Risk Profiling of Malaria Epidemiology in Rural Bangladesh

by

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Abstract

Background

Of 214 million estimated new malaria cases worldwide in 2015, 10% were from south-east Asia. Bangladesh is one of the 95 countries with ongoing malaria transmission. Of 13 malaria endemic districts in Bangladesh, Bandarban District—located in Chittagong Hill Tracts—has one of the highest malaria prevalence. This dissertation aimed to study risk factors associated with malaria endemicity and provide risk profiles of malaria epidemiology of the area.

Methods

This dissertation was conducted under *Mapping Malaria Epidemiology Project*, a prospectively surveillance project, in Bandarban, Bangladesh from 2009 to 2013. There were 5,006 households and 22,325 individuals resided in Bandarban Study Area, which included Kuhalong Union and Rajbila Union. We used logistic regressions to model field performance of FalciVax^{TM} Rapid Diagnostic Test (RDT) against Giemsa-stained microscopy (Chapter 3). How levels of *Plasmodium falciparum* density were associated with malaria symptoms was analyzed by logistic regressions (Chapter 4). Linear regressions were used to examine

the relationship between household building materials and average number of *Anopheles* mosquitoes found at households at night (Chapter 5). Finally, we conducted Generalized Estimating Equation (GEE) Poisson regression models to study how living standards (e.g. 33 durable assets and household building materials) would be associated with malaria incidence (Chapter 6).

Results

Among 616 *Plasmodium falciparum* tested individuals, 529 of them were malaria positive. Overall, sensitivity, specificity, positive and negative predictive values of FalciVax^{TM} RDT were 99.6% (527/529), 33.3% (29/87), 90.1% (527/585) and 93.5% (29/31), respectively. Being 30 years old or above, not having measured fever during malaria diagnosis, having symptom duration for more than 6 days, having self-reported fever at night, and not having self-reported fever with sweating were related to having lower level of *Plasmodium falciparum* parasite density (i.e. parasite density below median (5400 parasites/μl)). Approximately 5 *Anopheles* mosquitoes were found per night per household. Using mud as a building material (wall: N = 123 households (HHs), 95% CI: [0.31, 1.74]; partition: N = 119 HHs, 95% CI: [0.24, 1.70]; floor: N = 420 HHs, 95% CI: [0.15, 1.15]), comparing to the use of "bamboo", was associated with a higher number of *Anopheles* mosquitoes found at households at night. Having "bamboo" as a wall, partition and flooring material, having "corrugated tin or iron sheet" as a roofing material, as well as having "elevated ground floor at home" were related to elevated malaria incidence comparing to other types of building materials.

Discussion

With smaller sample size in stratum specific category, logistic regression could provide smoother estimates of sensitivity, specificity and predictive values. Sensitivity and specificity of rapid diagnostic devices were previously assumed to be independent from prevalence of malaria. Our field performance study may had shown otherwise. Future studies are needed to examine the assumption. How to utilize identified risk factors to integrate case awareness, reactive case search and hot spot analysis is essential to reduce malaria transmission in the study area. Although having some "*mud*" or "*Bamboo*" as part of building materials did not significantly change the overall *Anopheles* population, species specific preferences among *Anopheles* mosquitoes should be further studied. Although many factors were identified as risk factors for malaria incidence, the link among building materials, mosquitoes and malaria incidence, should be further explored.

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Introduction

1.1 Overview

1.1.1 Overview of Malaria

Malaria is a parasitic disease that is transmitted by mosquitoes.

Parasites that can cause human malaria include *Plasmodium falciparum (P. faciparum)*, *Plasmodium vivax (P. vivax)*, *Plasmodium ovale (P. ovale)* and *Plasmodium malariae (P. malariae)*. A zoonotic *Plasmodium* parasite, named *Plasmodium knowlesi (P. knowlesi)*, was found to have capability to infect human. Overall, *P. falciparum* and *P. vivax* are the major species in *Plasmodium* family that cause malaria.

Not all mosquitoes transmit malaria. Only female *Anopheles* mosquitoes serve as vectors to facilitate malaria transmission between two individuals. Approximately 70 out of 465 currently recognized *Anopheles* species can serve as a vehicle for *Plasmodium* parasites between humans [1, 2].

1.1.2 Overview of Malaria Worldwide

As of December 2015, 95 out of 194 (49%) World Health Organization (WHO) recognized countries and territories were malaria endemic [3]. This number was down from the 106 countries and territories with ongoing malaria transmission in 2000 [4]. With reduced risk worldwide, we saw estimated malaria cases have gone down from 262 million cases in 2000 to 214 million cases in 2015. Meanwhile, estimated malaria deaths dropped from 839,000 cases in 2010 to 438,000

cases in 2015. [5]

As of 2015, more than 40% of malaria endemic countries was located in Africa [3]. The continent encompassed 88% (188 million out of 214 million) of the estimated malaria cases and 90% (395,000/438,000) of the estimated malaria deaths worldwide [5]. Among 44 countries with malaria transmission in WHO African Regions, Democratic Republic of the Congo and Nigeria had the highest disease burden of malaria [4]. Outside of Africa, South-East Asia had the highest number of estimated cases (N = 20,000 (9%)) and estimated deaths (N = 32,000 (7%)) worldwide [5]. However, with discrepancy in population density, Africa had fewer number of people at risk (834 million people in 2015) than in South-East Asia (1.3 billion people) [3].

1.1.3 Overview of Malaria in Bangladesh

Bangladesh is one of the countries under WHO South-East Asia Region. Thirteen out of 64 districts in Bangladesh are endemic with malaria. These thirteen districts are located near the northeastern and southeastern Bangladesh, bordering India and Myanmar.

In 2000, 3.1 million malaria cases and 6,100 deaths were estimated in the country. The numbers were approximately 9% and 12% of the estimated cases (N = 33 million) and estimated deaths (N = 51,000) in WHO South-East Asia Region of the same year, respectively [5]. In 2007 and in 2009, grants received from *The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM)* and

Ministry of Health and Family Welfare in Bangladesh helped scale up malaria intervention. The *National Malaria Strategic Plan* was formed [6]. In 2014, Bangladesh joined *Asia Pacific Malaria Elimination Network (APMEN)* as the 16th country partner to combat malaria [7, 8].

In 2015, 700,000 malaria cases and 1,600 malaria deaths were estimated by WHO. The percentages of estimated cases and deaths in South-East Asia Region (cases: 20 million, deaths: 32,000) were decreased down to 5% and 3.5%, respectively [5]. Although malaria cases and deaths have decreased, to date, the three districts in Chittagong Hill Tracts in southeastern Bangladesh (i.e. Khagrachari District, Rangamati District, and Bandarban District) remain the biggest disease burden area of malaria in the country.

1.2 Malaria

1.2.1 Types of Malaria

Malaria is a parasitic disease caused by genus *Plasmodium* protozoa. Five species of *Plasmodium* parasite are currently known to be able to cause malaria in human: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. *P. falciparum* is currently known as the most virulent kind of malaria parasite. *P. vivax* and *P. ovale* can hibernate in human liver cells for weeks, and occasionally, years. *P. knowlesi* prefers macaque as its host; however, studies have shown its ability to jump from macaque to humans.

In Africa, malaria cases are almost exclusively from *P. falciparum*, except for cases in Eritrea and in Ethiopia—where 26% to 31% of cases were infected with *P. vivax*. Similarly, in Eastern Mediterranean Region, *P. falciparum* is most commonly seen in malaria patients, with the exception of cases identified in Afghanistan, Iran and Pakistan. *P. vivax* is the predominant species in these three countries. Unlike countries in African Region and in Eastern Mediterranean Region, over 70% of malaria cases in the Americas were infected with *P. vivax*. In South-East Asian Region and in Western Pacific Region, both *P. falciparum* and *P. vivax* can be seen in various WHO member countries. A wide range of mix percentage between *P. falciparum* and *P. vivax* can be found in countries with more than one circulating *Plasmodium* species. Some interesting findings in South-East Asian and Western Pacific countries included the exclusiveness of *P. vivax* in Democratic Peoples Republic of Korea ("North Korea") as well as the dominance of *P. vivax* in Republic of Korea ("South Korea"). In addition, *P. Knowlesi* was presence in approximately 40% of its malaria patients in Western Pacific Region. [3]

1.2.2 *Anopheles* Vector Mosquitoes

Anopheles mosquitoes are the vector for transmitting human malaria. Approximately 70 out of 465 formally recognized *Anopheles* species can carry and transmit malaria parasites. Hay [1], Sinka [2] and their colleagues reviewed previous studies done by White [9], Service [10, 11], Kiszewski [12] and Mouchet [13]. Then, they consulted technical advisory group to identify 41 dominant vector species (or species complexes) (DVS) in different geographic zones [14]. The 41

DVS were described as "main","dominant" or "principal" vectors in previous studies. These DVS are expected to be main contributors to malaria infection, have higher propensity to feed on humans and/or have longer adult longevity on average. Among the 41 DVS, 9 were identified in American group (Tables 1.1 and 1.2), 13 were identified in Africa, Europe and Middle East group (Tables 1.3 and 1.4) and 19 were identified in Asia Pacific group (Tables 1.5 to 1.9).

In Bangladesh, Alam et al. identified 20 different *Anopheles* species in Kuhalong Union from July 2009 to June 2010 [15]. Union is the smallest rural administrative and government unit in Bangladesh. Kuhalong Union is located within Bandarban District, Chittagong Hill Tracts in southeastern Bangladesh. The 20 identified *Anopheles (An.)* species were (1) *An. aconitus*, (2) *An. annularis*, (3) *An. baimaii*, (4) *An. barbirostris*, (5) *An. culicifacis*, (6) *An. jamesii*, (7) *An. jeyporiensi*, (8) *An. karwari*, (9) *An. kochi*, (10) *An. maculatus*, (11) *An. minimus*, (12) *An. nigerrimus*, (13) *An. nivipes*, (14) *An. subpictus*, (15) *An. tessellatus*, (16) *An. turkhudi*, (17) *An. umbrosus*, (18) *An. vagus*, (19) *An. varuna*, and (20) *An. willmori*.

Of which, *An. jeyporiensis* (N = 479), *An. vagus* (N = 429) and *An. kochi* (N = 369) were the most abundant female *Anopheles* species collected (N = 2,467) at the time of study. Within the 19 species that contained female *Anopheles* mosquitoes, 6 species were found to carry *P. falciparum* parasites (i.e. *An. barbirostris*, *An. jeyporiensis*, *An. kochi*, *An. maculatus*, *An. nigerrimus*, and *An. nivipes*) and 3 species were found to be positive with *P. vivax* (i.e. *An. nivipes*, *An. umbrosus* and *An. vagus*) [15].

1.2.3 Malaria Life Cycle

Although various species of *Plasmodium* parasites are linked to human malaria, their malaria life cycles are very similar. A malaria life cycle can be viewed in different stages: (1) The human liver stage, (2) the human asexual blood stage, (3) human sexual blood stage, and finally, (4) the mosquito stage.

A Blood Meal A malaria life cycle begins when a female *Anopheles* mosquito feeds on a human. If this *Anopheles* mosquito is malaria infected, *Plasmodium* parasites would be inoculated into the human host in the form of sporozoites during this blood meal process. Sporozoites then travel through the blood stream and move toward the liver. The human liver stage begins when sporozoites arrive in the liver.

Human Liver Stage Once in the liver cells, sporozoites begin to multiply and divide into merozoites. Tens of thousands of merozoites are now formed. Merozoites exit the liver cells and enter bloodstream of the human host.

Human Asexual Blood Stage When flowing through the bloodstream, merozoites begin to target red blood cells. Each red blood cell is invaded by a merozoite. After invading, a merozoite starts to multiply within the red blood cell. Once multiplied, merozoites burst out of the red blood cell. The newly formed merozoites continue to repeat the process of invading and multiplying the next targeted red blood cells.

Human Sexual Blood Stage During the asexual blood stage, some merozoites stop replicating and enter the sexual stage. At sexual stage, merozoites develop into male and female gametocytes in red blood cells.

Mosquito Stage When a blood meal is taken by a non-malaria infected female *Anopheles* mosquito, red blood cells are uptake and ingested. During this feeding phase, mosquito stage of a malaria life cycle begins. Male and female gametocytes that are in red blood cells begin to develop and mature in their female mosquito host. These matured male and female gametes can fuse into zygotes. Zygotes then transform into ookinetes. Ookinetes is an active form to help relocate *Plasmodium* parasite from a mosquito's midgut to its midgut wall. Once there, oocysts are formed. Oocysts serve as catalysts to produce thousands of sporozoites. When oocysts burst, sporozoites that were in the oocysts enter their mosquito host's body cavity and travel toward its salivary glands. When sporozoites arrive at the mosquito's salivary gland, they are ready to be inoculated through the next blood meal into the next human host.

Detailed malaria life cycle could be found on the *Centers for Disease Control and Prevention* website [16].

In general, a malaria life cycle takes 14 days to a month to complete. It takes, on average, 5-16 days at the liver stage, another 1-3 days at the blood stage and lastly, an additional 8-15 days to go through the mosquito stage. Many factors could affect the length of time in development of malaria parasites. Factors

such as temperature could affect the development process of *Plasmodium* parasite [17]. It also varies based on *Plasmodium* species. The relationship between temperature and time required for *P. falciparum* and *P. vivax* development inside a mosquito is shown in Figure 1.1 [17, 18].

With a warmer climate as predicted in the future and a common range of vector-borne diseases developed in 14°C-16°C to 35°C-40°C [19], many highlands at altitude—that were previously "protected" by the temperature threshold (i.e. under 18°C for *P. falciparum* and under 15°C for *P. vivax*)—may no longer have their edge in ceasing and slowing down the malaria transmission (Figure 1.2) [17].

1.2.4 Population at Risk

Children under 5 years of age, pregnant women and individuals with HIV infection are commonly considered as individuals with higher risk of malaria (Figure 1.3) [20]. However, at risk population varies based on malaria endemicity of an area. Malaria endemicity is an expression to quantify severity of malaria transmission. It will be defined in detail in section 1.3.1.

P. falciparum and *P. vivax* are the main *Plasmodium* parasites identified in malaria patients in Bangladesh. Therefore, we focused the general description of at risk population on these two species of *Plasmodium* infection.

P. falciparum At high malaria transmission areas, majority of the population is exposed to malaria during infancy or early childhood. Travelers and younger children who are naïve to malaria immunity are prone to severe *P. falciparum* malaria infection. In moderate malaria transmission area, highest parasite density is often found in older children and adolescents. In low malaria transmission area, similar risks of exposing to *P. falciparum* are observed in individuals of all ages. Severe *P. falciparum* malaria infection are not unique to young children in low and moderate malaria transmission zones. Nonetheless, pregnant women at second and third trimesters, HIV/AIDS patients and individuals with splenectomy have increased risk of acquiring severe malaria [21].

P. vivax Severe *P. vivax* can be found in younger children and in areas with chloroquine resistance (e.g. Indonesia). It is less common to find severe *P. vivax* infected cases in temperate areas [21].

1.2.5 Current Diagnosis

In the past, malaria diagnoses solely relied on the examination of clinical symptoms. Non-disease specific malaria symptoms, such as fever, made it hard to pin point the cause at the first sight. Therefore, having experienced health care providers was crucial in malaria diagnosis. Having limited number of experienced medical professionals and health care providers has been a concern in many rural corners of the world. Delayed malaria diagnosis and treatment could lead to more severe outcome.

Over the years, many laboratory tests have been developed to assist clinical diagnoses of malaria. This includes (1) microscopic examination of peripheral blood smears, (2) quantitative buffy coat (QBC), (3) rapid diagnostic tests (RDT), (4) immunofluorescence antibody testing (IFA), (5) polymerase chain reaction (PCR), (6) loop-mediated isothermal amplification (LAMP), (7) microarray, (8) flow cytometry (FCM), (9) automated blood cell counter (ACC) and (10) mass spectrometry (MS).

A brief comparison of these diagnostic tests can be found in Tables 1.10 to 1.12 [22]. In this dissertation, we focus on two of the methods in malaria diagnoses: Rapid diagnosis tests and microscopic examination. Description of these two methods are discussed below.

Rapid Diagnostic Tests

Currently, there are more than 200 commercial rapid diagnostic test (RDT) devices on the market. It is widely applied as a diagnostic tool in rural areas due to its portability, convenience, speed in malaria detection and easy interpretation by non-medical professionals.

FalciVax^{TM} Pv/Pf (Zephyr Biomedical Systems, India) is the RDT device used in Bangladesh. FalciVax^{TM} RDT uses "P. vivax-plasmodium Lactate Dehydrogrenase" (pv-pLDH) and "Histidine Rich Protein II" (HRP2) as target antigens to detect *P. vivax* and *P. falciparum*, respectively. The device requires 5 μl of

whole blood to perform the test and takes a minimum of 20 minutes to see the results [23]. FalciVax^{TM} RDT is designed in cassette format, as illustrated in Figure 1.4 [23].

Three lines can be shown on cassette format style of RDT devices. With the presence of "control" line and "*P. falciparum*" line, it shows the tested subject is infected with *P. falciparum* malaria. With the presence of "control" line and "*P. vivax*" line, it shows the tested subject is tested positive with *P. vivax* infection. With the presence of all three lines, it indicates the tested subject is infected by both *P. falciparum* and *P. vivax* parasites. If the "control" line does not appear during malaria diagnosis, the result is deemed invalid. A new FalciVax^{TM} RDT test should be repeated (Figure 1.5) [23].

To ensure the quality of rapid diagnostic test devices on the market, WHO partnered with *Foundation for Innovated New Diagnostics* (FIND), *Centers for Disease Control and Prevention* (CDC) and *Special Programme for Research and Training in Tropical Diseases* (TDR) to performed product testing of rapid diagnostic test devices. These laboratory based assessments test RDT performance with lower parasite density (200 parasites/μl) and with higher parasite density (2000 parasites/μl). A panel detection score would be assigned based on percent positive of malaria samples in given time. [24, 25, 26, 27, 28]

FalciVax^{TM} received a panel detection score of 95 for both low and high parasite density categories at Phase I (N = 20) for *P. falciparum*. At phase II (N = 98), panel detection score for low *P. falciparum* density was 98 and was 100 for high

P. falciparum density [23]. Other than laboratory based product performance examined by WHO, limited information have been provided with the field performance of FalciVax^{TM} RDTs [29, 30, 31, 32, 33, 34, 35]

Microscopic Examination

Microscopic examination ("microscopy") is the gold standard of malaria diagnosis. Due to its requirement of laboratory equipment (e.g. a microscope, slides, staining reagents) and at least one experienced microscopist, it is not available at all resource poor areas.

To conduct microscopy for malaria diagnosis, thin and thick blood films are needed. The blood films should be prepared from finger pricked blood of a tested subject. Thin and thick films serve different purposes. Thin films are used to identify *Plasmodium* species. Thick films are used to detect the presence of parasites and to estimate parasite density. The steps to prepare thin and thick blood films are shown in Figure 1.6 [36].

Under a microscope, red blood cells are usually normal in size in *P. falciparum* infections [37]; whereas, the size of red blood cells can be normal to 2 times the size in *P. vivax* infections [38]. The timing of producing blood films is crucial after finger prick blood is obtained. Delay in blood film production could lead to the change in parasite morphology [37, 38]. As parasite morphology was not the focus of this dissertation, the threat of changing its staining characteristics was minimized and not further discussed in this dissertation.

1.2.6 Current Treatment

Malaria is a treatable and preventable disease [39]. However, without prompt diagnoses and immediate treatments, uncomplicated malaria cases could become severe (Table 1.13) or even fatal. Treatment for malaria could vary depending on (1) *Plasmodium* species, (2) endemicity of the area that a patient acquired malaria, (3) drug-resistance status of an area, (4) symptoms and existence of accompanying diseases that a patient presents, (5) pregnancy status and (6) current medications taken by a patient [40].

Uncomplicated P. falciparum Beginning in 2011, 79 countries have adopted artemisinin-based combination therapies (ACTs) as their first-line treatment for uncomplicated *P. falciparum* infection [41]. ACT combined artemisinin derivatives and a companion drug to enhance the efficacy against local strains of *P. falciparum* malaria. ACT also aims to increase the patient compliance in taking both components of medicine and slow the drug resistance of each individual ingredient of the medicine. Currently, three types of artemisinin derivatives (dihydroartemisinin, artesunate and artemether) and six companion medications (lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine and chlorproguanil/dapsone) are used in ACTs [42]. For uncomplicated *P. falciparum* malaria (except for pregnant women in their first trimester), patients are recommended with one of the five ACTs: (1) artemether and lumefantrine (AL), (2) artesunate and amodiaquine (AS+AQ), (3) artesunate and

mefloquine (AS+MQ), (4) dihydroartemisinin and piperaquine (DHA+PPQ), or (5) artesunate and sulfadoxinepyrimethamine (AS+SP) [43].

Uncomplicated P. vivax Chloroquine is the first-line medicine for areas without known chloroquine-resistance [41]. For uncomplicated *P. vivax*, ACTs as shown in the previous paragraph are also recommended. In areas with chloroquine-susceptible infections, uncomplicated *P. vivax* cases (except for pregnant women in their first trimester) could have a choice between ACT and chloroquine [43]. However, in areas with chloroquine-resistant infections, chloroquine is not recommended. *P. falciparum* infected pregnant women in their first trimester should take quinine and clindamycin. *P. vivax* infected pregnant women in their first trimester should take chloroquine (if in areas with chloroquine-susceptible infections) or quinine (if in areas with chloroquine-resistant infections) [43]. Primaquine is another medicine aims to fight against dormant form of *P. vivax* in the liver stage, called hypnozoites. It is currently known as the only drug that prevents relapse of *P. vivax*. However, the use of primaquine can also trigger Glucose-6-phosphate dehydrogenase (G6PD) deficiency in patients without G6PD gene on their X chromosome. When the first-line chloroquine is not sufficient in clearing the blood-stage malaria, or when patients are not eligible in taking primaquine, it is recommended for patients to be treated with ACT [41].

Severe malaria infections Patients with severe malaria should be treated with intravenous or intramuscular artesunate (one of artemisinin derivatives), follow by a complete treatment course of ACT [41, 43].

1.2.7 Current Intervention Strategies

Vector control, preventive chemotherapy for malaria, case management, and vaccine development are current strategies in malaria control and prevention.

Vector Control

Vector control strategies include the use of insecticide-treated nets (ITNs), indoor residual spraying (IRS) and larval source management [44]. ITNs and IRS are discussed in detail below.

Insecticide-Treated Nets ITNs are designed to shorten the life span of a mosquito by contacting coated insecticide with bed nets. Recommended insecticides to create long lasting effects of mosquito bed nets are pyrethroids [45] derivatives, including permethrin and deltamethrin [46]. To reach its maximum performance, an ITN is best distributed for every 1.8 people. Figure 1.7 shows results from the household survey conducted by World Health Organization (WHO) in 2003-2011 in Africa. Overall, there was an increase in proportion of the population sleeping under an ITN in all age group. However, most households still have inadequate numbers of bed nets. Other findings include individuals that are (1) younger, (2) wealthier and (3) residing in urban areas are more likely to sleep under an ITN [44]. Due to more diversified malaria risk outside of Africa, it is difficult to estimate the number of people who are in need

of ITNs. To reach universal coverage of ITN, not only are more ITNs needed per household but also equity in ITN distribution should also be considered.

Indoor Residual Spraying IRS is recommended to be used in 80 countries. Pyrethroids is currently the primary insecticide used for spraying. Among the 24 countries with reported use of IRS, 75% of them use pyrethroids. The other 25% of the countries applies carbamates and dichloro-diphenyl-trichloroethane (DDT) as the agent. By the end of 2011, 11%, 5%, 4% and 2% of population in African region, American region, Southeast Asia region and Eastern Mediterranean region are estimated to be protected by IRS, respectively [44]. Figure 1.8 shows countries with reported insecticide resistance against at least one of Anopheles species. To allow IRS remain effective, studies on insecticide resistance is urgently needed from all applicable countries.

Preventive Chemotherapy

Preventive chemotherapy for malaria targets pregnant women (i.e. Intermittent preventive therapy for pregnant women, IPTp), infants (i.e. Intermittent preventive therapy for infants, IPTi) and areas with seasonal malaria transmission (i.e. Seasonal malaria chemoprevention, SMC—it was previously termed intermittent preventive therapy for children, IPTc) as preventive measures [47].

Intermittent Preventive Therapy (IPT) IPTp, IPTi and SMC are strategies used to prevent malaria infection by administering preventive medicine to vulnerable population. Recommendation revised in 2012 by WHO [47], it aims

to target pregnant women, infants and children aged 3-59 months predominantly in sub-Saharan Africa and in Sahel subregion in following ways.

Intermittent Preventive Therapy for Pregnant Women (IPTp) Pregnant women in moderate and high malaria transmission areas were previously suggested to receive IPTp with sulfadoxine-pyramethamine (SP) for at least 2 doses at antenatal care clinics during the second and third trimesters. Under this recommendation (used prior to 2012), the coverage of IPTp was low in sub-Saharan Africa compared to attendance at antenatal care services [48]. Uncertainty about sulfadoxine-pyramethamine administration for IPTp among health workers was speculated to be associated with declining efforts in scaling up IPTp [3]. Since 2012, recommendation has been updated and advises pregnant women living in moderate and high malaria transmission areas in sub-Saharan Africa to receive IPTp with SP at all antenatal care visits after the first trimester [47].

Intermittent Preventive Therapy for Infants (IPTi) Through Expanded Programme on Immunization (EPI) services, infants in moderate and high malaria transmission area in sub-Saharan Africa are scheduled to be vaccinated at 10 weeks, 14 weeks, and 9 months of age. WHO recommended infants in these areas that are without SP resistance in malaria parasites should receive IPTi with SP along with second and third doses of diphtheria-pertussis-tetanus (DPT) and measles vaccination. Burkina Faso was the first and the only country up till 2012 to adopt this recommendation as its national policy [47].

Seasonal Malaria Chemoprevention (SMC) Previously named Intermittent Preventive Therapy for Children (IPTc), SMC revised its recommendation in December 2012 and aims to target children aged 359 months in high malaria transmission and highly seasonal transmission area in Sahel subregion in Africa to receive full course of preventive medicine. The preventive medicine used is one of the five recommended ACTs: amodiaquine plus sulfadoxine-pyrimethamine (AQ+SP). The combination therapy should be administered at the beginning of the malaria transmission, and continue to be provided once a month, up to four doses, throughout the entire transmission season. Due to drug resistance of amodiaquine or sulfadoxine-pyrimethamine in some regions in Africa, only Sahel subregion is currently recommended to have SMC [47].

Case Management

Case management is considered as part of the malaria control program. As health facilities are not the only place for malaria diagnosis and treatment, strategies in regards to access to care and bringing effective malaria treatments to communities are the core principle of case management [49]. Examples of case management strategies include (1) patients of all ages should receive diagnostic test, (2) rapid diagnostic tests (RDTs) used at community level, (3) all cases detected in the private/public sector are microscopically confirmed, (4) nationwide microscopy quality assurance system covers public and private sectors, (5) artemisinin-based combination therapies (ACTs) are free for all ages in public sector, (6) radical treatment with primaquine for *P.vivax*, (7) treatment with ACT plus single dose primaquine for *P. falciparum*, (8) pre-referral treatment

with recommended medicines and (9) oral artemisinin-based monotherapies are not registered [50].

Having an effective Integrated Community Case Management (iCCM) program is also important [51]. The goal of a successful iCCM is to extend case management of malaria beyond health facilities. For example, by recruiting and training community health workers, diagnosis and referral of malaria could be done at the community level. More rural residents could have better access to care. More detailed information on iCCM could be found on the website of U.S. Agency for International Development (USAID) [51].

Vaccine

Vaccine development has been considered as one new tool in malaria control. Vaccine development has begun since 1970s. Currently, one vaccine—RTS,S/AS01 (RTS,S) (GlaxoSmithKline Biologicals (GSK))—that targets *P. falciparum* have finished its Phase III clinical trial. There are 20 other candidates in early phase I and phase II trials. Based on results of Phases I, II and III of RTS,S clinical trials, WHO created a position paper in January 2016 [5]. In sum, RTS,S vaccine is an additional method to current malaria prevention methods. The Phase III efficacy trial showed the need for four doses of RTS,S—where the first three doses should be administered one month apart and a fourth doses should be given 18 months later. Phase IV studies on safety and effectiveness are needed. Pilot studies will be rolled out in 3-5 countries in Africa. [5]

1.3 Malaria on the Global Scale

1.3.1 Disease Burden

Over the past century, the land cover of malaria has reduced by half [52]. Temperate areas, such as Russia and northern Europe, have no longer found human supported malaria cases since 1946. By 1965, most of the European countries, the United States of America and Australia have eliminated indigenous human malaria cases. Nowadays, regions and countries that are still suffering from malaria are roughly located within 30 degrees north and south of the equator (Figure 1.9). Nonetheless, the population growth rate in the past tens of decades outweighs the speed of shrinkage of the malaria territory. According to WHO Malaria Report in December 2015, there were 214 million malaria cases and 438,000 deaths worldwide in 2015 [3]. It was estimated 78% of deaths attributable to malaria were children under five [53].

To date, pneumonia, diarrhea and malaria remain the top causes of deaths for children aged 1-59 months (Figure 1.10 [53] and Table 1.14) [54]. In Africa, 15% of 3.552 million (0.540 million) deaths in children under 5 were attributed to malaria. In Southeast Asia, 1% of children under 5 years old died because of malaria [54]. To categorize severity of malaria endemicity, Lysenko [52] defined the endemicity of malaria into hypoendemic, mesoendemic, hyperendemic and holoendemic based on age-standardized proportion of *P. falciparum* parasites found in the 2-to-10-year age group (except for holoendemic) and in 1-year-old age group (holoendemic). When the age-standardized proportion of *P. falciparum* parasite rate found in the 2-to-10-year age group ($PfPR_{2-10}$) is less

than 10%, it is called hypoendemic. $PfPR_{2-10}$ in between 11% to 50% is defined as mesoendemic. $PfPR_{2-10}$ ranges in 51-70% is identified as hyperendemic. Last but not least, holoendemic is when age-standardized proportion of *P. falciparum* parasites found in 1 year old children ($PfPR_1$) reaches over 75%.

As shown in Figure 1.11, countries in Sub-Saharan Africa are mostly classified as mesoendemic or hyperendemic malaria areas. Countries in South and Southeast Asia, on the other hand, are mostly hypoendemic or mesoendemic. Although malaria endemicity is defined by parasite prevalence of *P. falciparum*, prevalence of *P. vivax* cannot be overlooked. Gething and colleagues updated the work from Geurra et al. and used 9,970 worldwide community survey results collected from 1985 to 2010 to estimate the global distribution of *P. vivax* [55]. The Bayesian based modeling method has shown the prevalence of *P. vivax* found in people aged 1 to 99 worldwide ($PvPR_{1-99}$) is in general 10% or lower (Figure 1.12). Countries that are heavily affected by *P. vivax* in 2010 are located in Central, South America and Southeast Asia.

The transmission risk of *P. vivax* can be stratified as stable risk and unstable risk based on the annual parasite incidence (API). If the annual parasite incidence of *P. vivax* ($PvAPI$) is less than 0.1 per 1000 people per annum, the transmission is considered as unstable. The *P. vivax* transmission is considered stable when annual parasite incidence is greater than 0.1 per 1000 people per annum. It was estimated 2.488 billion people were at risk of *P. vivax* malaria in 2010 worldwide. Among those, 964.90 million people lived in stable transmission areas (15,350,000 km^2) and 1.523 billion people lived in unstable transmission

areas (28,550,000 km^2). South and Southeast Asia, in particular, have 2.264 billion people—which consisted of 90% of the worldwide population—at risk of *P. vivax*. Central and South America came in second with 137.45 million people at risk of malaria. Africa was in third with 86.38 million people at risk of malaria.

One factor that affected global distribution of malaria infection was the existence of inherited blood disorders in certain population. Inherited blood disorders such as Sickle cell disease, Duffy negative and Glucose-6-phosphate dehydrogenase (G6PD) deficiency can affect one's susceptibility in malaria infections. Sickle cell disease is presented by two copies of mutated sickle hemoglobin (HbS), causing red blood cells retain a sickle shape. People who inherit only one copy of the HbS allele ("sickle cell trait") are an asymptomatic carrier to sickle cell anemia. These people are less vulnerable to malaria infection. Figure 1.13 demonstrates the global distribution of predicted HbS frequency. Individuals with Duffy negative are people who have mutated Duffy glycoprotein on their red blood cells. The mutated Duffy glycoprotein prevents its human host from being infected with *P. vivax*. However, researchers at American Society of Tropical Medicine and Hygiene (ASTMH) Conference 2013 (November 13-17) found *P. vivax* is potentially evolving and have more ways to infect people (personal communication). It is estimated 95% of the African people who were protected from *P. vivax* by having mutated Duffy glycoprotein could potentially be susceptible now (Figure 1.14 [56]. G6PD is in short of Glucose-6-phosphate dehydrogenase. It is a gene on X chromosome. As women has two copies of X chromosome, it is less likely for women than men to be G6PD deficient. G6PD

deficiency can be triggered by chemicals such as primaquine, a drug used to clear relapse of *P. vivax*. Individuals with G6PD deficiency may suffer from jaundice and hemolytic anemia. The global distribution of G6PD deficiency can be found in Figure 1.15 [57].

1.4 Malaria in Asia

Outside Africa, South and Southeast Asia consists of the largest number of malaria cases and deaths [3, 20, 45, 53]. There were two billion people at risk of malaria and approximately 525 million people live in areas with high risk of malaria in 2010 and 2011 (Table 1.15) [45, 58, 59, 60, 61, 62]. Control efforts done in the past decades have made Taiwan, Australia, Singapore and Brunei Darussalam malaria-free. The efforts also brought Sri Lanka and Republic of Korea (South Korea) into the elimination phase in 2012 (Table 1.16) [63].

Fast forwarded to 2015, 2.3 billion people were at risk of malaria in countries of WHO South-East Asian Region and Western Pacific Region. Of which, approximately 261 million people were at high risk of malaria infections. Countries that are in the pre-elimination phases are Malaysia, Bhutan and Democratic People's Republic of Korea ("North Korea"). Countries that are in the elimination phase in 2015 were China and Republic of Korea ("South Korea"). Sri Lanka is now at the malaria prevention of reintroduction phase. The rest of the countries in WHO South-East Asian and Western Pacific Regions (e.g. Bangladesh) remained in the control phase [3].

Overall, it is a global trend to have fewer countries with ongoing malaria transmission. However, malaria burden remains high in populous neighborhoods, remote forest areas, tribal groups and border areas [45]. Among countries in south and southeast Asia, India, Indonesia, Myanmar, Bangladesh and Timor-Leste have the highest burden in the region (Figure 1.16).

For the next 15 years (2016-2030), WHO set the vision for a malaria-free world. The goals to achieve it include: (1) Reduce malaria mortality rates globally compared with 2015 by at least 90%, (2) reduce malaria case incidence globally compared with 2015 by at least 90%, (3) eliminate malaria from countries in which malaria was transmitted in 2015 by at least 35 countries, and finally, (4) prevent re-establishment of malaria in all countries that are malaria-free (ie. re-establishment prevented) [64].

1.5 Malaria in Bangladesh

Overview

Bangladesh locates in South Asia, sharing its border with India and Myanmar. It is a country established after the Liberation War in 1971. Bangladesh currently has almost 161 million residents with an average population density greater than 1000 people per squared kilometer [58, 65]. Based on a nationwide survey conducted in 2007 [66], thirteen out of the 64 districts were identified as malaria endemic areas. In these 13 districts, 26.9 million people (17.8% of

the Bangladeshi population) were at risk of malaria. In 2010, its number of population at risk of *P. falciparum* ranked number 13 worldwide (Table 1.17) and ranked number 10 globally for population at risk of *P. vivax* (Table 1.18) [59, 60].

The thirteen endemic districts in Bangladesh include Kurigram, Sherpur, Mymensingh, Netrakona, Sunamgani, Sylhet, Maulvibazar, Habigani, Khagrachari, Rangamati, Chittagong, Bandarban and Coxs Bazar (Figure 1.18) [66]. All 13 districts are located along the Bangladeshi boarder, adjacent to India and Myanmar. Historically, this is, however, not the case. Bentleys malaria map in 1916 indicated western Bangladesh contained the highest malarial cases with a gradient of severity decrease from west to east (Figure 1.19) [67]. The change in geographic distribution of malaria endemic districts was presumed to be due to the ignorance of remote and hilly areas when malaria control and elimination efforts were first carried out in the country.

Over the past 50 years, Bangladesh has rolled out several malaria control programs which included Malaria Eradication Program (MEP), Primary Health Care and Control Program, Early Diagnosis and Prompt Treatment (EDPT) Program and programs carried out by the Malaria Research Group (MRG), Roll Back Malaria (RBM) and Global Fund for AIDS, TB and Malaria (GFATM), including the ongoing National Malaria Control Program (NMCP).

The first malaria program in the country—Malaria Eradication Program (MEP)—was executed from 1968 to 1974. Preparatory, Attack, Consolidation and Maintenance were the four phases in the program. During this time, the MEP brought down the malaria incidence from 11 to 5 malaria cases per 10,000 population a year [67]. With the waning incidence in malaria, the MEP efforts were loosened in 1971. In between 1972 and 1977, Liberation War in the country interrupted the MEP program and led to many homeless people. Development of the camp, population migration and replacement, change in livestock and mosquito habitats, and emergence of malnutrition became severe.

Malaria re-surged in the country and hit 65 malaria cases per 10,000 population per year [67]. MEP was then merged with the primary health care and control program in 1977. After 40 years of commonly using DDT as a mean of vector control, DDT was banned in Bangladesh in 1991. Malaria incidence quickly raised and reached a new record of 18.57 per 10,000 people per year in 1994 [67]. Same year, the government adopted and revised the Early Diagnosis and Prompt Treatment (EDPT) program. In 1996, the Malaria Research Group (MRG) emerged. In 1998, the Roll Back Malaria piloted the program in the country.

Starting in 2006, the Bangladesh government received financial assistant from Global Fund for AIDS, TB and Malaria (GFATM) and started National Malaria Control Program (2007-2014) focusing the efforts on the 13 endemic districts. Intervention includes the use of rapid diagnostic tests (RDT) at the local level and artemisinin-based combination therapy (ACT) as the first-line of treatment.

In 2015, the National Malaria Control Program (NMCP) was updated to match the goal of malaria elimination by 2020 worldwide, as well as, the development goal set by the Government of Bangladesh. Details of the Malaria National Strategic Plan can be found the NMCP website [6].

A Nation-wide Survey

A cross-sectional survey was done by the International Centre for Diarrhoea Disease Research, Bangladesh (icddr,b) and Building Resources across Communities (BRAC), a local non-government organization (NGO). This 2007 study found among the 13 endemic malaria districts, 89% of malaria infections came from *P. falciparum*, 5% of malaria infections were caused by *P. vivax* and 6% of the Rapid Diagnostic Tests (RDT) positive cases had mixed infections from both *P. falciparum* and *P. vivax* (Figure 1.18) [66]. Among 13 endemic districts, 3 of them (ie. Khagrachari District, Bandarban District and Rangamati District) locate in Chittagong Hill Tracts.

Chittagong Hill Tracts is at southeastern side of the country. The three districts had the highest malaria prevalence (13%) overall, comparing to all other districts during the 2007 survey. To be more specific, malaria prevalence was 15.5% in Khagrachari District, 10.7% in Bandarban District and 6.8% in Rangamati District [66]. Higher malaria prevalence in Chittagong Hill Tract could potentially be traced back to its historic background and health seeking behaviors of the residents.

Historic Background of Chittagong Hill Tract Chittagong Hill Tract (CHT) consists of 47% of the forest in Bangladesh. It is a place where Jummas live. Jumma is a collective term to call indigenous tribal people in Bangladesh. There are 12 tribes in Chittagong Hill Tracts: Bawm, Chak, Chakma, Khyang, Khumi, Lushai Marma, Mro, Pangkhoa, Tanchangya, Rakhaine and Tripura. Each one of them has their own cultures and languages. Along with Bangali immigrants, there were approximately 1.6 million people living in the Chittagong Hill Tracts according to the Bangladeshi census in 2011 [68, 69, 70]. The primary agricultural activities in the area is called "Jhum cultivation" (or "Jum cultivation") [71], where Jummas shift cultivation areas after growing crops in a plot of lands to allow time for the cultivated land to revert to its natural state. The creation of Kaptai Dam in Rangamati District (Figure 1.18) from 1957 to 1962 submerged 40% of the cultivable land in the area and displaced more than 18,000 Jumma families [71]. Followed by the Liberation War, the Government of Bangladesh and uncompensated Jummas had more than two decades of military activities against local armed organizations (Jana Samhati Samiti [72]). On December 2nd, 1997, Chittagong Hill Tracts Peace Accord—a peace treaty—was signed between the Government and Jana Samhati Samiti to return refugees, allow regional autonomy, and let BRAC to initiate development in Chittagong Hill Tracts [73].

Health Seeking behavior in Chittagong Hill Tract After the peace treaty [73] signed in Chittagong Hill Tracts (CHT) (Section 1.5), BRAC conducted a survey in summer 1998 to understand the level of development in CHT [71]. A stratified sampling was done based on the the ethnic group. A total of

2,550 household were selected. That is, among all ethnic groups that have more than 20,000 people (Bangali, Chakma, Marma, Mro and Tripura), 30 villages were randomly selected in each ethnic group. Within each village, 17 households were randomly chosen as survey participants. Which yields $5 * 30 * 17 = 2550$ households. Demographic information of the study population can be found in Table 1.20 [71]. Based on the 15-day recall history, the study found it was more common to see malaria in Bangali people (Malaria was self-reported and distinguished from fever by periodicity, the identification of shivering, and decreasing in body temperature with sweating) (Table 1.21) [71]. Para-professionals (i.e. village practitioners, medical assistants, para-medics, community health workers of government organization and non-government organization who have some formal exposure to allopathic medicine) and kabiraz (practitioners of Ayurvedic medicine) are the main health providers (Table 1.22) [71]. However, 40% of the ill population would seek unqualified allopaths as their first contact and 14% will not seek any medical treatment (Table 1.23) [71]. This situation is more common in tribal groups than in Bangali population (Figures 1.24 and 1.25) [71]. Females had less odds in seeking health care or allopathic care when compared to males. Wealthier household (have 50 decimals of lands) is more likely to seek medical care when ill. Distance from static health facilities and having self-perceived malaria have a significant impact on deciding whether seeking medical or allopathic care is necessary (Table 1.25) [71].

Risk Factors

Many malaria studies in Bangladesh were carried out by Haque and colleagues in Rajasthali under Rangamati District [74, 75, 76]. In Rajasthali, it was shown ethnic groups, materials of the floor, materials used for the wall, bed net ratios per person per household, forest densities and altitudes of household location were potential risk factors attributable to malaria risk. Gender differences, age groups, levels of education, occupations, bed net ownership and numbers of bed nets at home, all family sleep under bed nets, household density, distance to stream, aspect and wetness (a proxy for capability of water accumulation) are not dominant risk factors for malaria. (Figures 1.26 and 1.27)

In Khagrachari, researchers found locations of households being 3 kilometers or less from the forest or the water, precarious housing, less than 3 bed nets, and being younger than 17 years old were risk factors that were associated malaria transmission [77].

Conclusion

From 2012 to 2014, Bangladesh targeted high risk populations to distribute ITNs and antimalarial medicines. By 2014, at least 60% of the population at high risk had access to ITNs. From 2000 to 2014, Bangladesh along with 5 other countries (Bhutan, Democratic Peoples Republic of Korea, Nepal, Timor-Leste and Sri Lanka) in the WHO South-East Asian Region have achieved a $\geq$75% decrease in incidence of confirmed cases [3]. However, the threat to drug resistance (e.g. AS + MQ in Thailand) and insecticide resistance (e.g. pyrethroids)

have begun in the region. Meanwhile, the existence of many asymptomatic cases and highly seasonal malaria transmission remain a challenge.

To achieve a national goal (and contribute to the worldwide vision) of malaria elimination by 2020, more data are needed. We need information not only on how to scale up malaria prevention and control methods to the national level, but also on the know-how in targeting a small focal area where a higher malaria risk exists. Here, this dissertation focused on Bandarban District, a relatively high malaria transmission zone in Bangladesh. We aimed to find out what potentially contributed to the ongoing transmission in the area (i.e. "Bandarban Study Area").

1.6 Study Aims

Five Aims were explored in the dissertation. These aims looked at (1) field performance of FalciVax^{TM} rapid diagnostic tests, (2) relationship between clinical malaria symptoms and measured parasite density, (3) relationship between household building materials and the abundance of *Anopheles* mosquitoes, and finally, (4) relationship between living standards and malaria incidence.

1.6.1 Aim 1. Field Performance of FalciVax^{TM}] Rapid Diagnostic Tests

Motives FalciVax^{TM} rapid diagnostic test (RDT) is a front-line malaria diagnostic tool used in Bangladesh. Starting in 2008, WHO has provided laboratory

based performance tests for more than 200 commercial rapid diagnostic test devices. FalciVax^{TM} RDT was one of the models being tested. In both Phase I (N = 20) and Phase II (N = 98) tests, FalciVax^{TM} RDT has shown a score of 95 or above for accuracy. However, limited information has been documented with its field performance. Prior studies have shown the overall field performance under clinical settings, shorter time frames and/or smaller studied population. A field performance test of Falcivax^{TM} RDT under a multi-year population-based surveillance project at non-clinical facilities have not been done. We hoped to examine field performance of FalciVax^{TM} RDTs across years and seasons, between febrile and non-febrile individuals, and among individuals with various parasite density levels.

Hypothesis We hypothesized field performance of FalciVax^{TM} RDT would be the same across time. Its performance could be the same or better with febrile individuals and individual with higher parasite density, comparing to non-febrile individuals and individuals with lower parasite density, respectively.

Methods We used Giemsa-stain microscopy as our gold standard. We collected data from passive surveillance through home visits at Bandarban Study Area in Chittagong Hill Tracts, Bangladesh in 2009-2013. We created contingency tables to compare laboratory results from microscopy and field results from FalciVax^{TM} RDT. We further evaluated and modeled sensitivity, specificity, positive and negative predictive values by time, febrile statues and parasite density.

Results Findings from the study can be found in Chapter 3.

1.6.2 Aim 2. Relationship between Clinical Malaria Symptoms and Measured Parasite Density

Motives Although exception exists, people with higher parasite density tend to have a more serious malaria infection. However, how levels of parasite density are associated with malaria symptoms have not been fully explored. This is mainly due to malaria symptoms are not disease specific. For example, symptoms presented by malaria infected individuals include, but not limited to, fever, headache and chills. In a hypoendemic malaria setting such as Bandarban Study Area, improving case awareness and case detection of malaria on a population based level is crucial to reduce malaria incidence. We aimed to study the relationship between levels of parasite density and 17 malaria-related symptoms, symptom duration, fever status and basic demographic information among local residents in Bandarban Study Area. This information could hopefully (1) help local health professionals to conduct future reactive case search and (2) help locals be more aware of the symptoms and its severity in regard to malaria. With timely treatment of malaria cases, we can not only prevent cases from becoming more severe but also breaking potential transmission cycles for having additional cases.

Hypothesis We hypothesized levels of parasite density could be affected by following factors: (1) age and body mass index, but not gender, (2) presence of fever at the time of diagnostic test, (3) presence of specific malaria symptoms,

(4) number of (fever and non-fever related) symptoms, and (5) duration of malaria-related symptoms.

Methods We used population based passive surveillance system to enroll our study population from 2009 to 2013. During each home visit, we conducted malaria diagnostic tests (microscopy and FalciVax^{TM} RDT) and collected demographic and symptom information through standard questionnaires. Body temperatures were also measured at home visits. Measured body temperatures were used to define fever status of study participants. Logistics regressions were used to study the relationship between levels of parasite density (dependent variable) and following independent variables: (1) variables related to demographic information, (2) fever status, (3) 17 individual symptoms, (4) number of symptoms, (5) duration of symptoms, (6) joint effect of fever status and 17 individual symptoms.

Results Findings from the study can be found in Chapter 4.

1.6.3 Aim 3. Relationship between Household Building Materials and the Abundance of *Anopheles* Mosquitoes

Motives With the decline in malaria cases and the increase in at risk population for malaria, we hypothesized a growing asymptomatic malaria population existed in Bangladesh. To eliminate malaria, simply focusing on malaria treatment and control would not be enough. *Anopheles* mosquitoes are the vector for malaria transmission. Their resting surface, such as walls of a household,

could be distally related to the transmission cycle. Previously, only one study in Bangladesh examined relationship between household wall materials and clinical malaria. However, how household building materials (used across wall, roof, partition and floor) is related to population dynamics of *Anopheles* mosquitoes have yet to be documented. We aimed to used the relationship between numbers of mosquitoes and types of housing materials as a stepping stone to better understand the relationship among numbers of mosquitoes, various types of housing facilities and risk of human malaria in Bandarban study area.

Hypothesis We hypothesized different types of household building materials would have different effect on numbers of *Anopheles* mosquitoes in the household. We hypothesized mud and bamboo would be the two major factors associated with numbers of *Anopheles* mosquitoes. However, we did not hypothesize whether mud or bamboo would be more important in relation to population dynamics of *Anopheles* mosquitoes.

Methods From 2009 to 2013, we collected information on household building materials (i.e. type of materials used for wall, roof, partition and floor) from all households in Bandarban Study Area. We also gathered information on whether houses had elevated ground floor. From 2009 to 2012, we sampled households from the study area and conducted entomological survey. In entomological survey, *Anopheles* mosquitoes were collected by using CDC Light Traps. Linear regressions were done to see relationship between numbers of *Anopheles* mosquitoes and individual materials used at different household sections. In addition, we analyzed the combined effect of all materials used at a

single household and its relation to the abundance of *Anopheles* mosquitoes. Finally, we examined the areal effect of housing materials on numbers of *Anopheles* mosquitoes at the household level.

Results Findings from the study can be found in Chapter 5.

1.6.4 Aim 4. Relationship between Living Standards and Malaria Incidence

Motives Limited information on the relationship between malaria incidence and living standards has been discussed previously in Bangladesh. Prior studies have focused on the various species of *Anopheles* mosquitoes, demographic risk factors and weather in Chittagong Hill Tracts in relation to prevalence or odds of malaria. To transition from malaria control phase to malaria pre-elimination phase according to WHO standard, we cannot overlook any potential attributable factor from all aspects. Living standard could be seen as a way of life as well as risk of acquiring diseases. We hope to bridge the gap in knowledge by studying the relationship between malaria incidence and living standards (e.g. socioeconomic status, household building materials used).

Hypothesis We hypothesized people with higher living standards are at lower risks for acquiring malaria. We also hypothesized living standards could be represented by a combination of following factors: age, gender, marital status, length of education, occupation, employment status, source of income, land ownership and cultivation situation, source of water, light and fuel, type of toilet used, household building materials used and 33 durable assets (e.g. electricity,

fan, bed).

Methods We collected information on ownership of assets, household building materials and living environment from all households in Bandarban Study Area from 2009 to 2013. Meanwhile, individual's demographic information was also documented. We calculated each exposure time based on participants' entry dates. Malaria cases were identified through passive surveillance. We applied Generalized Estimating Equation (GEE) Poisson regression to analyze the relationship between malaria incidence and individual's (1) demographic information, (2) living environment, (3) assets, (4) household building materials, and (5) joint effect of assets (as represented by the first principle component) and building materials.

Results Findings from the study can be found in Chapter 6.

Table 1.1: Summary of bionomics of the DVS of the Americas - 1 of 2. Table was adopted from reference [78].

Species	**Larval site characteristics**
An. albimanus	Sunlit, brackish or fresh, clear, still or flowing water, containing higher plants or algae
An. albitarsis complex	Sunlit, fresh, clear or turbid, still water with some higher plants or algae
An. aquasalis	Sunlit, brackish or fresh, clear or turbid, still or flowing water with some higher plants or algae
An. darlingi	Shaded (occasionally sunlit), fresh (occasionally brackish), clear or turbid, still or flowing water with higher plants or algae
An. freeborni	Sunlit, fresh, clear, still water with higher plants or algae
An. marajoara	Sunlit (occasionally shaded), fresh, clear or turbid, still or flowing water with higher plants or algae
An. nuneztovari complex	Sunlit or shaded, fresh, clear or turbid, still or flowing water with higher plants or algae
An. pseudopunctipennis	Sunlit, brackish or fresh, clear or turbid, still or flowing water with higher plants or algae
An. quadrimaculatus complex	Sunlit, fresh, clear or turbid, still water with higher plants or algae. Occasionally no vegetation

Table 1.2: Summary of bionomics of the DVS of the Americas - 2 of 2. Table was adopted from reference [78].

Species	**Host**		**Biting**		**Resting**		**Other**
	Anthropophilic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. albimanus	•	•		•		•	Bites at dusk/night
An. albitarsis complex	•	•	•	•	○	•	Bites at dusk/night
An. aquasalis	•	•	•	•	-	•	An ability to utilise brackish coastal larval habitats raises this species from a relatively poor vector to a DVS. Has been found biting in the day but mostly bites at dusk/night
An. darlingi	•	○	•	•	-	•	Bites at dusk, night and dawn
An. freeborni	•	•	•	•	-	•	Bites at dusk, night and dawn
An. marajoara	•	•	•	•	-	•	Bites at dusk/night
An. nuneztovari complex		•		•	-	•	Bites at dusk, night and dawn
An. pseudopunctipennis	•	•	•	•	•	•	Larval habitats are strongly associated with filamentous algae, and species can exist at high altitudes (up to 3000 m). Bites at night
An. quadrimaculatus complex		•	-	•	-	•	Bites at dusk, night and dawn and occasionally in the day

Filled dot (•) typical behavior; Open dot (○): non-typical behavior but examples exist; Dash (−) no data

Table 1.3: Summary of bionomics of the DVS of Africa, Europe and Middle East group - 1 of 2. Table was adopted from reference [78].

Species	**Larval site characteristics**
An. arabiensis	Sunlit (occasionally shaded), fresh (occasionally brackish), clear or turbid, still or flowing water with higher plants or algae (occasionally without vegetation)
An. funestus	Sunlit or shaded, fresh (occasionally brackish), clear, still or flowing water with higher plants or algae (occasionally without vegetation)
An. gambiae	Sunlit (occasionally shaded), fresh (occasionally brackish), clear or turbid, still or flowing water with or without higher plants or algae
An. melas	Sunlit or shaded, fresh or brackish, clear or turbid, still water with higher plants or algae
An. merus	Sunlit or shaded, fresh or brackish, clear or turbid, still water with higher plants or algae
An. moucheti	Sunlit (occasionally shaded), fresh, clear (occasionally turbid), still or flowing water with higher plants or algae
An. nili complex	Sunlit or shaded, fresh, clear, still or flowing water with higher plants or algae

Table 1.4: Summary of bionomics of the DVS of Africa, Europe and Middle East group - 2 of 2. Table was adopted from reference [78].

Species	**Host**		**Biting**		**Resting**		**Other**
	Anthropophilic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. arabiensis	•	•	○	•	○	•	Bites at dusk/night and occasionally at dawn. Species shows high behavioral plasticity and readily adapts in response to control
An. funestus	•	○	•	•	•	○	Bites at dusk, but mainly during the night and to a lesser extent at dawn
An. gambiae	•	○	•	•	•	•	Larval site characteristics are influenced by molecular and/or chromosomal form
An. melas	•	•	•	•	○	•	Unlike other DVS, An melas densities tend to link to tides rather than rainfall
An. merus	•	•	○	•	○	•	Despite also being a coastal vector, An. merus is not influenced by tides like An. melas, nor can it tolerate the same levels of salinity.
An. moucheti	•	○	•	○	•	○	Range entirely restricted to equatorial forests. This vector is highly anthropophilic and endophilic.
An. nili complex	•	○	•	•	•	•	Behavior depends on sibling, with An. nili being highly anthropophilic and the most important vector of the complex

Filled dot (•) typical behavior; Open dot (○): non-typical behavior but examples exist; Dash (–) no data

Table 1.5: Summary of bionomics of the DVS of Asia Pacific group - 1 of 5. Table was adopted from reference [78].

Species	Larval site characteristics
An. aconitus	Sunlit, fresh, clear (occasionally turbid), still or flowing water with higher plants and algae (occasionally without vegetation)
An. annularis	Sunlit, fresh, clear (occasionally turbid), still or flowing water with higher plants and algae (occasionally without vegetation)
An. balabacensis	Shaded (occasionally sunlit), fresh, still water with or without higher plants or algae
An. barbirostris complex	Sunlit or shaded, clear or turbid, still or flowing water with higher plants or algae (occasionally without vegetation)
An. culicifacies complex	Sunlit, fresh (occasionally brackish) clear (occasionally turbid), still or flowing water with or without higher plants or algae
An. dirus complex	Shaded, fresh, clear or turbid, still water without vegetation
An. farauti complex	Sunlit or shaded, fresh or brackish, clear or turbid, stagnant (occasionally flowing) water with higher plants or algae (occasionally without vegetation)
An. flavirostris	Shaded, fresh, clear, flowing (occasionally still) water with higher plants or algae (occasionally without vegetation)
An. fluviatilis complex	Sunlit, fresh, flowing (occasionally still), water with higher plants or algae (occasionally without vegetation)
An. koliensis	Sunlit (occasionally shaded), fresh, clear, still water with higher plants or algae (occasionally without vegetation)
An lesteri	Shaded, fresh water with higher plants or algae
An. leucospyrus/latens	Shaded, fresh, clear or turbid, still water
An. maculatus (group)	Sunlit (occasionally shaded), fresh, clear (occasionally turbid), still or flowing water with higher plants or algae (occasionally without vegetation)
An. minimus complex	Shaded (occasionally sunlit), fresh, clear, still or flowing water with higher plants or algae (occasionally without vegetation)
An. punctulatus complex	Sunlit (occasionally shaded), fresh, clear or turbid, still water without vegetation (occasionally with higher plants or algae)
An. sinensis complex	Fresh, clear, still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)
An. stephensi	Sunlit or shaded, fresh (occasionally brackish), clear or turbid still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)
An. subpictus complex	Sunlit, brackish or fresh, clear or turbid, still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)
An. sundaicus complex	Sunlit (occasionally shaded, brackish (occasionally fresh), clear or turbid, still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)

Table 1.6: Summary of bionomics of the DVS of Asia Pacific group - 2 of 5. Table was adopted from reference [78].

Species	**Host**		**Biting**		**Resting**		**Other**
	Anthropophilic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. aconitus	○	●	●	●	○	●	Particularly favors both coast plain and upland rice fields as larval sites
An. annularis	○	●	●	●	●	○	Vector role depends on location. Possible complex of two (species A and B) siblings, but these do not appear to be linked to variable vector capacity
An. balabacensis	●	-	●	●	●	○	Primarily found in forested environments
An. barbirostris complex	○	●	○	●	○	●	The siblings within the complex are yet to be fully resolved and their distributions are unclear.
An. culicifacies complex	●	●	●	●	●	○	Bionomics dependent on sibling: Sp E = Anthropophilic; Sp A, B, C , D = Zoophilic)
An. dirus complex	●	○	●	●	-	●	Bionomics dependent on sibling but the two main vectors are An. dirus and An. baimaii. Anopheles scanloni is also anthropophilic but plays more focal role in transmission in Thailand

Filled dot (●) typical behavior; Open dot (○): non-typical behavior but examples exist; Dash (–) no data

Table 1.7: Summary of bionomics of the DVS of Asia Pacific group - 3 of 5. Table was adopted from reference [78].

