DENGUE INFECTION IN PUERTO MALDONADO, PERU: HUMAN MIGRATION AND ECONOMIC IMPACT

by

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Dissertation Abstract

Dengue virus (DENV) is an arbovirus with global distribution that affects more than 100 countries worldwide. This Flavivirus was reintroduced to Peru in the 1990s and has disseminated across several areas in the country, especially in the urban centers of the Amazon Basin. In Peru, the virus is transmitted by an anthropophilic mosquito, Aedes aegypti.

Dengue has been increasing in the southern Peruvian city of Puerto Maldonado since 2000, when it was first reported there. This region has the highest human migration rate in the country, and incomers to the city are mainly from non-endemic areas for DENV. The unique migratory movement situation of this city may pose a differential risk for between recent migrants (RM) and long-term residents (LTR).

In this scenario, this study was designed to a) describe the seroprevalence of dengue infection among residents of Puerto Maldonado and evaluate the influence that migration history and knowledge, attitudes and practices (KAP) related to dengue control and prevention may have on serostatus for DENV, b) to identify spatial patterns of risk for DENV infection for residents in Puerto Maldonado, considering migration background, services and infrastructure, socioeconomic status and to assess the proportion of household income that is diverted to costs incurred due to dengue illness and to compare these expenses between RM and LTR, defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. A secondary objective was to describe the demographic and socioeconomic characteristics of RM and LTR in Puerto Maldonado.
To achieve this we conducted a cross-sectional serosurvey and administered a KAP questionnaire to members of randomly selected households, 2012. Sera were screened for antibody to DENV by ELISA and confirmed by plaque reduction neutralization test (PRNT). We also measured other socioeconomic variables and income. To assess the household income diverted to dengue expenses we administered a standardized questionnaire to persons diagnosed with dengue disease. We compared costs incurred between RM and LTR. In order to evaluate the spatial patterns of DENV infection collected the information and geographic location where the serosurvey and questionnaire were administered. We explored the data for clustering patterns, identified clusters and tested the distance of some features in the city with DENV infection.

The results of this study showed a seroprevalence of 54% (95% CI: 49.6; 58.5), which was similar among RM and LTR. RM comprised 11% of this study population. Multivariate analysis indicated that higher values of KAPi (p=0.020) and household monthly income (p=0.009) were associated with antibody positivity, while migration status remained non-significant. For our findings regarding DENV expenses, twenty-eight of the 80 participants (35%), were RM. Each dengue disease episode cost the household an average of US$ 105 (SD=107), representing 24% of their monthly income. Indirect costs were the greatest expense (US$ 56, SD=87), especially lost wages.

The outcomes from spatial pattern analysis broadened the perspective of DENV infection in this city. We located areas where DENV infection clustered. As before, higher income and KAP score were predictors of infection, but the distance of the households to flooding areas was negatively associated to DENV prevalence. Therefore,
with each meter away from these areas the prevalence decreased about 1% (OR: 0.999; 95% CI: 0.998; 0.999).

From the serosurvey study, we concluded that the seroprevalence of DENV showed an association with risk behaviors and socio-economic status. The uncommon lower seroprevalence that is portrayed in Puerto Maldonado, as compared with other Peruvian cities like Iquitos, in Loreto, is likely due to the relative recent emergence of DENV in the city. Nonetheless, when exploring further the findings of this study with the spatial patterns, we were able to determine areas of higher risk in the urban area as well as depicting a relationship between time of residence in Puerto Maldonado and positive antibodies to DENV. In contrast, costs did not differ significantly between RM and LTR households. However, the study did highlight the significant financial burden incurred by households when a family member suffers dengue disease.

Despite the limitations of this study in terms of such as the lack of longitudinal information or entomological data, these findings can help guide DENV transmission prevention and vector control. Especially since this re-emergent virus is becoming a global concern and the efforts to develop an efficient vaccine continue.
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Chapter One

INTRODUCTION

Dengue is generally recognized as the most common and widely distributed arthropod-borne virus (or arbovirus). While the virus has been present in South East Asia for several decades, the resurgence of dengue and dengue hemorrhagic fever (DHF) in the Americas began to occur in the 1980s following a reintroduction of the vector after efforts to eradicate it. The main factors related to this reemergence have been attributed to changes in the demographic composition of human populations, increasing urbanization without appropriate planning and the lack of or inadequate provision of drinking water, waste disposal and sewage services.

This situation is similar in Peru, where cases of dengue fever (DF) and DHF have been reported since the early 1990s. The region of Madre de Dios has been reporting cases of dengue since 2000 and the circulation of serotypes 1 through 4 has been documented. The area is the least populated region in the country (approximately 100,000 inhabitants), but more than 73% of the population is located in urban areas. Additionally, it is the region portraying the most dramatic population growth, varying around 3.5% per year with a distinctive driver of this growth being migration. Seventy percent of the inhabitants of the entire region are immigrants from the neighboring high altitude regions of Cusco and Puno. Neither of these locations is dengue endemic, primarily due to a lack of vector presence, placing these populations at risk of DF and DHF following migration to endemic zones due to lack of previous exposure to the virus.
The purpose of this study was to assess the role of migration in dengue virus (DENV) infection risk in urban areas in the Southern Peruvian rainforest. Migration may pose differential biological and sociologic factors for DENV infection risk, such as the settling location within the urban landscape, attitudes and practices regarding prevention and control. The study also comprises a section to describe the economic impact of dengue disease on households in Puerto Maldonado, and compare these pressures between migrants as compared to long-term residents of the city. Therefore, Chapter Two of this document sets the environment for this task, explaining the biological aspects of DENV, the epidemiology and the role of the vector, *Aedes aegypti*. It also gives further background into the migration phenomenon and specifically the issue in the study area. It also provides an overview of the burden of the disease and the existing information regarding economic impact of this virus and finally describes the justification and significance of this research.

The study was designed as two cross-sectional investigations in the population of Puerto Maldonado. The details of this methodology are described in Chapter Three. Briefly, data collection comprised a serosurvey applied to residents from Puerto Maldonado, the capital city of Madre de Dios, to assess the seroprevalence of dengue according to their location in the city. Participants were also interviewed to collect information about past dengue infections, knowledge attitudes and practices about dengue, to gather information regarding socioeconomic status and migration history. These data were used to map past DENV infections and evaluate their location in Puerto Maldonado. The questionnaire also included information regarding the presence of certain facilities and services (*i.e.* sewage, running water, garbage collection sites). Sera
were screened using enzyme-linked immunosorbent assay (ELISA) and confirmed through plaque reduction neutralization test (PRNT) to all four serotypes. Finally, another cross-sectional study was performed to investigate the economic burden of symptomatic dengue at the household level. Information was collected from patients who were diagnosed with the disease at local health centers. Chapter Three also details the conceptual framework that guided this research, the specific location where the study was conducted and the study population.

The data collected allowed for answering the three main hypotheses under study (B. Specific aims). Consequently, Chapter Four, Chapter Five and Chapter Six are devoted to the findings and conclusions of each study question. Therefore, the focus of Chapter Four is on the effect of migration status on DENV seroprevalence and risk factors for acquisition. Chapter Five is centered on the economic burden of dengue disease in the households. The spatial patterns of DENV infection in Puerto Maldonado are evaluated in Chapter Six.

Chapter Seven reviews the findings of these research questions to provide an integrated discussion, considering the specific features of the study area and population and extrapolating it to similar environments and future steps in research. Ultimately, this study was designed to help understand the risk of DENV transmission and dengue disease burden in the context of rapidly growing urban environments shaped mainly by migration, in a resource limited, tropical area. We expect these findings to contribute to the body of knowledge of DENV control efforts, to better manage this re-emerging pathogen that threatens urban and peri-urban settings in tropical and sub-tropical areas across the globe. The generalizability of this information will be useful for settings that
are similar and also at risk for dengue endemicity. Currently, there is an unsatisfied need for solid data on the risk of dengue as it relates to demographic changes and urban sprawl, climate change, and the movement of populations and vectors, and this study can contribute to this body of knowledge.
Chapter Two

BACKGROUND

Biology

Dengue is a Flavivirus from the family Flaviviridae and is usually referred to as the most common arthropod-borne virus (or arbovirus) [2]. The virus has four distinct serotypes that produce monotypic immunity and transient heterotypic immunity [3]. Most DENV infections, primary or secondary may present no or very mild symptoms. However, exposure to different serotypes (termed heterotypic infection) may lead to a severe form of the disease, known as dengue hemorrhagic fever (DHF).

Dengue virus (DENV) is a positively stranded RNA virus. The genetic material is surrounded by a lipid bilayer and the whole particle has a 500 Å diameter. The viral particles also have three structural proteins arranged in proportional amounts apportioned to the core, the membrane and the envelope, to form an icosahedral structure. These particles follow complex transformations at different environmental conditions allowing for the fusion of the virus with the host cell [4].

The existence of an enzootic forest cycle of DENV has led to speculation about the origin in Africa or Asia, but there is a clear evolution in the Asian continent [5]. The genotypes associated with enzootic transmission are ecologically and genetically distinct. Three (DENV-1, -2 and -4) of the four sylvatic DENV genotypes have been shown to maintain regular cycles in the wild in Asia and there is serologic evidence of the circulation of sylvatic DENV-3. DENV-2 also maintains an enzootic transmission cycle in West Africa. Phylogenetic analyses of DENV have led to the distribution of these
serotypes in different genotype groups [6]. Therefore, DENV serotype 1 (DENV-1) is grouped further into five genotypes, the same as DENV-2, while DENV-3 has been grouped in four genotypes, like DENV-4. Phylogeny is mainly inferred from the nucleotide sequences of the E gene.

**Diagram of DENV showing the basic monomers and the three domains (I, II and III) that build the viral external interface: I – red, II – yellow, III – blue; asterisks show junction residues and fusion peptides are in green; scale bar is 100 Å; taken from [4].**

**Figure 1: Structure of DENV**

Despite different suggestions regarding DENV origin [5, 6], the major controversial issue for elimination remains: with the existence of a sylvatic cycle, reintroduction events of the virus are bound to occur, potentially precluding or hampering efforts to control DENV transmission and consequently disease [7]. However, some researchers argue that the role of the zoonotic sylvatic disease is no longer relevant for DENV transmission among human populations and hence for control efforts [8].
**Pathogenesis**

DENV is known for commonly having asymptomatic or “silent” transmission which transmits with unapparent disease or very mild disease [9]. However, when symptomatic it presents with fever, therefore called dengue fever (DF), and sometimes progresses to more severe forms involving plasma leakage and sometimes shock. Until 2009, the presentations of the disease were defined as DF, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which were initially thought to be completely different entities [3]. Dengue is usually a self-limiting disease with a wide range of flu-like symptoms (ie. fever, headache, malaise) and some more specific signs (ie. retro-ocular pain, myalgia, rash). Among those patients infected with DENV a small proportion will develop hemorrhagic manifestations (i.e. epistaxis, petechiae, ulemorrhagia) and can lead to shock where plasma leakage leads to circulatory collapse and even death. The current classification from the World Health Organization redefines DENV infection as dengue with and without warning signs (i.e. abdominal pain, persistent vomiting, fluid accumulation, bleeding lethargy, restlessness, liver enlargement, increased hematocrit with decreased platelet count) and severe dengue. The purpose of this re-categorization is to identify early symptoms of disease progression to severe dengue and prevent DHF, shock and death. However, the definition of DHF and DSS is still widely used [10].

The specific process that leads to severe dengue is not entirely understood. The pathogenesis related to each one of these disease manifestations comprises the convoluted relationship of factors associated with DENV features and the host immune response. Different virus genotypes have been associated with different levels of disease
severity and these varied phenotypic presentations have been related to the progression to DHF/DSS. Specifically, in the Americas, the imported, more aggressive South East Asian DENV-2 strain has been incriminated in the displacement of the less virulent American genotype DENV-2 leading to the development of DHF in the continent in the past three decades [8, 11].

The more serious manifestations have also been related to the host immune response and an inflammatory immune response after DENV infection [12]. Elevated viral replication and concentration of viral particles in the host can potentially trigger a cascade of cytokines, some of which may have positive and negative effects in disease progression [13]. Another mechanism that is related to the host response is antibody dependent enhancement (ADE). The proposed pathway by Halstead et al was assessed in view of clinical and epidemiological evidence which pointed to more serious manifestations of disease among people who had heterologous secondary infection with DENV or among infants who had primary infection, but still had maternal antibodies to a different serotype than the one with which they were initially infected [14]. ADE is a phenomenon where the binding of antibodies to DENV to ease of access to target cells, facilitating increased virus titers in a secondary infection with a different serotype [15]. In vitro, in vivo and epidemiological evidence have continuously provided support for this hypothesis [14, 16, 17]. This purported pathway couples the host immune response and the evolutionary mechanisms of the virus to select for more appropriate traits to maintain DENV in the human reservoir [18]. However, it still remains unclear whether the ADE mechanism itself, DENV virulence factors or host genetic characteristics and background have the most influence on the development of DHF/DSS.
Vector and Transmission

DENV is transmitted by a vector, a mosquito of the Aedes genus. In Peru, the species Aedes aegypti is the vector for DENV [19]. The life cycle of Ae. aegypti occurs in four stages: eggs, larvae, pupae and adults. Female adults commonly lay eggs in water filled-containers allowing for eggs to hatch and the development of four stages for larvae, progressing then to pupae and the adult form.

The life span of a wild adult female mosquito has been estimated at approximately 9-10 days [20]. Each reproductive or gonotrophic cycle requires a protein-rich nutrition to produce eggs. This requirement is fulfilled with a blood meal and it has been shown that imbibing human blood may confer a fitness advantage for this vector [21, 22]. The female mosquito becomes infected when it ingests a viremic blood meal and remains infected for life. Viral replication and dissemination within the arthropod vector requires what is termed an “extrinsic incubation period” which has been estimated at 8-11 days before the viral particles travel to salivary glands and the mosquito becomes infective and can transmit the virus [23]. The extrinsic incubation period can be altered due to environmental temperature and it has been shown to shorten with increased temperature [24] and to vary depending on the genotype of the virus, thus affecting the vector capacity of the mosquito.

Figure 1: Schematics of the life cycle of A. aegypti. Taken from © 2011 Nature Education. All rights reserved [1]
There has been speculation and several studies have suggested vertical transmission of the virus [26-29]. Therefore, it is presumed that as with other Flaviviruses, DENV can maintain transovarial transmission through several generations of mosquitoes. Nonetheless, the specific role this purported strategy may play to maintain DENV through inter-epidemic periods and how it interplays with the human reservoir [30] still needs to be reckoned [31].

Several studies have shown that other mosquito species such as *Ae. albopictus* possess certain traits that make it a very adaptable and resilient vector for DENV [32] and several studies have conferred a better outcome for *Ae. albopictus* as a vector for DENV. However, recent analysis has shown that *Ae. aegypti* actually fares better in terms of vector competence (*i.e.* virus replication and dissemination in the arthropod) [33]. But there are also other features that make *Ae. aegypti* a very successful vector, especially in urban settings. *Ae. aegypti* is recognized as a very nervous feeder. It can interrupt feedings easily and therefore may transmit DENV to several household dwellers in one gonotrophic cycle, even while probing [20, 34]. Likewise, size and temperature are related to feeding behavior increasing the frequency of the latter with a decrease in wing span and augmenting as well with higher temperature [35]. Additionally, this haematophagous arthropod is mainly a domestic dweller [36]. *Ae. aegypti* has easily adapted to urban living, preferring clean water and artificial containers to reproduce [20]. Nonetheless, the vector has also been found in septic tanks, showing great adaptability and ubiquity [37]. Dispersal studies of the adult form have established a mean range of dispersion of approximately 30-200 m, although it has been shown that it may disperse up to 800 m [38]. The flight range varies according to the clustering or scattering of
houses (host availability), shade and wind [39-41]. Therefore, the accessibility to hosts, mates, resting areas and ovipositioning places have led this pervasive and adaptable vector to commonly move within 10 m or within one household for life [42].

**Prevention and Control**

As presented before, exposure to dengue virus is dependent on the presence of the *Aedes* mosquito. *Ae. aegypti* is an anthropophilic mosquito, which has adapted well to human living conditions [37, 39]. Traditionally, entomological indices such as household index (proportion of households found with mosquito larvae or pupae), container index (fraction of containers found with mosquito larvae or pupae) and Bretau index (proportion of containers with larvae or pupae per 100 houses) have been used for surveillance activities as a proxy to measure mosquito density in an area [10]. Some of these indices have been shown to be robust, though not very sensitive to population changes [43]. Therefore, how these indices actually relate to DENV transmission and dengue disease as well as the establishment of specific thresholds for control is still under debate [20]. Specifically in Puerto Maldonado, the study location, the presence of *Aedes aegypti* has a seasonal pattern with a peak in November and a smaller increase in entomological indices around March [44].

Despite these difficulties, DENV control efforts have been focused on vector management. Different strategies have been tested and are still under study with the objective of diminishing breeding sites for the mosquito as well as repelling adult mosquitoes from human dwellings [45-47], spraying to diminish the adult vector population and involvement of the community through different strategies (i.e. container collection campaigns, programs in schools involving students) to maintain low vector
indices [46, 48, 49]. Hence, community involvement is regarded as a cornerstone for vector control [50, 51]. However, participation and engagement of community members is a lengthy process that has not yet offered consistent outcomes [52]. To overcome this, several studies have addressed distinct cultural characteristics (i.e., housewives’ participation in vector control programs, schooling of household head) to identify flaws in the engagement of these management activities [47, 53]. Different levels of knowledge, attitudes and practices of dengue prevention and vector control have been shown to influence vector infestation [54-56]. Other strategies regarding vector control have also been proposed and enduring initial trials as well. Among these is the use of a specific strain of the bacteria of the genus Wolbachia. Infection of the vector with these bacteria has demonstrated diminished viral replication and dissemination in the mosquito, rendering this strategy a promising one for vector and DENV control [57]. A similar approach has been under evaluation using genetically modified mosquitoes with a lethal gene. Under this design the release of the genetically modified mosquito populations could potentially suppress the wild population and maintain mosquito numbers at bay [58]. Both methods would require the massive dissemination of mosquitoes into field conditions, a type of experiment that has several important issues to consider, such as the niche replacement of the target vector by another vector species [59].

Another important avenue of dengue prevention and control is vaccine development. There are currently at least five vaccine candidates identified that are undergoing different trial phases, including large scale phase III trials [60, 61]. However, given the complexity of the immune response elicited by the four virus serotypes the most recent efforts have provided inconsistent results [62, 63].
**Epidemiology**

DENV is responsible for DF, DHF and DSS. The virus, along with its vectors has expanded to over 100 countries and it is estimated to produce approximately 400 million infections, placing over 2 billion people at risk of disease [10, 64]. Since the isolation of the virus in 1943, the virus has spread and become hyperendemic in Asia, Africa, Oceania and the Americas [2, 65]. While the virus has been present in South East Asia for several decades, the resurgence of dengue in the Americas occurred in the 1980s after the vector, *Aedes aegypti*, was reintroduced after an elimination campaign, which started approximately forty years earlier [2, 66]. In this scenario, Cuba experienced an outbreak in the region after years of silence in 1977 [2], which led to subsequent dengue hemorrhagic fever (DHF) outbreaks [67, 68]. The situation in Cuba has been similar to other countries in the region where dengue and DHF has reemerged in the past decades [19, 69-72].

