respond. Secondary questions depended upon a specific response to a primary question, reducing eligibility to respond. Completeness of response for primary questions ranged from a low of 89.7% for question J, which asked about participant's income, to nearly everyone (99.6%) for question 4, which asked how often respondents checked for breeding sites in their homes. Among secondary questions, responses ranged from 77.5% (question 23a) for an open-ended question regarding why a participant would not denounce a neighbor who refused to clean-up an obvious breeding site, to 99.95% (question 3a), which asked those who said they knew what a mosquito breeding site was to identify such a site. Despite the relatively low participation rate (45.6% of those approached participated in the study), there were generally high survey item completion rates among those who did participate.

Table 4.3. Descriptive Statistics for KAPs Continuous Variables.

Variable*	**Respondents**	**Mean**	**Min**	**Max**	**SD**
Age (years)	2005	42.7	18	85	15.6
Distance from Supermarket (in blocks)**	980	6.4	0	80	6.7
Inhabitants in Household	2000	3.6	1	20	1.8
Inhabitants under age 18	1027	1.7	1	9	1.0
Monthly Household Income (in R$)	1654	2276.1	260	19,000	2018.1
Self-Rated Dengue Score (range 0-10)	1993	7.4	0	10	2.3
How many times one can get dengue**	1346	2.9	1	300	32.9
Calculated Knowledge Score	2031	40.2	15	58	7.0

*Did not respond/ refused to respond removed from analysis.
**Only includes numerical responses. Responses that could not be numerically coded were excluded.

To better understand the study population, basic descriptive analyses were done for continuous and dichotomous variables as seen in Table 4.3. Survey respondents' ages ranged from 18-85 years, with mean of 42.7 (s.d.=15.6) years. The mean distance between participant households and survey locations (markets/ supermarkets) was 6.4 (s.d.= 6.7) blocks among participants who provided numerical responses regarding this distance. Most participants reported having children living in their households, with a mean of 1.7 children per household. Monthly household income varied widely, from R$260 (US$128)—less than the legal monthly minimum wage of R$622.00—to R$19,000 (US$ 9,370), using exchange rates from the date of the first survey, October 20, 2012 (38). Participants said that individuals could get dengue anywhere between once and 300 times, with mean reported 2.93 possible infections. Mean self-rated dengue score (scale 0-10) was 7.4 (s.d.= 2.3). Mean knowledge score was 40.2 (s.d.= 7.0). Table 4.4 presents counts and percentages for responses to dichotomous questions.

Table 4.4. Descriptive Statistics for Dichotomous Knowledge and other Questions.

Variable*	Count	Yes (%)	No(%)
Sociodemographic			
Previous dengue**	1995	557 (27.9)	1438 (72.1)
Laboratory confirmed dengue infection[#]	513	447 (87.1)	66 (12.9)
Knowledge			
Do you know what a mosquito oviposition site is?	1981	1777 (93.0)	134 (7.0)
Can Mosquito lay eggs?	1921	1842(95.9)	79(3.9)
Can Eggs be infected?	1577	1037(65.8)	540 (34.2)
Do you know how one gets dengue?	1927	1854 (96.2)	73 (3.8)
Can you have dengue without presenting symptoms?	1808	724 (40.0)	1084 (60.0)
Do dengue symptoms differ from flu?	1867	1409 (75.5)	458 (24.5)
Is there a vaccine available?	1827	342 (18.7)	1475 (81.3)
Can dengue be more severe the second time?	1892	1746 (92.3)	146 (7.7)
Attitudes			
Does the risk of dengue vary with financial status?	1963	351 (17.9)	1612(82.1)
Do some neighborhoods have more dengue than others?	1984	1754 (88.4)	230 (11.6)
Is dengue a disease associated with poverty?	1970	374 (19.0)	1596 (81.0)
Should household-level oviposition-site removal be obligatory by law?	1988	1684 (84.7)	304 (15.3)
Would you talk to your neighbor if he/she has obvious oviposition sites	1977	1681 (85.0)	296 (15.0)
If you found a large breeding site not in your neighborhood that is not a household, do you have the responsibility to contact the proper authorities?	1995	1911 (95.8)	84 (4.2)
If your neighbor refuses to remove sites, would you denounce him/her (assuming anonymity)	1954	1742 (89.2)	212 (10.9)
If there were an available vaccine in healthcare centers, would you get vaccinated?	1977	1910 (96.6)	67 (3.4)
Should people who refuse to clean oviposition sites be fined	1991	1878 (94.3)	113 (5.7)
Does educating children have a positive result against dengue	2007	1997 (99.5)	10 (0.5)
Practices			
Are there screens on your windows?	2007	267 (13.3)	1740 (86.7)
Do you use repellent or protection on yourself or family members?	1993	1553 (77.9)	440 (22.1)
Other			
Is there an outdoor rain drain in your house?	1979	1363 (68.9)	616 (31.1)

*Did not respond/ refused to respond removed from these analysis.
**5 participants reported more than 1 previous dengue infection.
[#] Among 557 eligible participants.

4.4.2. Knowledge Level Assessment.

A T-test was conducted to see if the knowledge score mean differed in a statistically significant way from a score of 42 (the lower bound of the high knowledge category group as described earlier). It was seen that mean score was 40.2 (95% CI: 39.89-40.50, p-value<0.0001), thus differing from the predicted score of 42 described earlier, thus the study population's mean knowledge level was actually lower than the high category. Figure 4.2 is a histogram showing the distribution of the knowledge scores, suggesting that the distribution of scores follows a fairly normal distribution.

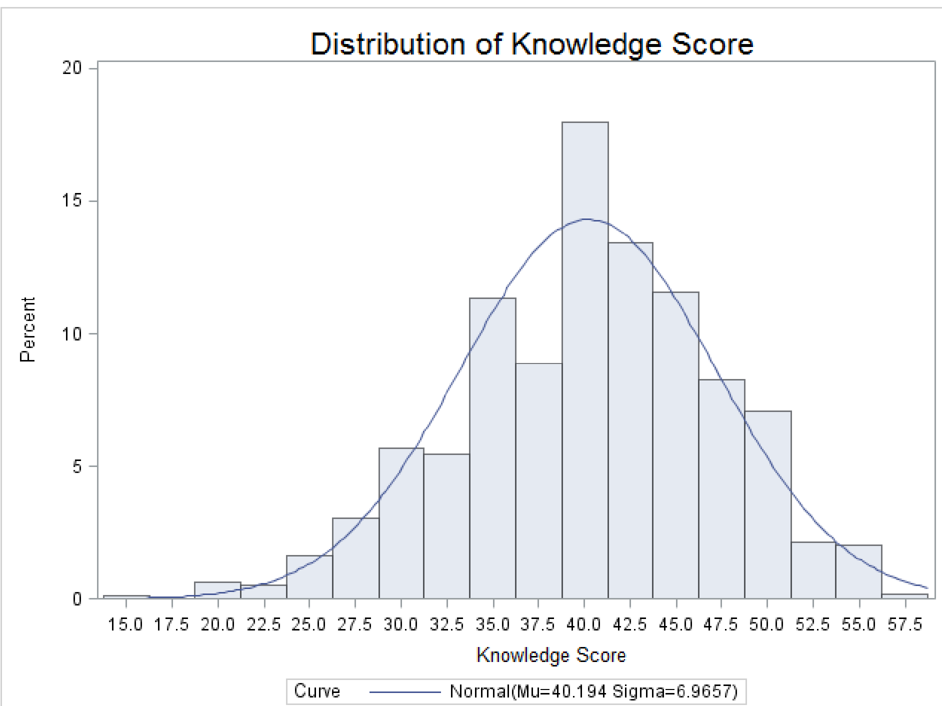

Figure 4.2 Distribution of Knowledge Score with Normal Line Plotted.