Species	**Host**		**Biting**		**Resting**		**Other**
	Anthropophilic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. farauti complex	•	○	•	•	○	•	Anopheles farauti, An. hinesorum and An. farauti No. 4 are the only siblings considered to be important malaria vectors
An. flavirostris	○	•	•	•	○	•	Historically confused/misidentified as An. minimus. All records of An. minimus from the Philippines, Sabah (Malaysia) and Indonesia are now considered to be An. flavirostris
An. fluviatilis complex	•	•	•	•	•	•	Bionomics dependent on sibling. Species S is the most anthropophilic and endophilic and is the main vector of the complex. Species T and U are primarily zoophilic, exophagic and exophilic and non or poor vectors in India.
An. koliensis	•	○	•	•	○	•	Currently considered a single species but new evidence suggests it may be a complex of two or more species

Filled dot (•) typical behavior; Open dot (○): non-typical behavior but examples exist; Dash (–) no data

Table 1.8: Summary of bionomics of the DVS of Asia Pacific group - 4 of 5. Table was adopted from reference [78].

Species	**Host**		**Biting**		**Resting**		**Other**
	Anthropophilic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An lesteri	•	•	?	?	•	-	Anopheles lesteri is synonymous with An. anthropophagus.
An. leucospyrus/latens	•	-	•	•	-	•	Most reported information for An. leucosphyrus probably actually refers to An. latens
An. maculatus (group)	○	•	•	•	○	•	Vector role of individual species is unclear due to previous misidentifications based solely on overlapping morphological characteristics and due to apparent variability within species depending on location
An. minimus complex	•	•	•	•	•	•	Within the complex, only An. minimus and An. harrisoni are current vectors of malaria
An. punctulatus complex	•	○	•	•	○	•	Within the complex, only An. punctulatus is a known vector of malaria
An. sinensis complex	○	•	-	•	-	•	Possibly refractory to P. falciparum but an important vector of P. vivax

Filled dot (•) typical behavior; Open dot (○): non-typical behavior but examples exist; Dash (–) no data

Table 1.9: Summary of bionomics of the DVS of Asia Pacific group - 5 of 5. Table was adopted from reference [78].

Species	**Host**		**Biting**		**Resting**		**Other**
	Anthropophilic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. stephensi	○	●	●	○	●	-	One of the few anophelines able to flourish in urban areas
An. subpictus complex	○	●	●	●	●	○	The complex is currently considered to consist of four siblings: Species A, B, C and D although there is some confusion in the identification of Sp. B in some localities (may be a member of An. sundaicus complex)
An. sundaicus complex	●	○	●	●	●	●	The complex is currently considered to consist of four allopatric siblings:

Filled dot (●) typical behavior; Open dot (○): non-typical behavior but examples exist; Dash (–) no data

Table 1.10: Summary of malaria diagnostic methods - 1 of 3. Table was adopted from reference [22].

Method	**Principle of Method**
Clinical Diagnosis	Based on presenting malarial signs and symptoms
Microscopy †	Visualization of morphological distinguishable stage of parasites under light microscope by thick and thin blood smear and staining
QBC ‡	Blood staining with acridine orange and detection by epifluorescent microscope
RDT $^{\alpha}$	Detection of parasite antigens or enzyme
IFA $^{\beta}$	Detection of antibodies against parasites
PCR $^{\gamma}$	Specific amplification of malaria DNA
LAMP $^{\delta}$	Detection of turbidity by a turbidity meter after amplifying DNA sequences
Microarray	Hybridization of DNA isolate and quantified by fluorescence-based detection
FCM $^{\epsilon}$	Detection of hemozoin by flow cytometer
ACC $^{\zeta}$	Detection of malarial pigment in activated monocyte
MS $^{\eta}$	Identification of heme by laser desorption mass spectrometry (LDMS)

† Microscopy: peripheral blood smears; ‡ QBC: quantitative buffy coat; $^{\alpha}$ RDT: rapid diagnostic tests
$^{\beta}$ IFA: immunofluorescence antibody testing; $^{\gamma}$ PCR: polymerase chain reaction
$^{\delta}$ LAMP: loop-mediated isothermal amplification; $^{\epsilon}$ FCM: flow cytometry
$^{\zeta}$ ACC: automated blood cell counter; $^{\eta}$ MS: mass spectrometry

Table 1.11: Summary of malaria diagnostic methods - 2 of 3. Table was adopted from reference [22].

Method	**Sensitivity and Specificity**	**Time Consumed** (minutes)	**Detection Limit** (parasites/μl)
Clinical Diagnosis	Depends on malarial endemicity	Depends on physician's skill	Undetermined
Microscopy †	Depends on good technique, good reagent and microscopist's skill	30-60	Expert: 5-10 Routinely >50
QBC ‡	Higher than microscopy	<15	>5
RDT $^{\alpha}$	Moderate if more than 100 parasite/μl	10-15	50-100
IFA $^{\beta}$	Relatively high but not correlate to clinical symptoms of patients	30-60	Undetermined
PCR $^{\gamma}$	Excellent	45-360 depending on the methods	$\geq$1
LAMP $^{\delta}$	Excellent	<60	>5
Microarray	Relatively high	<60	>5
FCM $^{\epsilon}$	Variable sensitivity, high specificity	Automated, <1 min/sample	Poor correlation with parasitemia
ACC $^{\zeta}$	Variable in both sensitivity and specificity	Automated, <1 min/sample	5-20
MS $^{\eta}$	Undetermined	Automated, <1 min/sample	100 for whole blood

† Microscopy: peripheral blood smears; ‡ QBC: quantitative buffy coat; $^{\alpha}$ RDT: rapid diagnostic tests;
$^{\beta}$ IFA: immunofluorescence antibody testing; $^{\gamma}$ PCR: polymerase chain reaction; $^{\delta}$ LAMP: loop-mediated isothermal amplification;
$^{\epsilon}$ FCM: flow cytometry; $^{\zeta}$ ACC: automated blood cell counter; $^{\eta}$ MS: mass spectrometry

Table 1.12: Summary of malaria diagnostic methods - 3 of 3. Table was adopted from reference [22].

Method	Expertise Required	Instrument Cost	Other Considerations
Clinical Diagnosis	High: in non-endemic areas	N/A	Easy to follow the diagnostic algorithm. Results in significantly over-treatment of malaria, especially in highly endemic areas but underestimate in hypoendemic areas. Mixed infection is still problematic
Microscopy †	High: in non-endemic areas	Low cost	Gold standard. Good for all Plasmodium species, except P. knowlesi. P. knowlesi need considerable expertise. Mixed infection and low parasitemia may cause misdiagnosis
QBC ‡	Moderate	Moderate	Simple and user-friendly. Electricity is needed. Limit for species identification and quantitative parasite. Cannot store capillaries for later reference
RDT $^{\alpha}$	Low	Moderate	First line diagnostic in all areas. Suitable in field work. May not possible for differentiation between P. vivax, P. ovale, P. malariae. Limited for quantifying parasites. low parasitemia may cause misdiagnosis
IFA $^{\beta}$	Moderate	Moderate	Results can be influenced by trained technicians. Possible for all Plasmodium species. Useful for Epidemiological surveys. Not useful for treatment decision making
PCR $^{\gamma}$	High	Expensive	Second line diagnosis in well-equipped laboratories. Useful for identifying the development of drug resistance, species identification and quantifying parasite density at low parasitemia
LAMP $^{\delta}$	High	Moderate	Limit for quantifying parasites. Possible for all Plasmodium species. Clinical trials are needed to validate feasibility and clinical utility
Microarray	High	Expensive	Still in the eary stages of development for diagnosis of malaria
FCM $^{\epsilon}$	High	Expensive	Useful for diagnosis of clinically unsuspected malaria. Clinical trials are needed to validate feasibility and clinical utility
ACC $^{\zeta}$	High	Expensive	Clinical trials are needed to validate feasibility and clinical utility
MS $^{\eta}$	High	Expensive	Still in the eary stages of development for diagnosis of malaria

† Microscopy: peripheral blood smears; ‡ QBC: quantitative buffy coat; $^{\alpha}$ RDT: rapid diagnostic tests;
$^{\beta}$ IFA: immunofluorescence antibody testing; $^{\gamma}$ PCR: polymerase chain reaction; $^{\delta}$ LAMP: loop-mediated isothermal amplification;
$^{\epsilon}$ FCM: flow cytometry; $^{\zeta}$ ACC: automated blood cell counter; $^{\eta}$ MS: mass spectrometry

Table 1.13: Common Complication of Malaria Infection. Table information was extracted from reference [21].

Risk Group	Children	Adults	Pregnant Women
Clinical Features	• Severe malaria • Cerebral malaria • Anemia • Respiratory distress (acidosis) • Hypoglycemia • Shock • Dehydration and electrolyte disturbance • Children unable to retain oral medication	• Cerebral malaria • Anemia • Acute kidney injury • Hypoglycemia • Metabolic acidosis • Pulmonary edema • Shock • Abnormal bleeding and disseminated intravascular coagulation • Hemoglobinuria	• Severe malaria • Hypoglycemia • Pulmonary edema • Anemia

Table 1.14: Percent Deaths Attributed to Top Causes of Death in Children under Five Globally, in Africa and in Southeast Asia. Table information was extracted from reference [54].

Age Group	World **N=7.6 million**		Africa **N=3.5 million**		SE Asia **N=2.1 million**	
Neonates (0-27 days)		**40%**		**30%**		**52%**
	Preterm	14%	Preterm	10%	Preterm	19%
	Intrapartum	9%	Intrapartum	8%	Intrapartum	10%
	Sepsis/Meningitis	5%	Sepsis/Meningitis	4%	Sepsis/Meningitis	7%
					Pneumonia	7%
Post-Neonates (28 days-59 months)		**60%**		**70%**		**48%**
	Pneumonia	14%	Malaria	15%	Pneumonia	15%
	Diarrhea	10%	Pneumonia	14%	Diarrhea	10%
	Malaria	7%	Diarrhea	11%	Injury	4%
					Measles	3%
					Meningitis	2%
					Malaria	1%

Table 1.15: Malaria Country Profile in South Asia and Southeast Asia. Table information was extracted from references [58, 59, 60, 61, 62].

Country	Population (million)	PAR Pf† in 2010 (million)	PAR Pv‡ in 2010 (million)	Malaria Cases in 2011	Malaria Deaths in 2011
South Asia					
Bangladesh	155.000	35.689	35.53	51,773	36
Bhutan	0.742	0.411	0.47	194	1
India	1,240.000	1,065.767	1134.71	1,310,367	753
Nepal	27.474	18.897	24.14	2,288	2
Pakistan	179.000	143.122	168.86	334,589	NA
Sri Lanka	21.098	10.231	11.79	124	NA
Southeast Asia					
Cambodia	14.865	13.123	13.12	57,423	94
Indonesia	247.000	132.878	129.28	256,592	388
Laos	6.646	5.435	1.89	17,835	17
Malaysia	29.240	27.845	27.88	4,164	NA
Myanmar	52.797	45.166	38.97	465,294	581
Philippines	96.707	50.154	50.34	9,552	12
Thailand	66.785	40.839	41.97	24,897	43
Viet Nam	90.796	53.857	49.63	16,612	14

† PAR Pf: The total population at risk (PAR) living in areas of P. falciparum malaria transmission
‡ PAR Pv: The total population at risk (PAR) living in areas of P. vivax malaria transmission

Table 1.16: Malaria Status of Countries and Territories in WHO Southeast Asia† and Western Pacific‡ Regions. Table information was extracted from reference [63].

Malaria Status	**Countries/Territories**
Elimination has been achieved	Taiwan (1965), Australia (1981), Singapore (1982), Brunei Darussalam (1987)
Elimination Phase	Sri Lanka (2012), S. Korea (2012)
Pre-Elimination Phase	Bhutan (2012), N. Korea (2012), Malaysia (2012)
Malaria never existed or disappeared without specific measures	Cook Islands (1963), Fiji (1963), Marshall Islands (1963), Micronesia (1963), Mongolia (1963), Nauru (1963), New Zealand (1963), Niue (1963), Palau (1963), Samoa (1963), Tonga (1963), Maldives (2012), Japan (2012), Kiribati (2012), Tuvalu (2012)

† Countries in Southeast Asia Region: Bangladesh, Bhutan, N. Korea, India, Indonesia, Myanmar, Nepal, Sri Lanka, Thailand, Timor-Leste

‡ Countries in Western Pacific Region: Cambodia, China, Laos, Malaysia, Papua New Guinea, Philippines, S. Korea, Solomon Islands, Vanuatu, Vietnam

Table 1.17: Population at risk of Plasmodium falciparum malaria in 2010: Top 60 Countries. Table information was extracted from reference [59].

Country	PAR_{all} (million)	Country	PAR_{all} (million)	Country	PAR_{all} (million)
India	1065.767	Côte d'Ivoire	21.571	Haiti	9.997
Nigeria	158.255	Cameroon	19.755	Somalia	9.356
Pakistan	143.122	Angola	18.982	Benin	9.219
Indonesia	132.878	Nepal	18.897	Dominican Republic	7.734
Congo	64.040	Madagascar	18.591	Burundi	6.894
Ethiopia	62.255	Colombia	16.975	Togo	6.774
Vietnam	53.857	China	16.646	Rwanda	6.570
Philippines	50.154	South Africa	16.561	Sierra Leone	5.837
Myanmar	45.166	Burkina Faso	16.25	Laos	5.435
Tanzania	43.607	Niger	15.885	Papua New Guinea	4.968
Sudan	43.202	Malawi	15.688	Guatemala	4.891
Thailand	40.839	Afghanistan	15.103	Ecuador	4.684
Bangladesh	35.689	Mali	13.362	Central African Republic	4.506
Brazil	35.310	Zambia	13.253	Eritrea	4.402
Uganda	32.299	Cambodia	13.123	Liberia	4.102
Malaysia	27.845	Zimbabwe	12.470	Congo	3.760
Ghana	24.339	Chad	11.509	Mauritania	3.099
Mozambique	23.404	Guinea	10.323	Peru	2.893
Yemen	21.922	Sri Lanka	10.231	Gambia	1.751

Table 1.18: Population at risk of Plasmodium vivax malaria in 2010: Top 60 Countries. Table information was extracted from reference [60].

Country	PAR_{all} (million)	Country	PAR_{all} (million)	Country	PAR_{all} (million)
India	1134.71	Sri Lanka	11.79	Kyrgyzstan	1.91
China	461.95	Mexico	11.77	Laos	1.89
Pakistan	168.86	Peru	8.08	Congo	1.55
Indonesia	129.28	Papua New Guinea	5.64	Turkey	1.41
Philippines	50.34	Madagascar	5.23	Panama	1.24
Viet Nam	49.63	Guatemala	5.14	Argentina	1.22
Brazil	45.22	Ecuador	4.52	Timor-Leste	1.15
Thailand	41.97	Nicaragua	4.5	Paraguay	1.02
Myanmar	38.97	Azerbaijan	4.19	Costa Rica	0.91
Bangladesh	35.53	Bolivia	3.89	Chad	0.88
Ethiopia	35.19	Tajikistan	3.71	Uganda	0.85
Malaysia	27.88	Honduras	3.59	Guyana	0.76
Afghanistan	24.27	South Africa	3.5	Mozambique	0.58
Nepal	24.14	El Salvador	3.17	Solomon Islands	0.53
Venezuela	22.87	S Korea	2.97	Zimbabwe	0.52
N Korea	22.08	Iran	2.68	Kenya	0.49
Colombia	19.15	Iraq	2.44	Georgia	0.48
Sudan	14.78	Nigeria	2.38	Bhutan	0.47
Cambodia	13.12	Eritrea	2.17	Angola	0.44
Yemen	13.06	Somalia	2.06	Niger	0.44

Table 1.19: Summary of Risk Factors in Rajasthali, Bangladesh [74, 75, 79]

Dominant Risk Factors	**Non-Dominant Risk Factors (or with Mixed Results)**
• Ethnic group: Tripura and Tonchonga have lower odds in malaria infection than Bengali • Bed Net – Bed Net Ratio per Person per Household: protected if having more than 0.5 bed nets/person/household • House Structure – Material of the Floor: Increasing odds with wood floor comparing to mud floor – Material of the Wall: Increasing incidence rate with mud wall comparing to bamboo wall • Geographical Feature – Forest Density: Higher odds of acquiring malaria when in denser forest (2nd and 3rd tertile) comparing to the first – Altitude of Household Location: Higher odds when living above 100 meters comparing to $\leq$ 50 meters	• Gender • Age Group • Level of Education • occupation • Bed Net – Ownership of Bed Nets – Number of Bed Nets at Home – All Family Sleep under Bed Nets • Geographical Feature – Household Density – Distance to Stream – Aspect – Wetness (a proxy for capability of water accumulation)

Figure 1.1: Relationship between Temperature and Malaria Parasite Development Time Inside the Mosquito [17]

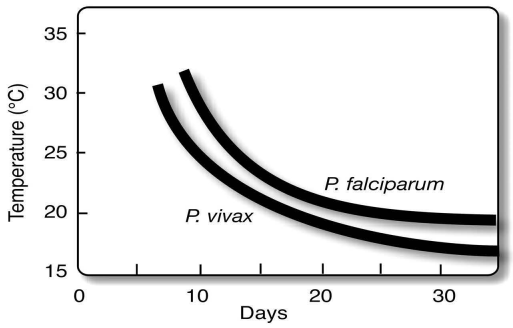


Figure 1.2: Climate Suitability for Stable Malaria Transmission in Zimbabwe (Orange-red indicates the suitability for malaria transmission) [17]

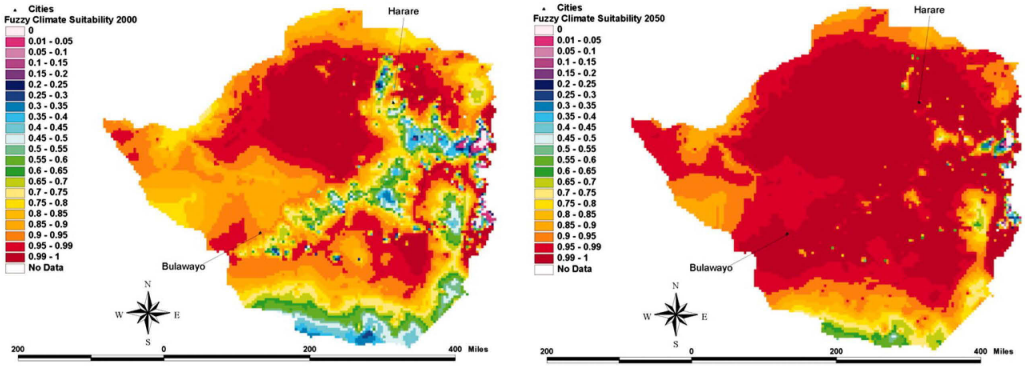


Figure 1.3: Vulnerable Population for Malaria Infection [20]

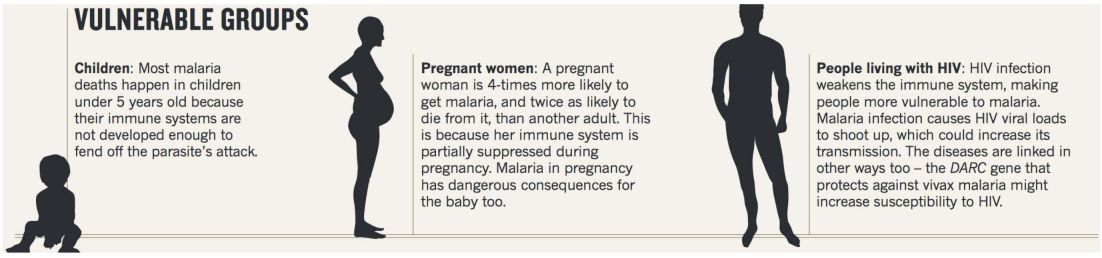


Figure 1.4: An Illustration of Cassette Format Type of Rapid Diagnostic Test [23]

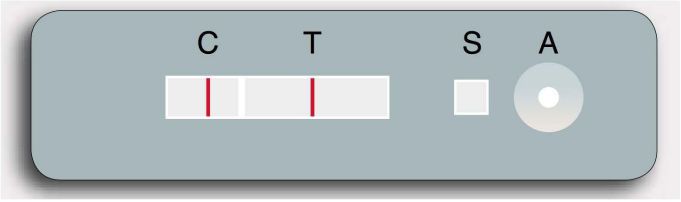


Figure 1.5: FalciVax^{TM} Pv/Pf RDT Result Interpretations [23]

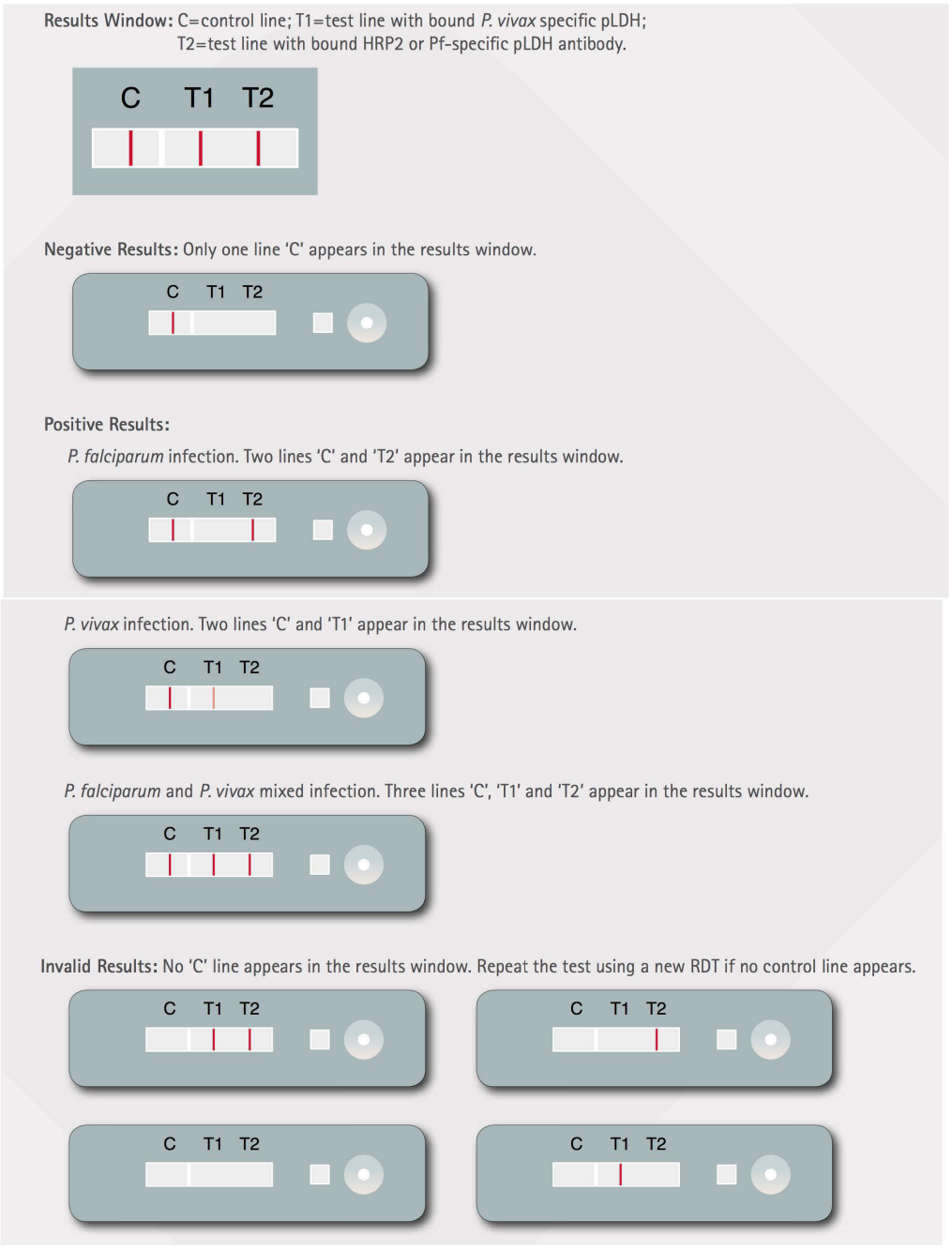
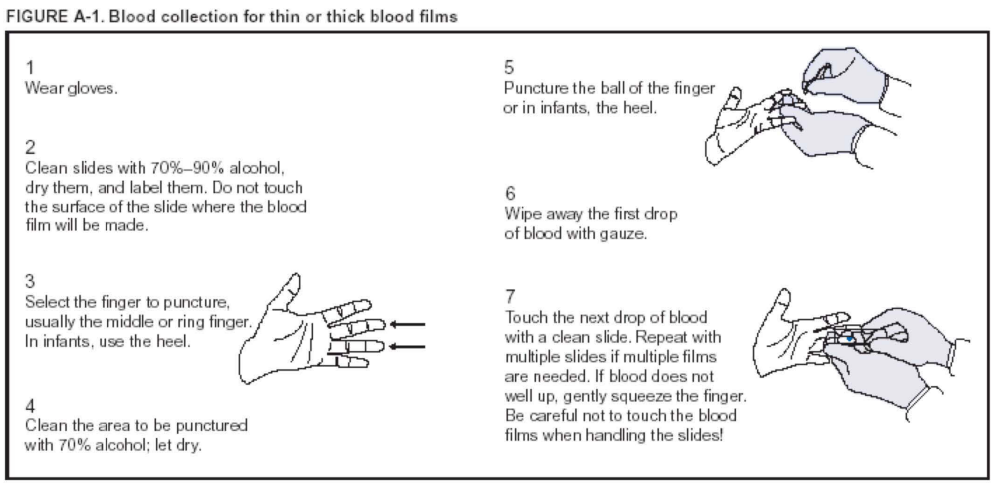


Figure 1.6: Preparation for Thin and Thick Blood Films [36]



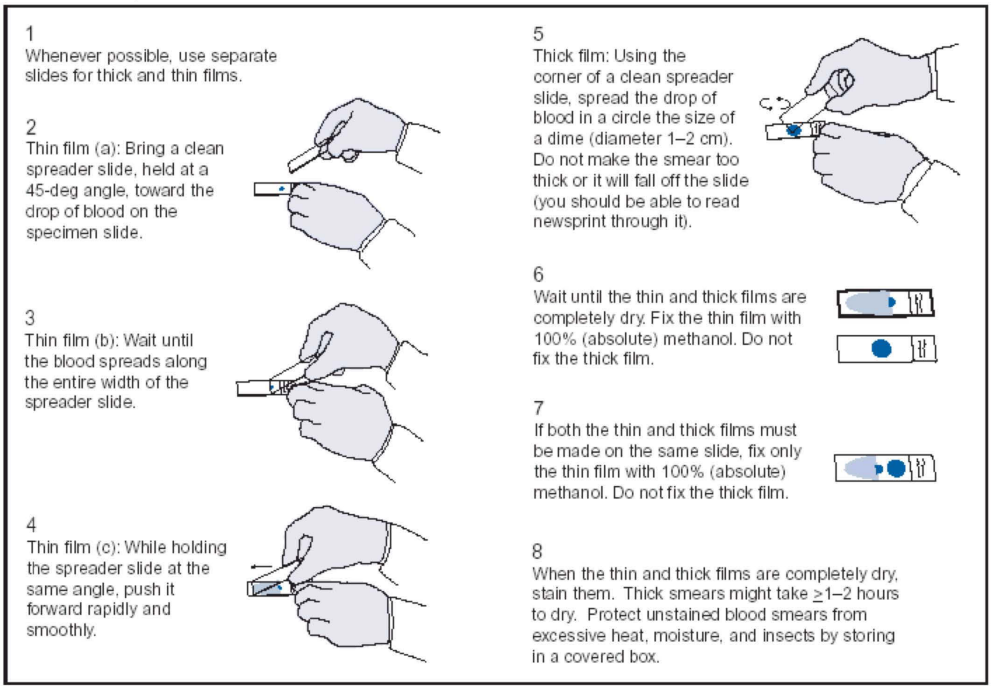


Figure 1.7: Proportion of the population sleeping under an ITN, by five-year age groups, 2003-2011 [44]

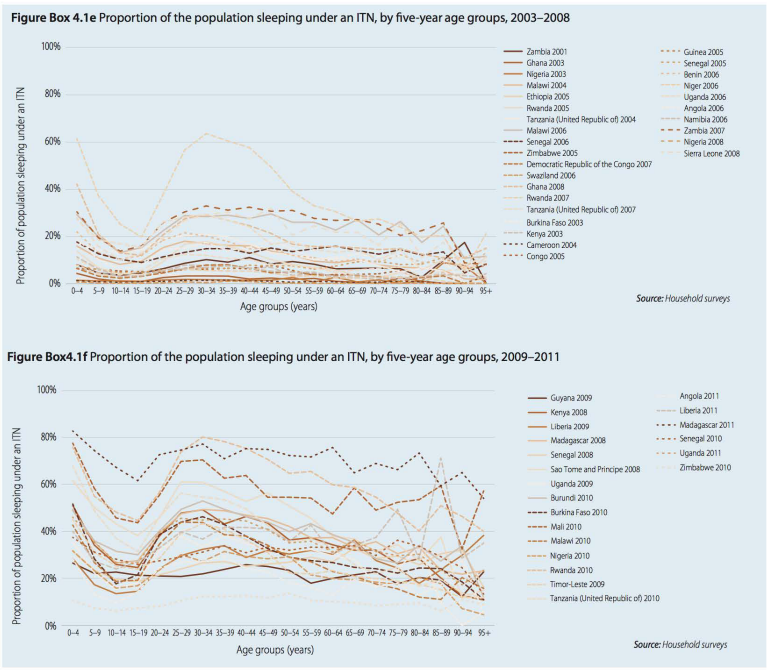
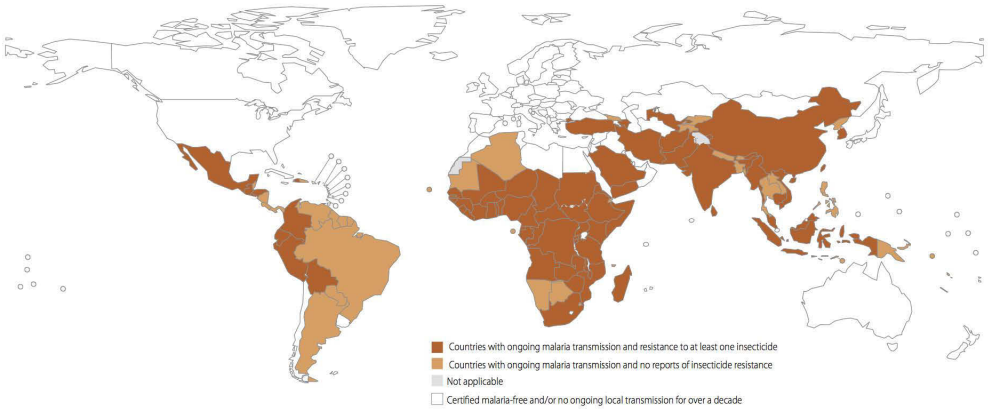


Figure 1.8: Countries with ongoing malaria transmission where insecticide resistance has been identified in at least one of their major vectors [44]



Source: Adapted from Global Plan for Insecticide Resistance Management in malaria vectors, WHO, Geneva, 2012. From WHO regional entomologists in WHO Regional Offices and literature review by the Global Malaria Programme. Map production: Global Malaria Programme (GMP), World Health Organization
Countries with ongoing malaria transmission and no reports of insecticide resistance include countries with confirmed susceptibility to all insecticides used and countries where susceptibility testing is not currently conducted or results are unknown. The map provides no indication of how widespread resistance is within a country; therefore, a single report of resistance would be sufficient to mark a country as having resistance

Figure 1.9: The global distribution of malaria [52]

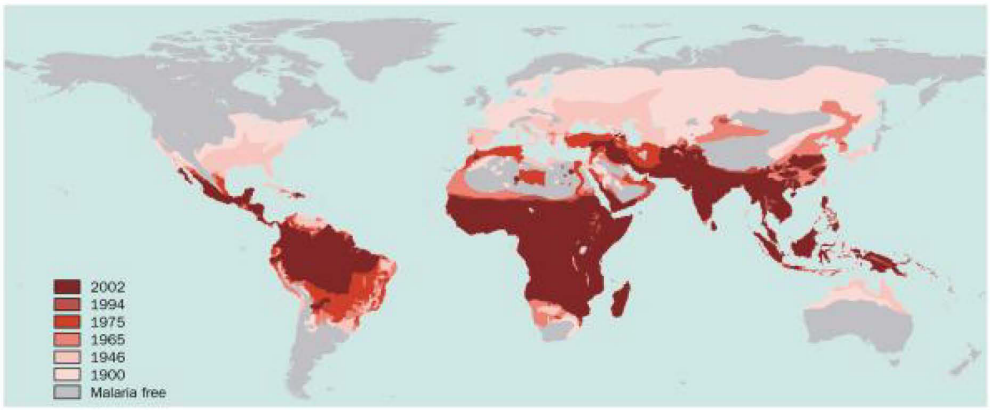


Figure 1.10: Global causes of childhood deaths in 2013 [53]

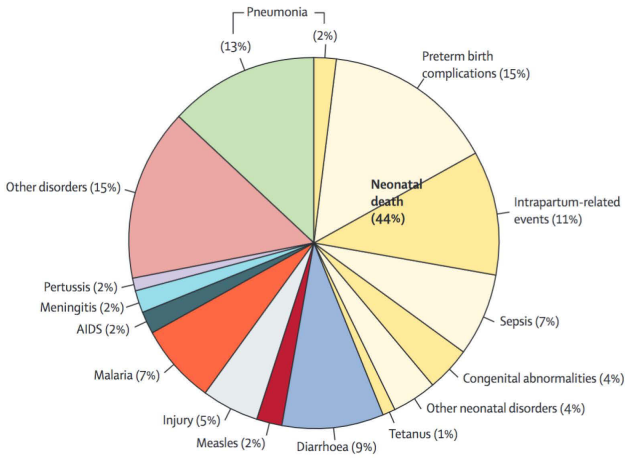


Figure 1.11: The spatial distribution of *Plasmodium falciparum* malaria endemicity in 2010 [80]

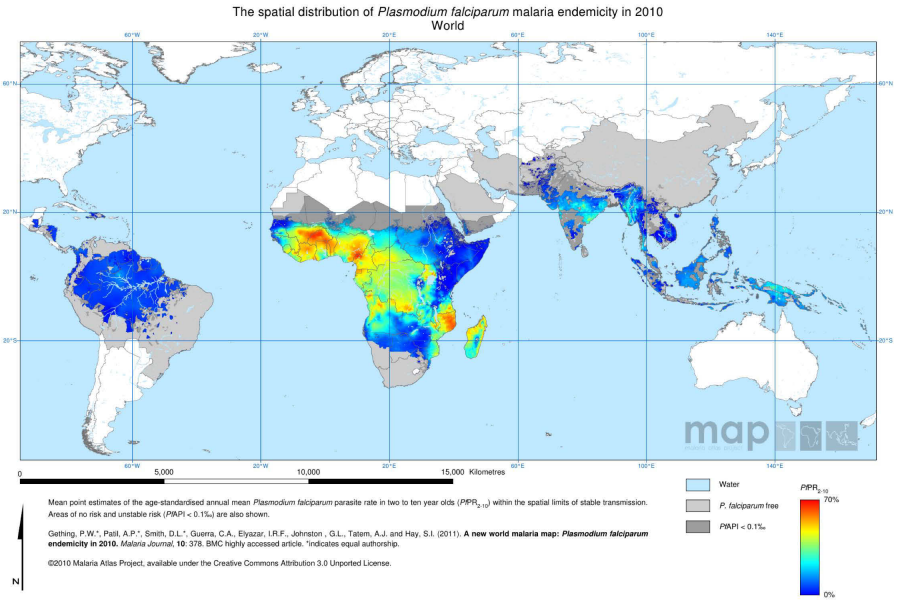


Figure 1.12: The spatial distribution of *Plasmodium vivax* malaria endemicity in 2010 [55]

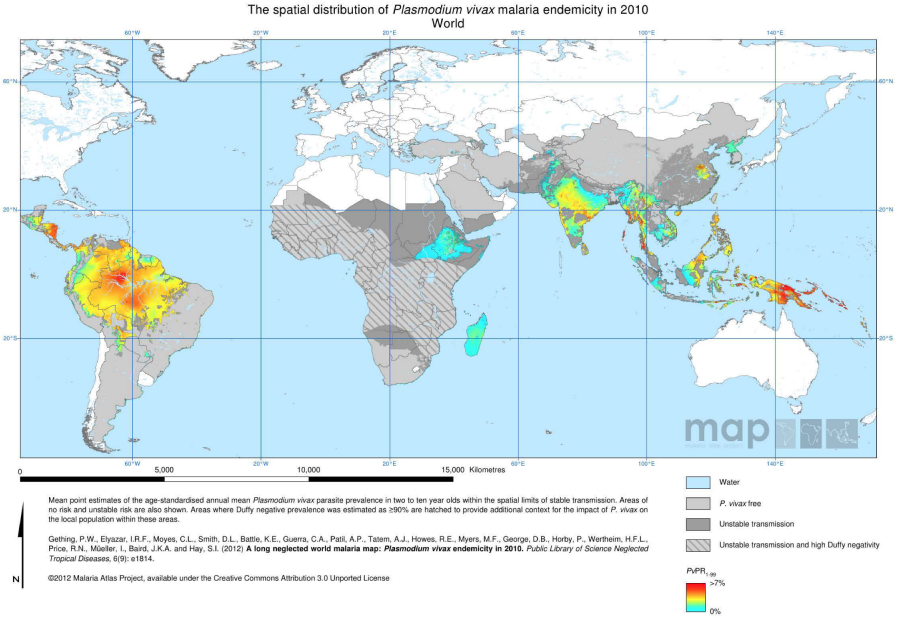


Figure 1.13: Mean Estimates of Sickle Hemoglobin Allele Frequency [81]

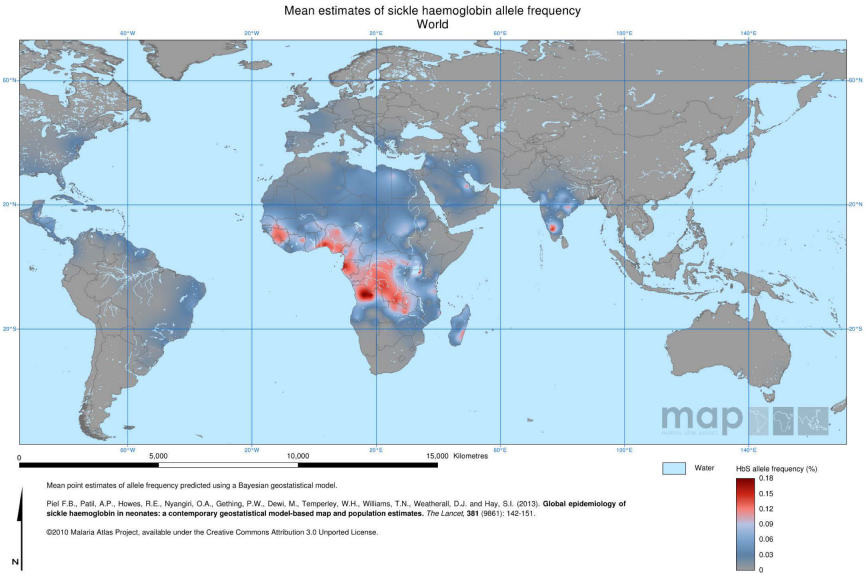


Figure 1.14: The Spatial Distribution of the Duffy Negative Phenotype [56]

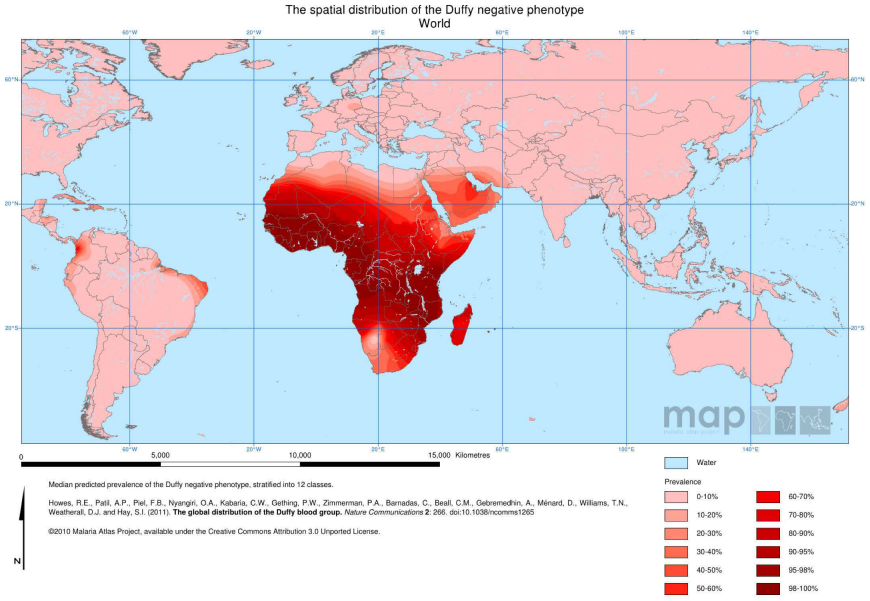


Figure 1.15: Predicted Allel Frequency for G6PD Deficiency in Malaria Endemic Countries [57]

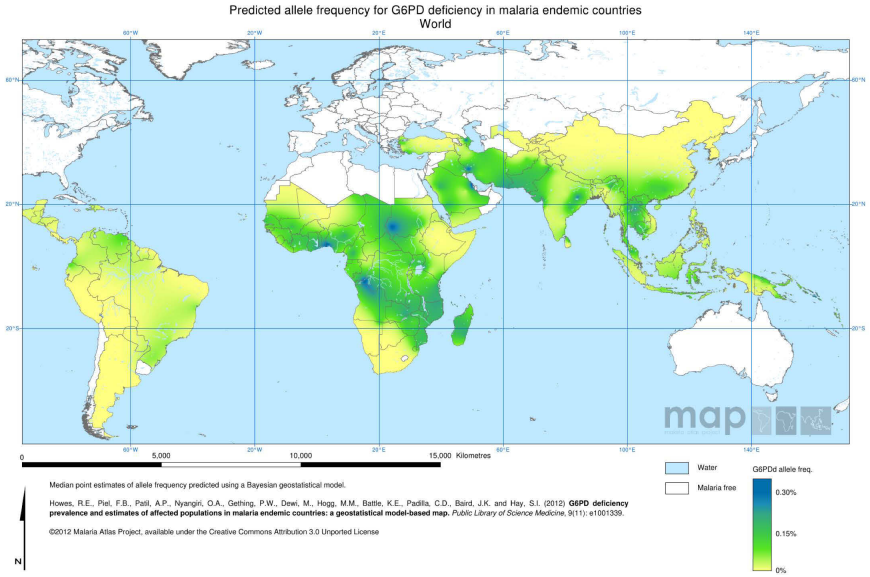
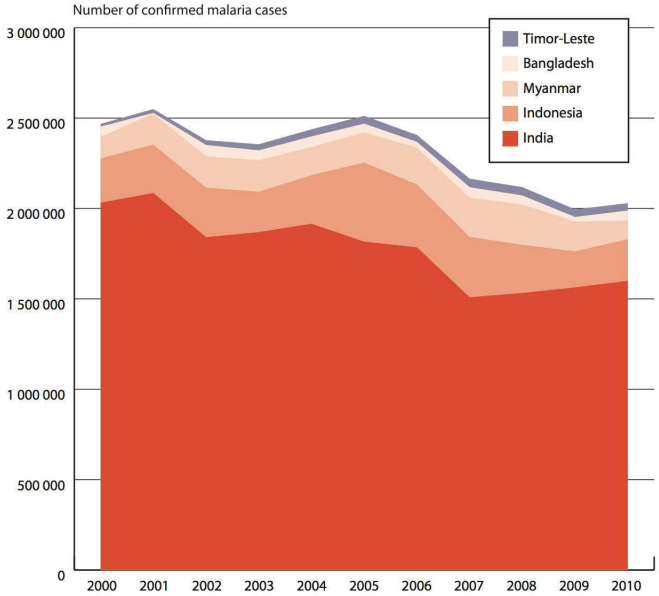


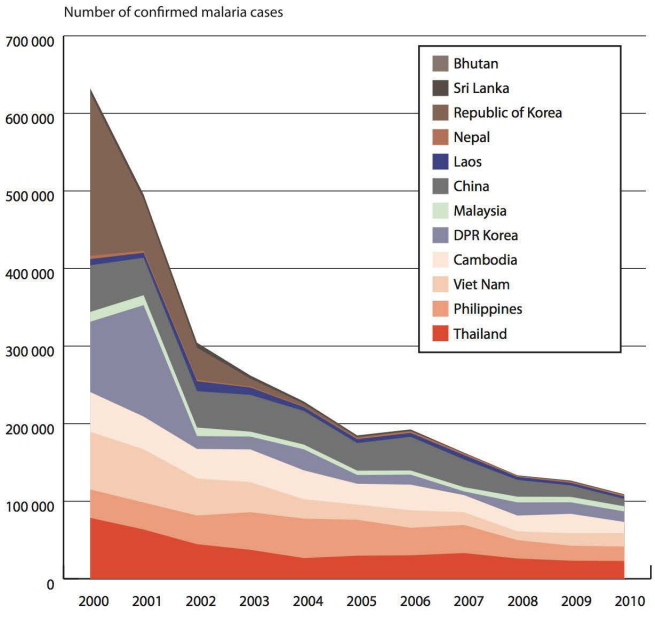
Figure 1.16: Trend in Number of Malaria Confirmed Cases in Countries located in South and Southeast Asia, 2000-2010 [45]

Figure 3.1
Trends in confirmed cases in South and South-East Asia

(a) Countries with more than 30 000 reported confirmed cases in 2010. India carries by far the heaviest burden of malaria among all countries in the region.

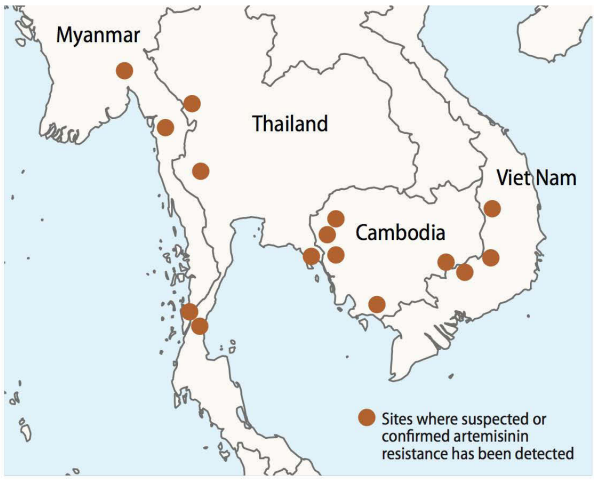


(b) Countries with less than 30 000 reported confirmed cases in 2010. Declines in confirmed cases from 2000 to 2010 were initially steep, particularly in Bhutan and the Democratic People's Republic of Korea, but by 2010 were more moderate.



Source: World Malaria Report 2011 (*16*).

Figure 1.17: Sites where suspected or confirmed artemisinin resistance has been detected in therapeutic efficacy studies, 2007-2012 [82]



Map production: *Global Malaria Programme (GMP), World Health Organization;* ***Source of data:*** *WHO Global Database on Antimalarial Drug Efficacy, as of November, 2012*

Figure 1.18: Distribution of *Plasmodium falciparum* and *Plasmodium vivax* in endemic areas of Bangladesh in 2007 [66]

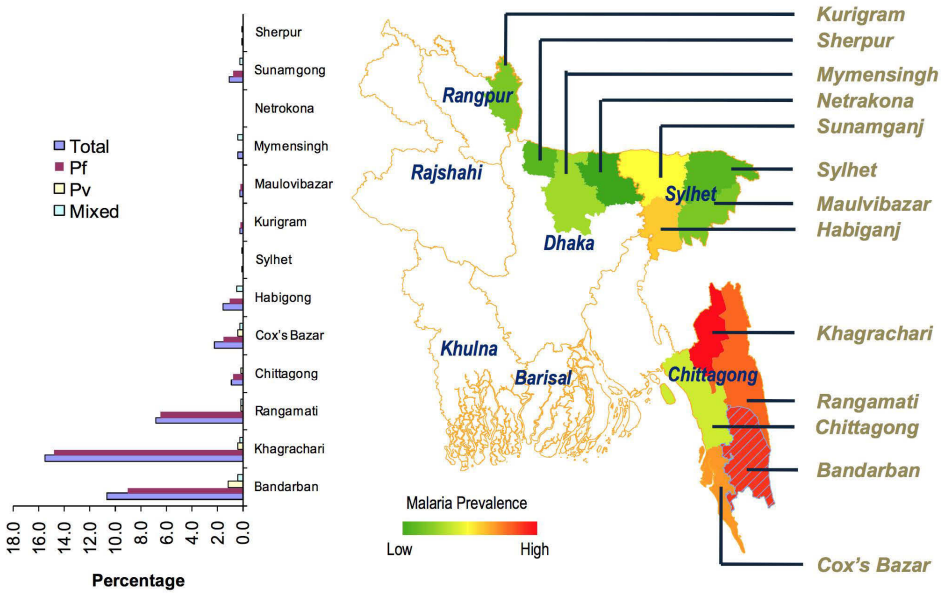


Figure 1.19: Bentleys malaria incidence map of 1916 [67]

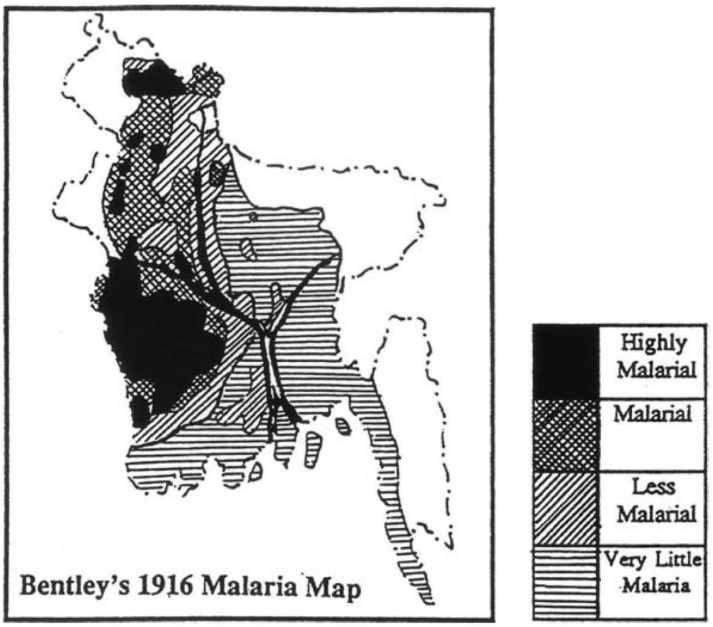


Figure 1.20: Morbidity Prevalence (15-day recall) among The Study Population by Age, Sex and Ethnic Group [71]

	Ethnic group (%)					
	Bangali	Chakma	Marma	Mro	Tripura	All (weighted)
Male	*21.6*	*12.0*	*14.0*	*8.2*	*17.0*	*17.5*
≤ 5 yrs	40.1	22.6	28.5	21.3	31.5	33.9
6 – 15 yrs	18.4	12.5	9.4	6.5	14.3	15.3
16 – 45 yrs	16.6	8.4	10.9	6.4	11.5	13.0
≥ 46 yrs	25.5	14.4	20.0	7.1	23.6	21.1
Female	*24.9*	*13.0*	*18.6*	*10.1*	20.3	20.5
≤ 5 yrs	38.9	26.8	39.7	23.0	41.6	36.5
6 – 15 yrs	21.1	10.1	14.2	9.0	15.5	17.0
16 – 45 yrs	23.0	10.1	15.3	5.9	14.2	17.6
≥ 46 yrs	24.3	18.5	20.9	10.7	31.9	22.1
Both	23.3	12.5	16.2	9.1	18.6	19.0
Education						
Illiterate	25.0	14.9	17.7	9.1	20.0	20.8
Literate	15.6	7.2	10.4	5.1	12.1	12.0
Household head's education						
Illiterate	24.2	13.7	16.3	9.1	18.1	19.5
Literate	21.9	10.5	15.6	9.1	19.9	17.9
Household's landholding size						
≤ 50 decimals	24.0	15.1	18.9	7.5	21.5	21.8
50+ decimals	22.5	11.8	14.7	10.0	16.4	17.2
Household's labour selling status						
Non-labour-selling	21.3	12.5	15.4	9.1	18.5	17.3
Labour-selling	27.9	11.9	19.6	-	19.0	24.9
Household's food security status						
Food insecure	25.0	12.8	17.5	9.1	19.8	20.0
Food secure	18.6	9.9	13.7	9.1	14.3	15.8
N	2718	2801	2613	2962	2659	13753

NB: The association between sociodemographic factors and illness prevalence was significant (χ^2 significance: $p<0.001$)

Figure 1.21: Three Most Commonly Occurring Illnesses among the Study Population by Age and Ethnic Group [71]

	Ethnic group (%)					
	Bangali	Chakma	Marma	Mro	Tripura	All (weighted)
Fever*	*31.8*	*48.7*	*58.4*	*46.1*	*58.0*	*39.7*
≤ 5 yrs	27.4	23.5	25.5	29	34.5	27.0
6 – 15 yrs	29.4	24.7	20.2	26.6	23.3	26.1
16 – 45 yrs	29.9	35.3	35.6	30.6	24.0	31.4
≥ 46 yrs	13.4	16.5	18.6	13.7	18.1	15.4
Gastrointestinal	*41.8*	*32.4*	*26.2*	*41.3*	*20.0*	*36.9*
≤ 5 yrs	31.4	19.5	24.3	43.2	21.2	29.0
6 – 15 yrs	25.0	26.5	22.5	22.5	33.3	25.2
16 – 45 yrs	34.8	36.3	34.2	23.4	34.3	34.8
≥ 46 yrs	8.7	17.7	18.9	10.8	11.1	11.0
Malaria	*21.0*	*8.0*	*2.6*	*2.2*	*12.3*	*15.9*
≤ 5 yrs	12.8	10.7	45.5	-	16.4	13.5
6 – 15 yrs	33.8	50.0	18.2	50.0	24.6	34.3
16 – 45 yrs	44.4	35.7	36.4	33.3	49.2	43.7
≥ 46 yrs	9.0	3.6	-	16.7	9.8	8.5
n	632	349	423	269	495	2168

* Of all types excluding malaria; NB: The association between types of illnesses and ethnic groups was significant at aggregate level (χ^2 significance: $p<0.001$).

Figure 1.22: Available Health Care Providers by Ethnic Identity of the Study Village of Chittagong Hill Tracts [71]

	Ethnic identity of the villages (%)				
	Bangali	Chakma	Marma	Mro	Tripura
Healthcare providers available					
MBBS doctors	3.3	13.3	-	-	3.3
Para-professionals*	**33.3**	20.0	16.7	-	3.3
Homeopath	**6.7**	6.7	-	-	-
Kabiraz**	**53.3**	70.0	90.0	93.3	90.0
Faith-healer	40.0	20.0	66.7	96.7	66.7
Dispensers at Medicine store	20.0	-	43.3	-	6.7
Total village	30	30	30	30	30

*Village practitioners, Medical Assistants, para-medics, CHWs of GO/NGO who have some formal exposure to allopathic medicine;
**practitioners of Ayurvedic medicine.

Figure 1.23: Health-Seeking Behavior (First Contact) of Study Population by Ethnic Group [71]

	Ethnic identity of the villages (%)					
	Bangali	Chakma	Marma	Mro	Tripura	All (weighted)
None	9.8	10.0	26.5	58.0	28.5	13.7
Home-remedies	17.0	16.3	12.7	63.6	5.1	13.9
Traditional*	4.2	7.9	7.8	9.1	2.8	4.5
Unqualified allopaths	37.0	59.9	70.6	9.1	55.2	40.0
Para-professionals**	15.4	4.4	2.9	-	0.6	9.9
Qualified allopaths	26.3	11.4	6.1	18.2	7.9	18.0
n	632	349	422	269	496	2168

*Kabiraji, Faith-healer, Herbalists etc. including homeopath, ** PCs, MAs, CHWs of GO/NGO etc. who have some formal training in allopathic medicine. NB: The association between types of health-care sought and ethnic groups is significant (χ^2 significance: $p<0.001$).

Figure 1.24: Distance of Nearest Static Health Facility by Ethnic Identity of the Villages [71]

	Ethnic identity of the villages (%)				
	Bangali	Chakma	Marma	Mro	Tripura
Distance from villages					
≤ 5 Km	83.3	73.2	63.3	23.3	23.3
6 – 10 Km	13.3	20.1	20.0	33.3	46.7
>10 Km	3.3	6.7	16.7	43.3	30.0

NB: The association between distance to nearest static health facilities and ethnic groups is significant (χ^2 significance: $p<0.001$).

Figure 1.25: Odds Ratios of Seeking Any Type, and Any Allopathic Type, of Healthcare in Last 15 days by the Study Population [71]

	Any type of health care (n=2049)		Any type of allopathic care (n=2049)	
	Odds	SE	Odds	SE
Age (years)				
≤ 5 yrs	1.00		1.00	
6 – 15 yrs	1.16	0.16	1.37*	0.15
16 – 45 yrs	1.11	0.15	1.19	0.14
≥ 46 yrs	1.20	0.18	1.21	0.16
Sex				
Male	1.00		1.00	
Female	0.74*	0.11	0.74**	0.10
Education				
Illiterate	1.00		1.00	
Literate	1.17	0.22	0.93	0.16
Education of household head				
Illiterate	1.00		1.00	
Literate	1.67**	0.17	1.28	0.13
Land-holding status of household				
Functionally landless (≤ 50 decimals)	1.00		1.00	
Have > 50 decimals of land	1.47**	0.13	1.27*	0.11
Labour-selling status of household				
Non labour-selling	1.00		1.00	
Labour-selling	0.74	0.15	0.86	0.13
Household's food security status				
Food-insecure household	1.00		1.00	
Food secure household	1.10	0.15	1.27	0.13
Types of illness				
Fever	1.00		1.00	
Gastro-intestinal diseases	0.79	0.13	0.33***	0.11
Malaria	2.16**	0.27	2.55***	0.22
Others	0.84	0.19	0.84	0.18
Ethnicity				
Bangali	1.00		1.00	
Chakma	0.80	0.24	0.76	0.16
Marma	0.30***	0.19	0.54***	0.15
Mro	0.08***	0.23	0.05***	0.25
Tripura	0.27***	0.20	0.68*	0.16
Distance from static health facilities				
≤ 5 km	1.00		1.00	
6 – 10 km	1.02	0.14	0.72*	0.12
10+ km	0.87	0.16	0.63**	0.16
-2log likelihood	1891.49		2266.72	
Model Improvement	332.22***		506.25***	
Overall predicted	79%		70%	

Significance levels: (*p<.05, **p<.01, ***p<.001).