This panorama has also replayed in Peru, where cases of DENV and DHF have been reported since the early 1990s [19, 73]. Currently, for this year there have been more than 11000 reported cases of DENV in Peru, between confirmed and probable cases, from 16 different administrative departments [74]. The area of Madre de Dios is particularly at risk for DENV and DHF for numerous reasons [75]. Dengue has been found in the region since 2000 and the circulation of serotypes 1 through 4 has been documented, but had presented only as DF [75-78]. Other areas, such as Maracay, Venezuela which has had endemic, longer term circulation of DENV and higher population density are more likely to have subsequent cases of symptomatic dengue and DHF [79].
Besides viral and vector features that have been discussed in previous sections, there are several other important factors that have been related to the reemergence of DF and DHF. Among these, some have been attributed to changes in the demographic composition of human populations, increasing urbanization without appropriate planning and the lack or inadequate provision of water, waste disposal and sewage services [20, 66, 80-82].

In terms of disease dynamics, recent studies on disease transmission dynamics have postulated that DENV is maintained not only in the mosquito vector, but also among the human population, acting as reservoirs and carriers of the virus [30, 83]. Likewise, years of data from areas with endemic transmission of DENV have displayed the movement of the virus through a region as “travelling waves” [84]. This behavior has been shown before with other diseases, such as measles. It requires the constant reintroduction of the virus in small communities and the maintenance of several “pockets” of transmission to keep the virus circulating in larger cities while garnering appropriate conditions for widespread dissemination from the larger urban centers [85].

**Burden and Economic Impact**

Previous studies of dengue seroprevalence and incidence in Latin America have provided a wide range of results. A study carried out in the border city of Matamoros in Mexico reported a 78% (95% CI 74%–83%) prevalence of dengue infection from a cross-sectional sample of all ages [86]. Balmaseda *et al* found a seroprevalence of 91% among school children in Managua, Nicaragua, and an incidence of 12 and 6 episodes / 100 person-year in different years of observation [87]. In the area of Recife, Brazil, seroprevalence among people older than 5 years old in economically distinct areas of the
city ranged from 73-91% with an incidence of 5.3 – 17.7 / 100 person-year [88]. Meanwhile, the region of Acre reported 18% prevalence and an incidence of 3.67 infections / 100 person-years of observation [89] among a rural population. Recently, Morrison et al reported a seroprevalence of 80% in the general population of Iquitos, Peru, as well as an incidence that ranged from 2 to 89 infections / 100 person-years during peak transmission periods [9]. In this latter study, the investigator suggests that certain temporal trends of the disease may be due to migratory movement within the population and a definite variation in seroprevalence of dengue infection by location within the city. All these regions within Latin America have reported the circulation of dengue at least since the 90s, while Madre de Dios has reported dengue in the region since 2000 [76, 77].

The economic impact of dengue has been mainly addressed at the institutional or regional level, identifying the losses of a specific area due to dengue cases or using disability adjusted life years (DALYs) for such estimates [90-93]. Few studies have investigated the impact of the disease at the household level. Clark et al. performed such a study in Thailand, where they found that in certain instances the expenses for the household surpassed its monthly income, placing such families in a vulnerable situation [94]. Additionally, studies in other settings have identified specific disadvantages faced by rural-urban migrants when joining the labor market and the gap in income between the newcomers and the urban residents [95, 96]. Specifically, some authors have portrayed the differential income earned by migrants when they first arrive at a new destination [97]. The lower wages that are offered to new migrant workers is usually balanced out with the local population after approximately two years in the area of study. However,
given the extensive migration from rural to urban areas in Peru [98] and in particular the intense population mobility reported for the Madre de Dios region, it is likely to find an economically vulnerable population. In particular, this situation has the potential of placing migrants in a more vulnerable situation, if they are facing diseases such as dengue that they are not familiar with.

**Migration**

Anthropogenic influences have been associated with the spread of disease in the past. A typical example is cholera and the spread beyond Asia in 1917 [99, 100] and also diseases like the bubonic plague [101] and smallpox in the Mexican colonies in the 17th century [102]. Conflicts have also been drivers of illness, sometimes causing more distress than was derived from actual combat [103-105]. Likewise, changes in economic activities have been related to increased incidence of sylvatic rabies in some endemic areas [106, 107] while urban land use change and development may influence disease vector abundance resulting in greater rates of human contact and subsequent disease transmission [108].

Demographic factors have also been previously linked with infectious diseases. The transmission of certain arboviruses, including dengue, is greatly influenced by the host, whether it is through immunity, abundance or diversity [109]. Recently, Cummings et al revealed that there is no incidence of dengue cases in certain months of the year in certain places in Thailand, depending on population size and the distance from a large urban area, like Bangkok [84]. The same author also showed that the growing older population in this area may be contributing to the changes in the epidemiology of dengue, with a slight trend to diminishing the incidence of the disease [80]. Likewise, Rodriguez-
Barraquer et al explained the increasing incidence of DHF in Recife, Brazil only after introducing the demographic changes (aging population and decreasing birth rate) the country has experienced in the past years [110].

The movement of people for work and to settle in different locations was not specifically addressed until the late 1970s when it was recognized that “the importance of migration as a component of population change has significance beyond its impact on the changing population size and composition of major regions, political subdivisions, and rural-urban places” [111]. Particularly, the movement in the rural-urban direction has been posed as the main explanation for growing urban centers in developing countries [112]. The causes and drivers for migration have been widely discussed, from economic models to behavioral ones [112-114] that focus on the individual or more recently the household [115, 116]. Currently, migration is a leading policy issue and a growing concern in public health around the world and especially in developing settings. In particular the movement of people has led to the introduction of diseases into new areas and the relocation of susceptible individuals [117].

The Region of Madre de Dios, Peru, is the least populated region in the country (approximately 100,000 inhabitants), but it is experiencing the most dramatic population growth due to migration in the country. The net migration rate is 32.1 x 1000 in 2002-2007, the highest in the country and almost four times the rate of Lima, which is also the capital city. Over 73% of this population is located in urban areas [118, 119]. Seventy percent of the inhabitants of the whole region are migrants from the neighboring regions of Cusco and Puno, neither of which are dengue endemic areas, placing this migrants at risk of developing DENV and DHF.
A few studies have highlighted the association between disease transmission and migration, such as the research by Marques et al among gold miners and farmers along the Trans-Amazon highway in Brazil [120]. Other investigations have looked at the migrant population to describe several disease processes within this group, such as cardiovascular disease and over nutrition, in Latin America and in Lima, Peru [121-123]. Finally, other authors have highlighted the risk of dissemination of disease from endemic areas through migrant populations and have proposed preventive measures. Such is the case for Chagas’ disease and tuberculosis [124, 125]. Despite the evident link of demographic factors, and specifically migration, with infectious disease and the recognition of its impact on the resurgence of diseases like dengue [2, 5] only scarce work has addressed migration and mobility among the hosts [83, 89]. Most of the studies on this virus in the region do not incorporate these social and demographic drivers to better understand DENV transmission.

**Significance**

The incorporation of demographic factors in this study provides an innovative approach to the research on DENV transmission. Despite the obvious influx of susceptible population through migration that may help maintain disease circulation in the city, their specific contribution to disease transmission has not been measured. A better understanding of the drivers for the transmission of DV and the role that population mobility and the concomitant factors related to relocation play in disease transmission can strengthen urban planning and vector control activities. This may also help target control activities for at-risk populations. Our findings may ultimately help guide
prevention strategies in areas where DENV has recently emerged or re-emerged, including the US [126]. Additionally, there are current efforts and advances in the discovery of a vaccine against dengue virus that can provide immunity to all four serotypes of the disease [127-129]. In light of these innovations, this study may help guide future vaccination strategies by identifying specific at-risk groups and their role in maintaining the circulation of the virus in endemic areas.

Considering the dynamic components of dengue transmission, it is fundamental to understand the demographic characteristics that are related to the risk of dengue infection in Madre de Dios. The purpose of this study is to better define DV prevalence and risk of transmission, considering the particular characteristics of population mobilization and settlement into an endemic area.
Chapter Three

METHODS AND OBJECTIVES

A. Conceptual Framework

Migration, and particularly internal migration from rural to urban areas, is conceptualized as a strategy of the rural household to adapt to socio-environmental challenges [114]. These households are probably not the poorest from rural areas, since migration itself requires some expenses, resources and time unemployed to establish in a new area [130]. Household members decide to move to an urban location, such as Puerto Maldonado, driven by better opportunities for work and therefore income, or expected income [113], education, health services and amenities [130]. This conceptual model assumes that when a new household is established in Puerto Maldonado, the members are not aware or do not have experience with endemic diseases such as dengue. They may get some information through networks established by prior migrants if they are in contact with these resources [130], but prevention strategies (i.e., mosquito nets, insect repellent, cleaning water containers) may still be unfamiliar or unavailable. Likewise, the areas chosen, or more likely, the only available sites for settlement are marginal areas in the periphery of the city, without adequate services such as running water, sewage or garbage collection associated with precarious housing [116]. These conditions together place recent migrants at higher risk of dengue exposure and disease. The conceptual framework underlying this study is presented in Figure 3.
There were three research components planned for this project: a seroprevalence study, an economic impact assessment and a geographical analysis. The design of the study was conceived to allow identifying variations in seroprevalence of DENV infection among the recent migrants (RM) vs. long term residents (LTR) in the city through serosurveys and household questionnaires for the first component. The second component evaluated the geographical aspects of the risk for DENV in Puerto Maldonado. The seroprevalence study across a random sample of the population provided information regarding past DV infections according to location in the city that was explored in this component. Finally, the third and last component was directed to the economic impact of DENV within households, was assessed to contrast households of...
RM and LTR and determine differences in the economic burden of these households. Information was collected from interviews with symptomatic cases identified at health care centers.

The assumption underlying these analyses is that aspects of DENV prevention and risk are different between RM and LTR. Migration and relocation may place RM households in a vulnerable situation. I proposed that RM in comparison with LTR may lack information regarding prevention practices or that a larger fraction of their income may be compromised with each illness episode. Finally, the location of settlement may introduce risk for DENV to these RM households.

B. Specific aims

The three main objectives of this study were to: (1) assess the role of migration in DENV infection risk in urban areas of the Southern Peruvian rainforest; (2) identify and describe specific characteristics of recent migrants as they established their household in the city that may be related to dengue risk, such as employment, urban land use and their knowledge, attitudes and practices about dengue; and (3) describe the economic impact of DENV at the household level and compare this influence by year of migration to Puerto Maldonado.

To address these objectives, we designed a cross-sectional seroprevalence study and a cross-sectional survey to collect economic impact data in the urban center of Puerto Maldonado, Peru. The Region of Madre de Dios and Puerto Maldonado, its capital city, are undergoing intense change with a substantial flow of migrants and increasing incidence of DENV transmission. We will test the following three hypotheses:
**Hypothesis 1**: the confirmed seroprevalence of dengue virus infection is higher among long term residents (persons who established in the city more than 5 years ago) compared to recent migrants (persons who established in the city in that past 5 years).

**Hypothesis 2**: the economic impact of an episode of symptomatic dengue at the household level in Puerto Maldonado is at least 10% of the total household monthly income.

**Secondary hypothesis 1**: the economic impact at the household level of symptomatic dengue infection is higher among recent migrants to Puerto Maldonado than long-term residents.

**Hypothesis 3**: the risk of dengue infection in Puerto Maldonado, Peru is not spatially random across the city and practices to prevent exposure to dengue vary by year of household establishment in the city.

**Secondary hypothesis 2**: areas of settlement in urban and peri-urban areas have differential characteristics that are associated with dengue seroprevalence.

**Secondary hypothesis 3**: recent migrants have different knowledge, attitudes and practices related to dengue prevention and exposure compared to long term residents.
C. Study Type

The study comprised a cross-sectional serosurvey and a cross-sectional questionnaire (Table 1). The cross-sectional serosurvey enrolled 505 residents from 307 households in Puerto Maldonado to evaluate their seroprevalence to dengue and their location to assess spatial arrangement of DENV risk across the city. A cross-sectional questionnaire was applied to 80 symptomatic cases of DENV who were diagnosed at local health centers in Puerto Maldonado.

Table 1: Components of dengue research in Puerto Maldonado by study design

<table>
<thead>
<tr>
<th>Study design</th>
<th>Outcome</th>
<th>Main variable</th>
<th>Details</th>
</tr>
</thead>
</table>
| Cross-sectional serosurvey | Dengue seroprevalence | Migration status
Geographical location | - 307 households randomly selected
- Application of questionnaire: migration history, knowledge and practices, socioeconomic status
- Blood draw to test for dengue antibodies |
| Cross-sectional questionnaire | Economic burden of dengue | Migration status | - 80 Participants identified in healthcare centers
- Application of questionnaire about household migration history and expenses due to dengue episode |

D. Study Location

This study was conducted in Puerto Maldonado, Madre de Dios Region, Peru. We took advantage of the existing infrastructure, workforce and logistics already in place from a Naval Medical Research Unit #6 (NAMRU-6) ongoing study: “Burden of illness and risk factors for transmission of seasonal influenza in four distinct regions of Peru”. Madre de Dios is a major corridor for the Interoceanic Highway connecting the Pacific Ocean in western Peru (Ilo and Matarani) with the Brazilian ports of Rio de Janeiro and Santos on the Atlantic coast (Figure 4). Road construction through Madre de Dios was
completed in 2011, with the construction of a bridge over Madre de Dios River and elevated platforms through the city of Puerto Maldonado.

Figure 4: Peruvian map and study site

E. Study Population

The population under study consisted of residents from the capital city, Puerto Maldonado in Madre de Dios, Peru. Madre de Dios is the least populated region in the country with a total (estimated) population of 84,383 in 2000 [131] and 109,555 in 2007. This region also has the lowest population density (1.3 inhabitants per square kilometer) and accounts for 0.4% of the Peruvian population [119]. However, Madre de Dios is a region undergoing the most dramatic population growth in Peru, with a total increase of 63.5% between 1993 and 2007 [119]. A large factor of this growth is due to migration. The total net migration rate is 32.1 x 1000 per year, the highest in the country [118]. More than 73% (80,309) of this population is located in urban areas and almost solely in
The city of Puerto Maldonado has almost doubled its population since 1993. The population in Puerto Maldonado is currently 65,756 people with an annual growth rate of 2.5%, which is among the highest of cities in Peru [132]. Migrants to Puerto Maldonado are primarily from Cusco (46.5%), Puno (8.1%) and other areas in Madre de Dios (9.9%) [118]. To emphasize even more the importance of migration in the composition of this population, the Demographic and Health Survey for this region reported permanent residence in urban areas of approximately 40% for women of reproductive age, while about half of these women have lived there for 20 years or less [133].

This study enrolled subjects from households in Puerto Maldonado and used the NAMRU-6 infrastructure and workforce already in place for the ongoing influenza study. The activities taking place already provided some preliminary information regarding the composition of the population under surveillance. About a fourth of the population are younger than 10 years old, 19% are between 10 to 17 years old, half of the population is between 18 to 60 years old and 5% are 60 years or older. There are more females (54.5%) than males (45.5%) and 6.6% of the population under study has underlying chronic conditions: 4.0% have asthma, chronic bronchitis, tuberculosis or rhinitis and 2.6% have hypertension, cancer or diabetes.

**F. Sample selection**

a) Seroprevalence and geographical location study

Except for a few residences, administrative buildings, private offices, educational facilities and hotels, most of the buildings in Puerto Maldonado are one story (less than
1% are apartment buildings) [133]. Therefore, a random sample of 300 blocks from Puerto Maldonado were selected using a multispectral, 1/100000 satellite image from July 2010. The field workers selected one household per block, starting with the northernmost corner of each appointed block and moving to the right (clockwise around the block) until a household from the block could be enrolled. If the block selected had a building with more than one story, a random number determined which apartment to try to enroll first. Every resident older than 6 months old was invited to participate. A household questionnaire collecting past dengue infection and migration history from all the members was collected.

b) Economic impact study

To evaluate the economic impact of symptomatic dengue infections field workers contacted outpatients and hospitalized patients in the city’s healthcare facilities to enroll them in the study (i.e., Hospital Santa Rosa, Laboratorio Regional, etc.). Participants were invited to participate and a questionnaire was applied. This was usually performed in the same health care facility or in the subjects household if that was more convenient.

G. Definitions

Recent migrant (RM) – a subject was considered a recent migrant to Puerto Maldonado if they had been living in the city for five year or less at the time of enrollment. This definition was initially adopted from one of the questions regarding migration from the population and household census in Peru [134].
**Long term resident (LTR)**—a participant will be considered a long-term resident if they have been living in the city for more than five years at the time of enrollment.

**H. Human Subjects Research**

Approval for this study was obtained from the Johns Hopkins Bloomberg School of Public Health Institutional Review Board (IRB) and the Naval Medical Research Unit No. 6 (NAMRU-6) IRB. NAMRU-6 participation was under Protocol NMRCD.2011.0003 at NAMRU-6 and #0367 at Johns Hopkins Bloomberg School of Public Health in compliance with all applicable federal regulations governing the protection of human subjects. Likewise, a permission to perform the study was granted by the local Ministry of Health from Madre de Dios (DIRESA, for the acronym in Spanish).
Chapter Four

EFFECT OF MIGRATION STATUS ON RISK OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

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Appendix: 2

Keywords: dengue, prevalence, migration, community-based, KAP

Running Title: Migration and dengue
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IV.1 ABSTRACT

Background: Dengue virus (DENV) affects more than 100 countries worldwide. DENV has been increasing exponentially in the southern Peruvian Amazon city of Puerto Maldonado (population ~65,000) since 2000. This region has the highest human migration rate in the country, mainly from non-endemic areas for DENV. The risk of DENV infection may vary between recent migrants (RM) and long-term residents (LTR) due to both biological and sociologic factors.

Objective: This study was designed to describe the seroprevalence of dengue infection among residents of Puerto Maldonado and evaluate the influence that migration history and knowledge, attitudes and practices (KAP) related to dengue control and prevention may have on serostatus for DENV.

Methods: We explored the prevalence of past DENV infection and KAPs of dengue control and prevention among RM and LTR, defined respectively as residency in Puerto Maldonado for five years or less or greater than 5 years. In 2012 we conducted a cross-sectional serosurvey and administered a KAP questionnaire to members of randomly selected households. Sera were screened for antibody to DENV by ELISA and confirmed by plaque reduction neutralization test (PRNT) against all four serotypes. We created indices for KAP (KAPi), household infrastructure and services (ISi) and assets (Ai). PRNT results were analyzed against migration status and these indices with an ordered logistic regression.
Results: Five hundred and five participants from 307 households provided a blood sample and completed a questionnaire. RM comprised 11% of the study population and was as likely to be DENV antibody negative as LTR on bivariate analysis. ISI, AI and KAPI were significantly associated with antibody status. However, after controlling for other variables in the multivariate analysis, higher values of KAPI (p=0.020) and household monthly income (p=0.009) were associated with antibody positivity, while migration status remained non-significant.

Conclusion: We conclude that risk of DENV infection in Puerto Maldonado relates more to risk behaviors and socio-economic status, than to migration status. The higher KAPI is likely consequence of experiencing the disease. These findings are available for the local health authorities in order to improve KAP in the area.
IV.2 BACKGROUND

Dengue virus (DENV) is the most common arbovirus, affecting people in more than 100 countries worldwide, placing between 2 and 4 billion [50, 66, 135, 136] at risk of disease. This RNA virus belongs to the *Flavivirus* genus and is responsible for approximately 500 000 cases of severe disease yearly [135, 137]. The immunological response elicited by each dengue serotype and subsequent exposures to a different serotype allows for more severe manifestations of the disease (*i.e.* dengue hemorrhagic fever, dengue shock syndrome), but also confers lifelong immunity to the infecting serotype [138]. The first infection with one of the four serotypes (DENV-1, -2, -3, -4) of the virus is commonly called “primary” infection, while subsequent infections with other serotypes is referred to “secondary” and is a known risk factor for the more severe forms of the disease [81].

