AEDES AEGYPTI DENSITY AND RISK OF DENGUE VIRUS SEROCONVERSION

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ABSTRACT

Elizabeth A. Cromwell: *Aedes aegypti* Density and Risk of Dengue Virus Seroconversion
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Routine entomological monitoring data are used as a surrogate for overall risk of dengue virus (DENV) infection and to trigger implementation of control interventions. Indicators that characterize *Aedes aegypti* abundance have not consistently been associated with an increased risk of dengue virus (DENV) seroconversion. Using longitudinal entomological and serological data from Iquitos, Peru, this dissertation estimated the risk of DENV infection for several entomological indicators to determine if any measure of *Ae. aegypti* abundance was associated with transmission.

Entomological survey data from two longitudinal cohort studies linked with 8,153 paired serological observations were analyzed. Indicators of *Ae. aegypti* density were calculated from entomological. The risk ratios (RR) estimating the association between *Ae. aegypti* abundance at the household and block levels and the six-month risk of DENV seroconversion were obtained. Cross-sectional *Ae. aegypti* densities were not associated with an increased risk of DENV seroconversion. Longitudinal measures of adult stage density resulted in adjusted RRs ranging from 1.01 (95% CI: 1.01, 1.02) to 1.30 (95% CI: 1.17, 1.46) and categorical immature indices (RRs ranging from 1.21 (95% CI: 1.07, 1.37) to 1.75 (95% CI: 1.23, 2.5)).

A total of 90,046 entomological monitoring observations were used to model the space/time covariance of ln(adult *Ae. aegypti* per m^2). Mosquito density modeled using the Bayesian Maximum Entropy (BME) geostatistical framework was associated with an increased

risk of DENV infection among densities ranging from 0.005 to 0.01 mosquitoes per m^2 (adjusted risk ratio: 1.14; 95% CI: 1.01, 1.28). A multi-level logistic model was used to test for heterogeneity of the association between DENV risk and longitudinal measures of *Ae. aegypti* density. The multi-level model results suggest that the population-level risk ratios are more appropriate estimates of the *Ae. aegypti*-DENV seroconversion association.

Ae. aegypti densities calculated from repeat entomological monitoring were associated with DENV seroconversion, whereas estimates of *Ae. aegypti* abundance measured cross-sectionally were not. It is possible that *Ae. aegypti* populations exhibit too much variability across space and time for periodic, cross-sectional measurement to adequately characterize entomological risk, in addition to having no correlation with true infection events due to human movement in space and time.

To Frank.

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LIST OF ABBREVIATIONS

AS	Activity Space
AIC	Akaike Information Criteria
BI	Breteau Index
BHK21	Baby Hamster Kidney Cell
BME	Bayesian Maximum Entropy
CI	Container Index; Confidence Interval
CDC	Centers for Disease Control and Prevention
DENV	Dengue virus
DENV1	Dengue virus (Serotype 1)
DENV2	Dengue virus (Serotype 2)
DENV3	Dengue virus (Serotype 3)
DENV4	Dengue virus (Serotype 4)
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
ECDC	Entomological Correlates of Dengue Control
G-KB	General Knowledge Base
GEE	Generalized Estimating Equation
GWR	Geographically Weighted Regression
HI	House Index
IQR	Inter-Quartile Range
LRT	Likelihood Ratio Test
MAUP	Modifiable Areal Unit Problem

NAMRU-6	Naval Medical Research Unit
PRNT	Plaque Reduction Neutralization Test
RR	Risk ratio
S-KB	Site-specific knowledge base
SES	Socio-economic status
SD	Standard Deviation
S/TRF	Space/time random field
UCD	University of California, Davis
WHO	World Health Organization
ZIKV	Zika virus

CHAPTER 1: SPECIFIC AIMS

1.1 Introduction

Dengue virus (DENV), an RNA virus transmitted by the bite of *Aedes aegypti* mosquitoes, causes more human morbidity and mortality than any other arthropod-borne virus worldwide. Since the 1950s, dengue has spread via the globalization of trade and travel, rapid urbanization and the expansion of vector habitats.[1] At least 128 countries are now considered to have endemic transmission, with the highest burden of dengue in the Americas and South-east Asia. The four serotypes (DENV1, DENV2, DENV3 and DENV4) occur throughout the tropics and infect approximately 390 million persons per year.[2] The most severe manifestation of dengue infection is dengue hemorrhagic fever, which can lead to dengue shock syndrome and can be fatal.

Ae. aegypti are daytime-biting, domesticated mosquitoes highly adapted to the human urban environment. This human-vector relationship is mediated by social and economic factors that govern human movement through time and space, as well as environmental factors that influence fluctuations in *Ae. aegypti* populations.[3, 4] In tropical and subtropical urban areas, the expansion of human habitats via urbanization has generally out-paced improvements in water, sanitation and housing infrastructure necessary to eliminate mosquito breeding sites and opportunities for human contact with *Ae. aegypti*.[5] High concentrations of *Ae. aegypti* within or around a household present an opportunity for DENV transmission among household members.

Monitoring vector abundance is recommended by the World Health Organization (WHO) for the deployment and evaluation of vector control interventions.[5] The public health utility of these indicators relies on the assumption that greater mosquito abundance increases the risk of DENV transmission, and therefore reducing exposure to the vector reduces infection incidence. Further, by identifying "hot spots" of *Ae. aegypti* infestation, targeted vector control would be an efficient use of limited intervention resources.[6] There is no established threshold of *Ae. aegypti* density associated with an increased risk of human DENV infection[7] and prior studies have shown no consistent association between various indices and dengue outcomes.[8]

To help predict risk and direct public health interventions, there is substantial interest in an improved understanding of the relationship between measures of mosquito density and DENV infection, according to mosquito life stage and unit of measurement. The objective of this dissertation was to use longitudinal data on mosquito density and human serology to test for quantifiable associations between *Ae. aegypti* indices and an estimated 6-month risk of DENV seroconversion. The analysis is an ancillary study of extant longitudinal data collected from 1999-2003 and 2008-2010 in Iquitos, Peru.[9] These longitudinal data offer a unique opportunity to improve understanding of the relationship between vector abundance and household-level characteristics in an endemic urban DENV transmission setting. While previous work has described the challenges inherent in monitoring mosquito populations and operational limitations of these indicators[10,11] as well as measuring the association with symptomatic dengue,[8] this analysis systematically compares measures of entomological risk with data on human DENV seroconversion.

1.2 Specific Aims

1.2.1 Compare Measures of Exposure to *Ae. aegypti* Constructed with Data from Routine Entomological Monitoring to Identify if any are Associated with the 6-month Risk of DENV Seroconversion

There are multiple indicators used to characterize exposure to *Ae. aegypti* and it is unknown which (if any) best correlate with DENV seroconversion. From a dengue control perspective, there is substantial interest in understanding how measures of mosquito density are associated with DENV infection, according to mosquito life stage and unit of measurement. In this Aim, entomological surveillance data will be used to estimate the association between indicators of *Ae. aegypti* abundance at the household-level and block-level and the risk of DENV seroconversion. *A priori*, adult mosquito indicators will be tested as DENV transmission occurs during the adult life stage; larval and pupae measures will be compared to determine which indicator has the strongest measure of association.

1.2.2 Estimate the association between *Ae. aegypti* densities and the 6-month risk of DENV seroconversion to determine if (1) their utility can be improved via spatial modeling and (2) if heterogeneities in the association exist within the community

In urban settings such as Iquitos, Peru, socio-economic status, housing quality and household-level exposure to *Ae. aegypti* are highly variable at fine spatial scales and may influence transmission at the household and beyond. In this aim, the association between adult *Ae. aegypti* per household area (m^2) and DENV seroconversion will be estimated using a space/time analysis to account for possible measurement error resulting from data collection. A multilevel approach will then be used to determine if heterogeneities in the association between mosquito density and DENV seroconversion exist among different levels of space (block and zone).

CHAPTER 2: DENGUE VIRUS BACKGROUND

2.1 Introduction

Dengue is a viral disease with established endemic and epidemic transmission throughout Asia, Central and South America, and Africa.[2] Dengue disease is characterized by symptoms ranging from a self-limiting fever, often accompanied by headache, arthralgia, myalgia and rash (dengue fever) to potentially life-threatening dengue hemorrhagic fever and dengue shock syndrome.[1, 12] The majority of individuals infected with DENV experience mild to no symptoms, commonly called "inapparent" dengue. The precise mechanism by which some individuals present with severe dengue disease is unknown. Treatment of dengue disease involves supportive care; there is no cure for dengue nor is there a widely-available vaccine.[13]

A recent review of country case reports and prevalence data suggest that a total of 390 million dengue infections occur annually, of which 50-100 million exhibit any form of apparent disease, and approximately 10,000 deaths each year result from dengue disease.[2,14] The geographic scope of dengue has expanded since the postwar period,[15] due to the globalization of trade and travel[16, 17], urbanization[18, 19] and the expansion of vector habitats.[20] At least 128 countries are now considered to have endemic transmission; approximately 14% of global dengue infections occur in the Americas, 16% in Africa and 70% in Asia.[2]

2.2 Transmission of DENV

DENV is transmitted by the bite of the female *Aedes aegypti* mosquito. *Ae. aegypti* is a domesticated mosquito that prefers to lay eggs in artificial containers found in and around

homes, such as automobile tires, rainwater collection buckets, trash, and storage containers.[21] The *Ae. aegypti* mosquito experiences aquatic and terrestrial life stages. Eggs are laid on the interior of containers (manmade or natural) and larvae emerge after the eggs are inundated with water. Larvae feed on organic matter and grow from first to fourth instars over a period of 7-9 days (at 25°C). Once the larvae are large enough (fourth instar), they metamorphose into pupae (2-3 days). Upon adulthood, the mosquitoes emerge from the water and mate, repeating the cycle, with female mosquitoes surviving 8-15 days and males 3-6 days.[22]

Ae. aegypti are daytime-biting mosquitoes and typically do not travel beyond 100 meters in urban settings.[23, 24] An infected female *Ae. aegypti* mosquito can feed on several persons during its gonotrophic cycle (adult stage), resulting in transmission of DENV among members of a household at approximately the same time. Female mosquitoes feed during daylight hours, typically 2-3 hours after dawn and in the afternoon for several hours.[25] Climatic factors, primarily temperature and precipitation, affect mosquito feeding and breeding patterns, and can shorten or lengthen the overall lifecycle.

In the mosquito, the DENV undergoes an extrinsic incubation period of 7-14 days, dependent on temperature.[7, 26] In humans, the virus incubates 3-15 days before symptoms of dengue disease are experienced. Once symptomatic, the acute phase begins and a viremic individual can infect susceptible mosquitoes, as DENV will circulate in human blood for an average of five days; individuals experiencing inapparent dengue are also infectious.[27, 28] Once the virus is re-introduced into the vector population, it will incubate again and the cycle repeats. Adult *Aedes* mosquitoes remain infected with DENV throughout their lifespan, which is on average 1-2 weeks.[22]

Human dengue transmission generally occurs in the urban setting where there are no other DENV hosts[3], and *Ae. aegypti* feed preferentially on human blood.[29] Using data from simulation studies, it has been estimated that a minimum population of 10,000 persons is required to maintain endemic human-to-mosquito DENV transmission; another model estimates a population of at least 150,000 persons is required to sustain transmission.[30, 31] The *Aedes albopictus* mosquito is also capable of transmitting DENV and has expanded its geographic range in the past decade. However, differences in human feeding behaviors suggest that *Ae. albopictus* may be a less competent vector of DENV than *Ae. aegypti,* and is not currently considered a threat for large-scale dengue outbreaks.[32] DENV transmission has also been documented among mosquitoes and primates in forested regions of rural Africa and Asia,[33] but this cycle rarely includes human populations; therefore, it is not of great importance in the establishment of endemic transmission.

The human-vector relationship is mediated by social and economic factors that govern human movement through time and space, as well as environmental factors that influence fluctuations in *Ae. aegypti* populations. In tropical and subtropical urban areas, the expansion of human habitats via urbanization has generally out-paced improvements in water, sanitation and housing infrastructure necessary to eliminate mosquito breeding sites and opportunities for human contact with *Ae. aegypti*.

2.3 Known Risk Factors for DENV Transmission

The primary risk factor for DENV infection is contact with the mosquito vector. Without contact, transmission cannot occur. Therefore, factors associated with an elevated risk of DENV infection are related to contact with the mosquito vector. These factors include housing quality (lack of screens, building type and construction materials), proximity to open breeding sites such

as open containers, and lack of vector control strategies such as personal insecticides and household or neighborhood spraying. Several studies have found an association with lower socio-economic status (SES)[34-36] and DENV infection, but SES is likely a proxy for poorer housing quality and reduced access to environmental sanitation in the urban environment.[37, 38] A study comparing the prevalence of dengue in Neuvo Laredo, Mexico, and Laredo, Texas, found a higher seroprevalence of DENV infection in Neuvo Laredo even though *Ae. aegypti* were more abundant in Laredo, suggesting better economic conditions (vis-à-vis improved housing quality, use of air conditioning, etc.) reduced DENV transmission.[39] Spatial analysis of DENV infection in Brazil has also demonstrated an association between low SES and infection.[36]

2.4 Interventions to Prevent DENV Transmission

All current public health efforts to prevent dengue outbreaks are aimed at vector control to reduce human contact with the mosquito and improve surveillance as the dengue vaccine is not widely available.[5] Interventions designed to reduce human-mosquito contact range from long-term infrastructure improvement at the community-level to household and individual behavior change. The WHO has adopted a target of reducing dengue incidence by 50% by 2020 through a suite of interventions.[40] Among these interventions, the improvement of public infrastructure requires large-scale municipal investment, such as improving public water system delivery and urban sanitation. Household level interventions include elimination of mosquito breeding sites through cleaning of water storage containers, gutters, and other sites where mosquitoes breed such as flower vases and removal of household trash.[5] The promotion of breeding site elimination includes removal or closure of containers that collect standing water. Improvement of housing quality, including the use of screens, is also promoted.

Insecticide-based interventions include indoor residual spraying to reduce interior *Aedes* populations and neighborhood-wide fumigation. The WHO recommends incorporating a monitoring framework to detect insecticide resistance in tandem with these interventions to ensure efficacy. Other novel approaches are under investigation, such as spatial repellents and lethal ovitraps.[40] Biological controls have also been promoted, including the introduction of fish or copepods to water containers (to digest *Aedes* larvae), the use of genetically modified mosquitoes to out-compete the native *Aedes* population and the introduction of the Wolbachia-infected *Aedes* to prevent infection of the vector with DENV.[41]

Some authors suggest that vector control interventions have not adequately reduced the spread of DENV transmission due to poor management of implementation, limited financial investment, poor geographic coverage, late deployment and lack of community involvement.[42] In some settings, community-led dengue control interventions have shown positive results.[43-46] Integration of dengue surveillance and interventions has also been shown effective.[47] The timing of vector control implementation may also play a role in their efficacy, with some researchers suggesting a "proactive" approach to break transmission before an epidemic occurs, rather than intervening after febrile cases have been detected.[48] Vector control interventions have been demonstrated to reduce the population of *Ae. aegypti*.[49] The impact of vector control is based on the hypothesis that reducing human contact with *Ae. aegypti* will reduce the likelihood of exposure to the virus, and therefore prevent transmission. Evidence of long-term efficacy of vector control interventions is varied. It is plausible that if vector control (in its various forms) is currently the only viable intervention available, then targeting these strategies to reach individuals most at risk of contact with *Ae. aegypti* should reduce transmission of DENV.

Targeting vector control interventions to households with high *Ae. aegypti* abundance would be beneficial given limited public health resources, or where large-scale interventions such as mass spraying of insecticides may have limited long-term efficacy due to the development of insecticide resistance.[6] Household level interventions may appeal to individuals in settings where public services may be limited or unreliable. However, as *Ae. aegypti* is a day-time biting mosquito, the relationship between exposure ascertainment at the household level via routine entomological monitoring, primarily of domestic premises, and risk of dengue infection is not clear-cut. Human movement and environmental factors may modify the relationship between household vector abundance and DENV seroconversion.

CHAPTER 3: *AEDES AEGYPTI* MONITORING INDICATORS

3.1 Indicators Used to Measure Exposure to *Aedes aegypti*

Use of *Ae. aegypti* monitoring indicators grew out of yellow fever control programs in the first half of the 20th century.[50] Since then, over twenty indicators have been proposed to quantify abundance of *Ae. aegypti*. Entomological data are typically collected from households over spatial units such as the neighborhood or block on a regular or ad hoc basis. Monitoring data vary by mosquito life stage (adults, larvae and/or pupae) and process of collection (ovitrap v. aspirator, identification of breeding sites, etc.).[51] Since it would be prohibitively expensive and logistically impossible to track individual human-mosquito contact over a large population, over the entire period of a dengue outbreak, entomological surveys are currently the only method available to generate data with which to quantify possible exposure to *Ae. aegypti*. Table 1 presents monitoring indicators that have appeared in the literature. These indicators vary by three primary characteristics: the life stage of mosquito measured (larval, pupal, adult); the level of measurement (household or community); and quantification of exposure (rates, proportions or scales).

3.2 Life Stage Measured

Ae. aegypti pass through three distinct life stages: larval; pupal; and adult. The larval and pupal (immature) stages can be identified and quantified at breeding sites as they cannot emerge from these sites until they reach adulthood. Larvae and pupae are fairly easy to count. Since breeding sites are generally well-characterized in dengue endemic settings, it is possible to

identify larvae and pupae by examining natural and man-made containers for their presence.[51] Among the indices used to measure the population of immature *Ae. aegypti*, the Breteau Index (BI), House Index (HI) and Container Index (CI) are among the most widely used.

Quantification of the adult female *Ae. aegypti* population is likely the most important in terms of measuring risk of DENV infection as adult females are responsible for transmission. Adult mosquitoes are capable of flight (albeit short distances); therefore, adult mosquitoes must be identified and counted within household interior and exterior spaces. Counting the number of adult mosquitoes requires equipment such as aspirators or ovitraps to capture adults and trained data collection personnel to ensure consistency of field measurements. The measurement of adult *Aedes* populations is also difficult to achieve under operational conditions and over a large geographic sample due to variability in mosquito behavior. Adult mosquitoes can move between households, hide in ceilings and interior spaces, so measurement on any given day reflects a spectrum of exposure, possibly from ranging from the true population of mosquitoes to one downwardly or upwardly biased due to factors such as time of day, season and adult mosquito behavior.[10] The potential for measurement error is therefore real.[52] Since adult mosquito measurement is difficult, most dengue surveillance and research rely on indices of larvae and/or pupae quantities as a proxy for adult measures.[6]

Pupal indicators have been proposed as proxy measures of future adult populations as pupal mortality is low compared to larval mortality and pupae are easier to count as well as identify from other species.[53, 54] However, pupal, larval and adult indices may not always correlate. A study in Trinidad observed a statistically significant negative correlation between the CI (a larval measure) and Pupae per Person, which is somewhat implausible.[55] In Iquitos, there was no significant correlation between pupal and adult measures in a 1998 cross-sectional survey.[24]

3.3 Household v. Group-Level Indicators

The level of measurement varies across these indicators as well, primarily at the household or block level. Most entomological surveys are conducted at residential locations. These data are then aggregated to describe block or neighborhood abundance in an attempt to account for entomological risk that might be shared across units of space. Non-residential sites have been proposed as a method to better describe neighborhood vector density but such sites are less commonly surveyed.[56] Aggregated measures of vector abundance are often used to compare one neighborhood to another to determine if differences in entomological patterns (and therefore DENV transmission) exist.

3.4 Quantification of Density

Exposure to *Ae. aegypti* is quantified as either a rate, a proportion or a scale. Mosquito densities are predominantly expressed in rates, either per geographic or population units. Note that some of these rate measures could be converted to a proportion; here they are listed as they have been described in the literature. Pupae per Hectare uses raw count data in the numerator to compare the magnitude of exposure at a specific spatial unit. Rates that employ population data in the denominator include the Pupa Index and Larval Density Index (households in the denominator); Pupae per Person; *Stegomiya* Larval Density Index; Larval Density Index; Pupae per hectare; Pupae per Person; and Pupa Index and the Single Larval Method. Mean Egg Density and Mean Adult Density calculate the rate of mosquito presence per trap. An important distinction among rate indicators is the use of a binary classification in the numerator ("infested/not infested" or "positive/not positive") and a population or spatial unit in the denominator. The following indicators all classify infestation dichotomously: Infested Receptacle Index; Stegomiya Index; BI; and the Potential Container Index. For example, when a mosquito is observed in a container, the entire household is defined as "positive" or "infested".

Therefore, an indicator such as the BI may capture the spatial distribution of *Ae. aegypti* as it accounts for the presence of the vector across multiple households, but it may fail to capture the risk of dengue transmission because it does not account for container productivity.[55]

The following indicators measure the proportion of exposure: the HI; the CI; and the Block Index. These three measures also use a binary classification of mosquito exposure in the numerator ("positive" or "infested"). Finally, two of the indicators used to characterize *Ae. aegypti* can be classified as "scales": the Density Index and the Adult Productivity Index. The Density Index classifies exposure discretely, on a scale of one to nine. The Adult Productivity Index places a value on the container type multiplied by the mean number of larvae observed, which is then summed across all container types found at the household (or premise). These indicators attempt to characterize the magnitude of mosquito infection by incorporating multiple measures of density.

Regardless of whether indicators are presented as a rate or a proportion, if a classification of "infestated" is used in the numerator, the indicator may not account for the productivity of single containers or the magnitude of infestation in a given household. For example, a container with one *Ae*. *aegypti* larva is given the same weight as a container with several. If the contribution of highly productive breeding sites is more important than the absolute number of positive containers, indicators that capture the variability of exposure may be more appropriate. For example, an indicator such as the Potential Container Index may more accurately represent risk compared to the BI or the CI. Although they are more difficult to estimate, indicators that do not rely on a classification of the numerator may better capture the productivity of different breeding sites and their contribution to risk of dengue transmission. Nevertheless, as a single mosquito can transmit dengue to multiple people, measures based on any presence of *Ae. aegypti* may still reflect risk of infection.

3.5 Limitations of Entomological Monitoring Data

Measuring *Ae. aegypti* densities requires large scale entomological data collection that is subject to a variety of limitations. Entomological survey techniques may not capture the fine spatial and temporal variability in an urban setting due to the dynamics of mosquito biology. Indices are effectively prevalence measures, calculated from cross-sectional vector surveillance, not derived from continuous monitoring. Entomological survey data do not capture the daily productivity of individual containers or the activity of individual mosquitoes over their lifespan.[10] Measurement error is also possible due to operational constraints and collection procedures such as skill of collection staff, time available per premise, size of premise, and degree of access permitted by residents.[51]

Adequate sampling of immature and adult populations requires consideration of vector dynamics[53] and spatial relationships.[57] Sampling techniques have been demonstrated to limit the power of longitudinal studies of DENV transmission, as household-level sample sizes used for entomological monitoring often do not exceed 100 households, which may result in bias due to sampling error.[57] A multi-country study of entomological surveys found sample size requirements varied by levels of vector dispersion, suggesting that spatial dynamics affect statistical power.[53] Per person measures may be unreliable due to inaccurate population data, which may vary by location at any given day or season, as humans move across time and space.[57] Rates that capture mosquito populations over a geographic area rather than per person may be better suited to capture the spatial dynamics of transmission, as observed in Brazil[58], but this may vary by local context. Finally, the role of herd immunity is not captured by these indices as entomological data cannot be directly linked in real time to individual seroconversions. Among households with few individuals susceptible to DENV, it is unlikely that any level of vector abundance will be associated with seroconversion.[11]

Despite these limitations, entomological surveillance data are promoted as proxies for exposure to *Ae. aegypti*.[40] Entomological survey measures are also a critical process indicator used to evaluate the effectiveness of vector control interventions in terms of reducing dengue disease, in order to compare pre- and post-intervention mosquito populations. Given these limitations, it is important to determine the potential use of entomological indicators of vector exposure calculated from both observed data as well as using methods to account for possible exposure misclassification arising from entomological data collection.

Table 1. Indices Used to Measure *Aedes aegypti* Density

Index	Formula	Mosquito Life Stage*
House Index/Premise Index/Aedes Index[50]	$\frac{\#\ of\ households\ infested\ with\ larvae\ or\ pupae}{total\ number\ of\ households} \cdot 100\%$	Larvae or Pupae
Container Index[50]/Receptacle Index	$\frac{\#\ of\ containers\ infested\ with\ larvae\ or\ pupae}{total\ number\ of\ containers\ inspected} \cdot 100\%$	Larvae or Pupae
Infested Receptacle Index[59]	$\frac{\#\ of\ positive\ containers}{total\ number\ of\ households\ (premises)}$	Larvae or Pupae
Stegomyia Index[60]	$\frac{\#\ of\ positive\ containers}{population} \cdot 1000\ persons$	Larvae
Breteau Index[61]	$\frac{\#\ of\ positive\ containers}{total\ households} \cdot 100\ households$	Larvae
Density Index[62]	Nine degrees of infestation derived from multi-country assessments of the House Index, Breteau Index and Container Index; use a reference table to match either the Breteau Index, House Index or Container Index to its corresponding value of 1 to 9*	Larvae or Pupae
Stegomyia Larval Density Index[63]	$\frac{\#\ of\ larvae}{population} \cdot 1000\ persons$	Larvae
Larval Density Index[64]	$\frac{\#\ of\ larvae}{total\ households}$	Larvae
Single Larval Method[65]	$\#\ of containers\ with\ \geq 1\ Aedes\ larva$	Larvae
Pupae per Hectare[55]	$\frac{\#\ of\ pupae}{hectare}$	Pupae
Pupae per Person[55]	$\frac{\#\ of\ pupae}{population}$	Pupae
Pupa Index/Pupal Density Index[66]	$\frac{\#\ of\ pupae}{total\ \#\ households\ inspected} \cdot 100$	Pupae
Potential Container Index[94]	$\frac{\#\ of\ potential\ breeding\ sites + \#\ positive\ breeding\ sites}{total\ \#\ households\ (premises)\ inspected}$	Larvae or pupae
Adult Productivity Index[82]	$\sum C_{i-\infty}\ (mean\ L_{i-\infty})$ Where $C_{i-\infty}$ is the frequency of container type and $L_{i-\infty}$ is the mean number of larvae in each container type	Larvae

Table 1 (Continued)		
Index	**Formula**	**Mosquito Life Stage***
Block Index[87]	$\frac{\#\ of\ blocks\ where\ houses\ show\ evidence\ of\ breeding}{total\ \#\ of\ blocks\ surveyed}$	Larvae and Pupae
Mean Adult Density	$\frac{\#\ of\ trapped\ adults}{total\ \#\ adult\ traps}$	Adult
Mean Egg Density	$\frac{\#\ of\ eggs\ collected}{total\ \#\ egg\ traps}$	Egg
Adult Premise Index[51]	$\frac{\#\ of premises\ with\ female\ Ae.aegypti}{total\ \#\ premises\ surveyed}$	Adult
Adult Density Index[51]	$\frac{\#\ of\ female\ Ae.aegypti}{total\ \#\ premises\ surveyed}$	Adult
Premise Condition Index[67]	Exposed defined as at least three infested containers	Larvae and Pupae
Ovitrap density index[51]	$\frac{\#\ of\ eggs\ found\ in\ 4\ weeks}{total\ \#\ premises}$	Egg
Ovitrap premise index[68]	$\frac{\#\ of\ premises\ posiitve\ for\ eggs}{total\ \#\ premises\ inspected} \cdot 100$	Egg
Index	**Index**	**Index**
Larval Premise Index[66]	$\frac{\#\ of\ premies\ positive\ with\ larvae}{total\ premises} \cdot 100$	Larval
Free Larval Index[69]	$\frac{\#\ of\ premies\ positive\ without\ larvae}{total\ premises} \cdot 100$	Larval

*No explicit formula for this indicator has been found in the literature.

CHAPTER 4: AIM 1 LITERATURE REVIEW

4.1 Introduction

If *Ae. aegypti* abundance and dengue outcomes were well-correlated or served as an adequate proxy for a substantial proportion of transmission events, then a strong positive association would be expected. Rather than demonstrating a consistently positive association between observed density and dengue outcomes, observational studies of the association between *Ae. aegypti* abundance and dengue outcomes have been inconclusive. A 2014 literature review identified 18 publications, of which most associations were inconclusive and the analytical methods were not robust.[8] An additional 14 studies not included in that review have also attempted to quantify this association; those results were also generally inconclusive. Of these studies, 26 estimated an association between vector abundance and apparent dengue only, while the remaining 6 studies included DENV seroconversion as an outcome. Since apparent dengue cases represent a fraction of all transmission events, these two outcomes warrant discussion separately.

4.2 *Ae. aegypti* Density and Apparent Dengue

A strong positive association between apparent dengue and *Ae. aegypti* abundance measured by monitoring data would arise from a scenario illustrated in Figure 1. This relationship, in which measured densities at a given time and place are used to represent true exposure to DENV, would be the result of a clear association with seroconversion, dengue disease, and case identification, with no measurement error or selection bias. In Figure 1, the

vector population measured at a given time and location is the same as the true value at that time and location, which is equal to or well-correlated with actual DENV virus exposure, which happens at a different time and likely a different location. Once exposed to DENV, seroconversion occurs, followed by apparent disease and presentation to a health facility. The relationship presented in Figure 1 assumes that all infections lead to apparent disease, and that all individuals with apparent illness seek treatment. These assumptions are violated in dengue endemic settings.