Figure 1.26: Frequencies and odds ratios for potential risk factors for malaria infections (Rapid Diagnostic Test positives) in Rajasthali [74]

			Univariate (Unadjusted)			Multivariate (adjusted)		
	Cluster	Non-cluster	OR	95% CI	P-Value	OR	95% CI	P-Value
Variables	**Frequency**	**Frequency**						
Sex								
Female	498	334	1					
Male	354	214	1.11	0.89–1.38	0.353			
Age (years)								
0–14	220	143	1					
>14–49	499	325	1.00	0.78–1.28	0.988			
>49	133	80	1.08	0.76–1.53	0.662			
Tribe								
Bengali	152	205	1			1		
Marma	356	237	2.03	1.56–2.64	0.001	1.13	0.77–1.66	0.528
Tripura	116	27	5.79	3.63–9.26	0.001	0.20	0.09–0.44	0.001
Tonchonga	153	77	2.68	1.90–3.78	0.001	0.53	0.33–0.84	0.007
Khiang & Chakma	75	2	50.58	12.23–209.21	0.001	3.85	0.71–20.92	0.118
Education (years)								
0	400	271	1					
1–5	190	122	1.06	0.80–1.39	0.702			
6–10	234	132	1.20	0.92–1.56	0.173			
>10	28	23	0 .82	0.47–1.46	0.510			
Occupation								
Service/Business	91	78	1			1		
Small business	78	40	1.67	1.03–2.72	0.039	0.99	0.55–1.82	0.988
Day labor	186	118	1.35	0.92–1.98	0.121	0.93	0.57–1.50	0.756
Agriculture	448	297	1.29	0.92–1.81	0.134	1.41	0.92–2.15	0.113
Unemployed	49	15	2.80	1.46–5.38	0.002	1.89	0.85–4.21	0.118
Number of bed net								
<2	487	302	1					
≥2	365	246	0.92	0.74–1.14	0.450			
Treated bed net or LLIN ownership								
No	31	37	1			1		
Yes	821	511	1.92	1.17–3.13	0.009	1.82	0.93–3.55	0.081
All family members sleep under bed net								
No	170	98	1					
Yes	682	450	0.87	0.66–1.15	0.337			
Forest								
1st Tertile	143	324	1			1		
2nd Tertile	298	169	4.00	3.04–5.25	0.001	3.42	2.40–4.88	0.001
3rd Tertile	411	55	16.93	12.01–23.87	0.001	17.28	7.97–36.75	0.001
Altitude (meter)								
≤50	496	387	1			1		
51–100	289	157	1.44	1.13–1.82	0.003	1.13	0.74–1.71	0.574
>100	67	4	13.07	4.72–36.15	0.001	3.44	1.06–11.12	0.039
Floor								
Mud	303	358	1			1		
Cement	62	50	1.47	0.98–2.19	0.063	1.43	0.89–2.29	0.134
Wood	487	140	4.11	3.23–5.24	0.001	1.98	1.32–2.96	0.001
Household density								
1–200	336	39	1			1		
201–500	184	143	0.15	0.10–0.22	0.001	0.22	0.11–0.45	0.001
501–1000	52	75	0 .08	0.05–0.13	0.001	0.46	0.18–1.15	0.097
>1000	280	291	0 .11	0.08–0.16	0.001	0.64	0.26–1.60	0.342
Malaria control program								
No	123	246	1			1		
Yes	729	302	4.83	3.74–6.23	0.001	6.82	4.71–9.89	0.001

Figure 1.27: Malaria Risk Factor in Rajasthali, Bangladesh [79]

Factor	No. households (%)	No. positive households (%)	Unadjusted IRR (95% CI)	*P*	Adjusted IRR (95% CI)	*P*
Bed net ratio (per person/per household)						
0–0.5	469 (28.7)	107 (22.81)	1		1	
> 0.5–1	971 (59.42)	211 (21.73)	0.99 (0.78–1.26)	0.951	0.71 (0.56–0.91)	0.006
> 1	194 (11.87)	38 (19.59)	0.84 (0.58–1.24)	0.388	0.42 (0.28–0.62)	0.0001
Wall						
Jute stick/bamboo	1,534 (93.88)	317 (89.04)	1		1	
Tin/concrete	40 (2.45)	13 (3.65)	2.07 (1.18–3.63)	0.011	1.63 (0.94–2.82)	0.081
Mud	60 (3.67)	26 (7.30)	2.88 (1.88–4.42)	0.0001	2.17 (1.45–3.26)	0.0001
House density (no.)						
1–200	821 (50.24)	115 (32.30)	1.00		1	
201–500	222 (13.59)	61 (17.13)	2.078 (1.51–2.87)	0.0001	1.80 (1.09–3.00)	0.022
501–1,000	60 (3.67)	11 (3.09)	1.697 (0.95–3.02)	0.072	1.43 (0.70–2.92)	0.326
> 1,000	531 (32.50)	169 (47.47)	3.00 (2.37–3.80)	0.0001	2.79 (1.70–4.56)	0.0001
First-order streams (km)						
< 2	1,527 (93.45)	326 (21.35)	1.00			
≥ 2	107 (6.55)	30 (28.04)	1.22 (0.93–2.23)	0.353		
Second-order streams (km)						
< 2	1,451 (88.80)	333 (22.95)	1.00		1.00	
≥ 2	183 (11.20)	23 (12.57)	0.46 (0.30–0.70)	0.0001	0.73 (0.32–1.71)	0.475
Third-order streams (km)						
< 2	240 (14.69)	60 (25.00)	1.00			
≥ 2	1,394 (85.31)	296 (21.23)	1.02 (0.75–1.38)	0.917		
Fourth-order streams (km)						
< 2	890 (54.47)	252 (28.31)	1.00		1.00	
≥ 2	744 (45.53)	104 (13.98)	0.40 (0.32–0.51)	0.0001	0.82 (0.49–1.37)	0.446
Aspect						
Eastern or southeastern	467 (28.58)	261 (22.37)	0.98 (0.77–1.24)	0.868		
Western or northwestern	1,022 (62.55)	95 (20.34)	1.04 (0.84–1.31)	0.696		
Elevation (meters)			0.996 (0.995–0.998)	0.0001	1.001 (0.997–1.003)	0.597
Convergence						
1 (wettest)	327 (20.01)	80 (22.47)	1.00			
2	327 (20.01)	78 (21.91)	0.96 (0.69–1.34)	0.826		
3	327 (20.01)	63 (17.70)	0.75 (0.53–1.07)	0.110		
4	327 (20.01)	62 (17.42)	0.90 (0.64–1.26)	0.536		
5 (driest)	326 (19.95)	73 (20.51)	0.90 (0.65–1.26)	0.548		
Wetness index						
1 (driest)	328 (20.07)	62 (17.42)	1.00			
2	328 (20.07)	69 (19.38)	1.16 (0.81–1.66)	0.406		
3	325 (19.89)	84 (23.60)	1.58 (1.12–2.22)	0.009		
4	329 (20.13)	68 (19.10)	1.15 (0.80–1.64)	0.451		
5 (wettest)	324 (19.83)	73 (20.51)	1.35 (0.96–1.92)	0.088		

*IRR = incidence rate ratio; CI = confidence interval. IRR for the reference category is 1.00

Chapter 2

Methods

The main goal of the dissertation was to create risk profiles of malaria epidemiology in rural Bangladesh. *Risk profiles* are quantitative analyses to identify variables that could pose threats or opportunities to residents living in malaria affected areas. The author partnered with *Mapping Malaria Epidemiology Project in Bangladesh* to conduct the research.

To understand threats or opportunities, we needed to start by knowing how many people were infected with malaria. Therefore, we tested the field performance of a locally used rapid diagnostic test (RDT) device, named FalciVax^{TM} RDT, against our comparative gold standard, Giemsa stained microscopy (Chapter 3). After looking at the field performance, we examined factors that drove the change in levels of parasite density (Chapter 4). Once the internal factors (symptoms, demographics) were examined, we turned our focus on the external factors. We began the analysis by looking at types of household building materials in relation to numbers of *Anopheles* mosquitoes found at households (Chapter 5). As housing structures are usually related to social economic status of a family, we examined how living standards could affect malaria incidence (Chapter 6). We then concluded the risk profiling analyses by summarizing the key findings and its public health implications in Chapter 7.

To accomplish these goals, we used the following methods:

2.1 Study Site

Site Selection Prior to the start of the study, a nationwide survey on malaria was conducted in Bangladesh in 2007. Results of the study showed Chittagong Hill Tracts, a hilly region located in southeastern Bangladesh, had the highest malaria prevalence in the country. Chittagong Hill Tracts borders Myanmar and India. It contains three districts: Khagrachari District, Rangamati District, and Bandarban District. The study team chose Bandarban, the remotest district in Bangladesh, to conduct the malaria surveillance.

Study Site Our study site was located in northern Bandarban District, Chittagong Hill Tracts, Bangladesh (Figure 2.1). It encompassed two Unions, Kuhalong Union and Rajbila Union, in Bandarban District. Unions are the smallest rural administrative and government unit in Bangladesh. The study site was approximately 17 kilometers by 17 kilometers in size. Based on our baseline demographic survey, Kuhalong Union had a population of 12,502 and Rajbila Union had a population of 9,823. Figure 2.2 showed the population pyramids in the study area. We categorized the study area into 24 *Clusters* (12 Clusters in each Union). Each Cluster had households close in distance and had a range of population from 660 to 1,321. (Table 2.2) *Cluster* was not an unit used by the government. It was created for easy execution of the study by field workers.

2.2 Study Time Frame

The study was conducted from 2009 to 2013. It began in Kuhalong Union in October 2009. The study was later rolled out in Rajbila Union in April 2010. For the dissertation, the data were collected till September 2013 in both Unions. Table 2.1 showed the detailed time frame of each survey.

2.3 Study Design

The *Mapping Malaria Epidemiology Project* was a 4-year perspective surveillance project. We followed the same five thousand households and twenty two thousand residents for the malaria study. Within the project, multiple study designs were used. There were active surveillance, nested longitudinal surveillance and passive surveillance. In addition, surveys on human and environmental risk factors were also collected. A full spectrum of the collected data was listed in Table 2.1. All questionnaires used for this project can be found in Appendix, located at the end of this chapter.

For the dissertation, we focused on data collected in following aspects:

- Population Information: Demographic survey; social economic status survey
- Human Malaria Information: Passive surveillance
- Mosquito Information: Entomological surveillance

Methods for data collection are described below.

2.3.1 Population Information

In *Baseline Demographic Survey*, every household and household member at the study area were assigned an unique identification code. The household identification code was created in the form of $\square - C\square\square - \square\square\square - \square\square\square$. For example, 1-C02-003-004. The first digit of the code indicated its *Union* location (1 for Rajbila and 2 for Kuhalong). The two digits followed the letter C were its *Cluster* number, ranging from 01 to 12. The next three digits (e.g. 003) were the *Para* ID. A *para* is a tribal village. The final three digits were the household number, unique to households within the same para. Each member of a household received a 2-digit individual number in conjunction with their household ID. For example, a person with a ID of 1-C002-003-004-05 indicated he or she came from "Household 1-C002-003-004". The person was the fifth member of the household. This identification number was used across all surveys and surveillance system in the study.

In demographic surveys, ethnicity, religion, education, occupation and marital status were collected for every person at the study site. Demographic surveys were followed up every 4 months after the initial interview. In *Social Economic Survey*, household respondents (i.e. heads of household) were asked about their household income, durable assets in the households, building materials used for the house, source of water, light, fuel and types of toilet used.

2.3.2 Human Malaria Information

Participants in passive surveillance were identified (1) through their contacts with study team members (1 of the 20 field workers, the medical officer or the field manager) when ill and (2) through cases identified by *Building Resources across Communities* (BRAC), a local non-government organization. All participants identified through passive surveillance were immediately visited by field workers at their residence (Day 0) and malaria tests were conducted. Two tests were performed: FalciVax^{TM} RDT and Giemsa stained microscopy. Positive cases diagnosed through the rapid diagnostic tests (RDTs) in passive surveillance were given malaria treatment right away. Blood films collected for microscopy were brought back to the field office for further examination. Confirmation of malaria was done by an experienced microscopist at the field office. In our study, two cases were RDT-tested negative, but were tested positive by microscopy at the field office. These two cases were treated the day after the initial visit (on Day 1). Follow up home visits to those positive cases were done on Day 2, Day 7 and Day 28. In addition to confirm malaria infection, the microscopist also identified *Plasmodium* species of malaria infection and measure its parasite density. All participants in passive surveillance also answered questions on their age, gender, height, weight, self-reported symptoms and symptom duration. Their body temperatures were also measured at home visits.

2.3.3 Mosquito Information

At the beginning of each year, a random sample of selected houses were chosen for entomological surveillance. Households were chosen based on stratified sampling. Five households were randomly chosen from each Cluster at each Union. There were two Unions (i.e. Kuhalong Union and Rajbila Union) and 12 Clusters. Therefore, 120 households were selected per year. Once selected, the household were visited once a month for a year for the entomological surveillance. When none of the members from a selected household was presented at the designated date of entomological surveillance, we turned to their neighbors. Prior to conducting entomological surveillance at the neighbor's house, we ensured an informed consent was reached. During each surveillance, a *CDC Light Trap* was set up for 12 hours at night (starting 6-7 o'clock in the evening). Once a light trap was collected the following morning, it was brought back to the field lab for analysis. The analysis was done by an entomologist at the Bandarban Field Office. A microscope was used to identify *Anopheles* species. Numbers of *Anopheles* mosquitoes were recorded immediately by species using Microsoft Office Access Database.

2.4 Malaria Diagnosis and Case Definition

2.4.1 Malaria Diagnosis

Two methods were used in malaria diagnosis: Giemsa-stained microscopy and FalciVax^{TM} rapid diagnostic tests. Giemsa-stained microscopy is the comparative gold standard in malaria diagnosis. It requires a microscope and at least a

skilled microscopist to read testers' thin and thick blood films. Therefore, the method is not practiced in all corners of the world. The advantage of using microscopy for malaria diagnosis is its ability to determine the presence, species and density of malaria parasites. FalciVax^{TM} RDT is one of the 200+ commercial rapid diagnostic devices on the market. A RDT is small and portable. It does not required a laboratory and a skilled scientist to understand the readings of diagnostic tests. Therefore, it is commonly used in resource poor settings. However, it only offers information on the presence and absence of certain species of *Plasmodium* parasites. In this case, FalciVax^{TM} RDT can differentiate malaria infection caused by *Plasmodium falciparum* and *Plasmodium vivax*, but not the ones caused by *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Because *Plasmodium falciparum* and *Plasmodium vivax* were the most commonly seen malaria parasites at our Bandarban study area, FalciVax^{TM} RDT and Giemsa-stained microscopy were chosen as our malaria diagnostic methods. Nonetheless, due to majority of malaria cases (95%) came from *Plasmodium falciparum* infection, this dissertation did not include *Plasmodium vivax* test results in the analysis.

2.4.2 Case Definition

Field Definition In the field, the timing of malaria treatment is critical. Therefore, if a participant was tested positive by Giemsa-stained microscopy or FalciVax^{TM} rapid diagnostic tests, the participant was considered as malaria positive. Treatment was provided to all who were tested positive.

Definition Used in Dissertation As more than 200+ commercial RDT devices are available on the market, we chose to use microscopy (the comparative gold standard) as our sole standard for defining malaria cases. It is a more stringent case definition. However, the analyses and implication wouldn't have changed if a different RDT device had been used during the study period. It also allows the comparison across like populations.

2.5 Quality Assurance

Our study site is predominantly composed of tribal groups. Therefore, it was crucial to understand local tribal culture and tribal languages when conducting surveillance and surveys. Therefore, all our staff members of the *Mapping Malaria Epidemiology* project were locally hired. Locally hired staff members included 1 field manager, 1 licensed physician, 1 field assistant and 20 field workers. As the official language in Bangladesh is Bengali, staff members were also required to speak Bengali in order to communicate with the study team located in Bandarban and in Dhaka. Written and oral exams were given to all applicants to ensure their ability in reading, writing and speaking Bengali and tribal languages. Staff members were chosen based on their performance at the exams.

Once on board, staff members underwent personnel training. This training started in June 2009. Topics covered at the training included, but not limited to, the national guidelines on malaria, process to obtain informed consent for the study, survey methods, preparation of blood films, interpret results from

rapid diagnostic devices and the use of CDC light traps. Questionnaires used during training were written in both English and Bengali. This was to ensure the mutual understanding of materials used for the surveillance project across the study team at JHMRI, icddr,b and at the field. After the personnel training was completed, surveillance workers were paired up by the field manager. Ten teams of two were formed. Each team would receive their daily assignments from the field manager. Performance of field personnel was oversaw and supervised by the field manager and the medical officer (i.e. the licensed physician).

2.6 Data Entry and Management

2.6.1 Data Entry

All questionnaires used in the study were designed in a standard format. The forms were then set up to be recognized by a form processing software ABBYY FlexiCapture 8.0. When field workers brought back surveyed questionnaires, the field assistant would scan the questionnaires. These scanned images of the pre-coded forms were first processed by the software. Initial data entry was completed. Field assistant then had to compare the data entry with handwritten answers on questionnaires. Correction would be made if any discrepancy was found. Once entry results were corrected and confirmed by the field assistant. This entry was exported from ABBYY FlexiCapture 8.0 to Microsoft Office Access 2007. Digitized questionnaires were stored in the field office. All scanned images from questionnaires along with data entry files were stored at *International Centre for Diarrhoeal Disease Research, Bangladesh* (icddr,b) in

Dhaka, Bangladesh and at the *Malaria Research Institute at the Johns Hopkins Bloomberg School of Public Health* (JHMRI) in Baltimore, Maryland, USA. Our microscopist and entomologist also work closely with icddr,b and JHMRI. Laboratory specimens from passive surveillance and from entomological surveillance were sent to icddr,b after the examination and data entry were completed at the Bandarban Field Office.

2.6.2 Data Management

Prior to data analysis, data cleaning and preliminary exploratory data analysis were performed in R 3.0.2 by the author to ensure data quality. Data cleaning and management process included, but were not limited to, reshaping the data, checking number formatting, checking data range, checking data distribution and missing data, generating and re-coding variables if needed, merging datasets when needed, creating and revising codebooks, and creating comments on R codes for future reference.

Reshape the data For baseline and follow-up demographic surveillance, individual data were collected. However, each row of data recorded in the database represented one household. Hence, the author reshaped the data from a wide form to a long form to showcase individual data by row. Mosquito data, on the other hand, were recorded based on visits for entomological surveillance. As households were visited at least once, it was feasible to have multiple lines of mosquito data per household. As our goal was to understand how household building materials were associated with number of *Anopheles* mosquitoes per

household, we collapse the mosquito data to display one row per household—with the calculation of average number of *Anopheles* mosquitoes found per household.

Check number formatting Some numeric variables were recorded as sting variables (i.e. text). Some categorical variables were represented by numeric numbers. Conversion between formats were needed to run-able or meaningful results in analyses.

Check data range If a recorded value is not within the range of designated variable values (e.g. a value of 7 in a categorical variable that only ranged from 1 to 6) or is not within a reasonable range (e.g. a height of 19 cm whilst having a weight of 100 kg), a request would be sent to confirm the data entry with the original value recorded on the questionnaires.

Check data distribution In addition to out-of-bound values, it was also important to check distribution of variables and missing data. For example, distribution of parasite density was skewed to the right. This indicated most individuals had relatively lower parasite density. Only selected few had high parasite density. We took natural logarithm of parasite density and found an approximately bimodal distribution between individuals with log(parasite density) above and below log(median parasite density). We generated a dichotomous variable on parasite density in paper 2 to represent the two modes (chapter 4).

Check missing data Having missing data could be unavoidable in surveillance. Therefore, understand the percentage of missing data and the reason

behind having missing data are important. For example, one of our study participants at passive surveillance were examined by microscopy but not by RDT. It was forgotten by field workers during the home visit. With only 1 out of 617 microscopy tested individuals missed the RDT test results, the person was removed from the analysis of testing field performance of FalciVax^{TM} RDT. However, if a large percentage of a variable of interest was missing, detailed inspection should be done prior to removing or imputing the missing data.

Generate variables if needed New variables were generated based on the need of the paper. For example, we were interested in knowing whether field performance of FalciVax^{TM} RDT differed by seasons. We generated a season variable based on the date the specimens were collected. We were also curious about the association between body mass index and levels of parasite density among malaria tested individuals. Therefore, we used height and weight to calculate BMI for each study participant.

Re-code variables if needed There were times, instead of generating new variables, where it was beneficial to group or re-code certain variables. For instance, we surveyed on the materials used to build walls, roofs, floors and partitions. Materials that were used by fewer than 5 households in the entire study areas were grouped into "Others" category. This helped prevent the number in each category spread too thin to provide useful statistical inference. Other times, we assigned new values to variables to provide consistency. For example, there were yes-no questions with answers of 0 (no) and 1 (yes); there were other yes-no questions with answers of 1 (yes) and 2 (no). To provide

consistency across all questions in questionnaires, we re-assigned all the "NOs" with a value of 0.

Merge datasets when needed In our study, all questionnaire was recorded and stored in separate database. For example, mosquito data from the *entomological surveillance* and household materials as part of the *social economic surveys* were store in two different database. As one example: To understand the association between household building materials and average number of *Anopheles* mosquitoes found per household, we need to merge these two datasets based on household identification numbers.

Create and Revise Codebooks Having a codebook is key for reproducibility. Questionnaires with variable names used for each question were typed up as shown in the Appendix. With initial codebooks provided by the study team, they were also modified based on the need of the dissertation.

Create comments on R codes As commends for data analysis could be visited at different point in time—even in the future, adding descriptions for commend lines and descriptions to why certain analyses were run are important. This was done throughout the analysis process.

2.7 Study Population

The study population used in the dissertation analyses was different in each chapter. However, they had some common features.

Eligibility All residents at the Bandarban Study Area (i.e. Kuhalong Union and Rajbila Union in Bandarban, Chittagong Hill Tracts in Bangladesh) during October 2009 and September 2013 were eligible for all surveys and surveillance projects carried by *Mapping Malaria Epidemiology Project*. All households located at the Bandarban Study Area were eligible to be drawn for entomological surveillance.

Informed Consent If informed consent was not received during surveillance, the test or survey would not be done. For example, if an individual provided consent for a rapid diagnostic test but not a microscopy test, only a rapid diagnostic test would be carried out.

Malaria Data Only test results and surveys recorded on Day 0 of home visits were included in the analyses. As previously mentioned, malaria treatments were provided to all individuals tested positive by Giemsa-stained microscopy or FalciVax^{TM} RDT. Follow-ups were done on later dates. Malaria test results conducted on Day 2, Day 7 and Day 28 would have varying degrees of influence from the medication. The degree of influence from medication could also vary by person. Therefore, we limited malaria test results to the ones performed on Day 0 to avoid the impact induced by medication.

2.7.1 Paper 1: Field Performance of FalciVax^{TM} RDT in rural Bangladesh

The goal of the study was to compare field performance of FalciVax^{TM} RDT against the comparative gold standard Giemsa-stained microscopy. Therefore,

our study population only included the 616 Bandarban Study Area residents who were enrolled in the passive surveillance from October 2009 to September 2013, and had malaria test results from both FalciVax™ RDT and Giemsa-stained microscopy on Day 0.

2.7.2 Paper 2: Association Between Levels of *Plasmodium falciparum* Density and Clinical Malaria Symptoms

The aim of the study was to study how demographic characteristics, measured body temperature, self-reported symptom, and symptom duration would be related to the level of parasite density. Having readings in parasite density was crucial for the paper. Therefore, all individuals without parasite density were excluded from the study. There were 617 study participants enrolled in passive surveillance and with microscopy test results on Day 0. Of those, only one individual was not tested by FalciVax™ . To allow the population be comparable across paper 1 and paper 2, we excluded the one individual. Therefore, the final study population is equivalent of the ones who were tested by both FalciVax™ RDT and Giemsa-stained microscopy on Day 0.

2.7.3 Paper 3: Association between Household Building Materials and the Abundance of *Anopheles* Mosquitoes in Rural Bangladesh

The objective of the paper was to examine the relationship between type of household materials used and the number of *Anopheles* mosquitoes found in the

household. Hence, "household" was our unit of interest. We included households that were selected for entomological survey and had information on their households building materials. Among 5,006 households located in Kuhalong Union and Rajbila Union, 1,079 households were selected for entomological survey. Of which, 1 household only had outdoor entomological data (whereas all other households had indoor data), and 15 households did not had information on building materials. Therefore, these 16 households were excluded from analyses. Our final analyses were based on the remaining 1,063 households that had indoor entomological surveillance data as well as information on building materials used on walls, roofs, floors and partitions.

2.7.4 Paper 4: Association between Living Standards and Incidence of Human Malaria in southeastern Bangladesh

We intended to understand how living standards (e.g. durable assets at households) could be related to malaria incidence. To achieve this goal, we required demographic information, human laboratory data and social economic survey data. Twenty two thousand four hundred and fifty (22,450) individuals from Bandarban Study Area were included in the analyses. Of whom, 529 were tested malaria positive by both FalciVax^{TM} RDT and Giemsa-stained microscopy on Day 0 of home visits.

2.8 Major Variables of Interest

2.8.1 Time Factors

Year and Season In paper 1, we compared field performance of FalciVax^{TM} RDT across time. In paper 3, we calculated the average number of *Anopheles* mosquitoes presented per year and per season. We used dates of specimen collection as our time stamp to create associated year and season variables. Year ranged from 2009 to 2013. Season included Spring (February-April), Summer (April-June), Monsoon (June-August), Autumn (August-October), Pre-Winter (October-December) and Winter (December-February). Each of the six local seasons was approximately 60 days in length.

Exposure Time from Malaria In paper 4, we calculated exposure time for each individual. As the population-based demographic surveillance was done at baseline and at follow-ups, it had the most comprehensive list of entry dates for all study participants. We utilized the initial entry date as the start date for malaria exposure. If a study participant was tested with malaria, he or she would use the malaria exam date as the end date of the exposure. If a study participant was never tested for malaria, he or she was administratively censored. In this case, the last day of the study would be the end day of the exposure.

2.8.2 Individual Factors

Age and BMI In papers 2 and 4, we examined the relationship between demographic characteristics (e.g. age and bmi) and the severity of malaria, and

between demographic characteristics (e.g. age and bmi) and malaria incidence. Age was calculated by the date difference in between the date of visit for passive surveillance and the person's date-of-birth. BMI was calculated by the person's height and weight (BMI $= \frac{\text{Weight}(kg)}{\text{Height}^2(m^2)}$).

Self-Reported Symptoms In paper 2, we discussed how self-reported symptoms were associated with different levels of *Plasmodium falciparum* parasite density. The symptoms included in the analyses and discussion were (1) fever with shivering, (2) fever at day time, (3) fever at night, (4) fever with sweating, (5) intermittent fever, and (6) remission of fever with sweating, (7) headache, (8) chills, (9) nausea, (10) vomiting, (11) diarrhea, (12) cough, (13) fatigue, (14) muscle ache, (15) muscle weakness, (16) convulsions / seizure, and (17) anemia.

Febrile Status In paper 2, other than self-reported symptoms, we also measured study participants' body temperature at home visits. Based on the body temperature, we created the febrile status for study participants. If oral temperature was taken, an individual was considered febrile if his/her body temperature was greater than or equal to ($\geq$) 37.5 °C. If axillary temperature was measured, an individual was considered febrile if his/her body temperature was greater than or equal to ($\geq$) 37.2°C.

Parasite density Parasite density was used in papers 1 and 2. It represented numbers of *Plasmodium falciparum* found per microliter (μl) of blood. In paper 1, rather than using parasite density as a continuous variable, we also created a categorical variable for it. The levels were: (1) no parasite, (2) 1-100 parasites/μl, (3) 101-500 parasites/μl, (4) 501-1000 parasites/μl, (5) 1001-5000

parasites/μl, and (6) more than 5000 parasites/μl. We used it to compared sensitivity and specificity of FalciVax^{TM} across different parasite levels. In paper 2, we created a dichotomous variable for parasite density: *parasite density above median* and *parasite density below median*. The two levels were designed to capture severity of malaria infection and to acknowledge its ability of having non-stationary estimates over time. We used this dichotomous parasite density as a dependent variable to analyze its relationship with clinical malaria symptoms.

2.8.3 Household Factors

Mosquitoes In paper 3, we analyzed the relationship between household building materials and average number of *Anopheles* mosquitoes per visit at a household level. As entomological surveys were recorded visit by visit, we first had to aggregate total number of *Anopheles* mosquitoes at each household. Meanwhile, we summarized the total number of visits each household had during the study time frame. Finally, we divided the two numbers and yielded an average number of *Anopheles* mosquitoes per visit at each household.

$$\begin{aligned}&\text{Average number of } \textit{Anopheles} \text{ mosquitoes at House}_i \\ &= \frac{\text{Total Number of } \textit{Anopheles} \text{ mosquitoes at House}_i}{\text{Total Number of Visits to House}_i}\end{aligned}$$

Building Materials In paper 3, we examined how materials used for building a house could be related to the size of mosquito population. In paper 4, we

used household building materials as part of the criteria for household living standard, and analyzed its relationship with malaria incidence. In both papers, we looked at common materials used for walls, roofs, floors and partitions. Below is a list of materials used for each section of the house.

- *Wall*: (1) Corrugated tin, iron sheet, (2) fired brick, cement, (3) tin, (4) pole and mud, (5) wood, (6) pole and grass, (7) stone, (8) unfired bricks, (9) bamboo, and (10) other
- *Roof*: (1) Straw, thatch, (2) asbestos, (3) pole and grass, (4) pole and mud, (5) bamboo, (6) mud tins, house of tins, (7) corrugated tin, iron sheet, (8) fired brick, cement, (9) concrete, cement, and (10) other
- *Partition*: (1) Jute stick, (2) wood, (3) concrete, cement, (4) mud, (5) tin, (6) bamboo, and (7) other
- *Floor*: (1) Mud, (2) bamboo, (3) semi-cement, (4) vinyl, (5) cement, (6) wood, and (7) other

Living Standard In paper 4, we discussed the living standards in Bandarban Study Area. Living standards was an index created by a collection by durable assets and household building materials. Durable assets included in the questionnaires were: (1) electricity, (2) television, (3) radio, (4) almirah, (5) bad, (6) clock, (7) refrigerator, (8) fan, (9) dining table, (10) telephone, (11) sofa set, (12) chair, (13) sewing machine, (14) blanket, (15) bednet, (16) power tiller, (17) rick mill, (18) rickshaw, (19) bicycle, (20) fishing boat, (21) modern agriculture machines, (22) shallow machine, (23) vehicles to rent out, (24) tube

well, (25) crushing mill, (26) khat, (27) reserved clothes, (28) dheki, (29) variety store, (30) fish hatchery, (31) fishing net, (32) live stocks, and (33) poultry.

2.9 Statistical Analysis

2.9.1 Paper 1: Field Performance of FalciVax^{TM} RDT in rural Bangladesh

We started the analyses by creating contingency tables for FalciVax^{TM} RDT and Giemsa-stained Microscopy. We calculated malaria prevalence, sensitivity, specificity, positive and negative predictive values were calculated. We then used logistic regression to model FalciVax^{TM} screening results given the comparative gold standard Giemsa-stained Microscopy results, and vice versa. Models used for adjusted and adjusted sensitivity, specificity, positive and negative predictive values are shown below:

Unadjusted Modeled:

Sensitivity and Specificity:

$$logit\ Pr(\text{RDT Positive} \mid \text{Microscopy Result}) = \beta_0 + \beta_1 * \text{Microscopy Result}$$

Positive and Negative Predictive Values:

$$logit\ Pr(\text{Microscopy Positive} \mid \text{RDT Result}) = \beta_0 + \beta_1 * \text{RDT Result}$$

Adjusted Modeled:

$$logit\ Pr(Y = 1|X) = \beta_0 + \beta_1 * X_1 + \sum_{k=2}^{K} \beta_k * X_k \tag{2.4}$$

where

Y is FalciVax^{TM} RDT status and X_1 is Giemsa-stained microscopy test results when calculating sensitivity and specificity; or,

Y is Giemsa-stained microscopy test results and X_1 is FalciVax^{TM} RDT status when calculating positive and negative predictive values;

and

X_k $(k = 2, 3, ...K)$ are the added categorical covariate of interest. Categorical variables (such as "seasons") were incorporated as dummy variables when we modeled sensitivity, specificity and predictive values using logistic regressions.

2.9.2 Paper 2: Association Between Levels of *Plasmodium falciparum* Density and Clinical Malaria Symptoms

To explore the relationship between self-reported symptoms and levels of parasite density, we chose to use a logistic regression model. A dichotomous dependent variable ("levels of parasite density") was used to acknowledge the non-stationary estimates of parasite density over time. Although blood films were taken at the same time as measurements of self-reported symptoms, a precised estimate of parasite density could be hard to achieve as a dependent

variable. Nonetheless, relative rank of parasite density among tested study participants should remain the same. Therefore, we chose to use a dichotomous variable to indicate measured levels of parasite density. To generate the variable, we began by examining distribution of parasite density. The distribution of parasite density was right skewed. We took natural log of parasite density ("*log*(parasite density)") to find its distribution was closer to a bimodal distribution. The two modes of *log*(parasite density) could be approximately separated by its median. Levels of parasite density—showcasing *parasite density above median* and *parasite density below median*—was then created.

We used logistic regression models to calculate the expected odds on acquiring high level of parasite density on a given exposure (e.g. measured fever status, self-reported symptom, symptom duration and demographic characteristics).

Exposure of Interest: A Continuous Variable

$$\begin{aligned} logit\ Pr(\text{High Density Level}) &= \beta_0 \\ &\quad + \beta_1 * \text{Exposure} \end{aligned}$$

Exposure of Interest: A Categorical Variable

$$\begin{aligned} logit\ Pr(\text{High Density Level}) &= \beta_0 \\ &\quad + \sum_{i=1}^{i=K-1} \beta_i * (\text{Dummy Variables of a Categorical Variable}) \end{aligned}$$

where K equaled to number of categories.

2.9.3 Paper 3: Association between Household Building Materials and the Abundance of *Anopheles* Mosquitoes in Rural Bangladesh

We first identified the top individual materials and material combination used for walls, roofs, floors and partition. Then, we calculated the average number of *Anopheles* mosquitoes per night at a household level. We used linear regressions to model the expected number of *Anopheles* mosquitoes per household per night given their choices of wall, roof, floor or partition material.

$$E(\text{Average Number of } \textit{Anopheles} \text{ per Visit}) = \beta_0 + \sum_{k=1}^{K-1} \beta_k * X_k \qquad (2.7)$$

where X_k $(k = 1, 2, ..., K-1)$ are $K-1$ dummy variables of one covariate of interest (i.e. wall, roof, partition, floor, or ground elevation status).

Followed by a regular linear regression model, we also used linear regression models with areal adjustment to take into account the background information, such as baseline number of *Anopheles* mosquitoes, provided by a certain type of materials (e.g. bamboo). Using excess (or deficient) number of *Anopheles* mosquitoes as a dependent variable, for example, we can better see the effect of mud on number of *Anopheles* mosquitoes without the areal effect from bamboo.

$$\begin{aligned} E(\text{Excess (or deficient) } \textit{Anopheles}) &= E(\text{Difference}_{mos_{ij} - \overline{mos_j}}) \\ &= \beta_0 + \sum_{p=1}^{P-1} \beta_p * X_{ip} \end{aligned}$$

where

- mos_{ij} is the average number of *Anopheles* mosquitoes per night at House i of Area j (j = 1,2,...,100);
- $\overline{mos_j}$ is the areal average of *Anopheles* mosquitoes per night per household at Area j, among households with *bamboo*; and
- X_{ip} $(p = 1, 2, ..., P-1)$ is one covariate of interest (i.e. wall, roof, partition, floor, or common combination of building materials) at House i represented by $P-1$ dummy variables.

2.9.4 Paper 4: Association between Living Standards and Incidence of Human Malaria in southeastern Bangladesh

Thirty-three types of durable assets were surveyed. As assets served as proxies to social economic status, we used principal component analysis to combined durable assets into one index. We further incorporated household building materials with the first principal component to form living standards in Bandarban Study Area. We applied Generalized Estimating Equation (GEE) Poisson regression to estimate log incidence rate of *Pf* malaria as a function of household durable assets and/or household building materials. Poisson regression enabled the calculation of malaria incidence, with provided malaria case counts and study participants' person-time exposures. On the other hand, GEE allowed us to minimize the unmeasured correlation among members of the same household.

2.10 Preliminary Data Analysis

2.10.1 Population Size

From the baseline demographic survey, we enumerated 22,325 people resided within the Bandarban Study Area. Of which, 12,502 people were from Kuhalong Union and 9,823 individuals were from Rajbila Union (Table 2.2). There were 11,064 males (49.6%) and 11,261 (50.4%) females (Table 2.3). Based on this enumerated data, we created a population pyramid as shown in Figure 2.2. The pyramid indicated the society was still expanding. Two things were seen from this population pyramid: (1) Many young adults aged 10-25 years old (Male: 10-15 years old; Female: 15-20 years old) were not present at Bandarban Study Area during the study period. (2) Population size for *children under 5* was smaller than expected (N = 2,765). This indicated a higher mortality or a lower birth rate was seen in an originally high birth and death rates society (Figure 2.2 and Table 2.5).

Detailed population structure by 5-year age groups can be found in Table 2.5.

2.10.2 Demographic Characteristics

In Bandarban Study Area, approximately 65% of the study population were 15 or above (N = 14,323 (64.2%)) (Tables 2.3 and 2.5). Among individuals aged 15 or older, 74.5% (N = 10,666) was married, 8.5% (N = 1,211) was divorced, separated or widowed, and only 17.1% (N = 2,446) were single.

Entering a marriage at a relatively young age also indicated years of education could be limited. An average of 2.8 years of education was observed in our study population older than 15 years of age. Most popular occupation for these individuals were farming (N = 5,469), day laboring (N = 2,263), being a housewife (N = 2,060) and doing Jhum cultivation (N = 1,001). (Table 2.3)

2.10.3 Study Population

At Bandarban Study Area, three types of human malaria surveillance systems were set up: Active surveillance, nested longitudinal surveillance and passive surveillance. At Month 0 and Day 0, we enrolled 2,727 individuals for active surveillance, 1,269 individuals for nested longitudinal surveillance, and 708 individuals for passive surveillance (Table 2.4). If tested positive by either FalciVax^{TM} RDT or microscopy, the study team would conduct followups on Day 2, Day 7 and Day 28. From Table 2.4, we can see 28 out of 2,737 had follow-ups on subsequent days. This showed the point prevalence of malaria from 2009 to 2013 was between 1-2%.

2.10.4 FalciVax^{TM} RDT and Microscopy

From 2009 to 2013, 10,521 individuals from Bandarban Study Area were enrolled for malaria diagnoses. This was a collective number from all three surveillance systems. Among them, 99% (N = 10,426) were tested by both FalciVax^{TM} RDT and Microscopy, 0.9% (N = 93) had malaria testing results from either FalciVax^{TM} RDT or Microscopy. Two of them did not receive either diagnostic

methods. (Table 2.6)

Of the 10,427 individuals tested by microscopy, 733 people were malaria infected (Table 2.7). We compared the overall results from FalciVax^{TM} RDT and Microscopy and found 9,210 out of 10,425 pairs had concordant results based on types of *Plasmodium* infection and their test results. (Tables 2.8 and 2.9)

2.10.5 *Plasmodium* Parasite

Parasite Density

Among the 9,210 concordant FalciVax^{TM} RDT-Microscopy pairs, the pairs with both positive *Plasmodium falciparum* and positive *Plasmodium vivax* had the highest parasite density on average (7,570 parasites/μl) (N = 4). The density (from high to low) was followed by concordant pairs with positive *Plasmodium falciparum* and negative *Plasmodium vivax* (7,402 parasites/μl) (N = 693), and then by pairs with both negative *Plasmodium falciparum* and positive *Plasmodium vivax* results (3,776 parasites/μl) (N = 24). Finally, majority of the concordant pairs were both negative on *Plasmodium falciparum* and *Plasmodium vivax* tests (0 parasite/μl) (N = 8,489). (Table 2.9)

Box plots comparing parasite density among individuals with different types of *Plasmodium* infection (i.e. *Plasmodium falciparum* and *Plasmodium vivax*) through different diagnostic methods (i.e. microscopy and FalciVax^{TM} RDT) could be found in Figure 2.3. Briefly, concordant pairs had higher parasite density; discordant pairs had lower parasite density. (Figure 2.3)

Parasite Stage

Of participants who enrolled and received for malaria diagnoses in 2009-2013 (N = 10,521), we found *early trophozoite stage* was the most common parasite stage during microscopy examination (N = 729)(Table 2.10). Within the early trophozoite stage, we found 95.5% of cases were from *Plasmodium falciparum* infection (N = 696), 4% came from *Plasmodium vivax* infection (N = 29), and less than 1% was from positive *Plasmodium falciparum* and *Plasmodium vivax* infection (Table 2.11).

2.10.6 Malaria Symptoms

Fever Status and Duration

Among individuals enrolled in active, nested longitudinal and passive surveillance, 4,717 individuals were visited on Month 0 and Day 0 (i.e. the initial date for home visit). Table 2.12 showed number of participants with fever status in relation to their microscopy results. Table 2.13 used self-reported fever duration as an indicator to show its relation with study participants' microscopy results. From both tables, we learned measured fever was presented at about half of the passive surveillance study participants. However, the fever population was much smaller in the active and nested longitudinal study (Table 2.12). Of participants claimed to have fever (i.e. "self-reported"), almost all of them were malaria tested within a week (Table 2.13).

Individual Symptoms

When looking at self-reported symptoms, cough (N = 422 (10.5%)), muscle weakness (N = 361 (9.0%)) and headache (N = 352 (8.8%)) were the top three individually reported symptoms on Month 0 and Day 0 (i.e. the initial date for home visit) among active and nested longitudinal surveillance study participants (N = 4010) (Table 2.14). Among passive surveillance group, headache (N = 568 (80.1%)), muscle ache (N = 441 (62.2%)) and fever with sweating (N = 413 (58.3%)) were reported the most on Day 0 (N = 709) (Table 2.15).

Patterns of Symptoms

Active and Nested Longitudinal Surveillance (Month 0 Day 0) There are 246 patterns of self-reported malaria symptoms reported by individuals in Active and Nested Longitudinal Surveillance. Top 10 patterns are listed in Table 2.16. Patterns that were not included in Table 2.16 had 16 or fewer people in each pattern. The most commonly reported pattern among the 246 scenarios was the one without any observed symptoms over the past 48 hours (N = 3038 individuals).

We further examined pattern frequency of self-reported malaria symptoms reported by malaria positive[1] individuals in Active and Nested Longitudinal Surveillance (N = 44) (Tables 2.17 and 2.18). There were 25 different symptom patterns among malaria positive individuals. Similar to results shown in Active

[1]To acquire malaria positive individuals, Active and Nested Longitudinal Database was merged with the Laboratory Database (at Month 0 Day 0). All malaria positive cases were confirmed by Blood Smear results.

and Nested Longitudinal data set for all individuals, "No reported symptoms" in the past 48 hours was the most commonly reported pattern among malaria positive individuals (N = 16).

Passive Surveillance (Day 0) There are 469 patterns of self-reported malaria symptoms reported by individuals in Passive Surveillance. Top 10 patterns are listed in Table 2.19. Patterns that were not included in Table 2.19 had 3 or fewer people in each pattern. The most commonly reported pattern among the 469 scenarios was the one without any observed symptoms (reported by 86 individuals).

Similar to examining pattern frequency of self-reported malaria symptoms reported in active and nested longitudinal study, we examined pattern frequency of self-reported malaria symptoms reported by malaria positive[2] individuals in Passive Surveillance (N = 556) (Tables 2.20 and 2.21). There were 428 different symptom patterns among malaria positive individuals. Unlike results from Passive Surveillance data set for all individuals, "No reported symptoms" was not the most common pattern among malaria positive individuals. Both top ranked patterns (reported by 7 individuals each) have shown fever, headache, chills, nausea, fatigue, muscle ache and muscle weakness as part of the observed malaria symptoms.

[2]To acquire malaria positive individuals, Passive Surveillance Database was merged with the Laboratory Database (at Month 0 Day0). All malaria positive cases were confirmed by Blood Smear results.

2.10.7 *Anopheles* Mosquitoes

Number of Houses

From 2009 to 2012, we visited 1079 unique households for entomological survey. We set light traps at selected households. Majority of selected households (N = 803 (74.4%)) were visited 1 to 5 times. Few households (N = 7 (0.6%)) were visited more than 20 times. Distribution of number of visits can be found in Table 2.22.

Number of Light Traps

Overall, 4,368 light traps were set and collected at selected household. More than 75% (N = 3,331) of the CDC mosquito light traps found 1-10 *Anopheles* mosquitoes overnight (i.e. a 12-hour period); 13% (N = 582) of the light traps didn't find any *Anopheles* mosquitoes during the 12-hour period. The rest (12%) of the light traps captured 11 to 283 *Anopheles* a night.(Table 2.23)

Number of *Anopheles* Mosquitoes

In 2009-2012, 22,224 *Anopheles* mosquitoes were collected in the study area by using the CDC light trap method. Each year, we collected approximately six to eight thousand *Anopheles* mosquitoes in the study area—with the exception in 2009, where only parts of the year were enrolled in the entomological study. In terms of absolute numbers of *Anopheles* mosquito caught, we have gathered the most *Anopheles* mosquitoes during Monsoon (N = 6,391) and Autumn (N = 5,385). During low malaria transmission seasons, we could still find one to

two thousand *Anopheles* mosquitoes per month. (Table 2.24)

By taking into account the number of light traps set at each household per night, we found the average number of *Anopheles* mosquitoes gathered per night (per light trap) ranged from 1.88 to 7.76. Interestingly, March (N = 7.76) trumped the average number of *Anopheles* mosquitoes per household per night (per light trap) over the ones gathering during high malaria transmission months: June (N = 6.43), July (N = 7.01) and August (N = 6.11). (Table 2.25)

This could be related to a wide spectrum of *Anopheles* species identified in the study area. Type of *Anopheles* species attributable to the population dynamics in March might not be the same as the ones in June to August. (Table 2.26)

2.10.8 Household Building Materials

During the study period, we surveyed household building materials used at different sections of the house, such as wall, roof, floor and partition. Overall, there were 5,006 households location within Bandarban Study Area from 2009 to 2013. Corrugated tin (N = 3,275 households (65%)) and thatch (N = 1,565 households (31%)) were the top two roofing materials used by the locals. Bamboo walls and bamboo partitions was the most common material used for walls (N = 4,179 households (83%)) and partitions (N = 3,964 households (79%)). Mud was the second most used wall and partition materials in the area (Wall: N = 634 households (13%); Partition: N = 620 households (12%))). As for flooring materials, more households were built with mud (N = 2,483 households

(50%)) than bamboo (N = 1,822 households (36%)) at the study site. (Table 2.27)

When looking at materials used at all sections of a house as a whole, we could identify the most popular combination of household building materials used in Kuhalong Union and Rajbila Union, as shown in Table 2.28. In Kuhalong (N = 2,710 households), the most commonly used material combination was using bamboo for wall, partition and floor, corrugated tin for roof and having elevated ground floor (N = 579 households (21%)). In Rajbila (N = 2,296 households), on the other hand, households were most commonly used bamboo as their wall and partition materials, mud as flooring material, corrugated tin as roofing material and were built without elevated ground floor (N = 470 (17%)). (Table 2.28)

2.10.9 Living Standards

During the study period, we used standardized questionnaires to collect information related to a person's living standard. Here we used information gathered on the *main water source of a household* and *near by water source of a household* as an example to understand the living situation in Bandarban Study Area in 2009-2013.

Thirty-one percent of the households (N = 1,585 households) in the study area used river or stream as the family's main source of water. Forty-three percent of the households (N = 2,131 households) used tube well. In Kuhalong, 608 households (22% of households in the Union) claimed to use ring wells as their

main source of water; whereas, only 328 households in Rajbila (14% of households in the Union) used ring wells. (Table 2.29)

Regardless the main source of water a family used, almost all households (N = 4,521 (90%)) indicated having a nearby river or stream from home. Having a pond near the house was another common feature identified by household members in Bandarban Study Area (N = 1,490 households (30%)). Ditches, irrigation and open drain were more accessible to households in Kuhalong than in Rajbila. (Table 2.30)

In Tables 2.31 and 2.32, we cross-tabulated and compared the main water source a household used and nearby water sources a household had. Most families used river, stream or tube well as a main water source indicated they did not have any pond, ditch, irrigation, open drain or uncovered water storage near the house. Majority of households using a ring well, private well or pump, uncovered water storage container and dam indicated having a nearby river or stream near the house (Tables 2.31 and 2.32). This indicated rivers and streams in Bandarban Study Area could be a potential hot spot for ongoing malaria transmission.

Table 2.1: Summary: Time Frame of Data Collection

Category	Data	Date	
		To	From
Weather	Weather Data	01/01/2009	12/31/2013
Mosquito	Entomological Data	07/20/2009	10/18/2012
Human	Active Surveillance Data		
	Active Surveillance	10/18/2009	09/30/2012
	Nested Longitudinal	10/18/2009	03/20/2013
	Passive Surveillance Data		
	Passive Surveillance	10/21/2009	09/24/2013
	Human Laboratory Data		
	from Active Surveillance	10/18/2009	09/30/2012
	from Nested Longitudinal	10/18/2009	07/04/2013
	from Passive Surveillance	10/21/2009	09/23/2013
Population Information	Demographic Surveillance Data—Initial Visit		
	in Kuhalong Union	10/18/2009	08/29/2013
	in Rajbila Union	04/18/2010	09/02/2013
	Demographic Surveillance Data—Followup Visits		
	in Kuhalong Union	03/01/2010	09/25/2013
	in Rajbila Union	05/13/2010	09/25/2013
	Social Economic Status Data		
	Social Economic Status	10/18/2009	09/02/2013

Table 2.2: Population Size in Each Cluster

	Population per Cluster (C)					
Union	C01	C02	C03	C04	C05	C06
Kuhalong	913	1150	847	892	1307	1100
Rajbila	892	730	820	1016	697	882
	Population per Cluster (C)					
Union	C07	C08	C09	C10	C11	C12
Kuhalong	1321	968	824	1223	804	1153
Rajbila	827	660	1075	742	769	713

Table 2.3: Basic Demographic Information of Population in Study Area

Population in Study Area				
Factor		All Ages N = 22,325		
Gender				
	Male	11064	(49.56	%)
	Female	11261	(50.44	%)
Age				
	$<$ 15 years old	8001	(35.84	%)
	$\geq$ 15 years old	14323	(64.16	%)
Population in Study Area				
Factor		Age $\geq$ 15 N = 14,323		
Education (Age $\geq$ 15)				
	No Education	7620	(53.20	%)
	1-6 years	3846	(26.85	%)
	7+ years	2857	(19.95	%)
	Average	2.83±3.60		
Marital Status$^{\alpha}$ (Age $\geq$ 15)				
	Married	10666	(74.47	%)
	Single	2446	(17.08	%)
	Other	1211	(8.45	%)
Occupation (Age $\geq$ 15)				
	Farming	5469	(38.18	%)
	Daily Labor	2263	(15.80	%)
	Housewife	2060	(14.38	%)
	Jhum Cultivation	1001	(6.99	%)
	Unemployed	973	(6.79	%)
	Student	890	(6.21	%)
	Other	1667	(11.64	%)

$^{\alpha}$ 4 people younger than 15 years old were married, widowed or separated

Table 2.4: Number of individuals visited in different months/days in Active, Nested Longitudinal and Passive Surveillance

Visit Month	**Visit Day**	**Surveillance Type** Active Surveillance	Nested Longitudinal Surveillance	Passive Surveillance
Month 0				
	Day 0	2737	1269	708
	Day 2	28	24	679
	Day 7	28	24	688
	Day 28	28	24	661
Month 3				
	Day 0	0	1202	0
	Day 2	0	7	1
	Day 7	0	7	0
	Day 28	0	6	0
Month 6				
	Day 0	0	1189	0
	Day 2	0	6	0
	Day 7	0	6	0
	Day 28	0	4	0
Month 9				
	Day 0	0	1172	0
	Day 2	0	8	0
	Day 7	0	8	0
	Day 28	0	7	0

Table 2.5: Population Size by Age Group

Age at Initial Demographic Survey	**Gender**		**Row Total**	**Group Total**
	Male	Female		
Children Under 5				2765
[0,5)	1372	1393	2765	
Age 5 to <15				5236
[5,10)	1445	1473	2918	
[10,15)	1150	1168	2318	
Age 15 to <35				7773
[15,20)	799	962	1761	
[20,25)	913	1245	2158	
[25,30)	1065	1117	2182	
[30,35)	883	789	1672	
Age 35 to <65				5612
[35,40)	757	681	1438	
[40,45)	595	581	1176	
[45,50)	516	468	984	
[50,55)	491	425	916	
[55,60)	323	295	618	
[60,65)	255	225	480	
Age 65+				938
[65,70)	196	139	335	
[70,75)	144	159	303	
[75,80)	74	62	136	
[80,85)	51	45	96	
[85,90)	21	18	39	
[90,95)	7	10	17	
[95,100)	2	2	4	
[100,115)	5	3	8	
Column Total	11064	11260		22324

Table 2.6: Number of Individuals Taken Rapid Diagnostic Test and/or Blood Smear

	Blood Smear Taken?		
RDT Taken?	Yes	No	**Row Total**
Yes	10426	92	10518
No	1	2	3
Column Total	10427	94	10521

Table 2.7: Comparison between malaria test status and results of RDT and Blood Smear

FalciVax™ RDT Taken?	**Blood Smear**									
	Yes			No			NA			
Results?	Positive	Negative	NA	Positive	Negative	NA	Positive	Negative	NA	**Row Total**
Yes										
Positive	726	1204	1	0	0	91	0	0	0	2022
Negative	6	8489	0	0	1	0	0	0	0	8496
NA	0	0	0	0	0	0	0	0	0	0
No										
Positive	0	0	0	0	0	0	1	0	0	1
Negative	0	0	0	0	1	0	0	0	0	1
NA	1	0	0	0	0	0	0	0	0	1
NA										
Positive	0	0	0	0	0	0	0	0	0	0
Negative	0	0	0	0	0	0	0	0	0	0
NA	0	0	0	0	0	0	0	0	0	0
Column Total	733	9693	1	0	2	92	0	0	0	10521

Table 2.8: Agreement on Type of Malaria Infection between Blood Smear and FalciVax™ RDT

	Blood Smear				
FalciVax™ RDT	Pf(−), Pv(−)	Pf(−), Pv(+)	Pf(+), Pv(−)	Pf(+), Pv(+)	**Row Total**
Pf(−), Pv(−)	8489	2	4	0	8495
Pf(−), Pv(+)	8	24	0	0	32
Pf(+), Pv(−)	1196	0	693	0	1889
Pf(+), Pv(+)	1	3	1	4	9
Column Total	9694	29	698	4	10425

Table 2.9: Parasite Count of Individuals who Received Results from Blood Smear and FalciVax ™ RDT Tests

Blood Smear	**FalciVax™ RDT**	**Parasite Count** N	Mean	Min	Median	Max	Range	SD	**Note**
Pf(−), Pv(−)	Pf(−), Pv(−)	8489	0.00	0	0	0	0	0.00	concordant Pair
Pf(+), Pv(−)	Pf(−), Pv(−)	4	340.00	40	360	600	560	249.80	
Pf(−), Pv(+)	Pf(−), Pv(−)	2	240.00	0	240	480	480	339.41	
Pf(+), Pv(+)	Pf(−), Pv(−)	NA	NA	NA	NA	NA	NA	NA	
Pf(−), Pv(−)	Pf(+), Pv(−)	1196	6.69	0	0	7400	7400	214.66	
Pf(+), Pv(−)	Pf(+), Pv(−)	693	7402.03	80	4880	144000	143920	10118.84	concordant Pair
Pf(−), Pv(+)	Pf(+), Pv(−)	NA	NA	NA	NA	NA	NA	NA	
Pf(+), Pv(+)	Pf(+), Pv(−)	NA	NA	NA	NA	NA	NA	NA	
Pf(−), Pv(−)	Pf(−), Pv(+)	8	0.00	0	0	0	0	0.00	
Pf(+), Pv(−)	Pf(−), Pv(+)	NA	NA	NA	NA	NA	NA	NA	
Pf(−), Pv(+)	Pf(−), Pv(+)	24	3775.83	80	2800	13800	13720	3493.20	concordant Pair
Pf(+), Pv(+)	Pf(−), Pv(+)	NA	NA	NA	NA	NA	NA	NA	
Pf(−), Pv(−)	Pf(+), Pv(+)	1	0.00	0	0	0	0	NA	
Pf(+), Pv(−)	Pf(+), Pv(+)	1	112000.00	112000	112000	112000	0	NA	
Pf(−), Pv(+)	Pf(+), Pv(+)	3	173.33	160	160	200	40	23.09	
Pf(+), Pv(+)	Pf(+), Pv(+)	4	7570.00	880	8000	13400	12520	5556.82	concordant Pair

Table 2.10: Malaria Parasite Stage Examined by Blood Smear

Malaria Infection	**Blood Smear** **Taken?**	Yes			No			
Parasite Stage	**Results?**	Positive	Negative	NA	Positive	Negative	NA	**Row Total**
Early Trophozoite		729	0	0	0	0	0	729
Late Trophozoite		0	0	0	0	0	0	0
Schizont		1	0	0	0	0	0	1
Gametocyte		2	0	0	0	0	0	2
None of the Above		1	9693	1	0	2	92	9789
Column Total		733	9693	1	0	2	92	10521

Table 2.11: Malaria Parasite Stage by Participants' P. falciparum and P. vivax Infection Status among Individuals Examined by Blood Smear and were Malaria Positive

	Blood Smear: Positive				
Parasite Stage	Pf(−), Pv(−)	Pf(−), Pv(+)	Pf(+), Pv(−)	Pf(+), Pv(+)	**Row Total**
Early Trophozoite	1	29	696	3	729
Late Trophozoite	0	0	0	0	0
Schizont	0	0	1	0	1
Gametocyte	0	0	1	1	2
None of the Above	0	1	0	0	1
Column Total	1	30	698	4	733

Table 2.12: Frequency Table of Fever Status by Study Type and Individuals' Blood Smear Results, at Initial Visit (Day 0) of Month 0

	Study Type and Blood Smear Status (Taken/Not Taken) and Results (+ / - / Not Applicable)																		
	Active						Nested Longitudinal						Passive						
	Taken			Not Taken			Taken			Not Taken			Taken			Not Taken			
Type																			**Row**
Fever Status	+	−	NA	+	−	NA	+	−	NA	+	−	NA	+	−	NA	+	−	NA	**Total**
Oral																			
No Fever—Cold																			
[32°C, 35°C)	0	3	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	5
[35°C, 36°C)	1	188	0	0	0	0	3	95	0	0	0	0	5	2	0	0	0	0	294
No Fever—Normal																			
[36°C, 37.5°C)	15	1470	0	0	0	0	13	856	0	0	0	0	209	28	0	0	0	0	2591
With Fever																			
≥37.5°C	1	19	0	0	0	0	1	3	0	0	0	0	202	17	1	0	0	0	244
Axillary																			
No Fever—Cold																			
[32°C, 35°C)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
[35°C, 36°C)	0	155	0	0	0	0	0	40	0	0	0	0	5	0	0	0	0	0	200
No Fever—Normal																			
[36°C, 37.2°C)	7	854	0	0	1	0	2	238	0	0	0	0	55	4	0	0	0	1	1162
With Fever																			
≥37.2°C	0	23	0	0	0	0	1	14	0	0	0	0	79	9	0	0	0	0	126
Not Taken																			
NA	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	90	91
Column Total	24	2712	0	0	1	0	20	1248	0	0	0	1	556	60	1	0	0	91	4714