Population studies focused on the seroprevalence of DENV antibodies across the globe have offered a wide range of results depending on the characteristics of the area and population immunity status. These have usually shown an increase with seroprevalence with increasing age and as DENV establishes in a new area. Therefore, different studies across time in Managua, Nicaragua, have accounted for seroprevalence of 13% in children in 1985 when dengue was first reported in the country, 66% in children in 1997 and 91% in children 4 to 16 years old in 2003 [87]. The presence of dengue in Peru was first reported in Iquitos in 1990 after decades of active efforts to eradicate the vector, *Aedes aegypti* [2]. This city is located in the Amazon Basin in northeastern Peru. In 1991 DENV-1 was identified in the area and in 1993, a longitudinal study among students 7-22 years old carried out by Watts *et al* estimated a
seroprevalence of 78%, 74% in 1994 and 94% in 1995, after the introduction of DENV-2 [139]. Studies carried out by Morrison et al starting in 1999 calculated the seroprevalence of infection at 80% across all the population and 56% for 5 year-old children, increasing to 87% by 14 years and 91% for adults (≥18 years old) [9].

All DENV serotypes have been reported to circulate in Puerto Maldonado, Madre de Dios. In 2001, DENV-1 was the first serotype identified and after a few years of apparent epidemiological silence DENV-3 was introduced in 2004 through 2005. DENV-2 was transmitted in 2007-2009, 2011 and recently in 2013. A few cases of DENV-4 have been reported in 2008, 2010, 2011 and 2013 as well. Both DENV-1 and DENV-3 reappeared in 2009, but DENV-3 persisted only until 2011 and DENV-1 has remained the predominant serotype since then. [78, 140-142]. Nonetheless, no population studies have enquired about the immunity profile of Puerto Maldonado.

This paucity of population-based data is particularly important since Madre de Dios is the least populated region in Peru (approximately 100 000 inhabitants), but in the past decade population has grown dramatically due to migration [118, 119]. The net migration rate estimated in the last Peruvian census in 2007 was 32.1 x 1000, the highest in the country and almost four times the rate of the following region, Lima, which is the capital city. Over 73% of this migrant population lives in urban areas [118, 119]. Seventy percent of the inhabitants of the whole region are migrants from the neighboring regions of Cusco and Puno, neither of which are DENV endemic areas [76, 77]. Noticeably, this pattern of human mobility also constitutes a flow of susceptible individuals into an area of DENV transmission.
Exposure to dengue virus is dependent on the presence of the *Aedes* mosquito. *Ae. aegypti* is an anthropophilic mosquito, which has adapted well to human living conditions [37, 39]. Activities to curb vector distribution are focused on the removal of larval habitats and spraying to diminish the adult vector population. This makes community involvement a cornerstone for vector control [46, 48, 49] and different levels of knowledge, attitudes and practices (KAPs) of dengue prevention and vector control have been shown to influence vector infestation [54-56]. Therefore, a community where its residents are unaware of dengue prevention and control measures may be at higher risk of DENV infection.

The local Ministry of Health (DIRESA, for the acronym in Spanish) has established a vector control program in the city [76]. However, given the short period of exposure to the disease and the relatively recent emergence of DENV, residents may not be familiar with the mode of transmission of the virus and the appropriate prevention control strategies, weakening vector control activities promoted by the DIRESA. Therefore, this study describes the seroprevalence of dengue infection among residents of Puerto Maldonado and evaluates the influence that their migration status and KAPs related to dengue disease control and prevention has on serostatus for DENV. This information will be used to help inform vector control programs in Madre de Dios and similar regions experiencing recent DENV emergence.
IV.3. MATERIALS AND METHODS

We conducted a cross-sectional survey applying a household questionnaire, an individual questionnaire and collecting a serum sample from each participant. Field work was performed between July and December 2012. The study was approved by the NAMRU-6 IRB (protocol NAMRU6.0003.2011) and Johns Hopkins School of Public Health IRB (protocol #0367).

Location

Puerto Maldonado is the capital city of Madre de Dios. It is located in the southern rainforest of Peru (S 12° 36' 12.3654", W 69° 11' 30.8682") in the junction of Madre de Dios and Tambopata rivers (Figure 5.) The population of the city is approximately 65 000 people, which has doubled since 1993 [134].

Figure 5: Madre de Dios, Peru
Household and participant selection, serosurvey and questionnaire application

Households were selected from city blocks identified using an IKONOS multispectral, 1/100 000 satellite image from July 2010 and a cadastral map obtained from the Municipality of Tambopata Province, Madre de Dios, January 2007. The cadastral map was updated with information from the satellite image as well as from current maps used by the DIRESA for the vector control program and by ground truthing on the field (checking accurate locations and updating information from the field). Blocks were selected randomly and maps of the areas were created to guide fieldwork. Only one household per block was enrolled for the study. Field workers began enrollment at the residence located in the northernmost corner of the selected block. If the lot was a commercial building or the household was not willing to participate, field workers continued moving around the block clockwise to the next household that was willing to participate. If the lot selected had several residences (i.e. apartment building, compound) they would select one of the houses or apartments using a random number table.

All members of the household were invited to participate, provided they were older than six months of age. The study was explained to the household heads and members. If they agreed to participate individual consent was obtained for participants who provided a blood sample and data. We administered a questionnaire to record household composition, migration history, assets, economic activities, access to public services and knowledge and practices regarding dengue to household heads or an adult informant. Additionally, every member who agreed to participate in the study and
provided a blood sample would complete an individual questionnaire regarding past DENV diagnosis, yellow fever vaccination status and usual places of dwelling.

Figure 6: Blocks selected from the study in Puerto Maldonado

Finally, participants were divided according to migration history – recent migrant (RM) were defined as current residents who had been living in the city for five years or less at the time of enrollment and long term residents (LTR) otherwise. Although this cut-off is consistent with migration questions and definitions used in the Peruvian population and household census [133], we conducted a sensitivity analysis to determine whether a more appropriate cut-off could be used to identify RM status. Results of this sensitivity analysis indicate no clear natural or specific cutoff to be associated with DENV infection; thus, the five-year cut-off was maintained.
Laboratory testing

Serum samples were collected from consenting participants and were taken using ice chests to the local Naval Medical Research Unit 6 (NAMRU-6) facilities. Samples were centrifuged and serum was kept at -70 °C until shipment to the NAMRU-6 laboratory in Lima with dry ice. Once in Lima, samples were tested for IgG and neutralizing antibodies against DENV using ELISA and plaque reduction neutralization (PRNT) assays, respectively.

**ELISA**

This assay was performed using a modified protocol from Kuno et al [143]. Briefly, ELISA plates were coated with 100 µl of antigen (whole inactivated virus) overnight at 4°C. The plates were washed five times with 300 µl of wash buffer. Sera samples were diluted 1/100 with ELISA buffer diluents. One hundred microliters of each serum dilution was placed into each plate well. Plates were incubated for 60 minutes at 37°C and washed again as described. Diluted 1/8000 KPL HP labeled goat anti-human IgG in ELISA buffer was added (100 µl) to each well. Again the plates were incubated and washed. ABTS substrate was added (100 µl) and the plates were incubated for 20 minutes at 37°C. Plates were read in Dynex ELISA MRX revelation reader. Cut off values were calculated averaging the negative values and adding three standard deviations.

**Plaque Reduction Neutralization Test (PRNT)**

Similarly, PRNT also benefits from the virus-antibody interaction in a plate. Sera samples aliquots of 200 µl and sera were inactivated in a water bath at 56°C for 30 minutes. Sera were then mixed in maintenance medium in a two-fold serial dilution from
1/20 to 1/320 and were placed in a 24-well plate. The target virus was grown in BHK21CL15 cell lines (5 days for DENV-2 and 7 days for DENV-1 and DENV-3, 6 days for DENV-4). The virus strains used for this assay were: DENV-1 (16007) C6/36 passage 9 (p-9), 02/12/2010 Asian strain, DENV-2 (16681) C6/36 p-9, 03/12/2010 Asian strain, DENV-3 (IQD1728) C6/36 p-7, 02/12/2010 Peruvian strain and DENV-4 (1036) C6/36 p-9, 02/12/2010 Asian strain. The virus was diluted to a final working dilution (1/2500 for DENV-1: 1.2x10^6 PFU, 1/40000 for DENV-2: 1.9x10^7 PFU, 1/1000 for DENV-3: 8.0x10^5 PFU and 1/1500: 1.1x10^6 PFU for DENV-4) using maintenance medium (E-MEM 2% FBS). Viral dilutions were added to the plate wells in the same volume as the sample dilutions (1:1) and were kept at 4°C overnight. The plaque assay was performed adding 0.5 ml of cell suspension at a concentration of 3 x 10^5 cells/ml. These were incubated for half an hour at 37°C and 5% CO₂. Fifty microliters of viral dilution was added to each well and incubated for 3 hours at 37°C and 5% CO₂. Inside a biological safety cabinet, 0.5 mL of semisolid media was added to each well and was incubated at 37°C and 5% CO₂: 5 days for DENV-2, 6 days for DENV-4, 7 days for DENV-1 and DENV-3. Media was then discarded and plates dried for color staining at room temperature for 30 minutes to 1 hour. Plates were rinsed and left to dry again, then they were placed in a light box for plaque counting. The percentage of neutralization was obtained for each sample dilution, using the negative controls for each sample. PRNT titer was calculated based on a 70% or greater reduction in plaque counts (PRNT₇₀) using probit analysis with SPSS 20.0 (IBM Corp, Armonk, NY, US). End point titers are expressed as the reciprocal of the last serum dilution showing 70% reduction in
plaque counts. The following cut-off values were used: DENV-1 and 3: greater than 1/60; DENV-2: 1/80; and DENV-4: 1/40 [9].

Data analysis

Through the questionnaire, we collected information regarding migration history, household assets, building materials, number of dwellers, family monthly income, access to running water, garbage collection and connection to sewage as well as other individual characteristics like age, education and occupation. The latter variable was categorized as student, household activities, blue collar (outdoors, ie. mototaxi driver, farmer) and white collar (indoors, ie. office, store). We created three ad hoc household indices for groups of variables attributed to KAP (KAPi), infrastructure and services (ISi) and assets (Ai). All variables included in the indices were assessed based on the variability described for this population and entered with different weights, according to the investigators’ criteria for KAPi and ISi and market value for Ai, for the final index. We compared different approaches to create these indices [144], including principal components analysis, but a final decision on which to use was based on ease of use and interpretation of the index. (See the for a detailed description of the indices.) A higher value means a better standing for all indices.

We were interested in examining DENV infection and infection status (ie. susceptible, primary or secondary infection). Therefore, we determined participants with primary infection those who had PRNT results positive for only one DENV serotype, secondary infection if they had a reaction for more than one serotype and susceptible if they showed a negative result to all DENV serotypes. The frequencies of the variables
gathered through the questionnaire were initially tested against DENV infection using chi square analysis for categorical variables and t-test for continuous variables. In order to compare household indices, households were also identified as RM or LTR, depending on whether the head of household or the spouse fell in either of these categories. Likewise, households were identified as positive if at least one member was positive for DENV with PRNT. Based on this preliminary analysis, variables were selected to enter a multilevel ordered logistic regression model to evaluate seroprevalence with three possible outcomes from a PRNT: susceptible or seronegative, primary infection or monotypic and secondary infection or multitypic. All analyses were performed using Stata 12.1 (StataCorp LP, College Station, TX).

IV.4 RESULTS

Overall description of the population

At least one member from each of the 307 households visited provided a blood sample and responded to an individual dengue questionnaire, for a total of 505 participants (Table 2). Only 11% of the study population was considered RM although almost half of the participants were not native to Puerto Maldonado. The study population was relatively young, with an average age of 33 years old and RM being significantly younger than LTR (29 and 34 years old, respectively; p=0.008). Consequently, almost a quarter of the participants in both groups were students, although commerce and home-keeping were also important activities. The sex and occupation distribution between RM and LTR was similar, as shown in Table 2.
Profile of the migrant population and evidence of DENV infection

Almost 50% (236/505) of the participants responding to the serosurvey were born outside of Puerto Maldonado (non-native). The majority of non-native household heads were from Cusco (38.4%), approximately a third had moved from other areas in Madre de Dios Region (35.9%), 5.8% were originally from Apurimac and the remaining were from diverse areas such as Arequipa, Lima and Moquegua. The areas from Cusco, Apurimac and several from Madre de Dios are non-endemic for DENV and the situation is similar for most locations where participants emigrated. The non-native population is relatively young; approximately 75% of them are 45 years old or less. When they moved to Puerto Maldonado they were on average 23 years old, ranging from 1 to 67 years old. Three quarters of this population had been living in Puerto Maldonado for at least 20 years, the mean length of residence in the city is 15 years with a maximum period of 55 years. Half of the non-natives have been in the city for ten years or less, since 2002, although migration was reported since 1957 for this group. In the same fashion, 23% (54/236) of these non-native participants were considered RM with the working definition.

All participants were screened for DENV antibodies with ELISA and those positive were further confirmed using the PRNT. Approximately 70% (355/505) of all participants had antibodies to DENV with ELISA. More than 270 participants were positive to DENV by PRNT, which accounts for 54% (95% CI: 49.6; 58.5) of the population surveyed. Fifty-five percent of non-natives were positive to DENV, compared to 53% of participants native to Puerto Maldonado (p=0.799). However, when examining the working definition, 46% of RM were positive to DENV by PRNT, in contrast to 55%
among LTR, but this difference was not statistically significant (p=0.226). We further explored the seroprevalence by age groups and by categorizing migrants across several groups depending on the time of their establishment in Puerto Maldonado. These estimates provided similar findings across all strata (see Table 3, Figure 7 and Table 4).

Table 2: General characteristics of the study population by migration status

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>RM</th>
<th>LTR</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>309 (61.2)</td>
<td>32 (59.3)</td>
<td>277 (61.4)</td>
<td>0.758</td>
</tr>
<tr>
<td>Male</td>
<td>196 (38.8)</td>
<td>22 (40.7)</td>
<td>174 (38.6)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, median, SD</td>
<td>33.3, 31.0, 17.3</td>
<td>28.8, 26.0, 13.9</td>
<td>33.9, 31.0, 17.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Time in Puerto Maldonado</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, median, SD</td>
<td>14.6; 10.0; 11.6</td>
<td>3.4; 4.5; 1.9</td>
<td>17.9; 15.0; 11.2</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home-keeper</td>
<td>92 (18.2)</td>
<td>12 (22.2)</td>
<td>80 (17.7)</td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>119 (23.6)</td>
<td>14 (25.9)</td>
<td>105 (23.3)</td>
<td></td>
</tr>
<tr>
<td>Professional/technical activity</td>
<td>86 (17.0)</td>
<td>5 (9.3)</td>
<td>81 (18.0)</td>
<td></td>
</tr>
<tr>
<td>Commerce</td>
<td>105 (20.8)</td>
<td>14 (25.9)</td>
<td>91 (20.2)</td>
<td>0.501</td>
</tr>
<tr>
<td>Agriculture / farming</td>
<td>59 (11.7)</td>
<td>4 (7.4)</td>
<td>55 (12.2)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>20 (4.0)</td>
<td>3 (5.6)</td>
<td>17 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>24 (4.8)</td>
<td>2 (3.7)</td>
<td>22 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17 (3.4)</td>
<td>2 (3.8)</td>
<td>15 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>126 (25.0)</td>
<td>12 (23.1)</td>
<td>114 (25.3)</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>228 (45.3)</td>
<td>25 (48.1)</td>
<td>203 (45.0)</td>
<td></td>
</tr>
<tr>
<td>Technical school</td>
<td>73 (14.5)</td>
<td>6 (11.5)</td>
<td>67 (14.9)</td>
<td>0.892</td>
</tr>
<tr>
<td>University</td>
<td>50 (9.9)</td>
<td>7 (13.5)</td>
<td>43 (9.5)</td>
<td></td>
</tr>
<tr>
<td>Post-graduate</td>
<td>1 (0.2)</td>
<td>0 (0.0)</td>
<td>1 (0.2)</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>8 (1.6)</td>
<td>0 (0.0)</td>
<td>8 (1.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Chi square, t-test or Fisher's exact.
Sixty-four percent of all DENV infections were primary infections, 90% of these occurring among natives to Puerto Maldonado. Although there seems to be a larger proportion of secondary infections among LTR, this difference was not statistically significant and the distribution of primary and secondary infections between RM and LTR was similar (p=0.444) (see Table 5). An unadjusted analysis contrasting time in Puerto Maldonado showed a trend towards an increase in the OR infection of ~3% (95% CI: 0.998; 1.055) with each increase of yearly residence (p=0.068).

### Table 3: Lifetime DENV infection across age groups

<table>
<thead>
<tr>
<th>Age category (years)</th>
<th>Individuals (n=505)</th>
<th>Seroprevalence [95% CI]</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0, 18</td>
<td>108</td>
<td>56.5 [46.6; 66.0]</td>
<td></td>
</tr>
<tr>
<td>&lt;18, 25</td>
<td>86</td>
<td>55.8 [44.7; 66.5]</td>
<td></td>
</tr>
<tr>
<td>&lt;25, 35</td>
<td>102</td>
<td>49.0 [39.0; 59.1]</td>
<td>0.836</td>
</tr>
<tr>
<td>&lt;35, 50</td>
<td>122</td>
<td>54.1 [44.8; 63.1]</td>
<td></td>
</tr>
<tr>
<td>&gt; 50</td>
<td>87</td>
<td>55.2 [44.1; 65.8]</td>
<td></td>
</tr>
</tbody>
</table>

* chi-squared

Individual characteristics such as sex, age and education level were evaluated, but were not related to PRNT results. In comparison, an unadjusted analysis showed that participants with an occupation within the household (i.e. housewives, retired) had higher odds of having antibodies to DENV compared to blue collar type jobs (OR: 2.117; p=0.018; 95% CI: 1.134, 3.953). However, when modeling the outcome of infection status and introducing sex, this association was no longer significant. Finally, when examining the serotypes of DENV for monotypic immunity (primary infection), the most common serotype was DENV-1 (88.6%), followed by DENV-2 (6.3%), DENV-3 (4.0%) and DENV-4 (1.1%).
Figure 7: Seroprevalence of DENV infection by age categories with SE bars

KAP and household variables

As described in the methods, we created three indices summarizing data on the infrastructure of the households and services (ISi), assets (Ai) and KAP (KAPi). Table 6 displays the distribution of these indices across the population and for RM and LTR. For further details regarding the calculation of the indices, see Table 9, Table 10, Table 11 in the Appendix 2).