Figure 1. Relationship between observed *Ae. aegypti* abundance and dengue outcomes if true association exists

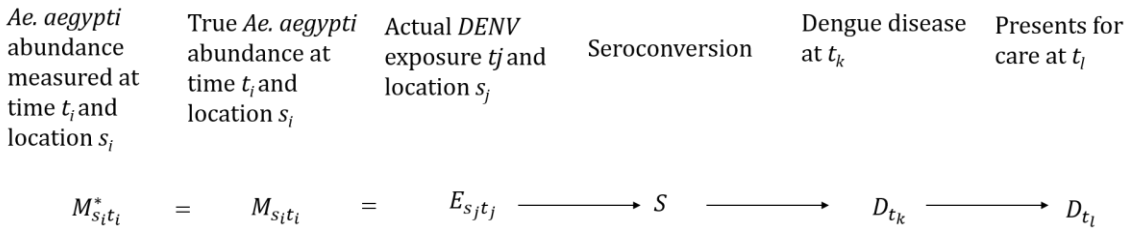
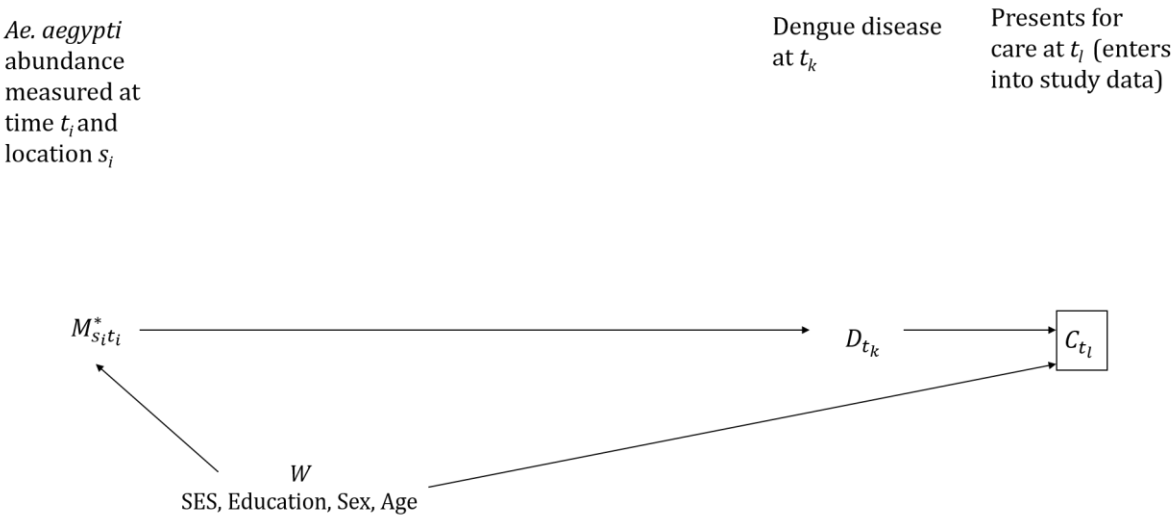


Table 2 presents a summary of publications quantifying an association between *Ae. aegypti* density and apparent dengue cases. Since only a fraction of dengue infections present as apparent illness, studies that use apparent dengue fail to capture the true distribution of dengue virus infection.[1] Apparent dengue is more likely the result of unmeasured individual host factors and may not follow the same distribution as DENV infection throughout the community. Twelve of these studies suggested positive associations between indicators of mosquito abundance and dengue disease using different indicators, data collection and analytical methods.

Figure 2 illustrates a possible selection bias mechanism when apparent dengue is used as a study outcome. The association of interest is the arrow from the measured value of *Ae. aegypti* to dengue disease. Only a subset of all cases will be captured by the health system, represented by C_{t_l}. In such a study, the analysis only includes individuals where $C_{t_l} = 1$, which can create an

association between entomological monitoring data and dengue disease because presentation to health centers and entomological exposure are likely associated with various demographic, economic and household factors. Conditioning on C_{t_l} introduces bias through the backdoor path from $M^*_{s_i t_i}$ to W to C_{t_l} to D_{t_k}.

Figure 2. Possible selection bias mechanism in reported apparent dengue disease as a study outcome



Variability in case detection methods could further bias associations with entomological data. Febrile dengue disease data are either obtained retrospectively through health system records or identified via prospectively recruited febrile patients from the community. Apparent dengue is classified by symptoms, which generally include fever, myalgia and headache as well as the more extreme presentation associated with DHF or DSS.[5] Of these 26 studies, 17 confirmed symptoms were due to DENV using serological and/or virological methods. In the studies conducted in Argentina[70] and Trinidad,[71-73] only proportion of the febrile cases included in analysis were confirmed by laboratory tests. In all other studies, febrile illness alone was used to determine outcome status.[74-79] Failure to include serological or virological confirmation of

febrile illness could result in other febrile illnesses being misclassified as dengue, especially in settings where Japanese Encephalitis Virus, malaria, chikungunya, influenza or yellow fever circulate.

Table 2. Studies that measure an association between *Ae. aegypti* density and apparent dengue

Study Site (Year)	Design	Statistical Analysis	Key Findings	Overall association
Argentina[70] (2002-2007)	Indicator(s): BI, HI Longitudinal evaluation of vector control intervention Baseline survey conducted in 1,808 households in 2002. Vector control interventions conducted every four months with immature measures taken at each visit prior to intervention.	Log-transformation of the BI was used to compare indices using t-test to test for differences across study visits.	No formal analysis of dengue outcomes and larval densities; authors compared trends in the community-level dengue incidence over time.	Inconclusive
Bangladesh[74] (2000)	Indicator(s): Total number of larvae Cross-sectional Collection of *Ae. aegypti* and *Ae. albopictus* larvae from containers inside, outside and on roofs of 9,284 households.	Spatial analysis using kernel estimation and kriging used to create surfaces to identify clusters of dengue cases. The surfaces were then used in a regression model. R^2 was used to compare reduction in variance in spatial regression. Contingency table analysis generated risk ratios.	The presence of *Ae. albopictus* in the household was associated with dengue disease (RR: 1.5, 95% CI: 1.14-1.79). *Ae. aegypti* was not associated with self-reported dengue (RR: 1.11, 95% CI: 0.91-1.35).	Positive (*Aedes albopictus*)
Brazil[80] (1986-1998)	Indicator(s): HI Longitudinal Monitoring of larval population at 3 month intervals of all households from 1986-1995, with interruptions (not described); from 1995-1998 HI calculated from a sample of 10% of households from each city block.	Descriptive and graphic comparison of dengue fever cases and HI results over the study period.	Dengue outbreaks occurred where HI >1%.	Positive

Table 2 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Brazil[75] (1996-2001)	Indicator(s): HI Longitudinal Infestation index calculated as the proportion of positive (larvae or pupae) properties among the total surveyed properties. Data from 13 surveys conducted during study period were analyzed.	Pearson correlation to measure association between monthly incident rate and HI; ANOVA to test four quantiles of the HI distribution.	Weak correlations (0.25, 0.21, 0.14) between various aggregated monthly incidence rates. No association found in ANOVA analysis.	Inconclusive
Brazil[81] (2007-2010)	Indicator(s): Average mosquito density per neighborhood Longitudinal Weekly monitoring of mosquito traps placed throughout the entire city.	A hierarchical Poisson regression used to predict weekly case data by average mosquito density, with a random effect term for neighborhood.	Models showed a weak association between weekly cases and adult mosquito densities; model fit statistics suggest limited predictive power even when accounting for space. Neighborhood effects improved model fit.	Inconclusive
Colombia[66] (1996-1997)	Indicator(s): Geometric mean of eggs, larvae, pupae, adults Longitudinal Monthly entomological data collected from 120 premises over a 7-month period.	Correlation statistics	No correlation between indices and incidence of dengue disease.	None
Colombia[82] (2002-2008)	Indicator(s): BI Longitudinal Entomological surveys were carried out by local health authority 2-3 times per year.	Ecological model constructed to generate predicted areas of *Ae. aegypti* infestation. Linear regression then used to test for association between apparent case rates and the proportion of neighborhood area predicted to have mosquito breeding.	Statistically significant positive correlation between the area predicted to be infested and apparent dengue rates. No association was observed with the BI.	Inconclusive Suggests spatial method could correct misclassification

Table 2 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Cuba[83] (2001)	Indicator(s): BI Longitudinal All household inspected monthly from May-July among 50 households and surrounding blocks; BI calculated at block level and neighborhood level.	A BI ≥4 in a neighborhood was used to classify exposure to mosquitoes. Sensitivity and specificity were calculated using the exposure definition.	A BI ≥4 predicted dengue cases with a sensitivity of 81.8% and a specificity of 73.3%.	Inconclusive
India[78] (1970-1989)	Indicator(s): HI Cross-sectional analysis of nine different dengue outbreaks. Entomological methods not adequately described.	Correlation of HI and apparent dengue cases.	When HI exceeds 20%, a higher dengue fever attack rate was observed.	Positive
India[77] (1996-2001)	Indicator(s): BI, CI, HI Longitudinal Entomological indicators calculated monthly at sentinel site locations.	Descriptive analysis in which case numbers and annual HI measures are visually compared over time.	Overall HI decline was observed from 1996-2001 but apparent dengue cases were did not decline from 1997-2001.	None
India[76] (2012)	Indicator(s): BI, CI, HI Longitudinal Weekly surveillance of entomological data and anti-larval and anti-adult mosquito interventions implemented.	Descriptive comparison of proportion of febrile cases and HI, BI and CI results.	Reduction in mosquito population across all indices after interventions; no clear trend in reduction of dengue cases.	None
Indonesia[69]	Indicator(s): BI, CI, HI, Pupa Index, Free Larval Index Longitudinal 4 villages (2 identified as endemic and 2 identified as non-endemic) surveyed twice; 100 households randomly sampled per village.	Chi-square test, t-test to compare differences in indices values comparing endemic to non-endemic villages.	No association observed between immature indices.	None

Table 2 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Malaysia[84]	Indicator(s): BI, HI Longitudinal 12 monthly container inspections of a 80-100 randomly selected households per month.	Correlation statistics to measure the association between number of incident cases and BI and HI.	Positive correlation (HI, r=0.432; BI, r=0.351)	Positive
Malaysia[85] (1994)	Indicator(s): BI, HI Longitudinal Monthly entomological surveys (unclear if they occurred at the same sites).	Correlation statistics to measure association between dengue cases and mosquito indicators.	Correlation statistics were generally statistically insignificant; only one of the six study sites demonstrated significant correlation between mosquito exposure and apparent dengue (r=0.60).	None
Peru[86] (2002-2004)	Indicator(s): Aedes Index, BI, CI Periodic entomological surveys conducted from 2002-2004 (months surveyed varied by year).	Correlation statistics to measure association between indices and dengue cases Regression analysis to use Aedes Index to predict number of dengue cases.	Correlation statistics between total and monthly cases of dengue (with intervention) and AI, BI, CI were >0.9. Linear regression of density indicators were statistically significant predictors of monthly dengue cases.	Positive
Thailand[87] (2007)	Indicator(s): BI, CI, HI Cross-sectional Dengue indices were collected from a total of 10 villages (one per province); febrile rates were derived from existing provincial data.	Descriptive GIS analysis, no statistical methodology. Visual comparison of BI, HI and CI for each village for low and high epidemic periods.	Substantial variability in village-level indices for low and high periods limits interpretation.	Inconclusive.

Table 2 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Taiwan[88] (1987-1988)	Indicator(s): Presence of breeding sites Case-control matched on age and sex Participant self-report of mosquito presence and breeding sites at household.	Univariable odds ratios.	No association, presence of breeding sites had an OR of 0.84 (95% CI: 0.47-1.50)	None
Taiwan[89] (2002)	Indicator(s): BI Longitudinal Monthly entomological surveys of 50 households.	Geographically weighted regression using ordinary least squares to determine spatial heterogeneity in the association between the BI and rate of dengue cases per 100,000.	Spatial heterogeneity for dengue-mosquito relationship was observed. BI correlates dengue incidence but not uniformly throughout study area.	Inconclusive
Taiwan[90] (2005-2012)	Indicator(s): Aedes Index, BI, CI, HI Longitudinal Weekly, monthly or bi-monthly entomological inspection carried out in one district for 50-100 randomly sample households per city neighborhood, collected May-December of each year. Frequency of inspection was determined by prior entomological data (high, medium or low risk).	A separate predictive Poisson regression model was fit for each indicator and climate variables, stratified by density status.	Crude associations with indicators and dengue incidence were statistically significant. Multivariable risk factor analysis of entomological indicators with meteorological variables associated with increased dengue incidence.	Positive

Table 2 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Taiwan[91] (2005-2012)	Indicator(s): Aedes Index, BI, CI, HI Case-crossover Cases were defined upon presentation with apparent dengue and their prior-weekly exposure was selected as a control at weeks 3, 6 and 9 before diagnosis. Entomological exposure derived from weekly surveillance data.	Conditional logistic regression to estimate the odds of becoming a dengue case controlling for vector indices and meteorological variables. Analysis was stratified by low, medium and high density status.	AI was significantly associated across density levels (OR: 1.29, 95% CI: 1.24-1.35; OR: 1.45, 95% CI: 1.36-1.55; OR: 1.32, 95% CI: 1.24-1.39).	Positive
Trinidad[92] (1998)	Indicator(s): BI Case-Control Larval collections at 87 case households (positive for apparent dengue) and 87 control households matched by age and sex.	G-test to compare differences in BI values.	Lower BI observed among control households.	Positive
Trinidad[93] (2002-2004)	Indicator(s): BI, HI Monthly household visits for container inspection.	Spearman rank, Chi-square and G-tests to compare BI values.	Higher range of density among case households.	Inconclusive
Trinidad[71] (2003)	Indicator(s): BI, HI Case-Control Larval collections at 30 case households (positive for apparent dengue) and 120 control households selected based on cardinal direction adjacent from case household (north, south, east, west).	Chi-square tests of association, G-test.	Primary finding that households to east and west of index cases were more likely to be positive for *Ae. aegypti* compared to households north and south.	Positive

Table 2 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Trinidad[72] (2003-2004)	Indicator(s): HI, BI, Pupa Index, CI, Pupae per Hectare Case-Control Larval collections at 33 case households (positive for apparent dengue) and 132 control households selected based on cardinal direction adjacent from case household (north, south, east, west) taken within 48 hours of case detection. Retrospective entomological survey data reviewed from previous year (4 visits).	G-test to compare location of case households with measures of infestation Prior densities compared with the measures taken within 48 hours of case detection.	Primary finding that households to east and west of index cases were more likely to be positive for *Ae. aegypti* compared to households north and south. Densities measured during case investigation higher than those reported through routine entomological surveillance.	Positive
Venezuela[94] (2000-2001)	Indicator(s): Adult measures Longitudinal Entomological measures taken by backpack aspirator among households and neighboring households where dengue cases were identified.	Pearson correlation	Positive correlation between abundance of *Ae. aegypti* (r=0.677, p=0.0078).	Positive
Vietnam[79] (2004-2008)	Indicator(s): BI, CI, HI, presence of adult mosquito Monthly larval data were collected from 100 households randomly selected from 8 districts. Households were randomly selected at each monthly visit.	Poisson regression model used to determine association between counts of dengue disease and mosquito density.	Rate ratios were statistically significant for: Per 5% increase: HI: 1.66 (1.62-1.70) Mosquito: 1.16 (1.14-1.18) CI: 1.78 (1.73-1.83). Per 5-unit increase: BI: 1.57 (1.53-1.60).	Positive

4.3 *Ae. aegypti* Density and Inapparent Dengue

Of the total 32 studies reviewed, only six evaluated an association between DENV infection and mosquito abundance, either by measuring seroconversion or seroprevalence. Instead of febrile outcomes, seroconversion to DENV should be used as the primary outcome as prevention of DENV transmission by vector control is the primary rationale for the use of entomological monitoring data. This outcome is more relevant for DENV control, increases sample size and is more representative of patterns of vector-human interaction. Furthermore, inapparent dengue is an outcome of additional public health importance, as there is some evidence that subsequent infections with DENV may increase the likelihood an individual experiences severe dengue disease.[1] Table 3 presents a summary of the studies in which inapparent DENV infection was the primary outcome of interest.

In terms of subject enrollment, inapparent dengue is more difficult to measure than apparent as it requires recruitment of subjects for serological sample collection, as opposed to reviewing dengue disease case reports or identifying individuals with febrile disease as they present at hospitals or clinics. Studies of seroincidence require longitudinal follow-up, which poses challenges with respect to subject retention. DENV serological evaluation procedures have been demonstrated to have variable performance depending on serotype and assay.[95, 96] Interpretation of longitudinal serological data could result in outcome misclassification if serotype-specific associations are of interest due to cross-reactivity between serotypes as a result of prior exposure, as well as possible cross-reaction with other flaviviruses.[95] If the outcome of interest is any DENV seroconversion, this misclassification is less of a concern.

Serologically-identified DENV infections are less likely than apparent dengue cases to be subject to selection bias by factors such as socio-economic status, type of employment, housing

quality, access to sanitation, age and sex. These individual and household characteristics likely influence health-seeking behavior, which may introduce selection bias when identifying febrile disease through passive surveillance. By including infection events, it is possible to avoid potential selection bias that occurs from case identification of dengue disease.

Table 3. Studies that measure an association between *Ae. aegypti* density and inapparent dengue

Study Site (Year)	Design	Statistical Analysis	Key Findings	Overall association
Brazil[97] (2006-2008)	Indicator(s): Mean Adult Density Mean Egg Density Longitudinal Weekly collection of eggs and adults from randomly sampled households.	Generalized Additive Model (allows for non-linearity of age to be included in a regression model). Spatial analysis employed household coordinates to generate a surface of dengue infections and mosquito densities.	Due to small sample size, only used visual inspection of contour maps to determine if greater numbers of mosquitoes were associated with dengue infection; No strong pattern observed.	None
Cuba[98] (2000)	Indicator(s): BI Case-control BI measured every 2 months in every household.	Analysis conducted at block and neighborhood level. Case blocks defined as any block with at least one confirmed dengue case; control blocks sampled from blocks with no dengue case; serology collected among all persons with history of a fever. Logistic regression to estimate odds ratio for BI measures.	Maximum BI ever reported found to be strongly associated with positive dengue case blocks (OR: 3.4, 95% CI: 1.2-9.6). Association varied at different time periods.	Positive
Mexico[99] (1980)	Indicator(s): CI, BI, HI Longitudinal Two areas in two separate cities selected for entomological surveillance in February and September 1980; every other house surveyed for larvae; CI data only collected in September.	Pearson correlation statistics (percentage of individuals infected with CI, BI, and HI).	No correlation in February between Breteau and House Index; Correlation coefficient of 0.95 (House Index and Container Index) and 0.90 (Breteau Index) in September.	Inconsistent

Table 3 (Continued)				
Study Site (Year)	**Design**	**Statistical Analysis**	**Key Findings**	**Overall association**
Puerto Rico[100] (1991)	Indicator(s): Number of female *Aedes aegypti* per person Cross-sectional Random sample of 98 households in a single neighborhood, adult mosquitoes collected in occupied bedrooms using backpack aspirators.	Forward selection to build predictive model based on personal and household risk factors, only included households with confirmed recent infections or households never infected.	An OR of 2.77 found for households with >1.5 *Aedes aegypti* per person (95% CI: 0.70-13.33).	None
Sudan[101] (2008-2009)	Indicator(s): BI, CI, HI, Pupae per person, Pupae per children Longitudinal Pupae, larvae and adult mosquitoes collected monthly from a sample of households (different households sampled each time).	Chi-square tests of association and ANOVA	Temporal and spatial pattern of dengue outcomes and entomological indices generally correlated; study did not include households for which no *Ae. aegypti* were observed in sampling for serological status.	Inconclusive
Thailand[102] (2004-2005)	Indicator(s): BI, CI, HI, Pupal and adult densities Case-control (febrile dengue cases were used to identify groups of households as either "positive" or "negative" dengue clusters). Adult female *Ae. aegypti* were collected at the time of case and control identification; larvae and pupae were collected from containers.	Independent t-tests to compare positive and negative clusters.	No statistically significant differences observed, but all mosquito indices were higher in positive clusters compared to negative clusters.	None

4.4 Limitations of Prior Studies

In addition to the choice of dengue outcome (apparent or inapparent), there is a wide range of other analytical limitations that could have introduced bias in the studies presented in Table 2 and Table 3. First, several studies employ small sample sizes of dengue-related outcomes. For example, a study in Brazil only had 28 infections[97]; one in India[76] included 79 apparent cases. Most studies recruited a larger sample of households for entomological data, although in Trinidad[72,92], Puerto Rico[100] and Taiwan[89] the number of households providing entomological data was less than 100 (the minimum sample size recommended[103]).

Second, study design methods were reported that may have biased results or limited generalizability. In Venezuela[82] and Sudan[101], entomological measures were only taken among households contributing dengue cases or DENV seroconversions. In the Venezuela study, the positive association observed may have been biased due to an inclusion of individuals with higher densities than those in the general population. The Sudan study, which generated extremely detailed data on immature population over time and space, provides evidence to support a dose-response relationship among those where *Ae. aegypti* were observed. The exclusion of outcome information from households with no exposure, however, does not account for dengue outcomes across the full distribution of *Ae. aegypti*. In Taiwan[88], participants self-reported household breeding sites and mosquito presence. Self-reported data on mosquito infestation are likely subject to recall and response bias, especially among individuals who experienced disease. In Sanchez 2006[98], only individuals with a history of fever were asked to provide serological samples; this excludes the proportion of individuals who were not febrile but may have been exposed to DENV from the analysis.

Third, many of these studies employed statistical analysis methods that provide limited inference. Most notably are studies that relied on Pearson correlation coefficients, g-tests or t-tests to ascertain an association between density and either number of cases or percentage of individuals positive for a dengue outcome.[66, 69-73, 75, 78, 80, 84-87, 92, 94, 99] Correlation coefficients can be misused to describe associations, particularly among cases in which a purely linear relationship may not exist, if there is heterogeneity of effect among subgroups within the dataset, or extreme outliers exist in the data that inflate coefficients.[104] Other inadequate statistical techniques include failure to account for dependency in regression analysis and use of linear instead of Poisson regression to model rates of dengue disease. Finally, four studies essentially presented visual or descriptive comparisons of vector density instead of formal statistical comparisons.[76, 77, 80, 97] The most robust statistical analyses used predictive modeling studies in Taiwan[90], and Brazil[81] and epidemiologic designs in Thailand[102], and Taiwan.[91]

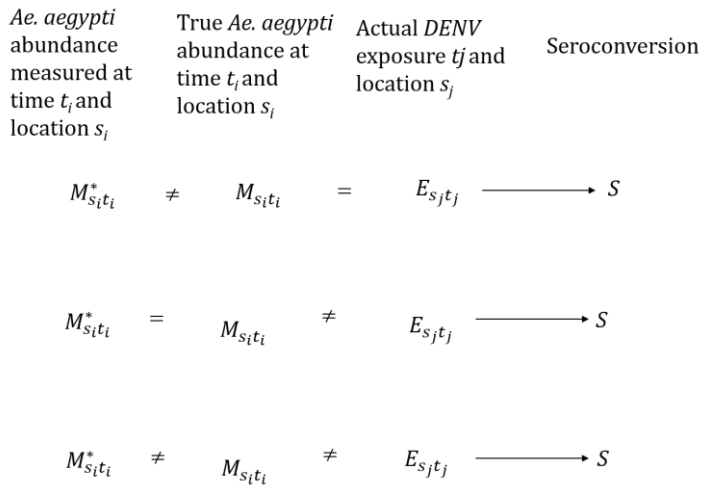
Finally, several studies[69, 76, 79, 86, 87] used aggregated disease outcomes such as district-level incidence which can induce bias due to the modifiable areal unit problem (MAUP). MAUP introduces statistical error when point-based events are aggregated into district or regional estimates.[105] This is similar to the ecological fallacy, which might also operate in these examples, in which inference on individuals is construed from group-level findings.[106] In these examples (notably, Argentina[70], Vietnam[79], Taiwan[89]), aggregated outcome data such as district incidence was compared to overall trends in entomological survey results to make statements about the role of entomological exposure on dengue outcomes. While these comparisons are useful for hypothesis generation or documenting community-wide trends, they make strong assumptions about the relationship between *Ae. aegypti* and dengue outcomes as the

entomological data may not relate to the exposure of individuals contributing to incidence rate or prevalence of dengue disease.

4.5 Entomological Data as a Proxy for True DENV Exposure

Failure to detect an association between inapparent DENV infection and mosquito abundance likely results from three scenarios, illustrated graphically in Figure 3. The first scenario exists when measured density is subject to error and does not reflect true density at a given location and time, but that true density is well-correlated with actual DENV exposure. *Ae. aegypti* indicators force cross-sectional measurement upon a highly time-varying, dynamic exposure. It is possible that current entomological survey techniques may not capture the fine spatial and temporal variability of vector populations in an urban setting, mainly due to operational limitations, differences in time of collection, and skill of study collection staff.[10]

Figure 3. Bias due to measurement error among studies with DENV seroconversion as a primary outcome



The second scenario posits that measurement is accurate, but that there is no equivalence or correlation between true density at the location and time of survey and true DENV exposure, which likely occurs at a different place and time. *Ae. aegypti* densities may fail to describe risk of DENV infection due to the complexity of transmission; there may be too much noise to detect

an association. Because *Ae. aegypti* are daytime-biting mosquitoes that are highly adapted to the human urban environment[39], their frequent biting contact with human hosts is mediated by social and economic[35] factors that govern human movement through times and spaces where they encounter mosquitoes.[107] Human movement in Iquitos has been demonstrated to drive DENV transmission.[107] The probability of transmission, however, is dependent on human movement to introduce DENV into mosquito populations and the presence of susceptible individuals that mosquitoes infect to perpetuate new rounds of transmission.[108]

Finally, it is possible that there is both measurement error and no association between a cross-sectional density measure and actual DENV transmission. Any observed association could be non-linear which may be difficult to detect. In all scenarios, it is likely that these relationships are not absolute and vary within a population.

4.6 Insights Gained from Prior Studies

Despite the limitations from the literature base, there are some relevant findings that should inform further investigation of the utility of monitoring data. First, these studies highlight the possibility that mosquito monitoring data can be associated with DENV but may be heterogeneous within the same study site. For example, in Cuba[98] the authors report that during one period the BI was strongly associated and not during another. This may be due to changes in population susceptibility that were not accounted for in the analysis. Alternatively, such heterogeneity may be the result of temporal variability in mosquito data, which could be a result of measurement error. The Taiwan[89] paper utilizing geographically weighted regression allowed for prediction to vary across space and found associations did not exist uniformly throughout the study site. Findings such as these suggest that there may be certain settings within finer spatial

scales or possibly socio-economic contexts in which mosquito density is more accurately measured and related to DENV risk.

None of these studies explicitly addressed the role of measurement error; however, taken together there is evidence that entomological monitoring data, combined with methods to address measurement error, could improve the utility of vector surveillance. In Cuba[98], a consistently strong association was found using the maximum BI ever observed. This approach likely corrects for possible measurement error that occurred during a single entomological collection survey, and may better correlate with individual exposure. In Bangladesh[74] and Colombia[82], spatial methods used to predict exposure improved the association of vector density measures.

4.7 Contribution of Proposed Research

Although indices of vector abundance have long been promoted as a means to identify areas of DENV transmission and target interventions, to date there is no known minimum threshold of *Ae. aegypti* exposure that predicts DENV transmission.[10, 68] The choice of *Ae. aegypti* indicator used to measure exposure to *Ae. aegypti* (and by extension, DENV risk) represents, to some extent, assumptions of the underlying transmission dynamics present in the community. For example, if household-level transmission is more successful in settings with a greater number of vectors relative to the number of people, then per person measures may be more useful.

Given the limitations of entomological data collection, a comparative approach of larval, pupal and adult monitoring indicators is warranted by first estimating the association with indicators calculated from entomological monitoring data. Tun-Lin *et al*[10] published a comparative literature review of these indicators but there has yet to be a formal comparison of

these indicators as they relate to DENV seroconversion using longitudinal data from a single study site.

Aim 1 will compare the performance of these indicators by linking them to the serological outcomes from individuals residing in households providing entomological data. The results of this Aim will have public health significance if indicators relevant to the transmission of DENV virus can be identified. If no association is seen across most indicators, the results will influence policy and program implementation by suggesting that resources would be better directed to monitoring other risk factors associated with transmission.

CHAPTER 5: AIM 2 LITERATURE REVIEW

5.1 Introduction

The lack of association between vector measures and dengue-related outcomes is possibly the result of measurement error. Given the difficulties inherent in field-based entomological surveillance, it is possible that measurement error results in a systematic downward bias in the number of adult *Ae. aegypti* observed, particularly among households with lower levels of infestation. If so, addressing such error might improve the utility of entomological monitoring data to identify an association with the risk of DENV infection. Since spatial clusters of mosquito density shift over time, it is possible that recent infestation, combined with information on density observed among neighboring households could improve cross-sectional estimates of mosquito abundance. This error could be addressed by leveraging spatiotemporal relationships.

Further, since the distribution of mosquito densities varies across space and time, then exploration of possible heterogeneities of the exposure-outcome association between units of space is warranted. There may be within-community factors that provide evidence for settings in which entomological data could be useful in identifying individuals with a higher risk of DENV infection. Failure to detect heterogeneity of an association would suggest that group-level (e.g., block) context did not contribute to differences in the risk of DENV seroconversion.

5.2 Spatial and Temporal Heterogeneity of *Aedes aegypti* Densities

Ae. aegypti dispersal, breeding and feeding habits likely contribute to spatial clustering of vector populations within the household or neighboring households as the vector is known to

have short dispersal patterns of <100 m.[109-111] In Iquitos, Peru, using data from 1998-1999, spatial clustering was estimated at a scale of approximately 30m.[24] A study in Ecuador[112] found that juvenile mosquitoes clustered within distances of 20m and adult mosquitoes within distances of 10m. Harrington's capture-release studies in Puerto Rico and Thailand found that among *Ae. aegypti* monitored for distance traveled, over 80% did not move beyond their release household, suggesting that presence of the dengue vector in a household could be relevant for characterizing DENV exposure that occurs within neighboring households or within a block.[23]

While *Ae. aegypti* spatial clusters have been previously documented, there is also evidence that the location of such clusters is highly variable over time. A subsequent analysis of Iquitos data (2009-2011) found no consistent temporal pattern among the location of *Ae. aegypti* clusters.[113] Adult and pupal measures rarely correlated. Clusters of adult measures were not likely to extend to distances beyond 100m, suggesting this scale for targeted intervention would be ineffective at reducing dengue transmission. The authors controlled for the background level of correlation among neighboring households but did not use spatial exposure to mosquitoes to evaluate an exposure-outcome relationship as described in this dissertation.

In Saudi Arabia,[114] the authors found that identification of *Aedes* "hot spots" were also sensitive to the temporal and spatial scales used to aggregate mosquito data. In this study, female adult *Aedes* mosquito samples were taken daily for a period of five years using traps. The authors use the Getis-Ord *Gi** statistic, which allows for the identification of spatial clusters at varying temporal and spatial scales. The temporal fluctuation in *Aedes* was modeled as 1) an average over five years, 2) over the entire year 2010, 3) monthly for the first quarter of 2010 and 4) weekly for January 2010. The 2010 data was used to compare spatial clustering at the sub-district, district and sub-municipality levels. The authors found that identification of *Aedes* "hot

spots" were sensitive to the temporal and spatial scales used in the analysis. A study in Argentina found the location clusters of *Ae. aegypti* immature populations to shift over the course of four entomological surveys.[70] Detection of clusters is sensitive to the geostatistical method used; it is possible that temporal variability also exists as a result of measurement error, in addition to seasonal fluctuations in vector populations.

5.3 Prior studies exploring the role of space

Very few studies have explored the role of space while estimating an "exposure-outcome" association such as household *Ae. aegypti* density and DENV seroconversion. Rather, most studies of the spatial distribution of dengue in the peer-reviewed literature are descriptive, focusing on either the spatial distribution of dengue outcomes or spatial characteristics of risk factors associated with DENV transmission (not the joint exposure-outcome relationship). Examples of these analyses have been presented from settings diverse as Argentina,[115, 116] Australia,[117] Bangladesh,[118] Brazil,[119, 120] Cambodia,[121] French Guiana,[122] Peru,[123] Puerto Rico,[124] Saudi Arabia,[125] Sudan,[101] Thailand[126] and Vietnam.[127] In a few of these studies, the spread of dengue through the community was attributed to specific factors, such as household water storage practices in Sudan[101]; landscape patterns in Thailand[126]; and road networks in Cambodia.[121] Many of the studies evaluating the spatial distribution of dengue only included apparent cases in the analysis.[117, 122, 128]

Only five studies included a spatial analysis of vector exposure in estimating an association with dengue outcomes. In Taiwan,[89] spatial analysis was performed using geographically weighted regression (GWR), a method that allows regression model parameters to vary across space.[129] The study found some neighborhoods in which the presence of higher vector indices was associated with an increase in dengue cases; although in other areas there was no association, suggesting heterogeneity of effect in terms of risk of apparent dengue. The

presence of heterogeneity in the association between vector measures and dengue cases could also be the result of other predictors that modify the association. The study did not control for demographic factors that might have been related to exposure to DENV transmission, such as improved housing quality, nor did it measure inapparent infections.