Table 4.5 Knowledge Score Category Distribution.

Knowledge Score Category	Count (%
Very Low	0 (0)
Low	121 (6.0)
Medium	1002 (49.3)
High	881 (43.4)
Very High	27 (1.3)

Most participants (49.3%) fell into medium knowledge level and 44.7% of the other participants fell in the two highest categories, with no participants in the lowest category. Thus, the local population is at least fairly aware of some aspects of dengue, however less than half of study participants would be considered highly knowledgeable Of the several knowledge questions asked to participants, more than half of participants demonstrated lack of knowledge for two questions: 72% of respondents responded incorrectly to questions involving the number of times one could get dengue and 53% incorrectly to whether symptoms of dengue could differ from those of the common cold.. Mean calculated knowledge score varied by multiple factors as seen in table 4.6.

Table 4.6 Independent T-tests for Differences in Mean Calculated Knowledge Score and Select Dichotomous Variables.

Variable	**Category**	**Count**	**Mean**	**SD**	**95% CI**	**T-Value**	**DF**	**P-Value**
Gender[2]	Male	943	40.5	7.3	40.5,40.9	1.41	1975	0.16
	Female	1074	40.0	7.2	39.6, 40.4			
Previous Dengue Infection[1]	Yes	557	41.0	6.5	40.5, 41.6	3.14	1993	**<0.01***
	No	1438	39.9	7.1	39.6, 40.3			
Children Present in Household[2]	Yes	1028	40.2	7.0	39.8, 40.6	0.45	1709	0.65
	No	787	40.4	6.8	39.9, 40.8			

1: Pooled/ Equal Variance. 2: Satterthwaite/ Unequal Variance. *p<0.05, **p<p0.0001.

Statistically significant differences were seen only among those who reported previous dengue infections compared to those who did not report previous infections. No statistically significant differences in mean calculated knowledge were seen between genders or when comparing participants who had children in their households to those who did not.

A one-way ANOVA test was conducted to assess the relationship between monthly income and knowledge scores, using income divided by quartiles. Higher income levels were associated with higher knowledge scores as seen in table 4.7. Mean knowledge scores increased with income level, and the difference was statistically significant overall.

4.7. One-Way ANOVA Testing Differences in Mean Calculated Knowledge Score by Income Quartiles.

Income Level	Count	Mean Score	SD	DF	F-Test	P-Value
<R$1,000	773	39.6	7.2	3	8.76	**<0.01****
R$1,000-1,700[a]	426	39.6	7.0			
R$ 1,700-R$2,900 [a]	418	40.5	6.5			
≥R$2,900	414	41.6	6.8			

*p<0.05, **p<p0.0001. a: Exclusive of upper value.

Table 4.8 assesses how mean calculated knowledge scores vary by educational levels. It was shown that mean score increased by educational level. There were no significant differences between those with less than primary education and those with only completed primary educations.

Table 4.8 ANOVA with Contrasts for Mean Calculated Knowledge Score among Different Educational Levels.

	DF	**Sum of Squares**	**Mean Square**	**F-Value**	**p-Value**
	3	2832.6	944.2	20.44	**<0.01****
Education	**Count**	**Mean**	**Maximum**	**Minimum**	**SD**
Level 1	456	39.0	66	27	7.9
Level 2	390	39.1	68	28	7.7
Level 3	806	41.0	71	23	7.6
Level 4	294	42.3	72	25	7.6
Contrast Levels	**DF**	**Contrast SS / Mean Square**		**F-Value**	**p-Value**
1st and 2nd	1	7.7		0.17	0.68
1st and 3rd	1	1167.6		25.3	**<0.01****
1st and 4th	1	1964.3		42.5	**<0.01****
2nd and 3rd	1	862.5		18.7	**<0.01***
2nd and 4th	1	1636.2		35.4	**<0.01****
3rd and 4th	1	862.5		18.7	**<0.01***

*p<0.05; **p<0.0001.
Contrast Levels—1: Incomplete/ No Primary Education; 2: Completed Elementary Education, No/ Incomplete Secondary Education; 3: Completed Secondary Education, No/Incomplete Tertiary Education; 4: Completed Tertiary Education.

4.4.3. Self-Rated Dengue Knowledge.

Table 4.9 presents results of self-reported dengue knowledge in relation to the respondents' gender, use of insect repellent, previous dengue infection, presence of window screen in households, poverty beliefs, and presence of children in the household. Independent T-Tests for differences in mean self-rated score were used in this test. For ease of comparability, the self-rated score was adjusted to the same overall range as the calculated knowledge score; that is, the new range was from 0-69, achieved by multiplying the current self-rating system (0-10) by 6.9. As this particular variable was not validated against the composite knowledge score, such comparisons conducted in this section are for exploratory purposes only, These analysis were conducted to see how self-rated knowledge varied by the socio-demographic factors present in the questionnaire.

Table 4.9. Independent T-tests for Differences in Mean Self-Rated Knowledge Score.

Variable	Category	Count	Mean	SD	95% CI	T-Value	DF	P-Value
Gender[1]	**Male**	926	49.5	16.6	48.5, 50.6	-3.51	1979	**<0.01***
	Female	1055	52.0	16.0	51.1, 52.9			
Previous Dengue Infection[1]	**Yes**	554	50.6	14.7	51.4, 53.9	3.08	1967	**<0.01***
	No	415	50.2	15.9	49.3, 51.0			
Children Present in Household[2]	**Yes**	1011	50.8	15.5	49.9, 51.8	0.43	1647	0.66
	No	777	50.5	15.9	49.4, 51.6			

1: Pooled/ Equal Variance. 2: Satterthwaite/ Unequal Variance. *p<0.05, **p<p0.0001.

Women rated themselves as having higher levels of dengue knowledge than men. Those who reported using insect repellent on themselves or other family members also tended to have higher self-rated dengue knowledge. Individuals who reported previous dengue infection also self-reported statistically significant higher dengue knowledge levels. No statistically significant differences were seen in self-rated dengue knowledge among those with and without window screens, who saw or did not see dengue as a disease of poverty, and between those with and without children in the household. Table 4.08 presents an ANOVA with contrasts to assess at mean self-rated knowledge score between different educational levels.

Table 4.10 ANOVA with Contrasts for Mean Self-Rated Knowledge Score among Different Educational Levels.

Schooling x Self-Rated Score					
	DF	**Sum of Squares**	**Mean Square**	**F-Value**	**p-Value**
	3	5226.7	1742.2	7.2	**<0.01****
Education	**Count**	**Mean**	**Maximum**	**Minimum**	**SD**
Level 1	438	49.0	69.0	0	2.7
Level 2	384	49.4	69.0	0	2.2
Level 3	802	51.9	69.0	0	2.1
Level 4	294	53.4	69.0	0	1.9
Contrast Levels	**DF**	**Contrast SS/ Mean Square**		**F-Value**	**p-Value**
1st and 2nd	1	28.8		0.1	0.7
1st and 3rd		2472.0		10.3	**<0.01***
1st and 4th		3446.7		14.3	**<0.01***
2nd and 3rd		1727.3		7.2	**<0.01***
2nd and 4th		2733.2		11.3	**<0.01***
3rd and 4th		466.4		1.9	0.2

*p<0.05; **p<0.0001.
Contrast Levels—1: Incomplete/ No Primary Education; 2: Completed Elementary Education, No/ Incomplete Secondary Education; 3: Completed Secondary Education, No/Incomplete Tertiary Education; 4: Completed Tertiary Education.