Table 2.13: Frequency Table of Self Reported Fever Duration by Study Type and Individuals' Blood Smear Results, at Initial Visit (Day 0) of Month 0

	Study Type and Blood Smear Status (Taken/Not Taken) and Results (+ / - / Not Applicable)																		
	Active						Nested Longitudinal						Passive						
	Taken			Not Taken			Taken			Not Taken			Taken			Not Taken			**Row**
Fever Status	+	−	NA	+	−	NA	+	−	NA	+	−	NA	+	−	NA	+	−	NA	**Total**
No Fever																			
0 Day	23	2702	0	0	1	0	13	1236	0	0	0	1	151	20	0	0	0	85	4232
Fever																			
1 Day	0	1	0	0	0	0	0	4	0	0	0	0	50	2	0	0	0	2	59
2 Days	0	3	0	0	0	0	2	4	0	0	0	0	100	14	0	0	0	1	124
3 Days	1	2	0	0	0	0	4	3	0	0	0	0	100	10	1	0	0	1	122
4 Days	0	3	0	0	0	0	0	0	0	0	0	0	81	4	0	0	0	0	88
5 Days	0	1	0	0	0	0	0	1	0	0	0	0	34	2	0	0	0	2	40
6 Days	0	0	0	0	0	0	0	0	0	0	0	0	9	2	0	0	0	0	11
7 Days	0	0	0	0	0	0	0	0	0	0	0	0	21	4	0	0	0	0	25
8 Days	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	3
9 Days	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3
10 Days	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	3
11 Days	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
12 Days	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
20 Days	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
25 Days	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Column Total	24	2712	0	0	1	0	20	1248	0	0	0	1	556	60	1	0	0	91	4714

Table 2.14: Active and Nested Longitudinal Surveillance: Self-Reported Symptoms at Month 0 and Day 0

	Presence of Symptoms					
Symptom	Yes	(%)	No	(%)	**Row Total**	(%)
Fever with Shivering	15	(0.37%)	3995	(99.63%)	4010	(100.00%)
Fever During the Day	53	(1.32%)	3957	(98.68%)	4010	(100.00%)
Fever During the Night	106	(2.64%)	3904	(97.36%)	4010	(100.00%)
Fever with Sweating	39	(0.97%)	3971	(99.03%)	4010	(100.00%)
Intermittent Fever	47	(1.17%)	3963	(98.83%)	4010	(100.00%)
Remission of Fever with Sweating	20	(0.50%)	3990	(99.50%)	4010	(100.00%)
Headache	352	(8.78%)	3658	(91.22%)	4010	(100.00%)
Chills	95	(2.37%)	3915	(97.63%)	4010	(100.00%)
Nausea	141	(3.52%)	3869	(96.48%)	4010	(100.00%)
Vomiting	98	(2.44%)	3912	(97.56%)	4010	(100.00%)
Diarrhea	33	(0.82%)	3977	(99.18%)	4010	(100.00%)
Cough	422	(10.52%)	3588	(89.48%)	4010	(100.00%)
Fatigue	244	(6.08%)	3766	(93.92%)	4010	(100.00%)
Muscle Ache	300	(7.48%)	3710	(92.52%)	4010	(100.00%)
Muscle Weakness	361	(9.00%)	3649	(91.00%)	4010	(100.00%)
Convulsions, Seizure	12	(0.30%)	3998	(99.70%)	4010	(100.00%)
Anemia	27	(0.67%)	3983	(99.33%)	4010	(100.00%)

Table 2.15: Passive Surveillance: Self-Reported Symptoms at Day 0

	Presence of Symptoms					
Symptom	Yes	(%)	No	(%)	**Row Total**	(%)
Fever with Shivering	304	(42.88%)	405	(57.12%)	709	(100.00%)
Fever During the Day	288	(40.62%)	421	(59.38%)	709	(100.00%)
Fever During the Night	300	(42.31%)	409	(57.69%)	709	(100.00%)
Fever with Sweating	413	(58.25%)	296	(41.75%)	709	(100.00%)
Intermittent Fever	236	(33.29%)	473	(66.71%)	709	(100.00%)
Remission of Fever with Sweating	177	(24.96%)	532	(75.04%)	709	(100.00%)
Headache	568	(80.11%)	141	(19.89%)	709	(100.00%)
Chills	383	(54.02%)	326	(45.98%)	709	(100.00%)
Nausea	382	(53.88%)	327	(46.12%)	709	(100.00%)
Vomiting	283	(39.92%)	426	(60.08%)	709	(100.00%)
Diarrhea	6	(0.85%)	703	(99.15%)	709	(100.00%)
Cough	141	(19.89%)	568	(80.11%)	709	(100.00%)
Fatigue	260	(36.67%)	449	(63.33%)	709	(100.00%)
Muscle Ache	441	(62.20%)	268	(37.80%)	709	(100.00%)
Muscle Weakness	403	(56.84%)	306	(43.16%)	709	(100.00%)
Convulsions, Seizure	2	(0.28%)	707	(99.72%)	709	(100.00%)
Anemia	11	(1.55%)	698	(98.45%)	709	(100.00%)
Do Not Know	87	(12.27%)	622	(87.73%)	709	(100.00%)
Other	1	(0.14%)	708	(99.86%)	709	(100.00%)

Table 2.16: Patterns Frequency of Self-Reported Malaria Symptoms from Individuals in Active and Nested Longitudinal Surveillance (Month 0 Day 0)

Pattern Rank	**Pattern of Self-Reported Malaria Symptoms**												**Number of People**
	Fever with Shivering	Fever During the Day	Fever During the Night	Fever with Sweating	Intermittent Fever	Remission of Fever with Sweating	Headache	Chills	Nausea	Vomiting	Diarrhea	Cough	Fatigue	Muscle Ache	Muscle Weakness	Convulsions, Seizure	Anaemia	
1	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	3038
2	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	No	No	No	No	196
3	No	No	No	No	No	No	No	No	No	No	No	No	No	**Yes**	**Yes**	No	No	71
4	No	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	No	No	No	48
5	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	**Yes**	**Yes**	No	No	41
5	No	No	No	No	No	No	No	No	No	No	No	**Yes**	**Yes**	No	No	No	No	41
7	No	No	No	No	No	No	**Yes**	No	No	No	No	No	No	No	No	No	No	31
8	No	No	No	No	No	No	**Yes**	No	No	No	No	No	No	**Yes**	**Yes**	No	No	22
8	No	No	No	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	No	22
8	No	No	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	No	No	22

Table 2.17: Patterns Frequency of Self-Reported Malaria Symptoms from Blood Smear Positive Individuals in Active and Nested Longitudinal Surveillance (Month 0 Day 0)

	Pattern of Self-Reported Malaria Symptoms													
Pattern Rank	Fever with Shivering	Fever During the Day	Fever During the Night	Fever with Sweating	Intermittent Fever	Remission of Fever with Sweating	Headache	Chills	Nausea	Vomiting	Diarrhea	Cough	Fatigue	Muscle Ache	Muscle Weakness	Convulsions, Seizure	Anaemia	**Number of People**
1	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	16
2	No	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	No	No	No	4
3	**Yes**	**Yes**	No	No	No	No	**Yes**	**Yes**	**Yes**	No	No	No	No	**Yes**	**Yes**	No	No	2
4	No	No	**Yes**	No	**Yes**	No	**Yes**	No	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	**Yes**	1
4	No	No	No	No	No	No	**Yes**	**Yes**	No	**Yes**	No	No	No	No	No	No	**Yes**	1
4	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	**Yes**	**Yes**	**Yes**	No	1
4	No	No	No	**Yes**	**Yes**	No	**Yes**	No	**Yes**	No	No	No	No	No	**Yes**	**Yes**	No	1
4	**Yes**	**Yes**	**Yes**	No	**Yes**	No	**Yes**	**Yes**	No	No	No	No	**Yes**	**Yes**	**Yes**	No	No	1
4	No	No	No	No	No	No	No	No	No	No	No	**Yes**	No	**Yes**	**Yes**	No	No	1
4	No	No	No	No	**Yes**	No	**Yes**	No	No	**Yes**	No	No	No	**Yes**	**Yes**	No	No	1
4	**Yes**	**Yes**	No	No	No	No	**Yes**	No	**Yes**	No	No	No	No	**Yes**	**Yes**	No	No	1
4	No	No	No	No	No	No	**Yes**	No	No	No	No	No	No	**Yes**	**Yes**	No	No	1
4	No	No	No	No	No	No	**Yes**	No	No	No	No	**Yes**	**Yes**	No	**Yes**	No	No	1

Table 2.18: Patterns Frequency of Self-Reported Malaria Symptoms from Blood Smear Positive Individuals in Active and Nested Longitudinal Surveillance (Month 0 Day 0) (Continued)

	Pattern of Self-Reported Malaria Symptoms												Number of People
Pattern Rank	Fever with Shivering	Fever During the Day	Fever During the Night	Fever with Sweating	Intermittent Fever	Remission of Fever with Sweating	Headache	Chills	Nausea	Vomiting	Diarrhea	Cough	Fatigue	Muscle Ache	Muscle Weakness	Convulsions, Seizure	Anaemia	
4	No	No	No	No	No	No	**Yes**	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No	1
4	No	No	**Yes**	No	No	No	**Yes**	No	**Yes**	**Yes**	No	No	No	No	**Yes**	No	No	1
4	No	No	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	No	No	No	1
4	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	No	No	No	No	1
4	No	No	No	No	No	No	**Yes**	No	**Yes**	No	No	No	**Yes**	No	No	No	No	1
4	No	No	No	No	No	No	**Yes**	No	No	No	No	**Yes**	No	No	No	No	No	1
4	No	No	**Yes**	No	**Yes**	No	No	No	No	No	No	**Yes**	No	No	No	No	No	1
4	No	No	**Yes**	No	No	No	No	No	No	No	No	**Yes**	No	No	No	No	No	1
4	No	No	No	No	No	No	No	No	No	No	**Yes**	No	No	No	No	No	No	1
4	No	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No	No	No	No	No	No	1
4	**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	No	No	No	No	No	1

Table 2.19: Patterns Frequency of Self-Reported Malaria Symptoms from Individuals in Passive Surveillance (Day 0)

Pattern Rank	Pattern of Self-Reported Malaria Symptoms												Number of People
	Fever with Shivering	Fever During the Day	Fever During the Night	Fever with Sweating	Intermittent Fever	Remission of Fever with Sweating	Headache	Chills	Nausea	Vomiting	Diarrhea	Cough	Fatigue	Muscle Ache	Muscle Weakness	Convulsions, Seizure	Anaemia	
1	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	86
2	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	9
3	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	8
4	No	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	6
5	No	**Yes**	No	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	No	No	No	5
5	No	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	**Yes**	No	No	No	No	**Yes**	No	No	5
5	No	**Yes**	**Yes**	No	No	No	**Yes**	No	**Yes**	No	No	No	No	**Yes**	**Yes**	No	No	5
5	No	No	No	**Yes**	**Yes**	No	**Yes**	**Yes**	**Yes**	No	No	No	No	**Yes**	**Yes**	No	No	5
5	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	5
10	**Yes**	No	**Yes**	No	No	No	**Yes**	**Yes**	No	No	No	No	**Yes**	No	No	No	No	4
10	No	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No	No	**Yes**	No	No	No	4
10	No	**Yes**	No	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	No	No	No	No	4
10	No	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	**Yes**	No	No	No	No	**Yes**	No	No	4
10	No	No	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	4
10	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	No	4

Table 2.20: Patterns Frequency of Self-Reported Malaria Symptoms from Blood Smear Positive Individuals in Passive Surveillance (Month 0 Day 0)

Pattern Rank	**Pattern of Self-Reported Malaria Symptoms**												**Number of People**
	Fever with Shivering	Fever During the Day	Fever During the Night	Fever with Sweating	Intermittent Fever	Remission of Fever with Sweating	Headache	Chills	Nausea	Vomiting	Diarrhea	Cough	Fatigue	Muscle Ache	Muscle Weakness	Convulsions, Seizure	Anaemia	
1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	No	7
1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	7
3	No	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	6
4	No	**Yes**	No	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	No	No	No	5
4	No	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	**Yes**	No	No	No	No	**Yes**	No	No	5
6	**Yes**	No	**Yes**	No	No	No	**Yes**	**Yes**	No	No	No	No	**Yes**	No	No	No	No	4
6	No	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No	No	**Yes**	No	No	No	4
6	No	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	**Yes**	No	No	No	No	**Yes**	No	No	4
6	No	**Yes**	**Yes**	No	No	No	**Yes**	No	**Yes**	No	No	No	No	**Yes**	**Yes**	No	No	4
6	No	No	No	**Yes**	**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No	No	**Yes**	No	No	4
6	No	No	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	No	4
6	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	No	**Yes**	**Yes**	**Yes**	No	No	4
6	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**	**Yes**	No	No	4

Table 2.21: Patterns Frequency of Self-Reported Malaria Symptoms from Blood Smear Positive Individuals in Passive Surveillance (Month 0 Day 0) (Continued)

	Number of People	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Pattern of Self-Reported Malaria Symptoms	Anaemia	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Convulsions, Seizure	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Muscle Weakness	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Muscle Ache	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Fatigue	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Yes
	Cough	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Diarrhea	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Vomiting	No	Yes	No	Yes	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Nausea	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Chills	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
	Headache	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
	Remission of Fever with Sweating	Yes	Yes	No	Yes	No	No	No	No	Yes	Yes	Yes	No	Yes	No	No	No	No	No
	Intermittent Fever	Yes	Yes	No	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No
	Fever with Sweating	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Fever During the Night	Yes	No	No	Yes	No	Yes	No	No	Yes	No	Yes	No	No	No	No	No	No	No
	Fever During the Day	Yes	No	Yes	No	Yes	No	Yes	No	No	Yes	No	No	No	No	Yes	No	No	No
	Fever with Shivering	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pattern Rank		14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14

Table 2.22: Mosquito Light Traps: Visit Frequency per Households

Number of Visits per Household	**Number of Households in Each Union** Rajbila Union	Kuhalong Union	**Row Total**
1-5 Times	380	423	803
6-10 Times	64	105	169
11-15 Times	37	35	72
16-20 Times	10	18	28
21-24 Times	4	3	7
Column Total	495	584	1079

Table 2.23: Summary of *Anopheles* Mosquitoes Found per Light Trap in Each Union

Number of *Anopheles* Found per Light Trap	**Number of Light Traps** in Rajbila Union	in Kuhalong Union	**Row Total**
No *Anopheles* Found			
0 *Anopheles*	266	316	582
1-100 *Anopheles* Found			
1-10 *Anopheles*	1503	1828	3331
11-25 *Anopheles*	192	179	371
26-50 *Anopheles*	25	42	67
51-100 *Anopheles*	9	5	14
More than 100 *Anopheles* Found			
101-283 *Anopheles*	2	1	3
Column Total	1997	2371	4368

Table 2.24: Number of *Anopheles* Caught by Season and Month, 07/20/2009—10/18/2012

	Month													
Year	Winter Jan	→← Feb	Spring Mar	→← Apr	Summer May	→← Jun	Monsoon Jul	→← Aug	Autumn Sep	→← Oct	Pre-Winter Nov	→← Dec	Winter	**Row Total**
2009							262	314	109	330	261	172		1448
2010	198	322	438	78	224	711	1029	1143	1001	788		305		6237
2011	605	491	4	375	220	1363	716	753	691	602	854	700		7374
2012	427	428	1056	609	337	577	1330	1034	975	392				7165
Column Total	1230	1241	1498	1062	781	2651	3337	3244	2776	2112	1115	1177		22224

	Season												
Year	Winter	→←	Spring	→←	Summer	→←	Monsoon	→←	Autumn	→←	Pre-Winter	→←	Winter	**Row Total**
2009							526		418		498		6	1448
2010	471		553		775		2028		1994		386		30	6237
2011	1021		343		1424		1635		1368		1537		46	7374
2012	670		1584		1104		2202		1605					7165
Column Total	2162		2480		3303		6391		5385		2421		438	22224

Local Ecological Seasons:
— Spring: February 18 to April 20
— Summer: April 21 to June 21
— Monsoon: June 22 to August 23
— Autumn: August 23 to October 23
— Pre-winter: October 23 to December 21
— Winter: December 21 to February 17

Table 2.25: Number of Light Traps Set Up by Union and Month, 07/20/2009—10/18/2012

Kuhalong Union	**Month**													
	W	→←	Sp	→←	Su	→←	M	→←	A	→←	PW	→←	W	**Row**
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		**Total**
2009							25	78	25	85	54	54		321
2010	54	54	52	53	68	37	70	63	64	63	0	71		649
2011	68	61	3	65	66	68	71	67	69	71	65	68		742
2012	68	68	67	70	71	74	73	61	73	34				659
Column Total	190	183	122	188	205	179	239	269	231	253	119	193		2371

Rajbila Union	**Month**													
	W	→←	Sp	→←	Su	→←	M	→←	A	→←	PW	→←	W	**Row**
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		**Total**
2009														
2010					61	88	77	82	74	69	0	61		512
2011	65	63	0	65	65	67	78	79	71	74	74	73		774
2012	72	72	71	70	84	78	82	80	78	24				711
Column Total	137	135	71	135	210	233	237	241	223	167	74	134		1997

Overall Union	**Month**													
	W	→←	Sp	→←	Su	→←	M	→←	A	→←	PW	→←	W	**Row**
Summary	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		**Total**
# of Light Traps	327	318	193	323	415	412	476	510	454	420	193	327		4368
# of *Anopheles*	1230	1241	1498	1062	781	2651	3337	3244	2776	2112	1115	1177		22224
Avg. # *Anopheles* per Light Trap	3.76	3.9	7.76	3.29	1.88	6.43	7.01	6.36	6.11	5.03	5.78	3.6		5.09

Local Ecological Seasons:

Spring (Sp): February 18 to April 20 / Summer (Su): April 21 to June 21

Monsoon (M): June 22 to August 23 / Autumn (A): August 23 to October 23

Pre-winter (PW): October 23 to December 21 / Winter (W): December 21 to February 17

Table 2.26: Number of Mosquitoes Caught per Month in Kuhalong Union, 7/20/2009—6/27/2010

	Month												
***Anopheles* Species**	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	**Row Total**
An. aconitus	0	0	0	0	4	1	3	0	1	1	2	1	13
An. annularis	0	0	0	0	0	0	0	2	0	0	0	0	2
An. baimaii	0	1	0	1	2	13	1	6	0	16	11	4	55
An. barbirostris	3	19	38	4	3	0	7	2	7	83	21	4	191
An. culicifacis	0	0	5	6	23	20	0	0	0	0	0	0	54
An. jamesii	7	11	5	0	0	7	0	2	1	1	10	3	47
An. jeyporiensis	60	32	11	15	8	54	41	33	5	46	97	60	462
An. karwari	5	4	1	0	0	13	2	2	0	0	9	9	45
An. kochi	47	109	104	9	4	17	2	23	2	14	6	35	372
An. maculatus	20	13	5	1	1	21	12	6	3	9	6	6	103
An. minimus	20	13	18	0	3	17	0	0	0	1	11	29	112
An. nigerrimus	1	19	23	13	16	5	8	1	1	17	0	1	105
An. pallidus	4	8	3	2	0	3	0	6	7	13	2	1	49
An. philippinensis	4	10	8	2	2	19	76	69	25	20	4	0	239
An. pseudowillmori	0	0	1	0	0	0	0	0	0	1	0	1	3
An. subpictus	0	0	0	0	0	0	2	4	1	1	0	0	8
An. tessellatus	0	0	0	2	0	0	5	0	0	0	0	0	7
An. turkhudi	0	0	0	0	0	0	1	0	0	0	0	0	1
An. umbrosus	7	4	3	7	1	1	7	2	0	46	35	5	118
An. vagus	14	54	171	13	14	20	23	48	32	22	17	5	433
An. varuna	1	1	1	3	1	0	20	14	10	9	0	0	60
An. willmori	5	6	1	0	1	23	5	19	0	3	10	3	76
Unidentified	0	3	1	0	0	0	0	7	0	9	0	0	20
Column Total	199	309	402	82	88	240	222	254	104	322	252	179	2653

Table 2.27: Housing Structure of Roof, Wall, Floor and Partition by Union

Roofing Material	Union		Row Total	Wall Material	Union		Row Total
	Rajbila	Kuhalong			Rajbila	Kuhalong	
Bamboo	4	6	10	Bamboo	1737	2442	4179
Pole and Grass	2	3	5	Pole and Grass	1	0	1
Pole and Mud	1	1	2	Pole and Mud	500	134	634
Mud Tins, House of Tins	19	97	116	Tin	0	1	1
Corrugated Tin, Iron Sheet	1668	1607	3275	Corrugated Tin, Iron Sheet	0	8	8
Fired Brick, Cement	7	8	15	Fired Brick, Cement	38	83	121
Concrete, Cement	5	8	13	Unfired Bricks	1	1	2
Straw, Thatch	588	977	1565	Wood	9	32	41
Asbestos	1	0	1	Stone	0	0	0
Other	1	3	4	Other	10	9	19
Column Total	2296	2710	5006	**Column Total**	2296	2710	5006

Partition Material	Union		Row Total	Flooring Material	Union		Row Total
	Rajbila	Kuhalong			Rajbila	Kuhalong	
Bamboo	1579	2385	3964	Bamboo	741	1081	1822
Mud	486	134	620	Mud	1279	1204	2483
Tin	0	2	2	Vinyl	0	2	2
Wood	11	37	48	Wood	159	240	399
Concrete, Cement	38	77	115	Cement	85	123	208
Jute stick	1	0	1	Semi Cemented	32	60	92
Other	181	75	256	Other	0	0	0
Column Total	2296	2710	5006	**Column Total**	2296	2710	5006

Table 2.28: Top 10 Material Combinations Used for Builing Wall, Roof, Partition, Floor and the Elevation Status of the House

Union Rank	Building Matrials				Elevated?	No. Households
	Wall	Roof	Partition	Floor		
Rajbila						
1	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Mud	No	470
2	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Bamboo	Yes	422
3	Pole and Mud	Corrugated Tin, Iron Sheet	Mud	Mud	No	409
4	Bamboo	Straw, Thatch	Bamboo	Bamboo	Yes	217
5	Bamboo	Straw, Thatch	Bamboo	Mud	No	217
6	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Wood	Yes	128
7	Pole and Mud	Straw, Thatch	Mud	Mud	No	52
8	Bamboo	Straw, Thatch	Other	Bamboo	Yes	47
9	Bamboo	Corrugated Tin, Iron Sheet	Other	Bamboo	Yes	42
10	Bamboo	Corrugated Tin, Iron Sheet	Other	Mud	No	41
Kuhalong						
1	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Bamboo	Yes	579
2	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Mud	No	545
3	Bamboo	Straw, Thatch	Bamboo	Mud	No	441
4	Bamboo	Straw, Thatch	Bamboo	Bamboo	Yes	412
5	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Wood	Yes	186
6	Pole and Mud	Corrugated Tin, Iron Sheet	Mud	Mud	No	55
7	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Semi Cemented	No	50
8	Pole and Mud	Straw, Thatch	Mud	Mud	No	43
9	Fired Brick, Cement	Corrugated Tin, Iron Sheet	Concrete, Cement	Cemented	No	43
10	Bamboo	Corrugated Tin, Iron Sheet	Bamboo	Cemented	No	38

Table 2.29: Water Source: Main Source of Water for Members of the Household, by Union

	Union					
	Rajbila (N=2295)		**Kuhalong** (N=2711)			
Symptom	Frequency	(%)	Frequency	(%)	**Row Total**	(%)
Dam	2	(0.09%)	15	(0.55%)	17	(0.34%)
Piped into Dwelling	2	(0.09%)	55	(2.03%)	57	(1.14%)
Uncovered Water Storage Container	1	(0.04%)	90	(3.32%)	91	(1.82%)
Private Well or Pump	114	(4.97%)	33	(1.22%)	147	(2.94%)
Public Well or Pump	1	(0.04%)	0	(0.00%)	1	(0.02%)
Public Stand Pipe	3	(0.13%)	4	(0.15%)	7	(0.14%)
River or Stream	871	(37.95%)	714	(26.34%)	1585	(31.66%)
Rain	8	(0.35%)	2	(0.07%)	10	(0.20%)
Irrigation	1	(0.04%)	23	(0.85%)	24	(0.48%)
Ring Well (Covered)	328	(14.29%)	608	(22.43%)	936	(18.70%)
Tube Well	964	(42.00%)	1167	(43.05%)	2131	(42.57%)
Column Total	2295	(100.00%)	2711	(100.00%)	5006	(100.00%)

Table 2.30: Water Source: Household's Nearby Water Source, by Union

	Union					
	Rajbila (N=2295)		**Kuhalong** (N=2711)			
Symptom	Frequency	(%)	Frequency	(%)	**Row Total**	(%)
Pond	460	(20.04%)	1030	(37.99%)	1490	(29.76%)
Ditches	59	(2.57%)	97	(3.58%)	156	(3.12%)
Irrigation	50	(2.18%)	226	(8.34%)	276	(5.51%)
Open Drain	17	(0.74%)	74	(2.73%)	91	(1.82%)
River, Stream	2001	(87.19%)	2520	(92.95%)	4521	(90.31%)
Uncovered Water Storage	8	(0.35%)	4	(0.15%)	12	(0.24%)

Table 2.31: Water Source: Main Water Source at Household vs. Household Identified Nearby Water Source

	Household's Main Water Source										
Perceived Nearby Facility	Dam	Piped into Dwelling	Uncovered Water Storage Container	Private Well or Pump	Public Well or Pump	Public Stand Pipe	River or Stream	Rain	Irrigation	Ring Well (Covered)	Tube Well
Have Nearby Pond											
Yes	4	41	25	51	0	0	234	2	1	325	807
No	13	16	66	95	1	7	1350	8	23	611	1324
Have Nearby Ditch											
Yes	0	1	3	0	0	4	69	0	4	31	44
No	17	56	88	147	1	3	1515	10	20	905	2087
Have Nearby Irrigation											
Yes	1	27	5	2	0	2	101	0	20	33	85
No	16	30	86	145	1	5	1484	10	4	903	2046

Table 2.32: Water Source: Main Water Source at Household vs. Household Identified Nearby Water Source (Continued)

	Household's Main Water Source										
Perceived Nearby Facility	Dam	Piped into Dwelling	Uncovered Water Storage Container	Private Well or Pump	Public Well or Pump	Public Stand Pipe	River or Stream	Rain	Irrigation	Ring Well (Covered)	Tube Well
Have Nearby Open Drain											
Yes	2	0	4	10	0	0	27	0	0	4	44
No	15	57	87	137	1	7	1556	10	24	932	2087
Have Nearby River or Stream											
Yes	13	22	82	146	1	4	1404	9	4	874	1962
No	4	35	9	1	0	3	180	1	20	62	169
Have Nearby Uncovered Water Storage											
Yes	0	0	1	0	0	0	8	0	0	0	3
No	17	57	90	147	1	7	1574	10	24	934	2128

Figure 2.1: Geographic Location of Kuhalong Union and Rajbila Union in Bandarban, Bangladesh and Household Distribution in the Study Area. Household locations were mapped based on the GPS data in May 2011

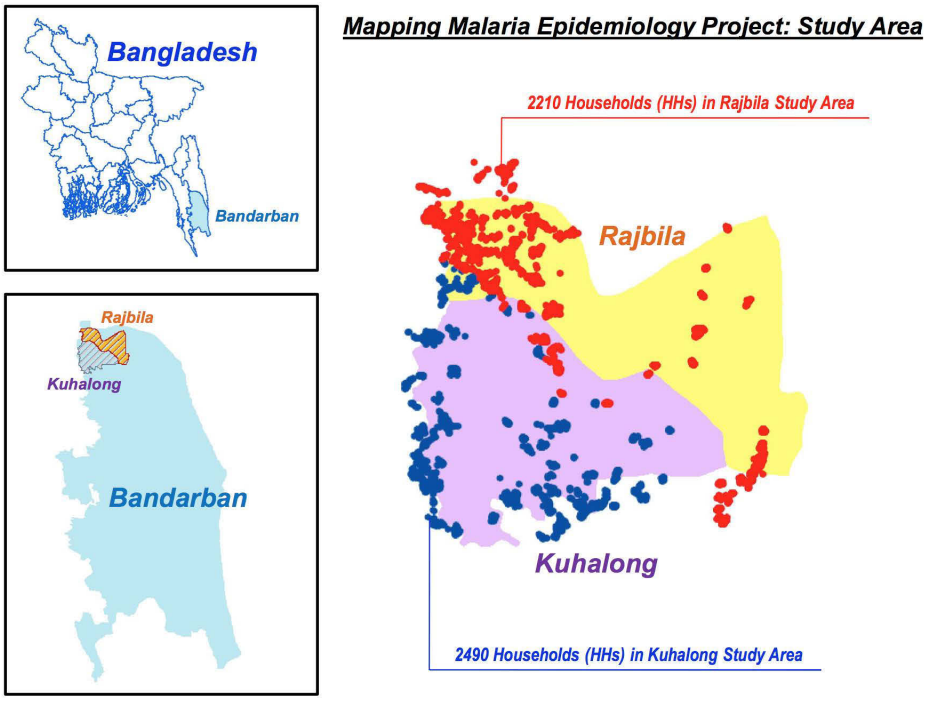
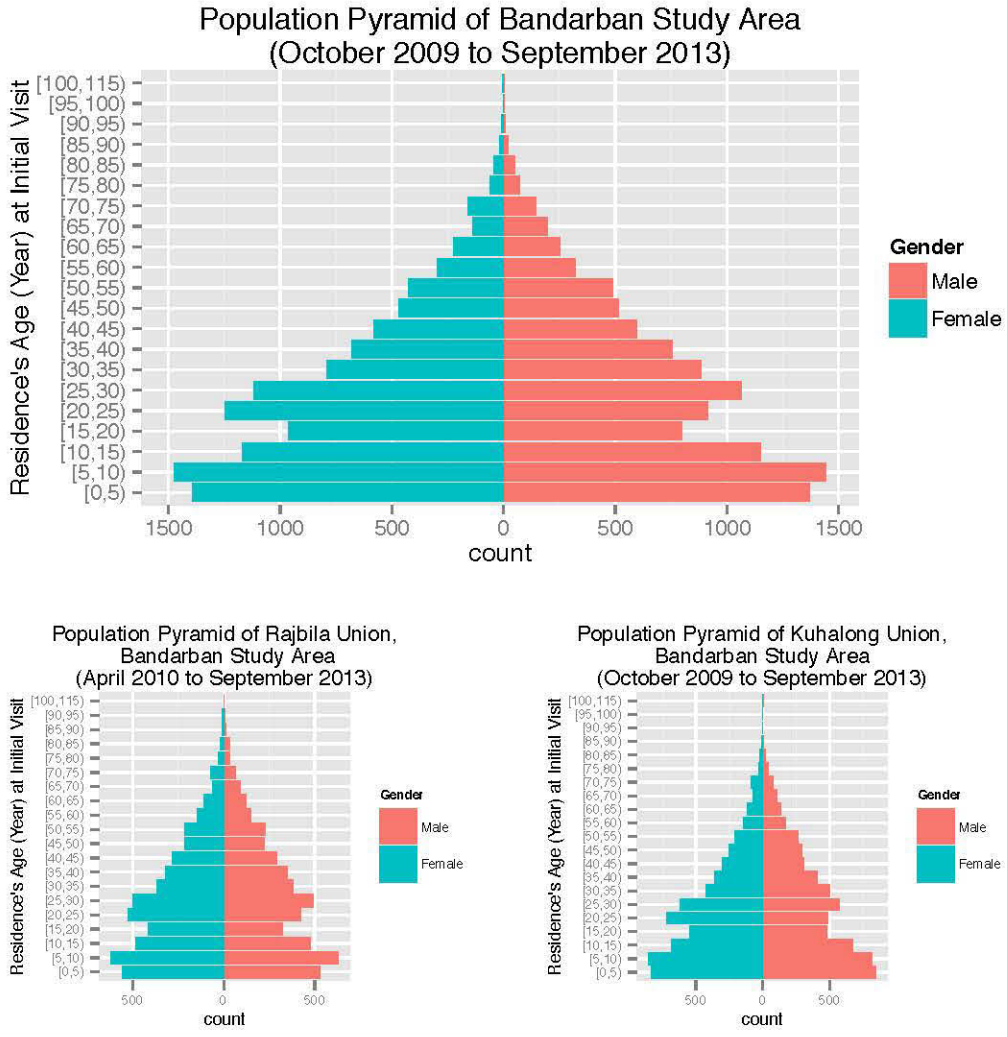


Figure 2.2: Population Pyramids of the Study Area in Mapping Malaria Epidemiology Project



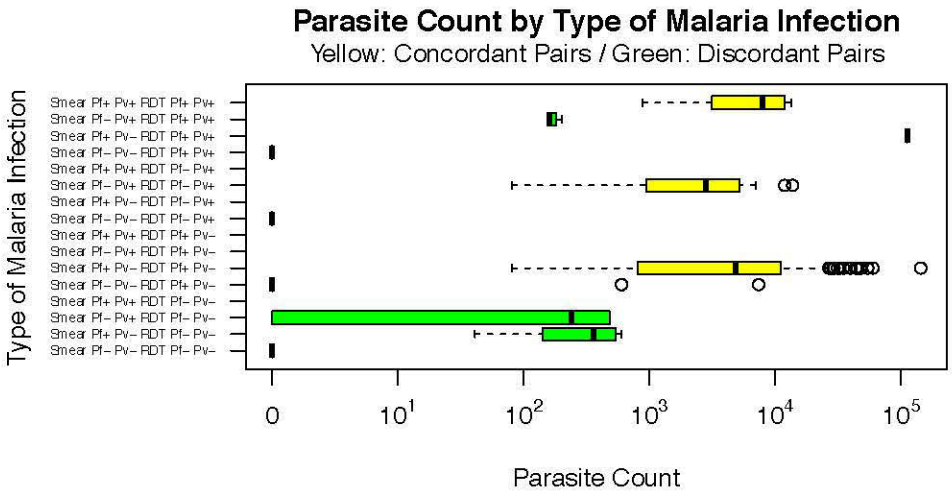


Figure 2.3: Parasite Count by Type of Malaria Infection

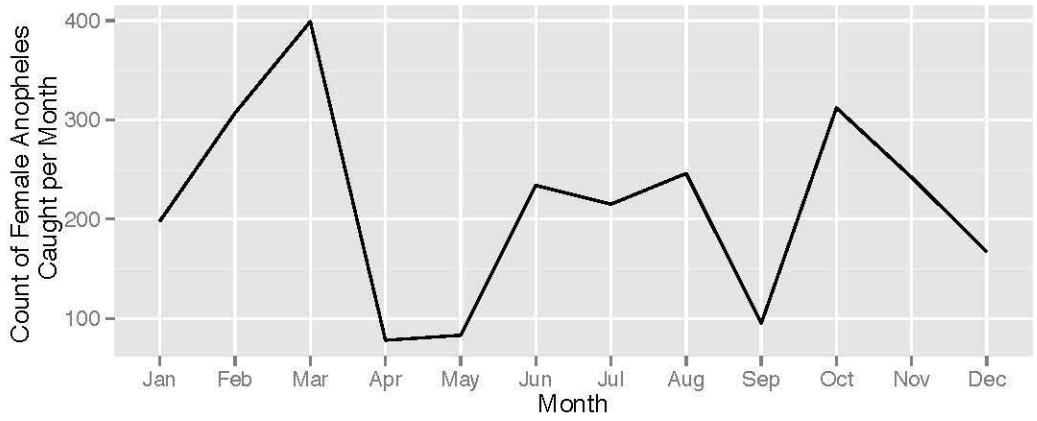


Figure 2.4: Count of Female Anopheles Caught per Month (Kuhalong Union, 7/20/2009 to 6/27/2010)

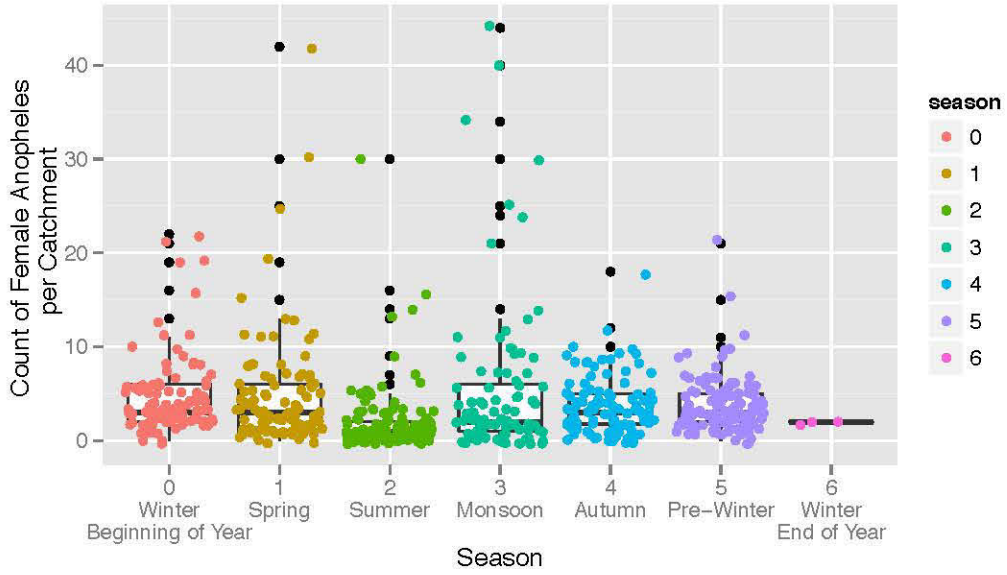


Figure 2.5: Count of Female Anopheles per Catchment, by Season (Kuhalong Union, 7/20/2009 to 6/27/2010)

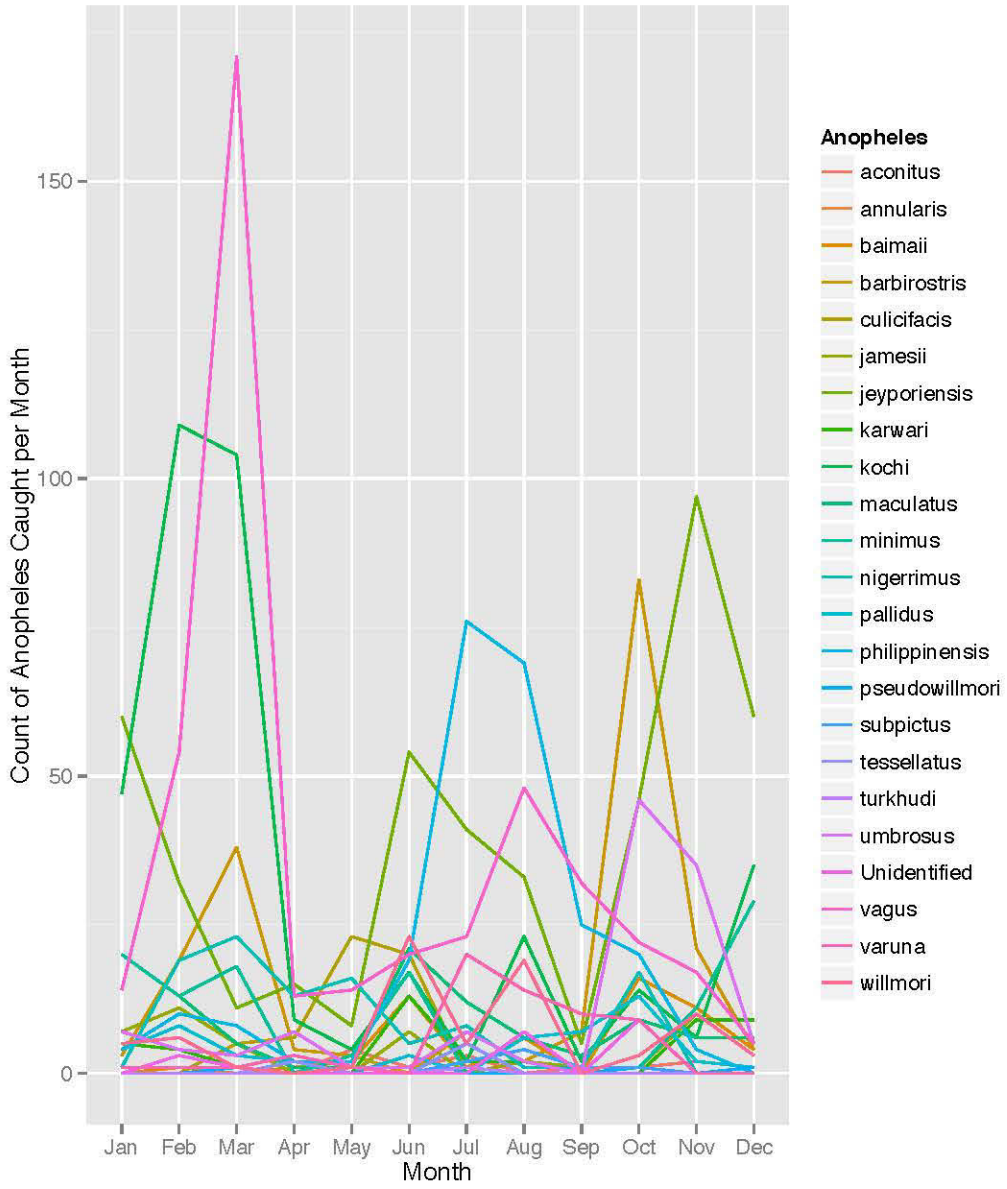


Figure 2.6: Count of Different Types of Anopheles Mosquitoes Caught per Month (Kuhalong Union, 7/20/2009 to 6/27/2010)

Appendix

A2.1 Questionnaires: Observed Indicators and Variable Names

A2.1.1 Demographic Survey—Initial Visit

1. Household ID (**HHID**)

2. Respondent's ID (**RID**)

3. Date of Visit (**DTVST**)

4. Religion (**RLGN**): Muslim / Hindu / Christian / Buddhist / No Religion

5. Race (**Race**): Tribal / Non Tribal

   (a) If Tribal, which Tribe? (**Tribe**): Marma / Chakma / Tripura / Tanchangya / Khyang / Bawm / Rakhaine / Mro / Pangkho / Lusai / Khumi / Chak

   (b) If Non-tribal, which one? (**NONTRB**): Bangali / Other, Specify (**NONTRBTXT**)

6. Language spoken (**LANG**): Marma / Chakma / Tripura / Tanchangya / Khyang / Bawm / Rakhaine / Mro / Pangkho / Lusai / Khumi / Chak / Bangla / Other, Specify (**LANGTXT**)

7. Household Census:

   (a) Person j (j=1,2,. . . ,12)

      i. RID (**RID[j]**)

ii. Name (**NAME[j]**)

iii. Date of Birth (**DOB[j]**)

iv. Sex (**SEX[j]**): Male / Female

v. Marital Status (**MARSTAT[j]**): Married / Single / Widowed / Separated / Divorced / Not Applicable

vi. Years of Education (**YREDUC[j]**)

vii. Occupation (**OCCPTN[j]**)

A. If Other, specify (**OCCPTXT[j]**)

viii. Relationship with the Household Head (**RLTNHH[j]**)

A. If Other, Specify (**RLTNHHTXT[j]**)

ix. Resident Status (**RSDNTSTS[j]**): Resident / Visitor

x. Place of employment (**PLCEMPL[j]**): Union of Residence / Other Union / Not applicable

xi. Fulltime employment within the union (**FTEMPLY[j]**): Yes / No / Not Applicable

8. Pregnancy—Any pregnant woman in the household (**PRGHH**): Yes / No

(a) Person j (j=1,2)

i. RID (**PRG[j]RID**)

ii. Last Menstrual Date of Pregnancy: Month (**PRG[j]LMPM**), Year (**PRG[j]LMPY**)

iii. Expected Date of Delivery (**PRG[j]EDD**)

iv. Prenatal Checking during the First (**PRG[j]PNC1**), Second (**PRG[j]PNC2**) and Third Trimester (**PRG[j]PNC3**)

A. Traditional Birth Attendant / Trained Traditional Birth Attendant / Nurse, Field Health Worker / Community Clinic, Family Welfare Clinic, Satellite Clinic / Maternal and Child Welfare Center / Upazila Health Complex / Government Hospital, Sub Center / Bandarban Sadar Hospital / Rajasthali UHC / BRAC Health Center / Private Clinic, Health Center / Kabiraj, Village Doctor / Did Not Seek Care / Other

B. Other, Specify (**PRG[j]PNC1TXT**, **PRG[j]PNC2TXT**, **PRG[j]PNC3TXT**)

9. Bednets

(a) Does your household have any mosquito bednets that can be used while sleeping (**BDNTHH**): Yes / No

i. If no, what is the reason you do not have a bednet in your house:

A. It is too hot under the net (**RSNTHOT**)

B. Not enough space between me and the net, I feel too closed in (**RSNNOSPC**)

C. It does not protect against mosquitoes, insects (**RSNNOTPRT**)

D. No mosquitoes around (**RSNNOMSQ**)

E. It is only for children, pregnant women (**RSNCHLPRG**)

F. It is too expensive (**RSNEXP**)

G. It is not the rainy/malaria season (**RSNNTMLRSN**)

H. It is difficult to maintain (**RSNDFMNTN**)

I. Can not hang it over my sleeping place / sleeping outside (**RSNSLPOUT**)

J. Change my sleeping place too often (**RSNCHGSLP**)

K. Do not know (**RSNDSK**)

L. Other (**RSNOTH**), Specify (**RBDNTTXT**)

(b) How many bednets (**NUMBDNT**)

i. Person j (j=1,2,. . . ,12)

A. RID (**BNRID[j]**)

B. Slept under a net last night (**SLPTBDNT[j]**): Yes / No

C. Brand, Color of Net (**BRNDBDNT[j]**): Untreated / Olyset net (light blue colour) with bigger holes—Sumitomo Co Ltd / Permanet (light blue colour) with bigger holes—Verstergaard Fradsen, Swizerland / Permanet (deep blue colour) with smaller holes—Verstargaard Fradsen / Other, Specify (**BRNDBNTXT[j]**)

D. How Long Owned, in Month? (**OWNBDNT[j]**)

E. Initially Treated (**TRTBDNT[j]**): Yes / No

F. Was it treated or re-treated: Month (**RTRTBN[j]M**), Year (**RTRTBN[j]Y**)

G. Where did you get the nets (**PLCBDNT[j]**): Purchased from the market / Government Source / BRAC, NGO Source / Other, specify (**PLCBNTXT[j]**)

A2.1.2 Demographic Survey—Follow-Up Visit

1. Household ID (**HHID**)
2. Respondent's ID (**RID**)
3. Date of Visit (**DTVST**)
4. Follow-Up Number (**FUPNM**)
5. Births (During the Last 4 Months) (**BIRTHS**): Yes / No
   (a) Child j Details (j=1,2)
      i. RID (**RID[j]**)
      ii. Name of Child 1 (**NMCHD[j]**)
      iii. Father's Name (**NMFTH[j]**)
      iv. Mother's Name (**NMMOT[j]**)
      v. Date of Birth (**DOB[j]**)
      vi. Sex of Child (**SEXCHD[j]**): Male / Female / Not Confirmed
      vii. Relationship with the Household Head (**RLTNHH[j]**)
         A. If Other, Specify (**RLTNHHTXT[j]**)
      viii. Resident Status (**RSDNTSTS[j]**): Resident / Visitor
      ix. Place of Birth:
         A. Village (**PLCBRTVL[j]**)
         B. Union (**PLCBRTUN[j]**)
         C. Upazila (**PLCBRTUP[j]**)

x. Prenatal Care—First Trimester [i=1] / Second Trimester [i=2] / Third Trimester [i=3]

A. Traditional Birth Attendant (**P[j]PNC[i]T**)

B. Trained Traditional Birth Attendant (**P[j]PNC[i]TT**)

C. Nurse, Field Health Worker (**P[j]PNC[i]N**)

D. Community Clinic, Family Welfare Clinic, Satellite Clinic (**P[j]PNC[i]CC**)

E. Maternal and Child Welfare Center (**P[j]PNC[i]MC**)

F. Upazila Health Complex (**P[j]PNC[i]UZ**)

G. Government Hospital, Sub Center (**P[j]PNC[i]GV**)

H. Bandarban Sadar Hospital (**P[j]PNC[i]BH**)

I. Rajasthali UHC (**P[j]PNC[i]RU**)

J. BRAC Health Center (**P[j]PNC[i]BC**)

K. Private Clinic, Health Center (**P[j]PNC[i]PC**)

L. Kabiraj, Village Doctor (**P[j]PNC[i]KV**)

M. Did Not Seek Care (**P[j]PNC[i]DN**)

N. Other (**P[j]PNC[i]OT**)

O. Other, Specify (**P[j]PNC[i]TXT**)

xi. Place of Delivery (**PLCDLV[j]**):

A. House / Community Clinic, Family Welfare Clinic, Satellite Clinic / Maternal and Child Welfare Center / Upazila Health Complex / Government Hospital, Sub Center / Bandarban Sadar Hospital / Rajasthali UHC / BRAC Health Center

/ Private Clinic, Health Center / Kabiraj, Village Doctor / Other

B. Other, Specify (**LV[j]TXT**)

xii. Birth Attended By:

A. Traditional Birth Attendant (**BRTAT[j]T**)

B. Family Welfare Visitor (**BRTAT[j]FW**)

C. Trained Traditional Birth Attendant (**BRTAT[j]TT**)

D. Nurse (**BRTAT[j]N**)

E. MBBS doctor (**BRTAT[j]D**)

F. Other (**BRTAT[j]OT**)

G. Other, Specify (**BRTAT[j]TXT**)

xiii. Mode of Delivery (**MDDLV[j]**): Normal / Operation C-Section / Instrumental

xiv. Litter Size (**LTRSZ[j]**): Single / Twin / Triplet

xv. Outcome of Delivery (**OTCMDLV[j]**): Live Birth / Stillbirth / Miscarriage Spontaneous / Miscarriage Induced

xvi. If multiple child, this child is (**BRTORDR[j]**): First / Second / Third / Not Applicable

6. Deaths (During the Past 4 Months) (**DEATHS**): Yes / No

(a) Deceased Person j (j=1,2)

i. RID (**RIDDTH[j]**)

ii. Name of Deceased (**NMDTH[j]**)

iii. Age at Death (**AGDTH[j]**)

iv. Case of Death, if known (**CSDTH[j]**)

v. Type of Death (**TYPDTH[j]**):

A. Natural (Due to Disease, Illness)

B. Unnatural (Accident, Injury, Drowning, Fell from High Place, Homicide, Suicide, Bitten by Animals)

vi. Relationship with the Household Head (**RLTNDTH[j]**)

vii. Resident Status (**RSDSTSDTH[j]**): Resident / Visitor

7. In-Migration (During the Past 4 Months) (**INMIG**): Yes / No

(a) Person j (j=1,2,. . . ,5)

i. RID (**RIDIM[j]**)

ii. Name (**NMIM[j]**)

iii. Date of Birth (**DOBIM[j]**)

iv. Sex (**SEXIM[j]**): Male / Female

v. Years of Education (**YREDUCIM[j]**)

vi. Occupation (**OCCPIM[j]**)

vii. Relationship with the Household Head (**RLTNIM[j]**)

viii. Marital Status (**MRTIM[j]**): Married / Single / Widowed / Separated / Divorced / Not Applicable

ix. Reason of Movement (**RSNIM[j]**):

A. Employment / Child Birth / Vacation, Holiday / Marriage / Education / Other

B. Other, Specify (**RSNIM[j]TXT**)

x. Resident Status (**RSDSTSIM[j]**): Resident / Visitor

xi. Place of Employment (**PLCEMPIM[j]**): Union of Residence / Other Union / Not Applicable

xii. Fulltime Employment within the Union (**FTEMPIM[j]**): Yes / No / Not Applicable

8. Out-Migration (During the Past 4 Months) (**OM**): Yes / No

(a) Person j (j=1,2,. . . ,5)

i. RID (**RIDOM[j]**)

ii. Name (**NMOM[j]**)

iii. Resident Status (**RSDSTSOM[j]**): Resident / Visitor

iv. Reason of Movement (**RSNOM[j]**):

A. Employment / Child Birth / Vacation, Holiday / Marriage / Education / Other

B. Other, Specify (**RSNOM[j]TXT**)

9. Pregnancy—Any pregnant woman in the household (**PRGHH**): Yes / No

(a) Person j (j=1,2)

i. RID (**PRG[j]RID**)

ii. Last Menstrual Date of Pregnancy: Month (**PRG[j]LMPM**), Year (**PRG[j]LMPY**)

iii. Expected Date of Delivery (**PRG[j]EDD**)

iv. Prenatal Checking during the First (**PRG[j]PNC1**), Second (**PRG[j]PNC2**) and Third Trimester (**PRG[j]PNC3**)

A. Traditional Birth Attendant / Trained Traditional Birth Attendant / Nurse, Field Health Worker / Community Clinic, Family Welfare Clinic, Satellite Clinic / Maternal and Child Welfare Center / Upazila Health Complex / Government Hospital, Sub Center / Bandarban Sadar Hospital / Rajasthali UHC / BRAC Health Center / Private Clinic, Health Center / Kabiraj, Village Doctor / Did Not Seek Care / Other

B. Other, Specify (**PRG[j]PNC1TXT**, **PRG[j]PNC2TXT**, **PRG[j]PNC3TXT**)

10. Bednets—Acquired new bednets during the past 4 months (**NWBDNTHH**): Yes / No

(a) Person j (j=1,2,3)

i. RID (**RIDBN[j]**)

ii. Slept under a net last night (**SLPBDNT[j]**): Yes / No

iii. Brand, Color of Net (**BRNDBDNT1**): Untreated / Olyset net (light blue colour) with bigger holes—Sumitomo Co Ltd / Permanet (light blue colour) with bigger holes—Verstergaard Fradsen, Swizerland / Permanet (deep blue colour) with smaller holes—Verstargaard Fradsen / Other, Specify (**BRNDBDNT[j]TXT**)

iv. How Long Owned, in Month? (**OWNBDNT[j]**)

v. Initially Treated (**TRTBDNT[j]**): Yes / No

vi. Was it treated or re-treated: Month (**RTRTBN[j]M**), Year (**RTRTBN[j]Y**)

vii. Where did you get the nets (**PLCBDNT[j]**): Purchased from the market / Government Source / BRAC, NGO Source / Other, specify (**PLCBNTXT[j]**)

A2.1.3 Social Economic Survey

1. Household ID (**HHID**)

2. Respondent's RID (**RID**)

3. Date of Visit (**DTVST**)

4. Description of Household Owned Land Holdings

   (a) Own land, Personally Cultivated (**LNDOWNPC**): Yes / No / Shared

      i. Amount of Land (**SZLNDOWNPC**)

   (b) Own land, Cultivated by Others (**LNDOWNCO**): Yes / No / Shared

      i. Amount of Land (**SZLNDOWNCO**)

   (c) Own land, Uncultivated (Abandoned) (**LNDOWNUC**): Yes / No / Shared

      i. Amount of Land (**SZLNDOWNUC**)

   (d) House on Own Land (**HSOWNLND**)

      i. Amount of Land (**SZHSOWNLND**)

   (e) Pond (**PND**)

i. Amount of Land (**SZPND**)

5. Number of Houses or Dwelling Units in Your Household (**NODWLUNT**)

6. Do You Own the Following Things? (Household Assets)

   (a) Electricity (**ASSELEC**): Yes / No

   (b) Television (**ASSTV**): Yes / No

   (c) Radio, Tape Recorder (**ASSRD**): Yes / No

   (d) Almirah, Showcase (**ASSALM**): Yes / No

   (e) Bed, Mattress (**ASSBDMT**): Yes / No

   (f) Clock, Watch (**ASSCLK**): Yes / No

   (g) Refrigerator (**ASSFGR**): Yes / No

   (h) Fan (**ASSFN**): Yes / No

   (i) Dining Table (**ASSDNTBL**): Yes / No

   (j) Mobile, Telephone (**ASSTEL**): Yes / No

   (k) Sofa Set (**ASSSFST**): Yes / No

   (l) Chair, Table (**ASSCHR**): Yes / No

   (m) Sewing Machine (**ASSSWMCH**): Yes / No

   (n) Blanket, Lep, Quilt (**ASSBLNK**): Yes / No

   (o) Bednet (**ASSBDNT**): Yes / No

   (p) Power Tiller (**ASSPWRTL**): Yes / No

   (q) Rice Mill (**ASSRCMLL**): Yes / No

(r) Rickshaw, Van (**ASSRCK**): Yes / No

(s) Bicycle, Motorcycle (**ASSCYCL**): Yes / No

(t) Fishing Boat (**ASSFSHBT**): Yes / No

(u) Modern Agriculture Machines (**ASSMDNMCH**): Yes / No

(v) Shallow Machine (**ASSSHLMCH**): Yes / No

(w) Vehicles to Rent Out (Taxi, Cab, Shallow Boat) (**ASSVHCLRNT**)

(x) Tube Well (**ASSTBWL**)

(y) Crushing Mill (**ASSCRSHML**)

(z) Khat, Chouki (**ASSKHT**)

(a) Reserved Clothes (**ASSRSVCLTH**)

(b) Dheki (**ASSDHK**)

(c) Variety Store (Mudi), Shop (**ASSVRTSTR**)

(d) Fish Hatchery (**ASSFSHHTCH**)

(e) Fishing Net (**ASSFSHNT**)

(f) Live Stocks (**ASSLVSTCK**)

(g) Poultry (**ASSPLTRY**)

(h) Other (**ASSOTH**), Specify (**ASSOTHTXT**)

7. Status of the Living Room:

(a) Walls (**STSLRWL**)

i. Corrugated Tin, Iron Sheet

ii. Fired Brick, Cement

iii. Tins

iv. Pole and Mud

v. Wood

vi. Pole and Grass

vii. Stone

viii. Unfired Bricks

ix. Bamboo

x. Other, Spcify (**STSLRWLTXT**)

(b) Roof (**STSLRRF**)

i. Straw, Thatch

ii. Asbestos

iii. Pole and Grass

iv. Pole and Mud

v. Bamboo

vi. Mud Tins, House of Tins

vii. Corrugated Tin, Iron Sheet

viii. Fired Brick, Cement

ix. Concrete, Cement

x. Other, Specify (**STSLRRFTXT**)

(c) Partition (**STSLRPRT**)

i. Jute Stick

ii. Concrete, Cement

iii. Tin

iv. Wood

v. Mud

vi. Bamboo

vii. Other, Specify (**STSLRPRTTXT**)

(d) Floor (**STSLRFLR**)

i. Mud

ii. Semi-Cemented

iii. Cemented

iv. Bamboo

v. Vinyl

vi. Wood

vii. Other, Specify (**STSLRFLRTXT**)

8. Is the Floor Elevated from Ground Level (**GRNDELV**): Yes / No

   (a) If Yes, Specify the Height (**GRNDELVHT**)

9. What is the Main Source of Water for Members of Your Household (**SRCWTR**)

   (a) Dam

   (b) Rainwater

   (c) River or Steam, Other Natural Body of Water

   (d) Irrigation Channel

(e) Uncovered House Water Storage Container

(f) Private Well or Pump

(g) Public Well or Pump

(h) Tube Well

(i) Ring Well (Covered)

(j) Public Stand Pipe

(k) Piped into Dwelling

(l) Do Not Know

(m) Other, Specify (**SRCWTRTXT**)