In a case-control study of severe dengue and mosquito exposure conducted in Trinidad[73], households with cases of confirmed symptomatic dengue were recruited via the public health system and control households were selected from the neighboring households at each cardinal direction (north, east, south, west). Cardinal direction was considered important factor due to meteorological patterns in Trinidad; this has not been cited as a relevant factor in dengue virus transmission in other settings. All enrolled households were surveyed for *Ae. aegypti*. The authors found that households to the east and west of case households were more likely to have *Aedes* positive containers. Overall, there was no statistically significant difference in mosquito exposure between case and control households using a Chi-square statistic. The design did not include collection of entomological data at the household beyond the nearest neighbor and inapparent dengue infections were not included as a study outcome.

A cross-sectional study of entomological indicators and self-reported apparent and severe dengue conducted in Bangladesh[74] found a significant association between *Ae. albopictus* (a dengue vector prevalent in parts of southeast Asia) populations but not *Ae. aegypti*. Although this study included over 9,000 households, it used self-reported symptomatic dengue as an outcome. Further, its cross-sectional design limits the ability to evaluate spatial trends over time. The authors used kriging and kerneling (common approaches to interpolate and aggregate the spatial data) to generate descriptive spatial surfaces for the exposure and outcome; the distance surface of dengue cases was used as an outcome variable in a regression analysis with vector

density as an independent variable. Distance surface refers to a value assigned using cluster maps to represent the distance an individual case was found from a cluster of dengue cases.

A hierarchical predictive model of apparent dengue based on weekly vector density describing transmission in a Brazilian city[81] explored the hypotheses that dengue cases were randomly distributed across space, that cases were correlated within neighborhoods, and that cases were correlated with neighborhood economic status and population density. The authors found that models that included neighborhood effects performed best at community-wide spatial scales, but the relationship between weekly mosquito density and dengue cases was weak. This suggests that dengue cases exhibit some spatial structure but that structure does not correlate with the distribution of mosquito abundance. In Colombia,[82] spatial methods were used to predict exposure improved the association of vector density measures. The authors used ecological niche modeling to predict larval density and then tested the association between model-predicted larval population estimates and apparent dengue. The larval population estimates predicted by the model outperformed the densities calculated form observed data on immature *Ae. aegypti* population counts.

None of the studies listed in Table 3 that used seroconversion to DENV as an outcome addressed spatial relationships or evaluated the variability of entomological indicators over different spatial scales (in terms of an association with DENV infection).

5.4 Contribution of the Proposed Research

Aim 2 benefits from data with sufficient temporal and spatial variability, over several years and multiple neighborhoods, to measure the performance of entomological indicators over different spatial scales. This Aim will explore two main areas of analysis. First, a robust spatiotemporal analysis of the mosquito-DENV seroconversion association will be conducted

using adult *Ae. aegypti* measures. Given the potential for measurement error in the collection of entomological data, of spatial information may improve the ability of measures of *Ae. aegypti* abundance calculated from entomological surveillance to distinguish individuals at a greater risk of DENV seroconversion. The Bayesian Maximum Entropy (BME) geostatistical framework will be used to model vector density to determine if spatial relationships can be used to improve estimates of *Ae. aegypti* populations and then identify an association with DENV.

Second, multilevel modeling will be used to test for within-community heterogeneities in the association between DENV and mosquito abundance. This work will determine if the 6-month risk of DENV seroconversion is associated with contextual factors, which may expand the possibility of spatially targeting interventions. If the association between household-level mosquito density and DENV seroconversion varies between spatial groups (block or zone), this such heterogeneity would suggest that measures of vector density do not consistently relate to DENV risk and that local contextual variables may influence the performance of entomological monitoring indicators.

CHAPTER 6: DESCRIPTION OF THE DATA SOURCE

6.1 Background: Dengue Cohort Studies, Iquitos, Peru

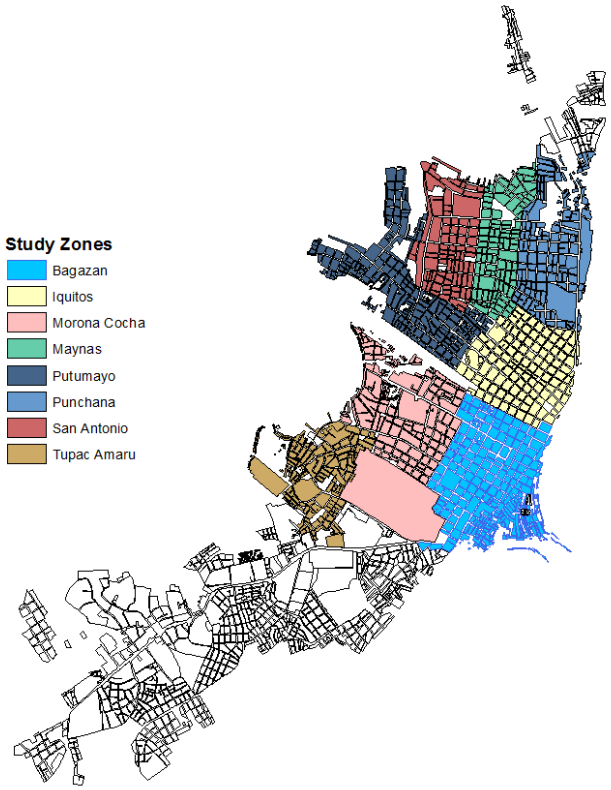
In Latin America, the *Ae. aegypti* population was successfully controlled through wide-spread vector control in the 1960s, thereby eliminating dengue virus transmission.[130] After the re-introduction of *Ae. aegypti* by globalization, trade and urbanization in the 1980s and 1990s, endemic dengue transmission in central and south America re-emerged as a major public health problem.[130]

Figure 4. Map of Peru

Iquitos is located in the Amazon Basin in northeastern Peru and is accessible only by air or boat (see Figure 4). An urban community, the population of Iquitos exceeds 400,000 (2007 census) and is the largest city in the Peruvian Amazon. Iquitos is divided into four administrative districts (San Juan, Maynas, Punchana and Belen). Each district is serviced by its own government and municipal authorities. DENV transmission is seasonal, with increased activity beginning in August and highest in January, and may persist through April. DENV1 is presumed

to have been introduced into Iquitos in 1990-1991[131], followed by DENV2 in 1995[132], DENV3 in 2001[133] and DENV4 in 2008.[134]

Figure 5. Map of ECDC study zones



The University of California at Davis (UCD) and the United States Naval Medical Research Unit in Peru (NAMRU-6), along with other partners, have supported dengue research in Iquitos since the late 1990s. Longitudinal research activities have been ongoing in the four districts. The dataset described in this proposal consists of research conducted primarily in Punchana and Maynas districts, which were divided into eight zones according to neighborhoods and district health center catchment areas as shown in Figure 5. Several different research cohorts have been assembled and followed since 1999. This research draws upon data generated by the ECDC cohort (1999-2003) and the Activity Space (AS) cohort (2007-2010). Each household was mapped using GPS coordinates.

6.2 Study Design: Iquitos Cohorts

The analytical cohort was constructed using entomological and serological data collected between 1999-2003 and 2008-2010. From 1999-2003, study activities were implemented in four city districts: Maynas, Punchana, Belen and San Juan. During the period 2008-2010, data were collected from two neighborhoods: Maynas and Tupac Amaru. Entomological and serological monitoring data from the period 2004-2007 were excluded due to city-wide implementation of vector control activities and low rates of DENV transmission.

6.2.1 ECDC cohort (1999-2003)

The ECDC study cohort[9] was assembled by subdividing each of the 8 study zones into a grid of 5 equal areas. Three blocks from each grid were randomly selected, resulting in a total of 15 sample blocks in each zone (120 blocks overall). In each zone, 50 individuals were recruited every month from the randomly selected blocks and 10 additional subjects were recruited from neighboring blocks. The family members of study subjects were also enrolled until a base cohort of approximately 2,400 individuals was obtained. As households dropped out of the cohort, they were replaced. Study participants provided serological samples every six months until the end of follow-up in 2003 or withdrawal from study participation. In total, 3,664 individuals from 1,259 households were enrolled. Subject age ranged from 2-86 years (mean: 15.2, SD: 11.57), and 57.7% were female. Each individual contributed an average of 4 blood draws (range 1-14), resulting in 12,993 observations in total. Households contributed a mean of 4.8 visits (SD: 3.7, range 1-39). A total of 62,653 containers were observed in during this period.

6.2.2 Activity Space (2008-2010)

Data were collected from 2008-2010 in the Tupac Amaru and Maynas neighborhoods (Figure 6).[107, 117] The study aimed to enroll as many individuals per neighborhood block as

possible, with a target of 1,200 individuals per neighborhood. Individuals provided blood samples for dengue virus seroconversion every 6 months and allow entomological surveys in their household every four months. Additional individuals were recruited in a febrile surveillance cohort. These participants did not provide serological samples every 6 months, but did participate in entomological surveys every four months. Data from these households was used to characterize block and neighborhoods. DENV4 was the predominant serotype in circulation during this period.

Figure 6. Map of Activity Space study zones

Overall, a total of 2,951 households were surveyed for entomological and container data, contributing 80,199 container-related data points. A total of 3,612 individuals provided serological data from 813 households, of which 1,142 seroconverted to DENV4 during follow-up. Average household size was 6 individuals, of which an average of 4.7 individuals per household (range 1-21) contributed to the study dataset. The mean number of serological data points collected was four per person (range 1-9 visits), resulting in 14,135 total serological data points.

6.3 Entomological Surveys

Once households were enrolled, two-person study teams collected entomological data following a circuit to survey neighboring households on the same block (and/or neighboring block) within an approximately two week period. The entire study area required approximately four months of data collection to complete, upon which entomological surveys resumed again following the same schedule. Adult *Ae. aegypti* were collected using CDC backpack aspirators (1999-2009)[135] or Prokopack aspirators (2009-2010)[136] in both the exterior and interior of the household by passing the vacuum tube over common *Ae. aegypti* resting sites, outside walls, vegetation, and the entrance of potential larval habitats. Pupal and larval populations were collected via enumeration of all wet containers or other larval development sites that contained water upon inspection. During surveys all observed pupae and a sample of larvae were collected in small plastic Whirlpack bags; larval density was estimated as one of four levels (0, 1-10, 11-100, >100). All adult, larval and pupal samples were examined at the study laboratory, counted and identified to species and sex. Pupal data were recorded as observed counts. Total numbers of adult male and female *Ae. aegypti* collected by interior and exterior of the dwelling were recorded. Household demographic data were collected for variables including water source, sanitation facility, presence of electricity, type of building material, roof structure, and any reported use of insecticide or larvacide.

6.4 Assessment of DENV Seroconversion

Members of households selected for entomological monitoring were asked to provide blood samples every six months.[137] Samples were collected at the participant's home using standard aseptic techniques, stored in ice and transported to the study laboratory within four hours of collection. Sera were tested at two (1999-2003) and four (2008-2010) serum dilutions

plaque reduction neutralization test (PRNT[138]) at the United States NAMRU-6 laboratory in Lima, Peru. Briefly, a serum sample is positive for dengue virus if a dilution neutralizes 70% of the test virus ($PRNT_{70}$). Serum samples were tested for DENV neutralizing antibodies by serotype-specific PRNT in baby hamster kidney BHK21 cells using carboxymethyl cellulose overlay. To identify seroconversion to DENV, a serum sample was declared positive for DENV if a dilution neutralized 70% of the test virus ($PRNT_{70}$). The $PRNT_{70}$ was used on final serum dilutions of 1:60 for DENV1 and DENV3, 1:80 for DENV2 and 1:40 for DENV4. PRNT is the most specific serological test for dengue infection, but results may be biased due to cross-reactions from multiple serotypes present in a single sample. The $PRNT_{70}$ assay has the following serotype-type specific sensitivity and specificity, respectively: DENV1[139], 95%, 62%; DENV2[139], 91%, 70%; DENV3[140], 88%, >95%; DENV4[140], 85% and >95%.

The primary outcome of interest in this analysis was seroconversion to any circulating DENV as determined by $PRNT_{70}$. $PRNT_{70}$ is the most specific serological test for dengue infection, but results from this assay may be biased due to cross-reactions from antibodies directed against multiple serotypes present in a single sample. Serological outcomes used in this analysis were previously evaluated.[137] To minimize misclassification of serological data, the full serological profile of subjects was reviewed as follows: if the increase in titer that reduced DENV plaques between a negative sample and a subsequent sample was at least 20% and all subsequent samples were positive, the subject was determined to have seroconverted. However, if subsequent PRNT results were not consistent with respect to seroconversion (e.g., negative-positive-negative), the subject was classified as not having seroconverted. For this study, serological results for all paired samples were classified as a binary outcome (any seroconversion versus no seroconversion) within the 6-month at risk interval.

6.5 Household-level Data

During entomological surveys, household-level demographic data were collected on the type of dwelling. Variables available for analysis include the household area (square meters), own/rent status, number of rooms, presence of screens, type of construction, type of cooking fuel, construction material, electricity, telephone, source of household water, water storage locations, animals present, type of waste disposal, and any reported use of insecticide or larvicide.

6.6 Ethical Considerations

Adult study participants provided written consent for serology. Informed consent for serological data collection among children younger than 18 years of age was obtained from parents or guardians and informed assent was obtained from children 8-17 years of age. Written consent was obtained from an adult head of household for entomological surveys. Data collection procedures were approved by the University of California, Davis (Protocols 2002-10788 and 2007-15244), Instituto Nacional de Salud, and Naval Medical Research Center Institutional Review Boards (Protocols NMRCD.2001.0008 and NMRCD2007.0007) in compliance with all federal regulations governing the protection of human subjects. This ancillary analysis was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill (Study # 14-3151).

CHAPTER 7: THE RELATIONSHIP BETWEEN ENTOMOLOGICAL INDICATORS OF *AEDES AEGYPTI* ABUNDANCE AND DENGUE INFECTION

7.1 Introduction

Dengue virus (DENV), which is transmitted by the bite of female *Aedes aegypti* mosquitoes, causes more human morbidity and mortality than any other arthropod-borne virus.[2] Since the 1950s, dengue has spread via the globalization of trade and travel, rapid urbanization and expansion of vector habitats.[1] The four serotypes (DENV1, DENV2, DENV3 and DENV4) occur throughout the tropics and infect approximately 390 million persons per year.[2] Until effective DENV vaccines become broadly commercially available, vector control will remain the primary prevention strategy in most dengue endemic settings[40] and even as vaccines become accessible vector control will be needed to supplement vaccine efforts, as well as control of other arboviruses also vectored by *Ae. aegypti*.[141]

The World Health Organization recommends monitoring vector abundance for the targeting and evaluation of vector control interventions.[5] *Ae. aegypti* monitoring was first employed in yellow fever control programs in the first half of the 20th century.[50,8] Since then, over two dozen indicators have been proposed to quantify abundance of *Ae. aegypti*. Entomological monitoring data are typically collected from households sampled from neighborhoods or blocks on a routine or ad hoc basis.[10] Monitoring indicators vary by mosquito life stage (adults, larvae and/or pupae), availability of larval development sites (container index), and process of collection (fixed trap or human-based surveys such as adult aspirator collections, household inspection for larvae).[51] The public health utility of these indicators is based on the

assumption that greater mosquito abundance increases the risk of DENV transmission, and therefore reducing exposure to the vector decreases incidence of infection. Further, by identifying "hot spots" of *Ae. aegypti* infestation, targeted vector control would be an efficient use of limited intervention resources.[16]

To date, studies have not shown a consistent association between various indices and infection or disease outcomes.[8] This may be due to several limitations inherent to the large-scale measurement of *Ae. aegypti* densities. First, there is no established threshold of *Ae. aegypti* density associated with an increased risk of human DENV infection.[7] Second, entomological survey techniques may not capture the fine spatial and temporal variability in an urban setting due to the constraints dictated by household-based monitoring, and the fact that indices are calculated from cross-sectional prevalence measures, not derived from continuous monitoring. Third, while adequate sampling of immature and adult populations requires consideration of vector dynamics[53] and spatial relationships,[57] the data do not capture the daily productivity of individual larval development sites or the activity of individual mosquitoes over their lifespan.[10,55] Finally, previous attempts to quantify the association between vector abundance and dengue outcomes may also have been biased due to measurement error caused by operational constraints and collection procedures,[51] and methodological issues, such as restricting the analysis outcomes to infected people who sought treatment or small sample size.[8]

Ae. aegypti densities may also fail to describe risk of DENV infection due to the complexity of transmission. The probability of transmission is dependent on human movement to introduce DENV into mosquito populations and the presence of susceptible individuals that mosquitoes infect to perpetuate new rounds of transmission.[108] Because *Ae. aegypti* are daytime-biting mosquitoes that are highly adapted to the human urban environment,[142] their frequent

biting contact with susceptible human hosts is mediated by social and economic[35] factors that govern human movement through times and spaces where they encounter mosquitoes.[107] While high concentrations of *Ae. aegypti* within or around a household present an opportunity for clustered DENV transmission, it ignores transmission occurring in other places.[124, 11]

To help predict risk and direct public health interventions, there is substantial interest in an improved understanding of the relationship between measures of *Ae. aegypti* density and DENV infection, according to mosquito life stage and unit of measurement. This study aimed to systematically examine measures of entomological risk with human DENV infection using longitudinal entomological and human serology data to test associations between *Ae. aegypti* indices and the 6-month risk of DENV seroconversion.

7.2 Methods

7.2.1 Study Site

The analytical cohort was constructed using entomological and serological data collected between 1999-2003 and 2008-2010 from two longitudinal cohort studies implemented in Iquitos, Peru. Iquitos, the largest city in the Peruvian Amazon, has a population of approximately 350,000.[9] DENV1 is presumed to have been introduced in 1990-1991,[131] followed by DENV2 in 1995,[132] DENV3 in 2001,[133] and DENV4 in 2008.[134] From 1999-2003, study activities were implemented in four city districts: Maynas, Punchana, Belen and San Juan. During the period 2008-2010, data were collected from two neighborhoods: Maynas and Tupac Amaru (located within the Maynas and Punchana districts).

7.2.2 Entomological Data Collection

Procedures for entomological data collection were previously described.[57, 9] Briefly, once households were enrolled, two-person study teams collected entomological data following a

circuit to survey neighboring households on the same and/or neighboring block (with block defined as a group of households that shared a common perimeter defined by city streets) within an approximately two week period. The entire study area required approximately four months to complete data collection, upon which entomological surveys resumed following the same schedule.

Adult *Ae. aegypti* were collected using CDC backpack aspirators (1999-2009)[135] or Prokopack aspirators (2009-2010)[136] in both the exterior and interior of the participating household by passing the vacuum tube over common *Ae. aegypti* resting sites, outside walls, vegetation, and the entrance of potential larval habitats. Pupae and larvae were collected via enumeration of all wet containers or other larval development sites that contained water upon inspection. During surveys, all observed pupae and a sample of larvae were collected in small plastic Whirlpack bags; larval density was estimated as one of four levels (0, 1-10, 11-100, >100). All adult, larval and pupal samples were transported to and examined at the study laboratory, counted and identified to species and sex. Pupal data were recorded as observed counts. The total number of adult male and female *Ae. aegypti* mosquitoes collected in the interior and exterior of the dwelling were recorded. Household demographic data were collected for variables including enumeration of household residents by age and sex, household water source, sanitation facility, presence of electricity, type of building material, roof structure, and any reported use of insecticide or larvacide.

7.2.3 Indicators for *Ae. aegypti* Density

The indicators were classified by scale (household or block) and life stage (adult, pupal and/or larval). Household-level indicators were calculated using the observed survey data. To construct block-level indicators, all household survey data were first aggregated by block using a

unique block identification number and circuit schedule. Indicators were then calculated using the aggregated block-level *Ae. aegypti* data. Block-level measures were then linked back to individual households by matching on block identifier and date of collection. The household-level indicators and their definitions are summarized in Table 4 and block-level indicators are summarized in Table 5. To test categorical versions of continuous indicators, a preliminary analysis was conducted to identify cut-off values by estimating the sensitivity and specificity of the mosquito density in terms of DENV infection at different levels (data not presented). Categorical classification of continuous indicators tested is listed in Table 4 and Table 5. Data on eggs or exact larval counts were not collected in the parent study; therefore, indices relying on this information could not be tested.

Longitudinal household-level indicators were calculated as an average of entomological data observed within the 12 months preceding the start of the seroconversion interval (up to three survey visits collected approximately every four months). If a paired sample interval began before any entomological data collection, the cross-sectional measure of mosquito density was used. For block measures, indicators were calculated by averaging block-level densities calculated from surveys conducted within 12 months from the start of the seroconversion interval.

7.2.4 Serological Data Collection and Outcome Classification

In the parent study, members of households selected for entomological monitoring were asked to provide blood samples every six months.[9, 137] Samples were collected at the participant's home, stored in ice and transported to the study laboratory within four hours of collection. Sera were tested at two (1999-2003) and four (2008-2010) serum dilutions plaque reduction neutralization test (PRNT[138]) at the United States Naval Medical Research Unit No. 6

laboratory in Lima, Peru. To identify seroconversion to DENV, a serum sample was considered positive for DENV if a dilution neutralized 70% of the test virus ($PRNT_{70}$).[9, 137]

The primary outcome of interest in this analysis was seroconversion to any circulating DENV serotypes as determined by $PRNT_{70}$. The longitudinal serological samples used in this analysis were previously reviewed to determine seroconversion.[137] In brief, to minimize misclassification of serological data, the full serological profile of subjects was reviewed as follows: if the increase in titer that reduced DENV plaques between a negative sample and a subsequent sample was at least 20% and all subsequent samples were positive, the subject was determined to have seroconverted. However, if subsequent PRNT results were not consistent with respect to seroconversion (e.g., negative-positive-negative), the subject was classified as not having seroconverted. For this study, serological results for all paired samples were classified as a binary outcome (any seroconversion versus no seroconversion).

7.2.5 Construction of Analytical Cohort

Serological data were reviewed to identify paired sample observations taken approximately six months apart that could be linked to household entomological data. To account for operational constraints around serology collection, the at-risk interval was defined as 140 to 220 days. Each paired sample interval for which a subject was susceptible to any of the circulating DENV serotypes (DENV1 and DENV2: all study years; DENV3: 2001-2010; DENV4, 2008-2010) was included in the risk set.

For household-level indicators, entomological data were matched by the date nearest the end of (but within) each paired serological sample interval. For block-level indicators, datasets were constructed by restricting to serological observations from blocks in which at least five households were surveyed, using the month and year of block data collection to anchor in time

block-level densities to serology. Finally, longitudinal densities were calculated by averaging entomological data collected in the 12 months preceding the start of the seroconversion interval.

7.2.6 Statistical Analysis

The association between each *Ae. aegypti* indicator and the 6-month risk of DENV seroconversion was estimated using a log binomial generalized estimating equation (GEE)[143], separately for each household-level and block-level indicator, and for both the cross-sectional and longitudinal scenarios. The log link with a binomial distribution allowed for estimation of risk ratio point estimates by exponentiating the beta coefficient for the indicator variable and calculation of 95% confidence intervals (CI).[106] For models using household-level densities, the GEE accounted for clustering due to repeated individual measures and dependence due to household membership using an exchangeable correlation structure; models for block-level densities accounted for repeated observations from individuals and block level membership. A directed acyclic graph (Appendix) identified *a priori* dengue transmission season, participant age and sex as confounding variables for use in all adjusted analyses of household-level indicators and season, participant age, and reported use of larvacide for adjustment of all block-level indicators. All analyses were conducted in SAS/STAT software, version 9.4 of the SAS system for Windows (SAS Institute, Cary, NC).

Sensitivity analyses were conducted to account for possible bias resulting from construction of the dataset (Appendix). Different inclusion criteria for serological observations were used to test more restrictive or relaxed scenarios. Additional sensitivity analyses included alternate strategies for linking serology to entomology, densities calculated from entomological data 6 months prior to serology, and stratification by aspirator type used during data collection.

Table 4. Summary of household-level indicators of *Aedes aegypti* tested for an association with seroconversion to DENV

Indicator	Definition/Formula	Variable type
Adult *Ae. aegypti* in the household	Number observed	Continuous
	Exposed defined as any adults observed (>0)	Categorical
Adult female *Ae. aegypti* in the household	Number observed	Continuous
	Exposed defined as any adult females observed (>0)	Categorical
Presence of adult *Ae. aegypti* indoors	Number observed	Continuous
	Exposed defined as any adult indoors (>0)	Categorical
Presence of adult female *Ae. aegypti* indoors	Number observed	Continuous
	Exposed defined as any adult female indoors (>0)	Categorical
Single Larval Method	# containers with ≥1 larvae	Continuous
	Exposed defined as SLM >0	Categorical
Presence of pupae in the household	Exposed defined as any pupae observed in containers (>0)	Categorical
Pupae per Hectare	# pupae/household area measured in hectare	Continuous
Pupae per Person	# pupae/household population	Continuous
Container Index (Receptacle Index)	# of containers infested with larvae or pupae/ total number of containers inspected x 100%	Continuous
	Exposed defined as CI >0	Categorical
Stegomyia Index	# positive containers (larvae or pupae)/population x 1000	Continuous
	Exposed defined as SI >0	Categorical

Parentheses signify different name for the indicator.

Table 5. Summary of block-level indicators of *Aedes aegypti* tested for an association with seroconversion to DENV

Indicator	Definition/Formula	Variable type
Breteau Index	(#containers infested/total households) x 100	Continuous
	Exposed defined as BI ≥5	Categorical
House Index (Premise Index; *Aedes* Index)	(# households infested /total households) x 100%	Continuous
	Exposed defined as HI ≥5	Categorical
Adult Premise Index	# premises positive for adult females/#premises x 100	Continuous
	Exposed defined as APrI ≥5	Categorical
Adult Density Index	# adult females / # of premises	Continuous
	Exposed defined as ADI >0	Categorical
Pupa Index	(# pupae/total number households inspected) x 100	Continuous
	Exposed defined as PI >5	Categorical
Pupae per Hectare	# pupae/household area (hectare)	Continuous
Pupae per Person	# pupae/household population	Continuous
Infested Receptacle Index	# positive containers/total number of households	Continuous
	Exposed defined as IRI >0	Categorical
Container Index (Receptacle Index)	# containers infested/total number of containers inspected x 100%	Continuous
	Exposed defined as CI ≥5	Categorical
Potential Container Index	(# potential breeding sites + # positive breeding sites)/households inspected	Continuous
	Exposed defined as PCI≥2	Categorical
Stegomyia Index	# positive containers (larvae or pupae)/population x 1000	Continuous
	Exposed defined as SI ≥5	Categorical

7.3 Results

In total, 13,526 households contributed 90,330 entomological observations and 25,755 paired serological samples (from 6,775 individuals). A total of 20,176 serological observations could be linked to entomological data. For the cross-sectional household-level analysis, 4,089 household entomological observations (from 1,377 unique households) were linked to 8,153 paired blood samples (from 3,824 individuals). For the longitudinal household-level analysis, 15,548 entomological observations from those 1,377 households were used to calculate average densities and matched to the 8,153 serological observations. The same set of serological and entomological observations were included in the block-level analyses, with the exception of 579 serological observations for which a block density could not be obtained (<5 households per block-visit were surveyed). A total of 7,574 serological paired samples (from 3,644 individuals) were used in the cross-sectional and longitudinal block-level analyses.

The mean age of individuals at first paired sample was 20.9 years (standard deviation: 16.3, range 2-96) and 57.7% of subjects were female. At first study visit, most households contributing any serological data reported access to electricity (99.7%), piped sanitation (77.1%), and potable water (75.2%), had open or partially open household roof structure (93.0%), and were constructed from either mud and/or wood (49.2%) or concrete and/or brick (50.8%). Only 28.2% of households reported using Abate (larvacide) at enrollment. There were a total of 1,191 seroconversions (14.6%) in the analysis of household level indicators and 1,129 seroconversions (14.9%) in the analysis of block-level indicators. Tables 6-8 present the distribution of entomological indicators.

7.4 Cross-sectional Densities

The adjusted RR point estimates and 95% CI are presented in Table 9. The household-level point estimates ranged from 0.75 (95% CI: 0.48, 1.34) to 1.05 (95% CI: 0.91, 1.21), suggesting no difference in the 6-month risk of DENV seroconversion based on *Ae. aegypti* density. At the block level, six indicators showed significant protective effects, which could be the result of higher background immunity, correlation with factors related to lower DENV risk, or chance. Compared to the adjusted RR estimates, crude risk ratio point estimates were similar for the household-level indicators and were slightly larger for block-level indicators (Appendix).

7.3.2 Impact of Repeated Measures on Household-level Indicators

Using the average of densities measured in the 12 months prior to the paired sample, the RR point estimate shifted above the null for categorical measures of adult density, adult female mosquitoes, and presence of adult mosquitoes indoors (any adults as well as only females), ranging from 1.25 (95% CI: 1.12, 1.39) to 1.30 (95% CI: 1.17, 1.46). This suggests that the observation of an adult female mosquito during a household survey performed during the 12 month period prior to collection of paired sera is associated with an approximately 25% increased risk in acquisition of DENV infection compared to the risk among individuals residing in households where no adult female was observed at any survey during the 12 months preceding the paired sera. In addition, four immature stage indicators suggested an elevated risk of DENV infection: any pupae observed; the Single Larval Method (categorical); Container Index (categorical) and the *Stegomiya* Index (categorical).

7.3.3 Impact of Repeated Measures on Block-level Indicators

Analysis of block-level indicators that incorporated repeated measures demonstrated a similar trend in which all measures calculated based on adult mosquito data shifted in comparison to the cross-sectional analysis: the Adult Premise Index (RR: 1.01; 95% CI: 1.01,

1.02 when continuous and RR: 1.24; 95% CI: 1.01, 1.48 as categorical) and the Adult Density Index (RR: 1.24; 95% CI: 1.02, 1.50 as continuous and RR: 1.72; 95% CI: 1.22, 2.43 as categorical). The Pupa Index (categorical) and the Infested Receptacle Index (categorical) were the only immature stage block-level indicators to demonstrate any association with DENV infection.

7.3.4 Sensitivity Analysis

Sensitivity analyses to compare different analysis datasets did not result in qualitatively different findings. Sensitivity analyses in which the inclusion of seroconversion events was relaxed and restricted did not alter interpretation of the main findings (Appendix). *Ae. aegypti* densities calculated from 6 months prior to the start of a seroconversion interval followed a similar pattern as results presented (Appendix). When analyzed separately, use of different aspirators over the course of data collection did not result in substantially different results for adult stage measures (Appendix).