Overall, mean self-rated knowledge increased as schooling levels increased similar to what was seen with calculated dengue knowledge scores. However, it was seen that differences in means between educational levels were greater in terms of self-rated scores versus calculated knowledge score, although the relative impact of differences among variables cannot be adequately assessed due to lack of previous validation of this variable against composite knowledge. Yet, a Pearson correlation score of 0.1342 (p-value <0.0001) was seen between self-rated knowledge and composite knowledge score, suggesting that the two variables are positively correlated.

4.4.4. Multivariate Analysis.

Multivariate linear regression model selection was conducted according to the guidelines in section 4.4.5. The dependent variable, calculated knowledge score, was considered to be nearly normally distributed and transformations to further normalize did not result in strong changes in model selection with any combination of dependent variables used. No potential independent predictors that were used in creating the KAPs score were used in the analysis as they are inherently associated with the independent variable.

Table 4.11. Knowledge Score-based on Calculated Knowledge Regressed on Age, Education, Previous Infection and Self-rated Score.

Variable	**Category**	**Estimate**	**Std. Error**	**T-Value**	**P-Value**
Intercept		36.4	0.6	63.8	**0<.01***
Previous Infection	Yes[a]	1.00	0.3	3.0	**<0.01***
Completed Education Level	Primary [b]	0.11	0.5	0.3	0.80
	Secondary [b]	1.8	0.4	4.7	**<0.01****
	Tertiary [b]	3.0	0.5	6.1	**<0. 01****
Self-Rated Dengue Knowledge Score[d]		0.05	0.0	5.0	**<0.01****
<u>Analysis of Variance</u>					**<u>Goodness of Fit</u>**
F-Value	**P-Value**			**Root MSE**	**R^2**
19.1	**<0.01****			6.8	0.05

*p<0.05; **p<0.0001.
A) Reference: No Previous Infection. b)Reference: Incomplete/ No Primary Education.
c) Using adjusted scale.

The strongest model predicting KAPs score based on these data was composed of dichotomous previous infection status, educational levels, and self-rated dengue score. Income level was found to not contribute to the model upon addition of participant educational level, nor did it improve model fit; as such, it was excluded from the final model. Previous infection (β=1.00, $p<0.01$, meaning an increase in one knowledge score point), education comparing those with completed secondary education to those with less than primary education (β =1,8, $p<0.01$) and those with completed tertiary education compared to those without education levels (β =3.0, $p<0.01$), and higher self-rated dengue knowledge scores (B=0.05, $p<0.01$) were associated with higher knowledge scores. There were no statistically significant differences in Knowledge score between the two lowest educational categories. Adjustment by previous infection status did not result in significant changes in composite score between those residing in a household with and without children nor within different educational groups, as such, only the unadjusted model is presented. Having had a previous dengue infection was associated with a 1.00 increase in mean calculated knowledge score above and beyond other variables in the model. Having a completed secondary education was associated with 1.8 increased points compared to having less than a completed primary education, whereas completed tertiary score was associated with having 3.0 points higher calculated score. Finally, self-rated knowledge was associated with a 0.5 increase in mean calculated score per increase in 1 point of the self-rated score. Monthly salary and age (continuous and categorical) were found to be non-significant and inclusion in the model resulted in poorer fit. Finally, a low R^2 was found in all models predicting KAPs scores; this was the case with numerous variable transformations and variable combinations, thus indicating that the variables used do not strongly explain the variability in KAPs scores.

4.4.5. Analyses of Survey Population.

Finally, analyses were conducted to characterize the survey participants (and refusals), and to assess how they may differ from the city of Ribeirão Preto as a whole, according to the 2010 census. To address potential differences between participants and those who refused to participate, vector control agents were instructed to note the gender of those who refused to participate in our survey. Table 4.12 shows the distribution by gender comparing refusals and participants (44,45).

Table 4.12. Chi-Square Test Assessing Gender-distribution Differences between Study Participants and People who were Approached but Refused Participation.

	No. KAPs Participants (%)	**No. Refused (%)**	**X^2 Value**	**DF**	**P-value**
Male	943 (46.8%)	1120 (46.3%)	0.162	1	0.68
Female	1074 (53.2%)	1299 (53.7%)			
Total	2017 (100%)	2419 (100%)			

Missing/refused/did not know=14.

There was no statistically significant difference between the male-female ratios of participants and those who refused to participate. Although we do not know other characteristics of the refusal population, this particular similarity provides basis that, on gender alone, the two populations are indeed similar.

Comparisons between the survey sample and inhabitants of Ribeirão Preto, according to the 2010 Census conducted by the Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística*)(17) are shown in table 4.13.

Table 4.13. Comparison between KAPs Survey Sample and 2010 Census, by Gender and Educational Levels.

Gender	**Frequency**	**Percent KAPS (%)**	**Census (%)**
Male	1074	53.25	52.01
Female	943	46.75	47.99
Educational Level[a]	**Frequency**	**Percent KAPS (%)**	**Census (%)**
Incomplete/ No Primary Education	456	22.85	36.74
Completed Elementary Education, (No or Incomplete Secondary Education)	390	19.54	18.29
Completed Secondary Education, (No or Incomplete Tertiary Education)	806	40.38	28.10
Completed Tertiary Education	294	14.73	16.22
Illegible	27	1.35	
Did not know	17	0.85	
Refused	6	0.30	
Undetermined			0.64

a. X^2 test for difference, p-value<0.001

It was seen that the distribution by gender comparing the study sample and the 2010 IBGE Census for Ribeirão Preto was overall very similar with nearly identical male: female ratios. In fact, a binomial test conducted showed that the proportion of female participants did not differ in a statistically significant way from the 2010 census (not shown). Conversely, distribution of completed educational levels between the study population and the 2010 Ribeirão Preto census differed according to a Chi-Square test with X^2 value of 220, 3 degrees of freedom, resulting in a p-value of <0.0001. It was seen that, overall, the survey sample had higher levels of education than the city of Ribeirão Preto, although a slightly lower proportion of those with completed tertiary education was seen in the survey sample.

4.5. Discussion.

4.5.1. Overall Discussion.

This dengue Knowledge, Attitudes, and Practices study conducted in Ribeirão Preto Brazil produced various new insights into dengue risk and prevention. First, it was shown that residents of Ribeirão Preto are knowledgeable regarding several aspects of dengue. High levels of knowledge were also reported in other dengue KAP studies conducted in Brazil (21,39). A study conducted by Neto et al in 2006 reported high levels of identification of *Aedes* oviposition site among inhabitants of the city of Catanduva (~150 km from Ribeirão Preto), but low levels of knowledge about transmission and clinical manifestations of the disease(40). In our study, mosquito biology knowledge was also high (in particular oviposition-site identification), whereas higher knowledge of clinical manifestations was seen in this study conducted in Ribeirão Preto. In contrast, low knowledge on vector biology was seen in a study in São Sebastião, ~ 450 km Southeast of Ribeirão Preto(41).