Table 4: Lifetime DENV infection across migration groups

<table>
<thead>
<tr>
<th>Migration category (years)</th>
<th>Individuals (n=505)</th>
<th>Seroprevalence [95% CI]</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>269</td>
<td>53.5 [47.4; 59.6]</td>
<td></td>
</tr>
<tr>
<td>&lt;0, 5]</td>
<td>54</td>
<td>46.3 [32.6; 60.4]</td>
<td></td>
</tr>
<tr>
<td>&lt;5, 10]</td>
<td>67</td>
<td>53.7 [41.1; 66.0]</td>
<td>0.170</td>
</tr>
<tr>
<td>&lt;10, 15]</td>
<td>30</td>
<td>46.7 [28.3; 65.7]</td>
<td></td>
</tr>
<tr>
<td>&gt; 15</td>
<td>85</td>
<td>63.5 [52.4; 73.7]</td>
<td></td>
</tr>
</tbody>
</table>

* chi-squared
Bivariate analysis evaluating the distribution of KAPi informed that LTR households had a higher score than RM households (p=0.003). Higher KAPi values were also associated with a positive household (p<0.001). The ISi was similar between RM and LTR households, but a higher ISi score was related to having a household with a positive PRNT result (p=0.035). In contrast, Ai was related to migration status: LTR had higher Ai (p=0.004) and higher Ai was also related to a household where at least one member showed antibodies to DENV (p=0.049). Household monthly income ranged from ~70 to approximately 3200 US dollars and was US$ 670 on average. RM households had a lower monthly income than LTR (~ US$ 540 and ~ US$ 725, p=0.038) and also a higher household income was related to positive households (p<0.001)

<table>
<thead>
<tr>
<th>Type of infection*</th>
<th>Total n (%)</th>
<th>RM n (%)</th>
<th>LTR n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>232 (45.9)</td>
<td>29 (53.7)</td>
<td>203 (45.0)</td>
<td>0.444</td>
</tr>
<tr>
<td>Primary</td>
<td>175 (34.7)</td>
<td>17 (31.5)</td>
<td>158 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>98 (19.4)</td>
<td>8 (14.8)</td>
<td>90 (20.0)</td>
<td></td>
</tr>
</tbody>
</table>

* primary or secondary infection is considered if participants show monotypic or multitypic immunity, respectively

We formulated a multilevel ordered logistic model considering the three possible outcomes from a PRNT: susceptible, primary infection or secondary infection. The variables evaluated in the model were: age, sex, occupation, KAPi, ISi, Ai, income (transformed with the natural logarithm), education level and migration status. Throughout the analysis, KAPi and monthly income were associated with increased log odds of having a positive PRNT. Therefore, a unit increase in the natural log income values will raise the odds of a primary or secondary infection by 1.6 compared to not
having infection. KAP was entered in the final model as a categorical variable, displaying increased cumulative odds of infection for households with higher scores (≥ 0.46). The cumulative odds of having a primary or secondary infection vs. being susceptible among RM was 0.697, compared to LTR of similar income and KAPs, but this was not statistically significant (p=0.341), similarly to the bivariate analysis. The parameters of the final model are shown in Table 7.

<table>
<thead>
<tr>
<th>Table 6: Household indices by migration status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index</strong></td>
</tr>
<tr>
<td>KAPIi</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ISIi</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ai</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

As mentioned above, another model that was also assessed included occupations categorized as white collar, blue collar, students and household activities (ie. housewives, infants, unemployed). These categories were based mainly on the location of jobs, for example, considering white collar jobs those developed in an office or similar environment and blue collar jobs those carried out mainly in an open area. Occupations that comprised time spent in the household had increased cumulative odds of infection compared to blue collar type of labor (p=0.023). However, it should be noted that this association was no longer significant when sex was included in the model (data not
Similarly, ISi was significant (p=0.036) until monthly household income was considered in the model, hence removing this variable from the final model.

Table 7: Mixed effects ordered logistic model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>SE</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>REF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.740</td>
<td>0.157</td>
<td>0.157</td>
<td>[0.488; 1.122]</td>
</tr>
<tr>
<td>Age</td>
<td>1.001</td>
<td>0.006</td>
<td>0.838</td>
<td>[0.989; 1.014]</td>
</tr>
<tr>
<td>Migration status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTR</td>
<td>REF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM</td>
<td>0.697</td>
<td>0.264</td>
<td>0.341</td>
<td>[0.331; 1.465]</td>
</tr>
<tr>
<td>Income*</td>
<td>1.613</td>
<td>0.297</td>
<td>0.009</td>
<td>[1.125; 2.313]</td>
</tr>
<tr>
<td>KAPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (0.17-0.37)</td>
<td>REF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2 (0.38-0.45)</td>
<td>1.350</td>
<td>0.465</td>
<td>0.383</td>
<td>[0.687; 2.654]</td>
</tr>
<tr>
<td>Q3 (0.46-0.78)</td>
<td>2.305</td>
<td>0.826</td>
<td>0.020</td>
<td>[1.142; 4.653]</td>
</tr>
<tr>
<td>Q4 (0.79-1.00)</td>
<td>1.949</td>
<td>0.695</td>
<td>0.061</td>
<td>[0.969; 3.919]</td>
</tr>
<tr>
<td>Cut 1: primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infection</td>
<td>3.707</td>
<td>1.381</td>
<td>0.007</td>
<td>[1.000; 6.415]</td>
</tr>
<tr>
<td>Cut 2: secondary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infection</td>
<td>5.678</td>
<td>1.419</td>
<td>&lt;0.001</td>
<td>[2.896; 8.458]</td>
</tr>
</tbody>
</table>

* The variable was transformed with the natural logarithm
The proportional odds / parallel lines assumption was tested with a Wald test. The final model does not violate these assumptions (p=0.511)

IV.5 DISCUSSION

Approximately half of the participants from this study had evidence of a past DENV infection. The information collected from Puerto Maldonado differs from seroprevalence data from other studies, which have shown a range between 70-90% in Brazil and Nicaragua [87, 88], 78% in Mexico [86], and about 60% in Vietnam [145]. Most of these areas have had DENV circulating for over twenty years compared to 12 years in Puerto Maldonado. A few studies have reported lower seroprevalence in specific
situations such as US population on the US-Mexico border (40%), which reported different risk behavior in contrast to their Mexican counterparts [86]. This difference was mainly due to the contrasting demeanor of both communities. Very similar seroprevalence was reported from Singapore in 1998, approximately 45% for adults in 1998 and 2002, and lower values (7%) among children 6-15 years old in 1997 [146, 147].

Da Silva-Nunes reports a baseline seroprevalence of 18% for rural areas of Acre, the Brazilian state bordering Madre de Dios on the Peruvian side, with a yearly increase of 3%, related to travel to endemic areas [89]. This study reported an effect of migration on serostatus for DENV, but showed higher seroprevalence among migrants from endemic areas, in contrast to the findings from Puerto Maldonado.

The prevalence we have found in Puerto Maldonado is lower than what has been reported for other Peruvian cities, such as Iquitos [9]. This is probably due to the relatively recent introduction of DENV in the area, however reports from Iquitos have also portrayed a very rapid dissemination of the virus across the city only two years after DENV was introduced [139]. Consequently, it is likely that the constant influx of migrants from regions without the circulation of DENV like Cusco and Puno also influence these findings providing a large pool of susceptible hosts and maintaining a constant seroprevalence [76]. Likewise, Puerto Maldonado has increasing exchange with Bolivia and Brazil due to the paving of the Interoceanic Highway, completed in 2012 [148]. This poses another challenge for dengue control as potentially naïve individuals are constantly entering the area, while potentially infected people acting as reservoirs of the disease also visit the region and the city continues to expand geographically [9, 30].

Previous studies conducted in Kamphaeng Phet, Thailand and Iquitos, Peru have shown
the marked effect of human movement on the local dynamics and transmission of the virus [97, 149]. The serostatus profile in Puerto Maldonado indicating similar distributions between LTR and RM also suggest the ostensible role of migrants in DENV transmission dynamics, possibly “dampening” the effect of DENV among the local population as new members without infection are introduced to the community.

The findings of this study suggest that the risk for DENV infection between RM and LTR is linked to the KAPs and monthly income of the participating households, regardless of their migration status. In our sample, RM and LTR were equally likely to have a positive PRNT result. In contrast, other characteristics such as higher KAPI and monthly household income were related to having a seropositive outcome. Other bivariate analysis of the data shows that home-keeping activities are a risk factor for DENV among residents of Puerto Maldonado. It is feasible that this type of work during the day, when mosquitoes bite may pose a risk for infection. Likewise, the specific location of these households [150, 151] and the heterogeneous transmission of DENV that has been reported before [152] may likely play a role, but these issues have not been assessed in this paper.

The relationship of positive PRNT with household income is in contrast to what has been reported in other studies, where lower socioeconomic status has been related to higher DENV infection risk [86, 88]. We postulate that our finding is describing the social quandary of recent migrants to a new location where they are faced with lower salaries compared to natives. This situation has been described before and portrays how migrants usually start off with lower wages when they arrive to a new location, but gradually match the natives’ income [96, 98]. Likewise, it is likely that as newcomers
establish in the city and perceive a higher income they may improve their living conditions and physical environment. If we consider the constant incursion of migrants to Puerto Maldonado and the trend to a yearly increase in the OR for positive PRNT results, this finding is not so surprising. It would also explain the initial association of DENV positive antibody results to higher ISi and Ai, which later dilute in a multivariate analysis. Though there did not seem to be a lot of correlation among these variables, it is possible that some features measured through these indices may also be associated with household income, therefore becoming unimportant in the multivariate analysis. Nonetheless, the influence of other factors such as the geographical location of positive participants and migrants should not be ruled out and would need further investigation. Spatial patterns of DENV infection will be further developed in Chapter Six.

We found somewhat contradictory evidence of a higher score of KAPi and its relationship with increased cumulative odds of infection. We explain this relationship possibly from the awareness generated about dengue after having the disease or seeing it in members from the same household. Likewise, correct knowledge about DENV and its vector does not necessarily relate to applying preventive measures [153, 154]. Results portraying this situation have been reported before in Thailand where better knowledge of Ae. aegypti breeding sites was associated to more development sites in the premises [155]. Nonetheless, it should be highlighted that the factors that seem more closely related to DENV infection risk (i.e. knowledge, attitudes and practices) can be targeted through outreach programs in the community. More so since there is a relationship with activities carried out in the household of the participants and having higher risk of a positive PRNT. Health authorities can focus efforts on specific strategies to improve the
awareness and prevention practices of vector breeding, especially at areas of new urban development or where lower income residents congregate to involve them in control activities and practical demonstrations at their own homes. Larger programs of city planning can also be devised to improve infrastructure and services provided across the urban area, especially for newly developing areas.

An important limitation to this study is the lack of longitudinal data that could allow understanding the specific role of KAP in transmission dynamics in this area. Likewise, there is potential for selection bias as households were not identified previous to enrolment. Households that were not available to participate or that chose not to do so may have had different characteristics than those who did. Sample size may also limit some of the inferences addressed in this study, since several variables like $A_i$ or $IS_i$ may behave differently across a wider pool of participants with different migratory background. Likewise, the lack of entomological information leaves a gap regarding the potential effect of some of these variables on the presence of the vector in the household.

IV.6 CONCLUSION

The prevalence of DENV in Puerto Maldonado was 54%. PRNT results were similar between RM and LTR. Similar findings have been reported elsewhere, with the more ostensible example in the US and Mexico border [86]. Higher risk of infection was mainly associated with KAPs regarding DENV. However, risk was also associated with higher income which is in contrast to what has been reported before. These findings portray similar risk of DENV infection for all residents of Puerto Maldonado regardless of their migration background. More importantly, it reveals the particular challenges that
this emerging infection poses on the residents of Puerto Maldonado and its health authorities, since the population data does not seem to correspond to previous knowledge about DENV infection. Therefore, these findings should help target more detailed vector and incidence studies that can address spatial heterogeneities in DENV transmission in Puerto Maldonado [152]. Likewise, KAP findings can provide information to tailor specific prevention and control strategies for the area involving the community. Such interventions should consider the cultural backgrounds of city dwellers in order to achieve desired participation and commitment [56, 154].
Appendix 1: Age distribution of the study population

Table 8: Age distribution of the study population

<table>
<thead>
<tr>
<th>Age category</th>
<th>Total n (%)</th>
<th>RM n (%)</th>
<th>LTR n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0 - 5]</td>
<td>7 (1.4)</td>
<td>0 (0.0)</td>
<td>7 (1.6)</td>
</tr>
<tr>
<td>&lt;5 - 10]</td>
<td>22 (4.4)</td>
<td>3 (5.6)</td>
<td>19 (4.2)</td>
</tr>
<tr>
<td>&lt;10 - 15]</td>
<td>31 (6.1)</td>
<td>2 (3.7)</td>
<td>29 (6.4)</td>
</tr>
<tr>
<td>&lt;15 - 20]</td>
<td>58 (11.5)</td>
<td>9 (16.7)</td>
<td>49 (10.9)</td>
</tr>
<tr>
<td>&lt;20 - 25]</td>
<td>65 (12.9)</td>
<td>9 (16.7)</td>
<td>56 (12.4)</td>
</tr>
<tr>
<td>&lt;25 - 30]</td>
<td>53 (10.5)</td>
<td>11 (20.4)</td>
<td>42 (9.3)</td>
</tr>
<tr>
<td>&lt;30 - 35]</td>
<td>54 (10.7)</td>
<td>3 (5.6)</td>
<td>51 (11.3)</td>
</tr>
<tr>
<td>&lt;35 - 40]</td>
<td>46 (9.1)</td>
<td>7 (13.0)</td>
<td>39 (8.6)</td>
</tr>
<tr>
<td>&lt;40 - 45]</td>
<td>43 (8.5)</td>
<td>4 (7.4)</td>
<td>39 (8.6)</td>
</tr>
<tr>
<td>&lt;45 - 50]</td>
<td>32 (6.3)</td>
<td>1 (1.9)</td>
<td>31 (6.9)</td>
</tr>
<tr>
<td>&lt;50 - 55]</td>
<td>27 (5.3)</td>
<td>2 (3.7)</td>
<td>25 (5.5)</td>
</tr>
<tr>
<td>&lt;55 - 60]</td>
<td>22 (4.4)</td>
<td>1 (1.9)</td>
<td>21 (4.7)</td>
</tr>
<tr>
<td>&lt;60 - 65]</td>
<td>19 (3.8)</td>
<td>0 (0.0)</td>
<td>19 (4.2)</td>
</tr>
<tr>
<td>&gt; 65</td>
<td>26 (5.1)</td>
<td>2 (3.7)</td>
<td>24 (5.3)</td>
</tr>
</tbody>
</table>
Appendix 2: Indices

1. Household knowledge, attitude and practices index (KAPi)

This index was created using information collected using the questions listed in the following table:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Question</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabedengue</td>
<td>Have you heard about dengue?</td>
<td>Yes, No</td>
</tr>
<tr>
<td>Mosquitotrans</td>
<td>How is dengue transmitted?</td>
<td>Do not know, Food or water, Direct contact, Mosquito bite</td>
</tr>
<tr>
<td>Infodengue</td>
<td>Who informed you about dengue?</td>
<td>Friend or family, Mass media (radio, TV), Health care personnel, Other</td>
</tr>
<tr>
<td>Buscoinfo</td>
<td>Did you look for more information?</td>
<td>No, Yes, Friend or family, Health care personnel, Internet, Other</td>
</tr>
<tr>
<td>Limpieza</td>
<td>How frequently do you clean the household premises?</td>
<td>Once a month, Twice a month, Once a week, Twice a week, Thrice a week, Daily</td>
</tr>
<tr>
<td>Drena</td>
<td>How do you drain stagnant water in the premises?</td>
<td>Do not drain, Sewer drain, ditches, Drain manually</td>
</tr>
<tr>
<td>Proteccion</td>
<td>Do you do anything to protect yourself from dengue?</td>
<td>Do not take measures, Use repellent, Use insecticide, Use repellent and insecticide</td>
</tr>
</tbody>
</table>

The values for each answer were standardized in order to get a maximum of 1 as the highest score for each question. Furthermore, the total sum of the values of each question was divided in order to achieve the same standardization. The weights used were decided upon by the investigators based on whether the KAP could have more direct impact on dengue control and prevention:
\[ KAP_i = \left( \frac{\text{limpieza}}{6} \times 2 + \frac{\text{drena}}{3} \times 2 + \frac{\text{sabedengue}}{2} \times 1 + \frac{\text{infodengue}}{4} \times 1 + \frac{\text{buscoinfo}}{6} \times 3 \right) + \frac{\text{proteccion}}{4} \times 2 + \frac{\text{mosquitotrans}}{3} \times 3 \right) / 14 \]

The findings from each of the questions from the administered questionnaire are in Table 9:
Table 9: Information assessed for household KAPi

<table>
<thead>
<tr>
<th></th>
<th>Total n (%)</th>
<th>RM n (%)</th>
<th>LTR n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you heard about dengue?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>299 (97.4)</td>
<td>28 (100.0)</td>
<td>271 (97.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>8 (2.6)</td>
<td>0 (0.0)</td>
<td>8 (2.9)</td>
<td></td>
</tr>
<tr>
<td>How is dengue transmitted?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not know</td>
<td>9 (2.9)</td>
<td>1 (3.6)</td>
<td>8 (2.9)</td>
<td>0.657</td>
</tr>
<tr>
<td>Food / water</td>
<td>2 (0.7)</td>
<td>0 (0.0)</td>
<td>2 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Direct contact</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Mosquito bite</td>
<td>296 (96.4)</td>
<td>27 (96.4)</td>
<td>269 (96.4)</td>
<td></td>
</tr>
<tr>
<td>Who gave you information about dengue?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10 (3.3)</td>
<td>2 (7.1)</td>
<td>8 (2.9)</td>
<td>0.294</td>
</tr>
<tr>
<td>Friend or family</td>
<td>30 (9.8)</td>
<td>4 (14.3)</td>
<td>26 (9.3)</td>
<td></td>
</tr>
<tr>
<td>Mass media (radio, TV)</td>
<td>41 (13.4)</td>
<td>2 (7.1)</td>
<td>39 (14.0)</td>
<td></td>
</tr>
<tr>
<td>Health care personnel</td>
<td>226 (73.6)</td>
<td>20 (71.4)</td>
<td>206 (73.8)</td>
<td></td>
</tr>
<tr>
<td>Did you seek more information?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>187 (60.9)</td>
<td>23 (82.1)</td>
<td>164 (58.8)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>120 (39.1)</td>
<td>5 (17.9)</td>
<td>115 (41.2)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (4.2)</td>
<td>0 (0.0)</td>
<td>5 (4.3)</td>
<td>0.272</td>
</tr>
<tr>
<td>Internet</td>
<td>12 (10.0)</td>
<td>0 (0.0)</td>
<td>12 (10.4)</td>
<td></td>
</tr>
<tr>
<td>Friend or family</td>
<td>6 (5.0)</td>
<td>0 (0.0)</td>
<td>6 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Health care personnel</td>
<td>97 (80.8)</td>
<td>5 (100.0)</td>
<td>92 (80.0)</td>
<td></td>
</tr>
<tr>
<td>How do you drain stagnant water?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not drain</td>
<td>77 (25.1)</td>
<td>8 (28.6)</td>
<td>69 (24.7)</td>
<td>0.830</td>
</tr>
<tr>
<td>Sewer drain / ditch</td>
<td>169 (55.0)</td>
<td>14 (50.0)</td>
<td>155 (55.6)</td>
<td></td>
</tr>
<tr>
<td>Manually</td>
<td>61 (19.9)</td>
<td>6 (21.4)</td>
<td>55 (19.7)</td>
<td></td>
</tr>
<tr>
<td>How frequently do you clean household surroundings?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once or twice a month</td>
<td>107 (34.9)</td>
<td>16 (57.1)</td>
<td>91 (32.6)</td>
<td>0.047</td>
</tr>
<tr>
<td>Once a week or more but not daily</td>
<td>128 (41.7)</td>
<td>8 (28.6)</td>
<td>120 (43.0)</td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>72 (23.5)</td>
<td>4 (14.3)</td>
<td>68 (24.4)</td>
<td></td>
</tr>
<tr>
<td>What do you do to protect yourself from mosquito bites?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>134 (43.6)</td>
<td>14 (50.0)</td>
<td>120 (43.0)</td>
<td></td>
</tr>
<tr>
<td>Use repellent</td>
<td>10 (3.3)</td>
<td>3 (10.7)</td>
<td>7 (2.5)</td>
<td>0.090</td>
</tr>
<tr>
<td>Use insecticide</td>
<td>160 (52.1)</td>
<td>11 (39.3)</td>
<td>149 (53.4)</td>
<td></td>
</tr>
<tr>
<td>Use repellent and insecticide</td>
<td>3 (1.0)</td>
<td>0 (0.0)</td>
<td>3 (1.1)</td>
<td></td>
</tr>
</tbody>
</table>
2. Household physical infrastructure and services index (ISi)