Table 6. Distribution of continuous entomological monitoring indicators for serological observations: cross-sectional densities

Indicator	Total	Mean	Standard Deviation	Min	Max
Continuous Measures					
Household level [a]					
Adult *Ae. aegypti*	8153	0.7	3.6	0	163.0
Adult female *Ae. aegypti*	8153	0.4	1.7	0	51.0
Single Larval Method	8153	0.2	0.6	0	9.0
Pupae in household containers	8153	1.7	15.9	0	642.0
Pupae per Hectare	8153	114.6	1154.4	0	62879.5
Pupae per Person	8139	0.24	2.5	0	78.0
Container Index	8153	4.3	13.0	0	100.0
Stegomiya Index	8133	0.03	0.1	0	2.0
Block level [b]					
Breteau Index	7574	22.1	23.3	0	214.3
House Index	7574	14.4	12.4	0	63.6
Adult Premise Index	7574	15.3	12.4	0	88.9
Adult Density Index	7574	0.3	0.4	0	8.8
Pupa Index	7574	186.0	418.4	0	9312.5
Pupae per Hectare	7574	123.2	279.9	0	7669.7
Pupae per Person	7574	0.3	0.6	0	11.9
Infested Receptacle Index	7574	0.2	0.2	0	2.1
Container Index	7574	5.5	4.9	0	33.3
Potential Container Index	7574	2.8	1.4	0.2	9.2
Stegomiya Index	7574	34.1	36.2	0	389.6

[a] Sample size for household-level indicators varies due to missing data on number of residents reported and missing data base on linkage of serology to entomology.
[b] Sample size for block-level analysis does not change as these are aggregated measures summarized over a group of households.

Table 7. Distribution of continuous entomological monitoring indicators for serological observations: longitudinal densities

Indicator	Number	Mean	Standard Deviation	Min	Max
Continuous Measures					
Household level [a]					
Adult *Ae. aegypti*	8153	0.5	1.8	0	84.0
Adult female *Ae. aegypti*	8153	0.3	1.04	0	47.0
Single Larval Method	8145	0.2	0.5	0	6.8
Pupae in household containers	8148	2.1	11.1	0	289.0
Pupae per Hectare	8153	180.6	1639.9	0	62276.3
Pupae per Person	8143	0.3	3.0	0	108.7
Container Index	8153	0.4	3.9	0	100.0
Stegomiya Index	8133	0.03	0.1	0	1.5
Block level [b]					
Breteau Index	7574	26.1	22.7	0	404.5
House Index	7574	16.3	10.7	0	63.0
Adult Premise Index	7574	14.0	8.3	0	50.0
Adult Density Index	7574	0.3	0.3	0	3.8
Pupa Index	7574	227.9	345.1	0	5283.3
Pupae per Hectare	7574	154.7	242.1	0	2611.3
Pupae per Person	7574	85.2	170.2	0	2556.6
Infested Receptacle Index	7574	0.3	0.2	0	4.0
Container Index	7574	6.2	4.0	0	34.5
Potential Container Index	7574	5.9	4.0	0	34.5
Stegomiya Index	7574	40.3	35.8	0	679.4

Table 8. Distribution of categorical indicators for serological observations

	Cross-sectional		Within 12 months	
	Number exposed	(%)	Number exposed	(%)
Household level[a]				
Any adult *Ae. aegypti*	1924	23.6	3543	43.5
Any adult female *Ae. aegypti*	1319	16.2	2578	31.6
Any adult *Ae. aegypti* indoors	1829	22.4	3364	41.3
Any adult female *Ae. aegypti* indoors	1249	15.3	2432	29.8
Single Larval Method	1143	14.0	2546	31.2
Any pupae in household containers	610	7.5	1589	19.5
Container Index	1146	14.1	2548	31.3
Stegomiya Index	1146	14.1	2537	31.1
Block level[a]				
Breteau Index	5699	752	6713	88.6
House Index	5543	73.2	6451	85.2
Adult Premise Index	5976	78.9	6690	88.4
Adult Density Index	6464	85.3	7198	95.1
Pupa Index	5245	69.3	6729	88.9
Infested Receptacle Index	6404	84.6	7219	95.3
Container Index	3603	47.6	4426	58.4
Potential Container Index	5129	67.7	6424	84.8
Stegomiya Index	6148	81.2	6924	91.4

[a] Analytical sample size as indicated in Tables 6 and 7.

Table 9. Adjusted risk ratios: association between *Ae. aegypti* and DENV seroconversion

	Cross-sectional			Longitudinal		
Indicator	**Risk Ratio**	**95% CI**		**Risk Ratio**	**95% CI**	
Household level[a]						
Adult *Ae. aegypti* (continuous)	1.00	0.99	1.01	1.02	1.00	1.05
Adult *Ae. aegypti* (categorical)	1.02	0.90	1.15	1.25	1.12	1.39
Adult female *Ae. aegypti* (continuous)	0.99	0.97	1.02	1.04	1.00	1.09
Adult female *Ae. aegypti* (categorical)	1.03	0.90	1.18	1.29	1.16	1.44
Adult *Ae. aegypti* indoors (categorical)	1.04	0.92	1.18	1.26	1.13	1.40
Adult female *Ae. aegypti* indoors (categorical)	1.05	0.91	1.21	1.30	1.17	1.46
Single Larval Method (continuous)	0.97	0.88	1.06	1.07	0.98	1.16
Single Larval Method (categorical)	0.92	0.79	1.08	1.23	1.11	1.38
Pupae in household containers (continuous)	0.99	0.99	1.00	1.00	1.00	1.00
Pupae in household containers (categorical)	0.96	0.78	1.18	1.21	1.07	1.37
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.96	0.92	1.01	1.00	0.98	1.02
Container Index (continuous)	1.00	1.00	1.00	0.98	0.95	1.00
Container Index (categorical)	0.92	0.78	1.08	1.23	1.11	1.38
Stegomiya Index (continuous)	0.75	0.42	1.34	1.06	0.61	1.82
Stegomiya Index (categorical)	0.92	0.78	1.08	1.24	1.11	1.38
Block level[b]						
Breteau Index (continuous)	1.00	0.99	1.00	1.00	1.00	1.00
Breteau Index (categorical)	1.03	0.91	1.17	0.89	0.76	1.05
House Index (continuous)	0.99	0.99	1.00	1.00	0.99	1.00
House Index (categorical)	1.04	0.92	1.17	0.91	0.79	1.00
Adult Premise Index (continuous)	1.00	0.99	1.00	1.01	1.01	1.02
Adult Premise Index (categorical)	0.87	0.76	0.98	1.24	1.01	1.48
Adult Density Index (continuous)	0.96	0.84	1.10	1.24	1.02	1.50
Adult Density Index (categorical)	0.83	0.72	0.95	1.72	1.22	2.43
Pupa Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupa Index (categorical)	1.00	0.89	1.12	1.30	1.08	1.57
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.76	0.65	0.87	1.00	1.00	1.00
Infested Receptacle Index (continuous)	0.62	0.46	0.82	0.93	0.72	1.20
Infested Receptacle Index (categorical)	0.98	0.85	1.14	1.75	1.23	2.50
Container Index (continuous)	0.99	0.98	1.00	1.01	0.99	1.02
Container Index (categorical)	0.96	0.86	1.07	1.00	0.90	1.11
Potential Container Index (continuous)	0.91	0.87	0.96	1.01	1.00	1.03
Potential Container Index (categorical)	0.76	0.67	0.85	0.99	0.86	1.15
Stegomiya Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Stegomiya Index (categorical)	0.99	0.86	1.13	1.13	0.93	1.39

[a]Adjustment variables: DENV transmission season (May-Aug, reference group; Sept-Dec, Jan-Apr); participant sex (Male; Female, ref); Participant Age (<18 years (ref), ≥18 years). [b]Adjustment variables: Season; reported use of larvicide; participant Age (<18 years, ≥18 years).

7.4 Discussion

The principal finding of this analysis is that a higher household level *Ae. aegypti* density calculated from cross-sectional entomological data was not associated with an increase in the risk of DENV infection. Compared to cross-sectional measures, the average *Ae. aegypti* density in the past 12 months resulted in more plausible effect estimates, especially for adult indices which monitor the life stage relevant to DENV transmission. Entomological evidence suggests that *Ae. aegypti* populations in Iquitos are highly variable in time and space[113] and the indices obtained from trimestral surveys are unlikely to capture all of the fine-scale temporal variation that occurred. It is thus possible that cross-sectional entomological survey procedures in which adult data are measured over a short period of time may result in lower or higher densities being attributed to the entire risk period.[103] This may be due to households with low levels of infestation being misclassified as having no *Ae. aegypti* present when relying on a single measurement.

Immature stage indicators were not associated with risk of DENV infection, with the exception of a few categorical indicators calculated from longitudinal data. This could be due to high larval mortality, the short lifespan of larvae and pupae, and brief time interval of data collection, resulting in immature population measures that do not always correlate in space and time with the biologically relevant adult measures.[24] For block-level indicators, aggregating household data could skew calculation of the indicator if the distribution of larval and pupal counts was concentrated in only a few households. Block-level indicators such as the Breteau Index and the House Index, which classify containers or households as "infested" if any larvae or pupae are observed, may not capture the contribution of container productivity. The pupae per

person and pupae per hectare measures are sensitive to bias from inaccuracies in population or area data, as well as sampling error as the pupal life stage is ephemeral.

7.4.1 Strengths

The major strengths of this analysis include the use of DENV infection (not disease) as an outcome, examination of longitudinal data, and its generalizability to similar settings in which routine, periodic entomological surveillance is conducted. While dengue disease is relevant from a public health perspective and easier to quantify, DENV infection, measured as seroconversion, is more important in terms of understanding patterns of transmission from mosquitoes to humans. Most prior studies of entomological indicators and dengue outcomes,[8] used symptomatic disease as the outcome. Symptomatic cases represent the small fraction of all infections that were severe enough to seek medical evaluation, thus introducing selection bias. This analysis also benefitted from longitudinal serological data, which enabled exclusion of paired sample observations once an individual was determined to no longer be at risk of infection by circulating serotypes.

Most prior studies used cross-sectional entomological data. Longitudinal entomological monitoring allowed the use of multiple (1 to 3 per household) mosquito measures per household. This may overcome some of the measurement error of entomological assessment and account for the temporal variability associated with entomological data collection. These results suggest the possibility that in any single entomological survey, a household with low-levels of *Ae. aegypti* infestation may be misclassified as having no infestation, at least for adult stage measures of abundance, which would bias the RR downwards.

Since these data were generated as part of a research study, they were subjected to rigorous monitoring of field collection procedures, reducing the extent of measurement error

compared to data collected in a programmatic context. Nevertheless, the findings are likely generalizable to similar dengue-endemic settings as the timing of serological and entomological collection employed are representative of the routine periodic monitoring used in dengue control programs.

7.4.2 Limitations

These results should be interpreted in light of the several limitations. First, a large proportion (9,739 of 20,176) of serological data failed to meet the 6-month inclusion criteria, which could have resulted in bias due to their exclusion. Results from sensitivity analyses to include paired samples taken more than six-months apart did not qualitatively change the findings (Appendix). Second, the entomological and serological monitoring data relevant for DENV transmission did not perfectly coincide temporally, possibly leading to bias due to time of measurement. In sensitivity analyses, results were not sensitive to different approaches to link entomology and serology (Appendix). While the use of averages is not the most sophisticated method to incorporate temporal lags and does not account for the spatial distribution of *Ae. aegypti*, it is implementable in basic statistical software and may be of utility to dengue program managers.

Third, while $PRNT_{70}$ is the most specific serological test for dengue infection, results from this assay may be biased due to cross-reactions from antibodies directed against multiple serotypes present in a single sample or with closely related viruses. The algorithm used to classify seroconversions was conservative, possibly underestimating the number of seroconversions, but this bias is likely non-differential with respect to mosquito density.

In this analysis, continuous indicators were tested as linear terms to maintain consistency with their definitions in the literature. It is possible that log-transformation or inclusion of

polynomial terms could improve model fit, but such manipulation would reduce interpretability. For continuous indicators, the RRs measure the relative risk for a one-unit change in the indicator value; these measures are likely not informative for targeting interventions. From a public health perspective, categorical indicators are more useful to trigger vector control activities. In Iquitos, levels of infestation were heavily dispersed and binary classification (any v. none) was most informative.

7.4.3 Conclusions

The risk ratios presented in this analysis should be interpreted as population-level effect estimates. There are likely differences in the RRs if measured among smaller groups in the community, such as the block or neighborhood that these measures do not capture. None of the RRs presented in this analysis represent a causal relationship between household or block-level mosquito density and true exposure to DENV. It is logistically impossible to monitor human-vector contact to establish where and when mosquito-human interaction and infection occurs. Therefore, *Ae. aegypti* indicators serve as surrogates of exposure, which will always remain unmeasured. Although adult measures that incorporated longitudinal data demonstrated an association, it is possible that some unmeasured variable associated with social network patterns, housing quality and day-time human movement modifies dengue risk. Entomological monitoring indicators were not developed to account for these additional factors. If this modification is present, it would further undermine the utility of these indicators as household-based measurement of *Ae. aegypti* would be insufficient to represent the entire range of exposure to DENV.

DENV transmission is complex and time-varying; the relationship between vector density and risk is not static nor adequately characterized through periodic entomological surveillance. While entomological monitoring will continue to serve a role in the evaluation of vector control

interventions (e.g., comparing pre- and post-intervention abundance), this analysis challenges the validity of most *Ae. aegypti* indicators as adequate proxies for true DENV exposure risk. In urban settings such as Iquitos, single cross-sectional measures of adult mosquito density and the immature stage indicators commonly used by dengue control programs, such as the Breteau Index and Container Index, may fail to measure risk of DENV infection. Measuring adult mosquito density over multiple occasions may be the best option, but is difficult to implement. Because cross-sectional entomological monitoring is unlikely to provide the information needed to target interventions based on levels of DENV infection risk, dengue control programs should consider whether the resources required to implement household monitoring of *Ae. aegypti* might be better deployed elsewhere.

CHAPTER 8: APPLICATION OF BAYESIAN MAXIMUM ENTROPY TO ESTIMATE THE ASSOCIATION BETWEEN ADULT *AEDES AEGYPTI* DENSITY AND SIX-MONTH RISK OF DENGUE VIRUS SEROCONVERSION

8.1 Introduction

Dengue virus (DENV) is transmitted by *Aedes aegypti* mosquitoes. Globalization, urbanization and climate change have expanded the geographic scope of vector habitats, and at least 128 countries are now believed to have established endemic transmission.[2] The four serotypes of DENV (DENV1, DENV2, DENV3 and DENV4), an RNA virus, infect approximately 390 million persons per year.[1] While the majority of DENV infections are inapparent or present as febrile illness, severe cases result in dengue hemorrhagic fever, which can be fatal. *Ae. aegypti* are of particular public health importance as they are also the vector of Zika virus, chikungunya, and yellow fever.[144]

Ae. aegypti are daytime-biting mosquitoes well-adapted to the human urban environment. In most dengue-endemic settings, mosquito breeding sites are prevalent due to lower quality housing conditions, poor container management and local climate, as well as substandard public water and sanitation infrastructure.[145] Since a vaccine for DENV is not widely available, most dengue control programs rely on vector control to decrease Ae. aegypti populations and thereby prevent transmission to humans. The World Health Organization (WHO) recommends entomological monitoring to target vector control interventions[5,6] to areas with greater abundance of *Ae. aegypti*. Abundance is typically characterized using a range of vector indices, measured from pupal, larval or adult life stages collected cross-sectionally at residential premises.[8, 10] Despite the widespread use of entomological monitoring data to identify

individuals at an elevated risk of DENV exposure, there is no established threshold of *Ae. aegypti* density that predicts transmission,[7] and no consistent association between various indices of infestation and dengue outcomes has been reported.[8]

The lack of association between vector measures and dengue-related outcomes may be the result of measurement error related to the choice of field collection methods used to measure infestation (e.g., traps, aspirators, inspection of breeding sites),[51, 136] the skill of field personnel, and extent of household access provided by residents.[146] In addition, each *Aedes* life stage poses unique challenges in terms of quantifying the population. Pupal and larval *Ae. aegypti* survive for only a few days and their mortality is high,[51] resulting in highly variable population estimates within short periods of time. Adult mosquitoes are capable of flight and difficult to capture,[52] and the choice of adult collection methods (aspirators and traps)[144, 136, 146] may underestimate their population size. It is possible that measurement error further results in a systematic downward bias in the number of adult *Ae. aegypti* observed, particularly among households with lower levels of infestation. If this measurement error is non-differential with respect to DENV infection status, then an association between density and dengue outcomes could be biased towards the null.

Alternatively, entomological monitoring measures may be poor proxies for true exposure to DENV due to the complexity of transmission between vector and human populations.[107] *Ae. aegypti* dispersal, breeding and feeding habits likely contribute to spatial clustering of vector populations within the household or neighboring households as the vector is known to have a short flight range of <100 m.[109,110] Harrington's[111] capture-release studies in Puerto Rico and Thailand found that over 80% of *Ae. aegypti* did not move beyond their release household. In Iquitos, Peru, spatial clustering was estimated at a scale of approximately 30m.[24] A study in

Ecuador found that juvenile mosquitoes clustered within distances of 20m and adult mosquitoes within distances of 10m,[112] suggesting that presence of the dengue vector in a household could be relevant for characterizing DENV exposure that occurs within neighboring households or within a block.

While *Ae. aegypti* spatial clusters have been previously documented, there is also evidence that the location of such clusters is highly variable over time. In Iquitos no consistent temporal pattern among the location of *Ae. aegypti* clusters was found.[113] In Saudi Arabia,[147] identification of *Aedes* "hot spots" were sensitive to the temporal and spatial scales used to aggregate mosquito data. The location clusters of *Ae. aegypti* immature populations in Argentina were found to shift over the course of four entomological surveys.[116]

In this study, information on household *Ae. aegypti* density combined with information on mosquito density observed among neighboring households is hypothesized to improve cross-sectional estimates of mosquito abundance. Therefore, the objective of this analysis is to apply the Bayesian Maximum Entropy (BME) geostatistical framework for a space/time analysis of adult *Ae. aegypti* density to determine if these predicted mosquito densities are more strongly associated with the six-month risk of DENV seroconversion than the observed densities.

8.2 Methods

8.2.1 Study Site

The analytical dataset was constructed using entomological and serological data collected in Iquitos, Peru. Data collection occurred between 1999-2003 and 2008-2010 during the implementation of two longitudinal cohort studies. Iquitos has been dengue-endemic since the early 1990s, with DENV1 and DENV2 introduced in the 1990s, DENV3 in 2001 and DENV4 in

2008. Data collection activities occurred throughout residential areas. Figure 7 presents a map of Iquitos and the location of all households contributing data.

8.2.2 Entomological Data Collection

Procedures for entomological data collection have been previously described.[9, 57] Once households were enrolled, two-person study teams conducted entomological surveys by following a circuit to sample households on the same block (and/or neighboring block) over a two week period. After approximately four months, the entire circuit was completed and entomological surveys resumed again following the same geographic pattern. The number of adult *Ae. aegypti* were measured by collection with CDC backpack aspirators (1999-2009, John W. Hock Company, Gainesville, FL)[52] or Prokopack aspirators (2009-2010)[136] throughout the premise by passing the vacuum tube over common *Ae. aegypti* resting sites, outside walls, vegetation, and the entrance of potential larval habitats. Household characteristics on water source, sanitation facility, presence of electricity, type of building material, roof structure, and any reported use of insecticide or larvacide were documented at each study visit.

8.2.3 Serological Data Collection Procedures

Serological procedures have also been previously described in detail.[9, 137] After households were selected for entomological monitoring, individuals were invited to provide blood samples approximately every six months for the duration of follow-up. Briefly, samples were collected in the household using standard techniques and transported within four hours via cold chain to the study laboratory. Sera were tested at two (1999-2003 samples) and four (2008-2010 samples) serum dilutions plaque reduction neutralization test (PRNT[138]) at the United States Naval Medical Research Unit No. 6 laboratory in Lima, Peru. The primary outcome of interest in this analysis was seroconversion to any circulating DENV as determined by $PRNT_{70}$. To minimize misclassification of serological data, the full serological profile of subjects was

reviewed as follows: if the increase in titer that reduced DENV plaques between a negative sample and a subsequent sample was at least 20% and all subsequent samples were positive, the subject was determined to have seroconverted. If subsequent PRNT results were not consistent with respect to seroconversion (e.g., negative-positive-negative), the subject was classified as not having seroconverted. For this study, serological results for all paired samples were classified as any seroconversion to DENV versus no seroconversion.

If the time between serological paired samples was approximately six months (within 140 and 220 days) it was included in the analysis; this window was used to account for operational constraints that resulted in variability in the amount of time between sample collections. Longitudinal serological samples were reviewed to determine prior DENV exposure. Each six-month paired sample interval during which a subject was susceptible to any of the circulating DENV serotypes (DENV1 and DENV2: all study years; DENV3: 2001-2010; DENV4, 2008-2010) contributed an observation to the dataset.

8.2.4 Statistical Analysis

The main objective was to determine if a space/time analysis could improve the association between mosquito density and DENV seroconversion by allowing neighboring observations in space and time to inform an estimate of mosquito density. As such, the study estimated the association between the predicted mosquito density and risk of DENV seroconversion and compared it to the association between the observed (original) density and DENV seroconversion.

First, the entomological and serological paired sample observations were matched by identifying the entomological visit closest to the end of the paired sample interval. The observed adult density was then calculated as the number of adult *Ae. aegypti* divided by the household area (m^2), using a GIS database and estimates of household area from entomological survey

observations. Adult mosquitoes per area was chosen as the primary variable of interest as the adult stage is most relevant to DENV transmission and to account for variations in household area across Iquitos. Crude risk ratios of the association between observed density and DENV seroconversion were estimated using a log binomial generalized estimating equation (GEE) to account for clustering among household members and repeat observations per subject. Risk ratios were adjusted for participant age (≥18 years v. <18 years), sex and dengue transmission season (3 levels).

To determine if space/time prediction of mosquito density reduced measurement error, and in turn resulted in a positive association between *Ae. aegypti* density and DENV seroconversion, the Bayesian Maximum Entropy (BME) geostatistical framework[148-150] was used to generate adult mosquito densities for household entomological observations in which no adult mosquitoes were observed, under the assumption that a proportion of those household observations were misclassified as "unexposed" to *Ae. aegypti*. *Ae. aegypti* densities in Iquitos have been previously described as highly over-dispersed, with an overwhelmingly large proportion of entomological survey observations reporting no adult mosquitoes.[18] While there is likely measurement error in the collection of adult *Ae. aegypti* across all observations, it is possible that some of these observations were not truly zero, rather were the result of measurement error due to the process of data collection and the biology of the vector. In this analysis, it was assumed that a household survey visit in which at least one mosquito was observed was far less likely to be incorrectly classified as "exposed" to *Ae. aegypti* compared to a survey observation in which no mosquitoes were observed. For example, measurement error in which one mosquito was observed instead of a true value of greater than one mosquito was less important than error which occurs for an observed value of zero where the true value was greater

than zero. In the analysis, using BME was limited to predict mosquito density for observations in which no adult *Ae. aegypti* were observed (observations for which uncertainty exists) and used the observed density as reported from entomological survey visits for which at least one mosquito was measured (observations that have some degree of certainty).

The BME method is a non-linear estimation approach that extends the linear kriging method by using Bayesian knowledge blending and Maximum Entropy information processing to account for a much wider knowledge base than that processed by kriging. In particular, BME accounts for the space/time variability and auto-correlation in the data and can process non-Gaussian distributions (e.g. uniform, etc.). Hampton *et al*[151] has shown that the BME platform is appropriate for mapping space/time processes that suffer from sparse data as is the case with *Ae. aegypti* abundance. For notation, let capital letters, e.g. X, represent random values, let lower case letters represent their realization, e.g. x, let bold face letters represent vector of values, e.g. $\boldsymbol{x}=[x_1, \ldots, x_n]$, and let $X(\boldsymbol{p})$ represent a space/time random field (S/TRF) describing the random value X at space/time location $\boldsymbol{p} = (\boldsymbol{s}, t)$, where $\boldsymbol{s}$ represents the spatial coordinates and t is the time measured in months since data collection began in January 1999.

Let $\boldsymbol{z}_d$ be the measured mosquito densities at sampled household space/time points $\boldsymbol{p}_d$. To generate a more normally distributed data, the log-transformed data was defined as $\boldsymbol{y}_d$ as $\boldsymbol{y}_d = \log \boldsymbol{z}_d$, and the log-transformed offset removed data $\boldsymbol{x}_d$ as $\boldsymbol{x}_d = \boldsymbol{y}_d - m_y$, where m_y is the mean of $\boldsymbol{y}_d$. In order to take the log transform, the over-dispersion of adult mosquito data (in which over 80% of entomological observations had no mosquitoes collected) had to be accounted for. For observations for which no adult mosquitoes were observed, 0.25 was substituted for zero, allowing for the mosquito density to be driven by the household area, not the mosquito count. This substitution is analogous to methods used to model data collected at a limit

of detection.[152] For all other observations (>0 adult mosquitoes observed), the log-transform of the density was used. $X(\boldsymbol{p})$ was defined as a homogeneous/stationary S/TRF for which the data $\boldsymbol{x}_d$ is a realization, and the log-mosquito density S/TRF was defined as $Y(\boldsymbol{p}) = X(\boldsymbol{p}) + m_y$ and the mosquito density S/TRF as $Z(\boldsymbol{p}) = \exp(Y(\boldsymbol{p}))$.

BME was used to model the space/time distribution of $X(\boldsymbol{p})$ and obtained that of the log-mosquito density using $Y(\boldsymbol{p}) = X(\boldsymbol{p}) + m_y$. BME incorporates the general knowledge base (G-KB) as the mean and variance of $X(\boldsymbol{p})$ across the S/TRF as well as site-specific knowledge (S-KB) that relates to locations for which data $\boldsymbol{x}_d$ were collected at $\boldsymbol{p}_d$. Under BME, the G-KB for the S/TRF $X(\boldsymbol{p})$ is described by the space/time mean function $m_x(\boldsymbol{p}) = E[X(\boldsymbol{p})]$, and covariance function $C_X(\boldsymbol{p}, \boldsymbol{p}') = E[[X(\boldsymbol{p}) - m_x(\boldsymbol{p}')][X(\boldsymbol{p}') - m_x(\boldsymbol{p}')]$. The G-KB is represented by $G = \{m_X(\boldsymbol{p}), C_X(\boldsymbol{p}, \boldsymbol{p}')\}$, and S-KB represented as $S = \{\boldsymbol{x}_h, \boldsymbol{x}_s\}$, where the hard data $\boldsymbol{x}_h$ consists of measured values where at least one mosquito was detected, and the soft data $\boldsymbol{x}_s$ corresponds to sampling events were no mosquito were found and therefore the mosquito density is below detection. The BME posterior pdf describing $X_k = X(\boldsymbol{p}_k)$ at an estimation points $\boldsymbol{p}_k$ coinciding with a household observation with zero mosquitoes reported is

$$f_K(x_k) = A^{-1} \int_{-\infty}^{\infty} dx_s f_G(\boldsymbol{x}_h, \boldsymbol{x}_s, x_k) f_S(\boldsymbol{x}_s, x_k)$$

where x_k is a realization of X_k, $\boldsymbol{x}_h$ are observed offset removed log-mosquito density values at surrounding households where the mosquito count was at least 1, $f_S(\boldsymbol{x}_s, x_k)$ is the S-KB pdf describing the uncertainty associated with the offset removed log-mosquito density at households where the mosquito count was zero, $f_G(\boldsymbol{y}_h, \boldsymbol{y}_s, y_k)$ is a multivariate Gaussian pdf with mean and covariance specified by the G-KB, and $A = \int_{-\infty}^{\infty} dy_k \int_{-\infty}^{\infty} dy_s f_G(\boldsymbol{y}_h, \boldsymbol{y}_s, y_k) f_S(\boldsymbol{y}_s, y_k)$ is a normalization constant.

The mean m_X of $X(\boldsymbol{p})$ was modeled as being zero since the $\boldsymbol{x}_d$ data is offset removed and therefore has a zero mean. The space/time covariance function $C_X(\boldsymbol{p}, \boldsymbol{p}')$, where $\boldsymbol{p} = (\boldsymbol{s}, t)$ and $\boldsymbol{p}' = (\boldsymbol{s}', t')$, was modeled by estimating experimental covariance values at various spatial lags $r = ||\boldsymbol{s}, \boldsymbol{s}'||$ and temporal lags $\tau = |t - t'|$, and fitting a covariance model consisting of three nested space/time exponential models.

The soft data at sampling points where no mosquito were detected were modeled using the approach described in Hampton *et al.*[151] to adjust in sampling variability in sparse data. In this approach the uncertainty associated with the log-mosquito density $Y_{s,i}$ at point $\boldsymbol{p}_{s,i}$ is described by a uniform probabilistic distribution of log-mosquito density in an interval, i.e. $\left\{Y_{s,i} \in I_i = \left[\log\left(\frac{min}{area_i}\right), \log\left(\frac{max}{area_i}\right)\right]\right\}$, where the minimum and maximum values represent lower and upper bounds of mosquito counts, and $area_i$ is the household area over which no mosquito was detected. This model accounts for the sampling variability by assigning wider intervals (i.e. more uncertainty) to zero counts in households with small sampled areas (i.e. where it is more likely that the area was not large enough to measure a low mosquito density).

The expected value of the BME posterior pdf at estimation $\boldsymbol{p}_k$ is the mean estimator of X_k, defined as

$$\hat{X}_k = E_K[X_k] = \int_{-\infty}^{\infty} dx_k f_K(x_k)$$

The variance of the BME posterior pdf provides an assessment of the associated estimation uncertainty. The mean m_y was then added back to $\hat{X}_k$ to obtain the mean estimate of log-mosquito density, and this value was further back log-transformed to the linear scale to obtain the median estimate of mosquito density. To implement BME, a range of user-defined parameters were identified *a priori* to find the model that best improved the ability of adult

density per household area to differentiate risk of DENV seroconversion. These parameters and the range of values tested are described in the Appendix.

The BME-generated estimates of mosquito density were substituted for the observed mosquito density for all household observations for which no mosquitoes were originally observed. These measures were then used to estimate the association between household *Ae. aegypti* density and DENV seroconversion using the same log binomial GEE model that was used to test for an association with the observed density. The updated mosquito densities were tested continuously as a linear term (with and without a log-transform) and with a restricted quadratic spline. Model-based predicted risks were generated to visualize the absolute risk difference. Quantiles of mosquito density were used to determine thresholds for categorization. Four categorical variables were constructed to represent exposure: binary density (≥0.01 v. <0.01); tertiles (3 levels: <0.005, 0.005 to <0.01, ≥0.01); indicator variables to compare densities ≥0.01 to those <0.005 (reference category) and 0.005-0.01 to <0.005; and a back-transform of the predicted adults per household area to extract the numerator in terms of mosquito counts (≥1 v. 0 mosquitoes).