Dengue knowledge differed by several factors assessed in the study. First, no difference was seen in calculated dengue score between men and women. In chapter II, it was seen that the incidence rates of dengue were similar for men and women and, as such, findings from this study further emphasize that disease is “democratic” in relation to gender. Dengue, however, was shown to, overall, not be a “democratic disease” in terms of the relation between calculated score and both income and education One-way ANOVA showed that KAPs score differed among income as higher incomes were significantly associated with higher knowledge scores. This finding is consistent with other studies in Brazil, where better/ higher KAPs scores were also detected among higher socioeconomic groups (42,43). However, similar studies conducted in other countries have found no association between socioeconomic position and KAP levels(23–25) and this term was found to be non-significant and did not improve model fit in this study. Accordingly, we believe that while dengue may have several "democratic" aspects as a whole, individuals in higher SES levels may have more access to dengue knowledge as a whole and, as such, may be better educated in preventive behaviors—in fact, education levels were strongly associated with dengue, suggesting that this factor may have a bigger role in dengue knowledge than income. In contrast, it was seen that educational levels were positively associated with higher composite knowledge scores, even upon adjustment by reported previous infection status. This suggests that those with higher educational levels in this study sample are indeed more informed about dengue than those with lower levels; this indicates that greater efforts may be needed to increase dengue knowledge among individuals with lower educational levels. This is also in-line with findings from Chapter II, where it was seen that dengue disproportionally affects lower education individuals. As such, the Municipal Secretariat of Health of Ribeirão Preto should consider tailoring the language and content of future dengue campaigns keeping in mind individuals of lower educational levels.

Higher knowledge scores were seen among residents who have previously had dengue. These findings are similar to those from a dengue KAPs conducted in Jeddah, Saudi Arabia, where study participants with family history of dengue had higher scores than those who did not have such history (26), as well as in another study conducted in Puerto Rico (12). Relatively few studies have assessed this factor and, as such, literature where this association was not found is sparse. As such, it is not possible to compare our findings to that of locations where this association was not seen.

Finally, adults with children living in their households were not found to have higher knowledge scores than those without children as no differences in mean knowledge scores were detected via T-test. Presence of children in the household also was not significant in multivariate models, even upon adjustment. Although the dengue KAPs literature does not appear to contain information regarding presence of children in the household, we hypothesized that awareness of dengue and dengue prevention would be higher among adults residing with children as they would be additionally concerned about dengue risk in their child. These findings set a basis for future studies to assess whether this is the case in other locations. Furthermore, this particular finding suggests that an individual’s knowledge may be independent of relationships with other individuals and that campaigns and interventions should indeed focus on the impact of dengue on individual themselves for greater efficacy. `

4.5.2. Discussion of Strengths and Weaknesses.

Our study is the largest dengue KAPs study ever conducted in Brazil, providing novel insights into knowledge levels in Ribeirão Preto, but has several limitations. One limitation was the use of non-probability-based, convenience sampling to recruit study participants, as this method is prone to selection bias due to many reasons. The sample consisted completely of volunteers who agreed to participate and may not be representative of the city's population. Furthermore, participants may have differed from those who were approached but refused to participate (44,45). More than half of individuals approached in the 26 markets and supermarkets refused to participate, but it is unknown how they may have differed from those who agreed to be part of the survey. Table 4.10 showed that individuals who refused to participate in the study showed similar gender patterns to those who participated. It is not known how the refusal population differed in terms of other sociodemographic criteria, nor is it known how participants and refusals may differ in terms of dengue knowledge.

Another problem related to the use of convenience sampling is that one cannot actually know how the study population differs from the intended sampling population(44–46). As such, sampling errors cannot be estimated from convenience sampling methods thus real-world validity of such findings cannot be determined, (45,47,48). In our study, it is not known how representative the KAPs study population is of the general adult population of Ribeirão Preto at risk of dengue. Given this and the previously mentioned limitation, the onus falls on the researcher to clearly justify using this non-probability sampling methodology(30–33,45). The American Association for Public Opinion Research (AAPOR) recommends comparing available sociodemographic characteristics of surveys to those of well-established assessments (e.g. local censuses) as one potential method for assessing if proper population coverage may be present (45). Results presented in table 4.11, in the population analyses section, provide criteria comparing participants to the city population. As previously mentioned, the survey sample's gender distribution did not vary by gender. Most other dengue KAPs studies have reported very high proportions of female participants(>70% female), especially in Latin America (39,49–51), so we indeed achieved a more gender-balanced sample than seen elsewhere.

The study population had higher overall completed education levels than those of Ribeirão Preto residents, while under-sampling the highest educational category. As such, educational level differences were seen between this sample and the targeted source population, and the Secretariat of Health must consider educational levels in the development of interventions. . Yet, we believe the approach employed in this study was ideal given that the previously mentioned budget and staff constraints would likely have limited the efficacy of probability-based sampling methods; convenience sampling is the easiest, least time-intensive, and cheapest method for survey sampling (44,52); other sampling methods may not have been as effective in this setting. Furthermore, this study does not suffer from two of the three “major problems” generally associated with non-probability samples according to the AAPOR. The AAPOR lists the three problems as exclusion of large numbers of people from the sampling process, reliance on volunteers, and high non-response. As such, findings from our study have applicability to Ribeirão Preto and should be used in shaping dengue preventive policies. First, it can be argued that few people were excluded from the sampling process as almost all potentially eligible adults in Ribeirão Preto have the opportunity to shop at supermarkets on Saturdays; those excluded would likely be individuals who shop at supermarkets not included in the supermarket selection process. Whereas our sample did rely solely on volunteers, we believe that past dengue outbreaks have led to general interest and enthusiasm in participating in preventing dengue as suggested by interactions between Vector Control Agents and Medical personnel in other dengue-related settings in the Ribeirão Preto. Finally, high-response rates were seen in the survey as mentioned in section 4.1.1. To further validate these findings, other methods such as probability-based sampling should be employed in future dengue knowledge assessments in Ribeirão Preto.

Another limitation was the cross-sectional nature of this study. People's knowledge levels at the moment of the survey were measured, making it impossible to know how these levels may have changed over time. This study was conducted during a period of low-transmission immediately following a non-epidemic dengue season. It is possible that this period of lower transmission may have affected participants KAPs, particularly their attitudes and practices, thus potentially leading to altered measurements of the KAPs scores.

As previously mentioned, questions used in this study were either developed from other dengue KAPs or by the research team if no such questions in the dengue KAPs literature had ever addressed them. Although the questions were tested extensively between and within the survey development team and selected vector control agents, no formal validation of the questionnaires was conducted. The studies considered in the questionnaire development process either did not explicitly state if validation was conducted or, sometimes, disclosed that while testing may have been conducted, formal validation was not undertaken (43,53,54). Formal validation benefits the researcher as it allows for weaknesses and strengths of the questionnaire to be assessed, thus contributing to applicability and validity of the survey instrument(55,56). To our knowledge, validation of dengue KAPs questions has only been done in a handful of studies (25,56,57). As our study did not conduct such a validation, caution should be considered when assessing the generalizability of our findings.

Another limitation of our study is that it is subject to response bias as participants may have felt coaxed to respond in a certain manner to appear to be correct to the agent. The may have also unknowingly coaxed the participant into a particular response. To limit this possibility, agents who administered the survey were trained thoroughly to avoid leading participants to a particular response. In addition, three questions had to be removed from the analyses due to unforeseen problems with how the data were recorded: these questions did not allow for differentiation of certain responses. We intended to include these questions in the assembling of the KAPs score and, consequently, were not used in this process.

Few potential associations were able to be assessed in regression analyses, reducing the ability to assess factors such as effect measure modification or confounding. Future iterations of this survey should include more detailed bio-socio-demographic variables so that in-depth epidemiological assessments can be conducted, providing further insights for combating dengue in Ribeirão Preto.