A similar approach as for KAPi was used for the ISi. The variables conveyed in this index are:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Question</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mate pared</td>
<td>Wall building material</td>
<td>Other, Wood, Bricks / cement</td>
</tr>
<tr>
<td>Piso</td>
<td>Flooring material</td>
<td>Dirt/sand/clay, Wood, Cement / bricks, Tiles</td>
</tr>
<tr>
<td>Techo</td>
<td>Roof material</td>
<td>Plastic tarp or other, Tin roof, Concrete / cement</td>
</tr>
<tr>
<td>Cocinapropia</td>
<td>Do you share a kitchen with other family or business?</td>
<td>No, Yes</td>
</tr>
<tr>
<td>Banhopropio</td>
<td>Do you share the bathroom with other family or business?</td>
<td>No, Yes</td>
</tr>
<tr>
<td>Excrementos</td>
<td>How do you dispose of excreta?</td>
<td>Open field, Latrine, Septic tank, Sewer system</td>
</tr>
<tr>
<td>Aguacorriente</td>
<td>What is the source of running water for this household?</td>
<td>Other, Plumbing into the house</td>
</tr>
<tr>
<td>Basura</td>
<td>How do you dispose of garbage?</td>
<td>Burn or bury residue, Routine pick-up by municipality</td>
</tr>
</tbody>
</table>

The values from each of the responses were standardized to add up to 1 and then the total sum was divided by 12 for the same purpose. The weights applied to this index were selected according to the relationship with vector breeding risk that has been reported for each of the elements included in the index:

\[
CFSi = \left(\frac{Mate pared}{3}\times 1 + \frac{Piso}{4}\times 1 + \frac{Techo}{3}\times 1 + \frac{Excrementos}{4}\times 2 + \frac{Basura}{2}\times 2 + \frac{Aguacorriente}{2}\right)
\times 3 + \left(\frac{Banhopropio}{2}\times 1 + \frac{Cocinapropia}{2}\times 1\right)/12
\]

The responses for the variables are detailed in Table 10 below:
### Table 10: Information assessed for household ISi

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>RM</th>
<th>LTR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Flooring material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dirt</td>
<td>46 (15.0)</td>
<td>5 (17.9)</td>
<td>41 (14.7)</td>
<td>0.516</td>
</tr>
<tr>
<td>Wood</td>
<td>5 (1.6)</td>
<td>1 (3.6)</td>
<td>4 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Cement / brick</td>
<td>253 (82.4)</td>
<td>22 (78.6)</td>
<td>231 (82.8)</td>
<td></td>
</tr>
<tr>
<td>Tiles</td>
<td>3 (1.0)</td>
<td>0 (0.0)</td>
<td>3 (1.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Wall material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>19 (6.2)</td>
<td>2 (7.1)</td>
<td>17 (6.1)</td>
<td>0.616</td>
</tr>
<tr>
<td>Wood</td>
<td>147 (47.9)</td>
<td>11 (39.3)</td>
<td>136 (48.7)</td>
<td></td>
</tr>
<tr>
<td>Bricks/cement</td>
<td>141 (45.9)</td>
<td>15 (53.6)</td>
<td>126 (45.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Roof material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic tarp</td>
<td>12 (3.9)</td>
<td>0 (0.0)</td>
<td>12 (4.3)</td>
<td>0.858</td>
</tr>
<tr>
<td>Tin roof</td>
<td>280 (91.2)</td>
<td>27 (96.4)</td>
<td>253 (90.7)</td>
<td></td>
</tr>
<tr>
<td>Concrete</td>
<td>15 (4.9)</td>
<td>1 (3.6)</td>
<td>14 (5.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Shared kitchen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30 (9.8)</td>
<td>6 (21.4)</td>
<td>24 (8.6)</td>
<td>0.042</td>
</tr>
<tr>
<td>No</td>
<td>277 (90.2)</td>
<td>22 (78.6)</td>
<td>255 (91.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Shared bathroom</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40 (13.0)</td>
<td>4 (14.3)</td>
<td>36 (12.9)</td>
<td>0.771</td>
</tr>
<tr>
<td>No</td>
<td>267 (87.0)</td>
<td>24 (85.7)</td>
<td>243 (87.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Removal of excreta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open field</td>
<td>6 (2.0)</td>
<td>1 (3.6)</td>
<td>5 (1.8)</td>
<td>0.455</td>
</tr>
<tr>
<td>Latrine</td>
<td>57 (18.6)</td>
<td>5 (17.9)</td>
<td>52 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Septic tank</td>
<td>117 (38.1)</td>
<td>8 (28.6)</td>
<td>109 (39.1)</td>
<td></td>
</tr>
<tr>
<td>Sewer system</td>
<td>127 (41.4)</td>
<td>14 (50.0)</td>
<td>113 (40.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Water source</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>17 (5.5)</td>
<td>0 (0.0)</td>
<td>17 (6.1)</td>
<td>0.382</td>
</tr>
<tr>
<td>Piped service inside the house</td>
<td>290 (94.5)</td>
<td>28 (100.0)</td>
<td>262 (93.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Garbage disposal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burn or bury</td>
<td>20 (6.5)</td>
<td>0 (0.0)</td>
<td>20 (7.2)</td>
<td>0.235</td>
</tr>
<tr>
<td>Routine pick-up</td>
<td>287 (93.5)</td>
<td>28 (100.0)</td>
<td>259 (92.8)</td>
<td></td>
</tr>
</tbody>
</table>
3. Household assets indices (Ai)

To create this index we used weights obtained from market value in US $ and the amount of each of the items listed below:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Question</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q01_1</td>
<td>Have you got a radio?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_1_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_2</td>
<td>Have you got a TV set?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_2_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_3</td>
<td>Have you got a mobile phone?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_3_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_4</td>
<td>Have you got a sewing machine?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_4_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_5</td>
<td>Have you got a refrigerator?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_5_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_6</td>
<td>Have you got a bicycle?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_6_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_7</td>
<td>Have you got a motorcycle?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_7_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_8</td>
<td>Have you got a car?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_8_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
<tr>
<td>Q01_9</td>
<td>Have you got a canoe?</td>
<td>No</td>
</tr>
<tr>
<td>Q01_9_CUANTOS</td>
<td>How many?</td>
<td>Number</td>
</tr>
</tbody>
</table>

With this information, we took the natural logarithm of the number obtained.

Therefore, Ai was calculated as follows:

\[
Ai = \ln(Q01_1 \times Q01_1_{CUANTOS} \times 46 + Q01_2 \times Q01_2_{CUANTOS} \times 124 + Q01_2 \times Q01_3_{CUANTOS} \times 50 + Q01_4 \\
\times Q01_4_{CUANTOS} \times 183 + Q01_5 \times Q01_5_{CUANTOS} \times 312 + Q01_6 \times Q01_6_{CUANTOS} \times 82 + Q01_7 \\
\times Q01_7_{CUANTOS} \times 1504 + Q01_8 \times Q01_8_{CUANTOS} \times 10160 + Q01_9 \times Q01_9_{CUANTOS} \times 100)
\]

The responses for the variables included in this index are presented in Table 11:
Table 11: Information assessed for household Ai

<table>
<thead>
<tr>
<th>Item</th>
<th>Total</th>
<th>RM</th>
<th>LTR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>The household has got:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radio</td>
<td>272</td>
<td>(88.6)</td>
<td>25</td>
<td>(89.3)</td>
</tr>
<tr>
<td>TV set</td>
<td>294</td>
<td>(95.8)</td>
<td>26</td>
<td>(92.9)</td>
</tr>
<tr>
<td>Mobile phone</td>
<td>295</td>
<td>(96.1)</td>
<td>27</td>
<td>(96.4)</td>
</tr>
<tr>
<td>Sewing machine</td>
<td>28</td>
<td>(9.1)</td>
<td>2</td>
<td>(7.1)</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>232</td>
<td>(75.6)</td>
<td>16</td>
<td>(57.1)</td>
</tr>
<tr>
<td>Bicycle</td>
<td>87</td>
<td>(28.3)</td>
<td>4</td>
<td>(14.3)</td>
</tr>
<tr>
<td>Motorcycle</td>
<td>219</td>
<td>(71.3)</td>
<td>15</td>
<td>(53.6)</td>
</tr>
<tr>
<td>Car</td>
<td>17</td>
<td>(5.5)</td>
<td>0</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Canoe</td>
<td>11</td>
<td>(3.6)</td>
<td>0</td>
<td>(0.0)</td>
</tr>
</tbody>
</table>
Chapter Five

HOUSEHOLD LEVEL ECONOMIC BURDEN OF DENGUE INFECTION AMONG RESIDENTS OF PUERTO MALDONADO, PERU

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Running Title: Economic burden of dengue
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The Naval Medical Research Unit-6 (NAMRU-6) participation was under Protocol NMRCD.2011.0003 at NAMRU-6 and #0367 at Johns Hopkins Bloomberg School of Public Health in compliance with all applicable federal regulations governing the protection of human subjects. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.
V.1 ABSTRACT

Background: Dengue virus (DENV) is an arbovirus with global distribution. DENV was reintroduced to Peru in the 1990s and has been reported in Puerto Maldonado (population ~65,000) in the Peruvian southern Amazon Basin since 2000. This region also has the highest human migration rate in the country, much of it from areas where DENV is not endemic. Several studies have reported on the economic burden of dengue disease from societal, health care system or governmental perspectives but few have focused on the financial burden at the household level.

Objective: The objective of this study was to assess the proportion of household income that is diverted to costs incurred due to dengue illness and to compare these expenses between recent migrants (RM) and long term residents (LTR), defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. A secondary objective was to describe the demographic and socioeconomic characteristics of RM and LTR.

Methods: We administered a standardized questionnaire to persons diagnosed with dengue and dengue with warning signs at Hospital Santa Rosa in Puerto Maldonado from December 2012 to March 2013. We compared direct and indirect medical costs between RM and LTR. Demographic data, socioeconomic characteristics and assets were also contrasted between RM and LTR.
Results: Eighty participants completed the survey. Twenty-eight (35%) were RM. Each dengue disease episode cost the household an average of US$ 105 (SD=107), representing 24% of their monthly income. Indirect costs were the greatest expense (US$ 56, SD=87), especially lost wages. LTR had a higher average monthly household income than RM (p=0.041) and were significantly more affluent based on a wealth index (p=0.003.)

Conclusion: The proportion of household income diverted to dengue did not differ significantly between RM and LTR households. However, the study highlights the very significant financial burden incurred by households when a family member suffers dengue disease, especially for RM since their overall monthly income is lower.
V.2 BACKGROUND

Dengue (DENV) is a Flavivirus with broad global distribution; it is considered the arbovirus with the most important public health impact [51]. Infection with DENV is usually characterized by fever and other nonspecific symptoms, although it is estimated that a large proportion of infections are asymptomatic [9, 156, 157]. Infection causes mild to severe disease, which may lead to hospitalization. Hospital stay may last for six days on average, especially among children and infants who are burdened with the most severe forms of the disease in hyperendemic areas (ie. circulation of the four serotypes of the virus) [158]. DENV is transmitted by the mosquito Aedes aegypti mainly in urban settings, while the vector Aedes albopictus has been related to the transmission in areas with rural characteristics [32, 159]. The distribution of DENV and its vectors have continued to expand across diverse environments worldwide [5, 10], changing its epidemiology and evolving into an important cause of morbidity, especially in developing countries [51, 93]. Meanwhile, the main control strategy remains focused on vector control [10, 46, 54, 160, 161], despite growing efforts to develop a vaccine [128, 129].

Studies of cost of illness became increasingly important in the past two decades to assess the use of resources in health care and prioritize diseases with larger burden [162]. Previous assessments that measure the economic impact of DENV have mainly focused on the burden for the health care system, which can ascend to US $27.4 million per year, approximately US $400 per patient, in countries like India [91]. Smaller areas like Zulia in Venezuela have reported total costs at the societal level of more than 1 million US$ in the period between 1997 and 2005 [90]. Likewise, other studies have measured the
burden using disability-adjusted-life-years (DALYs), ranging from 427 to 658 DALYs per million people per year [92, 94, 163].

The perspectives of these studies, either at the societal, health care system, or governmental level [164] do not reflect or describe clearly the economic burden of this disease on the patients or their households nor what differential characteristics of them may influence variability of such burden. There is limited research that has taken this approach. Studies in Thailand have estimated that the amount of expenses incurred by families with dengue cases without hospitalization range between US $10 to $24 (3-8 % of the monthly household income) [94, 163]. Nonetheless, when indirect costs are introduced and multiple members within the family are sick, the total average cost could be as much as the net household income per month, approximately US $60 [94]. Likewise, a similar study in Cambodia [165] estimated the average dengue episode at US$ 31 and documented that a large proportion of households incurred in debt due to dengue-related expenses. Moreover, the costs of care were still a barrier to hospitalization among the poorest families [94].

DENV was reintroduced into Peru in the 1990s in the northern Amazonian region of Loreto [19, 73]. The current countrywide incidence of DENV without complications has been estimated to be 94.2 per 100 000 people. However, due to the disparate geography and distribution of the vector and virus transmission, incidence can increase to 375.5, 1572.3, 2134.4, per 100 000, in regions like Ucayali, Madre de Dios and Loreto, respectively [166-169].

The city of Puerto Maldonado, where this study was performed, is located in Madre de Dios region. Madre de Dios has the highest rate of migration in Peru mainly
from the neighboring regions of Cusco and Puno, for occupational perspectives [133], [98]. These areas are mostly non-endemic for dengue. Previous studies have shown the disadvantages and vulnerabilities faced by migrants in comparison to native workers in different settings, ranging from lack of health awareness, disproportionally lower income, and invisible costs shouldered by migrants such as barriers to access better paid jobs [96, 97]. The sparse information existing assessing the complex process of migration and health related outcomes make Puerto Maldonado and its particular conditions an interesting area of study for DENV, specifically in terms of the differential economic pressure the disease may exert across the social structure of the city.

**Figure 8: Puerto Maldonado in Madre de Dios, Peru**

In Peru, health care is provided through the public and private sector. The public sector is divided in subsidized (or indirect contribution) and with direct contribution. The
former is directed to people living in poverty (covers 36% of the population) and the latter for households that contribute to social security through their employers or directly as autonomous workers (covers 23% of the population). The private sector and armed forces covers 5% of the population. The population with no coverage is approximately 36% [170, 171].

In this context, the objective of this study was to assess the proportion of household income that is diverted to cover the costs incurred due to dengue illness and to compare these expenses between persons who have recently moved to the city, or recent migrants (RM) as compared to long term residents (LTR.) Results from this study will provide essential information on the health burden of dengue at the household level, especially in light of current vaccine efforts [128, 129, 172-174] and continuing vector control strategies. As a secondary objective, we described the demographic and socioeconomic features of the population of Puerto Maldonado according to their migration status. Conceivably, this will allow a better understanding of the role out-of-pocket payments play in dengue disease prevention and access to treatment and how this information could be used for current dengue control efforts as well as for dengue vaccination introduction efforts in the future.

V.3 METHODS

Data collection

Potential research subjects were identified at the local hospital where dengue cases are referred. A field worker contacted patients of all ages who had been diagnosed with dengue fever, dengue with warning symptoms or dengue hemorrhagic fever and
briefly explained the study and retrieved contact information if the patient was interested in participating. Once the potential participant was at home the field worker would contact them and set up an appointment to explain the study, perform the informed consent process, if the subject was willing to participate, and administer the questionnaire. The standardized questionnaire collected data on household characteristics, income and financial expenses. The survey was applied from December 2012 to March 2013, which is the yearly period when most dengue cases occur in the region of Madre de Dios.

RM were defined as persons who had been living in Puerto Maldonado for fewer than five years while residents who had been stationed in the city for five years or more were identified as LTR. The use of this definition provided a means to calculate sample size based on the expected outcomes. This cut-off was selected with a basis on the more recent information available on migration and place of residence from the Peruvian census from 2007, which had a specific question regarding place of residence in the previous five years from the census [133].

Cost-of-illness estimation

The perspective selected was that of participants and their families [164]. As explained previously, data were collected with a standardized questionnaire (see Data collection). We estimated direct cost of medical treatment, other non-medical direct costs, as well as indirect costs. Direct medical costs included out-of-pocket payments for items such as medical appointments, laboratory exams, cost of hospitalization and medicines. Direct non-medical costs comprised transportation to the healthcare facility and indirect
costs were lost wages from the patient or the caregiver in the case of children. Participants were asked to report these lost wages. We estimated daily lost wage based on minimum wage in the case of non-paid activities. These estimates were obtained for participants lacking social security or similar insurance and were calculated using data collected on days lost due to illness or as caregiver and weekly salary to obtain a daily income.

We used the exchange rate from the Banco Central de Reserva del Peru for the period December 1, 2012 – March 1, 2013. www.bcrp.gob.pe. The rate equaled 2.56 Peruvian nuevos soles to 1 US dollar.

**Statistical analysis**

Data were initially assessed using descriptive statistics. We used Shapiro-Wilk test to assess for normality. K-sample and Mann-Whitney non-parametric tests were performed to assess differences in median and average income and cost-of-illness between RM and LTR. Likewise, chi square analysis, with Fisher’s exact adjustment as appropriate, was used to test the occupation of participants as well as the severity of dengue disease. A wealth index (WI) was created to assess wealth indicators of the participating households. WI included variables related to resources, construction materials of the house, and access to services such as running water, sewer and garbage collection. For details on the creation of this index go to Appendix 3. The higher values indicated more affluence. The proportion of the household income put towards or diverted to dengue expenses by migration status was appraised with the same
aforementioned tests. All statistical analysis was performed using Stata version 12.1 (StataCorp LP, College Station, TX, USA.)

V.4 RESULTS

General characteristics of the study population

A total of 80 subjects participated in the survey; Table 12 shows the general demographic characteristics of the study population, approximately. Twenty-six (33%) of the study subjects are native to Puerto Maldonado, but only 28 (35%) of those who are non-native to Puerto Maldonado meet the criterion for RM classification.

Among the participants who are non-native to Puerto Maldonado, the majority migrated from Cusco (33.3%), other areas in Madre de Dios (21.6%) and Puno (9.8%). On average, current residents who are non-native to Puerto Maldonado have been living in the city for 9.5 years (median: 5.2 years, SD 11.4). There was no difference in mean age regarding migration status (p=0.117). However, median age was lower among RM (median 23.5, SD 20.3) than LTR (median 31.5, SD 16.1), which was significant using the K-sample test for the equality of medians (p=0.035).