To explore the contribution of space and time via the three nested covariance structures, predicted densities were generated for the covariance with a short spatial and temporal range, a short spatial and long temporal range and a long spatial and temporal range. The variance estimates of these densities were compared to determine whether variability in the predicted densities changed based on structure of the covariance model (Appendix). Estimation of spatial and temporal covariance and BME prediction were performed in Matlab using the BMElib software package.[150] Regression analysis was performed using SAS/STAT software, version 9.4 of the SAS system for Windows (SAS Institute, Cary, NC).

8.2.5 Sensitivity Analyses

A number of sensitivity analyses were performed to validate the main study findings. First, when using adult mosquito counts as a discrete variable, a binary variable of mosquito density was defined as "exposed" if adult *Ae. aegypti* were detected among any of its adjacent neighbors as well as any household (boundaries defined by GIS database) within 30m, 50m and 100m of the observation (Appendix), compared to density observed for the only the household in the main analysis. Second, whereas in the main analysis in which 0.25 was used to substitute for zero values, a range of values from 0.1 to 0.9 were used as substitute values for zero mosquitoes to model the covariance of ln(*Ae. aegypti* per household area) in a sensitivity analysis. Third, to explore reliability of the predictions, BME estimation was conducted 14 times for uniform priors and eight times for triangular priors using different user-defined settings in the numeric implementation of BME (Appendix). Fourth, approximately 6% of study households either split or merged over the course of observation. The main analysis used the household boundary as defined in the GIS database, rather than merging or dividing households. In a sensitivity analysis, these households were excluded (Appendix). Finally, to estimate the sensitivity of risk ratio estimates to the categorical definitions, other cut-off values for classifying household mosquito exposure were tested (Appendix).

8.3 Results

8.3.1 Characteristics of Study Sample

The parent study provided 90,330 entomological observations that could be linked by household study code to the GIS database. Among these, 128 observations were removed due to possible date errors and 156 because the household area was reported to be greater than 1000 m^2. The remaining 90,046 entomological survey observations from 13,484 households were used to

model the distribution of adult *Ae. aegypti* in space and time using BME. Characteristics of all households contributing entomological data are presented in Table 10.

Among the complete set of entomological survey observations, 17,739 household observations were reported positive for adult *Ae. aegypti* with at least one adult mosquito observed. The remaining observations (n=72,307) reported no presence of adult *Ae. aegypti.* The mean adult mosquito density (adult *Ae. aegypti* per household area) for observations with at least one mosquito was 0.012 (SD: 0.06, Min: 0.001, Max: 3.22). The mean household area was 159.2 (SD: 97.4, IQR: 95-198) and did not differ between households that ever and never reported presence of adult *Ae. aegypti (*158.2m^2 vs 159.2m^2).

Of the 90,046 entomological observations, 4,087 were obtained from 1,375 households linked to subjects who provided serology. Overall, 3,819 individuals contributed one or more (median 2.1, SD: 1.3, IQR: 1-3) paired sample serological observations that met the inclusion criteria totaling 8,145 paired samples for the final analysis. Individuals providing serology were predominantly female (57.6%) and younger than 18 years of age at first sample (62.0%).

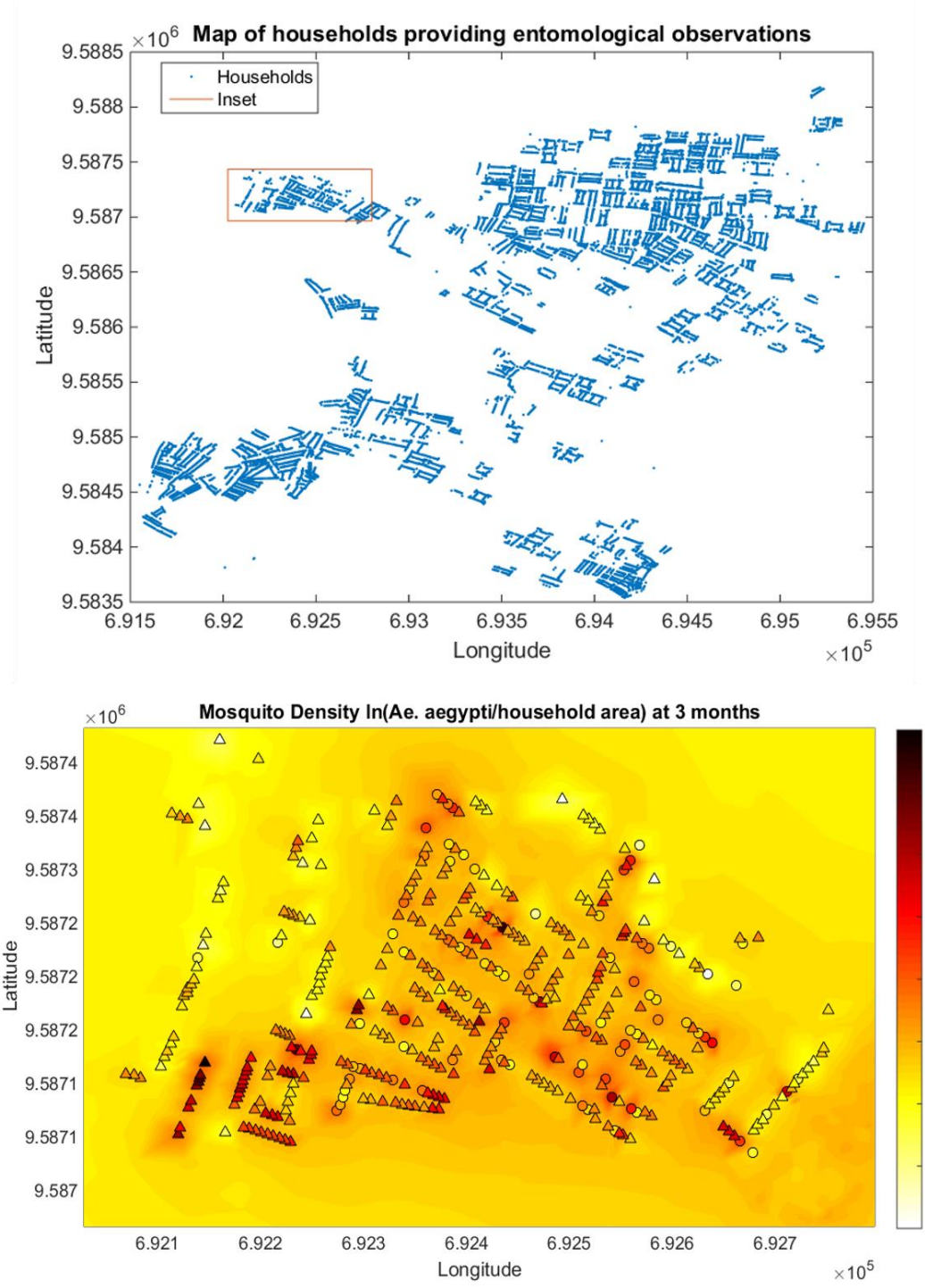
Table 10. Characteristics of the study participants and household

	Mean	SD	IQR*
Households contributing data to the BME analysis (N=13,484)			
Number of entomological observations per household	6.70	5.00	1-11
Household Area (m^2)	159.2	97.4	95-198
Number of rooms surveyed	4.2	1.7	3-5
Number of residents reported	6.3	2.8	4-8
Number of adult *Ae. aegypti* observed	0.5	3.7	0-620*
Number of adult female *Ae. aegypti* observed	0.3	1.6	0-210*
Adult *Ae. aegypti* per m^2 (pre-BME estimation)	0.004	0.03	0-3.2*
Adult female *Ae. aegypti* per m^2 (pre-BME estimation)	0.002	0.01	0-0.89*

	N	Percent (%)
*Characteristics of households from which individuals providing serology reside (N=1,375)***		
Wood/Mud construction	726	52.8
Wood stove	489	35.9
Lack of piped sanitation	250	18.3
Lack of potable water	256	18.6
Reported Abate use	640	46.6
Characteristics of household entomological observations linked to serology (N=4,087)		
≥ 1 adult *Ae. aegypti* observed	982	24
≥ 1 adult female *Ae. aegypti* observed	673	16.5
≥ 1 container positive for larvae or pupae	651	15.9

*Min and Max presented where IQR would have been 0-0.
**Characteristics at first survey visit.

Figure 7. Study data across space



A map of households providing observations (above) with an inset highlighting density prediction for March 1999 (below). In the bottom figure, households for which at least one mosquito was observed are identified by circles; triangles represent households for which no mosquitoes were observed. The ln(mosquitoes/area) was predicted using BME.

8.3.2 BME Estimation Results

As shown in Figure 8, the experimental covariance calculated from the observed data suggest that co-variability in space between any two observations was negligible beyond a distance of approximately 25m. Temporal covariance dropped to roughly 40% of the total variance at a temporal distance of approximately 5 months, but remained constant over a longer temporal range. A model of three nested, space-time separable exponential structures was fitted to the experimental covariance values. The space/time covariance model used for BME estimation was defined as:

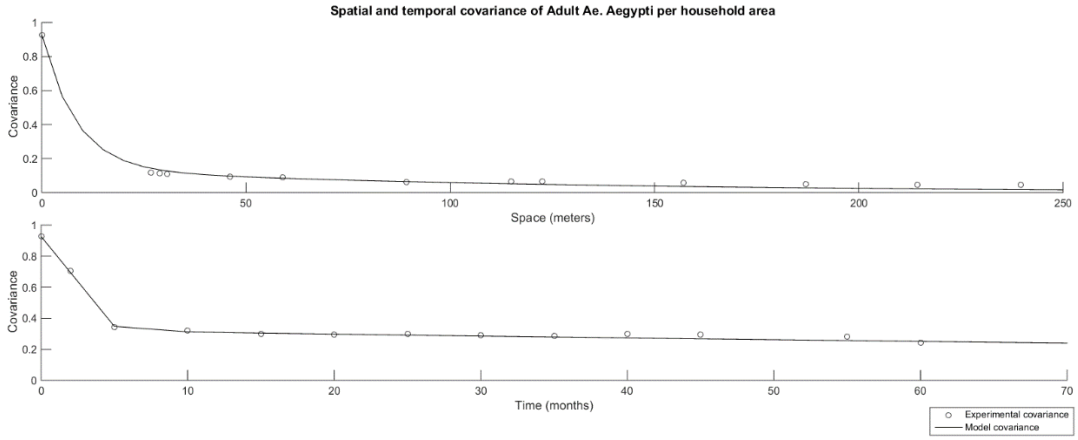
$$c_X(r,\tau) = var_X\left[0.65\exp\left(-\frac{3r}{a_{r1}}\right)\exp\left(-\frac{3\tau}{a_{t2}}\right) + 0.2\exp\left(-\frac{3r}{a_{r1}}\right)\exp\left(-\frac{3\tau}{a_{t2}}\right) + 0.15\exp\left(-\frac{3r}{a_{r2}}\right)\exp(-\frac{3\tau}{a_{t2}})\right]$$

where r represents the spatial lag (distance in space) and τ represents the temporal lag (distance in time). The spatial range values, a_{r1} and a_{r2}, were defined as 25m and 350m, respectively. The temporal ranges, a_{t1} and a_{t2}, were defined as 5 months and 700 months.

To illustrate the impact of BME on the distribution on mosquito density, Figure 7 presents a map of the households for which entomological data were observed as well as BME-predicted densities for a sub-set of households. The predicted density values for those areas surrounding the hard data observations were similar in magnitude, suggesting a reasonable fit to the data. By design, the density predictions for observations for which no mosquitoes were reported were generally larger than the observed density. The distribution of ln(*Ae. aegypti* per household area) before and after BME estimation for the complete dataset of 90,046 observations is presented in Figure 9. BME estimation shifted the overall distribution of the soft data towards larger densities, with a mean ln(*Ae. aegypti* per household area) of -5.2 (SD: 0.51; Min:-6.7;

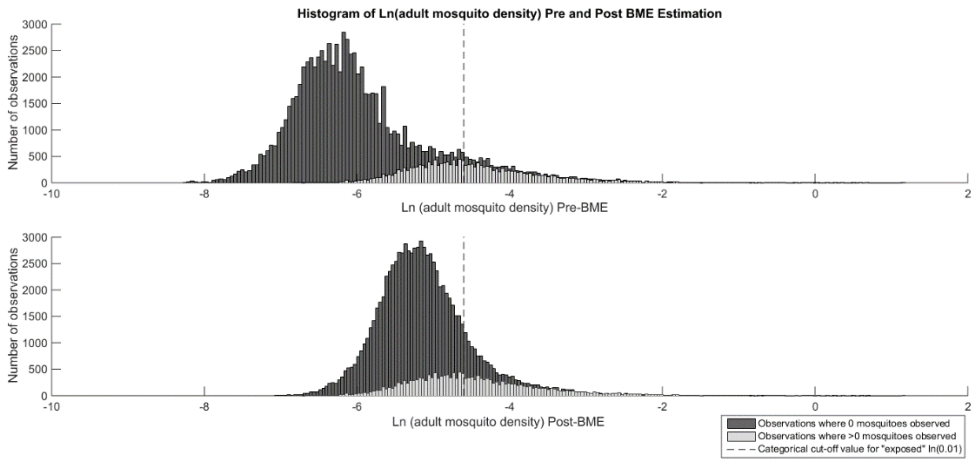
Max: -3.2) compared to a distribution of mean -6.2 (SD:.59; Min:-7.8; Max:-3.6) pre-BME estimation for the soft data points.

Figure 8. Spatial and temporal covariance of Adult *Ae. aegypti* per household area



In Figure 8, the spatial experimental covariance is plotted (top figure) alongside the temporal covariance (bottom figure). The covariance among observations with a distance beyond 25m is negligible. The temporal covariance drops from zero to five months, but is consistent over large temporal distances.

Figure 9. Histogram of Ln (Adult Mosquito Density) Pre and Post BME Estimation



In Figure 9, The histogram (top) shows the distribution of log-transformed density values for the data prior to BME estimation. In dark grey, the observations for which zero mosquitoes ("soft data") were observed are plotted. Log-transformed density values were calculated by dividing 0.25 by the household area (m^2) as a log transformation of the original values would be undefined. In light-grey, the observations for which at least one mosquito was observed ("hard data") are displayed. The histogram (bottom) of post-BME mosquito density shows the predicted densities for the soft data in dark grey contrasted with the density values for the hard data points remain unchanged. A reference line at ln(0.01) illustrates a possible categorical cut-off value for mosquito "exposure".

8.3.3 Mosquito Density and Risk of DENV Seroconversion

No association was found between the observed adult *Ae. aegypti* density and the risk of DENV seroconversion. As shown in Tables 11-13, adjusted risk ratios show null associations for four categorical approaches to classifying household mosquito exposure. After implementation of BME, no association was observed between mosquito density and DENV seroconversion for the indicators listed in Table 12, with the exception of the variable comparing a density ranging from 0.005 to <0.01 (approximately the first and third quartiles): RR 1.14 (95% CI: 1.01, 1.28). This suggests that while BME did account for some measurement error, it was likely effective only for observations with consistently low density. The 95% CI for the BME-predicted densities were narrower than the 95% CI for the original risk ratios, demonstrating that BME improved precision of the effect estimates across all comparisons. A sensitivity analysis utilizing a triangular distribution for the soft data priors also observed no association, but found slightly larger effect estimates with similar precision (Tables 11-12). Fourteen different sets of

parameters were tested to optimize the prediction of mosquito density in BME; all generated similar null results (see Appendix).

In order to compare the association with DENV risk between the observed mosquito density and BME predicted density as continuous variables, a restricted quadratic spline was used to model the association between adult *Ae. aegypti* per household area. In Figure 10, the pre-BME mean association is essentially null, as illustrated by the flat trend in predicted risk of DENV seroconversion. In contrast, the post-BME densities show an increase in risk for densities between approximately 0.005 and 0.01 after which the predicted risk shows no difference compared to a density of zero. Although the range of density categorized by this comparison is relatively small, it accounts for 41.3% of the serological observations in the analysis (see Figure 11). Individual and household demographic characteristics among these observations were similar to characteristics among observations with lower or higher densities (data not shown), suggesting that the difference in predicted risk is not the result of housing-related variables that modify true exposure to DENV, whether that exposure occurred at the household or elsewhere.

Table 11. Association between household *Ae. aegypti* density and 6-month risk of DENV seroconversion: pre-BME Estimation

Indicator	Risk Ratio[a]	95% CI	
Adult *Ae. aegypti* per area (m^2)			
Continuous (log scale)[b]	-	-	-
Binary (≥0.01 v.<0.01)	1.00	0.86	1.17
≥1 mosquitoes v. zero mosquitoes[c]	1.02	0.90	1.15
Tertiles (3 levels)[d]	1.01	0.94	1.09
Categorical indicator variables			
≥0.01	1.05	0.88	1.27
0.005 to <0.01	1.07	0.85	1.34
<0.005 (reference group)	1.00	-	-

[a]Adjustment variables: Dengue virus transmission season (May-Aug, reference group; Sept-Dec; Jan-Apr); Participant sex (Male, Female); Participant Age (<18 years, ≥18 years).
[b]RRs estimating the relative risk on the log scale because the log of zero is undefined.
[c]Count of mosquitoes was back-transformed from BME-mean estimates by multiplying the density by the household area
[d]Tertiles defined as 0= <0.005; 1=0.005 to <0.01; 2= ≥0.01. Assumes the same magnitude of effect comparing a value of 1 to 0 and 2 to 1.

Table 12. BME-Estimated Density Uniform Prior

Indicator	Risk Ratio[a]	95% CI	
Adult *Ae. aegypti* per area (m^2)			
Continuous (log scale)[b]	1.00	0.92	1.09
Binary (≥0.01 v.<0.01)	0.91	0.80	1.04
≥1 mosquitoes v. zero mosquitoes[c]	1.06	0.93	1.21
Tertiles (3 levels)[d]	1.00	0.94	1.07
Categorical indicator variables			
≥0.01	0.97	0.84	1.13
0.005 to <0.01	1.14	1.01	1.28
<0.005 (reference group)	1.00	-	-

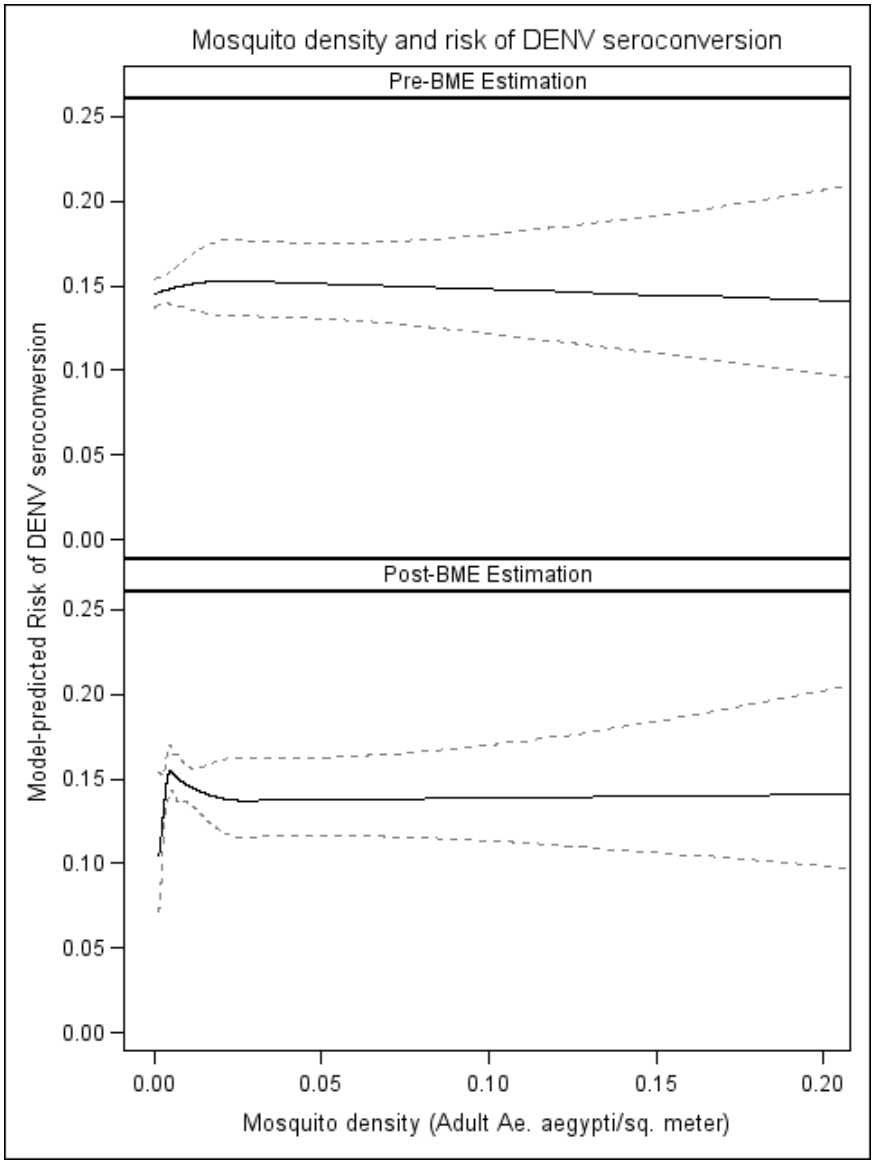
[a]Adjustment variables: Dengue virus transmission season (May-Aug, reference group; Sept-Dec; Jan-Apr); Participant sex (Male, Female); Participant Age (<18 years, ≥18 years).
[b]RRs estimating the relative risk on the log scale because the log of zero is undefined.
[c]Count of mosquitoes was back-transformed from BME-mean estimates by multiplying the density by the household area.
[d]Tertiles defined as 0= <0.005; 1=0.005 to <0.01; 2= ≥0.01. Assumes the same magnitude of effect comparing a value of 1 to 0 and 2 to 1.

Table 13. BME-estimated Density Triangular Prior

Indicator	Risk Ratio[a]	95% CI	
Adult *Ae. aegypti* per area (m^2)			
Continuous (log scale)[b]	1.00	0.92	1.09
Binary (≥0.01 v.<0.01)	0.94	0.83	1.05
≥1 mosquitoes v. zero mosquitoes[c]	1.04	0.92	1.19
Tertiles (3 levels)[d]	1.02	0.95	1.09
Categorical indicator variables			
≥0.01	1.04	0.90	1.21
0.005 to <0.01	1.18	1.04	1.35
<0.005 (reference group)	1.00	-	-

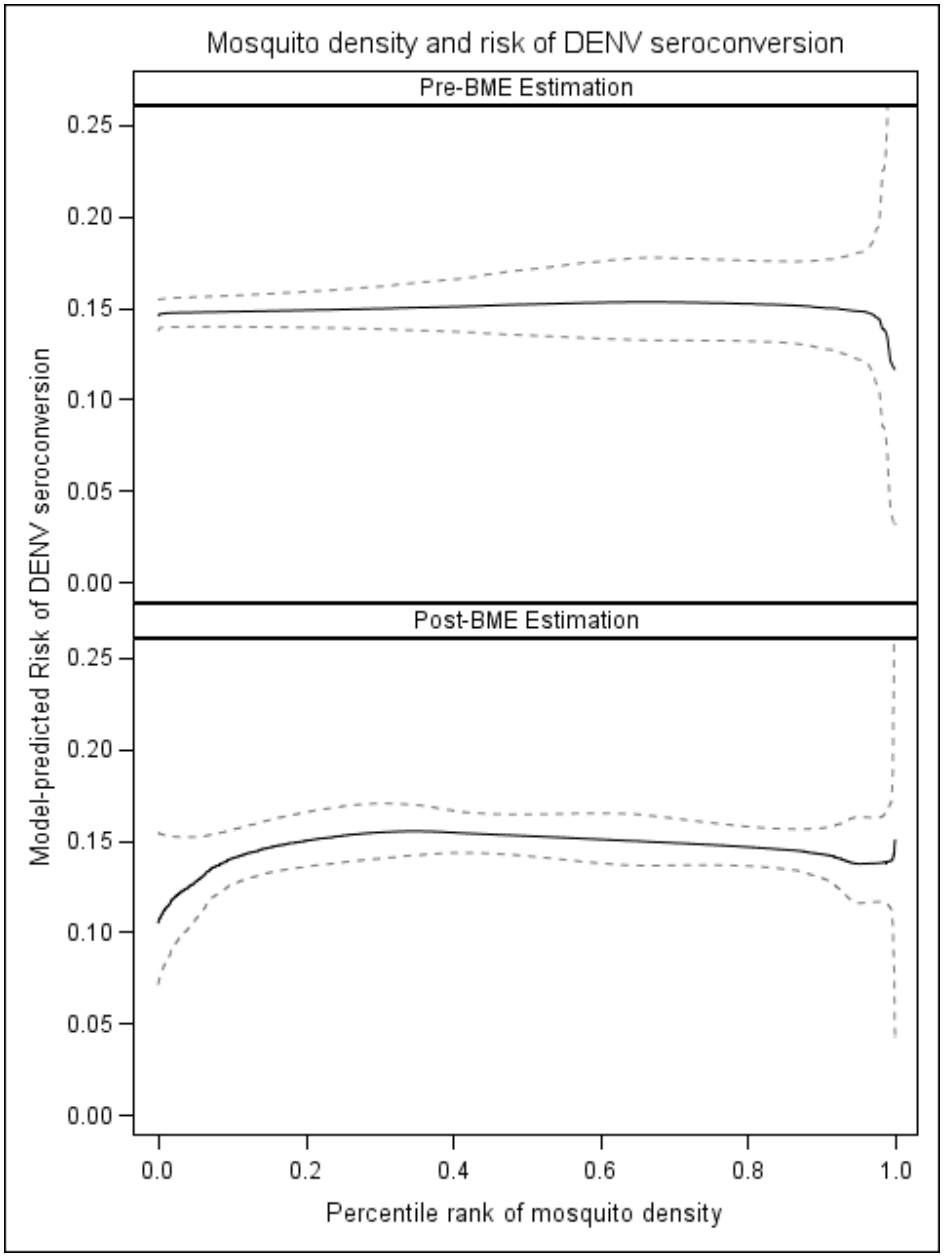
[a]Adjustment variables: Dengue virus transmission season (May-Aug, reference group; Sept-Dec; Jan-Apr); Participant sex (Male, Female); Participant Age (<18 years, ≥18 years).
[b]RRs estimating the relative risk on the log scale because the log of zero is undefined.
[c]Count of mosquitoes was back-transformed from BME-mean estimates by multiplying the density by the household area.
[d]Tertiles defined as 0= <0.005; 1=0.005 to <0.01; 2= ≥0.01. Assumes the same magnitude of effect comparing a value of 1 to 0 and 2 to 1.

Figure 10. Mosquito density and risk of DENV seroconversion



The model-predicted risks were generated using a restricted quadratic spine to visualize the relationship between mosquito density as a continuous variable and DENV seroconversion. On the left, the original density estimates were used to model the probability of seroconversion, with an essentially flat trend. On the right, the BME-estimated densities showed a marked increase in risk of DENV seroconversion for a range between 0.005 and 0.01, suggesting that an elevation in risk was detected for these low level densities.

Figure 11. Mosquito Density and Risk of DENV Seroconversion



To visualize the distribution of the densities across the observations, the percentile rank of mosquito density was plotted against the model-predicted risk of DENV infection. Estimates of risk were generated using a restricted quadratic spine to visualize the relationship between mosquito density as a continuous variable and DENV seroconversion. On the top, the original density estimates were used to model the probability of seroconversion, with an essentially flat trend. The densities that correspond with the percentiles are: 0.0; 0.0053; 0.0176; 0.0382; 06.569. On the bottom, the BME-estimated densities showed a marked increase in risk of DENV seroconversion for a range between 0.005 and 0.01, suggesting that an elevation in risk was detected for these low densities but account for a large proportion of the data. The corresponding densities are: 0.0013; 0.0037; 0.0067; 0.0099; 0.6569.

8.4 Discussion

These results demonstrate that an association between *Ae. aegypti* density and risk of DENV seroconversion can be detected by incorporating space/time information for observations in which no mosquitoes were observed; however, this association between mosquito density and DENV seroconversion was only observed within a strata of density (0.005 to 0.01 *Ae. aegypti* per household area), not across the entire range of densities. Treating household mosquito density as a categorical variable, the relative risk of DENV infection was estimated to be 1.14 (95% CI: 1.01, 1.28) among observations with a density of 0.005 to <0.01 per household area compared to observations with a density of <0.005 adult mosquitoes per household area; the highest density category (≥0.01) was not associated with DENV seroconversion. This relationship is illustrated in Figure 11, in which the predicted risk among observations with a density ≥0.01 was essentially equivalent to no density, although the small sample size at this range of the data reduces the precision of the predicted risks. If imputation of BME-predicted densities for households with no mosquitoes observed resulted in a dose-response relationship, then an increasing risk of DENV seroconversion as household mosquito density increased would have been observed.

The absence of a dose-response relationship mosquito density and DENV seroconversion could be due to several factors. First, given the extreme variability in space and time of high density observations, observations at these densities may not have had sufficient information to support estimation via BME. As illustrated by the comparison of variance estimates based on prediction using the nested covariance models, it is possible that the observations in time and space among high density locations were too variable to enable prediction at higher densities. If high density observations were not fairly consistent over time, the covariance model would have down-weighted prediction based on densities previously observed, which may have been much lower. The

comparison of variance (see SI) from the different covariance model structures also suggest that the ability of a space/time analysis to predict *Ae. aegypti* density may depend on repeated measures to inform prediction rather than information across space collected cross-sectionally. Nevertheless, the majority of study observations fell within the range of improved prediction, suggesting that while BME did not improve risk prediction for the full range of mosquito density, it did improve the measurement of entomological risk for a large subset of observations. Second, observations in which high adult mosquito densities were reported may have been collected among households in which variables related to their residents (such as daytime movement patterns, occupation, etc.) resulted in a distribution of DENV outcomes that was truly non-differential with respect to *Ae. aegypti* abundance as measured by routine entomological survey. Third, the smaller sample size of events and survey observations contributing higher densities introduces uncertainty due to poor precision for that subset of the data.

8.4.1 Strengths

The BME methodology was used to reduce possible measurement error. By considering the zero count observations through the lens of measurement error, mosquito density was imputed, treating density as a random variable. By allowing the density for mosquito-negative observations to have uncertainty, densities were modeled for these locations using the information provided by neighboring observations in space and time. The spatial covariance model, in which observations change together over distance, demonstrated a short spatial range of 25m after which the covariance between observations was low. This covariance model is consistent with the size of *Ae. aegypti* clusters previously reported in Iquitos and other settings, and is also consistent with the short flight range of the vector.

BME was chosen because it out-performs cluster identification methods, alternate methods to classify household observations as "exposed" to *Ae. aegypti* by accounting for spatial relationships, as it is less sensitive to missing data and observations that are irregularly measured in space and time.[153] Had cluster detection methods been used, mosquito density would have been modeled as a point process, and the location of observed densities would have been modeled as the random variable.[153, 105] Under such an approach, household vector exposure would then be determined categorically by cluster membership status (yes v. no) and then used as an exposure variable in an epidemiologic model. While cluster-based geostatistics provide evidence to inform the potential scale of spatial correlation, BME allows the direct estimation of density for each observation of interest by simultaneously accounting for space and time. Thus, density could be modeled as both continuous and categorical variables in terms of epidemiologic risk. This would not have been possible using cluster methods (i.e., local Moran, G-*i(d)*, spatial scan statistic, etc.).