The independent variables that were analyzed explained less than 5% of the variation in the model. This suggests that future questionnaires should incorporate more sociodemographic variables that could be considered potential dependent variables. This may be related to the fact that the knowledge score that was used is an artificial construct and is dependent on the quality and accuracy of the questionnaires used to assemble the score. Accordingly, it is also subject to the errors and problems associated with those questions. It is likely that these issues may have limited the amount of overall regression analyses present in the KAPs literature. Very few reports have been published that use regression analysis in KAPs studies. A search for “knowledge attitudes and practices KAP” yielded 848 articles, but the addition of the keyword “regression” results in just 52 articles (representing 6% of previously found articles). It is no different in the dengue KAPs literature: a “dengue Knowledge Attitudes Practices” keyword search yields 124 articles, but restricting it to articles containing regression analysis keywords resulted in only 8 articles (6.5%)(58). In place of traditional regression analysis, several KAPs studies use a confirmatory analytical technique known as structural equation modeling(59–61). Currently, there is only one known dengue KAPs study that employs this analytical technique(60). Future iterations of this questionnaire, accordingly, should involve fine-tuning of the questions, addition of new relevant sociodemographic questions, and potentially use of structural equation modeling.

Secondly, our study is the largest dengue KAPs studies conducted in the region of Ribeirão Preto, both in terms of participants and number of questions ascertained. Other studies conducted in this region of Brazil had less than 900 participants and primarily involved house-house sampling (8,21,28,40). In fact, we are only aware of one dengue KAPs study conducted world-wide with more participants than this study (26). It has been reported elsewhere that large sample sizes can improve the quality of non-probability based sampling surveys(29), accordingly this likely improved the applicability of the study’s findings. The large sample size allowed participation of people from diverse backgrounds, SES statuses, and regions within the city of Ribeirão Preto, thus improving the characterization of the population’s dengue knowledge. Our study also had a high level of completeness, with most questions having response rates of 90% or greater, so there was overall little missing data.

4.5.3. Conclusions.

Findings from this study suggest that many inhabitants of Ribeirão Preto are informed about several biological and entomological aspects of dengue. It is recommended that the Municipal Secretariat of Health conduct more KAPs studies in the future to assess how said campaigns and other preventive measures are affecting inhabitant's dengue KAPs levels. The Secretariat could use the findings presented here to create new iterations of the questionnaire to assess population KAPs in greater depth while also including using potentially more representative sampling methods using probability-based methods. Because our study population generally had higher educational levels than that seen in the city census, future sampling methods should account for these differences.

We hope that by sharing the findings from the survey with the Municipal Secretariat of Health of Ribeirão Preto, preventive campaigns will be enhanced and dengue risk will be eventually reduced. Our finding that higher education and higher income levels are associated with higher knowledge in Ribeirão Preto suggests that targeted campaigns among those with lower income and educational levels could be useful. By conducting further studies, the Secretariat can adjust their policies and potentially combat and prevent dengue in a more effective way.

Overall, this was study successful in assessing the dengue knowledge of the inhabitants of Ribeirão Preto, Brazil. This study was not only the largest dengue-KAPs study conducted so far in Brazil, but it also stands out for its novel participant sampling methodology, amount of questions asked, and particular questions asked in the survey.

The findings in this study suggest that the inhabitants of Ribeirão Preto have moderate levels of dengue knowledge and future campaigns should continue to focus on improving dengue knowledge, Finally, we believe our study contributes new insights to the body of dengue KAPs literature and hope that it will be used as a base or in consideration for other KAPs studies elsewhere.

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Chapter V

Dengue in Ribeirão Preto: Findings and Implications of Epidemiological Analyses, 2003-2012.

5.1. Introduction.

Dengue fever represents an ensemble of clinical manifestations caused by an one of four serotypically distinct viruses transmitted by the bite of an infected female *Aedes* mosquito. Infection with one serotype does not provide lasting immunity against other serotypes and, in fact, may actually lead to more severe disease (1–3). In 2013 alone, the Pan-American Health Organization (PAHO) reported that over 2.3 million cases of dengue were detected in the Americas(4). Approximately 65% of those cases occurred in Brazil, where all four viral serotypes are known to circulate(4,5). The etiology of dengue is complex, with dengue risk being affected by factors ranging from population structure to meteorological patterns and persona-level factors(Biswas et al., 2012; Blanton et al., 2008; Fried et al., 2010; Halsey et al., 2012; Montoya et al., 2013; Sierra, García, Pérez, & Morier, 2006; Silva et al., 2010; Souza et al., 2013; Al-Dubai et al., 2013; Ali-Risasi, Mulumba, Verdonck, Vanden Broeck, & Praet, 2014; Behnaz, Mohammadzade, Mousavi-E-Roknabadi, & Mohammadzadeh, 2014; Ibrahim et al., 2009(6–13)). To assess the relation between these three factors and dengue, we conducted three independent studies in Ribeirão Preto Brazil.

Ribeirão Preto is a city of ~650,000 inhabitants located in southeastern Brazil (21.2° S, 47.8° W)(14). Ribeirão Preto suffers from quasi-yearly outbreaks of dengue virus transmission(15) and all four serotypes have been detected in the city during the past decade (16). To assess population structure in relation to dengue we analyzed 10 years of routine, mandatory dengue surveillance data compiled by the Municipal Secretariat of Health and compared it to various factors of the 2010 Brazilian National Census (17), as presented in Chapter II. In chapter III, we combined the surveillance dataset with a local agriculture-oriented meteorological dataset (18) to analyze the impact of weather on dengue,. Finally, a large survey was undertaken to explore dengue knowledge among residents of Ribeirão Preto in 2012, focusing on mosquito biology, disease recognition, and preventive measures. In this Chapter V, a summary and synthesis of results along with a discussion of the impacts of our findings is presented. We also discuss future research directions, and present suggestions that may help the Municipal Secretariat of Health of Ribeirão Preto improve dengue prevention and preparation for disease outbreaks.

5.2. Summary and Impact of Findings.

5.2.1. Chapter II: Dengue in Ribeirão Preto, 2003-2012: Understanding Population Structure and Dengue Susceptibility.

As previously mentioned, Ribeirão Preto has suffered much from the impact of dengue. Accordingly, analysis of a multi-year, extensive, surveillance database consisting of all reported dengue cases in the city to epidemiologically characterize the patterns of disease among residents of Ribeirão Preto was conducted. Data came from a public health surveillance database, known as SINAN, which is managed by the

Municipal Secretariat of Health of Ribeirão Preto (*Secretaria Municipal da Saúde de Ribeirão Preto*) as mandated per the Brazilian Ministry of Health (*Ministério da Saúde*). Patients deemed to have a suspected dengue infection as per the opinion of a qualified healthcare professional between January 1st, 2003 and December 31st, 2012, had information collected according to Municipal Secretariat of Health's standard guidelines at the aforementioned public health-centers, some with referrals from private-sector medical facilities.

Annual incidence rates varied from 0.85 to nearly 500 per 10,000 during the 10 years of the study. No statistically significant differences were seen in incidence rates between men and women in Ribeirão Preto. No statistically significant trends were seen in terms of age differences over the study period. Dengue also varied by racial/ethnic groups and by educational levels, with dengue risk being inversely related to educational levels. The final part of characterizing the surveillance database suggested that more severe clinical manifestations of dengue may be occurring in Ribeirão Preto than what was reported by the Municipal Secretariat of Health. Results suggested that approximately one-third of Ribeirão Preto residents were infected with dengue during the study period.