10. What Kind of Toilet Do the People in This Household Usually Use (**TYPTLT**)

(a) Modern, Flush, Pakka Toilet

(b) Pit Latrine

(c) No Facility, Bush, Field

(d) Kacha, Hanging Toilet, Hanging Latrine

(e) Slab Toilet with Boundaries

(f) Slab Toilet without Boundaries

(g) Other, Specify (**TYPTLTTXT**)

11. What is the Source of Light (**SRCLGHT**)

(a) Candle

(b) Lantern

(c) Solar Energy

(d) Electricity

(e) Oil Lamp, Kerosene Lamp

(f) Other, Specify (**SRCLGHTTXT**)

12. What Type of Fuel Does Your Household Mainly Use for Cooking (**TYPFUEL**)

(a) Electricity

(b) Kerosene

(c) LPG, Natural Gas

(d) Coal

(e) Biogas

(f) Charcoal

(g) Firewood, Straw

(h) Dung

(i) Solar

(j) Other, Specify (**TYPFUELTXT**)

13. Where Do You Usually Cook (**CKPLC**): Inside House / Outside House / Both Inside and Outside

14. What Was Your Household's Economic Status in Terms of Money After Observing Last Year's Income and Expenditure (**ECONSTSLSTY**): Deficient All Year / Deficient Sometimes / Neither Deficient nor Surplus / Surplus

15. What is the Income Source of Your Household—Agriculture (Own Land) / Agricuture (Lend Land) / Mortgaged Land / Day Labor / Fishing / Poultry / Handicraft / Tailor / Business / Service / Pension / Money from Inside Country / Money from Abroad / Food for Work / Fund for Poor, Elderly / Rent from House, Shop / Other:

    (a) Main Source (**MNINCSRC**), Other—Specify (**MNINCSRCSP**)

    (b) Secondary Source (**SCNDINCSRC**), Other—Specify (**SCNDINCSRC_1**)

16. How Much is Your Yearly Income (Taka) (**INCYRLY**)

17. Many Household Cannot Provide Food for All the Members Three Times a Day. In the Past Year, Could You Arrange Foods for All the Household Members of Yours Three Times a Day (**ARRGNFD**): Yes / No

    (a) If No, How Many Times Did One or More of Your Family Members Could Not Eat to His/Her Full Three Times a Day during the Past Year:

        i. Months (**HWMNMTH**)

        ii. Times a Day (**HWMNTM**)

18. Are You or Any Member of Your Family a Member of the Following Shomiti or NGO (**NGOMEM**): Yes / No

    (a) If Yes, Which One: BRAC (**BRAC**) / Grameen Bank (**G_Bank**) / ASHA (**ASHA**) / CARE (**CARE**) / UNDP (**UNDP**) / Other (**WHCHNGO**), Specify (**WHCHNGOTXT**)

A2.1.4 Passive Surveillance

1. Household ID (**HHID**)
2. Respondent's RID (**RID**)
3. Date of Visit (**DTVST**)
4. Gender (**SEX**): Male / Female
5. Age: Year (**AGEYY**), Month (**AGEMM**), Day (**AGEDD**)
6. Visit Day (**VSTDY**): 2 days / 7 days / 28 days
7. What Symptoms of Malarial / Fever You / Your Child Had:
   (a) Fever, Fever with Shivering (**SYMFVSHVR**)
   (b) Fever at Day Time (**SYMFVDY**)
   (c) Fever at Night (**SYMFVNGT**)
   (d) Fever with Sweating (**SYMFVSWT**)
   (e) Intermittent Fever (**SYMINTFV**)
   (f) Remission of Fever with Sweating (**SYMRMFVSWT**)
   (g) Headache (**SYMHDACH**)
   (h) Chills (**SYMCHL**)
   (i) Nausea (**SYMNAUS**)
   (j) Vomiting (**SYMVMT**)
   (k) Diarrhoea (**SYMDIAR**)
   (l) Cough (**SYMCGH**)

(m) Fatigue (**SYMFTG**)

(n) Muscle Ache (**SYMMSLACH**)

(o) Muscle Weakness (**SYMSMLWK**)

(p) Convulsions, Seizure (**SYMCNVL**)

(q) Anaemia (**SYMANMA**)

(r) Do not Know (**SYMDNK**)

(s) Other (**SYMOTH**), Specify (**SYMOTHTXT**)

8. How Long Have You / Your Child Had These Symptoms of Malaria (Days) (**SYMDURDY**)

9. Have You / Your Child Taken Any Medications for Malaria (**MDCTKNMLR**): Yes / No

   (a) What Medication Did You / Your Child Take for Treating Malaria and For How Long

      i. Medication 1 (**MDC1MLR**), Days (**MDC1DY**)

      ii. Medication 2 (**MDC2MLR**), Days (**MDC2DY**)

      iii. Medication 3 (**MDC3MLR**), Days (**MDC3DY**)

   (b) Where Did You / Your Child Get the Medication

      i. Health Post, Health Center, Hospital (**PLCHP**)

      ii. Traditional Healer (**PLCTH**)

      iii. Herbs, Leaves (**PLCHL**)

      iv. Had it in the Home (**PLCHM**)

v. Pharmacy (**PLCPH**)

vi. Relative, Friend (**PLCRF**)

vii. Local Shop, Market (**PLCSHP**)

viii. Do Not Know (**PLCDK**)

ix. Other (**PLCOTH**), Specify (**PMDC_TXT**)

10. How was Malaria Confirmed: RDT (**CNFR_RDT**) / Microscopy (**CNFR_MC**) / Not Confirmed (**CNFR_NC**)

11. When was it Confirmed (**WHNCNFR**)

12. Were Any Specimens Collected at This Visit (**SPCCLCT**): Yes / No

    (a) If Yes, What: Blood for RDT (**WSPB_RDT**) / Blood for Microscopy (**WSPC_MC**) / Blood on Filter Paper (**WSPC_FP**)

13. Body Temperature (**TYPTMP**): Oral / Axillary

    (a) Temperature (C) (**TMPC**)

14. Weight (**WTTKN**): Taken / Not Taken

    (a) If Taken, How Much (Kg) (**WTKG**)

A2.1.5 Human Laboratory Data

1. Household ID (**HHID**)

2. Respondent's ID (**RID**)

3. Date of Specimen (**DTSPEC**)

4. Study Type (**STDTYP**): Active / Active Nested Longitudinal / Passive

    (a) Visit Day (**VSTDY**): Initial / Day 2 / Day 7 / Day 28

5. Visit Month (**VSTMNTH**): Month 0 / Month 3 / Month 6 / Month 9

6. History

    (a) History of Fever (**HISTYFVR**): Present / Absent

    (b) If Present, for how many days? (**FVRDY**)

    (c) History of fever medication in last 8 hours (**FVRMDC**): Yes / No / Unknown

    (d) If Yes, record medication (**MDCCD**)

    (e) Are bed nets used at home (**BDNT**): Yes / No / Unknown

7. Sample Preparation

    (a) Was malaria RDT done (**MLRRDTDN**): Yes / No

        i. If Yes, Result (**RDTRSLT**): Positive / Negative

        ii. If Positive, type of parasite: P. vivax (**RDTPRSTPV**) / P. falciparum (**RDTPRSTPF**) / Other (**RDTPRSTOTH**), Specify (**RPRST_TXT**)

        iii. If RDT not taken, why not (**RDTNTKN**): Participant or Parent refuses / Lancet was attempted but blood was insufficient / Other, Specify (**RDTNTKNSP**)

        iv. RDT Sample Label (**RDTLBL**)

    (b) Was blood smear taken (**MLRBLDSM**): Yes / No

i. If Yes, Result (**BLDSMRST**): Positive / Negative

ii. If Positive, type of parasite: P. vivax (**BLDSMPRSTPV**) / P. falciparum (**BLDSMPRSTPF**) / Other (**BLDSMPRSTOTH**), Specify (**BPST_TXT**)

iii. Stage present: Early Trophozoite (**STGET**) / Late Trophozoite (**STGLT**) / Schizont (**STGSC**) / Gametocyte (**STGGM**)

iv. Parasite Count (**PRSTCNT**)

v. If blood smear not taken, why not (**BLDSMNTKN**): Participant or Parent refuses / Lancet was attempted but blood was insufficient / Other, Specify (**SMNTKNSP**)

vi. Blood Smear Sample Label (**SMLBL**)

(c) Was blood spot specimen taken on filter paper (**BLDFLTPR**): Yes / No

i. If Yes, Result (**FLTPRRST**): Positive / Negative

ii. If Positive, type of parasite: P. vivax (**FLTPRSTPV**) / P. falciparum (**FLTPRSTPF**) / Other (**FLTPRSTOT**), Specify (**FLTPRRST_OTH**)

iii. CT value: P. falciparum (**FLTCT1PF**) / P. vivax (**FLTCT2PV**) / P. malariae (**FLTCT3PM**)

iv. If spot specimen on filter paper not taken, why not (**FLTNTKN**): Participant or Parent refuses / Lancet was attempted but blood was insufficient / Other, Specify (**FLTNTKNTXT**)

v. Filter Paper Sample Label (**FLTPRLBL**)

8. Pregnancy Test Done (**PRGTST**): Yes / No / Not Applicable
    (a) If Yes, Result (**PRGRSLT**): Positive / Negative
9. Body Temperature (**TYPTMP**): Oral / Axillary / Not Taken
    (a) Recorded Temperature (°C) (**TMPC**)
10. Weight Taken (**WTTKN**): Yes / No
    (a) Recorded Weight (kg) (**WTKG**)
11. Height Taken (**HTTKN**): Yes / No
    (a) Recorded Height (cm) (**HTCM**)

A2.1.6 Entomological Surveillance

1. Household ID (**HHID**)
2. Date of Collection (**DTCLTN**)
3. Place of Collection (**PLCCLTN**): Indoor / Outdoor
4. Land Elevation (**LNDELVN**): Highland / Lowland
5. Time of Day (**TMDY**)
6. Period of Day (**PRDDY**): AM / PM
7. Type of Catch (**TYPCTCH**): Human Landing Catch / Light Trap / Resting Collection / Spray Catch / Animal Collection / Other
    (a) If Other, Specify (**TYPCTCHTXT**)

8. Catching / Trapping Number (**CTCHTRPNO**)
9. Number of Anopheles (**NMBANPH**)
10. Number of Beds (**NMBBD**)
11. Number of Treated Bednets (**NMBTRTBN**)
12. Number of Untreated Bednets (**NMBUTRTBN**)
13. Number of Males in the House (**NMBMLHS**)
14. Number of Females in the House (**NMBFMLHS**)
15. Number of Persons Not Under Bednets
    (a) Male (**Male**)
        i. Less than 6 Months (**NMLT6MBN**)
        ii. 6 Months to Less than 5 Years (**NM6_5BN**)
        iii. 5 Years to less than 15 Years (**NM5_15BN**)
        iv. 15 Years and Above (**NMGT15BN**)
    (b) Female (**Female**)
        i. Less than 6 Months (**NFLT6MBN**)
        ii. 6 Months to Less than 5 Years (**NF6_5BN**)
        iii. 5 Years to less than 15 Years (**NF5_15BN**)
        iv. 15 Years and Above (**NFGT15BN**)
16. Animal Counts of the Household

(a) Type of Animal: If Present, Check the Type of Animal Box. If Not Present, Kepp the Box Blank

i. Cows: Present (**COWPR**) / Numbers (**COWNUM**)

ii. Goats: Present (**GOATPR**) / Numbers (**GOATNUM**)

iii. Pigs: Present (**PIGPR**) / Numbers (**PIGNUM**)

iv. Dogs: Present (**DOGPR**) / Numbers (**DOGNUM**)

v. Other Animals: Present (**OTHANMPR**) / Numbers (**OTHANMNUM**)

vi. Poultry: Present (**PLTRYPR**) / Numbers (**PLTRYNUM**)

Chapter 3

Paper 1: Field Performance of FalciVax^{TM} Rapid Diagnostic Test in rural Bangladesh

3.1 Abstract

Background Rapid diagnostic tests (RDTs) of malaria are widely implemented. FalciVax^{TM} RDT is a commercial device used in rural Bangladesh. Its laboratory-based performance was previously evaluated. However, its field performance has not been fully assessed. This paper analyzed field performance of FalciVax^{TM} RDT across time, febrile status and parasite density in a hypoendemic malaria district in southeastern Bangladesh.

Methods This study was part of a population-based malaria surveillance project in Bandarban, Bangladesh (2009-2013). Study participants were enrolled via passive surveillance. Enrolled individuals were tested with malaria by FalciVax^{TM} RDT (screening test) and Giemsa-stained Microscopy (gold standard). We used logistic regression to model overall and factor-specific performance of FalciVax^{TM} RDT. This included its sensitivity, specificity, positive and negative predictive values. Modeled field performance of FalciVax^{TM} RDT was compared to results calculated directly from field data.

Results With 22,325 eligible participants, 616 individuals were qualified to enroll in the study. Overall, sensitivity, specificity, positive and negative predictive values of FalciVax^{TM} RDT were 99.6% (527/529), 33.3% (29/87), 90.1% (527/585) and 93.5% (29/31), respectively. Field performance of FalciVax^{TM} RDT did not differ across season and febrile status. Statistically significant change in field performance was found between years. Correlation between microscopy status and parasite density made modeling sensitivity, specificity, positive and negative predictive values unfeasible.

Discussion We compared calculated and modeled sensitivity, specificity, positive and negative predictive values of FalciVax^{TM} RDT, which was not previously done. With smaller sample size in stratum specific category, logistic regression could provide smoother estimates of sensitivity, specificity and predictive values. Our sensitivity was higher and specificity was lower than previous studies, which could had been resulted from spectrum bias. Sensitivity and specificity of rapid diagnostic devices were previously assumed to be independent from prevalence of malaria. Our field performance study may had shown otherwise. Future studies are needed to examine the assumption.

3.2 Background

Malaria rapid diagnostic test (RDT) has been widely implemented since its development in 1990s. It provides a relatively inexpensive and immediate way to detect malaria. In resource scarce areas, this has become an alternative to Giemsa-stained Microscopy—the gold standard— for malaria diagnosis. In 1999, *World Health Organization* (WHO) stated any RDT at good standing should have its sensitivity and specificity greater than or equal to ($\geq$) 95% and 90%, respectively [83].

Currently, there are more than 200 commercial RDT models on the market [84]. However, quality of RDT models varies. In 2008, WHO initiated laboratory-based evaluation on commercial RDTs [85]. It aimed to examine their quality under a controlled setting. This initiative was executed in collaboration with

Foundation for Innovated New Diagnostics (FIND), *Centers for Disease Control and Prevention* (CDC) and *Special Programme for Research and Training in Tropical Diseases* (TDR). From 2008 to 2014, five rounds of laboratory evaluation were made [24, 25, 26, 27, 28]. WHO used the results to provide procurement recommendations for malaria control programs worldwide. During the evaluation process, manufacturers were invited to submit and resubmit RDT devices for testing. Although test results have shown maintenance or improvement in the performance of malaria diagnosis, evaluation of RDT devices in hypoendemic field settings is limited. Therefore, along with our 4-year malaria epidemiological study conducted in southeastern Bangladesh, we evaluated field performance of a RDT device—FalciVax^{TM} (Zephyr Biomedicals, India)—in a population based setting.

FalciVax^{TM} RDT device has previously been evaluated by Singh et al., Sreekanth et al. and Alam et al. [32, 33, 34, 35]. Singh and colleagues studied 372 patients at 2 primary health centers in India for 4 months and found sensitivity and specificity of FalciVax^{TM} to be 94.0% and 72.8%, respectively, against microscopy as gold standard [32]. Later, the group reevaluated FalciVax^{TM} RDT device among 1,807 subjects in selected villages in India over a 8-month period. The sensitivity was 89.4% and the specificity was 84.1% comparing to microscopy [35]. In Sreekanth's study, one hundred patients were referred from 2005 to 2007 to have FalciVax^{TM} rapid diagnostic tests. At this hospital setting in India, Sreekanth et al. found its sensitivity to be 100.0% and its specificity to be 98.7% when comparing FalciVax^{TM} RDT against microscopy [34]. In a more recent study, Alam and colleagues recruited 338 febrile patients from an

Upazila Health Complex in Bangladesh for FalciVax™ RDT evaluation. This 15-month project showed FalciVax™ RDT had a sensitivity of 98.2% and a specificity of 97.0% [33].

Previous studies have shown overall performance of the FalciVax™ RDT device. However, its performance across years and seasons, its performance between febrile and non-febrile individuals, and its performance among an individual's parasite density remain unexplored. Previous papers also focused their efforts under clinical settings, shorter time frames and/or smaller studied population. In this paper, we examined overall performance of FalciVax™ RDT by utilizing a population based surveillance system in 2009-2013. In addition, we evaluated and modeled sensitivity, specificity, positive and negative predictive values by time, febrile statues and parasite density.

3.3 Methods

3.3.1 Study Location

Our study site was located in Kuhalong Union and Rajbila Union in Bandarban District, Bangladesh. Union is the smallest local government unit in rural area. Bandarban District was one of the three districts in Chittagong Hill Tracts in southeastern Bangladesh. It borders Myanmar and the region is relatively hilly compared to the rest of the country. The two Unions were chosen based on their relatively high malaria prevalence in a nationwide survey in 2007 [66, 86].

3.3.2 Study Time Frame

This study was conducted under a population-based *Mapping Malaria Epidemiology* surveillance project. It was first launched in Kuhalong Union in October 2009. Rajbila Union joined the study in April 2010. Both Unions were followed till September 2013.

3.3.3 Study Population

All residents resided in the Bandarban Study Area was eligible to participate in the study. According to our baseline demographic survey, more than twenty-two thousand individuals (N = 22,325) were identified in the study area. In particular, there were 12,502 people and 9,823 people in Kuhalong Union and Rajbila Union, respectively.

3.3.4 Study Design

This paper was part of a multi-year malaria surveillance project [87]. The overarching project incorporated active, nested longitudinal and passive surveillance systems along with baseline demographic survey. This paper focused on passive surveillance aspect of the study to examine the field performance of FalciVax^{TM} Rapid Diagnostic Tests (RDTs).

Passive surveillance was triggered when (1) an ill person or his/her family member contacted one of our field staff (including a project manager, a medical

officer, an assistant and 20 surveillance workers) or (2) a malaria case was identified by *Bangladesh Rural Advancement Committee* (BRAC). When passive surveillance was initiated, the project manager assigned the closest field surveillance workers to conduct home visits and diagnostic tests. If an individual was malaria positive during the initial home visit (Day 0), additional visits were done on Day 2, 7 and 28.

3.3.5 Malaria Diagnosis

To date, Giemsa-stained microscopy is considered the gold standard of identifying (1) presence, (2) type and (3) density of malaria parasites in an individual's blood stream. Rapid diagnostic tests are commonly used in resource poor settings to help diagnose the presence of malaria. During each home visit, a rapid diagnostic test was conducted. FalciVax^{TM} , manufactured by Zephyr Biomedicals in India, was the rapid diagnostic device used during the study. In addition, study participants' thin and thick blood films (also known as blood smears) were made for further examination using Giemsa-stained microscopy. Both diagnostic tests were conducted only if informed consents were received.

Both FalciVax^{TM} RDT and Giemsa-stained microscopy have the ability to identify the presence of *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) malaria. Due to relatively low prevalence of *P. vivax* in the study area, only *P. falciparum* was included in this paper.

Case Definition In the field, a malaria case was defined by a positive test result from either FalciVax^{TM} RDT or Giemsa-stained microscopy. This was to ensure timely treatment of malaria [87]. As our focus of this paper was to examine field performance of FalciVax^{TM} , we used a more stringent case definition. That is, only Giemsa-stained microscopy positive individuals were considered as confirmed malaria cases in the analyses.

3.3.6 Quality Control

Personnel

Recruitment All staff members of the *Mapping Malaria Epidemiology* project were locally hired. This included surveillance workers, a field assistant, a field manager and a licensed physician. As home visits were an essential part of the study, hiring locally ensured personnel's understanding of local customs and their ability to speak local tribal languages. All staff members were selected based on their performance through our written and oral exams. They were also required to speak Bengali (the official language in Bangladesh) to better communicate with the study team in Bandarban and in Dhaka.

Training After recruitment, personnel training began in June 2009 (four months prior to the start of the study). Skills taught at training including how to obtain informed consent, how to conduct survey, collect finger prick blood, prepare blood slides and how to interpret results from FalciVax^{TM} RDT kits [87]. National guidelines on referral of severe malaria cases and on the use of anti-malarial drugs were also taught during training sessions.

Study Execution Surveillance workers were divided into teams of two. They received their daily home visit assignments from the field manager. The field manager and medical officer oversaw and supervised all cases in the study area.

Laboratory

Field laboratory had the capacity of housing FalciVax^{TM} RDT devices. It also had a microscope and resources for staining blood smears for microscopy. Specimens were processed, labeled, examined and organized by a trained microscopist and personnel in the field office [87]. Our field laboratory worked closely with other laboratory facilities available for the study. This included laboratory facilities at *International Centre for Diarrhoeal Disease Research, Bangladesh* (icddr,b) in Dhaka, Bangladesh and at the *Malaria Research Institute at the Johns Hopkins Bloomberg School of Public Health* (JHMRI) in Baltimore, Maryland, USA. Once RDT and blood smear specimens were examined in the field, blood samples were transferred to icddr,b.

Data Management

All surveillance data and laboratory results collected through passive surveillance were written onto pre-coded forms. The pre-coded form was designed to be recognized by the ABBYY FlexiCapture 8.0 software when scanned. Once a pre-coded form was scanned, initial data entry was completed. Once completed, the field assistant compared entered data with handwritten forms. If any discrepancy was found, correction would be made manually. Corrected data files

were then exported to Microsoft Office Access 2007. These files along with scanned images of surveillances forms were stored at icddr,b and JHMRI. Prior to data analysis, data cleaning and preliminary exploratory data analysis were performed by the author to ensure data quality.

3.3.7 Statistical Analysis

Inclusion Criteria

Only information recorded on Day 0 of passive surveillance was used in the analyses. This was to minimize the influence from malaria treatment. Malaria treatment was given to all individuals who were FalciVax^{TM} RDT and/or Giemsa-stained microscopy positive individuals on Day 0. Using information exclusively from Day 0 ensured the comparison between FalciVax^{TM} RDT and Giemsa-stained microscopy were not affected by medication.

Exclusion Criteria

Individuals enrolled in the study were provided with two tests: (1) Giemsa-stained microscopy and (2) FalciVax^{TM} RDT. Both tests were executed during home visits unless informed consent was not received. As we aimed to compare field performance of FalciVax^{TM} RDT against Giemsa-stained microscopy, only individuals with both test results on Day 0 were included in analyses (N = 616).

Variables of Interest

Malaria Status Two tests were used in our study site: (1) Giemsa-stained Microscopy Test: the gold standard in malaria diagnosis, and (2) FalciVax™ RDT: a screening tool to detect malaria infection. To test field performance of FalciVax™ RDT, a 2-by-2 contingency table was created. This yielded numbers of true positive (TP), true negative (TN), false positive (FP), false negative (FN), concordant and discordant pairs.

Time of Diagnostic Test To compare consistency in field performance of FalciVax™ RDT over time, two time variables—*YEAR* and *SEASON*—were created based on date of initial visit (Day 0). *YEAR* had values ranging from 2009 to 2013. *SEASON* included six local seasons in Bangladesh: Spring (February-April), Summer (April-June), Monsoon (June-August), Autumn (August-October), Pre-Winter (October-December) and Winter (December-February). Each season was approximately 60 days in length.

Febrile Status To compare field performance of FalciVax™ RDT across different body temperatures, febrile status was created. Febrile status was created based on study participants' body temperature taken on Day 0. Body temperature was measured through (1) oral temperature or (2) axillary temperature. If oral temperature was taken, an individual was considered *FEBRILE* if his/her body temperature was greater than or equal to ($\geq$) 37.5 °C. If axillary temperature was measured, an individual was considered *FEBRILE* if his/her body temperature was greater than or equal to ($\geq$) 37.2°C.

Parasite Density Parasite density is the number of malaria parasites found per microliter (μl) of blood. It is an indication of severity in malaria. To compare test results between FalciVax^{TM} RDT and gold standard Giemsa-stained Microscopy across parasite density, we created a 6-level categorical variable (*PARASITE DENSITY CATEGORY*). The levels included in the variable were: (1) no parasite, (2) 1-100 parasites/μl, (3) 101-500 parasites/μl, (4) 501-1000 parasites/μl, (5) 1001-5000 parasites/μl, and (6) more than 5000 parasites/μl.

Data Description

Cross-tabulation of FalciVax^{TM} RDT and Giemsa-stained Microscopy test results were generated by season and by year. Overall and factor-specific malaria prevalence, sensitivity, specificity, positive and negative predictive values were calculated. We plotted percent concordant pairs, percent true positive pairs and percent true negative pairs recorded per day using calendar heatmaps. Last but not least, participants' parasite density was plotted and described by malaria status.

Modeling Sensitivity, Specificity, and Predictive Values

Regression Analysis We used logistic regression to model FalciVax^{TM} screening results versus the gold standard Giemsa-stained Microscopy test results. Equations for modeling unadjusted sensitivity, specificity, positive and negative predictive values are shown below.

Sensitivity and Specificity:

$$logit\ Pr(\text{RDT Positive} \mid \text{Microscopy Result}) = \beta_0 + \beta_1 * \text{Microscopy Result}$$

Positive and Negative Predictive Values:

$$logit\ Pr(\text{Microscopy Positive} \mid \text{RDT Result}) = \beta_0 + \beta_1 * \text{RDT Result}$$

We further expanded the above equations to incorporate covariates of interest. The general logistic regression for modeling adjusted sensitivity, specificity and predictive models [88] is:

$$logit\ Pr(Y = 1|X) = \beta_0 + \beta_1 * X_1 + \sum_{k=2}^{K} \beta_k * X_k \tag{3.3}$$

where

Y is FalciVax$^{\text{TM}}$ RDT status and X_1 is Giemsa-stained microscopy test results when calculating sensitivity and specificity; or,

Y is Giemsa-stained microscopy test results and X_1 is FalciVax$^{\text{TM}}$ RDT status when calculating positive and negative predictive values;

and

X_k $(k = 2, 3, ...K)$ are the added categorical covariate of interest. Categorical variables (such as "seasons") were incorporated as dummy variables when we modeled sensitivity, specificity and predictive values using logistic regressions.

Modeled results were further used to compare with crude sensitivity, specificity, positive and negative predictive values calculated directly from field surveillance data.

Software Results from Giemsa-stained Microscopy and FalciVax^{TM} RDT were recorded in Microsoft Access 2007 (Redmond, WA). Data cleaning and analysis were performed in R version 3.0.2 [89].

3.4 Results

3.4.1 Overview

From 2009 to 2013, 708 out of 22,325 residents were recruited via passive surveillance. Of which, 305 people were from Kuhalong Union (305/12,502) and 403 people were from Rajbila Union (403/9,823). Among the 708 participants, 616 individuals (87%) granted their consents for both FalciVax^{TM} rapid diagnostic test and Giemsa-stained microscopy test (Kuhalong Union: N = 267 (87.5%); Rajbila Union: N = 349 (86.6%)). Ninety-one (91) individuals only had one of the two test results; one individual had neither of the test result. Reasons for not willing to receive the test(s) can be found in Table 3.1. In general, if a test was previously done by another institute or health facility, these participants opted to not receiving the test again. As the aim of this paper was to examine field performance of FalciVax^{TM} RDT by comparing results from RDT and microscopy, participants without both test results were removed from further analyses (92/708).

Among 616 participants with both test results, 527 of them (85.6%) were confirmed with malaria by both tests (Table 3.2). Parasite density of these 527 individuals ranged from a minimum of 100 parasites/μl to a maximum of 144,000 parasites/μl, with a mean and median of 9,426 and 6,800 parasites/μl, respectively (Standard Error = 510 parasites/μl) (Table 3.3). Two (N = 2) out of 616 individuals (0.3%) were tested positive by microscopy, but the results were not confirmed by FalciVax^{TM} RDT. Their blood films had shown parasite density of 240 and 600 parasites/μl. Fifty-eight (N = 58) out of 616 individuals (9.4%) were only tested positive by FalciVax^{TM} RDT. Twenty-nine (N = 29) out of 616 individuals (4.7%) were confirmed malaria-free by both tests. As the latter two groups of study participants (N = 87 out of 616) were tested negative by microscopy, their parasite density was recorded as 0 and no variation was found. Figure 3.1 shows the distribution of parasite density by malaria status.

Year 2009 marked our first year of the malaria surveillance project. We tested 10 individuals on malaria via passive surveillance (Table 3.4). From 2010 onward, local community was familiar with our surveillance program. We saw growing numbers of individual being tested. As Passive Surveillance was designed to recruit individuals who felt ill, number of individuals tested with malaria on day 0 provides a crude annual and seasonal malaria trend (Table 3.4). Overall, year 2011 was our highest year and monsoon season was our peak season in malaria diagnoses. We had approximately three times the tested individuals in 2011 (N = 317) than in any other year (2010: N = 123; 2012: N = 95; 2013: N = 71). Meanwhile, monsoon season (N = 277) encompassed approximately twice the number of malaria tested individuals than in summer (N = 113), autumn

(N = 130) and other seasons combined (Pre-winter: N = 55; Winter: N = 28; Spring: N = 13). Due to relatively small numbers of malaria tested individuals in pre-winter, winter and spring seasons, we combined these three seasons as one level ("other") in further analyses. Sensitivity, specificity, positive and negative predictive values of FalciVax^{TM} RDT by season and by year could be found in Table 3.5.

3.4.2 Unadjusted Test Results

Overall, malaria prevalence was 85.9% (529/616) among tested individuals in passive surveillance (Table 3.2). By using FalciVax^{TM} RDT as a screening tool to compare against microscopy as gold standard, we found sensitivity and specificity of FalciVax^{TM} RDT to be 99.6% (527/529) and 33.3% (29/87), respectively. Positive predictive value of FalciVax^{TM} RDT was 90.1% (527/585). Its negative predictive value was 93.6% (29/31). Percentage agreement between microscopy and FalciVax^{TM} RDT was 90.3% (i.e. $100 * (527 + 29)/616$), as indicated by Table 3.2. Figure 3.2(a) shows the percent agreement between microscopy and FalciVax^{TM} RDT on a daily basis. On days with malaria testing activities, most of concordant pairs were contributed by higher pairs of true positive than true negative (Figure 3.2(b) and Figure 3.2(c)). Modeled results had shown the same sensitivity, specificity, positive and negative predictive values as the ones calculated directly from the field data.

3.4.3 Adjusted Test Results

We began the adjusted analysis by stratifying malaria diagnoses based on year, season, febrile status and parasite density (Tables 3.5 and 3.7). Factor specific malaria prevalence, sensitivity, specificity, and predictive values were then calculated. In brief, malaria prevalence among individuals recruited through passive surveillance were high, even within each factor specific stratum ($\geq$ 78.12%). The only exception was found in the microscopy negative group (i.e. Parasite Density = 0) where its prevalence was 0.00% (Tables 3.7). Among strata of which sensitivity and specificity could be computed, FalciVax^{TM} RDT had a narrow range in its sensitivity (94.00—100.00%) but a broader spectrum in its specificity (0.00—100.00%). Positive and negative predictive values were dependent upon disease prevalence. Both FalciVax^{TM} RDT's positive predictive value (PPV) and negative predictive value (NPV) ranged from 0.00% to 100.00%. (Tables 3.5 and 3.7)

Test Results by Year

Year 2011 was our biggest year in data collection (N = 317), followed by Year 2010 (N = 123) and Year 2012 (N = 95) (Table 3.7 and Table 3.9). Yearly sensitivity of FalciVax^{TM} RDT was 100.0% (8/8), 99.1% (110/111), 100.0% (258/258), 98.9% (86/87) and 100.0% (65/65) from 2009 to 2013. Specificity was found to be 50.0% (1/2) in 2009, 25.0% (3/12) in 2010, 27.1% (16/59) in 2011, 62.5% (5/8) in 2012 and 66.7% (4/6) (Table 3.7 and Table 3.9). In 2011, the chance of having a positive FalciVax^{TM} RDT result was 2.8 times the chance

of having a negative FalciVax^{TM} RDT result among malaria negative individuals ($e^{1.03} = 2.8$; 95% CI: 1.6 - 5.1). After adjusting for years, the odds ratio of having a positive FalciVax^{TM} RDT result comparing malaria positive to malaria negative individuals was 202.4 (OR: $e^{5.31}$, 95% CI: 53.5 - 1366.5). No evidence had shown difference in field performance of FalciVax^{TM} RDT comparing year 2011 to years 2009, 2010 and 2013. However, lower odds was found in detecting FalciVax^{TM} RDT positive patients in 2012 than in 2011 (OR: $e^{-1.65} = 0.19$; 95% CI: 0.04 - 0.76), after controlling for microscopy status. Meanwhile, the odds of having a positive microscopy result when an individual's FalciVax^{TM} RDT result was negative was 0.03 (Odds: $e^{-3.53}$; 95% CI: 0.0 - 0.1). Given the same FalciVax^{TM} RDT status, individuals were more likely to be diagnosed by microscopy in 2010 (OR: $e^{0.82} = 2.3$; 95% CI: 1.1 - 5.2), 2012 (OR: $e^{1.65} = 5.2$; 95% CI: 1.9 - 19.5) and 2013 (OR: $e^{1.49} = 4.4$; 95% CI: 1.5 - 18.5) than in 2011 (Table 3.8).

Test Results by Season

Summer (N = 113), monsoon (N = 277) and autumn (N = 130) were the three main transmission seasons of malaria (Table 3.7). Prevalence within this study population was greater than 78.1% (Summer: 91/113 (80.5%); Monsoon: 245/277 (88.4%); Autumn: 118/130 (90.8%); Other: 75/96 (78.1%)). We found sensitivity of FalciVax^{TM} RDT to be 98.9% or higher (Summer: 90/91 (98.9%); Monsoon: 245/245 (100.0%); Autumn: 117/118 (99.2%); Other: 75/75 (100.0%)). Specificity, on the other hand, were lower than 55.0% (Summer: 4/22 (18.2%); Monsoon: 9/32 (28.1%); Autumn: 5/12 (41.7%); Other: 11/21

(52.4%)). Modeled sensitivity was in between 99.3% and 99.8% (Table 3.7). Modeled specificity ranged from 21.9% to 49.9% (Table 3.9). Field performance of FalciVax^{TM} RDT were comparable across seasons. There was no statistically significant difference among them (Table 3.8).

Test Results by Febrile Status

Of 616 participated individuals, approximately half of them (N = 307, 49.8%) had fever on Day 0 (Table 3.7). This was indicated by their oral or axillary temperature measured at home visit. Within the group of febrile individuals, 271 out of 307 (88.3%) were malaria positive. These microscopy results were 100.0% confirmed by FalciVax^{TM} RDTs (i.e. sensitivity: 271/271 = 100.0%). Where only 99.2% (256/258) of non-febrile malaria positive individuals were tested positive by FalciVax^{TM} RDTs (Table 3.9). Specificity of FalciVax^{TM} RDT was at 27.8% (10/36) and 37.3% (19/51) among febrile and non-febrile participants, respectively. Given the same microscopy status, higher odds of being detected by FalciVax^{TM} RDT was recorded comparing febrile versus non-febrile group (OR: $e^{0.60} = 1.8$). However, this result was not statistically significant (95% CI: 0.8 - 4.6). Similar findings could be applied to positive and negative predictive values of the test (Table 3.8).

Test Results by Parasite Density

Five hundred and twenty-nine (529) out of 616 enrolled individuals were microscopy positive. The arithmetic means of parasite density among FalciVax^{TM}

RDT positive and negative individuals were 9,426 parasites/μl and 420 parasites/μl, respectively (Table 3.3). As the rest of the enrolled participants (N = 87) were microscopy negative, no parasite was found on their blood films. Prevalence among the group with 1 or more parasites per microliter on blood films was 100.0% (Table 3.7). Sensitivity of FalciVax^{TM} RDT was in between 95% and 99% for groups with 1-1000 parasites/μl (1-500 parasites/μl: 23/24 (95.8%); 501-1000 parasites/μl: 69/70 (98.6%)). Its sensitivity among groups with greater than 1000 parasites/μl was 100.0%. Specificity of FalciVax^{TM} RDT was found to be 33.3% (29/87) among individuals with 0 parasite/μl. Due to monotone results of parasite density for microscopy negative individuals, logistic regression comparing FalciVax^{TM} RDT and microscopy cannot be conducted. Therefore, no results from the modeled sensitivity, specificity and predictive values could be shown.

3.5 Discussion

The goal of this paper was to analyze the performance of FalciVax^{TM} RDT device under various conditions in the field. Conditions included its performance across years, seasons, febrile status and parasite density. One strength of the study was using a population based malaria surveillance system from 2009 to 2013. With long-term existence of the surveillance system and the study team's constant effort in malaria diagnosis, treatment and prevention, we were known by the locals as their front line contact for malaria. As the team rolled out

Mapping Malaria Epidemiology surveillance project [87], providing timely diagnosis and treatment was its primary focus. Field validation of FalciVax^{TM} RDT was not. Therefore, all Giemsa-stained blood films were only examined by a trained microscopist. This paper thus provided information on field performance of FalciVax^{TM} RDT. However, it cannot be deemed as a validation study.

Another strength of the study was the use of both FalciVax^{TM} RDT and microscopy as parallel diagnostic methods since 2009. None of the study participant was examined by devices other than FalciVax^{TM} RDT. This minimized the possibility of contamination in field performance with other commercial RDT devices. It also maximized the number of study participants we had throughout the study. Overall, 708 residents in Bandarban Study Area were recruited through our passive surveillance. Of which, 91 participants (12.8%) opted out of the microscopy test. Almost all who opted out (90/91) claimed to receive a microscopy test elsewhere (Table 3.1). Therefore, these individuals only received FalciVax^{TM} RDT at home visits. Choosing RDT over microscopy could have resulted from the difference in processing time. If a resident had already received a microscopy test at a different facility, he/she might want a diagnostic test that could provide a more immediate feedback as a second option. Rapid diagnostic tests provided just that. It allowed residents to know their malaria status during the same home visit. Whereas residents might need to wait for results to come back the same day or the next day for microscopy tests. To compare field performance of FalciVax^{TM} RDT against microscopy as gold standard, we need to have both test results. Thus, 616 (87%) individuals were included in the analysis.

The other strength of the study was to provide home visits to all residents who contacted our study team. The form of contact could be in person or via phone calls. Once a notification was received, the project manager at Bandarban field office would send field workers to conduct home visits. This has greatly increased access to care for local residents, especial for those who lived in communities without easy road access. With the introduction of cell phones, it also facilitated case detection for malaria. A previous study from our field site showed approximately half of the symptomatic malaria cases (265/509) were reported initially via cell phones [90]. Overall, year 2011 (N = 317) and monsoon season (N = 277) were the busiest year and season, respectively, for malaria detection (Table 3.4). Among study participants, febrile (N = 307) and non-febrile (N = 309) status were fairly even split. More than half of the study participants (N = 320) were found with parasite density greater than or equal to 5,000 parasites/μl in their blood (Table 3.7). Due to the design of passive surveillance, we were likely to recruit study participants who were potentially ill. Nearly 90% of the participants (N = 529) were malaria positive on Day 0 based on results provided by Giemsa-stained microscopy ("gold standard") (Table 3.2). Therefore, malaria prevalence derived from the passive surveillance would be greater than the general malaria prevalence for the entire study population (1-2%).

From our analysis, we found the odds of having a positive FalciVax^{TM} RDT result was 1.99 (Odds: $e^{0.69}$; 95% CI: 1.30 - 3.16) given a negative microscopy test result. On the other hand, the odds of having a positive microscopy test result was 0.07 (Odds: $e^{-2.67}$; 95% CI: 0.01 - 0.23) when an individual received

a negative FalciVax^{TM} RDT result (Table 3.6). In other words, it was likely to see RDT positive when microscopy was negative. However, when RDT was negative, it was unlikely to see a positive microscopy result. This affirmed our knowledge that microscopy is a more sensitive method than FalciVax^{TM} RDT in malaria detection. In addition, the odds of having a positive FalciVax^{TM} RDT among microscopy positive individuals was 131.63 times ($e^{4.88}$) the odds among microscopy negative individuals (95% CI: 38.47 - 828.82). Similarly, odds ratio of having a positive microscopy result comparing FalciVax^{TM} RDT positives to FalciVax^{TM} RDT negatives ($e^{4.88}$) was also 131.63 (95% CI: 38.47 - 828.82). This modeled result was expected due to a direct swap of dependent and independent variables (i.e. FalciVax^{TM} RDT and Giemsa-stain Microscopy Test) in an unadjusted logistic regression (Table 3.6).

We used the modeled results to derived modeled sensitivity, specificity and predictive values, as shown in Table 3.9 and Table 3.10. Overall, unadjusted sensitivity (99.62%), specificity (33.33%), positive (90.09%) and negative (93.55%) predictive values were the same between modeled values and values calculated directly from collected data (Table 3.9 and Table 3.10). This was because unadjusted logistic regression model used information gathered from the entire study population. Results from direction calculation also utilized information from the whole study population. Therefore, it was not surprising to see similarities. However, in stratum specific sensitivity, specificity and predictive values, we saw differences in modeled and calculated indicator results. It was due to the reduction in sample size in each stratum of a variable of interest. Modeled logistic regression borrowed information from other strata to provide smoother

results with confidence intervals.

As malaria intensity differed by time and severity, we calculated as well as modeled sensitivity, specificity and predictive values to minimize the level of uncertainty caused by small number in any given category [88]. It allowed us to produce smoothed estimates and reduce instability of direct calculation results caused by smaller numbers in each factor specific strata—which was yet another strength of the study. When sensitivity and specificity were modeled, the performance of FalciVax^{TM} RDT held across seasons and febrile status. The sensitivity and specificity were not significantly different from one and other. Modeling sensitivity and specificity in year, on the other hand, has shown statistically significant field performance in between 2011 and 2012. Given the same microscopy status, lower odds of having a positive FalciVax^{TM} RDT result was found in 2012 than in 2011 (OR: $e^{-1.65} = 0.19$, 95%CI: 0.04 - 0.76). This means, if two individuals have the same microscopy confirmed malaria results (i.e. both positive, or both negative), the participant who was tested in 2012 had a 81% lower chance of having a positive FalciVax^{TM} RDT result than the person who was tested in 2011. Modeling positive and negative predictive values in year had identified similar statistical significance. Under the same FalciVax^{TM} RDT results, individuals who were tested in 2010 (OR: $e^{0.82} = 2.27$), 2012 (OR: $e^{1.65} = 5.20$) and 2013 (OR: $e^{1.49} = 4.43$) had a statistical significant higher odds of having positive microscopy readings than the individuals tested in 2011 (Table 3.8).

As for the field performance of FalciVax^{TM} RDT at various parasite density,

we were unable to model estimates of sensitivity, specificity, positive and negative predictive values properly with concise standard errors and 95% confidence intervals. The reasons are as follows: (1) When modeling sensitivity and specificity, we focused on individuals who were microscopy positive. As shown in Table 3.3, 527 individuals were true positive in malaria diagnosis (i.e. microscopy $\oplus \cap$ RDT $\oplus$) and 2 individuals were false negative in malaria diagnosis (i.e. microscopy $\oplus \cap$ RDT $\ominus$). The sample size in the false negative group were too small to provide enough information on how parasite density differed between true positives and false negatives; (2) when modeling positive and negative predictive values, we looked at individuals who were microscopy negative. As we saw in Table 3.3, all 87 individuals in false positive (i.e. microscopy $\ominus \cap$ RDT $\oplus$) and true negative (i.e. microscopy $\ominus \cap$ RDT $\ominus$) groups had parasite density of zero. There was no variation between the two groups. Hence, modeled standard error and confidence interval of the estimates, if able, were not informative.

Compared to prior studies that used FalciVax^{TM} RDT for *P. falciparum* detection, our overall sensitivity was relatively high (99.6%). Our overall specificity, on the other hand, was significantly lower (33.3%) than others. Prior studies had a sensitivity ranging from 89.4% to 100.0% (Singh: 94.0% [32] and 89.4% [35]; Sreekanth: 100.0% [34]; Alam: 98.2% [33]), and a specificity in between 72.8% and 98.7% (72.8% [32], 84.1% [35], 98.7% [34] and 97.0% [33]). One reason for discrepancy could be attributed to spectrum bias [91]. Spectrum bias indicates prevalence of disease could have an impact on its sensitivity and specificity of a screening test [91, 92]. This is contradictory to a general concept in principle of epidemiology where sensitivity and specificity of a screening test are

independent from disease prevalence; only its predictive values are affected by disease prevalence [93, 94]. Ransohoff and Brenner argued that sensitivity and specificity of a screening tool would only be independent from the underlying prevalence of a disease when there is a clear cut in disease diagnosis [91, 92]. Diseases that are directed associated with a genetic mutation would be a good example in this scenario. However, in scenarios where presence or classification of disease is related to a measurable or immeasurable continuous factor, sensitivity and specificity of a screening tool would be affected by prevalence of a disease [92]. Malaria, which is defined by presence of *Plasmodium* parasite in the blood, belonged to the latter scenario. The detection limit of a rapid diagnostic test or a bad sample of blood could, in theory, trigger the spectrum bias.

In this study, we had 529 out of 616 individuals in the Passive Surveillance system who were *P. falciparum* positive. This translated to a prevalence of 85.9% within our study population. In Singh and colleagues' studies in 2010 and 2013, only 35.8% (133/372) and 26.6% (480/1807) of study participants were diagnosed with *P. falciparum* malaria, respectively [32, 35]. Meanwhile, Sreekanth et al. found 19 out of 100 participants (19.0%) and Alam et al. diagnosed 171 out of 338 individuals (50.6%) with *P. falciparum* malaria [33, 34]. As explained by Brenner and colleagues—per spectrum bias, when the prevalence of the disease increases, sensitivity of a screening tool is likely to increase; on the contrary, specificity of the screening tool is likely to decrease [92]. This phenomenon was seen in our study with FalciVax^{TM} RDT. This led to the limitation of our study: Generalizibality. This paper focused on the field performance of FalciVax^{TM} RDT through passive surveillance. Data were

collected from individuals who were prone to malaria infection. The prevalence calculated from passive surveillance (95.88%) cannot represent the overall malaria prevalence—which was 1-2%—among the general population in Kuhalong and Rajbila Unions in Bandarban, Chittagong Hill Tracts, Bangladesh. As our prevalence of *P. falciparum* malaria and its underlying distribution of parasite density were unique to this study population, the field performance of FalciVax^{TM} RDT cannot be assumed the same across all population. The effect of spectrum bias is expected to be stronger in diseases with lower prevalence [91, 92].

In a laboratory controlled setting, using sensitivity greater than or equaled to ($\geq$) 95.0% and specificity greater than or equaled to ($\geq$) 90.0% as a guideline for rapid diagnostic devices is a good practice [83]. However, discussion needs to be initiated for guidelines used in the field. Given the assumption of field performance of RDT is associated with prevalence of malaria, how could we best address the issue (especially in hypoendemic area) and ensure the quality of RDT in case detection in a rural and resource scarce environment. Decisions made based on field performance should be exercised with caution. As many areas in the world are phasing into hypoendemic malaria state, it is crucial not to overlook the effect associated with spectrum bias. Unless a target population has a similar population and prevalence profile as to our study population, results from field performance of FalciVax^{TM} RDT cannot be compared. It would be interesting to see another field performance study of FalciVax^{TM} RDT conducted from Bandarban Study Area. With the new performance study focusing

on active surveillance, we could randomly select participants with similar demographics as our current study. If both studies have comparable sample sizes and similar study time frames, we could compare field performance results of FalciVax^{TM} RDT. Given differences in prevalence for active and passive surveillance, it would be intriguing to see the magnitude of spectrum bias within this study population.

Table 3.1: Participant-Reported Reasons for Not Willing to Have FalciVax™ RDT and/or Microscopy Test Taken

		Microscopy				**Grand Total**
		Taken		**Not Taken**		
		Reason	*Subtotal*	Total	*Reason*	*Subtotal*	Total	
FalciVax™ RDT	**Taken**			616			90	706
					Done by BRAC †	*82*		
					Done by Health Center	*1*		
					Done by MARIB ‡	*2*		
					Done by Sadar Hospital	*1*		
					Done by Village Doctor	*1*		
					Done by Government Source	*2*		
					Not specified	*1*		
	Not Taken			1			1	2
		Forgot	*1*		*Done by BRAC* †	*1*		
	Grand Total			617			91	708

† BRAC: Bangladesh Rural Advancement Committee
‡ MARIB: Malaria Research Initiative Bandarban

Table 3.2: 2x2 Contingency Table Showing Number of Individuals in Each of the Microscopy—FalciVax™ RDT Result Category

		Microscopy "Gold Standard"		
		Positive	Negative	Total
FalciVax™ RDT "Screening"	Positive	527	58	585
	Negative	2	29	31
	Total	529	87	616

Table 3.3: Parasite Density in Individual's Blood Stream

		Parasite Density (parasites/μl)					
Microscopy	**FalciVax™ RDT**	N	Mean	Median	Min	Max	Standard Error
+	+	527	9426.1	6800	100	144000	510.0
−	+	58	0.0	0	0	0	0.0
+	−	2	420.0	420	240	600	180.0
−	−	29	0.0	0	0	0	0.0

Table 3.4: Number of Microscopy—FalciVax™ RDT Pair Tested for *P. falciparum* Malaria, by Season and Year

	Year					
Season	2009	2010	2011	2012	2013	**Total**
Winter (Beginning of Year)		2	7	7	2	18
Spring		0	5	7	1	13
Summer		22	65	16	10	113
Monsoon		68	136	37	36	277
Autumn	1	19	69	19	22	130
Pre-Winter	9	7	33	6		55
Winter (End of Year)	0	5	2	3		10
Total	10	123	317	95	71	616

Table 3.5: Calculated Sensitivity, Specificity, Positive Predictive Values (PPV) and Negative Predictive Values (NPV) of FalciVax™ RDT against Microscopy as Gold Standard by Year and Season

Year	**FalciVax™ RDT**							
Season	TP$^{\alpha}$	FP$^{\beta}$	FN$^{\gamma}$	TN$^{\delta}$	Sensitivity	Specificity	PPV	NPV
2009								
Autumn	0	1	0	0	NA	0.00	0.00	NA
Other †	8	0	0	1	100.00	100.00	100.00	100.00
2010								
Summer	19	2	1	0	95.00	0.00	90.48	0.00
Monsoon	62	5	0	1	100.00	16.67	92.54	100.00
Autumn	16	2	0	1	100.00	33.33	88.89	100.00
Other †	13	0	0	1	100.00	100.00	100.00	100.00
2011								
Summer	47	15	0	3	100.00	16.67	75.81	100.00
Monsoon	113	18	0	5	100.00	21.74	86.26	100.00
Autumn	64	3	0	2	100.00	40.00	95.52	100.00
Other †	34	7	0	6	100.00	46.20	82.93	100.00
2012								
Summer	15	1	0	0	100.00	0.00	93.75	NA
Monsoon	36	0	0	1	100.00	100.00	100.00	100.00
Autumn	16	0	1	2	94.12	100.00	100.00	66.67
Other †	19	2	0	2	100.00	50.00	90.48	100.00
2013								
Summer	9	0	0	1	100.00	100.00	100.00	100.00
Monsoon	34	0	0	2	100.00	100.00	100.00	100.00
Autumn	21	1	0	0	100.00	0.00	95.45	NA
Other †	1	1	0	1	100.00	50.00	50.00	100.00

† Other: It includes Spring, Pre-Winter and Winter
$^{\alpha}$ True positive (TP): Microscopy $\oplus \cap$ RDT $\oplus$
$^{\beta}$ False Positive (FP): Microscopy $\ominus \cap$ RDT $\oplus$
$^{\gamma}$ False Negative (FN): Microscopy $\oplus \cap$ RDT $\ominus$
$^{\delta}$ True Negative (TN): Microscopy $\ominus \cap$ RDT $\ominus$

Table 3.6: Overall Logistic Regression for Modeling Sensitivity, Specificity, and Predictive Values of FalciVax™ RDT—Coefficients, Standard Errors (SE), Lower and Upper 95% Confidence Limit (LCL, UCL)

	Model for Sensitivity and Specificity †				**Model for Positive and Negative Predictive Value** ‡			
			95% CI				95% CI	
Covariate	Coefficient	SE	LCL	UCL	Coefficient	SE	LCL	UCL
Intercept	0.69	0.23	0.26	1.15	-2.67	0.73	-4.50	-1.48
Microscopy $^{\alpha}$	4.88	0.74	3.65	6.72				
FalciVax™ RDT $^{\beta}$					4.88	0.74	3.65	6.72

† Dependent variable was defined to be FalciVax™ RDT (Screening Test)
‡ Dependent variable was defined to be Microscopy (Gold Standard)
$^{\alpha}$ Microscopy: Coded as Positive = 1, Negative = 0
$^{\beta}$ FalciVax™ RDT: Coded as Positive = 1, Negative = 0

Table 3.7: Factor Specific *P. falciparum* Malaria Prevalence and Calculated Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of FalciVax™ RDT

			FalciVax™			
Category	**Count**	**Prevalence** $^{\int}$	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Overall						
	616	85.88	99.62	33.33	90.09	93.55
Year						
2009	10	80.00	100.00	50.00	88.89	100.00
2010	123	90.24	99.10	25.00	92.44	75.00
2011	317	81.39	100.00	27.12	85.71	100.00
2012	95	91.58	98.85	62.50	96.63	83.33
2013	71	91.55	100.00	66.67	97.01	100.00
Season $^{\alpha}$						
Summer	113	80.53	98.90	18.18	83.33	80.00
Monsoon	277	88.45	100.00	28.12	91.42	100.00
Autumn	130	90.77	99.15	41.67	94.35	83.33
Other	96	78.12	100.00	52.38	88.24	100.00
Febrile						
Non-febrile	309	83.50	99.22	37.25	88.89	90.48
Febrile	307	88.27	100.00	27.78	91.25	100.00
Parasite Density (parasites/μl)						
0	87	0.00	NA	33.33	0.00	100.00
1-500	24	100.00	95.83	NA	100.00	0.00
501-1000	70	100.00	98.57	NA	100.00	0.00
1001-5000	115	100.00	100.00	NA	100.00	NA
> 5000	320	100.00	100.00	NA	100.00	NA

$^{\int}$ Prevalence: It was calculated based on number of microscopy positive individuals given all individuals tested through Passive Surveillance

$^{\alpha}$ Seasons: Summer — Mid-April to Mid-June, with a total of 62 days/year on average; Monsoon — Mid-June to Mid-August, with a total of 63 days/year on average; Autumn — Mid-August to Mid-October, with a total of 61 days/year on average; Other — Pre-Winter (Mid-October to Mid-December), Winter (Mid-December to Mid-February) and Spring (Mid-February to Mid-April), with a total of 179 days/year on average

Table 3.8: Factor Specific Logistic Regression for Modeling Sensitivity, Specificity, and Predictive Values of FalciVax™ RDT—Coefficients, Standard Errors (SE), Lower and Upper 95% Confidence Limit (LCL, UCL)

	Model for Sensitivity and Specificity †				**Model for Positive and Negative Predictive Value** ‡			
Factor			95% CI				95% CI	
Covariate	Coefficient	SE	LCL	UCL	Coefficient	SE	LCL	UCL
Season								
Intercept	1.04	0.39	0.31	1.86	-2.61	0.77	-4.47	-1.32
Microscopy $^{\alpha}$	4.94	0.75	3.68	6.79				
FalciVax™ RDT $^{\beta}$					4.94	0.75	3.68	6.79
Summer	0.24	0.63	-0.98	1.54	-0.67	0.34	-1.33	0.00
Monsoon	Ref	–	–	–	Ref	–	–	–
Autumn	-0.81	0.65	-2.11	0.49	0.53	0.44	-0.28	1.45
Other	-1.03	0.57	-2.17	0.07	-0.38	0.39	-1.12	0.41
Year								
Intercept	1.03	0.29	0.48	1.63	-3.53	0.80	-5.45	-2.18
Microscopy $^{\alpha}$	5.31	0.79	3.98	7.22				
FalciVax™ RDT $^{\beta}$					5.31	0.79	3.98	7.22
2009	-0.95	1.40	-3.79	2.28	0.26	1.04	-1.44	2.86
2010	-0.24	0.66	-1.49	1.16	0.82	0.39	0.10	1.65
2011	Ref	–	–	–	Ref	–	–	–
2012	-1.65	0.71	-3.14	-0.27	1.65	0.59	0.63	2.97
2013	-1.39	0.80	-3.01	0.20	1.49	0.63	0.40	2.92
Febrile								
Intercept	0.46	0.28	-0.09	1.03	-2.75	0.74	-4.58	-1.53
Microscopy $^{\alpha}$	4.85	0.74	3.62	6.69				
FalciVax™ RDT $^{\beta}$					4.85	0.74	3.62	6.69
Non-Febrile	Ref	–	–	–	Ref	–	–	–
Febrile	0.60	0.45	-0.27	1.52	0.21	0.27	-0.33	0.75

† Dependent variable was defined to be FalciVax™ RDT (Screening Test)
‡ Dependent variable was defined to be Microscopy (Gold Standard)
$^{\alpha}$ Microscopy: Coded as Positive = 1, Negative = 0
$^{\beta}$ FalciVax™ RDT: Coded as Positive = 1, Negative = 0

Table 3.9: Compared Modeled and Calculated Sensitivity and Specificity of FalciVax™ RDT

Factor	Sensitivity (%)			Specificity (%)		
Strata	Modeled[†,‡]	Calculated		Modeled[†,‡]	Calculated	
Overall						
	99.62	99.62	(527/529)	33.33	33.33	(29/87)
Season						
Summer	99.80	98.90	(90/91)	21.90	18.18	(4/22)
Monsoon	99.75	100.00	(245/245)	26.19	28.12	(9/32)
Autumn	99.43	99.15	(117/118)	44.41	41.67	(5/12)
Other	99.29	100.00	(75/75)	49.86	52.38	(11/21)
Year						
2009	99.54	100.00	(8/8)	48.16	50.00	(1/2)
2010	99.77	99.10	(110/111)	31.25	25.00	(3/12)
2011	99.82	100.00	(258/258)	26.34	27.12	(16/59)
2012	99.09	98.85	(86/87)	65.05	62.50	(5/8)
2013	99.29	100.00	(65/65)	58.99	66.67	(4/6)
Febrile						
Not Febrile	99.51	99.22	(256/258)	38.69	37.25	(19/51)
Febrile	99.73	100.00	(271/271)	25.74	27.78	(10/36)
Parasite Density (parasites/μl)						
0	NA	NA	(0/0)	NA	33.33	(29/87)
1-500	NA	95.83	(23/24)	NA	NA	(0/0)
501-1000	NA	98.57	(69/70)	NA	NA	(0/0)
1001-5000	NA	100.00	(115/115)	NA	NA	(0/0)
> 5000	NA	100.00	(320/320)	NA	NA	(0/0)

[†] Modeled: Sensitivity, specificity, positive and negative predictive values were estimated based on the logistic regression models presented in Table 3.8.

[‡] Sensitivity and Specificity Models: Dependent variable was defined to be FalciVax™ RDT (Screening Test); independent variables were Microscopy and one covariate of interest (season, year, febrile or parasite density). For categorical covariates of interest (e.g. season, year, parasite density), dummy variables were created for modeling purposes.