Finally, the results describe patterns of risk in terms of inapparent dengue, rather than symptomatic disease. Most prior studies[8] of the association between *Ae. aegypti* density and dengue outcomes rely on apparent dengue as an outcome, thus introducing selection bias as reported cases represent a fraction of transmission events. In addition, this analysis utilizes a large sample of entomological and serological observations that are generalizable to other dengue-endemic settings with similar patterns of *Ae. aegypti* abundance.

8.4.2 Limitations

Adult *Ae. aegypti* was chosen for the model because this life stage is responsible for DENV transmission. Although restricting to female *Ae. aegypti* may have been biologically more relevant, those measures were even more dispersed than total adult density, which would have reduced the available sample size and thus adversely impacted precision. It is also possible that in a space/time

analysis framework, the presence of infested containers or larval density could be incorporated as a prior to inform prediction even when immature indices derived from larval and pupal measure have not been shown to be associated with DENV seroconversion in standard analytic approaches.

There is likely some degree of error in the estimate of household size, although such error would not be differential with respect to DENV infection status. To remove the household area from the density variable, we back-calculated the numerator from the predicted densities and tested the predicted mosquito count as a categorical variable and still found no association with DENV risk. Excluding the 6% of the households surveyed that were recorded as having merged or split over the course of data collection also did not result in a qualitative difference in the results. In addition, mosquitos per room surveyed and mosquitoes per resident were tested as alternative methods of characterizing household size but these indicators were uninformative (data not presented).

8.4.3 Conclusions

Positive associations between vector density and risk of infection would be expected if household *Ae. aegypti* abundance as measured by routine entomological monitoring was an adequate proxy for true exposure to DENV, but such associations have not been consistently observed in prior studies.[8] This analysis attempted to correct for the possible measurement error in entomological sampling that may cause the observed lack of association by incorporation of spatial and temporal information into a predicted *Ae. aegypti* density using a BME methodology. While the results suggest the BME methodology can be used to generate better measures of mosquito density for a subset of the data in terms of risk of DENV, the magnitude of improvement may not be sufficient to justify implementation of vector control interventions. None of the other methods of generating a mosquito density variable for use in an epidemiologic model showed an association

with DENV, suggesting there are limitations in terms of what a space/time analysis can achieve in this context.

While the analysis may have addressed measurement error among observations in which no mosquitoes were detected, if household mosquito abundance is simply not correlated with actual DENV exposure, even complete correction of measurement error would not result in an association in terms of DENV infection risk. Even though these results suggested that a space/time approach did detect an association for a strata of mosquito density, overall the findings do not provide compelling evidence that entomological monitoring measures are suitable proxies for risk of DENV infection. The study does, however, suggest that the BME methodology may have applications in correcting for measurement error that warrant further exploration. The risk ratios presented are associational, and it is unknown whether intervention targeted at these predicted densities would result in a reduction in DENV transmission. It is possible that *Ae. aegypti* populations exhibit too much variability across space and time for periodic, cross-sectional measurement to adequately characterize entomological risk, in addition to having limited correlation with true infection events due to human movement in space and time.

CHAPTER 9: EXPLORING HETEROGENEITY IN THE ASSOCIATION BETWEEN VECTOR DENSITY AND DENV RISK: A MULTI-LEVEL ANALYSIS

9.1 Introduction

In Chapter 7, *Ae. aegypti* densities calculated from longitudinal data demonstrated a stronger association with DENV seroconversion than cross-sectional measures of mosquito abundance, primarily for adult stage indicators. In Chapter 8, *Ae. aegypti* densities using adult mosquito data were predicted using BME to account for possible measurement error; an association with DENV seroconversion was observed using model-predicted densities for density households with a density of approximately 0.005 to 0.01 adult *Ae. aegypti* per household area. In these two analyses, the risk ratios presented quantify the association between vector measures and DENV infection for the entire community of Iquitos, Peru, over the period of data collection (the population-level effect).

The objective of this analysis was to (1) quantify the magnitude of block-level and zone-level variation in odds of DENV seroconversion after controlling for household and block-level *Ae. aegypti* densities and (2) determine if the association between adult vector density and DENV varies within the community by testing a random slope term (block and zone). In order to determine whether the 'improved' association between vector abundance and DENV infection varies between groups within the community, a multi-level logistic model was employed to separately test block-level and zone-level random effects. In Iquitos, blocks were defined as groups of households bordered by the same streets and were identified using a GIS database. Membership in MOH catchment zones were identified through the study database. To account

for different periods of DENV invasion, modification by the period defined by the major serotype in circulation is explored via stratified analysis (1999-2000 DENV1, DENV2; 2001-2003 DENV3; 2008-2010 DENV4).

9.2 Methods

In order to quantify possible heterogeneities in the association between household-level *Ae. aegypti* abundance and risk of DENV seroconversion, a multi-level logistic model[154] was used to estimate odds ratios while accounting for dependencies in the data due to block and zone membership. The multi-level model approach sought to explore two specific objectives: (1) quantify the between-group variability in DENV risk by accounting for individual risk factors and group-level contextual variables to determine if the variability in risk of DENV could be explained by these predictors and (2) test a random slope term for household *Ae. aegypti* abundance to determine if between-group differences in the odds ratio existed. Separate two-level analyses were conducted, one for block membership and a second for MOH zone membership. *A priori*, the individual level was excluded as there was no variability to model in that level as determined by a likelihood ratio test (LRT). The analysis was repeated using the block as the second level of interest and the MOH zone as the level of interest to compare the association between DENV risk and vector density by these different administrative units.

To compare the impact of fixed effects on between-group variability in DENV risk as measured by the variance of the group-level random intercept, the following models were tested. First, an "empty" model with a random intercept was fit to quantify the group-level risk of DENV. Second, a model with random intercepts and fixed individual effects was fit, followed by a model with additional group-level contextual variables. The variables tested in the analysis were as follows. For individual fixed effects: age (≥18 years v. <18 years); sex (male v. female);

and season (September-December; January-April; May-August). For household-level fixed effects: any adult *Ae. aegypti* observed in the household in the past 12 months, any adult *Ae. aegypti* at the cross-sectional entomological survey visit matched to serology, reported use of larvacide (any v. none), presence of infested containers (any v. none), housing quality (any brick/concrete v. only wood/mud), and number of household residents reported (>5 residents v. ≤5 residents). At the block level, fixed effects included: the block Adult Premise Index (APRI) ≥5 in the past 12 months, majority of households in block with some brick or concrete housing (>50% of households v. ≤ 50% of households) and proportion of households with more than five residents (>50% of households v. ≤ 50% of households).

After fitting the fixed effect variables to the random intercept model, inclusion of a random slope term was tested by LRT. In all models, the outcome was any DENV seroconversion in a six-month period. The analysis was also repeated by stratifying on the era of DENV circulation to determine if the association was variable across period of DENV serotype transmission: (1999-2000: DENV1, DENV2; 2001-2003: DENV3; 2008-2010: DENV4).

Logistic multi-level regression was implemented in Stata 12 (College Station, TX: StataCorp LP) using the xtemlogit command with 20 integration points, which directly implements maximum likelihood via numerical integration. The random intercepts and slopes were estimated using an exchangeable covariance structure. Model comparisons were conducted using a likelihood ratio test to determine if addition of variables was warranted at an alpha of 0.10. The final model was chosen based on the lowest Akaike Information Criteria (AIC).

9.3 Results

This analysis was conducted using the block-level analytical dataset constructed in Chapter 7, in which 7,558 paired serological observations were matched with entomological

surveillance data. Overall, a total of 289 city blocks were identified in this dataset, nested within 18 MOH catchment zones. The distribution of covariates in the study sample are presented in Chapter 7.

9.3.1 Block-level Analysis

The results of the model building process are presented in Table 14. Sex, any adult *Ae. aegypti* observed during the survey visit matched to the serological interval (cross-sectional) and > 5 household residents were not retained in the final models. The null model variance was 0.128 (SE: 0.040) with an AIC of 6352.2. The variance of the random intercept term comparing Model 1 through Model 3 showed little change (0.104 to 0.092), suggesting that the inclusion of fixed effects did account for differences in the odds of DENV seroconversion that exist between blocks. The point estimates of the fixed effects did not change dramatically when comparing these models with Model 4, the model with a random slope term included for any *Ae. aegypti* observed within 12 months.

Inclusion of the random slope term slightly reduced the magnitude of the odds ratio for the primary vector variable of interest, any adult *Ae. aegypti* observed within 12 months (OR: 1.15, 95% CI: 0.98, 1.34). The random slope had essentially no impact on the fixed effect estimates for the other predictors in the model.

Table 14. Block-level Multilevel Model Results

	Model 1			Model 2		
Variable	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
Individual Predictors						
Age (≥18 yrs v. <18 yrs (ref))						
Season (Sep-Dec v. May-Aug)	1.54	1.34	1.76	1.44	1.26	1.66
(Jan-Apr v. May-Aug)	1.28	1.08	1.51	1.23	1.04	1.45
	1.10	0.92	1.31	1.06	0.89	1.26
Household predictors						
Any adult Ae. aegypti (w/in 12 months)						
Reported use of Abate				1.22	1.07	1.40
Presence of infested container(s)				1.78	1.54	2.06
Housing quality (brick/concrete v. wood/mud)				0.98	0.81	1.19
				0.93	0.81	1.06
Block predictors						
APRI*>5 w/in 12 months						
>50% of HH** with brick/concrete housing						
>50% of HH with >5 residents reported						
Random Effect (Block)						
Intercept						
Variance	0.104			0.089		
SE	0.037			0.035		
Slope (adult *Ae. aegypti* w/in 12 months)						
Variance						
SE						
AIC	6307.6			6238.7		

*APRI: Adult productivity index; **HH: Household.

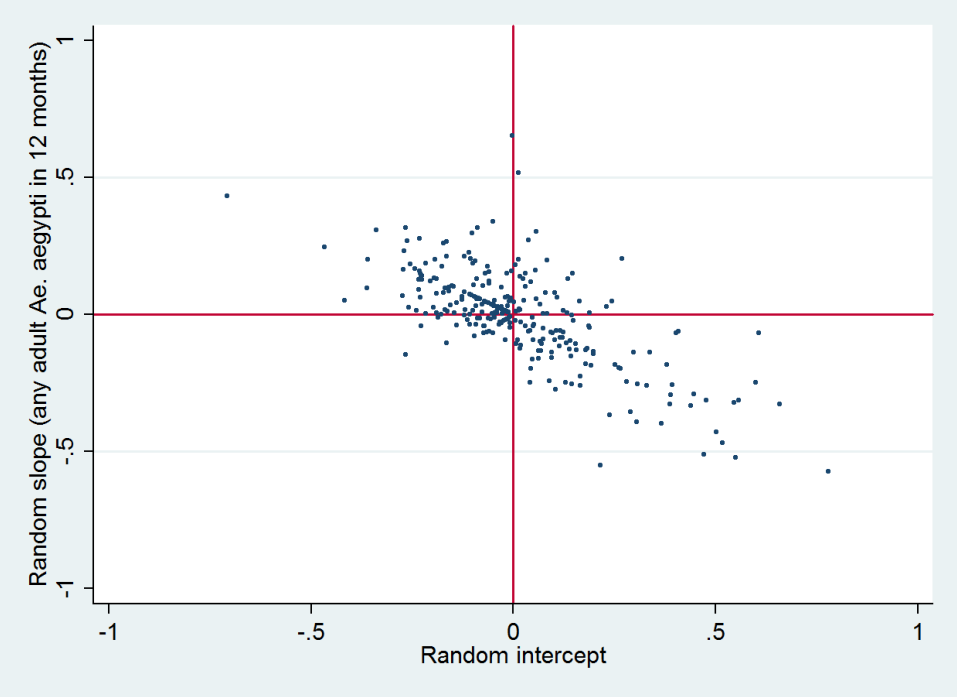
Table 14 (Continued)

	Model 3			Model 4		
	Odds Ratio	95% CI		Odds Ratio	95% CI	
Variable						
Individual Predictors						
Age (≥18 yrs v. <18 yrs (ref))	1.44	1.25	1.65	1.43	1.24	1.65
Season (Sep-Dec v. May - Aug)	1.24	1.05	1.47	1.23	1.04	1.46
(Jan-Apr v. May-Aug)	1.05	0.88	1.26	1.05	0.88	1.26
Household predictors						
Any adult Ae. aegypti (w/in 12 months)	1.19	1.04	1.36	1.25	1.05	1.48
Reported use of Abate	1.75	1.51	2.02	1.74	1.50	2.01
Presence of infested container(s)	0.97	0.80	1.17	0.96	0.79	1.17
Housing quality (brick/concrete v. wood/mud)	0.89	0.78	1.03	0.89	0.77	1.02
Block predictors						
APRI*>5 w/in 12 months	1.21	0.95	1.53	1.21	0.95	1.54
>50% of HH** with brick/concrete housing	1.15	0.96	1.39	1.15	0.95	1.40
>50% of HH with >5 residents reported	0.88	0.75	1.02	0.86	0.74	1.01
Random Effect (Block)						
Intercept						
Variance	0.092			-		
SE	0.036			-		
Slope (adult *Ae. aegypti* w/in 12 months)						
Variance	-			0.200		
SE	-			0.078		
AIC	6236.7			6229.7		

*APRI: Adult productivity index; **HH: Household.

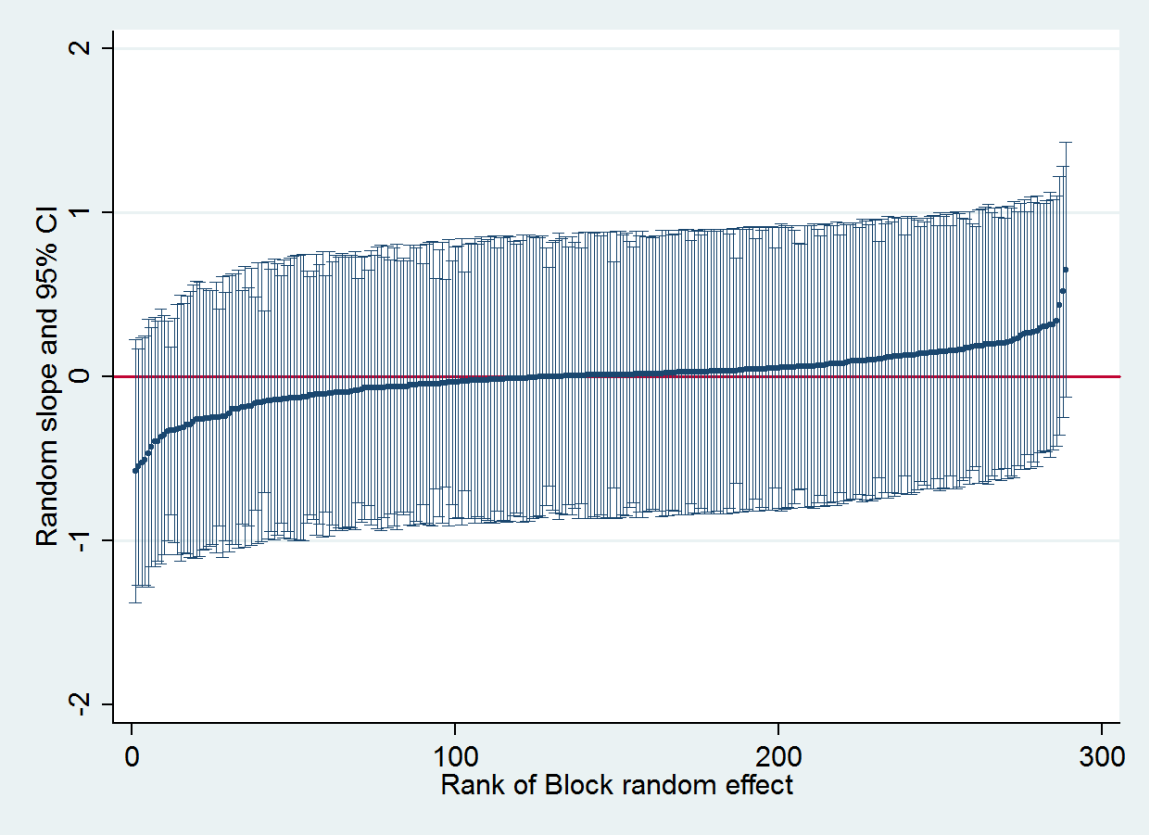
In Figure 12, the correlation between the block random intercept and random slope is illustrated. This figure shows that among blocks with a lower than average log odds of DENV seroconversion, the effect of household exposure to *Ae. aegypti* for the group is greater than the fixed effects odds ratio (the effect of household *Ae. aegypti* in an "average" block). The correlation between random slopes and intercepts is -0.685.

Figure 12. Correlation between random intercept and random slope



To compare the degree of heterogeneity in random slope estimates, the caterpillar plot in Figure 13 shows the lack of precision in these estimates. Figure 12 should be interpreted in light of Figure 13 to consider the possible amount of uncertainty that may exist in the correlation between random slopes and random intercepts. The random slope estimates have confidence bands that overlap zero, which would suggest no difference from the fixed effects odds ratios.

Figure 13. Caterpillar plot of random slope estimates



9.3.2 Zone-level Analysis

In the analysis of MOH zone between-group differences, a random slope term was not included in the final model to quantify the association between *Ae. aegypti* measured within 12 months and DENV infection. Table 15 presents the model results from the zonal analysis. In the final models, age and season were retained as individual predictors, any adult *Ae. aegypti* within the past 12 months, reported use of larvicide, presence of infested containers, and housing quality were included as household predictors. Most notably, the inclusion of block-level contextual variables (in Model 3) an APRI >5 and at least 50% of households reported at least 5 residents resulted in a 14% reduction in the variability of the random intercepts.

Table 15. MOH-level model results

	Model 1			Model 2		
	OR	95% CI		OR	95% CI	
Variable						
Individual Predictors						
Age (≥18 yrs v. <18 yrs)	1.50	1.31	1.72	1.41	1.23	1.62
Season of serological sample	1.28	1.08	1.49	1.22	1.03	1.44
	1.09	0.92	1.30	1.05	0.88	1.25
HH predictors						
Any adult *Ae. aegypti*				1.21	1.06	1.38
Reported use of Abate				1.74	1.51	2.01
Presence of infested container(s)				0.97	0.80	1.16
Housing quality				0.92	0.81	1.04
Block predictors						
APRI>5 w/in past 12 months						
>50% HH with >5 residents						
Random Effect (Zone)						
Intercept						
Variance	0.048			0.026		
SE	0.029			0.020		
AIC	6306.3			6241.6		

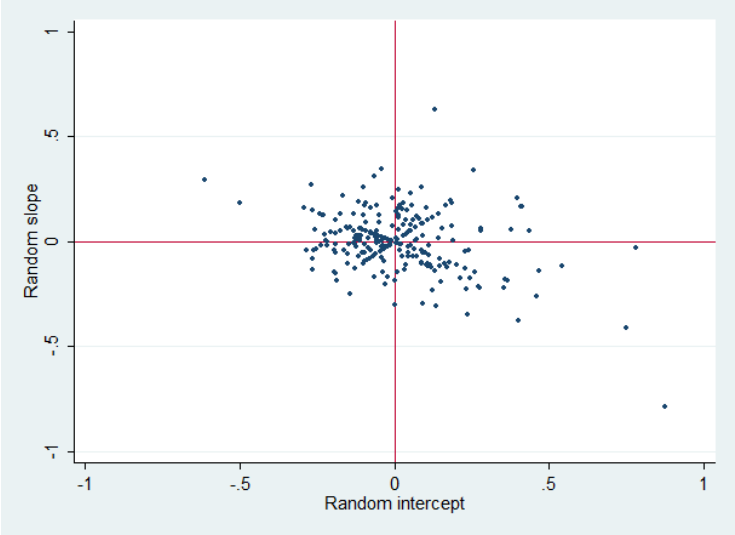
Table 15 (Continued)

	Model 3		
	OR	**95% CI**	
Variable			
Individual Predictors			
Age (≥18 yrs v. <18 yrs)	1.40	1.22	1.61
Season of serological sample	1.23	1.04	1.45
	1.05	0.87	1.24
HH predictors			
Any adult *Ae. aegypti* (w/in 12 months)	1.18	1.04	1.35
Reported use of Abate	1.72	1.49	1.99
Presence of infested container(s)	0.96	0.80	1.16
Housing quality (brick/concrete v. wood/mud)	0.91	0.80	1.03
Block predictors			
APRI>5 w/in past 12 months	1.24	0.99	1.55
>50% households with >5 residents reported	0.92	0.80	1.05
Random Effect (Zone)			
Intercept			
Variance	0.024		
SE	0.18		
AIC	6240.6		

9.3.3 Exploration of Time Period

Stratified analysis by period of DENV serotype circulation was performed for the block-level group as this was the only spatial group for which a random slope term was warranted in the primary multi-level analysis. In a model restricted to the first era (1999-2000), in which DENV1 and DENV2 primarily circulated, a random slope term was not included based on the findings of an LRT (p=0.72). For the period 2001-2003, when DENV3 was the dominated serotype in circulation, a random slope term was including based on an LRT (p=0.09). In Figure 14, the correlation between random intercepts and random slopes is illustrated for this period (correlation -0.55). The fixed effects odds ratio for the *Ae. aegypti* variable was 1.26 (95% CI: 1.0, 1.6). Finally, for the period in which DENV4 was in circulation, 2008-2010, a random effect term was not included in the final model (p=0.10).

Figure 14. Random Intercept v. Random Slope, 2001-2003



9.4 Discussion

The multilevel model approach was used to explore heterogeneity in the "improved" *Ae. aegypti* density measure, any adult *Ae. aegypti* observed at the household within the past 12 months, as well as explore contextual factors that might be associated with group-level odds of

seroconversion. In the block analysis, inclusion of variables describing household status such as housing quality, size and use of larvacide did not substantially reduce between-block variability, nor did inclusion of block-level characteristics, suggesting other unmeasured risk factors may be more relevant to explaining variability in the odds of seroconversion that occur over space. In the zone analysis, inclusion of block level contextual factors did reduce between group variability; it is possible that within the health zone catchment area the standard of living at the block level is relevant to odds of DENV infection. A random slope term was not warranted in the zone analysis, which provides evidence that the population-wide association between household *Ae. aegypti* abundance and DENV seroconversion adequately accounts for differences in risk across the population.

Multilevel models are inherently aspatial as they do not account for the distances between groups, nor does the method allow neighboring groups to influence the random effect estimates. That said, the approach does enable exploration of the variability of the odds ratio between groups commonly used to delineate intervention units (such as the MOH zone), which is relevant for programs. The analysis presented here demonstrates while there may be some evidence of heterogeneity in the association between measures of *Ae. aegypti* abundance and DENV infection, these results suggest that the population-average effect estimates presented in Chapter 7 and Chapter 8 adequately describe the association in Iquitos. Risk factors related to individual age (adults ≤18 at an elevated risk compared to children <18 years of age), season (peak period of transmission September through December) and household reported use of larvicide were strongly associated with the odds of DENV seroconversion. Surprisingly, contextual factors related to housing quality, density of household residents, and presence of infested containers were not associated with an increased odds of DENV infection.

CHAPTER 10: DISCUSSION

10.1 Summary of Findings

The objective of this research was to evaluate the utility of entomological monitoring indicators in terms of identifying an association with DENV seroconversion. In order for cross-sectional measurement of *Ae. aegypti* to serve as a proxy for DENV risk, vector density needs to be collected with little or no measurement error and density should correlate with true mosquito exposure for individuals at risk of infection. These assumptions are likely violated in most dengue endemic settings in which entomological monitoring is performed infrequently, primarily at domestic premises and prone to mismeasurement due to operational constraints.

If household *Ae. aegypti* abundance as measured by routine entomological monitoring was an adequate proxy for true exposure to DENV, consistently positive associations between vector density and risk of infection would have been observed. Given the lack of association with observed *Ae. aegypti* per household area in this study, this analysis attempted to correct for measurement error that may have occurred during entomological sampling. Incorporation of spatial and temporal information into estimates of *Ae. aegypti* density resulted in a positive association.

Adult densities that incorporated repeated entomological measures via a moving average demonstrated the strongest association with DENV seroconversion compared to cross-sectional densities and BME-predicted densities. If such measurement error was the principle source of bias in the association with DENV risk, the magnitude of the association would likely be greater

than the strongest association reported, an adjusted RR of 1.72 (95% CI: 1.22, 2.43) for block-level Adult Density Index (categorical). A comparison of the three nested space-time covariance structures revealed that the long range spatial and long temporal range covariance model resulted in less variability in prediction. Taken together, these results suggest that if vector surveillance is to provide any useful information with which to identify those at risk of DENV, longitudinal data collection is required to account for the variability of *Ae. aegypti* population estimates over time. Incorporation of prior density estimates likely accounts for some degree of measurement error that occurs during a single entomological survey visit, particularly in households with low levels of vector abundance. Without time series entomological data, the ability of BME to improve density estimates may be extremely limited as prediction would only be informed by the nearest neighboring observations. Dengue control program managers should consider whether the resources needed to sustain frequent entomological collection are worth the investment in terms of the absolute difference in infection risk.

To address the possibility of heterogeneity of effect in the association between household level *Ae. aegypti* density and DENV seroconversion, a multi-level model was used to test for the inclusion of a random slope. While a random slope term was warranted in a model of block-level random effects based on a likelihood ratio test, the distribution of random effects suggest that any heterogeneity of effect is not strong enough to change the overall findings. The correlation between the random slope and random intercepts does suggest that the effect of the variable any adult *Ae. aegypti* observed within 12 months has a greater magnitude among blocks with a higher odds of DENV seroconversion. At the zone level, inclusion of a random slope term was not warranted, suggesting no heterogeneity of effect at that scale. Ultimately, the results of the multi-

level model further confirm the population-level risk ratios adequately describe the relationship between vector density and DENV seroconversion.

None of the risk ratios presented in this analysis represent a causal relationship between household or block-level mosquito density and true exposure to DENV. In order to quantify the impact of vector control interventions on DENV incidence, causal risk ratios estimated from a randomized controlled trial would be more appropriate. A trial of vector control interventions, would likely monitor *Ae. aegypti* density over more frequent time periods and implementation of interventions would need to account for premises where individuals may spend more of their daytime hours.

In this dissertation, *Ae. aegypti* indicators were evaluated as surrogates of exposure, which will always remain unmeasured. It is possible that some unmeasured variable associated with social network patterns, household quality and day-time human movement modifies dengue risk. If this modification is present, it would further undermine the utility of these indicators by violating the assumption that all individuals residing in the same household or block experience the same level of risk.

10.2 Contribution of Findings

This study is the first analysis to comprehensively test associations between larval, pupal and adult measures of *Ae. aegypti* abundance and evidence of DENV infection. Unlike prior studies, these results demonstrate the limited utility of entomological monitoring data by linking individual seroconversion events to the households from which entomological monitoring data were collected. The longitudinal structure of the study dataset allowed for comparison of entomological measures over time, correcting for some degree of measurement error. The review of longitudinal serology measures allowed the analytical cohort to be constructed by excluding

individuals who had evidence of not being susceptible to circulating DENV serotypes. While the data used in this analysis were collected for research purposes, the frequency of entomological collection was similar to many other dengue-endemic settings in which vector surveillance is conducted quarterly or trimesterly. The findings presented in this dissertation are likely generalizable to many other settings.

This analysis is also the first to explicitly account for possible measurement error in mosquito data collection by constructing an estimate of vector density using a simple method (averaging prior *Ae. aegypti* observations) as well as predict density via a space/time analysis. Both approaches suggest that an association can be detected: adjusted RR estimates ranged from 1.02 (95% CI: 1.0, 1.05) to 1.72 (95% CI: 1.22, 2.43) for adult measures. By considering the nature of measurement error, this study provides more evidence that cross-sectional prevalence estimates of vector density are not sufficient proxies for DENV risk unless information on prior *Ae. aegypti* densities are available. Even if improvement of indicators is achieved with longitudinal measures, the magnitude of effect may not warrant the investment needed to conduct regular surveillance.

10.3 Future Research Directions

Entomological monitoring will continue to serve an important role in the evaluation of vector control interventions (e.g., comparing pre- and post-intervention abundance); however, in terms of monitoring risk of DENV infection, this analysis challenges the validity of *Ae. aegypti* indicators as adequate proxies for true DENV exposure. Alternate methods to generate more meaningful estimates of risk warrant development and evaluation. The research presented here could be extended in four specific areas: (1) comparison with other dengue settings; (2) development of improved entomological monitoring frameworks with the purpose of evaluating

vector control; (3) investigating other risk factors related to human movement (e.g., occupation) to serve as proxies for risk; and (4) to explore the role of entomological monitoring measures as a proxy for dengue transmission at the community-level.

Despite recent licensure of a dengue vaccine, vector control remains the only viable strategy to reduce transmission of pathogens vectored by *Ae. aegypti* available in most dengue endemic settings. In light of recent ZIKV outbreaks, it is likely that ministries of health will continue to monitor *Ae. aegypti* populations to forecast the spread of ZIKV as well as DENV. As such, this research provides a rationale for more frequent *Ae. aegypti* surveillance to better capture the variability in the vector population, as well as expand entomological monitoring to non-residential premises to better capture day-time mosquito-human contact. In terms of repeating this analysis in other contexts, there are settings such as Taiwan[155] in which more frequent (e.g., weekly) *Ae. aegypti* surveys are conducted. Nevertheless, as entomological monitoring activities are highly resource-intensive, data from such settings may not be representative of what is feasible for most dengue control programs. Any future evaluation of more frequent entomological monitoring strategies should include a cost-effectiveness component. In addition, since entomological monitoring is a key element of the global guidelines for dengue control and surveillance,[40] it may also be necessary to repeat this analysis using data from locations similar to Iquitos to confirm the findings from this dissertation research, thereby providing evidence from multiple settings with which to update global recommendations.

As this research suggests that estimates of *Ae. aegypti* populations from periodic entomological monitoring offer limited information in terms of predicting DENV infection risk, there is an opportunity to re-direct entomological monitoring to test vector control strategies in terms of human DENV infection events. In this scenario, estimates of vector density would serve

as process indicators to confirm vector populations were reduced as a result of intervention. As described in Chapter 3, entomological measurements were designed to serve as a proxy for true DENV exposure, not to represent a causal mechanism of infection. By designing studies to measure the effectiveness of specific vector control strategies, the intervention itself would be evaluated as a primary exposure of interest. Such an approach would be more meaningful to program managers as effect estimates from these studies would relate specific interventions to potential reductions in infections.

This analysis estimates the association between entomological monitoring and DENV infection at the individual level. While these results show that entomological monitoring does not predict dengue risk on an individual basis, it does not eliminate the possibility that entomological monitoring indicators may predict risk on a community basis. Furthermore, these measures of *Ae. aegypti* abundance may be an effective way to monitor interventions. To ensure that the findings of this dissertation are consistent with respect to the utility of mosquito density estimates calculated at the community-wide scale, the association between entomological monitoring measures of *Ae. aegypti* should be estimated comparing larger spatial scales such as the community to ascertain if a that spatial dimension can be used to detect an association between mosquito density and dengue outcomes.