Findings from Chapter II have contributed to a relatively sparse epidemiological literature for this city which is the largest metropolitan area in the northern portion of São Paulo state and the Brazilian "agri-business" capital (17,19). From a PubMed search that identified 60 studies on multiple aspects of dengue in Ribeirão Preto since 1991 (20), most had addressed clinical disease, virological characteristics of dengue, or potential anti-dengue vaccines (21–31). However, only three recent studies were found that focused on more traditional epidemiological aspects of dengue in Ribeirão Preto involving molecular epidemiology, a SARIMA model forecast, and a surveillance report for 1994-2003 (19,32,33). Our study builds on the findings of Hino et al.'s (2003) surveillance assessment study, going much more in depth into various epidemiological characteristics of dengue from 2003 through 2012. This study also showed that dengue is not always a democratic disease as it was seen that the distribution of various sociodemographic variables among reported cases of dengue differed from what would be expected solely on census results for the city of Ribeirão Preto. In particular, this study shows that those with lowest levels of education were disproportionately affected by dengue, demonstrating inequity in disease risk. As such, this further emphasizes the need to assess population structure to understand disease risk at a more detailed level. This study also shows the need for robust routine dengue surveillance systems to better understand how population structure is related to dengue, as analysis and interpretation of findings were limited by incomplete and/or missing data.

5.3.2. Chapter III: Relationship between Dengue Fever Incidence and Weather Variation in Ribeirão Preto, Brazil, 2003-2012.

Relationships between climatic and weather variables and the distribution and incidence of dengue have been the subject of many published studies. Some have demonstrated that climate patterns and weather variables affect *Aedes* mosquito life cycles and/or are associated with risk of dengue. Examples of such weather variables that have been studied include maximum and minimum temperatures, extreme and weekly average temperatures, relative humidity, vapor pressure, and rainfall (6–8,34–43). In order to evaluate the epidemiological importance of weather as a possible influence on patterns of disease in Ribeirão Preto, we analyzed relationships between the same dengue case data reported above and weather data from the Integrated Center for Agro-Meteorological Information, CIIAGRO (*Centro Integrado de Informações Agrometeorológicas*). These analyses explored various time lags associated with mosquito life-span and development. To address the different temporal scales over which data were reported, analyses were performed on one week intervals throughout the 10-year study period. Variables assessed included absolute maximum and minimum weekly temperatures, mean weekly temperature, and weekly total precipitation. A variety of statistical approaches to assessing weather-dengue relationships have been used previously, including time series approaches (such as spectral analysis and ARIMA) that allow for the identification and even isolation of seasonal effects and thus permit identification of patterns that may explain and predict unusual dengue disease outcomes. Other studies have also used autoregressive linear models, generalized estimating equations (GEEs), generalized additive models (GAMs), multivariate ARIMA models, and even Vector Autoregressive Models (44–52). Ultimately, we used a generalized linear model (GLM) with a negative binomial distribution, following methods similar to those found in other studies as this approach has been shown to be effective in assessing the dengue-weather relationship(6,7,10,53–56). It has also been shown to be successful in forecasting dengue cases(7,10). Finally, this model can be employed with relative ease by health agencies that may have computational or statistical limitations, which is often the case in developing nations.

Initial univariate descriptions of weather variables showed clear seasonality, especially with precipitation. Increases in minimum temperature and precipitation were the variables that showed the strongest explanatory power in the dengue-weather relationship. Multivariate regressions showed that minimum temperature at week 6 and precipitation at week 8 were related to more dengue, and a statistically significant interaction between temperature and precipitation was also seen in the regression. All predictors were positively associated with dengue incidence except for the interaction term. Inclusion of terms accounting for outbreak years and seasonality improved model fit. The next step will be to validate these findings against dengue surveillance data from 2013 and 2014 when available. Although our study did not use true time-series methods, similar methods have shown much success in forecasting dengue related-aspects, including disease outbreaks (7,10).

Results reported in Chapter III also contribute to the existing body of literature regarding dengue and weather-related predictors as the first one for the city of Ribeirão Preto. In particular, this study contributes to the literature for the dengue-weather relationship for the State of São Paulo (57–65). We hope that our study may be useful to the public's health in the city, as it may permit forecasting of dengue risk by the Municipal Secretariat of Health upon validation and fine-tuning.

5.3.3. Chapter IV. People's Knowledge of Dengue in Ribeirão Preto, Brazil, 2012: Implications for Risk Reduction.

In order to better understand the importance of individual-level risk factors for dengue, especially how individuals' knowledge may contribute to vector abundance and consequently dengue transmission, we evaluated dengue knowledge using a knowledge, attitudes, and practices (KAPs) survey among residents of Ribeirão Preto. The questionnaire was created by a multidisciplinary staff consisting of medical doctors, epidemiologists, nurses, and vector-control staff familiar with the study site and the local culture.

This study found that survey participants were knowledgeable about several aspects of dengue disease. Knowledge levels, measured via a calculated knowledge score, varied by various sociodemographic characteristics assessed in the survey. Knowledge scores were positively associated with educational levels, income, previous dengue infection, and self-rated dengue score. Scores did not vary by sex or whether children resided in the same household as survey participants. Similarly to the calculated knowledge score, mean self-rated scores also varied by educational levels and previous infection. In contrast to the calculated knowledge score, differences were seen in self-rated score by sex, with women having a higher mean calculated score than men. No statistically significant differences were seen by child residing in the household.

This study contributes new insights to the general dengue knowledge literature, and particularly to a better understanding of the situation in Brazil. This study used a super-market based sampling strategy in an effort to reach otherwise hard-to-reach populations such as high SES individuals residing in gated communities. However, unknown sources of bias may remain as a result of the non-random nature of the sampling strategy employed in this survey. However, the large number of participants and high response levels, combined with similarities with the study population and census-findings, suggest that findings may diminish these potential biases (66). Findings from this survey will contribute to the both local and general dengue knowledge assessment literature and will provide a basis for future government campaigns aimed at curbing dengue incidence, particularly in producing campaigns targeted as different sub-populations in Ribeirão Preto.

5.3.4. Overall Impact of Findings.

Research presented in chapters II through IV represents a wide range of findings and, consequently, many potential implications. First, these three studies add to the overall epidemiological literature of the city and the region and provide a basis for public health measures by the local Secretariat of Health. Furthermore, these studies provide a basis for both practice-driven analyses aimed at comparing generalized epidemiological findings and more theoretically-based efforts comparing different methodologies. This is particularly true for chapter III. Several methods are present in the dengue literature and comparing said methods would potentially provide essential insight into both the relationship between dengue and weather-related aspects, as well as further the understanding of complexity-applicability trade-offs associated with the different analytical methods. Furthermore, the knowledge survey creates a base for subsequent in-depth assessment of the applicability of a novel dengue-related survey-recruiting method, as well as for other diseases in similar situations.

These three studies also provide the Municipal Secretariat of Health of Ribeirão Preto with several opportunities that can potentially be integrated into short and long-term public health planning. Findings from Chapter II allow the Secretariat to better understand the population it serves while allowing the Secretariat to see how dengue has impacted the city in a more in-depth level than they would otherwise be able using their current methods. Findings from Chapter III, upon validation of the model, may provide the Secretariat with a tool to forecast dengue cases and properly prepare for future outbreaks of the disease. Accordingly, findings from these two chapters may allow for more fine-tuned development of preventive strategies, and better allocation of resources to properly prepare for the financial impact and personnel needs associated with dengue outbreaks. Chapter IV provides insight into population knowledge, which can serve as a base for future educational campaigns aimed at curbing mosquito ubiquity and dengue incidence. It also provides a basic framework that the Secretariat can use in future assessments of several factors related to dengue knowledge, attitudes, and practices in Ribeirão Preto. Finally, our findings might be used to improve relations between the Secretariat and city inhabitants, as divulging these findings further demonstrates the Secretariat's commitment to improving local public health.