Table 3.10: Compared Modeled and Calculated Predictive Values of FalciVax™ RDT

Factor Strata	Positive Predictive Value (%)			Negative Predictive Value (%)		
	Modeled[†,‡]	Calculated		Modeled[†,‡]	Calculated	
Overall						
	90.09	90.09	(527/585)	93.55	93.55	(29/31)
Season						
Summer	84.09	83.33	(90/108)	96.36	80.00	(4/5)
Monsoon	91.19	91.42	(245/268)	93.12	100.00	(9/9)
Autumn	94.62	94.35	(117/124)	88.84	83.33	(5/6)
Other	87.61	88.24	(75/85)	95.19	100.00	(11/11)
Year						
2009	88.48	88.89	(8/9)	96.33	100.00	(1/1)
2010	93.07	92.44	(110/119)	93.76	75.00	(3/4)
2011	85.56	85.71	(258/301)	97.14	100.00	(16/16)
2012	98.86	96.63	(86/89)	86.73	83.33	(5/6)
2013	96.33	97.01	(65/67)	88.49	100.00	(4/4)
Febrile						
Not Febrile	89.14	88.89	(256/288)	93.97	90.48	(19/21)
Febrile	91.00	91.25	(271/297)	92.67	100.00	(10/10)
Parasite Density (parasites/μl)						
0	0.00	0.00	(0/58)	100.00	100.00	(29/29)
1-500	100.00	100.00	(23/23)	0.00	0.00	(0/1)
501-1000	100.00	100.00	(69/69)	0.00	0.00	(0/1)
1001-5000	100.00	100.00	(115/115)	0.00	NA	(0/0)
> 5000	100.00	100.00	(320/320)	0.00	NA	(0/0)

[†] Modeled: Sensitivity, specificity, positive and negative predictive values were estimated based on the logistic regression models presented in Table 3.8.

[‡] Positive and Negative Predictive Value Models: Dependent variable was defined to be Microscopy; independent variables were FalciVax™ RDT (Screening Test) and one covariate of interest (season, year, febrile or parasite density). For categorical covariates of interest (e.g. season, year, parasite density), dummy variables were created for modeling purposes.

Figure 3.1: Boxplot of Parasite Density by Malaria Status

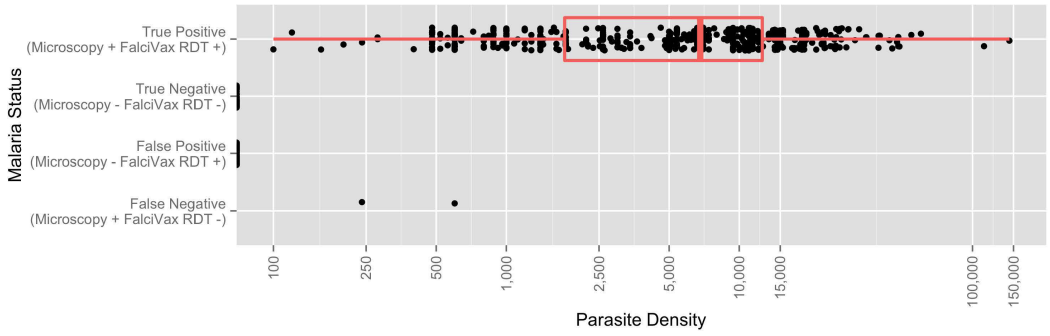
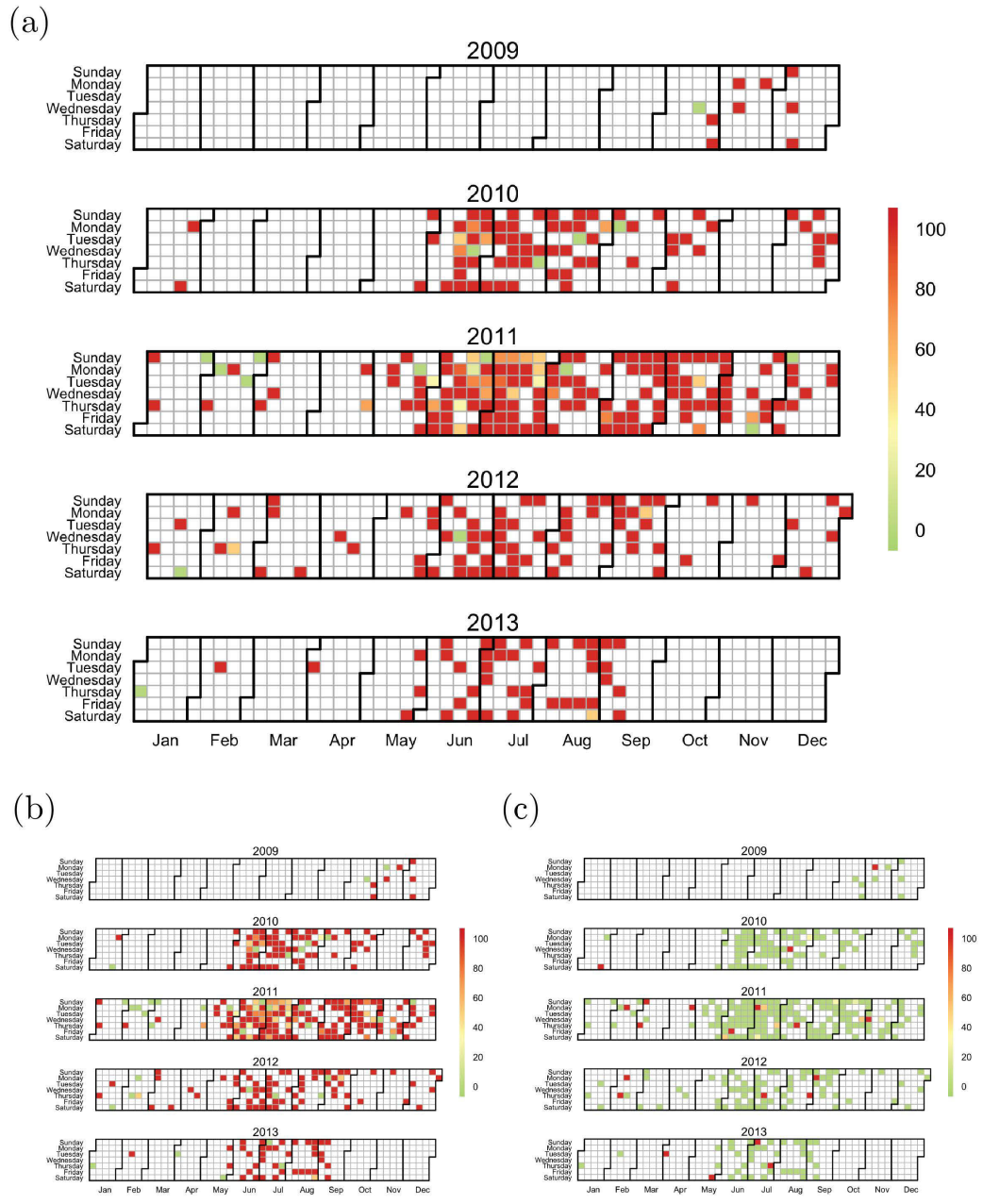


Figure 3.2: Calendar Heatmap: (a) Percent Concordant Pairs, (b) Percent True Positive Pairs and (c) Percent True Negative Pairs examined per day



Chapter 4

Paper 2: Association Between Levels of *Plasmodium falciparum* Density and Clinical Malaria Symptoms

4.1 Abstract

4.1.1 Background

To help move toward malaria elimination, broadening case search for malaria diagnosis is important. Prior studies used association between fever and malaria parasite density to gauge percent individuals with fever who were truly infected with malaria. However, how parasite density is associated with basic demographic characteristics and various malaria symptoms remained unclear. This study intends to study this association to provide a means to improve case awareness and case detection in rural Bangladesh.

4.1.2 Methods

A population-based passive surveillance on *Plasmodium falciparum* malaria was carried out in Bandarban District, Chittagong Hill Tracts, Bangladesh from 2009 to 2013. Home visits were done upon notifications from residents with malaria-like symptoms. Thin and thick blood films and rapid diagnostic tests were examined. Basic demographic information, self-reported symptoms and measured body temperature were recorded using standardized questionnaires. Logistic regression models were used to study the association between levels of *Plasmodium falciparum* parasite density and human risk factors (age, gender, body mass index, measured fever status, and malaria-related clinical symptoms).

4.1.3 Results

More than 22,000 residents were in the study area; 616 individuals were malaria tested. Three hundred (300) of tested individuals had high level of *Plasmodium falciparum* density (i.e. parasite density above median (5400 parasites/μl)). We found no statistically significant difference in acquiring higher level of parasite density between gender, body mass index and number of self-reported symptoms. Being 30 years old or above, not having measured fever during malaria diagnosis, having symptom duration for more than 6 days, having self-reported fever at night, and not having self-reported fever with sweating were related to having lower level of *Plasmodium falciparum* parasite density. Given having chills or not having self-reported fever at day time, diarrhea, cough and anemia, elevated odds of having *Plasmodium falciparum* parasite density above median was seen among febrile individuals.

4.1.4 Discussion

Delayed reporting would not only lead to delayed diagnosis and treatment, but also prolong the time a person could serve as a human host for malaria. To stop the malaria transmission, an integrated case awareness, reactive case search and hot spot analysis should be considered. We identified risk factors associated with increased odds of having higher level of *Plasmodium falciparum* parasite density. How to implement the findings to improve case awareness and case detection is essential to reduce malaria transmission in the study area.

4.2 Background

Malaria is a disease caused by *Plasmodium* parasites. Prior to the invention of the rapid diagnostic tests (RDTs), there wasn't an easy and reliable detection method of malaria in rural areas. This was largely due to the inaccessibility of microscopy services (the gold standard) in those resource scares areas. Therefore, health care providers relied on clinical symptoms of malaria to prescribe treatment. There is one caveat. Malaria symptoms are non-specific. Fever is the most commonly seen symptom among symptomatic malaria patients. However, not all fever patients were malaria infected. In the past, studies had focused on analyzing the odds of fever attributed by levels of parasite density [95, 96, 97]. It was used to guide clinical diagnosis, by estimating the percentage of fever patients that were malaria infected.

With improvement in malaria prevention, intervention and treatment methods, many endemic areas have symptomatic and asymptomatic cases. Both symptomatic and asymptomatic malaria infected individuals carry *Plasmodium* parasites; however, asymptomatic malaria cases do not present clinical symptoms. The more the *Plasmodium* parasite carriers are in a community, the harder it is to break the malaria transmission cycle. Although exceptions exist, in general, higher parasite density is associated with more severe malaria infection [98, 99, 100]. With the decline in parasite density, it's a sign an individual is recovering from malaria.

Previously, how parasite density could be affected by the wide spectrum of

malaria symptoms—including fever, headache, chills, sweating, fatigue, muscle ache, muscle weakness, cough, nausea, vomiting, diarrhea, anemia and convulsions—and basic demographic factors was not fully explored. As many countries are moving toward hypoendemic or pre-elimination phase, the incidence of malaria is decreasing. In Bangladesh, the country aims to be free of malaria in 2020.

As research facilities or health posts may not always be available in each rural corner of the world, how to do an effective case search is important. Passive surveillance relies on individuals to notify the study team. Active surveillance is too expensive to do on every person in the community on a regular basis. A viable option to broaden case detection without putting too much burden on a health system is to have a mixed method in combining passive and active surveillance.

Reactive case detection method is an example of combing active and passive surveillance. Reactive surveillance starts by identifying a malaria positive individual. Family members of the malaria positive case would then be tested with malaria. Reactive case detection helps broaden malaria case search. However, family members are often times not the only group that shares common space or time together with a malaria positive individual. Independently, hot spot analysis is an example of finding spatial clusters of a malaria-related risk factor. Active case search could focus on areas that are prone to malaria infection. The ability to integrate reactive case search and hot spot analysis would facilitate a more broadened and effective case search.

For example, a malaria case could be identified by passive surveillance. An active malaria case search could then be conducted around the index case based on fever and non-fever related malaria symptoms and demographic characteristics. In addition, with the knowledge on how malaria symptoms could be associated with malaria severity, we could create risk indices and risk maps for malaria. Areas with the higher density of fever and non-fever related malaria symptoms or characteristics could be potential hot spots for asymptomatic malaria cases.

People in a higher risk group were assumed to be more potent malaria carriers. Risk indices created based on passive surveillance could be used as a reference for future reactive case detection. In areas without microscopy service, rapid diagnostic tests (RDTs) could be the only way to detect malaria infection. Rapid diagnostic tests provide dichotomous yes-and-no results for tested individuals. However, it cannot provide reads of parasite density. If an individual is tested malaria positive by a rapid diagnostic test and is in a higher risk group (based on symptoms and demographic factors), their family members and co-workers who work and live in close proximity should be subsequently examined for malaria infection. This would broaden the malaria search for treatment and control, as well as prioritize the resource on those in need the most.

In Bandarban Study Area, it was hypothesized the number of asymptomatic malaria cases outweighed the number of symptomatic malaria cases. To move the study area from malaria control phase to malaria elimination phase, we

could not neglect asymptomatic malaria cases. As mentioned above, it is impractical financially and impossible physically to conduct malaria tests regularly on every resident in the study area. It is easier to identify symptomatic cases instead. Asymptomatic cases could go undetected. Therefore, how to broaden the case search by using an index case and search for potential asymptomatic malaria cases in the community are case detection lessons we could explore on. Moreover, how to transfer the knowledge we acquired to local residents is important. Case awareness is one thing we could pass on to the local residents. Local residents could benefit from knowing how to self diagnose with malaria based on malaria symptoms, as well as knowing their relationship with malaria severity. This awareness could shorten the gap between access to care and receiving proper treatment.

We aimed to use passive surveillance to identify human risk factors and clinical symptoms that were associated with levels of *Plasmodium falciparum* density. From there, we can utilize the findings in future studies to identify higher risk groups around the index case as a step toward effective reactive case detection for both symptomatic and asymptomatic cases. To understand how clinical symptoms and basic demographic characteristics are associated with levels of malaria parasite density, we nested this study under a four-year malaria surveillance system in Bandarban, Bangladesh. We hypothesized the level of parasite density could be affected by the following factors: (1) age and body mass index, but not gender, (2) the presence of measured fever status at the time of diagnostic test, (3) presence of specific malaria symptoms, (4) number of fever and non-fever related symptoms, and (5) duration of malaria-related symptoms.

4.3 Methods

4.3.1 Study Location

This study was part of the Mapping Malaria Epidemiology Surveillance project located in Chittagong Hill Tracts in Bangladesh. Chittagong Hill Tracts are home to 13 tribal groups and are divided into three Districts. Our study site was located at the southern most district, named Bandarban. Within the study site, there were two Unions (the smallest Government and Administrative Units in Bangladesh): Kuhalong Union and Rajbila Union. From 2009 to 2013, there were approximately 5,000 households accommodating more than 22,000 people in the study area.

4.3.2 Study Time Frame

This study was rolled out in two phases. The first phase was from October 2009 to March 2010. During this time, only residents in Kuhalong Union were recruited into the study. In April 2010, we expanded the study area to the neighboring Rajbila Union. The second phase began. We conducted the study in both Unions till September 2013.

4.3.3 Study Design

This is a population-based surveillance project located at Bandarban Study Area from 2009 to 2013. This paper focused on individuals recruited through the

passive surveillance system. That is, when an individual had fever or malaria like symptoms, the individual was advised to contact one of the Mapping Malaria Project team members. This included one of twenty field workers, a field medical doctor or a field project manager. All of which were locally hired and trained by the study team at *Johns Hopkins Malaria Research Institute* (JHMRI), *Johns Hopkins Bloomberg School of Public Health* (JHSPH) and *International Centre for Diarrhoeal Disease Research, Bangladesh* (icddr,b). In addition, the field project manager took referrals from a local non-government organization, *BRAC*, for potential malaria cases.

Once an individual was identified as a potential malaria case, the field project manager assigned 2 field workers that were in close proximity to conduct home visits. At each home visit, a FalciVax^{TM} rapid diagnostic test (RDT) was performed. Thin and thick blood films were made on the spot for further microscopy examination. Blood films collected during home visits were then analyzed by a microscopist at the Bandarban Field Office of the Mapping Malaria Epidemiology Project. The trained microscopist used Giemsa-stained microscopy to confirm malaria test results provided by FalciVax^{TM} RDT. Meanwhile, the microscopist utilized blood films to type the malaria infection, as well as to measure parasite density presented in an individual's blood.

If an individual was *Plasmodium falciparum* positive by either method, treatments were provided immediately (RDT) or the next day (microscopy). Follow-up home visits to malaria positive individuals were done on Day 2, Day 7 and

Day 28. During home visits, participants' age, gender, height, weight, self-reported symptoms, and symptom duration were recorded.

4.3.4 Study Population

All residents from Bandarban Study Area in between October 2009 and September 2013 were eligible to be part of our passive surveillance. Individuals contacted the study team for malaria diagnoses and individuals referred by BRAC were recruited into the study (N = 708). Participants without both FalciVax^{TM} RDT and Giemsa-stained microscopy test results on Day 0 were excluded from the analysis (N = 92). We further narrowed the study population for this paper down to the final 529 individuals who were *Pf* tested positive by the gold standard, Giemsa-stained microscopy (i.e. parasite density > 0 parasite/μl).

All analyses were conducted using (1) all 616 participants who were tested by RDT and microscopy, and (2) the 529 malaria positive individuals.

4.3.5 Malaria Definition

This paper aimed to study human risk factors associated with levels of *Plasmodium falciparum* parasite density. Individuals were considered as *Plasmodium falciparum* malaria positive only if the microscopist found *Plasmodium falciparum* parasites on their thick blood smears.

4.3.6 Statistical Analysis

Variables of Interest: Outcome

Our outcome of interest was a dichotomous variable derived from parasite density. Parasite density was measured from *Plasmodium falciparum* malaria tested individuals. As distribution of parasite density skewed to the right, we transformed individuals' parasite density by natural log. The natural log of individuals' parasite density demonstrated a rough bimodal distribution. The two modes could be separated by the median of parasite density among tested individuals (Figure 4.1).

Therefore, a dichotomous variable showing two levels of parasite density was created. Having *high parasite density* meant a person had *Plasmodium falciparum* density greater than median (recorded was 1). Having *low parasite density* meant a person had *Plasmodium falciparum* density less than or equal to median (recorded as 0).

Variables of Interest: Exposure

Exposures of interest included demographic information, measured fever status, self-reported symptoms and duration of symptoms.

In demographic information, we looked at age and gender of all individuals, as well as their body mass index (BMI). Age was calculated by the date difference in between the date of visit for passive surveillance and the person's date-of-birth. BMI was calculated by the person's height and weight (BMI =

$\frac{\text{Weight}(kg)}{\text{Height}^2(m^2)}$).

We measured participants' body temperature during the passive surveillance. Based on their oral or axillary temperature, we determined a person had fever if (1) his or hers oral temperature was greater than or equal to 37.5°C, or (2) his or hers axillary temperature was greater than or equal to 37.2°C.

Self-reported symptoms were recorded based on a standard questionnaire, designed by the study team of the Mapping Malaria Epidemiology Project. It included fever related and non-fever related symptoms. Fever related symptoms were (1) Fever with Shivering, (2) Fever at Day Time, (3) Fever at Night, (4) Fever with Sweating, (5) Intermittent Fever, and (6) Remission of Fever with Sweating. Non-fever related symptoms were (1) Headache, (2) Chills, (3) Nausea, (4) Vomiting, (5) Diarrhea, (6) Cough, (7) Fatigue, (8) Muscle ache, (9) Muscle weakness, (10) Convulsions / Seizure, and (11) Anemia.

Descriptive Analysis

We compiled the most commonly self-reported symptoms among our studied population. We further examined the relationship between levels of parasite density and participants' age group, individual symptoms, number of self-reported symptoms and measured fever status.

In addition, we explored potential spatial clustering of parasite density by plotting an areal summary of natural log of median parasite density. The areal

summary was calculated using the following steps:

1. The study area was evenly divided into a 10-by-10 grid (i.e. 100 grids).
2. Each household was assigned to one of the 100 grids based on its geographic location.
3. All members of a household were assigned to one of the 100 grids based on their household locations.
4. If an individual was malaria tested, his or her parasite density was linked to the household location. Therefore, it was linked to one of the 100 grids.
5. Median parasite density of each grid could be calculated.
6. A choropleth map showing the gradient of natural log of median parasite density could be found in Figure 4.2

Regression Analysis

We use logistic regression model to calculate the odds of having high level of parasite density on a given exposure, as described above.

For continuous and binary exposures of interest, the regression used was listed below.

Exposure of Interest: A Continuous Variable

$$logit\ Pr(\text{High Density Level}) = \beta_0 + \beta_1 * \text{Exposure}$$

For categorical variables, such as BMI categories, we used dummy variables to represent the exposure of interest.

Exposure of Interest: A Categorical Variable

$$logit\ Pr(\text{High Density Level}) = \beta_0 + \sum_{i=1}^{i=K-1} \beta_i * (\text{Dummy Variables of a Categorical Variable})$$

where K equaled to number of categories.

Software

Data recording and management were done by Microsoft Access 2007 (Redmond, WA). Data cleaning and analysis were performed in R version 3.0.2 [89].

4.4 Results

From October 2009 to September 2013, 616 passive surveillance participants were tested by FalciVax^{TM} RDT and Giemsa-stained microscopy. Of those, 529 individuals (86%) had malaria and 87 individuals (14%) were malaria-free.

Among 87 malaria-negative participants, half of them were male (N = 44). Age of this group ranged from 385 days to 79.1 years old. Their *Plasmodium falciparum* (*Pf*) density was recorded as 0. Among the 529 individuals who were tested positive, 298 of them were male (56%). On the day of malaria examination, the youngest and eldest *Pf* positive participants in the study area were 243 days old and 84.3 years old, respectively. The median age was 16.5 years old.

Among all *Plasmodium falciparum* malaria tested participants, the minimum and maximum recorded *Pf* density were 0 parasite/μl and 144,000 parasites/μl, respectively. The median *Pf* density was 5400 parasites/μl. Three hundred (300) individuals had *Pf* density higher than median. Three hundred and sixteen (316) individuals had *Pf* density lower than median. Distributions of parasite density and natural log of parasite density can be found in Figure 4.1. Geographically, there was no discernible pattern of natural log of median parasite density among all *Pf* malaria tested individuals across the study area (Figure 4.2).

From 2009 to 2013, the authors didn't find difference in the level of parasite density between female (N = 274) and male (N = 342) participants (95% CI: [0.60, 1.13]). Age was found to be a statistically significant factor in protecting participants from acquiring higher level of *Pf* parasites (Table 4.1). This was especially apparent among individuals aged 30 to 40 years old (N = 84), 40 to 50 years old (N = 46) and 60 to 90 years old (N = 18). Their odds of acquiring higher than median *Pf* parasite density was 0.55 (95% CI: [0.32, 0.91]), 0.45 (95% CI: [0.23, 0.88]) and 0.17 (95% CI: [0.04, 0.54]) times the odds of acquiring higher than median *Pf* parasite density among children under 10, respectively. With body mass index (BMI), we found individuals with BMI ranging [18.5, 25) (N = 174) had the lowest odds in having *Pf parasite density higher than median* than individuals with BMI lower than 18.5 (N = 412) and individuals with BMI greater than or equal to 25 (N = 12). However, this relationship was not statistically significant. (Table 4.1)

Based on body temperatures measured at home visits, 307 out of 616 (50%) study participants had body temperatures above 37.5°C (oral) or 37.2°C (axillary) (Table 4.2). Among febrile individuals, 53% (N = 163) of them had *Pf* density higher than median. Comparing to 44% (N = 137) of non-febrile individuals having *Pf* density higher than median, it showed a 42% increase in odds in acquiring higher level of parasite density for participants presenting fever during malaria diagnosis (95% CI: [1.04, 1.95]). (Table 4.2)

Other than measured body temperatures, self-reported symptoms were recorded on standardized questionnaires. Most individuals had 6 to 8 self-reported symptoms (Table 4.2). Of which, 2 to 3 symptoms were fever related, and the other 4 to 5 were not fever related (Table 4.3). Among malaria tested individuals, more than half of them (57%) reported having symptoms for no more than 3 days (N = 353) (Table 4.4). Fewer than 5% of all study participants had one or more self-reported symptoms for more than a week. This indicated majority of ill participants contacted and were seen by the study team soon after a symptom occurred.

Among 6 types of self-reported fever related symptoms, slightly more than half of all participants did not have *fever during day* or *night time* (Day: N = 329 (53%); Night: N = 321 (52%)), nor did they have *fever with shivering* (N = 315 (51%)). Not having *intermittent fever* (N = 382 (62%)) nor *remission of fever with sweating* (N = 441 (72%)) was also common. The only exception was the number people reported with *fever with sweating.* Only 34% (N = 209) of study population did not have *fever with sweating.* (Table 4.5).

Overall, odds of having higher level of parasite density was insignificantly different than odds of having lower level of parasite density among individuals with or without self-reported fever related symptoms (Table 4.5). Having *fever at night*, on the other hand, was the only exception. Among 295 study participants reported having *night time fever*, 128 of them (43%) had *Pf* density higher than median. This percentage was significantly lower than the one found in participants without fever at night (N = 172 out of 321 (54%)) (OR: 0.66, 95% CI: [0.48, 0.91]). (Table 4.5)

For non-fever related symptoms, 91% of study participants reported having headache (N = 563) and 71% of individuals had muscle ache (N = 438). Muscle weakness (N = 396), chills (N = 382) and nausea (N = 379), vomiting (N = 282) and fatigue (N = 259) were symptoms reported among 42 to 64 percent of malaria tested individuals. Cough (N = 140), diarrhea (N = 6), convulsions/seizure (N = 2), and anemia (N = 10) were not commonly seen. Individually, level of parasite density did not differ among individuals with or with a specific non-fever related symptom. (Tables 4.6 and 4.7)

If we count up the number of fever related symptoms a participant had, we found comparing to individuals having 2 fever-related symptoms in a logistic regression, the odds of having higher level of parasite density had a general upward trend as the number of fever-related symptoms went higher. This upward trend turned downward when a participant had 4 or more fever-related symptoms. Similar patterns of "dose response" could be found when comparing

odds of having higher level of parasite density among individuals having different numbers of non-fever related symptoms. However, this finding was not statistically significant. (Table 4.3)

When combining the effect of measured fever status and self-reported symptoms, we found the odds of having parasite density above median was consistently higher in fever group than in non-fever group when the referenced self-reported symptom did not occur (Tables 4.9 and 4.10). The most noteworthy increases in odds were the ones among individuals without *fever during the day*, without *diarrhea*, without *cough*, without *anemia* or with *Chills*.

To be more specific, given not having *fever at day time*, individuals who had fever during malaria diagnosis had 1.61 times the odds of having higher parasite density than individuals who did not have fever during malaria diagnosis (95% CI: [1.04, 2.49]). Likewise, given having *Chills* or not having *diarrhea*, *cough* and *anemia* statuses, individuals who had fever during malaria diagnosis had 1.55 times (95% CI: [1.03, 2.32]), 1.41 times (95% CI: [1.02, 1.94]), 1.55 times (95% CI: [1.08, 2.23]) and 1.43 times (95% CI: [1.04, 1.97]) the odds of having higher parasite density than individuals who did not have fever during malaria diagnosis, respectively. (Tables 4.9 and 4.10)

Among individuals with without measured fever during malaria diagnosis, we found having *fever at night* was protective against having parasite density above median (OR = 0.61, 95% CI: [0.39, 0.96]). On the contrary, not having *fever with sweating* lower the odds of having parasite density above median among

individuals without measured fever status (OR = 0.59, 95% CI: [0.35, 0.96]). Meanwhile, all interactions between individual self-reported symptom and fever status were not statistically significant. (Tables 4.9 and 4.10)

If we looked at *symptom duration* reported by study participants, we found percentage of *parasite density above median* hovered between 48% and 59% with 5 or fewer days of self-reported symptoms. When more than 6 days of preexisting symptoms were reported, percentage of *parasite density above median* dropped down to lower than 30% (Table 4.4). When analyzed by logistic regressions, we found individuals with 6 days of self-reported preexisting symptoms had 62% less odds in having higher level of parasite density than individuals with 3 days of self-reported preexisting symptoms. Similarly, having 7 and 8+ days of self-reported preexisting symptoms provided 57% and 64% reduction in odds of having higher level of parasite density comparing to individuals with 3 days of self-reported preexisting symptoms. Individuals with other symptom duration did not differ by level of parasite density. (Table 4.4)

Last but not least, when comparing the joint effect of symptom duration and measured fever status on levels of parasite density, we did not find having fever was significantly associated with elevated odds of having high level of parasite density with the adjustment of symptom duration (Odds Ratio = 1.70, 95% CI: [0.91, 3.23]). With controlled fever status, participants with 2 days of prior symptoms had 2.11 times the odds of having parasite density above median than individuals with 3 days of prior symptoms (95% CI: [1.08, 4.19]). (Table 4.8)

4.5 Discussion

Bandarban Study Area is considered as a low transmission, hypoendemic malaria zone. Prior to the 4-year study, a baseline malaria survey in 2007 showed *Plasmodium falciparum* (*Pf*) malaria prevalence of 10.97% in the entire District of Bandarban [66]. With the efforts in malaria control and prevention, prevalence of *Pf* malaria has gone down in the area [101]. Our study site, located in northern Bandarban District, has the *Pf* malaria prevalence of 1-2% (Data not shown). This result was documented by the active surveillance system of the Mapping Malaria Epidemiology Project [87].

During the study, we used a population based surveillance system to capture a full range of participants. In conjunction with the passive surveillance, our study team also carried out active and longitudinal surveillance, as well as other surveys. Therefore, the visibility of our locally hired field workers was high. This reliability of the team and the long term contribution to the community have benefited us with enrollment of the passive surveillance. Home visits were given to all individuals who contacted the study team. It reduced the chance of residents shying away from notifying the team due to inaccessibility to field office or due to other obligations.

Rapid diagnostic tests and microscopy tests were provided to study participants. Rapid diagnostic tests were used as the front line measurement. Treatment was

given to individuals with positive results at home visits. Blood films created for microscopy tests were examined by a microscopist at the field laboratory. The microscopy tests served as a confirmation. Individuals with positive microscopy results—but without being detected by rapid diagnostic tests—were also treated. The dual testing mechanism was the strength of the study. Having trained field workers administer rapid diagnostic tests also facilitated the diagnostic and treatment process. The ability to established parasite density among *Pf* positive participants was attributable to our experienced microscopist. With limited resources, however, only one microscopist was stationed at the field laboratory. Cross-examination of parasite density was not feasible.

Parasite density provided by the microscopist had shown a skewed distribution (Figure 4.1(a)). By skewing to the right, it indicated majority of participants had lower parasite density in their blood. In fact, fifty percent of the study participants had a parasite density of 5,400 parasites/μl or less. We chose to transform participants' parasite density by taking natural logarithm of all values (Figure 4.1(b)). The transformed distribution was closer to Gaussian distribution. These transformed values of parasite density showed a crude bimodal distribution. The two modes of transformed parasite density could be separated by natural log of median parasite density (i.e. $log(5,400 parasites/\mu l)$), as indicated by the red line shown on Figure 4.1.

We chose to create a dichotomous variable to represent the level of parasite density each malaria-tested individual had. Despite blood slides for measuring parasite density having been taken at the same time as the measurements for

body temperature, symptom status and symptom duration, we recognized parasite density could not have been at a steady state. Given the potential change in actual values of parasite density, we assumed the rank of parasite density each individual had remained approximately stationary. Therefore, we used median parasite density as a cutoff for the new dependent variable, "*level of parasite density*", in logistic regressions to preserve a rough rank (i.e. above/below median) of parasite density and to acknowledge the bimodal distribution of the natural logarithm of parasite density.

One limitation of the study was the inability to calculate parasite clearance rate and its association with self-reported symptoms. As body clears out malaria parasites over time, it would had been ideal to measure parasite density at multiple time points (e.g. from the onset of the disease to every hour thereafter). The study team had followed up malaria positive individuals on Day 2, Day 7 and Day 28. Parasite density was also measured during these time points. However, as treatment was given to malaria positive individuals on Day 0, parasite density was influenced by medications at follow-ups. Our focus was to examine the relationship between levels of parasite density and malaria-related symptoms—without the influence of treatment and medication. Therefore, information collected at follow-ups was not incorporated.

Although study team conducted home visits as soon as they were contacted by residents at Bandarban Study Area, there were still time gaps between onset of self-reported symptoms and collection of blood films. As shown in Table 4.4, majority of participants contacted our study team within 3 days of feeling ill.

Not all individuals contacted the study team immediately. It means parasite density could fluctuate before a home visit was done. Therefore, the magnitude of association between levels of parasite density and each self-reported symptom could change over time. Accuracy of this association could benefit from having a reduced time gap between onset of disease and self reported symptoms. It would also benefit from frequent measurements of parasite density and symptoms prior to treatment administration at a pre-determined time frame (e.g. hourly measurement up to 24 hours post disease onset).

In this study, we defined fever status based on measured body temperature. The advantage of using measured temperature is the objectiveness in defining fever. We used 37.5°C (oral temperature) and 37.2°C (axillary temperature) as our cutoffs for fever. However, there could be a hidden threat. Intermittent fever was reported by 38% of all tested participants (N = 234 out of 616). This indicated the possibility of seeing non-fever individuals with *P. falciparum* during home visit. In fact, 271 (51%) of *P. falciparum* positive individuals had fever during home visits; 87 $Pf(+)$ study participants (16%) reported having intermittent fever but had shown normal body temperature during home visits on Day 0. Although logistics regressions as shown in Table 4.2 indicated having fever had significantly increased the odds of having higher level of parasite density level (95% CI:[1.04, 1.95]), using fever as a sole standard to estimate the odds of having malaria should be executed with caution.

Since the mid-1980s, there was a trend in using parasite presence in human blood as a measure to differentiate malaria infected from non-infected cases

among febrile individuals [97, 102]. Pyrogenic threshold, defined by the odds of fever as a function of parasite density, was continuously discussed until the 2000s. One form of pyrogenic threshold is shown below.

$$logit(\gamma_i) = \alpha + \beta I(p_i > \tau)$$

where γ_i indicated whether fever was observed, p_i was log parasite density, τ was the threshold, I was an indicator variable, α and β are location and scale parameters [95].

Some studies questioned the ability to distinguish fever in malaria from other causes in areas with more than 20% of malaria prevalence [103]. It was likely due to the ability to acquire immunity against malaria at an younger age, as well as the common presence of asymptomatic individuals in this type of population. Although timely diagnosis and treatment are crucial in preventing the progression in malaria condition, overestimating the percentage of febrile patients whose fever can be attributed to malaria could induce antimalarial drug resistance and delay treatment to other febrile diseases (e.g. bacterial infections).

The era of calculating pyrogenic threshold faded with the introduction of rapid diagnostic tests (RDTs). The accessibility to RDTs and its minimal requirements in expertise training and in laboratory equipment had change the dynamics in malaria diagnosis. Although parasite density is no longer a main indicator for having fever, we have learned a few things from previous studies. First, the accuracy of predicting febrile malaria with parasite density is

dependent upon malaria endemicity. There is not a clear cutoff across studies to determine the parasite density level for estimating pyrogenic threshold [96]. Treating all fever cases as malaria cases unnecessarily increase the disease burden of malaria. Second, higher parasite density is associated with increased risk of fever. Lower parasite density ($< 2{,}000$ parasites/μl) is not associated with the occurrence of fever [104]. Many asymptomatic malaria cases had parasite count fall within this range. Third, asymptomatic cases and intervention methods, such as artemisinin-based combination therapy (ACT) and long-lasting insecticide treated nets (LLIN), could lower the pyrogenic threshold [97]. Fourth, parasite density is higher among younger individuals [105]. Body temperature of malaria infected individual's could be lower as the age goes up.

In regard to levels of parasite density among *Plasmodium falciparum* positive participants, our study results have shown the same conclusion in age and measured fever status as previous findings. We looked into level of parasite density by 10-year age groups and found, on average, older individuals had lower odds of having high level of parasite density compared to younger groups. Long term exposure to hypoedemic malaria environment and its immunity built in elder participants could have made them less susceptible to severe malaria. However, the change in odds were not significant among individuals younger than 30 years old. The protective effect of age was only apparent when comparing individuals who were 30 and above to children under 10.

By using 18.5 and 25 as Body Mass Index (BMI) cutoffs for underweight, normal and overweight, we found more than 60% of study participants were underweight

on days of home visits. Less than 2% of participants were overweight. We have found odds of having high level of parasite density was the lowest among individuals with normal BMI (Table 4.1). Underweight individuals had higher odds of having high level of parasite density; overweight individuals had the highest odds of having high level of parasite density. Due to a small sample size in the overweight group, this finding should be taken with grain of salt. Although insignificant, we cannot overlook potential nutritional impact on the association between BMI and parasite density.

Prior studies have shown malaria had negative impact on children's growth. Limited information was provided among adolescent or elder adults on this matter. One study found negative association between BMI and parasite density in young adults; however, it was not statistically significant [106]. A population based study on nutritional status, BMI and parasite density in Bandarban is worth looking into.

Earlier, we found individuals with fever (Oral temperature $\geq 37.5°C$; axillary temperature $\geq 37.2°C$) during home visits had significantly higher odds in having *parasite density above median* than those without fever (Table 4.2). We then analyzed this association with the addition of self-reported symptoms and symptom duration.

We found with symptom itself, having self-reported *fever at night* significantly lowered the odds of having high level of parasite density comparing to those

who didn't have the symptom (Table 4.5). Other self-reported symptoms, including *fever with shivering*, *fever at day time*, *fever with sweating*, *intermittent fever*, *remission of fever with sweating*, *headache*, *chills*, *nausea*, *vomiting*, *diarrhea*, *cough*, *fatigue*, *muscle ache*, *muscle weakness*, *convulsions/seizure* and *anemia*, did not affect the odds of having high level of parasite density (Tables 4.6 and 4.7). The protective effect of having self-reported *fever at night* persisted among individuals without measured fever at diagnosis. On the other hand, having self-reported *fever with sweating* exacerbated the odds of having *parasite density above median* among individuals without measured fever at diagnosis (Tables 4.9 and 4.10).

Among individuals with referenced symptom status, on the contrary, we found individuals who had fever during home visits had higher odds of having *parasite density above median* than individuals without fever. These symptoms included *fever at day time*, *chills*, *diarrhea*, *cough*, and *anemia* (Tables 4.9 and 4.10).

Similarly, the odds of having high level of parasite density significantly went down as self-reported symptom duration surpassed 6+ days (Table 4.4). However, the changed in odds by comparing having 6 or more days of prior symptoms and having 3 days of prior symptoms was not significant after controlling for participants' fever status. Given the same symptom duration, level of parasite density was not affected by measured fever status. The only exception to this multivariable logistic relationship between fever status, symptom duration and level of parasite density was the comparison between the odds of having *high level of parasite density* among tested individuals with 2 or 3 days of prior

symptoms, with the adjustment of their fever statuses.

In this paper, we compared odds of having high *level of parasite density* (i.e. odds of having *parasite density above median*) across all tested participants. By focusing on all tested participants, we learned the likelihood of having elevated parasite given certain demographic characteristics, fever status and symptoms in the general population.

As tested participants were recruited through passive surveillance, they were not a random sample of the study population. Therefore, incidence derived from the study would be considered as *reported incidence*. By recruiting a random sample of the study population for malaria testing, their *Plasmodium falciparum* density could better represent the overall residents. However, with the current 1-2% of malaria prevalence in Bandarban Study Area, sample size required to acquire informative results on association between levels of parasite density and various demographic characteristics and symptoms would be high. Hence, passive surveillance was chosen for this particular study.

With passive surveillance, tested participants had to be aware of the presence of malaria and inform the study team to be examined. In addition, if a local resident tended to seek help with presence of certain symptoms (e.g. seizures, diarrhea), the likelihood of this individual contacted the study team would be higher. This was a threat of the study design.

The advantage of using self-reported symptoms in this study was its self-perceptiveness.

If certain self-reported symptoms are incorporated as additional indicators for broadening case search or conducting reactive surveillance (as described in the following paragraphs), we will acquire reports from residents on whether they have certain symptoms. If the keywords used for self-reported symptoms remain the same (e.g. chills, headache, etc.) between this study and future surveillance efforts, we could assume the perceived understanding of those symptoms would likely align with each other. Therefore, the findings from this study could be more applicable in future endeavor.

The disadvantage of using self-reported symptoms in this study was its self-perceptiveness as well. As symptoms were self-reported, they were not based on clinical diagnoses. Hence, whether self-reported symptoms were perceived by the study team the same way the local residents were was unclear to the authors. Moreover, more than one languages were used by local residents in Bandarban Study Area (e.g. English, Bengali and tribal languages such as Marma). The preciseness of each symptom may or may not be fully translated or represented in another language.

To accommodate this caveat, our questionnaires were written in English and Bengali. Our field workers were locally hired and were tested on their understanding of Bengali. In addition to Bengali, each field worker also speaks Marma. Marma is the biggest tribal group in Bandarban Study Area. Their language "Marma" is the common language used to communicate across tribes. To understand how well study participants understand malaria symptoms and

how likely they would seek medical treatment with presence of certain self perceived symptoms, another study should be conducted. The findings of their knowledge, attitude and practice toward malaria would be a logical next step following this paper.

In resource poor settings, microscopy examination for malaria were often not readily accessible. The requirement for laboratory equipment and having an experienced microscopist sets a high bar for its accessibility. The introduction of rapid diagnostic tests lowered the bar for malaria diagnosis. However, rapid diagnostics tests only provide yes-no results to tested individuals. They do not offer a detailed parasite count like microscopy does. This paper studied the link between clinical symptoms and level of parasite density to help provide additional insights on broadening case awareness and care detection when rapid diagnostic tests are the only tool for malaria diagnosis.

Case Awareness

Symptoms for malaria are not disease specific. Recognizing the wide spectrum of malaria-related symptoms is important in low malaria transmission area. It is crucial for local residents not to overlook its health impact. This paper studied level of *Plasmodium falciparum* density given different demographics and malaria related symptoms. With provided demographic factors, symptoms and symptom duration, likelihood of having high level of parasite density could be calculated.

To help Bandarban Study Area achieve malaria elimination phase, we not only have to broaden malaria surveillance but also emphasize on health education. To broaden malaria surveillance, relying on local residents to be aware of clinical malaria symptoms is one way of approaching it. With awareness of malaria infection, residents should be encouraged to find facilities with rapid diagnostic tests for examination. Health education, on the other hand, could incorporate how demographic factors, malaria symptoms and symptom duration are associated with the likelihood of having higher level of parasite density.

Case Detection

It is not financially viable to examine every person in an area for malaria. This is especially true for resource scarce areas. How to find higher risk groups has become a quest for effective case detection. With passive surveillance, we tested subjects that contacted the study team. With home visits, inaccessibility to field office for malaria diagnosis was minimized. However, there were asymptomatic cases and under reported cases that we potentially missed. The ability to identify those individuals is essential to move toward malaria elimination phase.

Previous studies had used methods such as reactive surveillance. That is, to test all family members once a positive case was detected [107]. Testing all family members, no doubt, broadens the case search. However, in a population dense area such as Bangladesh, simply testing family members of a positive case might not be sufficient. As neighbors also share similar living environment (e.g. similar landscape with mosquito reservoirs, similar household building materials,

etc.), we cannot neglect the possibility of having symptomatic or asymptomatic malaria cases hidden in the neighborhood.

Which raises a question: How wide should the search be for reactive case detection once we found a malaria positive case? In addition, by testing family members of a malaria positive case, we assumed most family members spend majority of the day together. As mosquitoes do not recognize family units and are not limited to one location, mosquito bites and malaria infection could have happened outside of the family circle.

Theoretically, it would be helpful to test everyone who commonly go to the same living spaces as the malaria positive case (e.g. market, rice field, etc.). Practically, it would be impossible to efficiently trace everyone's whereabouts on a large scale in a resource poor setting. Therefore, this paper touched upon an idea for broadening the search for malaria case detection.

Hot Spot Analysis and Reactive Surveillance

To eliminate malaria in Bandarban Study Area, it's beneficial to combine the efforts of *case awareness* and *case detection*. Hot spot analysis and reactive surveillance are means to integrate the efforts.

Since 2008, malaria diagnosis is free of charge in public sectors in Bangladesh. If free diagnosis service could be extended to all medical related facilities (e.g.

clinics, drug stores) and designated market spaces and schools in the 13 endemic districts, these sentinel surveillance station could be very accessible to local residents. With examination on association between levels of *Plasmodium falciparum* density and risk factors (e.g. demographic factors, fever status, self-reported symptoms, symptom duration), we could create hot spots based on the results.

For instance, we can start by creating a density map across neighborhoods based on measured parasite density (Figure 4.2) ("*case detection*"). We contact sentinel stations located in neighborhoods with higher parasite density. Sentinel surveillance stations then notify local residents with certain demographic characters or malaria related symptoms to be examined by rapid diagnosis tests ("*case awareness*"). Meanwhile, regular passive surveillance is ongoing at each sentinel surveillance station.

Another example would be to start by creating a density map based on numbers of febrile cases in the neighborhoods. Numbers of febrile cases could be reported through sentinel stations ("*case awareness*"). This density map can be updated weekly or biweekly. Once a malaria positive person is diagnosed, not only could the person's family or co-workers be tested through a reactive surveillance system, nearby neighborhoods with higher density of febrile cases could also be swept and tested for malaria ("*case detection*").

With additional funding, home visits by community health workers could be arranged based on daily sentinel reports. Surveys on symptom related risk factors

and blood films for microscopy would be collected at each home visit. If mobile devices are incorporated in conducting surveys, survey results can be saved to a database immediately. Microscopy can be examined by trained microscopists at field laboratories or on mobile stations (e.g. trucks). With daily reports collected from sentinel stations and home visits, not only can malaria outbreak be detected in a timely manner, but also *Plasmodium falciparum* density maps and risk factors can be updated regularly. Updated density maps and symptom-related risk factors could further facilitate case awareness and case detection on a local level.

In sum, we used passive surveillance to study 616 malaria tested individuals in Bandarban Study Area in southeastern Bangladesh. We examined their malaria status and *Plasmodium falciparum* density. We also collected their basic demographic information, body temperature, self-reported symptoms and symptom duration. This paper looked at symptom-related risk factors in association with levels of *Plasmodium falciparum* parasite density. Under the overarching goal of malaria elimination in Bandarban, the main goal of this paper was to help broaden the malaria search in Bandarban study area. We approached this goal by finding symptoms that were associated with relatively severe malaria infected individuals. This study also provided a means to connect reactive case detection and hot spot analysis.

Symptomatic and asymptomatic malaria cases could be identified more thoroughly by improving case awareness and case detection in the study area via a streamlined reactive case detection and hot spot analysis. This is especially

true in areas with low prevalence of symptomatic cases and high prevalence of asymptomatic cases. With this study, challenges remain in the specificity of malaria symptoms, validity of self-reported symptoms, and the dynamic change in parasite density in malaria infected residents.

Table 4.1: Individual Level Single variable$^{\alpha}$ and Multivariable$^{\beta}$ Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Gender, Age and Body Mass Index (BMI)

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Gender								
Female	274	148	126	0.82	1.18	0.60	1.13	0.228
Male	342	168	174	Ref	—	—	—	—
Age Group (year)								
$[0, 10)$	187	86	101	Ref	—	—	—	—
$[10, 20)$	164	76	88	0.99	1.24	0.65	1.50	0.947
$[20, 30)$	80	41	39	0.81	1.31	0.48	1.37	0.431
$[30, 40)$	87	53	34	0.55	1.30	0.32	0.91	0.022
$[40, 50)$	46	30	16	0.45	1.41	0.23	0.88	0.021
$[50, 60)$	34	15	19	1.08	1.46	0.52	2.28	0.840
$[60, 90)$	18	15	3	0.17	1.91	0.04	0.54	0.006
BMI Category								
$[0, 18.5)$	412	210	202	Ref	—	—	—	—
$[18.5, 25)$	174	94	80	0.88	1.20	0.62	1.26	0.499
$[25, 100)$	12	4	8	2.08	1.86	0.64	7.89	0.238

$^{\alpha}$ Single Variable Logistic Regression was used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Gender*: $logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Gender}$

$^{\beta}$ Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Age, BMI*: $logit(Pr(\text{High Density Level})) = \beta_0 + \sum_{i=1}^{i=K-1} \beta_i * (\text{Dummy Variables of a Categorical Variable})$

Table 4.2: Individual Level Single variable[α] and Multivariable[β] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Measured Fever Status and Number of Self-Reported Symptoms

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Fever Status [α]								
Yes	307	144	163	1.42	1.18	1.04	1.95	0.030
No	309	172	137	Ref	—	—	—	—
Number of Symptoms [β] [γ]								
2	3	2	1	0.53	3.45	0.02	5.68	0.610
3	11	7	4	0.61	1.92	0.15	2.11	0.444
4	25	15	10	0.71	1.56	0.29	1.68	0.439
5	73	38	35	0.98	1.34	0.55	1.74	0.944
6	130	67	63	Ref	—	—	—	—
7	108	57	51	0.95	1.30	0.57	1.59	0.849
8	102	46	56	1.29	1.30	0.77	2.18	0.330
9	59	28	31	1.18	1.37	0.64	2.19	0.603
10	48	25	23	0.98	1.40	0.50	1.90	0.949
11	19	8	11	1.46	1.64	0.56	4.00	0.444
12	22	12	10	0.89	1.59	0.35	2.20	0.794
13	8	7	1	0.15	2.95	0.01	0.89	0.082
14	8	4	4	1.06	2.07	0.24	4.67	0.933

[α] Single Variable Logistic Regression was used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Fever*: $logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Fever}$

[β] Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Number of Symptoms*: $logit(Pr(\text{High Density Level})) = \beta_0 + \sum_{i=1}^{i=K-1} \beta_i * (\text{Dummy Variables of "Number of Symptoms"})$

[γ] Symptoms: (1) Fever with Shivering, (2) Fever at Day Time, (3) Fever at Night, (4) Fever with Sweating, (5) Intermittent Fever, (6) Remission of Fever with Sweating, (7) Headache, (8) Chills, (9) Nausea, (10) Vomiting, (11) Diarrhea, (12) Cough, (13) Fatigue, (14) Muscle ache, (15) Muscle weakness, (16) Convulsions / Seizure and (17) Anemia

Table 4.3: Individual Level Multivariable[α] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Number of Self-Reported Fever and Non-Fever Related Symptoms

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Number of Fever Related Symptoms [β]								
0	6	5	1	0.21	3.02	0.01	1.35	0.161
1	82	44	38	0.92	1.30	0.55	1.53	0.748
2	221	114	107	Ref	—	—	—	—
3	171	78	93	1.27	1.23	0.85	1.90	0.241
4	59	31	28	0.96	1.34	0.54	1.71	0.896
5	36	20	16	0.85	1.44	0.41	1.73	0.658
6	41	24	17	0.75	1.41	0.38	1.47	0.414
Number of Non-Fever Related Symptoms [γ]								
0	2	0	2	(Did Not Converge)				
1	5	3	2	0.82	2.53	0.11	5.09	0.832
2	24	14	10	0.88	1.56	0.36	2.08	0.774
3	100	54	46	1.05	1.29	0.64	1.73	0.848
4	163	90	73	Ref	—	—	—	—
5	153	73	80	1.35	1.25	0.87	2.11	0.183
6	110	50	60	1.48	1.28	0.91	2.41	0.114
7	46	24	22	1.13	1.40	0.58	2.18	0.715
8	12	7	5	0.88	1.83	0.25	2.87	0.834
9	1	1	0	(Did Not Converge)				

[α] Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Number of Symptoms*: $logit(Pr(\text{High Density Level})) = \beta_0 + \sum_{i=1}^{i=K-1} \beta_i * (\text{Dummy Variables of "Number of Symptoms"})$

[β] Fever related symptoms: (1) Fever with Shivering, (2) Fever at Day Time, (3) Fever at Night, (4) Fever with Sweating, (5) Intermittent Fever, and (6) Remission of Fever with Sweating

[γ] Non-fever related symptoms: (7) Headache, (8) Chills, (9) Nausea, (10) Vomiting, (11) Diarrhea, (12) Cough, (13) Fatigue, (14) Muscle ache, (15) Muscle weakness, (16) Convulsions / Seizure and (17) Anemia.