Income, occupation, assets and WI

Two-thirds of the dengue cases among RM and half of those among LTR had received payment for their main occupation in the week previous to the application of the questionnaire, but this discrepancy was not statistically significant (p=0.122). Data collected showed similar monthly household income of the cases interviewed as assessed by their migration status, among those participants who received remuneration (p=0.444).
Likewise, neither the household heads nor the cases interviewed showed a significant
difference in education level associated with their RM or LTR status ($p=0.651$ and
$p=0.283$, respectively). The average monthly household income for participants was US$
618.2, ranging from US$ 39.1 to 1562.6. Nonetheless, the average household income for RM was approximately US$ 507.8, about US$ 136.7 lower than that of LTR families (p=0.041). Participants had diverse occupations depending on their length of residence in Puerto Maldonado (p=0.012). More RM were students (36%), while the most common occupation among LTR cases was homemaker (27%).

| Table 13: Household characteristics and access to utilities by migration status |
|---------------------------------|--------|----------------|----------------|----------------|
|                                 | Total (n=80) | RM (n=28) | LTR (n=52) | p-value* |
| Services                        |         |        |        |         |
| In-house water plumbing         | 63 (79) | 18 (64) | 45 (87) | 0.020 |
| Garbage collection service      | 64 (80) | 18 (64) | 46 (88) | 0.010 |
| Sewage connection               | 56 (70) | 16 (57) | 40 (77) | 0.066 |
| Shared bathroom with other family or business | 12 (15) | 4 (14) | 8 (15) | 1.000 |
| Flooring material               |         |        |        |         |
| Wood                            | 6 (8)   | 5 (18) | 1 (2) | 0.018 |
| Dirt                            | 18 (23) | 8 (29) | 10 (19) | 0.340 |
| Cement/concrete                 | 49 (61) | 14 (50) | 35 (67) | 0.130 |
| Tiles                           | 7 (9)   | 1 (4) | 6 (12) | 0.412 |
| Roofing material                |         |        |        |         |
| Cement/concrete                 | 6 (8)   | 1 (4) | 5 (10) | 0.659 |
| Corrugated iron                 | 72 (90) | 26 (93) | 46 (88) | 0.706 |
| Palm trees                      | 2 (3)   | 1 (4) | 1 (2) | 1.000 |
| Wall material                   |         |        |        |         |
| Wood                            | 51 (64) | 18 (64) | 33 (63) | 0.942 |
| Cement/concrete                 | 23 (29) | 8 (29) | 15 (29) | 0.979 |
| Other                           | 6 (8)   | 2 (7) | 4 (8) | 1.000 |

*Chi squared or Fisher’s exact test

RM and LTR showed differences in access to public services and household construction materials: RM were more likely to lack running water (p=0.020) and
garbage collection services (p=0.010). They reported burning or burying residues and using a latrine in contrast to having a WC connected to the sewerage, although this was non-significant (p=0.066). Likewise, RM were more likely to have a wooden floor (p=0.018), but the use of dirt and cement flooring among RM showed no differences when compared with LTR (Table 13). These differences in household resources and services influenced the WI, which ranged between 0.410 and 1.868. The mean WI for RM was 1.319 while LTR had a WI of 1.471, the difference was statistically significant (p=0.003).

Dengue disease, use of and access to healthcare

About a quarter (26%) of all DENV episodes were classified as dengue with warning signs. There was also a case of severe dengue. A similar number of cases within both groups were classified as dengue with warning signs, regardless of migration status (p=0.158), and there were no differences in underlying illnesses (p=0.287). A discrepancy was detected in the need for caregivers between LTR and RM, with RM requiring more caregivers (p=0.082). Approximately 64% of participants reported being incapacitated due to illness. The number of days lost (from work, housework or school) for each dengue episode was on average 5.1, ranging from 1 to 30 days. Thirty-seven (46%) subjects required hospitalization, which lasted for 3.4 days on average, ranging from 1 to 10 days. About half (54.0%) of these subjects had dengue with warning signs and only a small proportion (16.2%) of hospitalized patients had underlying chronic conditions. In general, the severity, days of incapacitation and hospitalization was similar between RM and LTR.
More than 60% of respondents did not have health insurance, a slightly higher proportion among RM, but non-significant. Both groups were seen in similar health care facilities and they presented at a facility 1.5 times on average, ranging from 1 to 3 for the same dengue episode, showing no difference between the groups.

**Cost of illness**

Each dengue episode accounted for a mean cost of US$ 105.3. On average, direct costs summed to US$ 47.6, non-medical direct costs (transportation) were US$ 2.3 and indirect costs were US$ 55.5 (Table 13).
The larger proportion of expenses was aggregated in direct costs (49%) and indirect costs (35%). However, indirect costs were more than half of total expenses (52%) when these costs were reported. There were no significant differences between average costs incurred by RM and LTR (Table 13). The mean total cost for patients who were hospitalized was US$ 149.7, in contrast to US$ 68.8 for outpatients (p<0.001). Participants who had health insurance had fewer expenses than those who did not have it (p=0.010), but the difference was not relevant after controlling for severity (p=0.060). The main difference between insured and uninsured patients was direct costs, which came up to almost US$ 60 for those who did not have any kind of insurance compared to ~US$ 30 on average for participants who had coverage (p<0.001). The mean total cost for patients with dengue without warning signs was US$ 97.4 and those with warning signs accounted for US$ 127.5 (p=0.122) (Table 14).

The proportion of monthly household income that is diverted to dengue related expenses or lost wages because of illness is approximately 24% (SD 36.0), ranging from no expenses to spending all household income on a dengue episode. We did not find any difference in the proportion of expenses by severity of disease. There was no statistical difference in the proportion of income that was invested by LTR as compared to RM (p=0.462) although RM invested a slightly higher proportion of their income in each dengue episode (31%). Nonetheless, only one participant reported incurring in debt because of dengue illness.
Table 13: Detail of direct and indirect costs (in US$) by migration status

<table>
<thead>
<tr>
<th>Type of cost</th>
<th>Total</th>
<th>RM</th>
<th>LTR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Median</td>
<td>IQR*</td>
</tr>
<tr>
<td>Direct costs</td>
<td>47.6</td>
<td>42.5</td>
<td>42.3</td>
<td>67.3</td>
</tr>
<tr>
<td>Non-medical direct costs</td>
<td>2.3</td>
<td>1.6</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Indirect costs</td>
<td>55.5</td>
<td>86.5</td>
<td>24.4</td>
<td>67.1</td>
</tr>
<tr>
<td>Total costs</td>
<td>105.3</td>
<td>106.1</td>
<td>77.6</td>
<td>108.8</td>
</tr>
</tbody>
</table>

* IQR = interquartile range

Table 14: Detail of direct and indirect costs (in US$) by diagnosis

<table>
<thead>
<tr>
<th>Type of cost</th>
<th>Total</th>
<th>Classic Dengue</th>
<th>Dengue with warning signs or severe</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Median</td>
<td>IQR*</td>
</tr>
<tr>
<td>Direct costs</td>
<td>47.6</td>
<td>42.5</td>
<td>42.3</td>
<td>67.3</td>
</tr>
<tr>
<td>Non-medical direct costs</td>
<td>2.3</td>
<td>1.7</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Indirect costs</td>
<td>55.5</td>
<td>86.5</td>
<td>24.4</td>
<td>67.1</td>
</tr>
<tr>
<td>Total costs</td>
<td>105.3</td>
<td>106.1</td>
<td>77.6</td>
<td>108.8</td>
</tr>
</tbody>
</table>
Finally, we estimated the total cost of reported dengue cases for the region of Madre de Dios in 2012, which had the second highest annual incidence rate of the country: 1604/100,000 people [175]. Total costs amount to US$ 216,076 for 2052 dengue cases reported for Madre de Dios.

**V.5 DISCUSSION**

Despite previous larger studies to assess the economic burden of DENV in Latin America [93, 176], there is sparse information regarding DENV costs at the household level in the region. Likewise, there has not been a previous assessment of expenses incurred due to DENV in Peru, although certain regions, such as Madre de Dios, have a disproportionate burden of the disease when compared with national rates [169].

The study found no significant differences between the costs incurred by RM and LTR. Direct medical costs were approximately US$ 48, which is higher than total costs (US$ 27, approximately 23% of the average monthly income [177]) estimated in a study from Kampong Cham Province, Cambodia [178]. It is also in excess of the highest cost for hospitalization with dengue hemorrhagic fever (US$ 39.1, approximately 12% of the average monthly income [179]) as reported from a cohort of children in Thailand [163] or the average cost (approximately US$ 24) for a population of children and adults in the same country [94]. However, research conducted in Vietnam estimated total costs at US$ 167.8 per hospitalized case [180], closer to the findings in this study for patients who required hospitalization (US$ 135.8). The direct costs of dengue illness we calculated were similar to those estimated in the state of Zulia, Venezuela [90]. It is remarkable, though that research conducted in Southeast Asia reported a large proportion of
participants contracting debt [178]. This is in contrast to the findings in this study, in which only one patient from Puerto Maldonado contracted debt due to dengue illness. Similarly in both studies by Huy et al and Tam et al. [165, 180] a large proportion of expenses were for direct medical costs. The same situation appears within this population, but lost wages also constituted a large burden when indirect costs were estimated among participants from Puerto Maldonado. A recent study conducted to measure the economic burden of influenza in several regions in Peru, including Madre de Dios [181-183], estimated the average total expenditure per episode at US$ 33. While the total cost per episode of influenza is strikingly less on average than for dengue (~US$105), these are cases that were sought prospectively at the community level, and therefore, costs range from US$ 263 for hospitalized patients to US$ 19 for individuals who sought self-care. However, direct medical costs are still a fraction (US$ 8 vs. 47) of what has been assessed for DENV.

The total expenditure for DENV episode accounted for approximately a quarter of the monthly household income on average. This proportion was lower than 37% as reported for Thailand [94], but comparable to Vietnam [180]. This research however was unable to find or link a higher proportion of monthly household income diverted to DENV expenditures from RM households compared to LTR. Data collected from the investigation suggested that households of LTR a have higher income than RM, similar to previous findings [96]. It is possible, however that the main reason for this differential income is the age distribution of both groups, which may also influence the main occupations of participants. Median age for RM was 23 years, while that for LTR is 31 and the group of recent migrants had a third of participants considered students, which
consisted of less than 10% for LTR. In a similar fashion, the group of RM that had a higher need for caregivers during their DENV episode was students (10/27).

Availability of public services like garbage collection, running water and connection to sewers was different between RM and LTR, with insufficient coverage among RM. This is probably related to the settling areas of RM, which may be in newer locations in the city and grow in an unplanned fashion similar to other urban areas in developing countries [112, 184, 185]. Differential risk of dengue because of location in urban settings has been described before in other studies [82, 97, 150, 156, 186, 187] and has been assessed with countrywide data in Peru, linking poor access to running water as a risk factor for DENV [169].

The length of dengue illness per episode was comparable to what has been reported in other studies [94, 163]. Likewise, the number of contacts a dengue patient makes with healthcare facilities for each episode of dengue, 1.5 on average, is similar to what has been estimated before in different settings as well [178]. In terms of severity, almost half of dengue cases were hospitalized (46.3 %). Although this percentage is lower than what has been shown in similar studies [178], hospitalization was only related to having been diagnosed with dengue with warning signs, but not with the pre-existence of a chronic condition (ie. diabetes, hypertension, etc.). According to the World Health Organization Guidelines for Diagnosis, Treatment, Prevention and Control of Dengue, the presence of co-morbidities corresponds to admission criteria for dengue treatment [10]. Therefore, this concerning finding should be evaluated through different means to assess the protocol and practices in place for the hospitalization of patients with dengue.
This study has several limitations, including a small sample size, the lack of data to assess expenditures of the household for more comprehensive and detailed understanding of the impact of dengue illness. Similarly, the information collected for this study pertains to cases with enough symptoms to seek healthcare. Therefore, this is a lower bound estimate of the true impact since some illness may not be reported but may influence productivity and household income. Likewise, there may be economic barriers to accessing healthcare in the first place that this study was not designed to address, but should be evaluated.

This is the first study in this region to describe economic impact of dengue at the household level. The total cost for the dengue cases in the region for 2012 was US$ 216,076. Approximately half of these costs ~ US$ 106,000 corresponds to direct costs and ~US$ 75,500 are indirect costs. Similar studies are needed to improve our understanding of the burden of this disease, especially in the face of current efforts to develop a vaccine [188] and cost-effectiveness studies that may be needed to correctly assess the impact of these strategies [174].

V.7 Conclusion

This study has highlighted the extent to which resources from a household may be compromised due to a DENV episode. The economic burden of dengue at the household level in Puerto Maldonado accounts for approximately 24% of the monthly household income. The expenses incurred by RM or LTR are not significantly different nor is the fraction of monthly household income that may be affected by either group. However,
RM has a significantly reduced monthly income as compared to LTR so they are likely to experience a differential hardship.

This data has also presented some varying characteristics in these groups, such as access to running water or garbage collection services, which seem to be limited among RM. Likewise, RM had a lower WI indicating less affluence than LTR. These features may also contribute to a variation in economic stress between these two groups, although this was not portrayed in these specific dengue-associated expenses.
Appendix 3: Wealth Index

The wealth index (WI) was created using the same variables included in the services and infrastructure index (ISi) and the natural logarithm of the assets index (Ai) described in Appendix 2. The same weights were applied, based on the investigators decision.

Infrastructure and services index:

\[
CFS_i = \left[\left(\frac{pared}{3}\right) \times 1 + \left(\frac{piso}{4}\right) \times 1 + \left(\frac{techo}{3}\right) \times 1 + \left(\frac{excrementos}{4}\right) \times 2 + \left(\frac{basura}{2}\right) \times 2 + \left(\frac{aguacorriente}{2}\right) \times 3 + \left(\frac{banhopropio}{2}\right) \times 1 + \left(\frac{cocinapropia}{2}\right) \times 1\right]/12
\]

Assets index:

\[
AI = \ln(Q01_1 \times Q01_{1\text{CUANTOS}} \times 46 + Q01_2 \times Q01_{2\text{CUANTOS}} \times 124 + Q01_3 \times Q01_{3\text{CUANTOS}} \times 50 + Q01_4 \\
\times Q01_{4\text{CUANTOS}} \times 183 + Q01_5 \times Q01_{5\text{CUANTOS}} \times 312 + Q01_6 \times Q01_{6\text{CUANTOS}} \times 82 + Q01_7 \\
\times Q01_{7\text{CUANTOS}} \times 1504 + Q01_8 \times Q01_{8\text{CUANTOS}} \times 10160 + Q01_9 \times Q01_{9\text{CUANTOS}} \times 100)
\]

The values for ISi were standardized to provide a maximum of 1 and the values for Ai were divided by the maximum value obtained for this index (9.41) to obtain a maximum value of 1 for this index as well.

Finally, the WI was calculated:

\[
WI = CFS_i + AI
\]
Chapter Six

SPATIAL PATTERNS OF DENGUE RISK IN PUERTO MALDONADO, PERU

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Keywords: dengue, prevalence, migration, geospatial analysis

Running Title: Spatial patterns of dengue risk
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VI.1 ABSTRACT

Background: Dengue virus (DENV), from the Flavivirus genus, is considered the most widely distributed arbovirus in the world. The distribution of DENV and its vector population is an important area of epidemiological research due to the close relationship between the human host and the vector.

Objective: To identify spatial patterns of risk for DENV infection for residents in Puerto Maldonado, considering migration background, services and infrastructure, socioeconomic status.

Methods: We selected one household from each block where a serosurvey and questionnaire were administered to household members. Testing for DENV antibodies was performed in the NAMRU-6 laboratory. We explored the data for clustering patterns with average nearest neighbor analysis, inverse distance weighting and Getis-Ord General G. We identified clusters of DENV infection using Getis-Ord hotspot analysis. Finally, data were evaluated using an ordinal model in SaTScan to evaluate presence of clustering after adjustment for other covariates. We created a model introducing variables measuring the location of households to potential vector and infection sources such as markets, cemetery, hospitals flooding areas and river shore.

Results: We analyzed data from 270 households. Over 60% of households were migrants to the city. Almost 40% of households had primary infection and over a quarter had
secondary infection. We located five clusters of high prevalence for DENV infection. The most likely cluster had an extension of 0.75 Km and included 15% of total cases, but accounts for approximately 30% of secondary cases. Higher income and KAP score were predictors of infection and the risk for infection decreased with increasing distance (in meters) from flooding areas (OR: 0.999; 95% CI: 0.998; 0.999). The distance to other features in the city were not associated with DENV infection in the multivariate analysis.

**Conclusion:** The study has highlighted the heterogeneous risk of DENV in Puerto Maldonado and its relationship with socioeconomic variables as well as with geographic location of households, in particular, the distance to flooding areas. Additionally, it has also displayed the lack of appropriate housing infrastructure and public services in an area of urban expansion.
VI.2 BACKGROUND

Dengue virus (DENV), from the Flavivirus genus, is considered the most widely distributed arbovirus in the world [2]. The virus resurged in the Americas in the 1980s when the vector Aedes aegypti was reintroduced after a decades long elimination campaign ended [2, 66]. Infection with any dengue serotype will confer transient immunity to any of the four serotypes (DENV-1, DENV-2, DENV-3, DENV-4) and lifelong immunity to the infecting serotype [3].

The distribution of DENV and its vector population is an important area of epidemiological research. The close relationship between the human host and the vector is translated into a heterogeneous pattern of distribution of the disease in urban environments [9, 150, 189], particularly where Ae. aegypti has adapted extremely well. Varying conditions of human dwelling in urban settings have led to the association of dengue risk to several factors such as socioeconomic context, demographic characteristics and environmental features [159, 190, 191]. Therefore, spatial analytical methods are increasingly used to investigate focal areas of disease transmission and prevalence to improve vector and disease surveillance activities [43, 160, 192, 193], explain disease distribution [194] and better understand outbreak related factors [195].

The more detailed studies linking mosquito distribution and dengue cases have been performed in Iquitos, Peru and rural villages in Thailand [42, 150, 196]. This research has shown the focal dispersal of the vector and has strengthened the notion of human role in the dissemination of the virus [30, 197, 198]. In contrast, larger scale assessments have contributed with a different perspective to understand the context in which transmission occurs and the sociodemographic conditions that favor DENV
infection. Therefore, studies carried out in Brazil have identified a higher risk of infection in areas in Rio de Janeiro with poor public sanitation infrastructure [190], or related to low educational level and lower income in Goiana [82, 199], and also to a higher population density and a higher children and elderly women ratio in Belo Horizonte [189]. These types of studies have been carried out with area level data combining spatial analysis techniques and data collected at the individual and household level. Research carried out in Lao and Thailand has provided further insights into the local features that facilitate DENV infection. These studies have revealed that housing quality and human behavior or the geographical location within the city, in some cases related to the presence of agricultural land, may place some communities under different risk levels, which allows for focused prevention and control strategies [200, 201].