Hopefully, these studies will strengthen collaborations between academic scientists and government public health workers, something which has previously been recognized as important by other researchers in Ribeirão Preto (32). Our study involved a multidisciplinary team composed of medical doctors, epidemiologists, nurses, vector control agents, and many others. This diversity of professions and educational background was essential to the success of these studies. We also believe that our studies are a good example of how multidisciplinary international collaborations can produce new and useful knowledge. These efforts were only possible because of extensive collaborative work of researchers from two large research-oriented universities from the United States and Brazil: the University of Michigan School of Public Health and the Medical School of Ribeirão Preto of the University of São Paulo, respectively. Furthermore, the knowledge survey showed the potential impact of collaboration involving private companies and public groups: the privately-owned supermarkets made for effective recruiting locations as evidenced by how representative the study population appears to be of Ribeirão Preto. Finally, our studies show that such multi-pronged approaches can produce real-world, public health results without the need of extensive financial backing. The first two studies used already existing data and the third was financed through the use of piecemeal funding sources; no large governmental or privately funded grants were used in this study. For all of these reasons, we believe these three investigations have produced meaningful impacts both in terms of advancing knowledge as well as strengthening the opportunities and desirability for further similar collaborations.

5.4. Suggestions for the Municipal Secretariat of Health.

We hope that our findings and experiences are useful to the Municipal Secretariat of Health. To that end, various suggestions are offered. First, based on our experience and findings, the Secretariat should continue to regularly and systematically gather and analyze surveillance data. In fact, the Secretariat may consider expanding its routine surveillance data collection to include more sociodemographic variables needed for more in-depth dengue analyses. By doing so, the Secretariat could potentially detect changes in dengue patterns that might impact disease risk among residents of the city. For example, increases in the proportion of pediatric dengue cases to adult cases could suggest that the Secretariat should perhaps prepare by increasing spending on pediatric clinics and improving pediatric care. Second, we believe the Secretariat might engage in research involving determinants of healthcare use among individuals with suspected dengue infections. Third, the Secretariat is likely to improve its ability to serve people's needs by continuing collaborations with academic researchers to further their insights into and understanding of dengue and other public health problems. Fourth, it should be very useful to conduct studies aimed at characterizing the dengue mosquito vectors in Ribeirão Preto, even though severe limitations with current vector assessment methods have been shown (67). Fifth, public health strategies with the specific goal of improving dengue knowledge and diminishing dengue incidence among lower income and education groups should be employed as these groups were found to have the lowest levels of knowledge and individuals with the lowest educational levels were disproportionately affected by dengue. Finally, we also suggest conducting studies aimed at assessing geospatial patterns of dengue in Ribeirão Preto.

The knowledge survey found that, overall, respondents had moderate-high levels of knowledge related to several factors of dengue. As such, we suggest that the Secretariat pair with professional advertising and marketing agencies (e.g. for a *pro-bono publico* project) to produce more effective and targeted public-health campaigns to continue improving dengue knowledge in Ribeirão Preto, especially among populations with lower income and education levels. Furthermore, we believe the Secretariat would benefit by conducting KAPs and other knowledge-related surveys. In particular, it would be useful to undertake yearly assessments of how the city's knowledge levels are changing over time. As our study was cross-sectional in nature, we cannot evaluate whether and how peoples' knowledge has been changing. By conducting annual surveys, the Secretariat should have the ability to periodically adjust their preventive campaigns. Furthermore, by conducting such surveys in schools, proper age-targeted dengue preventive campaigns might be developed. School-based studies showed that students had inadequate knowledge levels of dengue and dengue prevention(68,69), making them an important population to target in dengue prevention (70). By continuing to explore populational dengue KAPs, we believe the Secretariat will improve the effectiveness of their dengue prevention campaigns.

5.5. Future Research Directions.

Although our studies resulted in numerous findings of interest, there are a number of areas of research that remain unexplored. First, we propose more research be done to assess weather-dengue relationships in Ribeirão Preto. As indicated in Chapter 3, we plan on validating our models against surveillance data from 2013 and 2014 when they become available. We also proposed to compare the GLM models we developed to other methods such as equations (GEEs), generalized additive models (GAMs), multivariate ARIMA models, and Vector Autoregressive Models, while using the same data. We also propose using different software programs that allow for complex time-series analysis such as WinRats and EViews (71,72), as well as open-source programs such as Cronos (73). By acquiring financial and economic data from the Ribeirão Preto government, and combining it with dengue surveillance data, it would be useful to assess the economic burden of dengue in the city. There are also plans to use our survey questionnaire in São José do Rio Preto, where a KAPs-like survey was conducted in 1996 (74). We also hope to conduct similar studies in other locations to allow for comparative analyses. Finally, use of other places of business for study population recruitment besides markets and supermarkets should also be assessed for future studies.

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APPENDIX A.

Questionnaire – Dengue Knowledge, Attitudes and Practices,

Ribeirão Preto, Brazil.

Sociodemographic Data:

A. Sex: [1] M [2] F [999] Refused

B. Place of Birth City ____ State ______ Other ____________________ [999] Refused

C. Age: __________ [888] Unknown [999] Refused

D. Profession

____

[888] Unknown [999] Refused

E. Address

____

Number_____________

[888] Unknown [999] Refused

F. Estimated Distance from Supermarket: _______ [444]Does not live near [888] Unknown [999] Refused

G.

H. Highest Completed Educational Level

____

[888] Unknown [999] Refused

I. Family Income

_____________ Monthly Minimum Salary or R$_____________ [888] Unknown [999] Refused

J. How many people live in your household?

_______ [888] Unknown [999] Refused

K. Are any of them under the age of 18? If yes, how many?
_______ [555] No tem [888] Unknown [999] Refused

KAPS:

1. Have you previously had dengue? [1] Yes [2] No [3] Yes, [888] Does not know [999] Refused
    a. If YES, was it confirmed via laboratory testing?
        [1] Yes [2] No [888] Does not know [999] Refused

2. On a scale of 0 to 10, what grade would you give yourself on your knowledge of dengue?
    ____________ [888] Unknown [999] Refused

3. Do you know what a mosquito breeding site is? [1] Yes [2] No [999] Refused
    a. Describe those you know:
    [__] drain [__] outdoor drain[__] gutter [__] tire [__] decorative plants [__] plant base (plate)

    other:____
    ____

4. How often do you check for the presence of breeding sites in your home?

    [1]More than once per week   [4] Does not check

    [2] Once per week   [888] Does not know

    [3] Less than once a week   [999] Refused

5. Does the dengue mosquito produce eggs [1] Yes [2] No [888] Does not know [999] Refused
    a. If Yes, do you believe the mosquito can produce an egg already contaminated with dengue?
        [1] Yes [2] No [888] Does not know [999] Refused

6. If you did not take care of your house, where do you believe the most likely locale for the mosquito to deposit its eggs would be?

____
_____________________________ [888] Does not know [999] Refused

7. What's the most likely place in your neighborhood that you believe that the mosquito would lay its eggs?

____
____

[888] Does not know [999] Refused

8. How many times can you get dengue?__________(number) [777] Other [888] Does not know [999] Refused

9. Do you know how one gets dengue? [1] Yes [2] No [888] Does not know [999] Refused
   How? [1] Mosquito Bite [2] Other [888] Does not know [999] Refused

10. Can you have dengue without exhibiting any symptoms? [1] Yes [2] No [888] Does not know [999] Refused

11. Do you know what dengue hemorrhagic fever is? [1] Yes [2] No [888] Does not know [999] Refused
    a. Please describe what it is:

____
____

[888] Does not know [999] Refused

12. Are there any symptomatic differences between dengue and the flu? [1] Yes [2] No [888] Does not know [999] Refused

13. Is there a vaccine against dengue?[1] Yes [2] No [888] Does not know [999] Refused

14. How does one treat dengue?

____

____

[888] Does not know [999] Refused

15. Do you think dengue can be more severe the second time you get it?
[1] Yes [2] No [888] Does not know [999] Refused

16. Do you believe the risk of dengue varies with people's financial status?
[1] Yes [2] No [888] Does not know [999] Refused

17. Do you believe some neighborhoods have more dengue than others?
[1] Yes [2] No [888] Does not know [999] Refused

a. Why?