Table 4.4: Individual Level Multivariable$^{\alpha}$ Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Self-Reported Symptom Duration

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Symptom Duration								
1 Day	51	25	26	1.11	1.38	0.59	2.09	0.750
2 Days	145	59	86	1.55	1.26	0.99	2.46	0.058
3 Days	157	81	76	Ref	—	—	—	—
4 Days	110	55	55	1.07	1.28	0.65	1.74	0.798
5 Days	64	31	33	1.13	1.35	0.63	2.04	0.671
6 Days	23	17	6	0.38	1.65	0.13	0.96	0.051
7 Days	38	27	11	0.43	1.48	0.19	0.91	0.033
8+ Days	28	21	7	0.36	1.59	0.13	0.85	0.026

$^{\alpha}$ Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Symptom Duration*: $logit(Pr(\text{High Density Level})) = \beta_0 + \sum_{i=1}^{i=K-1} \beta_i * (\text{Dummy Variables of Symptom Duration})$

Table 4.5: Individual Level Single variable[α] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Self-Reported Symptoms, I

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Individual Symptoms								
Fever with Shivering								
No	315	163	152	Ref	—	—	—	—
Yes	301	153	148	1.04	1.17	0.76	1.42	0.820
Fever at Day Time								
No	329	170	159	Ref	—	—	—	—
Yes	287	146	141	1.03	1.18	0.75	1.42	0.843
Fever at Night								
No	321	149	172	Ref	—	—	—	—
Yes	295	167	128	0.66	1.18	0.48	0.91	0.012
Fever with Sweating								
No	209	117	92	0.75	1.19	0.54	1.05	0.096
Yes	407	199	208	Ref	—	—	—	—
Intermittent Fever								
No	382	197	185	Ref	—	—	—	—
Yes	234	119	115	1.03	1.18	0.74	1.43	0.863
Remission of Fever with Sweating								
No	441	226	215	Ref	—	—	—	—
Yes	175	90	85	0.99	1.20	0.70	1.41	0.968

[α] Single Variable Logistic Regression was used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Symptom*: $logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Symptom}$

Table 4.6: Individual Level Single variable[α] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Self-Reported Symptoms, II

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Individual Symptoms								
Headache								
No	53	32	21	0.67	1.34	0.37	1.18	0.169
Yes	563	284	279	Ref	—	—	—	—
Chills								
No	234	110	124	1.32	1.18	0.95	1.83	0.096
Yes	382	206	176	Ref	—	—	—	—
Nausea								
No	237	127	110	0.86	1.18	0.62	1.19	0.369
Yes	379	189	190	Ref	—	—	—	—
Vomiting								
No	334	179	155	Ref	—	—	—	—
Yes	282	137	145	1.22	1.18	0.89	1.68	0.215
Diarrhea								
No	610	313	297	Ref	—	—	—	—
Yes	6	3	3	1.05	2.27	0.19	5.73	0.949
Cough								
No	476	247	229	Ref	—	—	—	—
Yes	140	69	71	1.11	1.21	0.76	1.62	0.588

[α] Single Variable Logistic Regression was used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Symptom*: $logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Symptom}$

Table 4.7: Individual Level Single variable[α] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Self-Reported Symptoms, III

Factor	**Density Level Sample Size**			**All (N = 616) Logistic Regression**				
Variable	All	Low	High	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Individual Symptoms								
Fatigue								
No	357	175	182	Ref	—	—	—	—
Yes	259	141	118	0.80	1.18	0.58	1.11	0.184
Muscle Ache								
No	178	96	82	0.86	1.20	0.61	1.22	0.405
Yes	438	220	218	Ref	—	—	—	—
Muscle Weakness								
No	220	119	101	0.84	1.18	0.60	1.17	0.302
Yes	396	197	199	Ref	—	—	—	—
Convulsions, Seizure								
No	614	315	299	Ref	—	—	—	—
Yes	2	1	1	1.05	4.12	0.04	26.72	0.971
Anemia								
No	606	312	294	Ref	—	—	—	—
Yes	10	4	6	1.59	1.92	0.45	6.28	0.475

[α] Single Variable Logistic Regression was used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Symptom*: $logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Symptom}$

Table 4.8: Individual Level Multivariable$^{\alpha}$ Logistic Regression: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Measured Fever Status and Self-Reported Symptom Duration

Factor	**All (N = 616) Logistic Regression**				
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Fever Status (F:Yes/No) and Symptom Duration (D:Days)					
F: Yes	1.70	1.38	0.91	3.23	0.099
F: No	Ref	—	—	—	—
D: 1 Day	0.32	1.97	0.07	1.07	0.090
D: 2 Days	2.11	1.41	1.08	4.19	0.029
D: 3 Days	Ref	—	—	—	—
D: 4 Days	1.28	1.41	0.65	2.52	0.473
D: 5 Days	1.74	1.51	0.78	3.93	0.179
D: 6 Days	0.37	1.99	0.08	1.30	0.153
D: 7 Days	0.48	1.64	0.17	1.22	0.136
D: 8+ Days	0.62	1.79	0.18	1.88	0.418
F: Yes and D: 1 Day	4.87	2.23	1.10	27.19	0.048
F: Yes and D: 2 Days	0.53	1.60	0.21	1.34	0.181
F: Yes and D: 4 Days	0.68	1.65	0.25	1.81	0.439
F: Yes and D: 5 Days	0.41	1.82	0.12	1.30	0.131
F: Yes and D: 6 Days	1.08	2.76	0.14	8.34	0.942
F: Yes and D: 7 Days	0.96	2.29	0.18	4.87	0.959
F: Yes and D: 8+ Days	0.26	2.71	0.03	1.69	0.175

$^{\alpha}$ Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Fever, Symptom Duration*:
$logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Fever}$
$+ \sum_{i=2}^{i=8} \beta_i * (\text{Dummy Variables of Symptom Duration})$
$+ \sum_{i=9}^{i=15} \beta_i * \text{Fever} * (\text{Dummy Variables of Symptom Duration})$

Table 4.9: Individual Level Multivariable[α] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Measured Fever Status and Self-Reported Symptoms, I

Factor	**All (N = 616)**				
	Logistic Regression				
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Fever Status and Individual Symptoms					
Fever (Ref: No)	1.51	1.26	0.97	2.36	0.072
Fever with Shivering (Ref: No)	1.06	1.26	0.68	1.67	0.791
Interaction	0.89	1.38	0.47	1.68	0.710
Fever (Ref: No)	1.61	1.25	1.04	2.49	0.033
Fever at Day Time (Ref: No)	1.17	1.26	0.74	1.83	0.501
Interaction	0.77	1.38	0.41	1.45	0.419
Fever (Ref: No)	1.24	1.25	0.80	1.93	0.342
Fever at Night (Ref: No)	0.61	1.26	0.39	0.96	0.034
Interaction	1.24	1.39	0.66	2.36	0.505
Fever (Ref: No)	1.26	1.22	0.85	1.86	0.245
Fever with Sweating (Ref: Yes)	0.59	1.29	0.35	0.96	0.035
Interaction	1.53	1.42	0.77	3.03	0.223
Fever (Ref: No)	1.33	1.23	0.89	2.00	0.162
Intermittent Fever (Ref: No)	0.92	1.27	0.57	1.47	0.729
Interaction	1.18	1.40	0.61	2.29	0.615
Fever (Ref: No)	1.39	1.21	0.96	2.03	0.083
Remission of Fever with Sweating (Ref: No)	0.92	1.30	0.55	1.55	0.763
Interaction	1.08	1.44	0.53	2.20	0.829

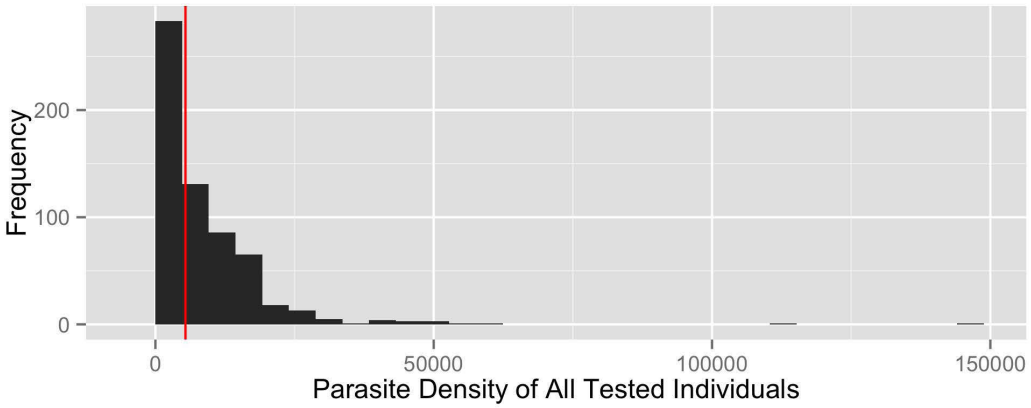
[α] Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Fever, self-reported Symptom*: $logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Fever} + \beta_2 * \text{Symptom} + \beta_3 * \text{Fever} * \text{Symptom}$

Table 4.10: Individual Level Multivariable[α] Logistic Regressions: Level of *Plasmodium falciparum* Parasite Density on Malaria Tested Participants' Measured Fever Status and Self-Reported Symptoms, II

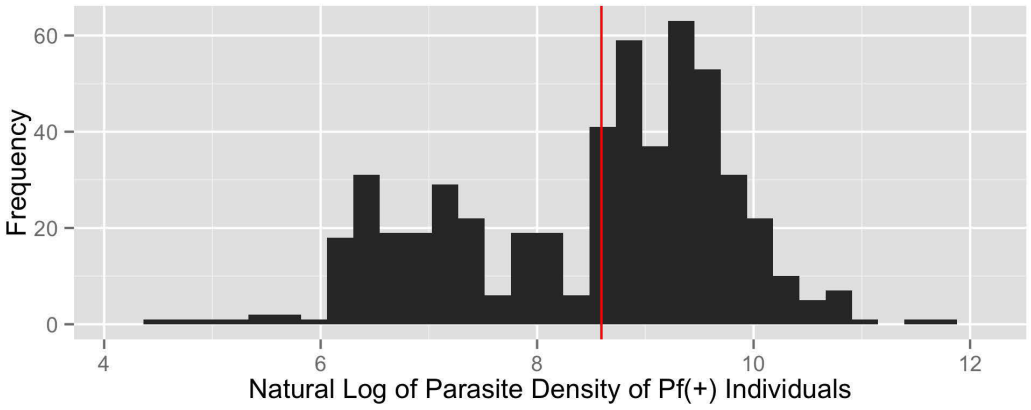
	All (N = 616)				
Factor	**Logistic Regression**				
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Fever (Ref: No)	1.36	1.18	0.98	1.89	0.070
Headache (Ref: Yes)	0.53	1.52	0.22	1.18	0.135
Interaction	1.64	1.82	0.51	5.41	0.410
Fever (Ref: No)	1.55	1.23	1.03	2.32	0.035
Chills (Ref: Yes)	1.48	1.27	0.93	2.36	0.102
Interaction	0.79	1.40	0.41	1.52	0.476
Fever (Ref: No)	1.25	1.23	0.83	1.87	0.281
Nausea (Ref: Yes)	0.72	1.27	0.45	1.14	0.166
Interaction	1.41	1.40	0.73	2.73	0.301
Fever (Ref: No)	1.24	1.25	0.81	1.92	0.326
Vomiting (Ref: No)	1.04	1.26	0.66	1.64	0.872
Interaction	1.30	1.39	0.68	2.47	0.424
Fever (Ref: No)	1.41	1.18	1.02	1.94	0.036
Diarrhea (Ref: No)	0.63	3.42	0.03	6.59	0.702
Interaction	2.84	5.70	0.11	149.49	0.548
Fever (Ref: No)	1.55	1.20	1.08	2.23	0.017
Cough (Ref: No)	1.34	1.31	0.79	2.30	0.279
Interaction	0.68	1.47	0.32	1.45	0.322
Fever (Ref: No)	1.23	1.24	0.81	1.87	0.325
Fatigue (Ref: No)	0.71	1.26	0.45	1.11	0.135
Interaction	1.36	1.39	0.71	2.61	0.347
Fever (Ref: No)	1.39	1.21	0.96	2.03	0.086
Muscle Ache (Ref: Yes)	0.83	1.29	0.50	1.36	0.465
Interaction	1.08	1.43	0.54	2.18	0.833
Fever (Ref: No)	1.35	1.22	0.91	2.01	0.132
Muscle Weakness (Ref: Yes)	0.79	1.27	0.49	1.26	0.330
Interaction	1.14	1.40	0.59	2.21	0.704
Fever (Ref: No)	1.44	1.18	1.05	1.98	0.024
Convulsions, Seizure (Ref: No)	(Did Not Converge)				
Interaction	(Did Not Converge)				
Fever (Ref: No)	1.43	1.18	1.04	1.97	0.029
Anemia (Ref: No)	2.53	3.42	0.24	54.85	0.450
Interaction	0.47	4.27	0.02	7.73	0.600

[α] Multivariable Logistic Regressions were used for analyzing the association between levels of *Plasmodium falciparum* parasite density and *Fever, self-reported Symptom*:
$logit(Pr(\text{High Density Level})) = \beta_0 + \beta_1 * \text{Fever} + \beta_2 * \text{Symptom} + \beta_3 * \text{Fever} * \text{Symptom}$

Figure 4.1: Distribution of *Plasmodium falciparum* Parasite Density

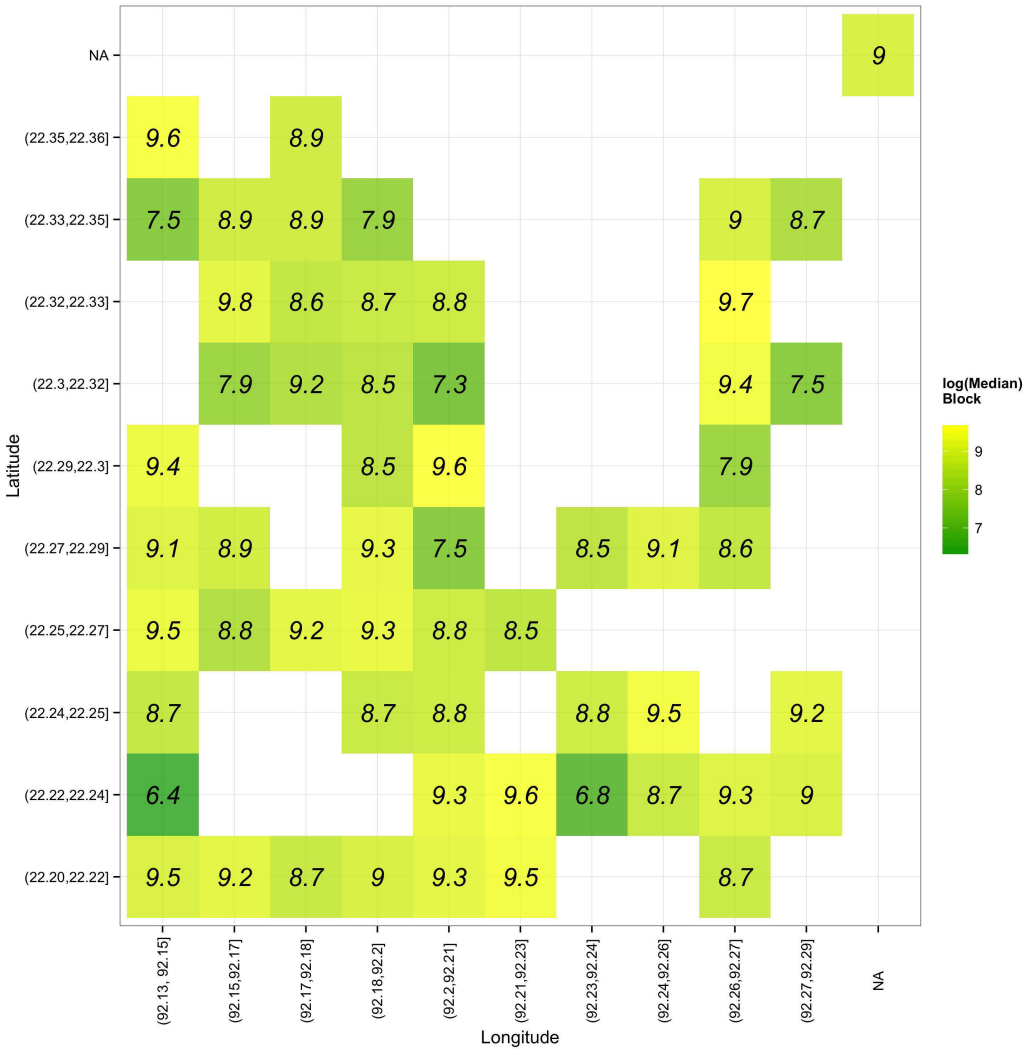


(a) Parasite Density. Red Line: median parasite density



(b) Natural Log of Parasite Density. Red Line: log(median parasite density)

Figure 4.2: Areal Summary of Natural Log of Median *Plasmodium falciparum* Parasite Density of All Malaria Tested Individuals by Their Household Location



Chapter 5

Paper 3: Association between Household Building Materials and Abundance of *Anopheles* Mosquitoes in Rural Bangladesh

5.1 Abstract

5.1.1 Background

Anopheles mosquitoes are key to malaria transmission. Types of household building materials in association with abundance of *Anopheles* mosquitoes has not been fully explored in rural Bangladesh. This paper studied this relationship to better understand the underlying risk of acquiring human malaria among residents lived in houses with various types of building materials.

5.1.2 Methods

The study took place in Bandarban, Bangladesh from 2009 to 2013. One thousand and seventy-nine households (N = 1,079, 21.6%) were sampled from all households in the study area. Information on household building materials and ground elevation status was surveyed through standard questionnaires. Numbers of *Anopheles* mosquitoes at each selected house was collected by standard CDC mosquito light traps. Linear regression and areal maps were analyzed to study individual and combined impact of building materials on average number of *Anopheles* mosquitoes found per night at household level.

5.1.3 Results

Approximately 5 *Anopheles* mosquitoes were found per night per household. "*Bamboo*" was the most commonly used building material for wall (N = 907), partition (N = 867) and floor (N = 450). Meanwhile, "*corrugated tin, iron sheet*" was the most favorable roofing material (N = 749). More than half (N =

574) of the selected households in the study area were built off ground. Linear regression had shown the use of *mud* as a building material (wall: N = 123, 95% CI: [0.31, 1.74]; partition: N = 119, 95% CI: [0.24, 1.70]; floor: N = 420, 95% CI: [0.15, 1.15]), comparing to the use of "*bamboo*", was associated with a higher number of *Anopheles* mosquitoes found at households at night. In addition, houses *with elevated ground floor*" (N = 574) were related to a lower number of *Anopheles* mosquitoes found per night during entomological surveys (95% CI: [-1.03, -0.12]). After adjusting for areal variability, having some "*mud*" or "*bamboo*" as part of building materials did not provide significant difference in numbers of *Anopheles* mosquitoes found at houses.

5.1.4 Discussion

The change in average number of *Anopheles* mosquitoes could seem small when comparing the impact of building materials on numbers of mosquitoes found at households at night. However, this could not be overlooked. With an average of 5 *Anopheles* mosquitoes presented at households at night, a fluctuation of ± 1 to ± 2 *Anopheles* mosquitoes would be equivalent to a 20-40% change in mosquito population. Although having some "*mud*" or "*Bamboo*" as part of building materials did not significantly change the overall *Anopheles* population, species specific preferences among *Anopheles* mosquitoes should be further studied.

5.2 Background

In 2013, 198 million malaria cases and 584,000 deaths were recorded worldwide [48]. In World Health Organization's (WHO) South-East Asia Region, 352 million people were in high risk of acquiring malaria. Approximately 1.4 billion people lived with some risk of malaria [48]. Bangladesh is one of the countries in South-East Asia. To date, it is still listed as a malaria endemic country by WHO [3]. Over the past decade, multiple intervention policies and strategies were implemented. Implementation included (1) malaria diagnostic tests provided for patients at all ages, (2) freely distributed insecticide treated bednets, and (3) free Artemisinin-based Combination Therapy (ACT) available at public sectors. The intervention brought the number of microscopy confirmed cases in Bangladesh down from 58,894 people (505 deaths) in 2004 to 3,249 people (45 deaths) in 2014 [3]. In addition, the at risk population in Bangladesh also dropped from an estimated 60.5 million people in 2010 to 16.5 million people in 2014 [3, 108].

With a much faster decline in malaria cases than in at risk population, the underlying asymptomatic population could have grown much greater. In a hypoendemic country like Bangladesh, we hypothesized symptomatic cases were a tip of an iceberg. To understand malaria burden at present state, it might not be enough to just focus on treatment and prevention methods. Environmental aspects such as household building materials could be distally associated with malaria infection. *Anopheles* mosquitoes that carry *Plasmodium* parasites are key to malaria transmission. *Anopheles* mosquitoes could have preferred

types of resting surface. Resting surfaces, in this case, are household building materials. Understanding the association between abundance of *Anopheles* mosquitoes and household building materials could provide an indirect insight on relationship between *Anopheles* mosquitoes and human malaria. Some studies have reported quality of housing structure and materials used for houses are associated with malaria incidence [109]. However, the link between household building materials and the abundance of *Anopheles* mosquitoes have not been widely analyzed. In Bangladesh, only one prior study examined the relationship between household wall materials and clinical malaria [76]. None has looked at the relationship between household building materials and population dynamics of *Anopheles* mosquitoes. Their relationship in Bangladesh remained unclear.

As part of our 4-year population based malaria surveillance project in southeastern Bangladesh, we explored different types of building materials used at different sections of a house. This included materials used for walls, roofs, partitions and floors. Records of ground elevation status of a house was also included. This is the first paper providing a broader view of building materials and their association with the number of *Anopheles* mosquitoes in a household. We aim to use this study as a stepping stone to better understand under various types of housing facilities, the risk of human malaria in association with population dynamics of *Anopheles* mosquitoes.

5.3 Methods

5.3.1 Study Location

This study was part of the overall Mapping Malaria Epidemiology project in Bangladesh [87]. It was located in Bandarban District, the southern most district of Chittagong Hill Tracts in southeastern Bangladesh. The study site was composed of two adjacent Unions within the District: Kuhalong Union and Rajbila Union. During the study period, the study site housed more than 5,000 households and 22,000 people.

Union was the smallest rural government unit in Bangladesh. In order to more efficiently serve the local residents and conduct surveillance, an administrative unit— called "Cluster"— was created by the study team. Each Cluster was similar in population size. Households located within the same Cluster were close in proximity. Kuhalong Union and Rajbila Union each consisted of 12 Clusters. A total of 24 Clusters were assigned in the study area.

5.3.2 Study Time Frame

Information on household building materials and ground elevation status was recorded from October 2009 to September 2013. Meanwhile, entomological surveillance was carried out from July 2009 to October 2012 in Kuhalong Union, and from May 2010 to October 2012 in Rajbila Union.

5.3.3 Study Population

All 5,006 households in the study area were recruited for household survey. We randomly selected 1,079 out of 5,006 households (21.6%) for entomological surveillance (Kuhalong: N = 584; Rajbila: N = 495).

5.3.4 Data Collection

Among selected households, two types of data were collected: Household data and mosquito data.

In household survey, we used a standardized questionnaire to record building materials and ground elevation status of a house. Building materials included materials used for wall, roof, partition and floor. Ground elevation status indicated whether a household was built off ground. Many households in the study area were built off ground to accommodate the uneven terrain and flooding in monsoon season. Some used the space underneath the elevated ground floor for their domesticated animals. As mosquitoes could potentially rest at the damped and/or shaded surface area beneath the elevated ground floor, household elevation profile was recorded.

Five households were randomly selected for entomological survey from each Cluster at the beginning of each year. Therefore, 60 households were randomly chosen for each Union, and a total of 120 households were selected for entomology survey each year. Once a household was selected for the entomological

surveillance, it was visited once a month by the study team. When the household representative of a selected house was not present at the time of entomological survey, a neighboring house would be selected as an alternative. With informed consent, an entomological survey would be performed at this neighboring house.

We utilized the Light Trap Method introduced by Centers for Disease Prevention and Control (CDC) to collect mosquitoes [87]. During an entomological survey, a light trap was hung at participant's house for a period of 12 hours. It started at 6 to 7 PM and ended at 6 to 7 AM the following morning. Mosquitoes trapped inside a light trap were brought back to the field laboratory for examination. Mosquitoes were killed at the beginning of examination procedure. A trained entomologist, then, separated *Anopheles* and non-*Anopheles* mosquitoes. Species of *Anopheles* mosquitoes were identified and counted. Information on *Anopheles* species and their numbers were immediately recorded in Microsoft Access Database 2007. After documentation, *Anopheles* mosquitoes were preserved separately and sent to our collaborated parasitology laboratory at International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

5.3.5 Statistical Analysis

Inclusion Criteria

The 1,079 households located in Kuhalong Union and Rajbila Union with informed consent for both household survey and entomological surveillance were included.

Exclusion Criteria

CDC light traps could be used either indoor or outdoor. As majority (99.9%) of our light traps were set indoor, we excluded outdoor entomological survey results (N = 5 records, 0.1%). This was to ensure comparability across all records. Among 1,079 surveyed houses, one of them had exclusively outdoor entomological survey records. This household was thus removed from the analysis. We further excluded 15 additional households without household information on building materials (Kuhalong: N = 12; Rajbila: N = 3). This led us to a total of 1,063 out of 1,079 households for the final analysis.

Variables of Interest

***Anopheles* mosquitoes** Some households were visited more than others. To ensure building materials used at each household were equally weighted, we calculated average number of *Anopheles* mosquitoes per visit at a household level. To do so, we first aggregated total number of *Anopheles* mosquitoes collected at each household. Then, we calculated number of times each household was visited. Finally, we divided the two numbers and yielded an average number of *Anopheles* mosquitoes per visit at each household.

$$\begin{aligned}&\text{Average number of } \textit{Anopheles} \text{ mosquitoes at House}_i \\ &= \frac{\text{Total Number of } \textit{Anopheles} \text{ mosquitoes at House}_i}{\text{Total Number of Visits to House}_i}\end{aligned}$$

As light traps were set up at night, we used "average number of *Anopheles*

mosquitoes per visit" and "average number of *Anopheles* mosquitoes per night" interchangeably for easy understanding.

Individual Building Materials Below is a list of materials listed on the questionnaire. It showed a full spectrum of building materials used for wall, roof, partition and floor in the study area. In the analysis, we kept categories of building materials used by 5 or more households. Materials used by fewer than 5 households were combined with "*other*" category.

- *Wall*: (1) Corrugated tin, iron sheet, (2) fired brick, cement, (3) tin, (4) pole and mud, (5) wood, (6) pole and grass, (7) stone, (8) unfired bricks, (9) bamboo, and (10) other
- *Roof*: (1) Straw, thatch, (2) asbestos, (3) pole and grass, (4) pole and mud, (5) bamboo, (6) mud tins, house of tins, (7) corrugated tin, iron sheet, (8) fired brick, cement, (9) concrete, cement, and (10) other
- *Partition*: (1) Jute stick, (2) wood, (3) concrete, cement, (4) mud, (5) tin, (6) bamboo, and (7) other
- *Floor*: (1) Mud, (2) bamboo, (3) semi-cement, (4) vinyl, (5) cement, (6) wood, and (7) other

Ground Elevation Two aspects of ground elevation were recorded: (1) *Ground elevation status*—whether a house was built off ground (Yes/No), and (2) *ground elevation height*—how far up a house was built off ground (recorded in centimeters (cm)).

Common Combination of Building Materials and Ground Elevation Status A house is a multifaceted structure. It is a combination of wall, roof, floor and partition. We first looked at how one type of materials used for a single structure (e.g. wall) was associated with number of *Anopheles* mosquitoes. However, this would not provide a whole picture on how combined housing materials used at all sections of a house was associated with numbers of *Anopheles* mosquitoes. To address this, we created a categorical variable based on popular combination of building materials and ground elevation status in the study area.

- *Common Combination 1*: Wall—Bamboo; Roof—Corrugated Tin, Iron Sheet; Partition—Bamboo; Floor—Bamboo; Elevation—Yes
- *Common Combination 2*: Wall—Bamboo; Roof—Corrugated Tin, Iron Sheet; Partition—Bamboo; Floor—Mud; Elevation—No
- *Common Combination 3*: Wall—Pole and Mud; Roof—Corrugated Tin, Iron Sheet; Partition—Mud; Floor—Mud; Elevation—No
- *Common Combination 4*: Wall—Bamboo; Roof—Straw, Thatch; Partition—Bamboo; Floor—Bamboo; Elevation—Yes
- *Common Combination 5*: Wall—Bamboo; Roof—Corrugated Tin, Iron Sheet; Partition—Bamboo; Floor—Wood; Elevation—Yes
- *Common Combination 6*: Wall—Bamboo; Roof—Straw, Thatch; Partition—Bamboo; Floor—Mud; Elevation—No
- *Other*: Any combination used by less than 5% of households in the study area

Data Description

We calculated distribution of *Anopheles* mosquitoes by year, month and season. Household building materials used for wall, roof, partition and floor were summarized by Union. Top 10 combinations of building materials and ground elevation status were also tabulated. In addition, box plots and scatter plots were presented to compare average number of *Anopheles* mosquitoes per visit at household level. Last but not least, we used areal maps in grids to examine factor-specific spatial variation on average number of *Anopheles* mosquitoes per visit across the study area.

Regression Analysis

We used linear regression to model average number of *Anopheles* mosquitoes per night at household level, as a function of materials used for a section of the house. Similarly, we modeled the relationship between abundance of *Anopheles* mosquitoes and ground elevation status using linear regressions.

The general form of our regression model is shown below.

$$E(\text{Average Number of } \textit{Anopheles} \text{ per Visit}) = \beta_0 + \sum_{k=1}^{K-1} \beta_k * X_k \qquad (5.2)$$

where X_k $(k = 1, 2, ..., K-1)$ are $K-1$ dummy variables of one covariate of interest (i.e. wall, roof, partition, floor, or ground elevation status).

As an example, the model below analyzes the association between wall materials and average number of *Anopheles* mosquitoes found per visit:

$$E(\text{Average Number of Anopheles per Visit}) = \beta_0 + \beta_1 * \text{Fired Brick and Cement} + \beta_2 * \text{Pole and Mud} + \beta_3 * \text{Wood} + \beta_4 * \text{Other}$$

where "Bamboo" is the reference group.

Reference group was selected based on the popularity. The most commonly used material was assigned as reference. Once ground elevation status and building materials at each section of the house were modeled, we analyzed the association between ground elevation height and the abundance of *Anopheles* mosquitoes, and the association between common combination of building materials and the abundance of *Anopheles* mosquitoes.

Linear Regression with Areal Level Adjustment

We wanted to eliminate potential areal influence of *material A* when discussing the association of *Anopheles* and *material B*. Therefore, we incorporated areal level adjustment into linear regression. To achieve this goal, we first divided the study area into a evenly spaced 10-by-10 grid. All households were assigned

to one of the grids based on their geographic coordinates, recorded in latitudes and longitudes. We followed by calculating areal average per grid of *Anopheles* mosquitoes found per night among households with presence of *material A*. This areal average per grid was used as our background information. The difference between average number of *Anopheles* mosquitoes and the background information was the excess (or deficient) number of mosquitoes found per night per household. This excess (or deficient) number of mosquitoes was attributable to materials other than *material A*. We used this difference (i.e. excess (or deficient) number of mosquitoes) as our dependent variable in the linear regression. Independent variables, on the other hand, were building materials contributed by all households without *material A*.

The areal level adjustment was used in two specific scenarios: (1) the effect of *Mud* without the attribution of *Bamboo* at each section of the house, and (2) the effect of *some Mud* without the attribution of *no Mud*. Below is the generalized equation used for scenario 1 and scenario 2.

$$E(\text{Excess (or deficient) Number of } \textit{Anopheles}) = E(\text{Difference}_{mos_{ij} - \overline{mos_j}}) = \beta_0 + \sum_{p=1}^{P-1} \beta_p * X_{ip}$$

where

- mos_{ij} is the average number of *Anopheles* mosquitoes per night at House i of Area j (j = 1,2,...,100);
- $\overline{mos_j}$ is the areal average of *Anopheles* mosquitoes per night per household at Area j, among households with *bamboo* (scenario 1) or *no mud* as part

of the common combination of household building materials (scenario 2); and

- X_{ip} $(p = 1, 2, ..., P-1)$ is one covariate of interest (i.e. wall, roof, partition, floor, or common combination of building materials) at House i represented by $P-1$ dummy variables.

Software

Microsoft Access Database 2007 (Redmond, WA) was used to document and store the entomological surveillance data. R version 3.1.2 [89] was used to clean and perform data analyses.

5.4 Results

5.4.1 Overview

Overall, 1,079 households were surveyed and 4,368 visits were made. Among 1,079 selected households, 584 households were located in Kuhalong Union and 495 households were located in Rajbila Union. Table 5.1 showed the number of unique households surveyed in each Cluster across both Kuhalong and Rajbila Unions. Figures 5.1 to 5.3 represented geographic distribution of surveyed households and frequency of visits each household received during the study period. In sum, 41.15% of households were visited once; 33.27% of households were visited 2-5 times; 15.57% of households were visited 6-10 times; 10.01% of households were visited 11 times or more. For the analysis, we included visits with indoor entomological surveillance data (N = 4,363 visits; 99.9%). From

July 2009 to October 2012, we identified 22,214 *Anopheles* mosquitoes over 4,363 visits. That is, an average of 5.1 *Anopheles* mosquitoes per household visit (Table 5.2). Its Union-specific boxplot can be found in Figure 5.4.

Average number of *Anopheles* mosquitoes found per visit were similar across years, with an average of 4.5 to 5.2 mosquitoes per visit. The month of May had the lowest average number of *Anopheles* mosquitoes per visit (N = 1.9 mosquitoes). Whereas, March (N = 7.8 mosquitoes) and July (N = 7.0 mosquitoes) had the highest average numbers of *Anopheles* mosquitoes per visit. On average, *Anopheles* mosquitoes were most abundant in Monsoon season (Mid-June to Mid-August; N = 6.6 mosquitoes) and were least populated in Winter and Summer (N = 3.8 mosquitoes). (Table 5.2 and Figure 5.5)

In terms of building materials, *Bamboo* (N = 907 households, 84.1%) and *mud* (N = 123 households, 11.4%) were most commonly used wall materials. *Corrugated Tin* (N = 749 households, 69.4%) and *straw* (N = 299 households, 27.7%) were the top roofing materials. *Bamboo* (N = 867 households, 80.4%) and *cement* (N = 119 households, 11.0%) were the most popular partition materials. *Bamboo* (N = 450 households, 41.7%) and *mud* (N = 420 households, 38.9%) were the most used flooring materials (Tables 5.3 and 5.4 and Figures 5.6 and 5.7). Approximately 53% of the selected households (N = 574 households) were built with elevated ground (Table 5.5 and Figure 5.7).

Households built with the same combination of building materials could provide similar environment for *Anopheles* mosquitoes. Therefore, based on household

building materials and ground elevation status, we tabulated overall and Union-specific common combinations used in the study area (Table 5.6 and Table 5.7). Throughout the study area, *bamboo*, *corrugated tin* and *off ground settlement* are the most popular material combination for building a single household (N = 260 households, 24.1%). This was held true in both Kuhalong Union (N = 156 households, 26.7%) and Rajbila Union (N = 104 households, 21.0%).

5.4.2 Linear Regression

Two sets of linear regression were conducted. The first set was run with all surveyed household in the study area (N = 1,063 households). The second set was run with non-extreme data points (N = 1,054 households). Extreme outliers (N = 9 households) were diagnosed by leave-one-out method in linear regression (Table 5.8). Studentized residuals were calculated from modeling the relationship between average number of *Anopheles* mosquitoes per visit at household level and building materials. Households with high studentized residuals (i.e. households with an average of $\geq$ 31 *Anopheles* mosquitoes per visit) were removed form the second set of analyses. Table 5.9 and Table 5.10 compared results from linear regression analyzed with both sets of data.

When households with extreme average numbers of *Anopheles* mosquitoes were removed, we found households using *mud* as part of their building materials attracted a significantly higher number of *Anopheles* mosquitoes than houses built by *bamboo* (Wall— 1.03, 95% CI: [0.31, 1.74]; Roof— 2.93, 95% CI: [0.29, 5.57]; Partition— 0.97, 95% CI: [0.24, 1.70]; Floor— 0.65, 95% CI: [0.15, 1.15]) (Table

5.9). Meanwhile, households with elevated ground floor was associated with significantly lower average number of *Anopheles* mosquitoes per visit than the ones without ground elevation (-0.58; 95% CI: $[-1.03, -0.12]$). With one centimeter increase in ground elevation height, the average of *Anopheles* mosquitoes per visit would decrease by 0.004 *Anopheles* mosquitoes (95 CI%: $[-0.008, 0.001]$) (Table 5.10).

Among common combinations of households building materials, settlements that were built on the ground with *mud* and *corrugated tin* as building materials (i.e. *common combination 3*, N = 95 households) were found to have an extra mosquito per night on average than households that were build off ground and using *bamboo* and *corrugated tin* as building materials (i.e. *common combination 1*, N = 260 households) (N = 1.02 mosquitoes, 95% CI: [0.13, 1.92]). (Table 5.10)

After areal level adjustment, *mud* was found to attract extra 0.5 to 0.6 *Anopheles* mosquitoes per night at different sections of the house (i.e. wall, partition and floor). However, this finding was not statistically significant (Tables 5.11 and 5.12). Without the attribution of *bamboo*, all materials— including *mud*, *cement* and *wood*— were not associated with having excess (or deficient) number of *Anopheles* mosquitoes at houses at night. As *bamboo* was not used as a roofing material, the roofing section was not included in the areal adjusted linear regression analysis.

Common combination 3 was the only combination of building materials without

Bamboo. Hence, the linear regression to examine excess (or deficient) number of *Anopheles* mosquitoes without the attribution of *bamboo* couldn't not be performed. However, its expected mean difference could be calculated. On average, households using *no bamboo* (i.e. *Common Combination 3*) as building materials had an average of 1.53 additional *Anopheles* mosquitoes at houses at night comparing to those using *bamboo* as parts of building materials in the same geographic area (Table 5.12).

Last but not least, if we compared households using *some mud* as part of its combination of building materials to those without using any *mud* in the same geographic region, we found having *some mud* would significantly increased the number of *Anopheles* mosquitoes at houses at night (N = 0.64 mosquitoes, 95 CI%: [0.04, 1.25]) (Table 5.13).

5.5 Discussion

This study was part of an overall population-based malaria surveillance project in southeastern Bangladesh [87]. All households in the study area were enumerated. This baseline information provided us with an advantage in stratified sampling. Equal number of households under each Union and Cluster could, hence, be selected. Housing structures were assumed to remain the same throughout the study. Survey on building materials were only surveyed once. This limitation prohibited us from studying any potential association between temporal change (2009-2013) in housing structure and population dynamics of *Anopheles*

mosquitoes.

Previously, mud was the most studied building material in relation to malaria. Studies on mud mainly took place in Africa. The ones in Asia were found in India, Malaysia and Sri Lanka. Focuses of these studies were on effect of mud in relation to indoor residual spraying (IRS), mosquito control as well as people's Knowledge, Attitude and Practice (KAP) toward mud [110, 111, 112, 113, 114, 115, 116, 117, 118, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128]. Others were analyzing the association between mud and malaria incidence [76, 109, 129, 130, 131, 132, 133, 134, 135, 136]. Only one prior study, by Kirby and colleagues, focused on the association between mud and abundance of *Anopheles* mosquitoes within a household [137]. This gave our study a niche in studying the association between building materials and *Anopheles* mosquitoes. We provided information not only on mud, but also on a wider variety of building materials. In addition, building materials were recorded at different sections of selected houses (e.g. wall, roof, floor and partition). This supplied our readers with a more comprehensive view on their relationships with *Anopheles* mosquitoes.

Our entomological surveillance was conducted at night, for a period of 12 hours each time. This has brought strength to the study as night time activities were prominent among mosquitoes [138]. During the study, very limited light trap information was gathered outdoors (N = 5 traps). Therefore, outdoor information was discarded. We focused the analysis based on indoor entomological surveillance (N = 4,363 traps). By excluding outdoor light traps, we underestimated numbers of *Anopheles* mosquitoes in the entire community. If more

outdoor light traps were set for the study, a community level analysis could be conducted. For example, we could analyze the association between density of certain type of building material used in the community and the numbers of *Anopheles* mosquitoes in the same area. Nonetheless, indoor housing materials could better reflect the abundance of *Anopheles* mosquitoes that shared the same space than if we had collected *Anopheles* mosquitoes outdoors. The timing of the mosquito surveillance also coincided with the time most residents were home. As we used this paper as a stepping stone to study the relationship between number of *Anopheles* mosquitoes and the number of malaria cases at households with various indoor building materials, setting indoor light traps at night was a logical choice to reduce the unmeasured variability.

There were times household representatives of a selected household were not present during the time of entomological survey. In a situation like this, we gathered informed consent from one of their neighbors to conduct entomological survey. This was based on an assumption that houses in close proximity shared similar housing structures and *Anopheles* profile than houses located further apart. Throughout the study, some households had more opportunities in receiving entomological surveys (Figure 5.2). To ensure building materials of each household shared equal weights during regression analyses, we calculated average number of *Anopheles* mosquitoes found per visit for each household as dependent variables. This was used in replace of the total number of *Anopheles* mosquitoes found at each surveyed household.

Previously, Kirby and colleague found *Anopheles* mosquitoes in Gambia (mainly

Anopheles gambiae sensu lato) were prevalent in houses with mud walls than houses with concrete walls (OR = 1.44, 1.10-1.87) [137]. In our study, we did not find similar relation in mud and cement walls (Table 5.9). This could be because different species were circulating in Bangladesh and in Gambia. Preference toward building materials could differ by *Anopheles* species. During entomological surveillance, we not only recorded the total number of *Anopheles* mosquitoes collected, we also logged the number collected for each *Anopheles* species. From 2009 to 2013, we found more than 20 different species of *Anopheles* mosquitoes in the study area. Of those, 17 species were documented by Alam et al. [139]. This was different than the situation in Africa, where majority of the *Anopheles* mosquitoes identified belonged to a few major species (e.g. *Anopheles gambies*) [140]. As numbers of individual *Anopheles* species spread too thin in analyzing their association with housing materials, we only kept the total number of mosquitoes collected per visit per household for final analyses.

Prior to adjusting for areal influence, we found having *mud* as a building material— either on wall, roof, partition or floor— was related to a significantly higher number of *Anopheles* at home at night. This change in average number of *Anopheles* mosquitoes per night could seem small; however, it could not be overlooked. With an average of 5 *Anopheles* mosquitoes presented at households at night, a fluctuation of ± 1 to ± 2 *Anopheles* mosquitoes would be equivalent to a 20-40% change in mosquito population. Although having some "*mud*" or "*Bamboo*" as part of common building material combination did not significantly change the overall *Anopheles* population, species specific preferences among *Anopheles* mosquitoes should be further studied. We also

found few households with an extreme average number of mosquitoes at night (Table 5.8). For instance, one household was visited twice. The first mosquito surveillance identified 114 *Anopheles* mosquitoes. The second surveillance found another 86 *Anopheles* mosquitoes. Extreme outliers such as this one were removed from the regression analysis (Tables 5.9 and 5.10). However, for future studies, detailed characteristics of these households could be looked into. With repeated entomological surveillance on targeted households, we could potentially address its reason behind a higher number of *Anopheles* mosquitoes in the household.

Another limitation of the study was the sole collection of adult *Anopheles* mosquitoes. Without information on larval habitats, we could not provide a comprehensive conclusion on how building materials are associated with *Anopheles* mosquitoes. Association between building materials different stages of mosquitoes may vary. Granted, it was difficult for household sections, such as walls or partitions, to be formed as larvae reservoirs. Materials like *mud*, on the other hand, could serve as building materials as well as a source to form larval reservoir. For instance, Rohani et al. found shallow pools (5 to 15 cm deep) with mud substrate are common habitat for *Anopheles maculatus* in Malaysia [141]. How building materials are associated with larvae population could be a focus for future study. Despite the limitation on adult only *Anopheles* mosquito collection, we increased the breadth of the study by incorporating multiple types of building materials used at multiple section of the houses—which was not previously achieved. In addition, we used an areal level adjustment to tease out potential baseline influence by *mud* and *bamboo* (Figure A5.28 to Figure A5.32).

Results from these linear regressions with areal level adjustment were shown in Tables 5.11 to 5.13. Although an alternative method— such as Kriging— may generate a smoother estimate and a narrower variance, the focused area created by our areal level adjustment was able to provide a least biased method in conducting linear regression on association between building materials and their expected number of *Anopheles* mosquitoes.

The association between household construction materials and abundance of *Anopheles* mosquitoes cannot be viewed as causation. Household construction materials by itself cannot solely explain the abundance of *Anopheles* mosquitoes. R-squares of the linear regression analysis, as shown in Tables 5.9 and 5.10, indicated housing materials could only explain 1 to 2 percent of the variation in abundance of *Anopheles* mosquitoes. With areal level adjustment on linear regression (Tables 5.11 to 5.13), the percentage explained by housing materials could go up to 4%. Future studies are needed to further identify other factors associated with abundance of *Anopheles* mosquitoes in Bandarban, Bangladesh.

Table 5.1: Number of Unique Households Surveyed for Anopheles Mosquitoes

	Cluster												
Union	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	**Total**
Rajbila	33	44	24	28	36	35	47	60	49	43	61	35	495
Kuhalong	31	52	45	34	60	48	72	87	42	31	28	54	584
Total	64	96	69	62	96	83	119	147	91	74	89	89	1079

Table 5.2: Average Count of Anopheles per Year, Month and Season

Time	Count		Average
	Anopheles	Household Visit	Anopheles per Visit
Overall			
	22,214	4,363	5.09
Year			
2009	1,448	321	4.51
2010	6,237	1,161	5.37
2011	7,367	1,512	4.87
2012	7,162	1,369	5.23
Month			
January	1,230	327	3.76
February	1,241	318	3.90
March	1,498	193	7.76
April	1,062	323	3.29
May	781	415	1.88
June	2,648	411	6.44
July	3,337	476	7.01
August	3,237	506	6.40
September	2,776	454	6.11
October	2,112	420	5.03
November	1,115	193	5.78
December	1,177	327	3.60
Season			
Spring	2,480	488	5.08
Summer	3,303	863	3.83
Monsoon	6,381	965	6.61
Autumn	5,385	923	5.83
Pre-Winter	2,421	531	4.56
Winter	2,244	593	3.78

Table 5.3: Housing Materials Used in the Study Area

Wall	Union		Total	Roof	Union		Total
	Rajibila	Kuhalong			Rajibila	Kuhalong	
Corrugated Tin / Iron Sheet	0	1	1	Straw / Thatch	115	184	299
Fired Brick / Cement	2	18	20	Asbestos	0	0	0
Tins	0	0	0	Pole and Grass	1	0	1
Pole and Mud	100	23	123	Pole and Mud	1	0	1
Wood	2	8	10	Bamboo	0	0	0
Pole and Grass	1	0	1	Mud Tins / House of Tins	2	6	8
Stone	0	0	0	Corrugated Tin / Iron Sheet	371	378	749
Unfired Bricks	0	0	0	Fired Brick / Cement	2	3	5
Bamboo	386	521	907	Concrete / Cement	0	1	1
Other	1	1	2	Other	0	0	0
Unknown	3	12	15	Unknown	3	12	15
Total	495	584	1079	**Total**	495	584	1079

Partition	Union		Total	Floor	Union		Total
	Rajibila	Kuhalong			Rajibila	Kuhalong	
Jute stick	0	0	0	Mud	238	182	420
Wood	3	7	10	Bamboo	178	272	450
Concrete / Cement	2	16	18	Semi Cemented	8	18	26
Mud	96	23	119	Vinyl	0	1	1
Tin	0	0	0	Cemented	15	23	38
Bamboo	355	512	867	Wood	53	76	129
Other	36	14	50	Other	0	0	0
Unknown	3	12	15	Unknown	3	12	15
Total	495	584	1079	**Total**	495	584	1079

Table 5.4: Housing Materials and Their Running Titles in this Paper

Wall		**Roof**	
Recorded Name	**Shortened Name**	**Recorded Name**	**Shortened Name**
Corrugated Tin / Iron Sheet	Corrugated Tin	Straw / Thatch	Straw
Fired Brick / Cement	Cement	Pole and Mud	Mud
Pole and Mud	Mud	Mud Tins / House of Tins	Mud Tins
Wood	Wood	Corrugated Tin / Iron Sheet	Corrugated Tin
Bamboo	Bamboo	Fired Brick / Cement	Cement
Other	Other	Other	Other
Partition		**Floor**	
Recorded Name	**Shortened Name**	**Recorded Name**	**Shortened Name**
Wood	Wood	Mud	Mud
Concrete / Cement	Cement	Bamboo	Bamboo
Mud	Mud	Semi-Cemented	Semi-Cemented
Bamboo	Bamboo	Cemented	Cemented
Other	Other	Wood	Wood
		Other	Other

Table 5.5: Ground Elevation of Households in the Study Area

	Union		
Ground Elevation	Rajibila	Kuhalong	**Total**
Yes	228	346	574
No	264	226	490
Unknown	3	12	15
Total	495	584	1079

Table 5.6: Top 90% Most Common Combination of Building Materials and Ground Elevation Status Used in Selected Households

Union	**Building Materials**				**Elevated**	**Combination**	**No. of**
Rank	Wall	Roof	Partition	Floor	**Ground**	**Number**	**Household**
Overall							1079
1	Bamboo	Corrugated Tin	Bamboo	Bamboo	Yes	1	260
2	Bamboo	Corrugated Tin	Bamboo	Mud	No	2	189
3	Bamboo	Straw	Bamboo	Bamboo	Yes	4	152
4	Bamboo	Corrugated Tin	Bamboo	Wood	Yes	5	106
5	Mud	Corrugated Tin	Mud	Mud	No	3	95
6	Bamboo	Straw	Bamboo	Mud	No	6	94
7	Bamboo	Corrugated Tin	Bamboo	Semi-Cemented	No	Other	20
8.5	Bamboo	Straw	Other	Bamboo	Yes	Other	14
8.5	Bamboo	Corrugated Tin	Other	Bamboo	Yes	Other	14
10.5	Mud	Straw	Mud	Mud	No	Other	13
10.5	Bamboo	Corrugated Tin	Bamboo	Cemented	No	Other	13

Table 5.7: Top 10 Combination of Building Materials and Ground Elevation Status Used in Selected Households, by Union

Union	**Building Materials**				**Elevated**	**No. of**
Rank	Wall	Roof	Partition	Floor	**Ground**	**Household**
Rajbila						495
1	Bamboo	Corrugated Tin	Bamboo	Bamboo	Yes	104
2	Bamboo	Corrugated Tin	Bamboo	Mud	No	93
3	Mud	Corrugated Tin	Mud	Mud	No	84
4	Bamboo	Straw	Bamboo	Bamboo	Yes	54
5	Bamboo	Corrugated Tin	Bamboo	Wood	Yes	42
6	Bamboo	Straw	Bamboo	Mud	No	34
7	Bamboo	Corrugated Tin	Other	Bamboo	Yes	13
8	Bamboo	Corrugated Tin	Bamboo	Cemented	No	9
9	Bamboo	Straw	Other	Mud	No	7
10	Mud	Straw	Mud	Mud	No	6
Kuhalong						584
1	Bamboo	Corrugated Tin	Bamboo	Bamboo	Yes	156
2	Bamboo	Straw	Bamboo	Bamboo	Yes	98
3	Bamboo	Corrugated Tin	Bamboo	Mud	No	96
4	Bamboo	Corrugated Tin	Bamboo	Wood	Yes	64
5	Bamboo	Straw	Bamboo	Mud	No	60
6	Bamboo	Corrugated Tin	Bamboo	Semi-Cemented	No	16
7	Mud	Corrugated Tin	Mud	Mud	No	11
8	Cement	Corrugated Tin	Cement	Cemented	No	11
9	Bamboo	Straw	Other	Bamboo	Yes	9
10	Mud	Straw	Mud	Mud	No	7

Table 5.8: Linear Regression Diagnostics: Studentized Residuals from Modeling Average Number of *Anopheles* per Visit per Household by Building Materials and Ground Elevation Status

	Count		**Average**	**Linear Regression: Studentized Residuals**†					
HHID	Collected *Anopheles*	Total Visits	Number of *Anopheles* per Visit	Wall	Roof	Partition	Floor	Ground Elevation	Common Combination
1-C06-201-019	40	1	40.0	6.012	6.164	6.014	6.141	6.135	6.043
1-C11-304-013	31	1	31.0	4.584	4.552	4.762	4.585	4.603	4.535
1-C11-306-007	37	1	37.0	5.657	5.668	5.653	5.657	5.675	5.721
1-C11-306-016	54	1	54.0	8.810	8.767	8.808	8.809	8.827	8.754
1-C11-308-014	317	5	63.4	10.653	10.605	10.652	10.722	10.578	10.604
2-C03-108-016	31	1	31.0	4.395	4.595	4.395	4.527	4.522	4.535
2-C05-115-003	200	2	100.0	19.043	18.960	19.050	19.037	19.051	18.965
2-C05-116-006	39	1	39.0	6.018	5.983	6.014	6.019	6.037	5.966
2-C08-218-009	67	2	33.5	5.029	5.040	5.024	4.971	4.966	5.047

† Studentized Residuals (*rstudent*): Re-normalize the residuals to have unit variance, using leave-one-out measure of the error variance

Table 5.9: Linear Regression Diagnostics: Average Number of Anopheles Mosquitoes per Visit on Housing Materials

	Linear Regression Models							
	All Data Points *(N = 1,063)*				**Extreme Outliers Removed**† *(N = 1,054)*			
Factor			95% CI				95% CI	
Covariate	Coef	SE	LCL	UCL	Coef	SE	LCL	UCL
Wall								
Intercept	4.67	0.19	4.29	5.04	4.30	0.13	4.06	4.55
Bamboo	Ref	—	—	—	Ref	—	—	—
Cement	-1.37	1.31	-3.94	1.20	-1.01	0.85	-2.68	0.67
Mud	1.15	0.56	0.06	2.25	1.03	0.37	0.31	1.74
Wood	-1.60	1.84	-5.21	2.02	-1.23	1.20	-3.59	1.12
Other	-1.00	2.91	-6.70	4.69	-0.64	1.89	-4.35	3.07
F-Test	**1.65 (p = 0.159)**				**2.76**‡ **(p = 0.027)**			
Roof								
Intercept	4.80	0.21	4.38	5.21	4.39	0.14	4.12	4.67
Corrugated Tin	Ref	—	—	—	Ref	—	—	—
Cement	-1.01	2.61	-6.13	4.10	-0.61	1.70	-3.94	2.72
Mud Tins	2.53	2.07	-1.52	6.58	2.93	1.34	0.29	5.57
Straw	-0.22	0.40	-1.00	0.56	-0.11	0.26	-0.62	0.40
Other	2.01	3.36	-4.58	8.60	2.41	2.19	-1.88	6.71
F-Test	**0.60 (p = 0.663)**				**1.61 (p = 0.171)**			
Partition								
Intercept	4.71	0.20	4.33	5.10	4.37	0.13	4.11	4.62
Bamboo	Ref	—	—	—	Ref	—	—	—
Cement	-1.74	1.38	-4.45	0.97	-1.39	0.90	-3.15	0.37
Mud	1.13	0.57	0.02	2.24	0.97	0.37	0.24	1.70
Wood	-2.07	1.84	-5.68	1.54	-1.72	1.20	-4.07	0.63
Other	-0.77	0.84	-2.43	0.88	-0.98	0.55	-2.06	0.11
F-Test	**2.06 (p = 0.085)**				**3.88**‡ **(p = 0.004)**			
Floor								
Intercept	4.64	0.27	4.11	5.18	4.11	0.18	3.76	4.46
Bamboo	Ref	—	—	—	Ref	—	—	—
Cemented	-0.23	0.98	-2.15	1.70	0.31	0.64	-0.94	1.56
Mud	0.33	0.39	-0.44	1.10	0.65	0.26	0.15	1.15
Semi-Cemented	0.02	1.17	-2.28	2.31	0.55	0.76	-0.94	2.04
Wood	-0.19	0.58	-1.33	0.95	-0.11	0.38	-0.86	0.63
Other	11.67	5.82	0.27	23.07	12.20	3.77	4.81	19.60
F-Test	**1.04 (p = 0.393)**				**3.61**‡ **(p = 0.003)**			

†: Removed HHIDs were shown in Table 5.8

‡: Statistically significant at 5% error rate

Table 5.10: Linear Regression Diagnostics: Average Number of Anopheles Mosquitoes per Visit on Ground Elevation and Common Combination of Building Materials and Ground Elevation Status

	Linear Regression Models							
	All Data Points *(N = 1,063)*				**Extreme Outliers Removed**† *(N = 1,054)*			
Factor			95% CI				95% CI	
Covariate	Coef	SE	LCL	UCL	Coef	SE	LCL	UCL
Ground Elevation Status								
Intercept	5.00	0.26	4.49	5.52	4.70	0.17	4.36	5.04
No	Ref	—	—	—	Ref	—	—	—
Yes	-0.46	0.36	-1.16	0.24	-0.58	0.23	-1.03	-0.12
F-Test	**1.66 (p = 0.198)**				**6.08**‡ **(p = 0.014)**			
Ground Elevation Height								
Intercept	4.96	0.25	4.48	5.44	4.57	0.16	4.26	4.89
Height (cm)	-0.004	0.003	-0.011	0.003	-0.004	0.002	-0.008	0.001
F-Test	**1.49 (p = 0.222)**				**2.85 (p = 0.09)**			
Common Combination of Building Materials and Ground Elevation Status ∫								
Intercept	4.95	0.36	4.24	5.65	4.25	0.24	3.79	4.71
Combination 1	Ref	—	—	—	Ref	—	—	—
Combination 2	-0.32	0.56	-1.41	0.77	0.38	0.36	-0.33	1.09
Combination 3	0.69	0.79	-0.68	2.06	1.02	0.46	0.13	1.92
Combination 4	-0.60	0.59	-1.77	0.56	-0.12	0.39	-0.89	0.64
Combination 5	-0.86	0.67	-2.17	0.46	-0.16	0.44	-1.02	0.70
Combination 6	-0.29	0.70	-1.66	1.08	0.09	0.46	-0.81	0.99
Other	0.01	0.58	-1.12	1.14	0.04	0.38	-0.70	0.78
F-Test	**0.83 (p = 0.222)**				**1.29 (p = 0.259)**			

∫: List of common combination of building materials and ground elevation status can be found in Table 5.6

†: Removed HHIDs were shown in Table 5.8

‡: Statistically significant at 5% error rate

Table 5.11: Linear Regression on Effect of Mud without the Attribution of Bamboo: Difference in between Average Number of *Anopheles* per Visit per Household for Households without Bamboo as Building Materials and Areal Average Number of *Anopheles* per Visit per Household with Bamboo as Building Materials by Latitude and Longitude on Types of Housing Materials (9 Households with Extreme Average Number of *Anopheles* were Removed)

		Linear Regression Models Extreme Outliers Removed†					
					95% CI		
Factor	**Covariate**	N	Coef	SE	LCL	UCL	
Wall							
	Bamboo	899					*(Removed from Regression)*
	Intercept		0.51	0.35	-0.18	1.20	
	Mud	121	Ref	—	—	—	
	Cement	20	-1.47	0.92	-3.29	0.34	
	Wood	10	-1.47	1.25	-3.94	1.00	
	Other	4	-0.75	1.93	-4.57	3.06	
	F-Test	**1.22 (p-value = 0.304)**					
Partition							
	Bamboo	860					*(Removed from Regression)*
	Intercept		0.51	0.35	-0.18	1.19	
	Mud	117	Ref	—	—	—	
	Cement	18	-1.75	0.94	-3.61	0.11	
	Wood	10	-2.25	1.23	-4.67	0.17	
	Other	49	-1.15	0.64	-2.41	0.10	
	F-Test	**2.59 (p-value = 0.054)**					

†: Removed HHIDs were shown in Table 5.8

‡: Statistically significant at 5% error rate

Table 5.12: Linear Regression on Effect of Mud without the Attribution of Bamboo: Difference in between Average Number of *Anopheles* per Visit per Household for Households without Bamboo as Building Materials and Areal Average Number of *Anopheles* per Visit per Household with Bamboo as Building Materials by Latitude and Longitude on Types of Housing Materials (9 Households with Extreme Average Number of *Anopheles* were Removed) (Continued)

		Linear Regression Models Extreme Outliers Removed†					
					95% CI		
Factor	**Covariate**	N	Coef	SE	LCL	UCL	
Floor							
	Bamboo	444					*(Removed from Regression)*
	Intercept		0.58	0.23	0.12	1.03	
	Mud	417	Ref	—	—	—	
	Cemented	38	-0.48	0.76	-1.98	1.02	
	Semi-Cemented	26	0.85	0.83	-0.79	2.49	
	Wood	128	-0.81	0.42	-1.65	0.02	
	Other	1	11.54	3.93	3.81	19.27	
	F-Test	**3.63‡ (p-value = 0.006)**					
Common Combination of Housing Materials							
	with Bamboo	960					*(Removed from Regression)*
	without Bamboo	94	Regression cannot be done: Combination 3 is the only level without Bamboo. Mean (Expected Differences) = 1.53				

†: Removed HHIDs were shown in Table 5.8

‡: Statistically significant at 5% error rate

Table 5.13: Linear Regression on Effect of Some Mud without the Attribution of No Mud: Difference in between Average Number of *Anopheles* per Visit per Household for Households with Mud as part of Building Materials and Areal Average Number of *Anopheles* per Visit per Household without any Mud as Building Materials by Latitude and Longitude on Types of Housing Materials (9 Households with Extreme Average Number of *Anopheles* were Removed)

		Linear Regression Models Extreme Outliers Removed†					
					95% CI		
Factor	**Covariate**	N	Coef	SE	LCL	UCL	
Common Combination of Housing Materials							
	without Mud	678					*(Removed from Regression)*
	with Mud:						
	Intercept		0.64	0.31	0.04	1.25	
	Combination 2	189	Ref	—	—	—	
	Combination 3	94	0.22	0.55	-0.87	1.30	
	Combination 6	93	-0.56	0.55	-1.65	0.53	
	F-Test	**0.79 (p-value = 0.453)**					

†: Removed HHIDs were shown in Table 5.8

‡: Statistically significant at 5% error rate

Figure 5.1: Geographic Distribution of Selected Households

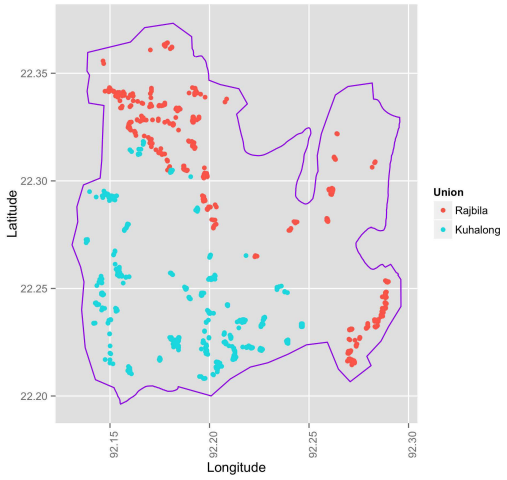


Figure 5.2: Number of Visits per Household

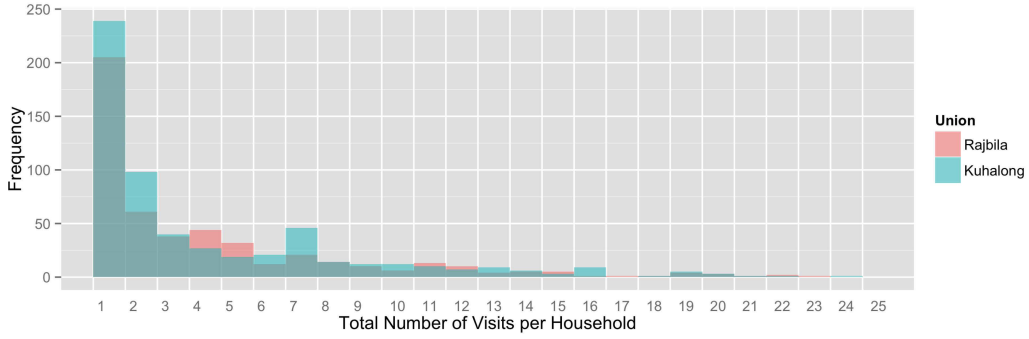
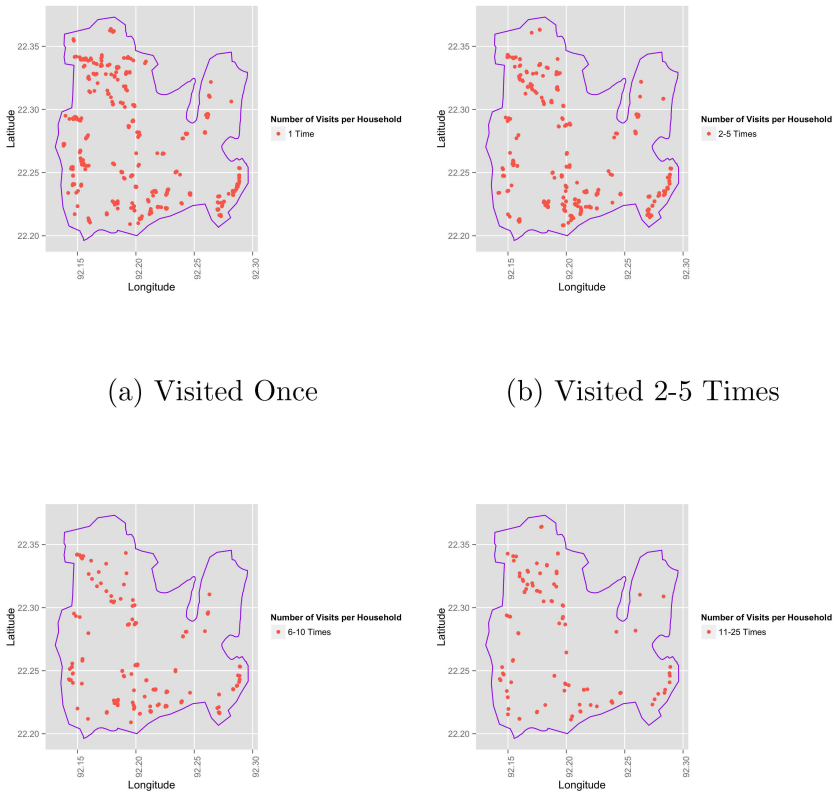