Puerto Maldonado, the capital city of Madre de Dios Region, is a rapidly expanding urban center; it is located in the southern rainforest in Peru. One of the main causes of urban expansion for Puerto Maldonado is migration [118]. In 2002, a study from the Instituto Nacional de Defensa Civil (INDECI, equivalent to the Federal Emergency Management Agency in the United States) reported that the city lacked approximately 1300 housing units, which required more than 32 hectares of city expansion to meet this demand [202]. Likewise, the region reports the highest migration rate of the country, 32.1 x 1000 per year [133], portraying a very dynamic flow of people into this area. Remarkably, migrants to Puerto Maldonado are mainly from areas from Cusco, Puno, Apurimac and other areas of Madre de Dios that are not endemic for DENV (Chapter Four).
Accompanying these rapid demographic and geographic changes in Puerto Maldonado was the detection of *Aedes aegypti* in 1999 and the subsequent circulation of DENV, first reported in 2000 [78, 140]. Since then, all four serotypes have been identified in the region, specifically in this city. The purpose of this study was to identify and analyze spatial patterns of risk for DENV infection for residents in Puerto Maldonado, considering aspects such as migration background, availability of services and infrastructure quality, variables of socioeconomic status collected at the household level and the distance of households to potential high risk areas (*ie.* flooding areas, cemeteries, markets), which has shown to be relevant for other vector-borne diseases [203, 204]. A better understanding of the underlying structure of these attributes will likely help identify and explain why the aggregation of seropositive residents in the city exists and suggest more focused strategies for DENV vector control and disease prevention.

**VI.3 METHODS**

**Location**

This study was part of a cross-sectional serosurvey performed on 307 households of Puerto Maldonado from July-December 2012. The city is located in the southern rainforest of Peru (S 12° 36' 12.3654", W 69° 11' 30.8682") in the junction of Madre de Dios and Tambopata rivers. The population of the city is approximately 65 000 people [132].

**Household and participant selection and questionnaire application**

City blocks were randomly selected using a multispectral, 1/100 000 IKONOS satellite image from July 2010 and a cadastral map obtained from the Municipality of
Tambopata, Madre de Dios, from January 2007. The cadastral map was updated with information from the satellite image as well as from current maps used by the local Ministry of Health (DIRESA) for vector control and from information gathered by field workers while conducting the study (Figure 10).

Once a selected block from the map was located, field workers systematically invited households to participate starting from the northernmost household in the block, moving clockwise to the next household if the first one was not suitable (i.e. empty lot, unwilling to participate, commercial facilities, governmental offices, schools, etc.). One household was selected from each block. Every member older than six months living in the household was invited to provide a blood sample. A detailed questionnaire was administered to household heads regarding household composition, household assets, access to public services (including running water, sewage, garbage collection, etc.), migration history and knowledge attitudes and practices (KAP) regarding dengue.

Laboratory testing

Serum samples were collected from consenting and assenting participants and were transported using coolers to the local Naval Medical Research Unit 6 (NAMRU-6) facilities. Samples were centrifuged and serum was kept at \(-70^\circ\text{C}\) until shipment to the NAMRU-6 laboratory in Lima with dry ice. Once in Lima, samples were tested for IgG to dengue virus using enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT). Sera samples were initially screened with ELISA and those positive were confirmed with PRNT. The methodology for the laboratory tests performed for this study has been described before in Chapter 4 (IV.3. MATERIALS AND METHODS).
Data and spatial analysis

We determined our primary outcome, “household status”, based on the most severe results from PRNT testing obtained from any member of the household. Therefore, if a household had three members who were tested and one had history of a secondary infection, another had had a primary infection while the other one was susceptible the household was considered as secondary infection. Likewise, we considered a household as positive if at least one of the tested members in the household had a positive PRNT for DENV infection. Finally, as migration status is an important demographic variable in Puerto Maldonado, migrant households were considered if the
household head or spouse were not from Puerto Maldonado. Therefore, we also created different categories of migrant households considering when the household head or spouse moved to the city. The following strata for household migration background were included: 7 years or less, 8 to 15 years, more than 15 years and native population.

We performed an initial assessment of spatial clustering using the average nearest neighbor (ANN) analysis for positive and negative households and for households that were considered migrant or native households. This method is adequate for binary outcomes and is oriented to detect clustering in a fixed area [205]. We also explored geographical patterns of household status using an inverse weighted distance method (IDW) [206]. IDW is a non-parametric interpolation method that estimates the influence each spatial observation may have among the others under study, considering the influence decay across distance and using the inverse squared distances between points to allocate weights [206].

After assessing the patterns highlighted by IDW we created an ordered logistic model using Stata 12.1 (StataCorp LP, College Station, TX) that included spatial coordinates. Along with the information on location we included three household indices which have been used in a previous study and that have been explained before in more detail (IV.3. MATERIALS AND METHODS, Appendix 2). These indices were designed to a) measure knowledge attitudes and practices (KAPi), b) infrastructure and services of the residence (ISi) and c) household assets (Ai). Likewise, the proportion of migrants in the household, using the information regarding when they moved to Puerto Maldonado, was included in the analysis.
In a following assessment, we used incremental spatial autocorrelation (ISA) to estimate the thresholds where clustering of positive household members may occur in our study area. ISA performs a test called local Moran’s I at different distances to help determine where aggregation of spatial data may occur. Moran’s I evaluates whether a set of objects are located randomly, clustered or dispersed using the following formula:

\[ I = \frac{n \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} (x_i - x_m)(x_j - x_m)}{\left( \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} \right) \sum_{i=1}^{n} (x_i - x_m)^2} \]

Where \( n \) is the number of points, \( x_m \) is the mean of the variable, \( x_i \) and \( x_j \) correspond to the variable at the specific locations \( i \) and \( j \) and \( w_{ij} \) is the weight correlating the location of \( i \) relative to \( j \). If neighboring objects are clustered \( I \) will be positive, while if dispersed \( I \) is negative. A value of \( I=0 \) signifies randomness. ISA is a tool that assumes an environment of homogenous risk at increasing radius from the events under study [205]. Though the premise of homogeneous risk underlying ISA may be unrealistic we used it as a starting point to assess point pattern analysis with Getis-Ord General G. This statistic assesses the relationship among the events (DENV infection) at different distances, in order to determine if there is a global pattern of clustering, dispersion or spatial randomness. The General G also provides information whether the spatial clustering involves areas where high values or low values concentrate (hot and cold spots):

\[ G = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} x_i x_j}{\sum_{i=1}^{n} \sum_{j=1}^{n} x_i x_j} \]

For this statistic, \( n \) is the number of points to study, \( x \) is the variable located in different locations \( i \) and \( j \), and \( w_{ij} \) is the weight correlating \( j \) relative to \( i \). For this index, if the p-value is significant and the z score is positive it indicates a clustering of high
values, the opposite occurs for negative z scores and a non-significant p-value prevents from rejecting a null hypothesis of a spatial random process [206]. The General G was used on continuous household variables: number of members positive to DENV per household, proportion of household members who are migrants, household monthly income, KAPi, ISi and Ai.

The Getis-Ord hot spot analysis (Gi*) was used to locate where the clumping of data occurred. This statistic for spatial association is a variation of the General G, focusing on defining the actual location of clusters:

$$G_i^* = \frac{\sum_j w_{ij}(d)x_j}{\sum_j x_j}$$

Global clustering patterns and cluster identification was performed with ArcGIS 10.0 (Esri, Redlands, CA).

We introduced the significant variables identified by the ordered logistic model and the global and local tests described above into SaTScan v9.3 in order to adjust for covariates [207]. SaTScan is a spatial scan statistic that tests for spatial randomness using a moving circular window of variable radii across the study area. The scan statistic compares the events inside and outside the moving window and possible clusters to determine where the most likely clusters are located and the relative risk for the outcome in each cluster compared to the other areas. P-values were obtained using Monte Carlo hypothesis testing with 999 iterations. The test was set for an ordinal model and to include a maximum of 50% of the population in a cluster. We selected clusters that had a p-value of 0.001.

Finally, we reproduced a map from the threats analysis conducted by INDECI in 2002 [202] that identified areas with temporal flooding during the rainy season (i.e.
November - March). We also included features identified in the city that could potential be sources for the vector or infectious individuals: markets, the cemetery, hospitals and riverine border. We estimated the distance from each study point to these areas and included this variable to the ordered logistic model to assess whether these features had any influence on the location of positive households.

VI.4 RESULTS

Migration and seroprevalence status

We obtained geographic locations for 285 households, with 270 households providing complete survey data and sera samples. Of the total households, 168 (62.2%) were migrant households (ie. household head or spouse was born outside of Puerto Maldonado) and 102 (37.8%) were native to Puerto Maldonado. Based on the time of arrival to the city of the household head or spouse and the time frames before and after the introduction of DENV (12 years ago), migrant categories were arranged as is shown in Table 15:

<table>
<thead>
<tr>
<th>Years living in Puerto Maldonado</th>
<th>Number of households (n=270) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0; 7]</td>
<td>45 (16.7)</td>
</tr>
<tr>
<td>&lt;7; 15]</td>
<td>33 (12.2)</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>90 (33.3)</td>
</tr>
<tr>
<td>Native</td>
<td>102 (37.8)</td>
</tr>
</tbody>
</table>
Sixty three percent of households from Puerto Maldonado were positive for DENV infection (171). Of these, 37.0% were considered as having primary infection, 26.3% secondary infection and the rest (36.7%) were the susceptible households.

Spatial patterns and cluster location

ANN indicated spatial clustering among positive households (p<0.001). This spatial pattern was explored using IDW, which identified areas with higher risk for DENV infection, Figure 11, areas in red. Large scale spatial trends were also suggested using an ordered logistic model for the outcome of household status (ie. households with susceptible members, households with at least a member with a primary infection or households with at least a member with a secondary infection). For this analysis we compared a model that incorporated categories of migrants (based on time living in Puerto Maldonado), monthly household income and KAPI, against a model with the same variables plus the coordinates for location. The likelihood-ratio test was significant (p=0.045) (see Table 16).

The ISA tool using the number of positive members in the household was used to determine the distance at which clustering may be occurring in this spatial pattern. The analysis output a peak distance at ~417 m (p<0.001). This information was used for the further analysis of spatial patterns using the global test, General G.

General G statistic suggested clustering for the number of positive members in the households (p=0.002), the household monthly income (p=0.012) and the ISi (p=0.006). The proportion of migrants in the households (p=0.181), the KAPI (p=0.272) and Ai (p=0.358) were non-significant with this test.
Figure 11: Inverse distance weighting (IDW) of DENV infection

Gi* was applied on the same variables described before (number of positive members in the household, proportion of household members who are migrants, ISi, KAPi, Ai and household monthly income). All of them rendered areas defined as hot spots, except for ISi which provided cold spots. Although not a lot of hotspots were identified, it is remarkable that the aggregation of high values of positive cases, household income and KAP score occur in the central area (~centroid) of the city. In contrast, low values of ISi aggregate in cold spots in what could be considered as city border areas (see Figure 15).
Table 16: Ordered logistic model and likelihood-ratio test including variables of spatial location

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>SE</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years living in Puerto Maldonado</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 7 years</td>
<td>1.814</td>
<td>0.632</td>
<td>0.087</td>
<td>[0.917; 3.590]</td>
</tr>
<tr>
<td>8 to 15 years</td>
<td>0.601</td>
<td>0.245</td>
<td>0.212</td>
<td>[0.271; 1.337]</td>
</tr>
<tr>
<td>More than 15</td>
<td>1.198</td>
<td>0.334</td>
<td>0.517</td>
<td>[0.693; 2.070]</td>
</tr>
<tr>
<td>Monthly income*</td>
<td>1.657</td>
<td>0.307</td>
<td>0.006</td>
<td>[1.153; 2.382]</td>
</tr>
<tr>
<td>KAPI score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (lower)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2</td>
<td>1.445</td>
<td>0.508</td>
<td>0.296</td>
<td>[0.725; 2.878]</td>
</tr>
<tr>
<td>Q3</td>
<td>2.289</td>
<td>0.774</td>
<td>0.014</td>
<td>[1.180; 4.443]</td>
</tr>
<tr>
<td>Q4</td>
<td>2.684</td>
<td>0.957</td>
<td>0.006</td>
<td>[1.335; 5.397]</td>
</tr>
<tr>
<td>Northing (Km)</td>
<td>0.768</td>
<td>0.081</td>
<td>0.012</td>
<td>[0.625; 0.943]</td>
</tr>
<tr>
<td>Easting (Km)</td>
<td>0.942</td>
<td>0.098</td>
<td>0.057</td>
<td>[0.768; 1.156]</td>
</tr>
<tr>
<td>cut 1: primary infection</td>
<td>3.748</td>
<td>1.369</td>
<td></td>
<td>[1.064; 6.432]</td>
</tr>
<tr>
<td>cut 2: secondary infection</td>
<td>5.497</td>
<td>1.394</td>
<td></td>
<td>[2.764; 8.229]</td>
</tr>
</tbody>
</table>

* The variable was transformed with the natural logarithm

Finally, as part of the exploration tools, we used ANN to identify global clustering regarding migration status and across different levels of migrant households. This analysis indicated that households characterized as migrants were clustered (p=0.01). Therefore, although the Global G analysis did not give significant results for the proportion of household members that were migrants we examined the local Gi* with this variable and identified several hotspots especially to the west of the city (See Figure 14). We portray a map with the location of migrants according to their migration time to
Puerto Maldonado to visualize these patterns in Figure 14. The mapping of these categories display newer migrants (< 7 years) located across the city, similar to native residents. However, migrants between 7 and 15 years are located mainly to the west and north of the city.

We applied the SaTScan analysis to the outcome by itself and identified 6 significant clusters (p=0.001). The most likely cluster had an extension of 0.77 Km and a prevalence rate (PR) for primary infection of 1.01 and 2.57 for secondary infection (Figure 12). Subsequently, we assessed the location of spatial clusters for household infection status adjusting for categories of time living in Puerto Maldonado, income and KAPI, which were shown to be significant in the ordinal model (Table 16). This analysis provided five significant clusters. Some of these cluster areas were superimposed with the clusters obtained from the model without other variables, in particular the most likely cluster for both analyses. After adjusting for income, KAPI and migration time, the cluster in the northern area of the city was not identified, but a cluster in the southern area was evident (Figure 12). The most likely cluster had a radius of 0.57 Km and PR of 1.14 and 2.31 for primary and secondary infection, respectively.

Following, we analyzed if there were specific features of the city that could be a source for exposure to the vector and generate clusters. We evaluated several models including variables of the distance from each study point to 3 markets, 2 hospitals, 1 cemetery, distance to the river shore and previously identified flooding areas. Only the last variable improved the model, portraying a significant decrease in the cumulative OR of primary or secondary infection with DENV to having no infection, of approximately
1% with each meter. Finally, the incorporation of this measure of distance into the model was significant (p=0.003) (Table 17).

Figure 12: Clusters detected with scan statistics

VI.5 DISCUSSION

The spatial pattern and distribution of DENV cases has been examined before in different locations [82, 190, 195, 201]. In a study in Thailand, Van Benthem et al reported that in rural areas infection occurred mainly in village centers. Likewise, they found an association with housing quality and preventive measures in less urban areas. The authors inferred that the landscape characteristics in urban areas favored transmission while specific household features may play a more important role in rural
areas [208]. In contrast to these findings, in Puerto Maldonado the association with housing quality and services was not evident, despite spatial clustering of low values that was portrayed through the hotspot analysis. Furthermore, the center of the city of Puerto Maldonado suggested higher risk of DENV transmission with the interpolation (Figure 11) and indicated hotspots for DENV positive seroprevalence in bivariate analysis using Gi* (Figure 14). However, after adjusting for KAPi, monthly household income and migration categories, the scan statistic revealed five clusters, locating the most likely cluster to the central eastern area of the city. Despite adjustment for variables that showed local indices for aggregation when incorporated in the ordinal model for DENV infection, DENV infection still seems to disseminate unevenly across the different areas of Puerto Maldonado. Aspects such as KAPi and household income appear to be the most important factors related to DENV infection. Akin to what has been reported in Thailand, these features are also linked to DENV infection.

The study by Siquiera et al, in Goiana, Brazil, explained higher seroprevalence for DENV with head of household income [82]. This is a similar finding to what has been reported in this study. However, it contrasts what has occurred in other locations [86], including Rio de Janeiro, Brazil, where a study of DENV risk and spatial aggregation indicated that the presence of certain assets was negatively correlated to DENV positive outcomes [190]. In the same study, though the most important variable for DENV infection was the presence of sanitation services, in contrast to what we found in this analysis where ISi was not relevant, though it initially showed clustering in the local hotspot analysis.
Table 17: Ordered logistic model and likelihood-ratio test including distance to flooding areas

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>SE</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years living in Puerto Maldonado</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 7 years</td>
<td>1.987</td>
<td>0.70</td>
<td>0.052</td>
<td>[0.994; 3.973]</td>
</tr>
<tr>
<td>8 to 15 years</td>
<td>0.623</td>
<td>0.258</td>
<td>0.254</td>
<td>[0.277; 1.404]</td>
</tr>
<tr>
<td>More than 15</td>
<td>1.111</td>
<td>0.312</td>
<td>0.707</td>
<td>[0.640; 1.929]</td>
</tr>
<tr>
<td>Monthly income*</td>
<td>1.593</td>
<td>0.297</td>
<td>0.012</td>
<td>[1.106; 2.296]</td>
</tr>
<tr>
<td>KAPI score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (lower)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2</td>
<td>1.515</td>
<td>0.539</td>
<td>0.243</td>
<td>[0.754; 3.044]</td>
</tr>
<tr>
<td>Q3</td>
<td>2.245</td>
<td>0.767</td>
<td>0.018</td>
<td>[1.149; 4.386]</td>
</tr>
<tr>
<td>Q4</td>
<td>2.663</td>
<td>0.958</td>
<td>0.006</td>
<td>[1.315; 5.391]</td>
</tr>
<tr>
<td>Distance to flooding (m)</td>
<td>0.9992</td>
<td>0.0002</td>
<td>0.004</td>
<td>[0.9988; 0.9998]</td>
</tr>
<tr>
<td>Northing (Km)</td>
<td>0.745</td>
<td>0.079</td>
<td>0.006</td>
<td>[0.604; 0.918]</td>
</tr>
<tr>
<td>Easting (Km)</td>
<td>0.902</td>
<td>0.098</td>
<td>0.340</td>
<td>[0.729; 1.115]</td>
</tr>
</tbody>
</table>

| cut 1: primary infection                | 2.887| 1.407 |         | [0.129; 5.645]  |
| cut 2: secondary infection             | 4.676| 1.427 |         | [1.879; 7.473]  |

The heterogeneity of DENV transmission has been reported and discussed before [152], in some cases with finer detail related to the presence of the vector *Ae. aegypti* [42, 150]. As shown previously in this discussion, there are similar and contradictory patterns regarding DENV risk and socioeconomic variables. Specifically, in Puerto Maldonado, our findings from the data analysis suggested a higher risk of infection related to higher income and values of KAPI. The specific characteristics underlying the interrelation of these variables may not be evident in a cursory assessment. Seropositive results to DENV
are related to the time of exposure with higher chances of being positive with age [110, 209]. However, Puerto Maldonado has a very important driver of population renovation which is migration and may be influencing this more common temporal risk relationship.

Figure 13: High risk clusters for DENV infection and assessed features

Also, the economic conditions of migrants in the areas where they relocate tend to improve through time [97, 210]. These relationships could help describe the association between higher risk of DENV infection and higher household income. Additionally, if we look at the findings from the hotspot of household income and period of migration (Figure 16), we have a clearer illustration of these issues. As such, families with longer residence in the city are located in areas of clustering of higher income values, in the
central area of the city, where also households with DENV positive serostatus aggregate, while some groups of migrants tend to establish in the outskirts of the urban surface, specifically to the west (Figure 14).