Finally, these results suggest that a new paradigm of entomological and epidemiologic surveillance is needed to understand patterns of contact between *Ae. aegypti* and human populations with which to inform the implementation of vector control and enhance surveillance for apparent dengue disease. Risk factors that better correlate with human-mosquito contact (e.g. gender, age, occupation, socio-economic status) need to be identified. Ideally, dengue control program managers will use these findings advocate for new monitoring strategies with consideration to the resources required for data collection.

APPENDIX A: SUPPLEMENTAL MATERIALS TO CHAPTER 7

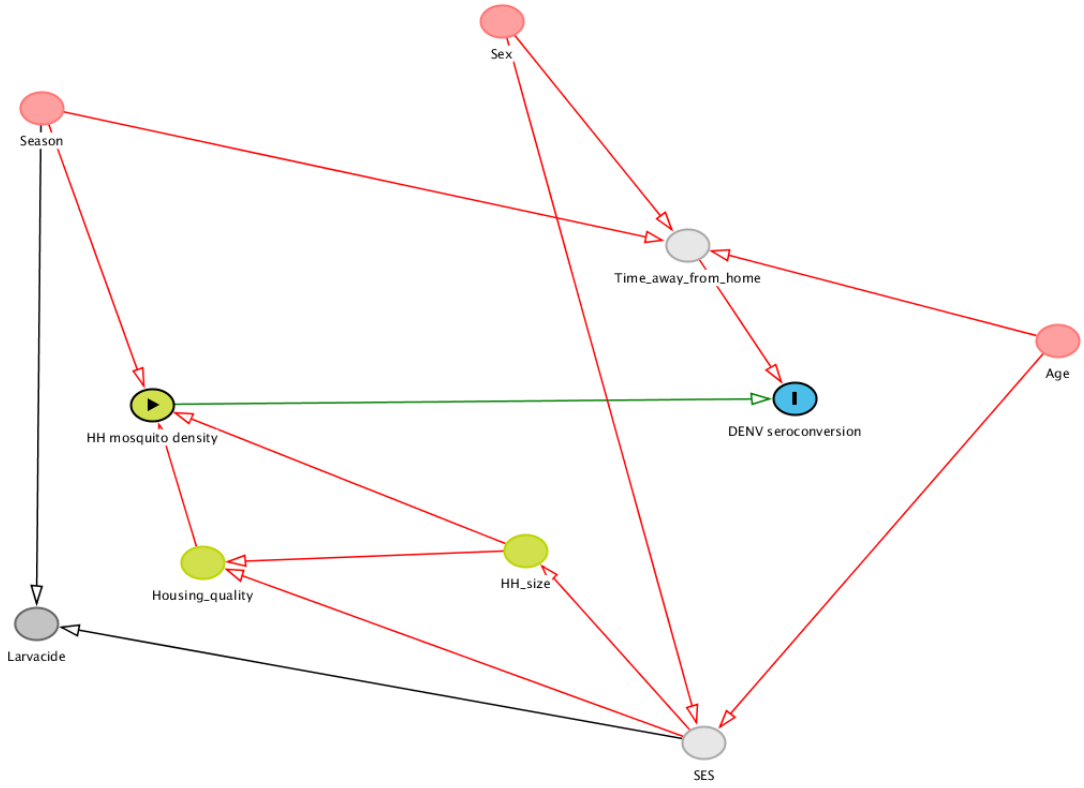
Entomological and serological data were not collected at the same time in the parent study. Seroconversion was measured within an approximately six-month interval and entomological data were collected trimesterly. Sensitivity analyses were conducted to confirm that the results were consistent over several different approaches that could be used to link serology to entomology and to explore the impact of possible misclassification in the evaluation of serological outcomes.

Table A-1: Crude RRs and 95% CI

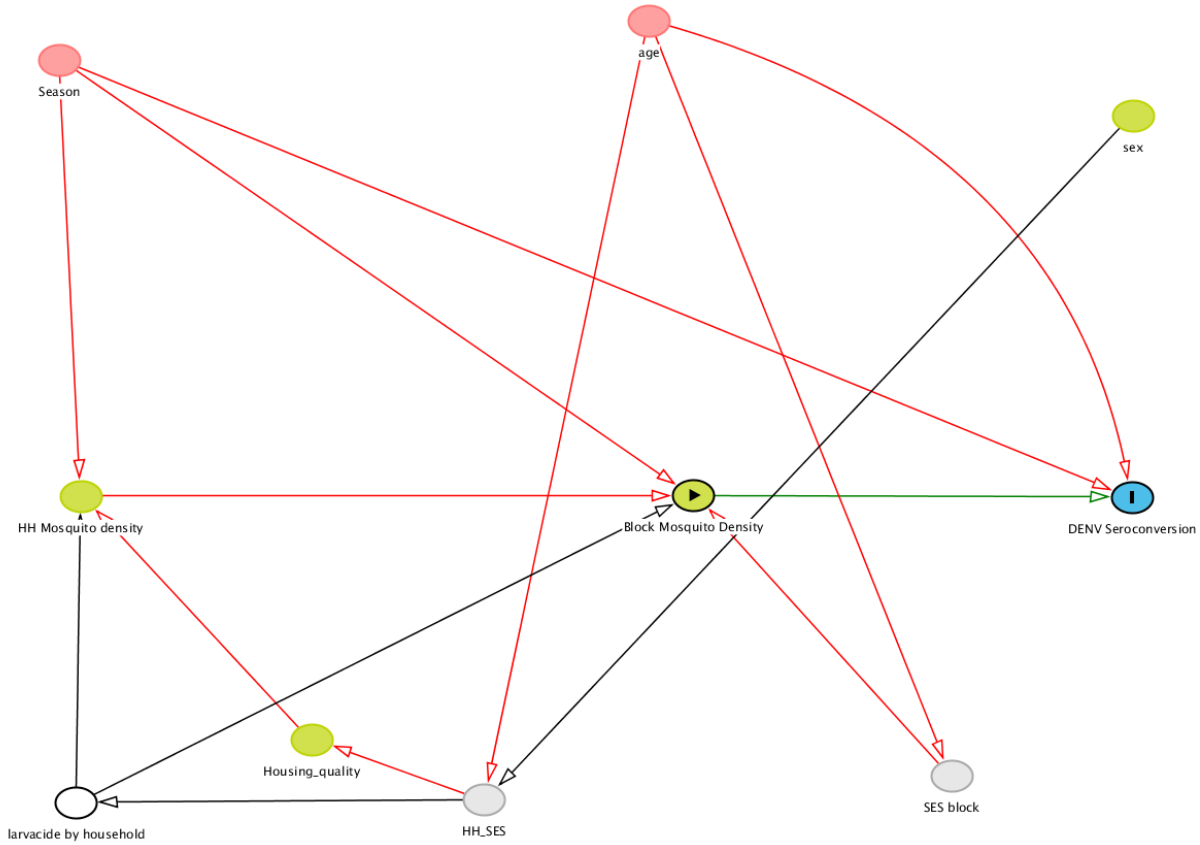
Indicator	Cross-sectional Risk Ratio	95% CI		Longitudinal Risk Ratio	95% CI	
Household level						
Adult *Ae. aegypti* (continuous)	1.00	0.99	1.01	1.02	0.99	1.04
Adult *Ae. aegypti* (categorical)	1.03	0.91	1.16	1.23	1.11	1.37
Adult female *Ae. aegypti* (continuous)	0.99	0.97	1.02	1.04	0.99	1.08
Adult female *Ae. aegypti* (categorical)	1.04	0.90	1.19	1.26	1.13	1.41
Adult *Ae. aegypti* indoors (categorical)	1.06	0.93	1.19	1.25	1.12	1.39
Adult female *Ae. aegypti* indoors (categorical)	1.06	0.92	1.22	1.28	1.15	1.43
Single Larval Method (continuous)	0.94	0.85	1.04	1.03	0.95	1.13
Single Larval Method (categorical)	0.88	0.75	1.03	1.18	1.06	1.31
Pupae in containers (continuous)	0.99	0.98	1.00	1.00	1.00	1.01
Pupae in containers (categorical)	0.91	0.74	1.13	1.14	1.01	1.30
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.96	0.91	1.01	1.00	0.98	1.03
Container Index (continuous)	1.00	0.99	1.00	0.97	0.95	1.00
Container Index (categorical)	0.87	0.75	1.03	1.18	1.06	1.31
Stegomiya Index (continuous)	0.68	0.37	1.25	0.92	0.52	1.64
Stegomiya Index (categorical)	0.87	0.74	1.02	1.18	1.06	1.32
Block level						
Breteau Index (continuous)	0.99	0.99	0.99	1.00	0.99	1.00
Breteau Index (categorical)	0.95	0.84	1.07	0.93	0.79	1.09
House Index (continuous)	0.99	0.98	0.99	0.99	0.99	1.00
House Index (categorical)	0.93	0.83	1.05	0.89	0.78	1.03
Adult Premise Index (continuous)	1.00	0.99	1.00	1.01	1.00	1.01
Adult Premise Index (categorical)	0.87	0.77	0.98	1.29	1.06	1.56
Adult Density Index (continuous)	0.96	0.85	1.09	1.13	0.93	1.38
Adult Density Index (categorical)	0.82	0.72	0.95	1.91	1.35	2.71
Pupa Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupa Index (categorical)	0.91	0.81	1.02	1.32	1.09	1.60
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.69	0.58	0.81	1.00	1.00	1.00
Infested Receptacle Index (continuous)	0.45	0.34	0.60	0.66	0.51	0.85
Infested Receptacle Index (categorical)	0.98	0.84	1.13	2.09	1.47	2.98
Container Index (continuous)	0.98	0.97	0.99	0.99	0.98	1.01
Container Index (categorical)	0.86	0.77	0.96	0.89	0.79	0.99
Potential Container Index (continuous)	0.86	0.82	0.90	1.00	0.99	1.02
Potential Container Index (categorical)	0.64	0.57	0.71	0.99	0.85	1.15
Stegomiya Index (continuous)	1.00	0.99	1.00	1.00	1.00	1.00
Stegomiya Index (categorical)	0.95	0.83	1.09	1.23	1.00	1.51

Figure A-1: Directed Acyclic Graphs

Household-level indicators



Block-level indicators



Figures A-2-5: Cross-Sectional Sensitivity Analyses

Three additional serological outcome datasets were constructed:

S1) Any paired samples taken within 335-395 days apart (approximately 12 months) was split into two six-month intervals. If a seroconversion occurred during that interval, it was assigned to the first six-month interval. These observations were added to the original analysis set.

S2) Any paired samples taken within 335-395 days apart (approximately 12 months) was split into two six-month intervals. If a seroconversion occurred during that interval, it was assigned to the second six-month interval. These observations were added to the original analysis set.

S3) Any paired samples taken within 210-335 days that was originally excluded was included based on the range of dates coinciding with the annual estimated epidemic curve as described in Stoddard *et al* 2014 PLoS NTDs. These observations were added to the original analysis set.

Cross-sectional entomological data was matched to the serology data as follows:

E1) The last entomological data point to be observed within a paired sample interval.

E2) The first entomological data point to be observed within a paired sample interval.

All possible combinations of serological and entomological datasets were analyzed (six in total). The following comparisons are presented in Figures 1-3: S1-E1; S2-E1; S3-E1. The E2 results were similar to the E1 scenarios (data not shown).

The analysis was repeated for all household and block-level indicators; block-level indicators (data not shown) show a similar pattern to the household-level indicators shown as Figures 1-3. Original refers to the RR and 95% CI reported in the main study analysis.

Figure A-2: Cross-sectional SI_E1 Household-level indicators

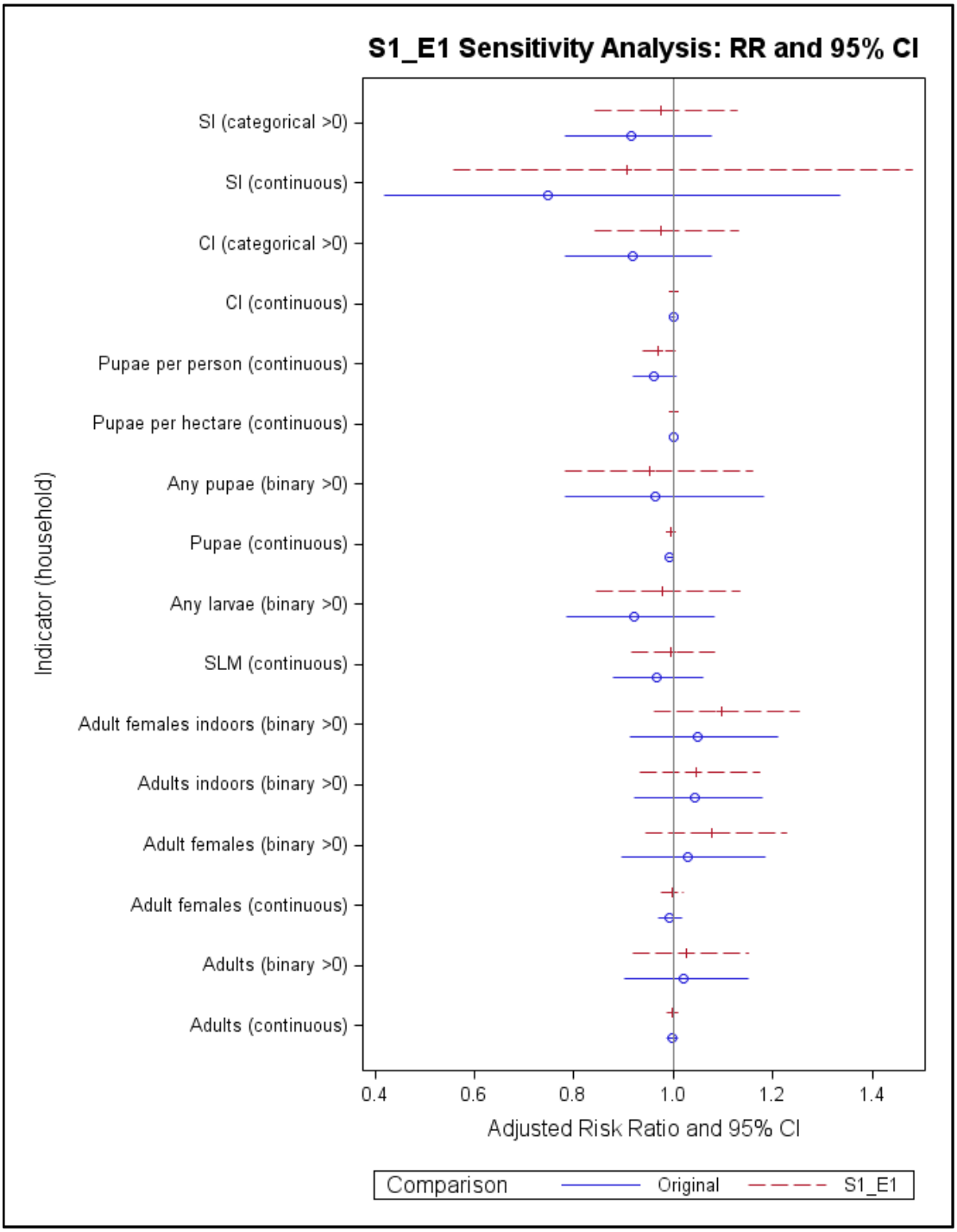


Figure A-3: Cross-Sectional S2_E1 Household level indicators

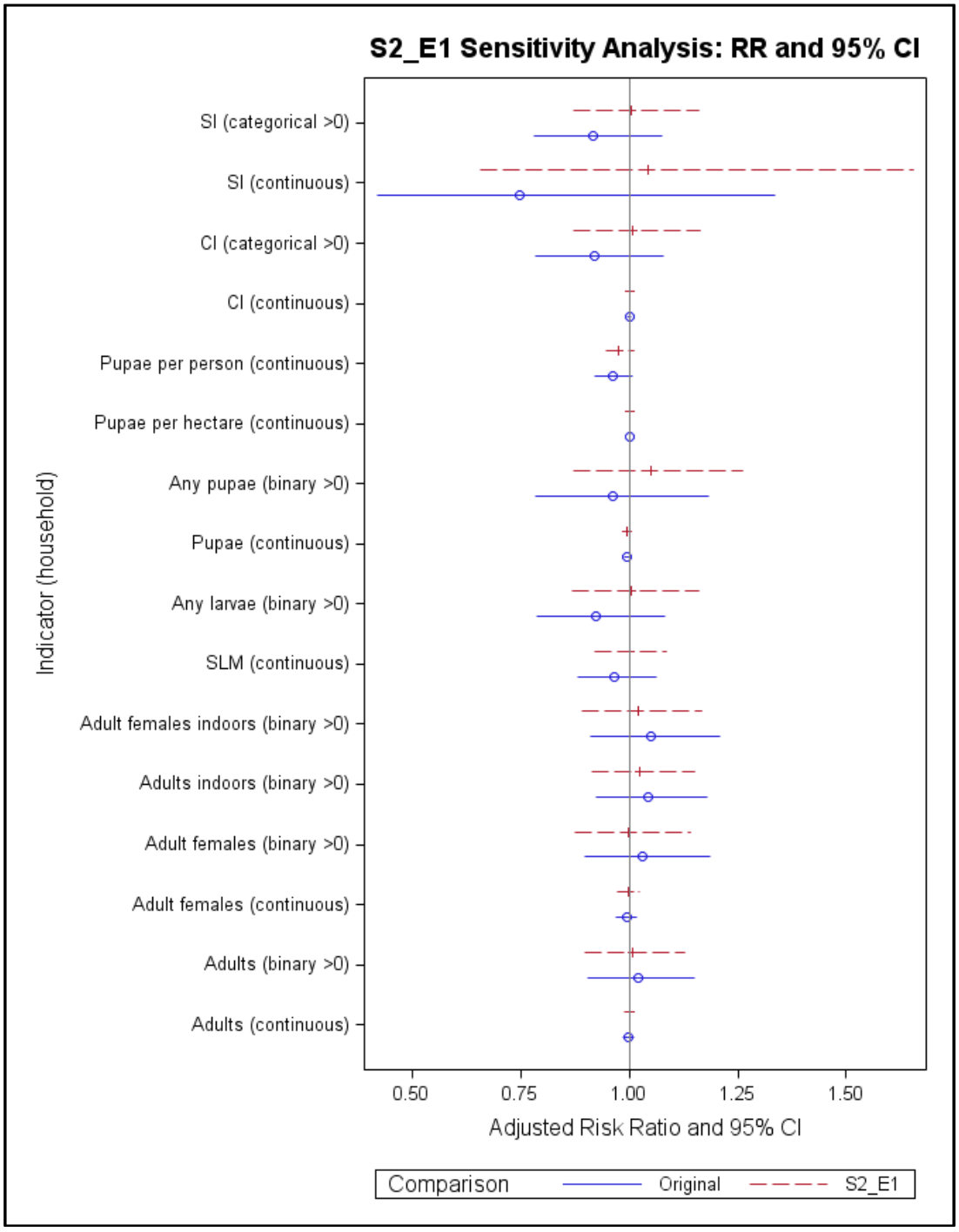
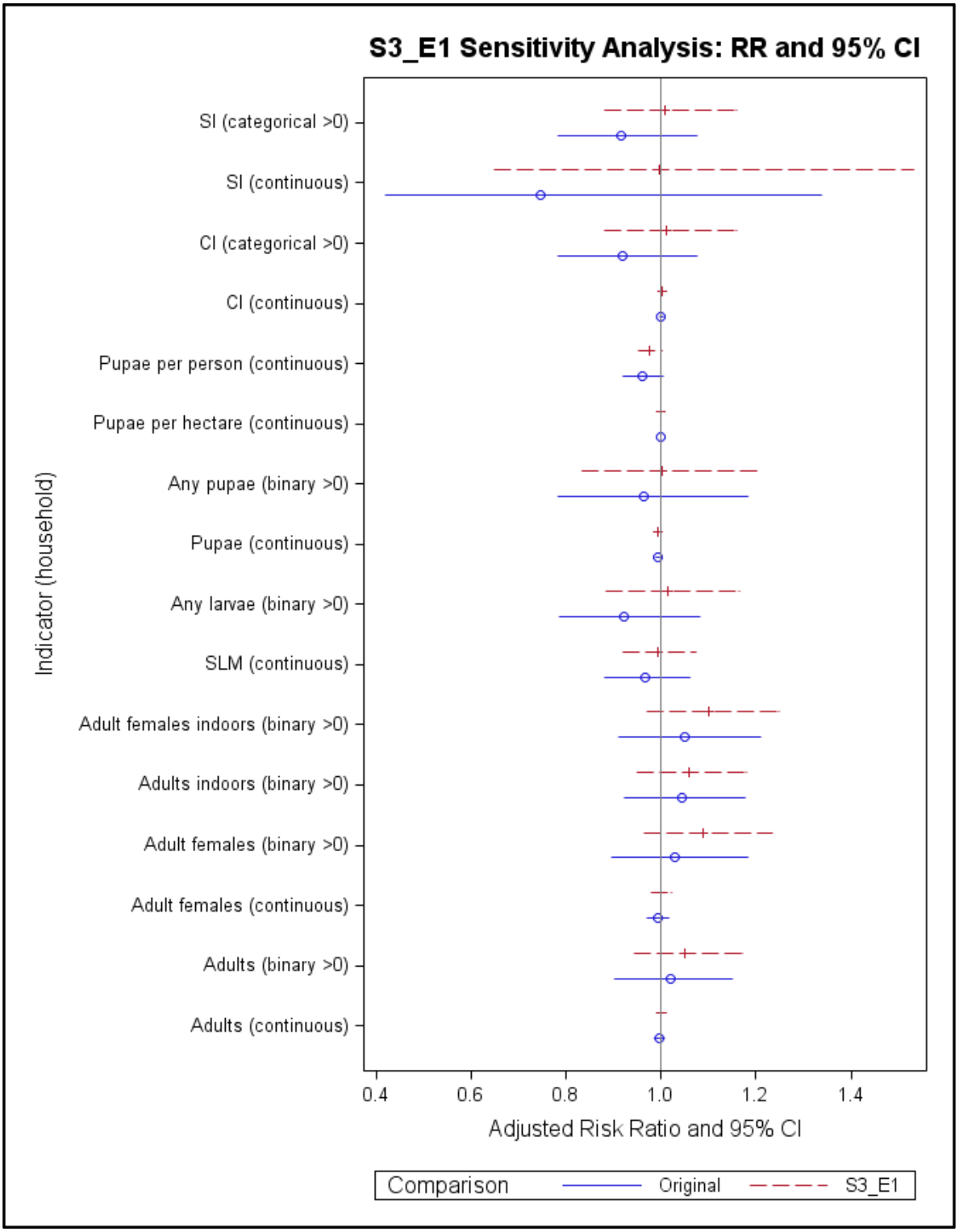


Figure A-4: Cross-sectional S3_E1 Sensitivity Analysis



Figures A-5-7: Longitudinal Sensitivity Analyses

The three serological outcome datasets were constructed:

S1) Any paired samples taken within 335-395 days apart (approximately 12 months) was split into two 6-month intervals. If a seroconversion occurred during that interval, it was assigned to the first six month interval. These observations were added to the original analysis set.

S2) Any paired samples taken within 335-395 days apart (approximately 12 months) was split into two 6-month intervals. If a seroconversion occurred during that interval, it was assigned to the second six month interval. These observations were added to the original analysis set.

S3) Any paired samples taken within 210-335 days that was originally excluded was included based on the range of dates coinciding with the annual estimated epidemic curve as described in Stoddard *et al* 2014 PLoS NTDs.

Longitudinal entomological data was calculated from survey data as follows:

L1) Average of entomological data points occurring within 12 months before the start of the seroconversion interval.

L2) Average of entomological data points occurring within 6 months before the start of the seroconversion interval.

All possible combinations of serological and entomological datasets were analyzed (six in total).The following comparisons are presented in Figures 4-6: S1-L1_L2; S2-L1_L2; S3-L1_L2. This analysis was repeated for all household and block-level indicators; block-level indicators (data not shown) show a similar pattern to the household-level indicators shown in Figures 4-6. Original refers to main study analysis.

Figure A-5: S1_L1_L2 Comparison with Original Results

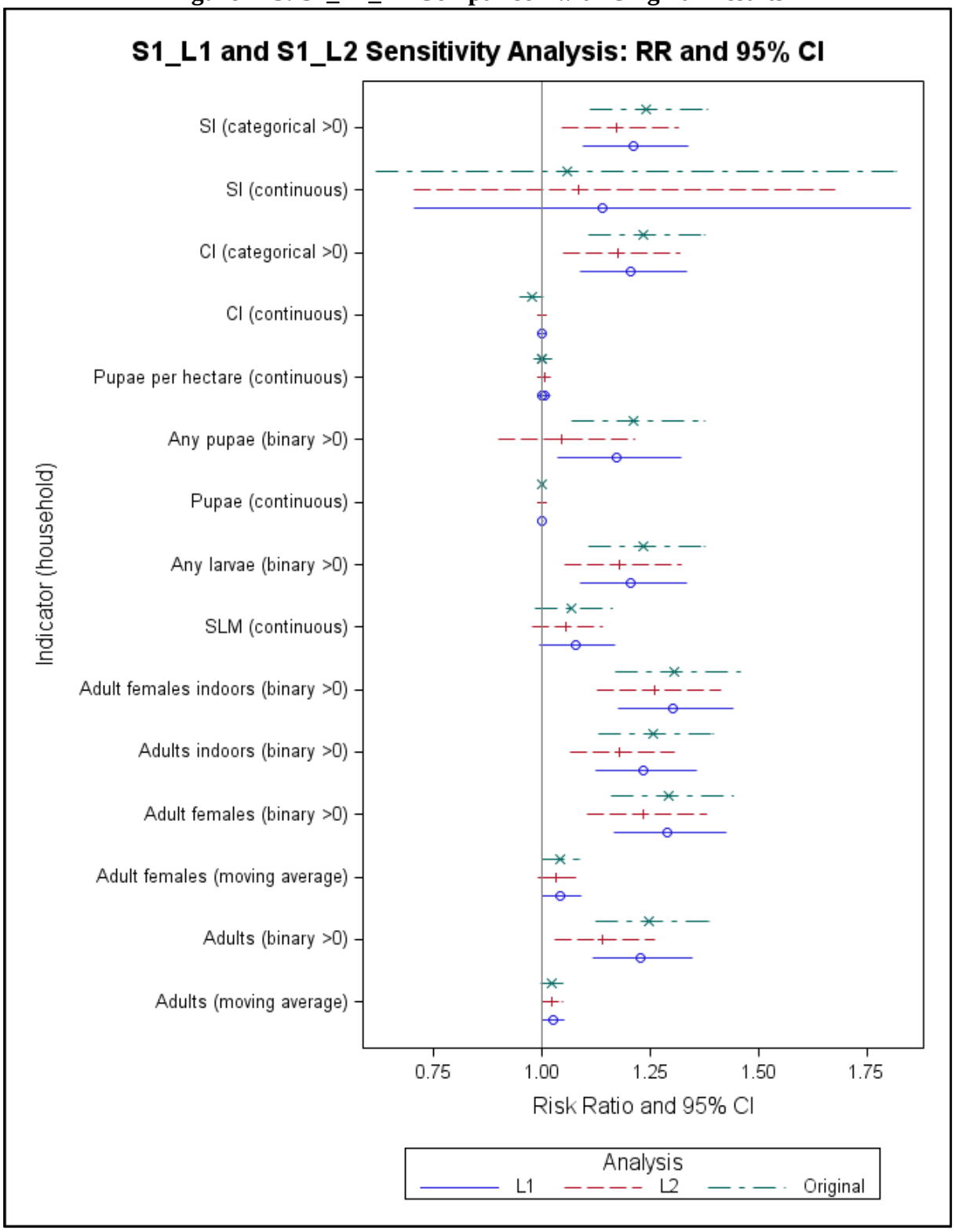


Figure A-6: S2_L1_L2 Comparison with Original Results

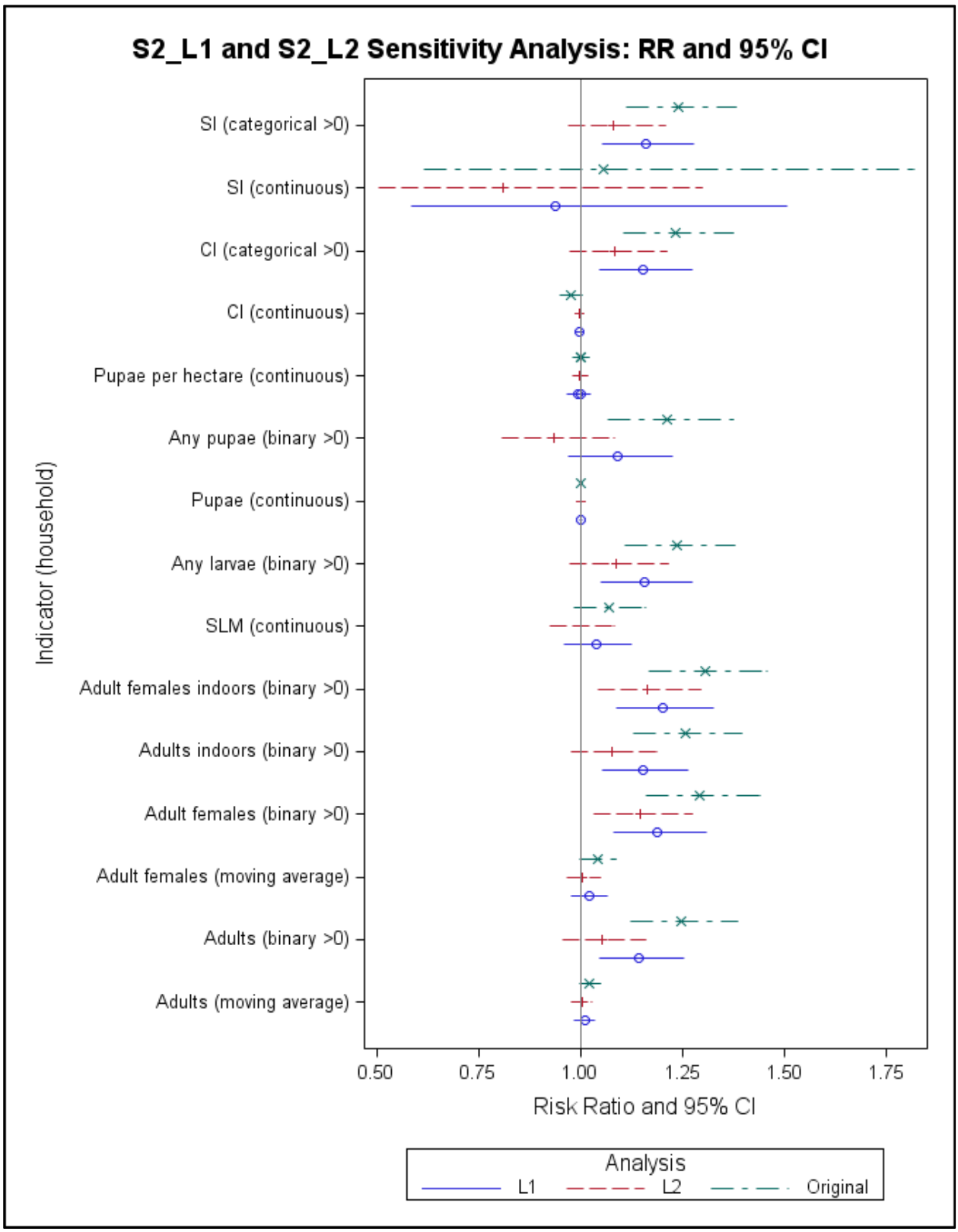


Figure A-7: S3_L1_L2 Comparison with Original Results

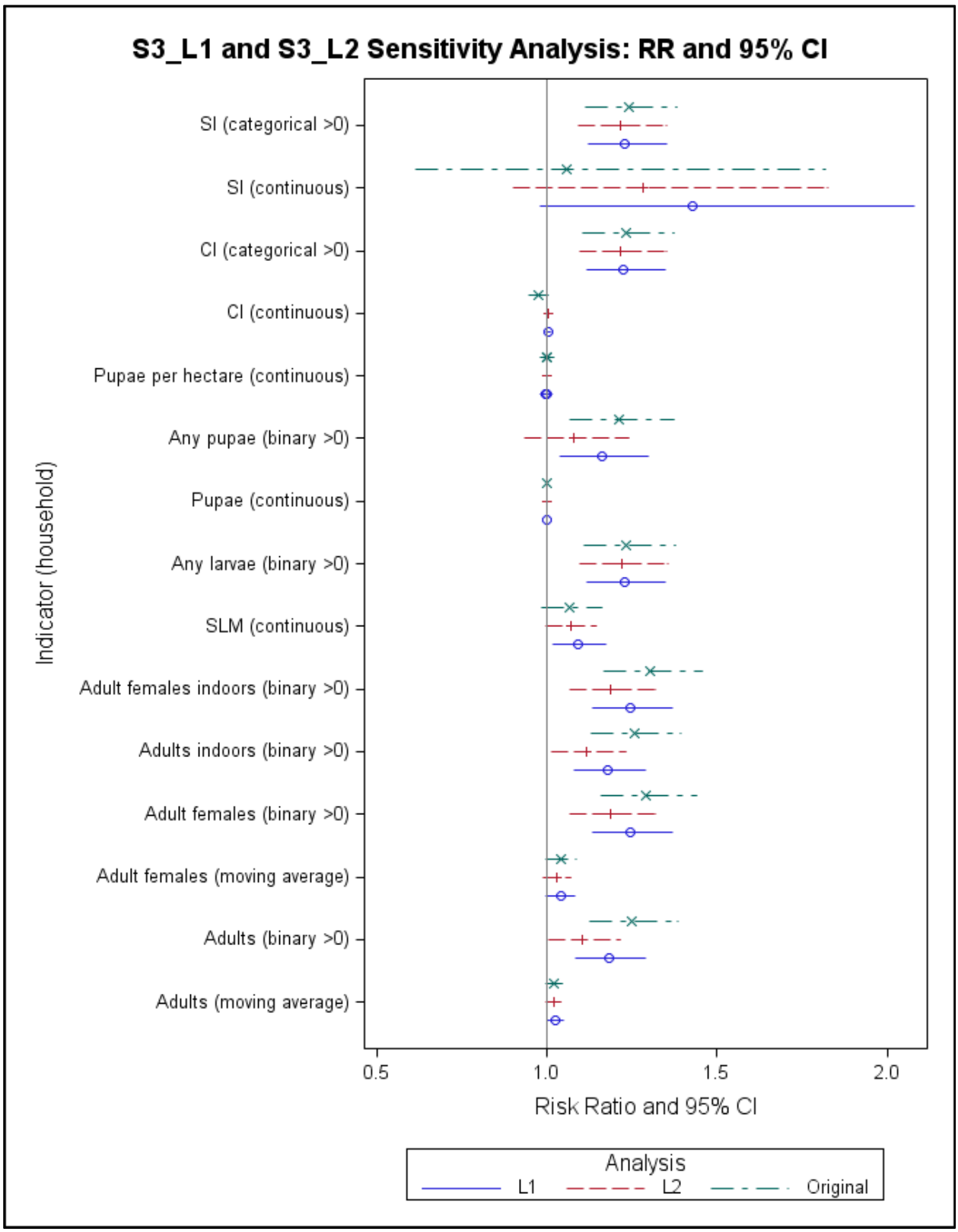


Table A-2 Comparison of indicators calculated by averaging data collected 6 and 12 months of start of seroconversion interval