____

____

18. Do you consider dengue to be a disease associated with poverty?[1] Yes [2] No [888] Does not know [999] Refused

a. Why?

____

____

19. How does one prevent dengue?

____

____________________ [888] Does not know [999] Refused

20. Who is responsible for preventing dengue?
[1] Government [2] Citizen [3] Both [4] Other [888] Does not know [999] Refused

21. Do you believe that removal of potential breeding sites at the household level should be obligatory by law to prevent dengue?
[1] Yes [2] No [888] Does not know [999] Refused

22. If your neighbor has obvious breeding sites, would you talk to him/her?
[1] Yes [2] No [888] Does not know [999] Refused

23. If your neighbor refuses to remove the breeding site, would you denounce him/her (assuming it could be done anonymously)?
[1] Yes [2] No [888] Does not know [999] Refused

a. If not, why?

____

24. Who should you contact if there are breeding sites present in your neighborhood?

____

[888] Does not know [999] Refused

25. If you were to find a large breeding site in your neighborhood that is not in a household, do you have the responsibility to contact the sanitary authorities? [1] Yes [2] No [888] Does not know [999] Refused

26. Whose responsibility is it to clean abandoned lots with breeding sites?
[1] Owner of the lot [2] Government [3] Both [4] Other [888] Does not know

[999] Refused

27. Do you wash the sidewalk in front of your house?
[1] Yes [2] No [888] Does not know [999] Refused

a. If yes, how frequently?

[1] More than once per week [4] Less than once per month

[2] Once per week [5] Other

[3] <u>Less</u> than once per <u>week</u>, but *more* than once per *month*

28. How frequently do you verify the cleanliness of your gutters?

[1] Once per month or more frequently [5] Only when it rains

[2] Between once per month & once every 6 months [6] Does not do it

[3] Between once every six months and a year [888] Does not know

[4] Once a year or less [999] Refused

29. Are the screens on your windows? [1] Yes [2] No [888] Does not know [999] Refused

30. Why do you believe there were less diagnosed cases of dengue in Ribeirão Preto this past year?

____
____

31. If you believed you maybe had dengue, how long would you wait before visiting a healthcare professional?

____

[888] Does not know [999] Refused

32. Do you use some sort of repellent or natural protection on either yourself or your family members to not get bitten? [1] Yes [2] No [888] Does not know [999] Refused

a. If yes, what do you use?

____
____

[888] Does not know [999] Refused

33. Is there an outdoor (rain) drain in your house? [1] Yes [2] No [888] Does not know [999] Refused

a. If Yes, how often do you clean it? _______________
Does not know [999] Refused

34. If there were a dengue vaccine available in the healthcare centers, would you get vaccinated?
[1] Yes [2] No [888] Does not know [999] Refused

35. Are you in favor of fines for people who refuse to clean mosquito breeding sites in their property?
[1] Yes [2] No [888] Does not know [999] Refused

36. Do you believe that educating children at school about dengue could have a positive result in the fight against dengue? [1] Yes [2] No [888] Does not know [999] Refused

APPENDIX B.

Calculated Knowledge Score Assembly.

A2.1. Description: Points attributed to composite questions (questions consisting of primary and secondary questions, as described in Chapter 4) as well as individual questions (those that did not consist of more than one part) could range from -3 to +3 points. Within composite questions, points are distributed within primary and secondary questions. Note that language of questions presented here is based on the English-language version of the questionnaire and does not reflect the linguistic aspects employed in the Portuguese-version. Scores were assembled by addition of the Knowledge sub-scores, offset by 27 points as -27 points was lowest possible score and, as the idea of negative “KAPs” score was deemed impossible by research staff, all scores were offset by +27 points. *Correctness* in this appendix refers to questions decided as being true/ correct or false/incorrect based on decision of a multi-collaborative team of epidemiologists, clinicians and epidemiological nurses as well as use of clinical guidelines(1–3). No points were added or deduced if participants did not answer, refused to answer, or replied they did not know the response.

A2.2. Key:

Question (Number): Refers to question and its number within the questionnaire (Appendices 1 and 3). *Points:* Description of how points were awarded for this question.

.**A.2.3. Knowledge Questions and Knowledge Points:**

Question 3: Do you know what a mosquito breeding site is? [1] Yes [2] No [999] Refused

Describe those you know:

[__] drain [__] outdoor drain[__] gutter [__] tire [__] decorative plants [__] plant base (plate)

other:____
Points: 0 points for primary; up to 3 points for secondary question—each correct site was awarded 1 point up to 3 points.

Question 5: Does the dengue mosquito produce eggs [1] Yes [2] No [888] Does not know [999] Refused

a. If Yes, do you believe the mosquito can produce an egg already contaminated with dengue? [1] Yes [2] No [888] Does not know [999] Refused
Points: 2 points for answer Yes in primary question; -2 points for answering No in primary question; 1 point for answering Yes to secondary question 5a, -1 for answering No.

Question 8. How many times can you get dengue?__________(number) [777] Other [888] Does not know [999] Refused
Points: 3 points for responding 4. -3 points for incorrect responses.

Question 9. Do you know how one gets dengue? [1] Yes [2] No [888] Does not know [999] Refused

How? [1] Mosquito Bite [2] Other [888] Does not know [999] Refused
Points: 3 points for responding Mosquito bite, -3 points for Other

Question 10. Can you have dengue without exhibiting any symptoms? [1] Yes [2] No [888] Does not know [999] Refused
Points: 3 points for Yes, -3 points for No

Question 11. Do you know what dengue hemorrhagic fever is? [1] Yes [2] No [888] Does not know [999] Refused .a. Please describe what it is:

---

[888] Does not know [999] Refused
Points: 3 points for a correct answer to question 11a , -3 for an incorrect answer to question 11 a.

Question 12. Are there any symptomatic differences between dengue and the flu? [1] Yes [2] No [888] Does not know [999] Refused
Points: 3 points for Yes; -3 points for No

Question 13. Is there a vaccine against dengue?[1] Yes [2] No [888] Does not know [999] Refused
Points: 3 points for Yes; -3 points for No

Question 14. How does one treat dengue?

---

[888] Does not know [999] Refused
Points: 3 points for clinically correct treatment. 1 point for partially clinically correct treatment (meaning mostly correct treatment and presence of incorrect treatment), -1 point for partially clinically correct treatment meaning mostly incorrect treatment and presence of some correct treatment), and -3 for completely incorrect answer.

Question 15. Do you think dengue can be more severe the second time you get it?

[1] Yes [2] No [888] Does not know [999] Refused
Points: 3 points for Yes; -3 points for No

Question 19. How does one prevent dengue?

____

[888] Does not know [999] Refused
Points: 3 points for correct preventive methods. 1 point for partially correct preventive methods (meaning mostly correct preventive methods and presence of incorrect correct preventive methods), -1 point for correct preventive methods meaning mostly incorrect correct preventive methods and presence of some correct preventive methods), and -3 for completely incorrect answer.

Total Possible Points: 33.

A2.4. Bibliography

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