A remarkable finding was the increase in DENV infection risk with closeness to areas of temporal flooding (Figure 13). Including this variable in the model explained better the relationship with DENV risk. A study in the highlands of Kenya found similar results with malaria vectors and the distance of households to breeding sites [203]. These findings highlight the need to make use of spatial analysis tools to better target vector control. Additionally, it is distressful to note that in Puerto Maldonado the areas of constant flooding were identified more than ten years ago and the requirement for improved drainage facilities has not been properly addressed to date [202].

Finally, it is worth mentioning that this study also portrayed the cold spots for ISi (clusters of low values for ISi). Although this finding was not relevant for DENV outcome, this clustering occurs in areas that are considered for urban expansion and the lack of appropriate services or adequate housing could trigger higher risk areas in the future (Figure 13) as has been found in other settings [201].

The lack of temporal data for DENV infection or dengue cases and chronological features of city development preclude us from further assessing the spatial relationship with DENV infection risk in Puerto Maldonado. Likewise, vector information is very important for the study of this virus and in particular related to its geographical distribution. This information could strengthen the associations and inferences from this study to a finer scale as has been done in Iquitos, Peru and in Thailand [42, 150, 196].
VI. CONCLUSION

The findings of this study have pointed to a clear spatial pattern in DENV risk and its association with household income and KAP score of the household. In particular, the study has highlighted the risk of DENV in specific areas through the location of clusters of high prevalence. Likewise, it has portrayed the relationship of DENV infection to the geographic location of the households from flooding areas. As an additional finding, the analysis has also displayed the lack of appropriate housing infrastructure and public services in a sensible area of the city due to urban expansion. Therefore, we expect these findings to further guide urban planning in this area and admonish local authorities to take action in the recommendations that have been previously presented regarding environmental threats for Puerto Maldonado, specifically considering the relationship to DENV prevalence that this study has depicted.
Appendix 4: Maps of hot spots, cold spots and migration

1. **Figure 14**: Hot spots for migrant members, KAPi, income and positive households

2. **Figure 15**: Cold spots for ISi using Getis-Ord Gi* and expansion areas in the city

3. **Figure 16**: Time of residence in Puerto Maldonado
Figure 14: Hot spots for migrant members, KAPI, income and positive households
Figure 15: Cold spots for ISi using Getis-Ord Gi* and expansion areas in the city
Figure 16: Time of residence in Puerto Maldonado
Chapter Seven

INTEGRATED DISCUSSION

VII.1. CONCLUSION

The findings from this research have shown to different degrees the burden of DENV among RM to Puerto Maldonado, compared to LTR or to the native community. Initially I had set out to test a conceptual framework regarding the process of establishing a new household in Puerto Maldonado and the implications this has in terms of risk for DENV infection. In particular, I had focused on the specific aspects of migrant condition that may place someone at increased risk, such as KAP, socioeconomic status or location, and the economic vulnerability related to this group. I am confident that we have consistently addressed these research questions and revealed different findings pertaining to DENV risk related to migration in Puerto Maldonado.

Our seroprevalence estimates of DENV infection for the population in Puerto Maldonado was 54% (95% CI: 49.6; 58.5) and was similar across the working definitions of RM and LTR. However, there was a trend to an increase in OR (OR: 1.026; 95% CI: 0.998; 1.055) with each year living in the city (p=0.068). Approximately 35% of these infections were monotypic or primary and about 20% were heterotypic or secondary. The most important serotype diagnosed among monotypic infections was DENV-1 (88.6%), followed by DENV-2 (6.3%), DENV-3 (4.0%) and DENV-4 (1.1%). DENV-1 was the first serotype identified in Madre de Dios in 2001; therefore it has been circulating in the region for several seasons: 2009, 2011 and 2012. DENV-3 was introduced in 2005 and
reappeared again in 2009-2011. In 2012 the circulation of DENV-2 Asian/American genotype was reported. In contrast, DENV-4 has only been identified in 2009 with very limited circulation [78, 140, 141].

In terms of risk of DENV infection, the first noteworthy result is the association of better household income and increased prevalence of DENV infection. This conclusion, which was consistent in several different analyses, is also at odds with other studies that have demonstrated that lower socioeconomic status was highly associated with DENV risk, even in cross-sectional studies [86, 189]. However, the conditions that spur DENV transmission in Puerto Maldonado are likely unique due to its human population dynamics. A relatively recent study by Adams et al purported that humans constitute the reservoir and main “vector” in the dissemination of the virus within an area and to different regions [30]. Field work in the city of Iquitos, in the northern Amazon Basin in Peru has also supported this concept [197, 198]. Considering these assumptions, I perceive DENV dissemination in Puerto Maldonado almost solely maintained by the constant introduction of susceptible hosts and fueled by the reintroduction of the virus from endemic and hyperendemic locations (Brazil and Bolivia). Researchers before have described and termed this behavior as “sink populations” within metapopulation dynamics and have tested how maintenance of vector-borne diseases require human movement to become endemic in some areas [85, 211]. The coupling of these complex human and vector conducts in a relatively small community such as Puerto Maldonado likely favors dissemination (ie. force of infection) of DENV across all city strata, including wealthier groups.
Another factor related to higher DENV infection risk is increased knowledge regarding dengue disease and transmission. This finding is also contradictory at first assessment. However, due to the cross-sectional design of the study this outcome may just be showing the link with increased awareness after developing the disease within the household. Previous studies have also reported outcomes where knowledge was unrelated to lower risk of dengue disease or breeding sites, similar to our findings [153-155]. This finding obviously draws attention to the need for refining strategies for outreach and involving the community in preventive activities.

This research also has revealed the positive relationship of wealth and assets with the extent of time spent in the city. This phenomenon has been described before in very different settings like the United States of America [210]. Nonetheless, in an area where DENV is endemic, such disparity acquires a distinct accent. The economic burden of dengue disease at the household level is similar between RM and LTR in Puerto Maldonado. The total costs account for US$105.3, approximately a quarter of the average monthly income in Puerto Maldonado [177]. This could imply that lower income households may have a more severe impact from this disease and though it has not been reported in this study, it could lead to barriers to access healthcare services in other locations. It should be noted also that income reported in this city is approximately 30% higher than the average across the country, so similar direct costs in other areas may have a more acute impact in the household economy in other regions [132]. However, we identified a discrepancy in the household income of RM, which was lower compared to LTR (p=0.041). The findings of the WI between both groups is also in concert with the lower income among RM. This finding portrays the potential of a differential burden of
disease based on time of residence in the city, although it was not depicted with these data. Moreover, access to running water or garbage collection services, seem to be limited among RM, which has shown to influence risk of DENV infection in other settings adding to a likely more vulnerable situation for this group [190, 191].

It is also remarkable that hospitalization was associated with having been diagnosed with dengue with warning signs, but not with the pre-existence of a chronic condition, as recommended by the World Health Organization Guidelines for Diagnosis, Treatment, Prevention and Control of Dengue [10]. This is an important issue regarding clinical protocols that will require detailed review by local health authorities.

Spatial clustering of DENV risk areas was evident with the findings from this research. It was initially suggested when modeling the individual factors for DENV risk with the seroprevalence data and these hinted towards an association with household activities. Likewise, information gathered through the economic burden survey proposed the differential access of RM to specific services and potentially a spatial pattern in the location of some of these households. Therefore, it is not surprising that we identified clusters areas of high values for the proportion of migrants in the household and the aggregation of low values for ISi in specific zones as well. Hence, as an additional finding, the analysis performed in this study has pointed to a lack of appropriate housing infrastructure and access to public services within areas subject to urban expansion.

After adjusting for household income, time of residence in Puerto Maldonado and KAPi score, we identified significant clusters with high prevalence in some areas in the city. More importantly, we estimated an association with distance to flooding areas that have been located in the city for over a decade. Previous studies have shown before the
link of vector-borne diseases with features in the landscape that may increase or diminish risk of infection in the exposed population [201, 203].

Overall, this work exhibits the important influence of time living in Puerto Maldonado and migrant status on DENV infection risk and other associated impacts of dengue disease. Seroprevalence data were collected from a geographically distributed sample of the population of Puerto Maldonado. Also, it included residents with different time living in the city. The research also allowed collecting detailed information from the individual and combining it with household level data that was not available for Puerto Maldonado.

A particular strength of this study is the use of PRNT to test for past DENV infection, which – despite issues that have been addressed before [212, 213] - is still the gold standard. Additionally, this test has been validated before at the NAMRU-6 laboratory. Also, it allows identifying asymptomatic infections which are usually ignored [9, 196].

The specific angles of the studies: economic burden, seroprevalence and spatial patterns, facilitated obtaining information from different sources and aspects related to DENV transmission and the disease, allowing contrasting and comparing the data and providing a very comprehensive picture of the situation of DENV infection in Puerto Maldonado. There is extensive data about dengue and DENV transmission from other areas in South America [9, 189, 190, 214], but there is very scarce information from this particular zone, with the exception of the data provided by Forshey et al and da Silva-Nunes et al. However, in the first article the numbers for Puerto Maldonado were aggregated and analyzed within a larger region [141] and the latter refers to a rural area in
Brazil, near the border with Madre de Dios, where Puerto Maldonado is located [89]. Therefore, this study describes with a lot of detail the situation of DENV in Puerto Maldonado and suggests the dynamics that underlie the complex interplay between vectors and hosts.

The particular situation of Puerto Maldonado with a continuous flow of susceptible subjects to a city with the presence of DENV can also give insights regarding the emergence or reemergence of this virus in other places. DENV and dengue are increasingly disseminating across different regions where the virus and its vector were considered eradicated or where it had not been reported before [65, 215]. Furthermore, the most recent vaccine developments merit detailed knowledge of the epidemiology of the virus across different settings in order to make an informed policy decision regarding immunization against the virus [60-63, 216].

Ultimately, in terms of public health strategies for disease prevention, this work capitalizes on the need for an interdisciplinary approach to a) improve KAPs for this specific community and measuring its translation in decreased disease incidence [52], b) enhance and tailor adequate urban infrastructure that can prevent the development of high risk areas for DENV transmission [217] and c) provide specific services oriented to integrate and promote migrants into the city, considering their particular needs and gaps in knowledge, using appropriate cultural cues [97]. These three axes require careful coordination with diverse governmental offices, ranging from the municipality, the regional government, the local ministry of health and health care facilities. However, this research has clearly depicted the distinct areas that intervene in DENV infection risk and
the task of decreasing incidence and improving treatment will not be achieved by one entity by itself.

VII.2. LIMITATIONS

An important limitation of this study is the cross-sectional nature of the research. Collection of data at one point in time, though valuable, prevents from addressing aspects from causality, such as the relationship of KAP scores and positive DENV infection or temporal information about DENV introduction and dissemination across the city [9]. Incidence information from a longitudinal study would be ideal, in particular to identify the risk factors at play at the time of infection and also to determine the circulating serotype within an area and the temporal patterns these present [214].

Selection bias may also have been present in the seroprevalence study when inviting participating households and household members. We did not conduct a census previous to the enrollment, therefore, are unable to identify if the population under study had different characteristics.

For the purposes of this study, another limitation was sample size. The evidence from Puerto Maldonado portrays increasing prevalence for migrants as they spend different periods of time in the city. However, despite information collected from a varied group of people with different migratory background, we still lacked power to measure a significant trend in DENV infection measured through prevalence. Likewise, the identification of symptomatic cases of dengue for the economic burden study may have some misclassification. Cases were included in the study with either a laboratory or a
clinical diagnosis. We decided on this approach because until recently, most cases did not have a laboratory diagnosis for DENV. As was the usual procedure, once the circulation of the virus was established a large proportion of cases were confirmed by epidemiological link. Nonetheless, a previous study in Thailand comparing dengue costs with other febrile illnesses allocated greater expenses and duration of disease [163]. Therefore, we expect that these findings are comparable with other febrile illnesses that may circulate in Puerto Maldonado or at least not overestimate them.

Finally, another obvious key limitation in this study was the lack of entomological information. This is a weakness from this assessment which we hope to address properly in our future work (see below).

VII.3. DIRECTIONS OF FUTURE RESEARCH

A. Understand the introduction and dissemination of DENV serotypes in the Southern Amazon Basin

As has been presented throughout the document, Puerto Maldonado has particular challenges that make it a unique location for the prevention and control of DENV infection. At the same time, NAMRU-6 has an ongoing febrile surveillance project in the city [141]. Therefore, samples collected since 2004 could provide meaningful data on the serotype and phylogeny of the circulating viruses and its relationship with strains sampled in other locations in the region.
Likewise, in order to achieve better knowledge of the circulating serotypes and genotypes in this region I propose this would require a longitudinal population-based study. A prospective design would also be oriented to locating non-symptomatic contacts of dengue cases. This would not only address the lack of serotype specific information but also provide sufficient data regarding risk factors across different personal and household characteristics, including migratory history. Additionally, this study should be focused also in obtaining information from other locations in the region where DENV is introduced (ie. Iberia, Laberinto, Mazuko) in order to explain the modes of dissemination that take place and identify potential control strategies.

**B. Understand vector distribution and measure the effects of current and improved vector control strategies**

The lack of entomological information is a major flaw from this research. However, it should not lack in a prospective study. The collection of vectors in different stages, specifically pupae and adults is essential to target prevention and control strategies [218]. Additionally, it can help link the serotype identified in the vector with the circulating strain in the area or within a cluster of cases. Such information would also provide finer scale data on spatial patterns of *Ae. aegypti* dispersal and DENV transmission [196, 219].

Current control strategies have been based on indoor residual spraying and inspections of household premises to discard or treat potential breeding sites. In January 2013 the regional governments from Madre de Dios, Puno and Cusco launched a concerted plan to prevent and control dengue [140]. This plan includes vector control
activities which require periodic measurements to validate existing strategies. However, some of these comprise the collection of data on adult mosquitoes which pertains specific equipment (i.e. mosquito aspirators). Therefore, the synergy with ongoing efforts from the local government could provide an ideal field of research to test and tailor different interventions for vector control [37].

C. Design and evaluate specific strategies for community mobilization

This last area of research is a hinge between the two topics that have been described above. Previous studies have described the importance of culturally sensible approaches to invoke community participation and establish involvement [47, 54, 220]. The region of Madre de Dios and Puerto Maldonado are constantly being exposed to diverse background experiences and perceptions of incoming migrants from areas that are non-endemic for DENV. Therefore, it is particularly important to understand the cultural cues that are relevant to incorporate newcomers in community mobilization activities.

Aspects of health promotion and prevention have been included also in the macroregional plan for dengue prevention and control [140]. However, there is not a clear description of the steps to develop a culturally-sensible and motivating strategy to commit the participation of the community at large. This poses a specific gap in knowledge that requires appropriate undertaking to achieve the desirable long-term objectives. There is a wide spectrum of strategies that have been applied to involve the community in these tasks ranging from social influences to mass media campaigns [221-224]. It is likely that a multi-tiered approach would be required to maintain a long-term commitment with the community regarding dengue prevention and control. Nonetheless,
these efforts are useless if they are not based in a proper ethnographic research that can critically appraise past interventions and identify focus areas, networks or social arrangements to strengthen or encourage.

Likewise, there is little information regarding how the impact of these efforts have been measured and those that have been assessed provide varied results [52]. This emphasizes the need to articulate the research directed at measuring vector populations and incidence of disease (A and B).
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CURRICULUM VITAE

Summary
Gabriela Salmon-Mulanovich is a researcher at the Naval Medical Research Unit No. 6 (NAMRU-6) in Peru. She has master’s level studies in epidemiology and is currently pursuing her PhD at the International Health Department, Global Disease Epidemiology and Control track from the Johns Hopkins Bloomberg School of Public Health. Her dissertation project is “Dengue infection in Puerto Maldonado, Peru: human migration and economic impact”. Ms. Salmon’s research/academic interests are the epidemiology of infectious diseases and the use of modeling tools to understand the dynamics of disease transmission within human populations, reservoirs and vectors to apply evidence-based public health policy.

Employment
Naval Medical Research Unit No. 6 (NAMRU-6)
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Research Scientist, Vector-borne and Zoonotic Diseases Unit, Virology and Emerging Infections Department
• Participate in human, animal and human-animal interface research activities
• Coordinate and manage various epidemiological studies on infectious diseases
• Coordinate activities and information flow with the Peruvian Ministry of Health and local epidemiology office

• Establish and maintain partnerships with private, public, national and international institutions that work in the area of public health and epidemiology

• Design and conduct research in vector-borne and zoonotic diseases

Naval Medical Research Unit No. 6 (NAMRU-6)


Program Assistant, Emerging Infections Program

• Coordinated and taught of courses for NAMRU-6’s Public Health Training Program: biosafety, use of geographic information systems tools and outbreak investigation

• Coordinated of yearly field activities of the Tropical Medicine Training from the Naval Medical Education and Training Command

• Performed research on occupational health, food safety, zoonotic diseases, outbreak control and responses and use of geographic information systems in public health
Asociación Peruana para la Conservación de la Naturaleza – APECO  
Feb 2000 – Feb 2002  
(Peruvian Association for the Conservation of Nature)  
Research Assistant

- Revised and edited the documentation for the Regional Biodiversity Strategy of the Andean Countries
- Revised and published a CD Rom with supporting documentation for the Regional Biodiversity Strategy of the Andean Countries
- Coordinated the “Experts Meeting for the Final Design of the Regional Biodiversity Strategy of the Andean Countries”, for the Comunidad Andina – CAN (Andean Community)
- Developed the Second National Biodiversity Report, for the Consejo Nacional del Ambiente – CONAM (National Environmental Council)
- Updated of the First National Biodiversity Report for the Consejo Nacional del Ambiente – CONAM (National Environmental Council)

Comité Peruano de la Unión Mundial para la Naturaleza - IUCN  
Feb 2000 – Feb 2001  
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Johns Hopkins Bloomberg School of Public Health, Maryland, US

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2007 - 2008

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Masters’ Program in Clinical Epidemiology and Quantitative Methods

2003 - 2004

Universidad Nacional Agraria La Molina, Lima, Peru

Specialization Program on Environmental Management and Audit

1994 – 1999

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School of Science, Biological Sciences Program – Biotechnology Track

Bachelor in Science
Publications

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Books and book chapters
• Book Chapter. Riesgos a la salud de la vida en una megametrópoli (Health risks to life in a mega metropolis), published by the Universidad Nacional Autónoma de México (2010)

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Co-investigator in the project *Effects of anthropogenic habitat perturbation on rodent population dynamics and risk of rodent-borne diseases*

Department of Defense/Global Emerging Infections Surveillance
Oct 2013 – Sep 2014

Co-investigator on the following projects being implemented or in collaboration with the Virology and Emerging Infections Department of the U.S. Naval Medical Research Unit No. 6 in Peru:


NAMRU6.2011.0004. *Migration along the Interoceanic Highway in Peru: Exploring linkages between population, health and environment*

NAMRU6.2013.0002. *Epidemiology of rickettsiosis and leptospirosis in four geographically distinct regions of Peru*

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- 2013 – present: Member, American Society of Tropical Medicine and Hygiene
- 2013 – present: Member, Ecological Society of America
- 2013 – present: Board member, Asociacion Peruana para la Conservacion de la Naturaleza (APECO), Lima, Peru
- 2008 – present: Invited Lecturer; Masters in Epidemiology, Universidad Peruana Cayetano Heredia, Lima, Peru
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