	12 months			6 months		
Indicator	**Risk Ratio**	**95% CI**		**Risk Ratio**	**95% CI**	
Household level						
Adult *Ae. aegypti* (continuous)	1.02	1.00	1.05	1.02	0.99	1.04
Adult *Ae. aegypti* (categorical)	1.25	1.12	1.39	1.14	1.02	1.27
Adult female *Ae. aegypti* (continuous)	1.04	1.00	1.09	1.02	0.98	1.07
Adult female *Ae. aegypti* (categorical)	1.29	1.16	1.44	1.23	1.09	1.38
Adult *Ae.aegypti* indoors (categorical)	1.26	1.13	1.40	1.18	1.06	1.32
Female *Ae. aegypti* indoors (categorical)	1.30	1.17	1.46	1.25	1.11	1.41
Single Larval Method (continuous)	1.07	0.98	1.16	1.03	0.95	1.12
Single Larval Method (categorical)	1.23	1.11	1.38	1.16	1.02	1.31
Pupae in containers (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae in containers (categorical)	1.21	1.07	1.37	1.04	0.89	1.22
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	1.00	0.98	1.03	1.00	0.98	1.03
Container Index (continuous)	0.98	0.95	1.00	0.99	0.97	1.00
Container Index (categorical)	1.23	1.11	1.38	1.16	1.02	1.31
Stegomiya Index (continuous)	1.06	0.61	1.82	0.93	0.56	1.55
Stegomiya Index (categorical)	1.24	1.11	1.38	1.15	1.02	1.30
Block level						
Breteau Index (continuous)	1.00	1.00	1.00	1.00	0.99	1.00
Breteau Index (categorical)	0.89	0.76	1.05	1.07	0.92	1.23
House Index (continuous)	1.00	0.99	1.00	1.00	0.99	1.00
House Index (categorical)	0.91	0.79	1.00	0.96	0.85	1.09
Adult Premise Index (continuous)	1.01	1.01	1.02	1.01	1.00	1.01
Adult Premise Index (categorical)	1.24	1.01	1.48	1.05	0.90	1.22
Adult Density Index (continuous)	1.24	1.02	1.50	1.22	1.04	1.44
Adult Density Index (categorical)	1.72	1.22	2.43	1.40	1.09	1.80
Pupa Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupa Index (categorical)	1.30	1.08	1.57	1.04	0.91	1.20
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Infested Receptacle Index (continuous)	0.93	0.72	1.20	0.79	0.62	1.02
Infested Receptacle Index (categorical)	1.75	1.23	2.50	1.15	0.92	1.43
Container Index (continuous)	1.01	0.99	1.02	0.99	0.88	1.10
Container Index (categorical)	1.00	0.90	1.11	0.88	0.79	0.98
Potential Container Index (continuous)	1.01	1.00	1.03	1.00	0.99	1.01
Potential Container Index (categorical)	0.99	0.86	1.15	1.09	0.95	1.25
Stegomiya Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Stegomiya Index (categorical)	1.13	0.93	1.39	1.03	0.87	1.22

Table A-3: Adjusted RRs and 95% CI allowing any positive serology result to be classified as a seroconversion event (most inclusive)

	Cross-sectional			Longitudinal		
Indicator	**Risk Ratio**	**95% CI**		**Risk Ratio**	**95% CI**	
Household level						
Adult *Ae. aegypti* (continuous)	1.00	0.99	1.01	1.00	0.98	1.03
Adult *Ae. aegypti* (categorical)	1.09	0.98	1.21	1.13	1.03	1.24
Female *Ae. aegypti* (continuous)	1.00	0.98	1.02	1.00	0.96	1.05
Adult female *Ae. aegypti* (categorical)	1.11	0.99	1.25	1.15	1.04	1.27
Adult *Ae. aegypti* indoors (categorical)	1.11	1.00	1.23	1.14	1.04	1.25
Female *Ae. aegypti* indoors (categorical)	1.14	1.01	1.28	1.16	1.05	1.28
Single Larval Method (continuous)	0.92	0.84	1.01	0.97	0.89	1.06
Single Larval Method (categorical)	0.86	0.74	1.00	1.11	1.00	1.22
Pupae in containers (continuous)	0.99	0.98	1.00	1.00	1.00	1.00
Pupae in containers (categorical)	0.86	0.71	1.05	1.09	0.97	1.22
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.94	0.88	1.00	1.00	0.98	1.02
Container Index (continuous)	1.00	1.00	1.00	0.97	0.94	1.00
Container Index (categorical)	0.86	0.74	1.00	1.10	1.00	1.22
Stegomiya Index (continuous)	0.65	0.37	1.14	0.76	0.42	1.37
Stegomiya Index (categorical)	0.86	0.74	1.00	1.11	1.00	1.22
Block level						
Breteau Index (continuous)	0.99	0.99	0.99	0.99	0.99	1.00
Breteau Index (categorical)	0.96	0.86	1.07	0.78	0.68	0.89
House Index (continuous)	0.98	0.98	0.99	0.98	0.98	0.99
House Index (categorical)	0.93	0.84	1.03	0.79	0.71	0.89
Adult Premise Index (continuous)	1.00	1.00	1.01	0.99	0.99	1.00
Adult Premise Index (categorical)	1.01	0.90	1.14	1.02	0.88	1.19
Adult Density Index (continuous)	1.07	0.97	1.18	0.87	0.68	1.09
Adult Density Index (categorical)	1.00	0.87	1.14	1.48	1.08	2.03
Pupa Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupa Index (categorical)	0.92	0.83	1.01	1.10	0.94	1.29
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.89	0.79	1.01	1.00	1.00	1.00
Infested Receptacle Index (continuous)	0.40	0.30	0.53	0.47	0.35	0.63
Infested Receptacle Index (categorical)	1.01	0.89	1.15	1.61	1.11	2.33
Container Index (continuous)	0.98	0.97	0.99	0.98	0.97	0.99
Container Index (categorical)	1.01	0.89	1.15	0.81	0.74	0.89
Potential Container Index (continuous)	0.82	0.79	0.86	0.98	0.97	0.99
Potential Container Index (categorical)	0.58	0.53	0.65	0.89	0.79	1.01
Stegomiya Index (continuous)	0.99	0.99	1.00	1.00	0.99	1.00
Stegomiya Index (categorical)	1.01	0.89	1.15	0.96	0.81	1.14

Table A-4: Adjusted RRs and 95% CI excluding any serological result to test positive for more than one serotype in a single sample (restrictive)

	Cross-sectional			Longitudinal		
Indicator	**Risk Ratio**	**95% CI**		**Risk Ratio**	**95% CI**	
Household level						
Adult *Ae. aegypti* (continuous)	1.00	0.98	1.01	1.05	1.02	1.08
Adult *Ae. aegypti* (categorical)	1.01	0.88	1.16	1.37	1.22	1.55
Adult female *Ae. aegypti* (continuous)	0.99	0.97	1.02			
Adult female *Ae. aegypti* (categorical)	1.04	0.89	1.22	1.36	1.20	1.54
Adult *Ae. aegypti* indoors (categorical)	1.03	0.89	1.18	1.39	1.24	1.57
Adult female *Ae. aegypti* indoors (categorical)	1.06	0.90	1.25	1.41	1.24	1.60
Single Larval Method (continuous)	1.02	0.93	1.12	1.10	0.99	1.21
Single Larval Method (categorical)	1.04	0.88	1.24	1.15	1.01	1.31
Pupae in containers (continuous)	0.99	0.99	1.00			
Any pupae in containers (categorical)	1.07	0.86	1.34	1.21	1.04	1.41
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.97	0.94	1.01	1.00	1.00	1.01
Container Index (continuous)	0.85	0.46	1.58	1.00	1.00	1.01
Container Index (categorical)	1.00	1.00	1.01	1.15	1.01	1.31
Stegomiya Index (continuous)	1.00	0.57	1.76	1.23	0.70	2.15
Stegomiya Index (categorical)	1.04	0.87	1.23	1.15	1.01	1.31
Block level						
Breteau Index (continuous)	1.00	0.99	1.00	1.00	1.00	1.00
Breteau Index (categorical)	1.00	0.86	1.15	0.95	0.79	1.15
House Index (continuous)	0.99	0.99	1.00	1.00	0.99	1.01
House Index (categorical)	1.01	0.88	1.16	0.95	0.80	1.12
Adult Premise Index (continuous)	1.00	0.99	1.00	1.01	1.01	1.02
Adult Premise Index (categorical)	0.87	0.75	1.01	1.27	1.03	1.58
Adult Density Index (continuous)	0.96	0.83	1.12	1.36	1.12	1.67
Adult Density Index (categorical)	0.88	0.75	1.05	1.48	1.03	2.14
Pupa Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupa Index (categorical)	0.98	0.86	1.13	1.32	1.05	1.64
Pupae per Hectare (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Pupae per Person (continuous)	0.96	0.91	1.01	1.00	1.00	1.00
Infested Receptacle Index (continuous)	0.65	0.46	0.91	1.07	0.81	1.42
Infested Receptacle Index (categorical)	0.95	0.80	1.12	1.88	1.22	2.89
Container Index (continuous)	0.99	0.97	1.00	1.01	0.99	1.03
Container Index (categorical)	0.95	0.80	1.12	1.04	0.92	1.19
Potential Container Index (continuous)	0.93	0.88	0.98	1.02	1.00	1.03
Potential Container Index (categorical)	0.80	0.70	0.92	1.01	0.85	1.20
Stegomiya Index (continuous)	1.00	1.00	1.00	1.00	1.00	1.00
Stegomiya Index (categorical)	0.95	0.80	1.12	1.15	0.91	1.47

Table A-5: Adjusted RRs and 95% CI stratified by type of aspirator used (cross-sectional)

	1999-2008 (CDC)			2010 (Prokopack)		
Indicator	**Risk Ratio**	**95% CI**		**Risk Ratio**	**95% CI**	
Household level						
Adult *Ae. aegypti* (continuous)	1.00	0.97	1.02	0.99	0.97	1.01
Any *Ae. aegypti* (categorical)	0.98	0.84	1.16	0.79	0.63	0.99
Adult female *Ae. aegypti* (continuous)	0.98	0.95	1.03	0.99	0.95	1.03
Any female *Ae. aegypti* (categorical)	0.96	0.80	1.16	0.96	0.75	1.22
Any *Ae. aegypti* indoors (categorical)	1.01	0.86	1.19	0.81	0.64	1.01
Any female *Ae. aegypti* indoors (categorical)	0.99	0.82	1.20	0.95	0.75	1.22
Block level						
Adult Premise Index (continuous)	0.99	0.98	0.99	1.01	1.00	1.02
Adult Premise Index (categorical)	0.76	0.66	0.88	1.26	0.77	2.05
Adult Density Index (continuous)	0.79	0.62	1.01	1.05	0.85	1.30
Adult Density Index (categorical)	0.74	0.63	0.86	1.40	0.49	4.01

Adult collection with Prokopack aspirators began in 2009; these aspirators are more efficient at sampling adult mosquitoes. To determine if aspirator performance affected adult density indicators, we stratified the analysis by year of data collection, excluding 2009 as this is the year the change was implemented. The point estimates for household-level RRs are similar; for block measures the poor precision of these estimates for the Prokopack era limits comparability.

APPENDIX B: SUPPLEMENTAL MATERIALS TO CHAPTER 8

Table B-1: Categorical mosquito exposure defined by presence of *Ae. aegypti* among households of varying proximity

Categorical density measures constructed using distance*	Risk Ratio**	95% CI	
Any adjacent household ≥1 adult *Ae. aegypti*	1.10	0.99	1.22
Any adjacent household ≥1 adult female *Ae. aegypti*	1.03	0.92	1.16
Household within 30m ≥1 adult *Ae. aegypti*	0.96	0.83	1.11
Household within 30m ≥1 adult female *Ae. aegypti*	0.95	0.83	1.08
Household within 50m ≥1 adult *Ae. aegypti*	0.93	0.77	1.12
Household within 50m ≥1 adult female *Ae. aegypti*	0.87	0.74	1.01
Household within 100m ≥1 adult *Ae. aegypti*	0.79	0.62	1.01
Household within 100m ≥1 female *Ae. aegypti*	0.82	0.66	1.02

Distribution of sampled households within space	N	Mean (SD)	Min	Max
Number of adjacent neighbors	13,354	3.8 (1.5)	1	19
Number of adjacent neighbors sampled	13,354	2.5 (1.5)	0	17
Number of households within 30m	13,298	30.9 (9.4)	1	75
Number of households within 30m sampled	13,298	19.2 (8.1)	0	52
Number of households within 50m	13,380	63.7 (15.9)	10	157
Number of households within 50m sampled	13,380	34.4 (15.1)	0	94
Number of households within 100m	13,376	175.9 (44.8)	26	438
Number of households within 100m sampled	13,376	78.0 (36.5)	0	206

*Reference category: No adult mosquitoes observed within that distance.

**Adjustment variables: age (<18 yrs v. ≥18 yrs); sex; dengue season.

Table B-2: BME results for adult mosquitoes/square meters for different implementation parameters: Uniform prior

BME Parameters								Model Results		
Lower	Upper	Nhmax	Nsmax	Space	Time	s/t metric	Order	Risk Ratio	95% CI	
0.25	2.00	10	2	1000	18	1.1	NaN	1.01	0.92	1.10
0.25	2.00	12	2	500	12	1.1	NaN	1.01	0.92	1.10
0.25	2.00	10	2	1000	12	1.1	0	1.00	0.92	1.10
0.25	2.00	10	2	1000	12	5.0	0	1.00	0.92	1.10
0.50	2.00	6	2	500	12	3.0	NaN	1.00	0.91	1.10
0.50	2.00	4	2	100	18	2.0	NaN	1.00	0.91	1.09
0.25	3.00	8	2	500	12	5.0	NaN	1.00	0.91	1.10
0.25	1.50	8	2	500	12	1.1	NaN	1.01	0.93	1.10
0.25	1.50	6	0	500	10	1.1	NaN	1.07	0.97	1.17
0.25	1.50	12	2	1000	12	1.1	NaN	1.01	0.92	1.10
0.25	1.50	12	2	1000	18	5.0	NaN	1.01	0.93	1.10
0.25	0.75	4	0	1000	12	1.1	0	1.00	0.93	1.08
0.25	0.75	4	0	1000	6	1.1	NaN	1.08	0.99	1.17
0.25	2.00	6	0	1000	6	5.0	NaN	1.07	0.98	1.17

Space: Maximum distance used to select points from nhmax and nsmax.
Time Maximum time period used to select points.
s/t metric: Parameter used to weight time v. space (lower value gives more weight to temporal proximity than spatial proximity).
Order for polynomial estimating a local mean: NaN=zero; 0=constant mean.

Table B-3: Preliminary BME results for adult mosquitoes/square meters: triangular prior

BME Parameters									Model Results		
Lower	Mid-Point	Upper	Nhmax	Nsmax	Space	Time	s/t metric	Order	Risk Ratio	95% CI	
0.25	1.00	2.00	8	2	1000	12	1.1	NaN	1.00	0.92	1.09
0.25	1.00	2.00	6	0	1000	6	5.0	NaN	1.06	0.97	1.16
0.25	1.25	2.00	6	0	500	18	1.1	NaN	1.05	0.95	1.15
0.25	1.75	2.00	4	0	1000	6	1.1	NaN	1.06	0.97	1.15
0.25	1.75	3.00	6	2	1000	12	1.1	NaN	1.10	0.86	1.40
0.25	1.75	3.00	6	0	500	6	5.0	NaN	1.23	0.96	1.56
0.25	1.5	3.00	6	0	1000	12	1.1	NaN	1.16	0.91	1.49
0.25	2.25	3.00	6	2	1000	12	1.1	NaN	1.10	0.86	1.40

Space: Maximum distance used to select points from nhmax and nsmax.
Time Maximum time period used to select points.
s/t metric: Parameter used to weight time v. space (lower value gives more weight to temporal proximity than spatial proximity).
Order for polynomial estimating a local mean: NaN=zero; 0=constant mean.

Table B-4: Results excluding households that merged or divided

Indicator	BME-Estimated Density Uniform Prior			BME-estimated Density Triangular Prior		
	Risk Ratio[a]	95% CI		Risk Ratio[a]	95% CI	
Adult *Ae. aegypti* per area (m^2)						
Continuous (log scale)[b]	1.03	0.94	1.12	1.02	0.94	1.11
Binary (≥0.01 v.<0.01)	0.96	0.85	1.08	0.97	0.86	1.09
≥1 mosquitoes v. zero mosquitoes[c]	1.07	0.94	1.20	1.06	0.93	1.21
Multi-level categorical variable (3 levels)[d]	1.01	0.94	1.09	1.03	0.96	1.10
Categorical indicator variables						
≥0.01	1.02	0.88	1.19	1.07	0.92	1.24
0.005 to <0.01	1.11	0.98	1.27	1.16	1.02	1.33
<0.005 (reference group)	1.00	-	-	1.00	-	-

[a]Adjustment variables: Dengue virus transmission season (May-Aug, reference group; Sept-Dec; Jan-Apr); Participant sex (Male, Female); Participant Age (<18 years, ≥18 years).
[b]RRs estimating the relative risk on the log scale because the log of zero is undefined.
[c]Count of mosquitoes was back-transformed from BME-mean estimates by multiplying the density by the household area
[d]Tertiles defined as 0= <0.005; 1=0.005 to <0.01; 2= ≥0.01. Assumes the same magnitude of effect comparing a value of 1 to 0 and 2 to 1.

Table B-5: Comparison of categorical cut-point values to define variables of mosquito exposure

	Observed Density			BME-Estimated Density Uniform Prior		
	Risk Ratio**	**95% CI**		**Risk Ratio****	**95% CI**	
Binary classification (lower density is reference group)						
≥0.01 v.<0.01*	1.00	0.86	1.17	0.91	0.80	1.04
≥0.005 v.<0.005	1.04	0.92	1.19	1.08	0.97	1.21
≥0.007 v.<0.007	1.02	0.89	1.17	0.90	0.81	1.01
≥0.015 v.<0.015	1.06	0.89	1.27	1.05	0.89	1.24
≥0.02 v.<0.02	1.03	0.83	1.27	1.00	0.82	1.23
Categorical indicator variables						
≥0.01*	1.05	0.88	1.27	0.97	0.84	1.13
0.005 to <0.01*	1.07	0.85	1.34	1.14	1.01	1.28
<0.005 (reference group)*	1.00	-	-	1.00	-	-
≥0.02	1.03	0.83	1.28	1.05	0.85	1.31
0.005 to <0.02	1.05	0.90	1.22	1.08	0.97	1.21
<0.005 (reference group)	1.00	-	-	1.00	-	-
≥0.005	1.02	0.89	1.17	1.01	0.81	1.26
0.003 to <0.007	1.03	0.84	1.27	1.14	0.92	1.41
<0.003 (reference group)	1.00	-	-	1.00	-	-
≥0.015	1.06	0.88	1.27	1.00	0.84	1.19
0.007 to <0.015	0.97	0.80	1.18	0.87	0.77	0.98
<0.007 (reference group)	1.00	-	-	1.00	-	-

*Comparison presented in main analysis.

**Adjusted risk ratios.

Figure B-1: Full covariance model of predicted mosquito density

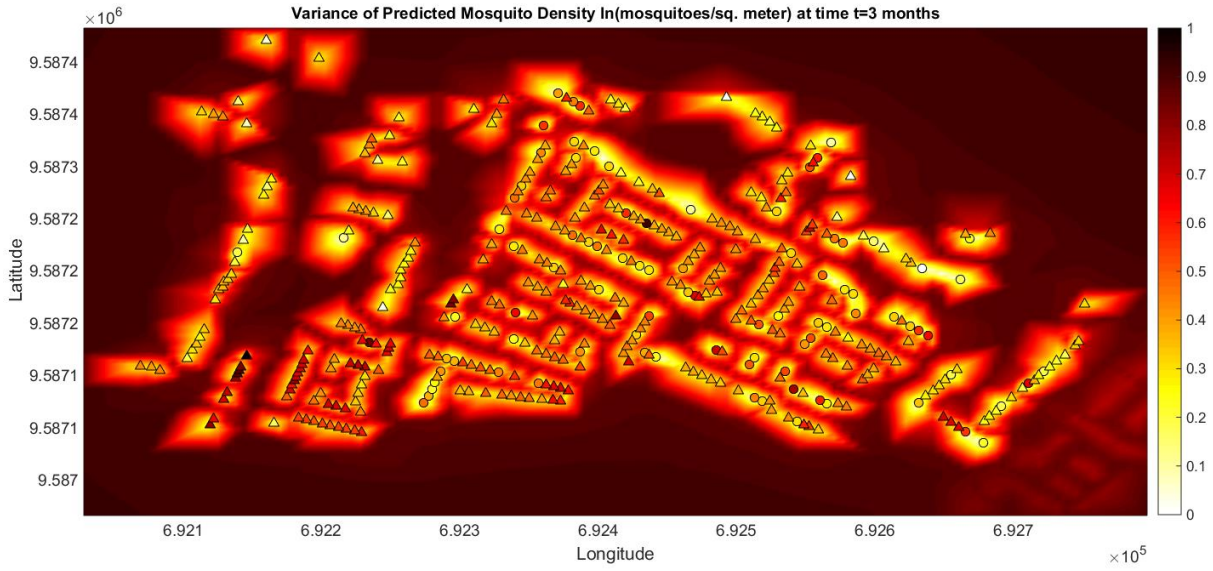


Figure B-2: Variance from covariance structure: short spatial range (25m) and short temporal range (5 months)

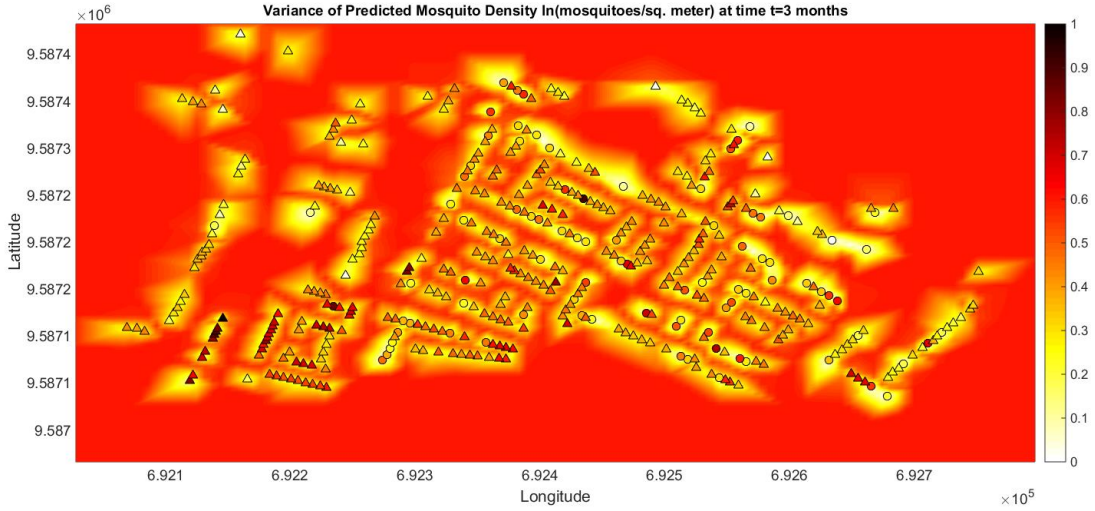


Figure B-3: Variance from covariance structure: short spatial range (25m) and long temporal range (700 months)

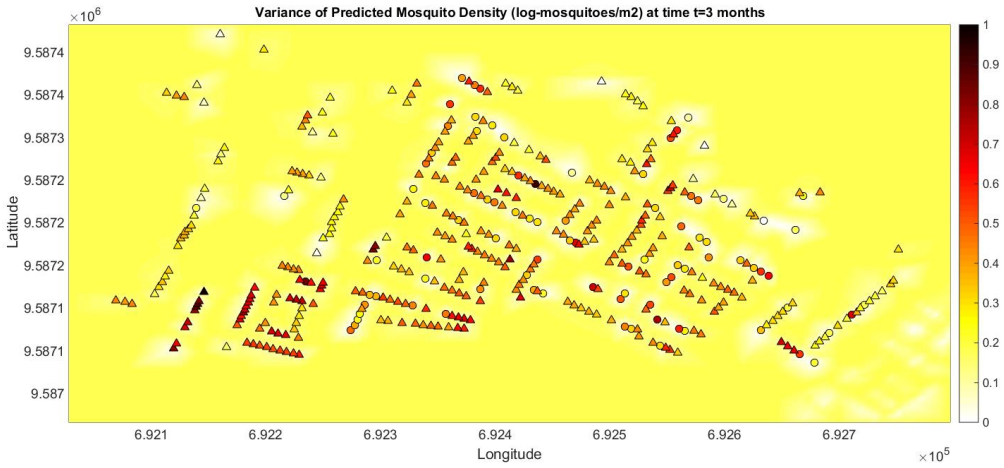
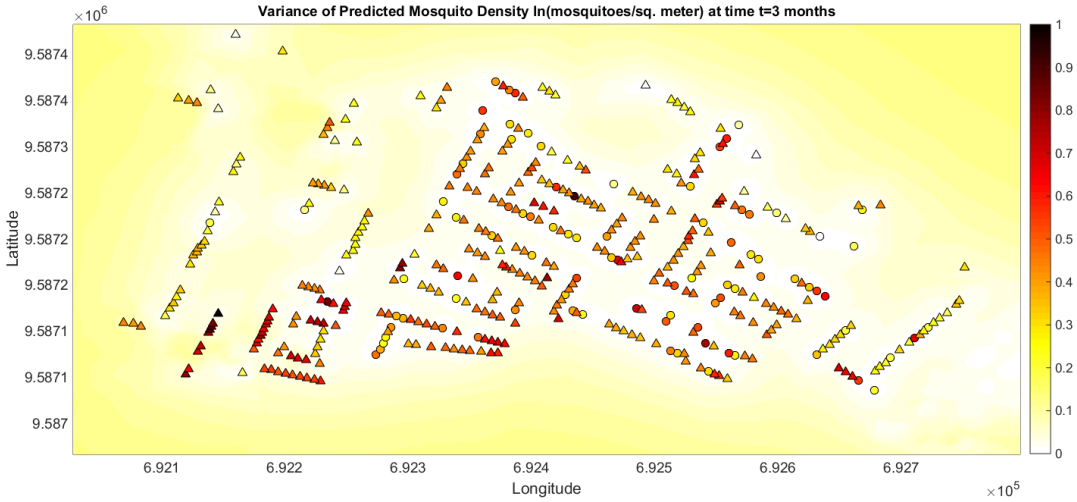


Figure B-4: Variance from covariance structure: long spatial range (350m) and long temporal range (700 months)



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