Figure 5.3: Geographic Distribution of Selected Households, by Number of Visits per Household



(a) Visited Once

(b) Visited 2-5 Times

(c) Visited 6-10 Times

(d) Visited > 10 Times

Figure 5.4: Boxplot: Average Number of *Anopheles* Mosquitoes per Visit per Household, by Union

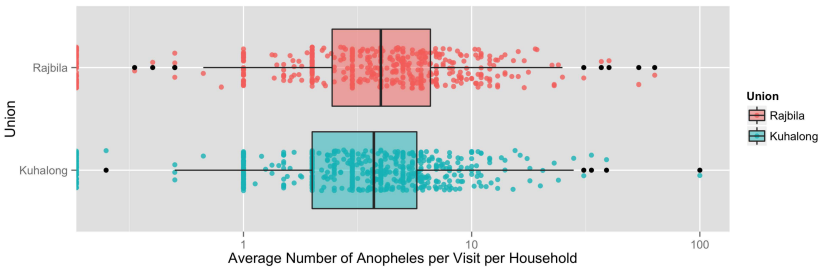


Figure 5.5: Average Number of *Anopheles* per Month and per Season

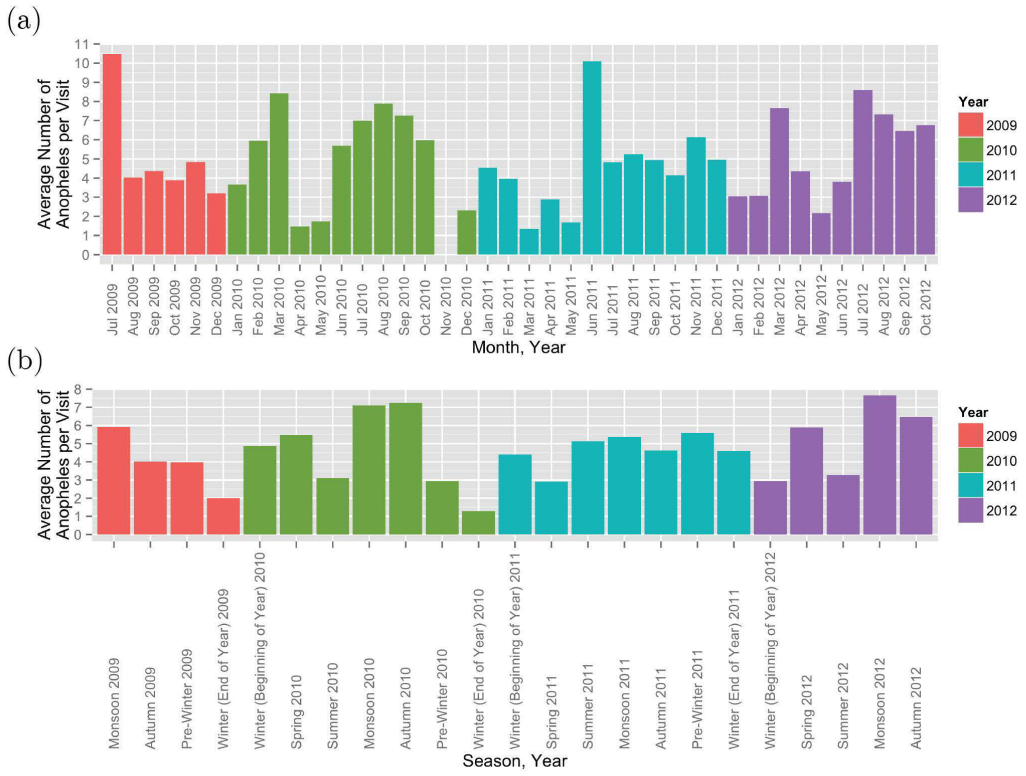
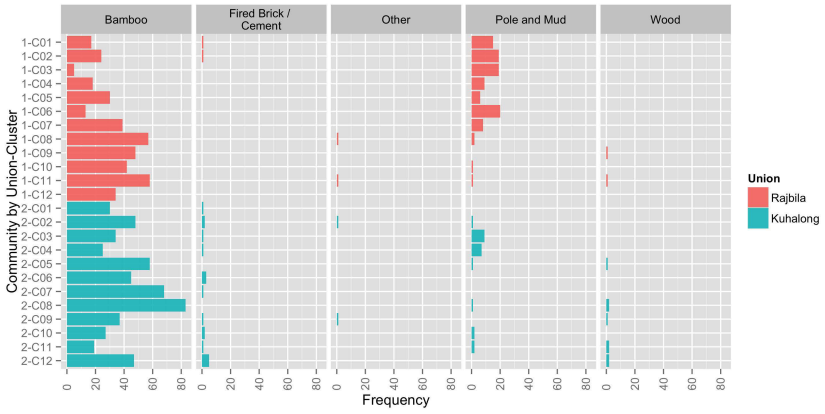
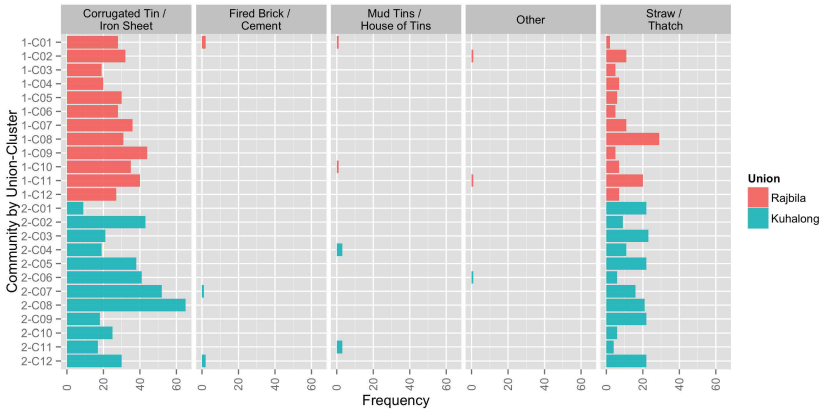


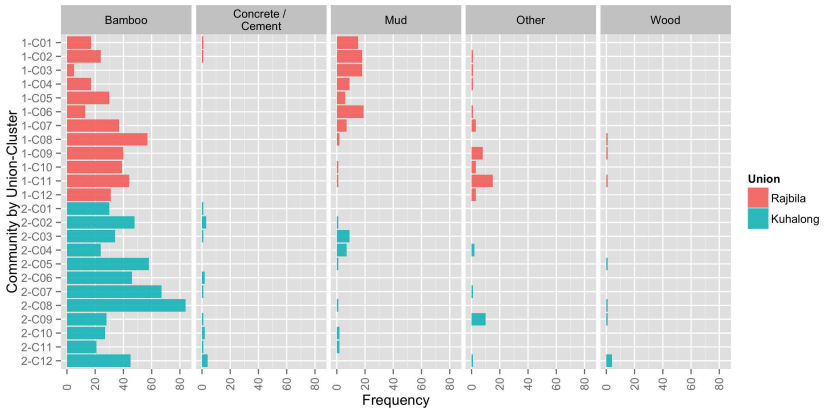
Figure 5.6: Histogram: Distribution of Different Types of Building Materials by Geographic Locations



(a) Wall

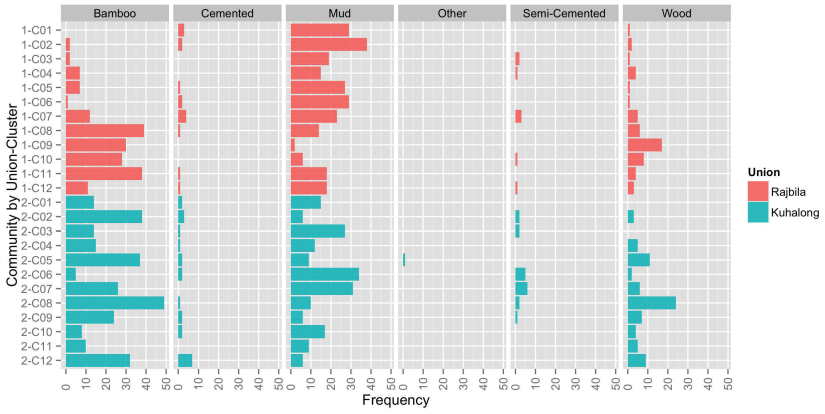


(b) Roof

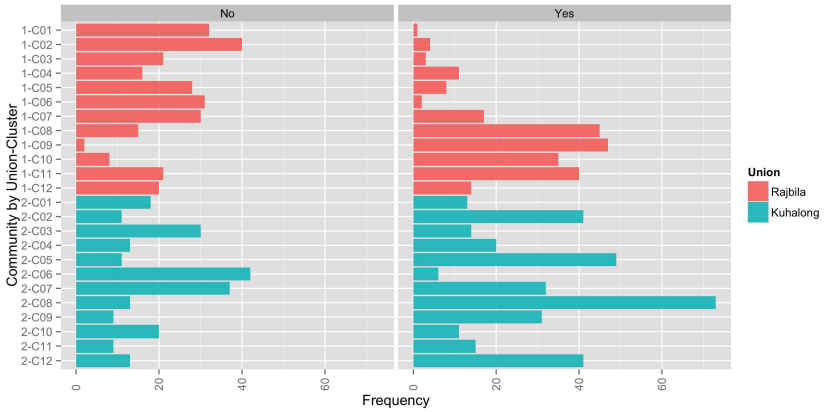


(c) Partition

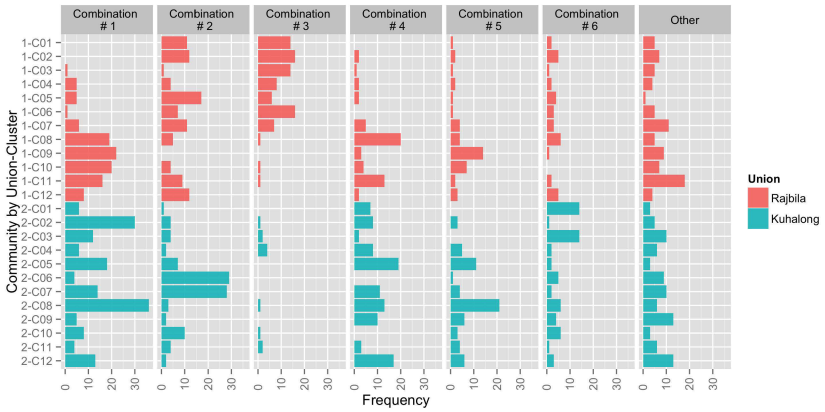
Figure 5.7: Histogram: Distribution of Different Types of Building Materials by Geographic Locations (Continued)



(a) Floor



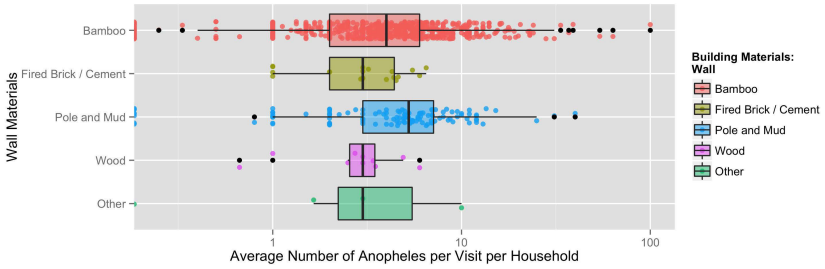
(b) Ground Elevation Status



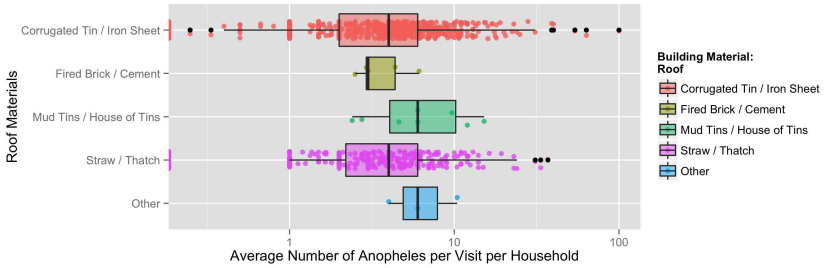
(c) Common Combination

Appendix

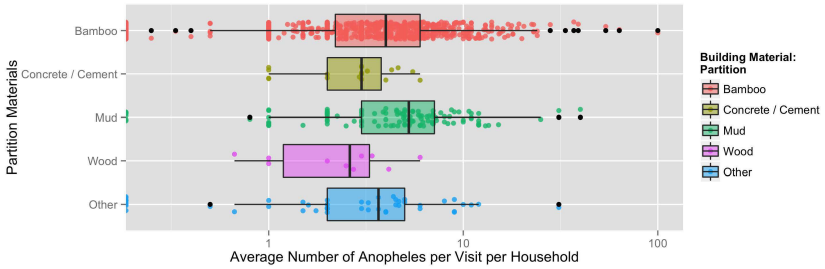
Figure A5.1: Boxplot: Average Number of *Anopheles* Mosquitoes per Visit per Household, by Building Materials



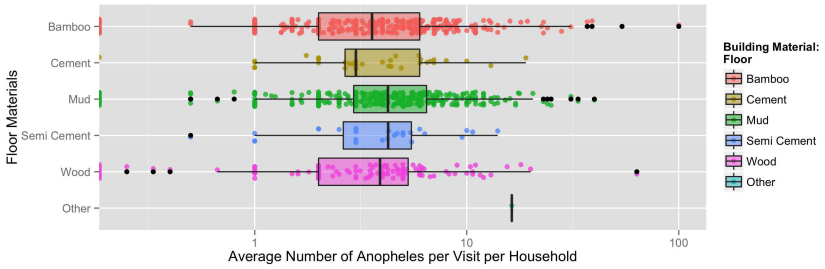
(a) Wall



(b) Roof



(c) Partition



(d) Floor

Figure A5.2: Scatter Plot: Average Number of *Anopheles* Mosquitoes per Visit per Household, by Household Ground Elevation Height

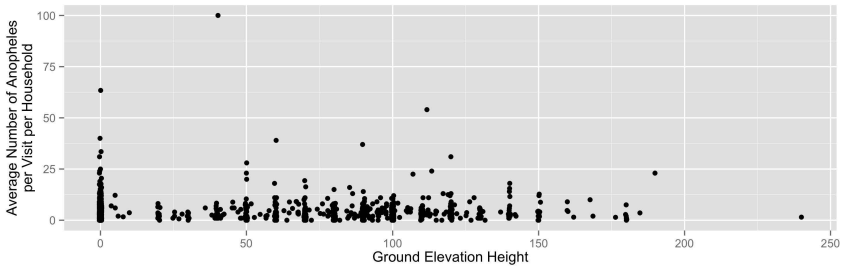
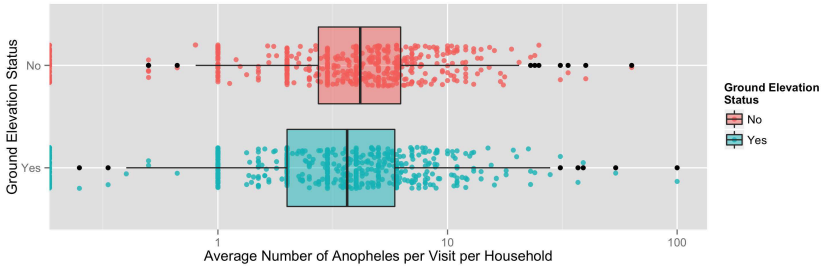
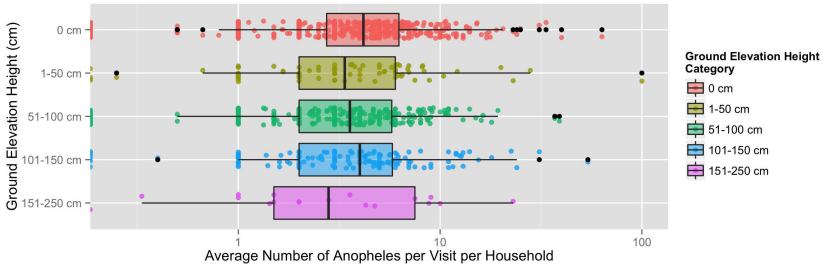


Figure A5.3: Boxplot: Average Number of *Anopheles* Mosquitoes per Visit per Household, by Ground Elevation Status and Height Category



(a) Ground Elevation Status



(b) Ground Elevation Height (Category)

Figure A5.4: Boxplot: Average Number of *Anopheles* Mosquitoes per Visit per Household, by Common Combination of Household Building Materials and Ground Elevation Status

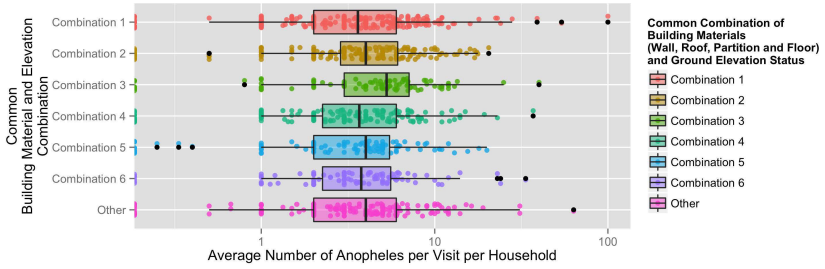


Figure A5.5: Geographic Distribution of Selected Households, by Average Number of *Anopheles* per Visit per Household

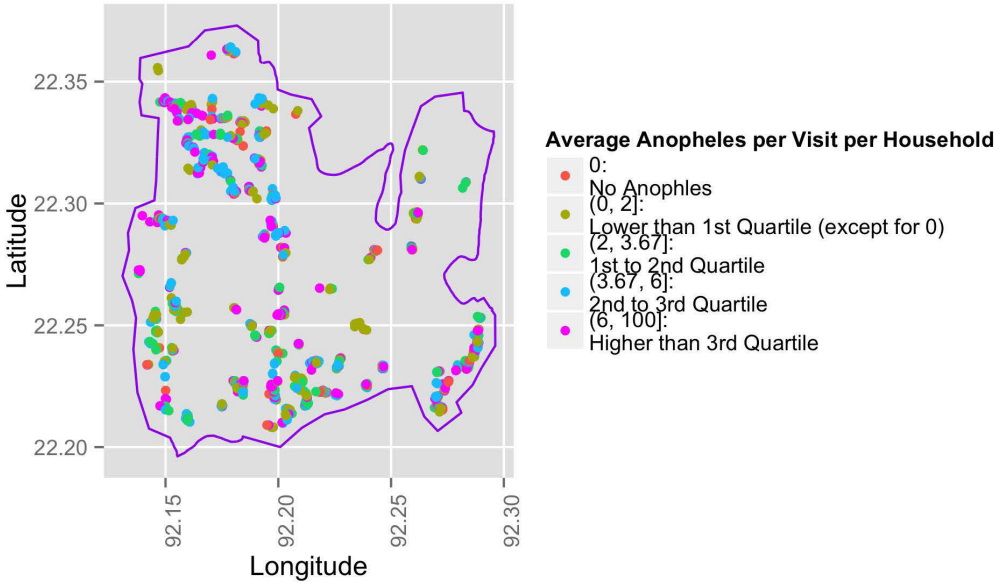
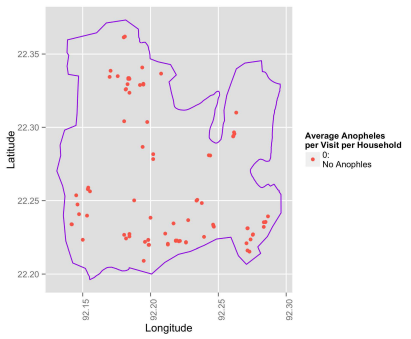
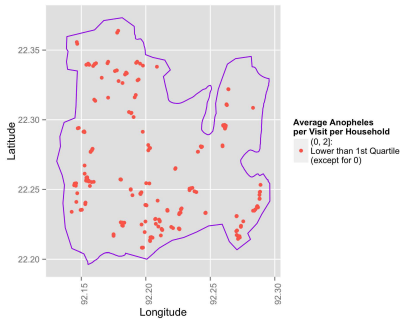


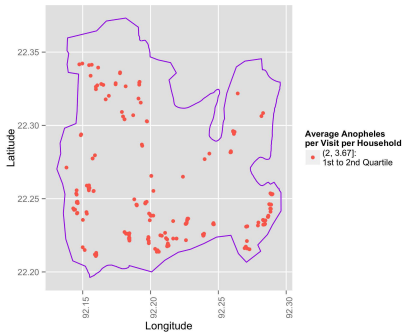
Figure A5.6: Geographic Distribution of Selected Households, by Average Number of *Anopheles* per Visit per Household (continued)



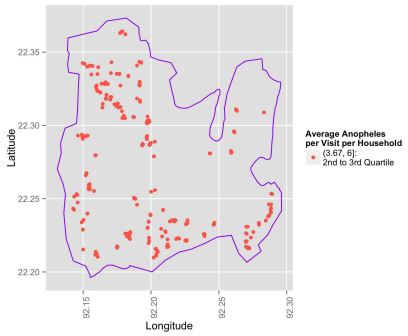
(a) No Anopheles



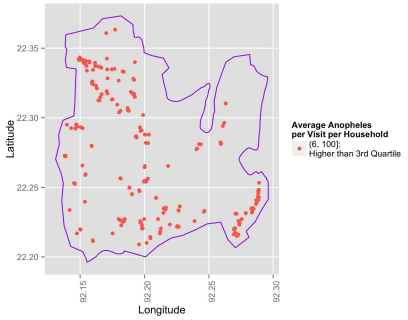
(b) 1st Quartile and Below (Except for 0)



(c) 1st to 2nd Quartile

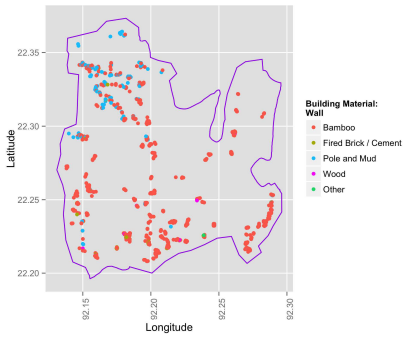


(d) 2nd to 3rd Quartile

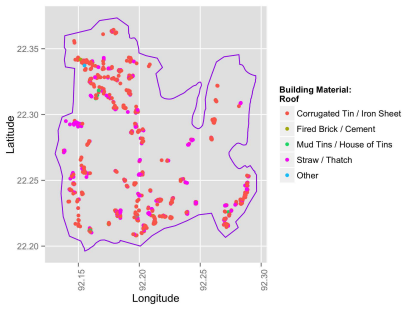


(e) Greater than 3rd Quartile

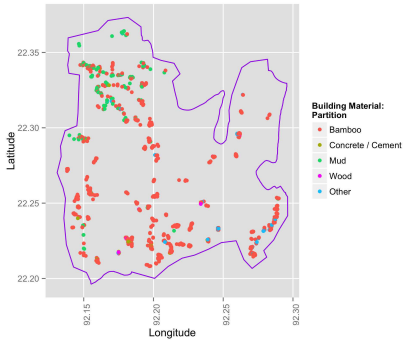
Figure A5.7: Geographic Distribution of Different Types of Building Materials



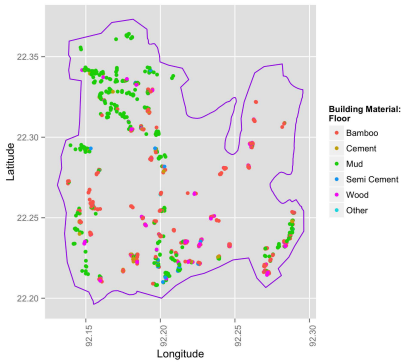
(a) Wall



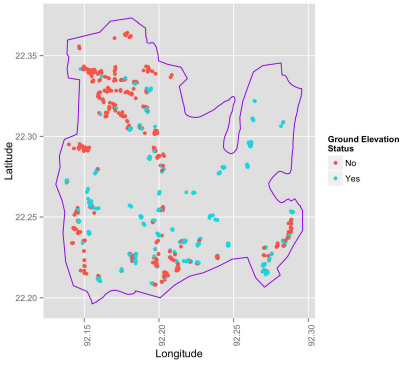
(b) Roof



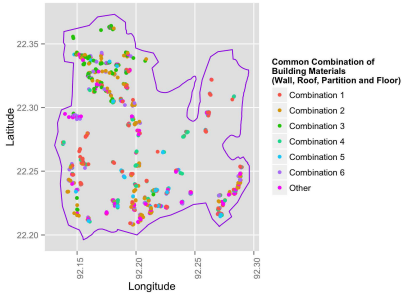
(c) Partition



(d) Floor

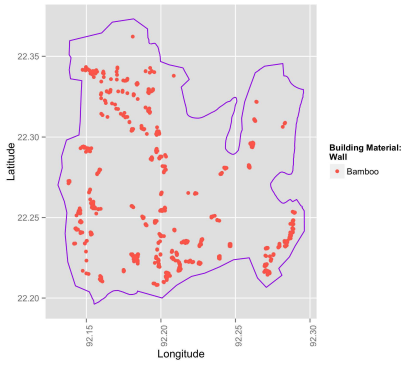


(e) Ground Elevation Status

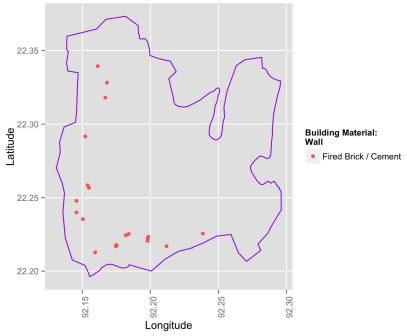


(f) Common Combination

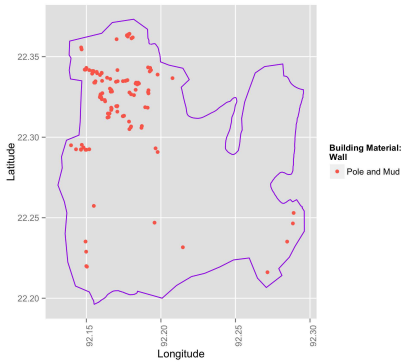
Figure A5.8: Geographic Distribution of Different Types of Wall



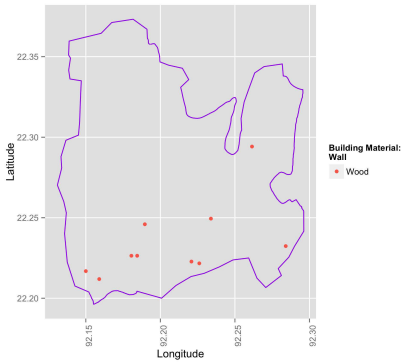
(a) Wall—Bamboo



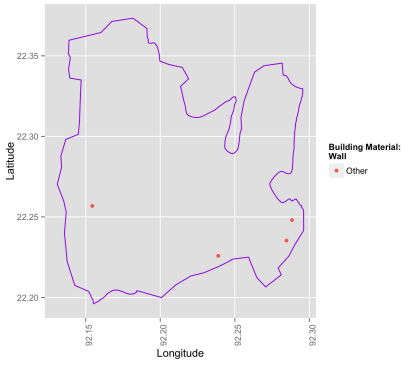
(b) Wall—Cement



(c) Wall—Mud

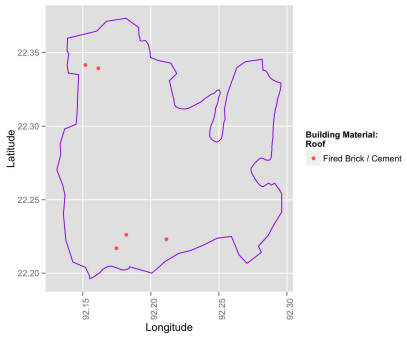


(d) Wall—Wood

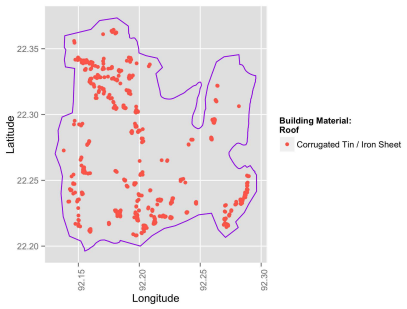


(e) Wall—Other

Figure A5.9: Geographic Distribution of Different Types of Roof

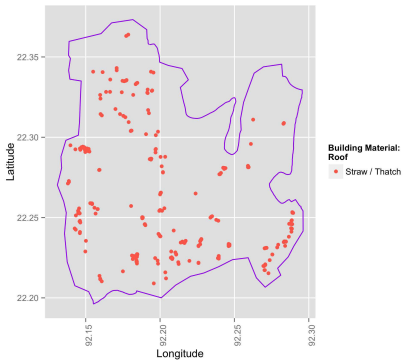


(a) Roof—Cement

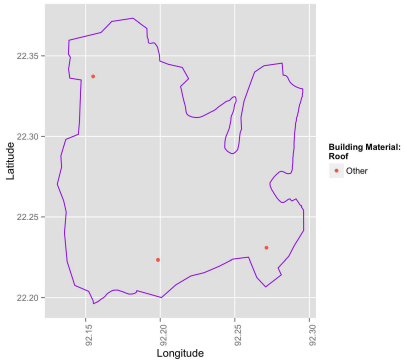


(b) Roof—Corrugated Tin

(c) Roof—Mud Tins

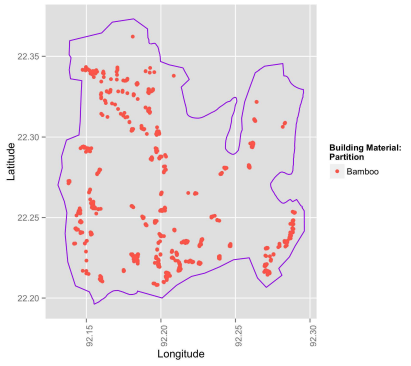


(d) Roof—Straw

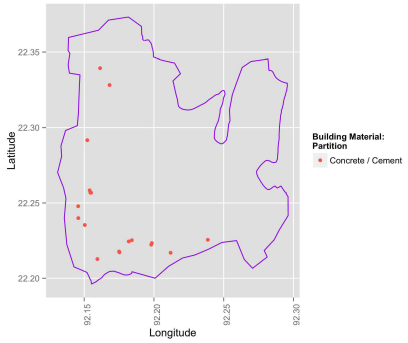


(e) Roof—Other

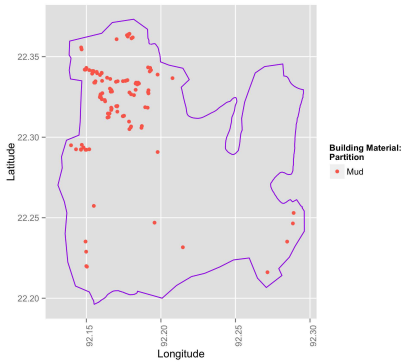
Figure A5.10: Geographic Distribution of Different Types of Partition



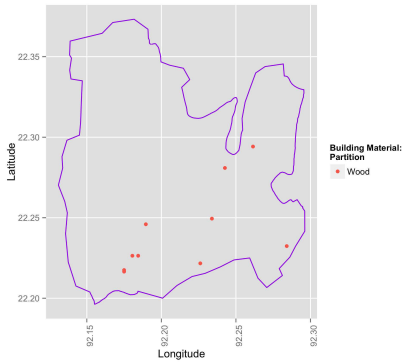
(a) Partition—Bamboo



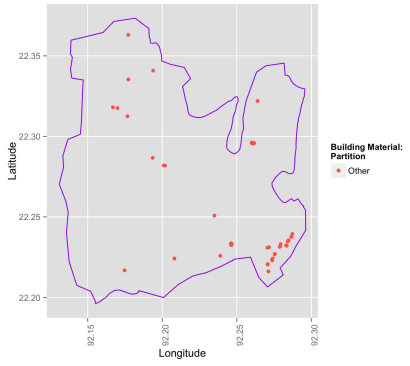
(b) Partition—Cement



(c) Partition—Mud

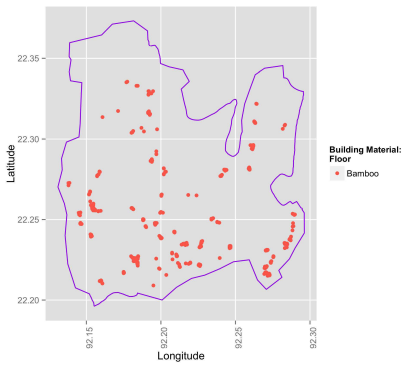


(d) Partition—Wood

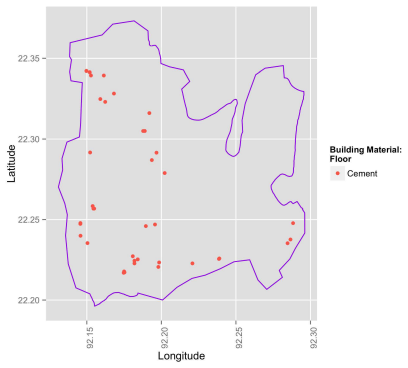


(e) Partition—Other

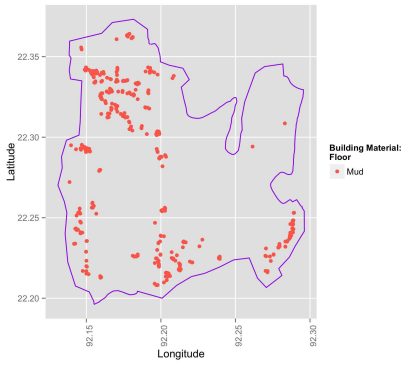
Figure A5.11: Geographic Distribution of Different Types of Floor



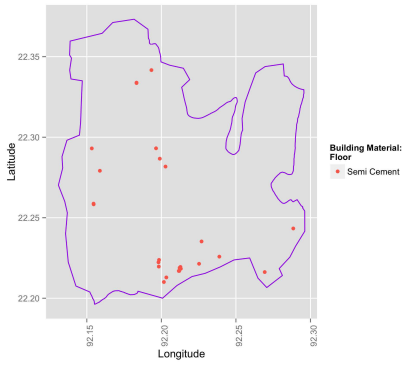
(a) Floor—Bamboo



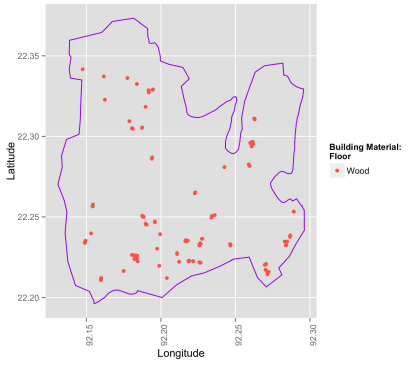
(b) Floor—Cemented



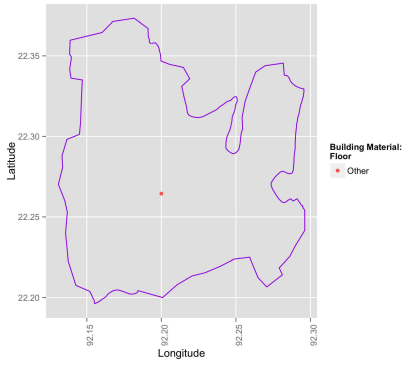
(c) Floor—Mud



(d) Floor—Semi-Cemented

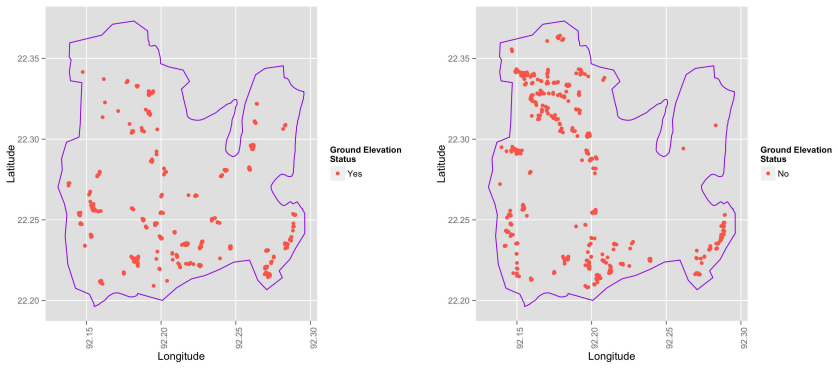


(e) Floor—Wood



(f) Floor—Other

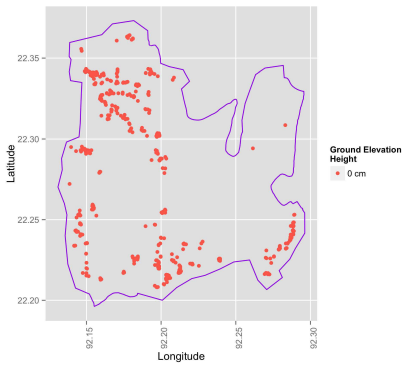
Figure A5.12: Geographic Distribution of Ground Elevation Status



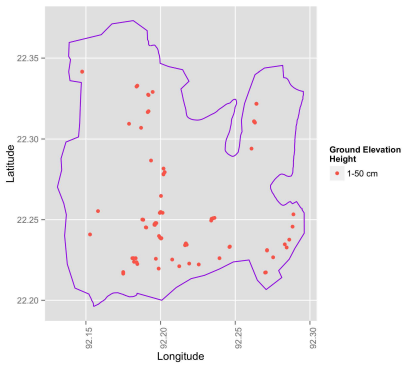
(a) Elevation—Yes

(b) Elevation—No

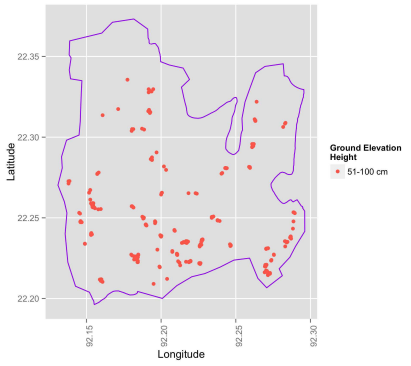
Figure A5.13: Geographic Distribution of Ground Elevation Height



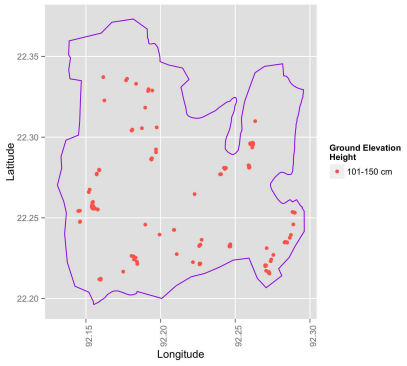
(a) Elevation—0 cm



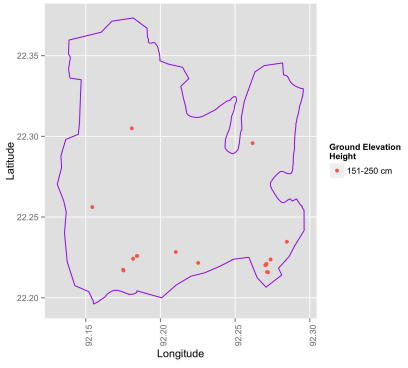
(b) Elevation—1 to 50 cm



(c) Elevation—51 to 100 cm

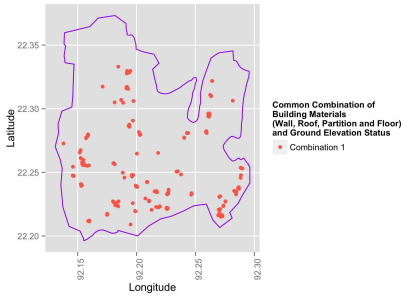


(d) Elevation—101 to 150 cm

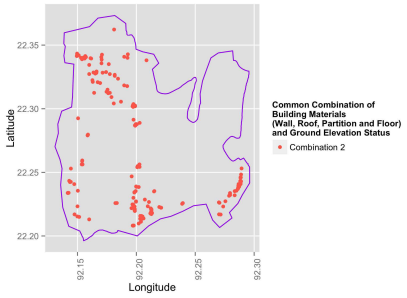


(e) Elevation—151 to 250 cm

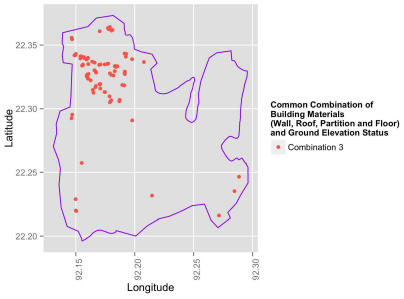
Figure A5.14: Geographic Distribution of Common Combination of Building Materials and Ground Elevation Status



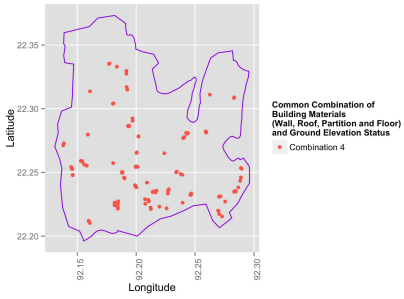
(a) Common Combination—1



(b) Common Combination—2

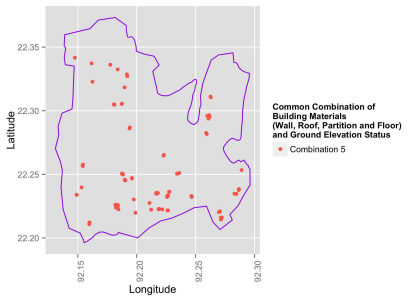


(c) Common Combination—3

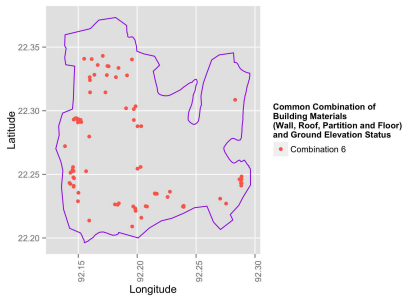


(d) Common Combination—4

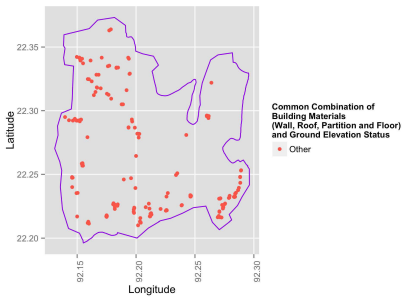
Figure A5.15: Geographic Distribution of Common Combination of Building Materials and Ground Elevation Status (Continued)



(a) Common Combination—5



(b) Common Combination—6



(c) Other Common Combinations

Figure A5.16: Summary of Average Number of *Anopheles* per Visit per Household by Latitude and Longitude Grids (Extreme Outliers were Removed)

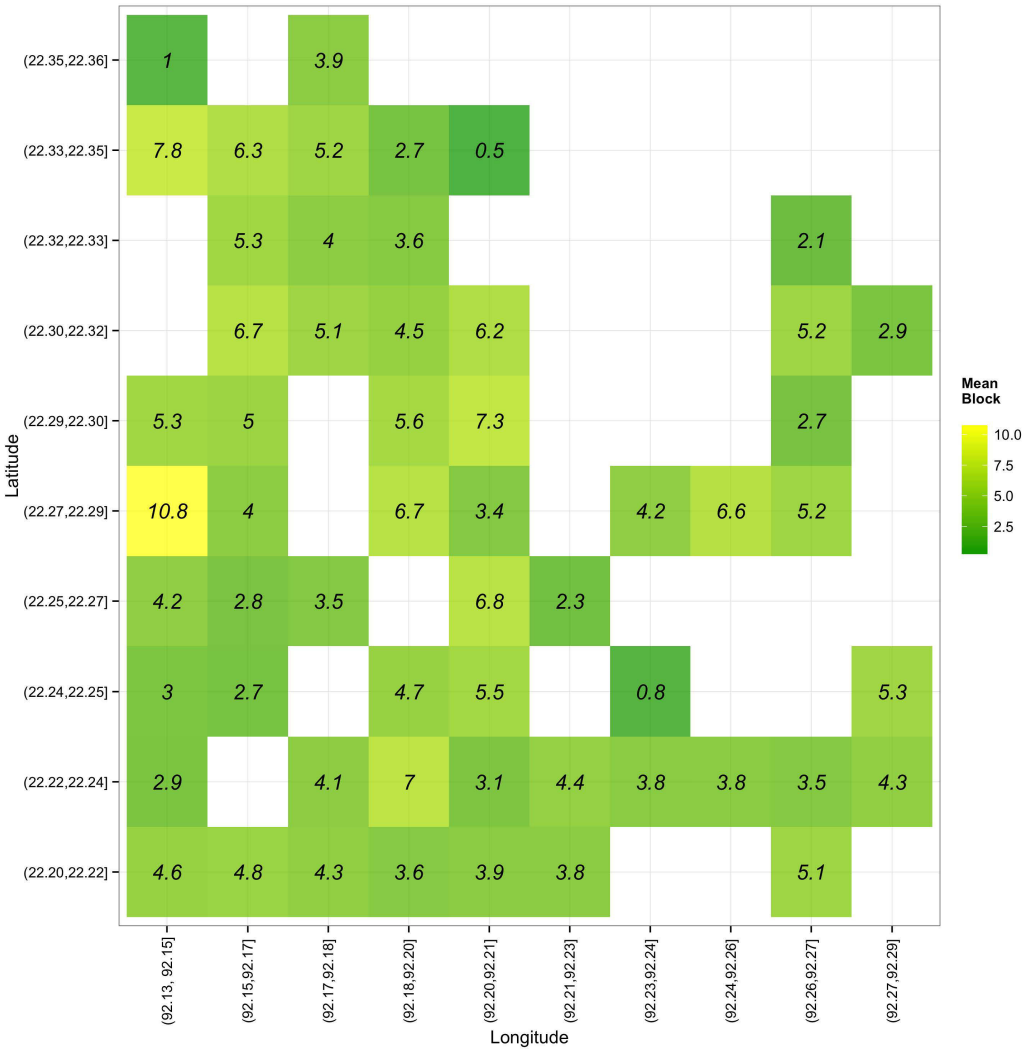
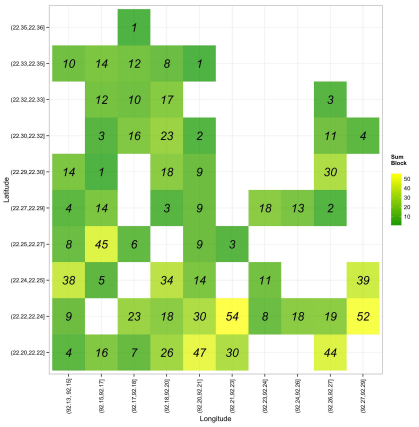
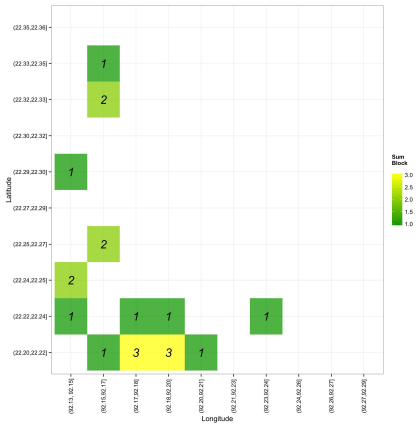


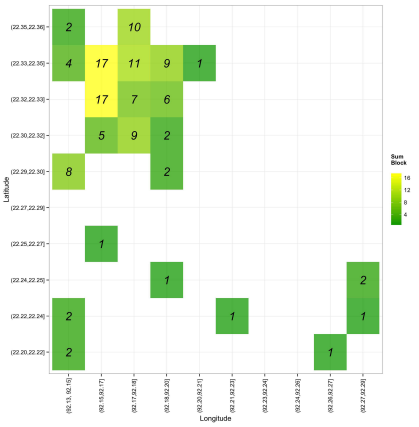
Figure A5.17: Total Number of Households with Different Types of Wall Materials by Latitude and Longitude Grids — Bamboo



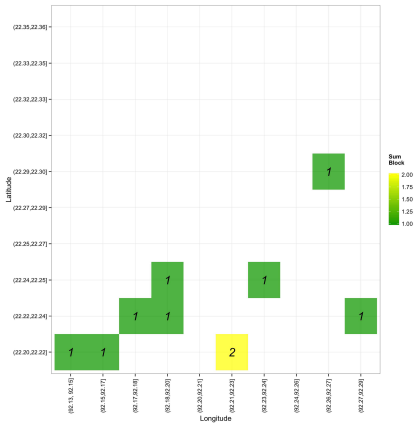
(a) Bamboo



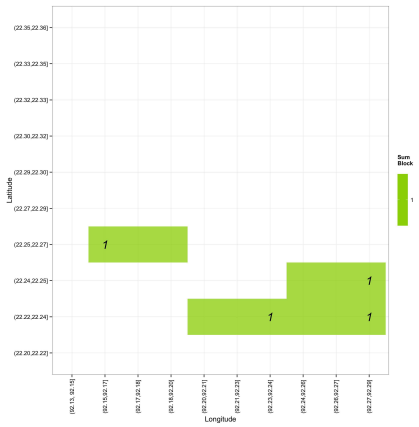
(b) Fired Brick / Cement



(c) Pole and Mud

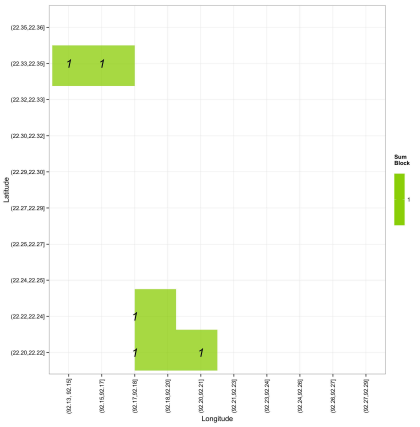


(d) Wood

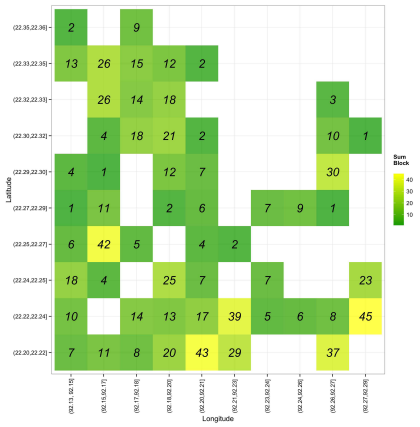


(e) Other

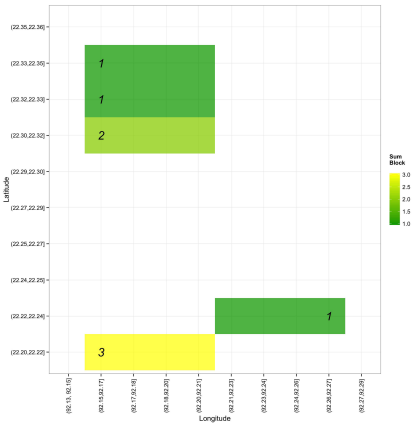
Figure A5.18: Total Number of Households with Different Types of Roof Materials by Latitude and Longitude Grids



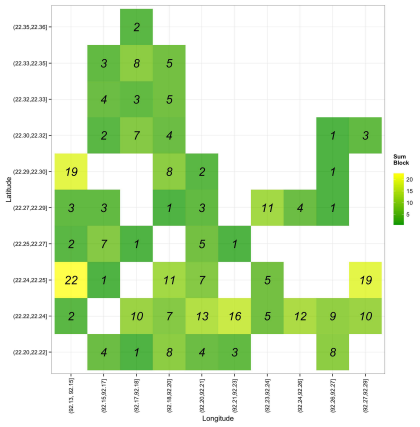
(a) Fired Brick / Cement



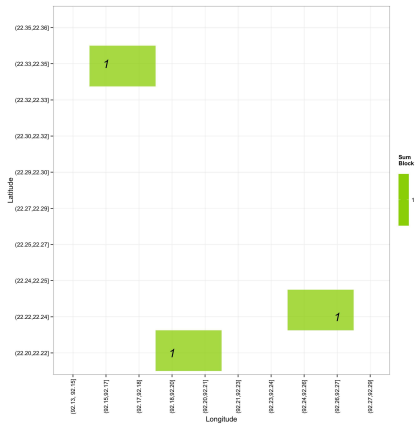
(b) Corrugated Tin / Iron Sheet



(c) Mud Tins / House of Tins

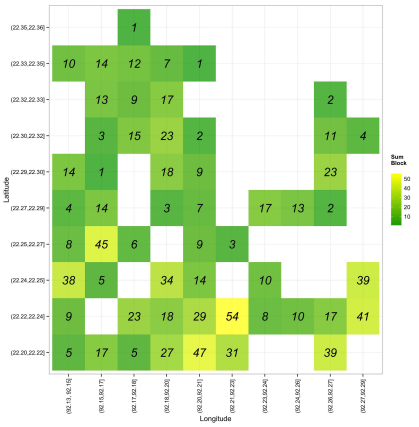


(d) Straw / Thatch

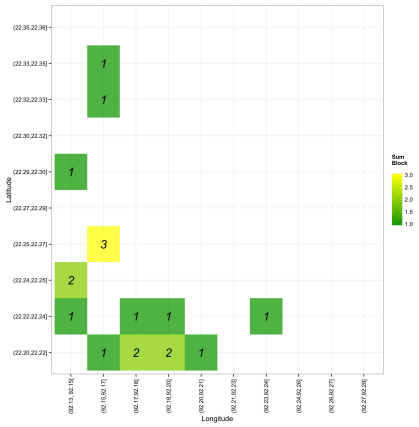


(e) Other

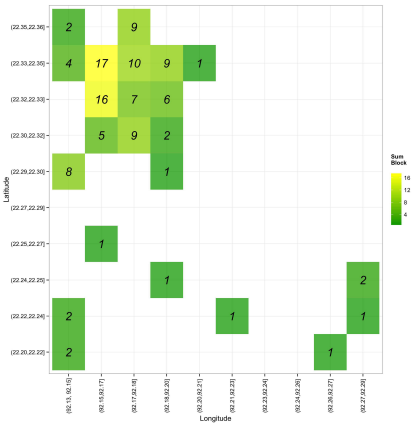
Figure A5.19: Total Number of Households with Different Types of Partition Materials by Latitude and Longitude Grids



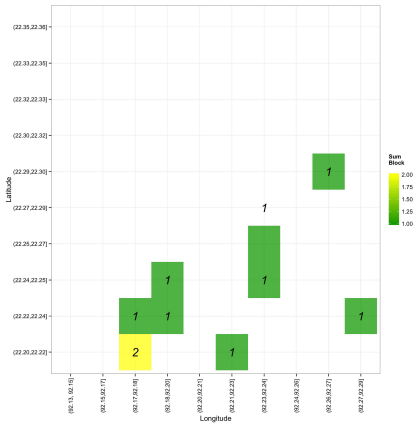
(a) Bamboo



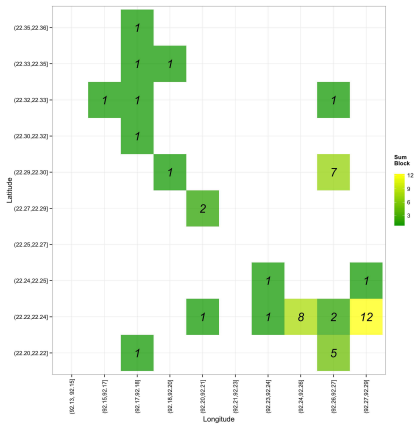
(b) Concrete / Cement



(c) Mud

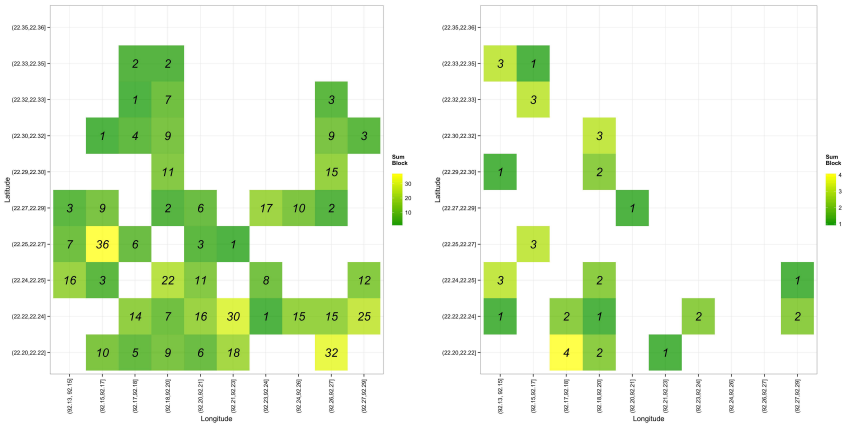


(d) Wood



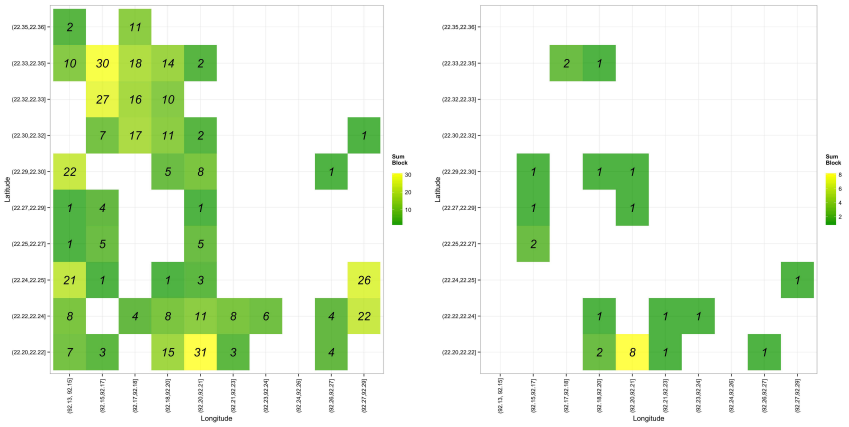
(e) Other

Figure A5.20: Total Number of Households with Different Types of Floor Materials by Latitude and Longitude Grids



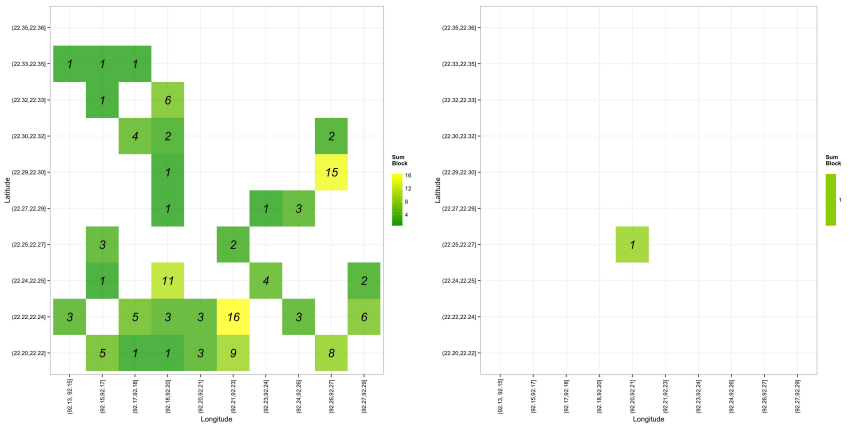
(a) Bamboo

(b) Cemented



(c) Mud

(d) Semi-Cemented



(e) Wood

(f) Other

Figure A5.21: Summary of Ground Elevation Height of Households by Latitude and Longitude Grids

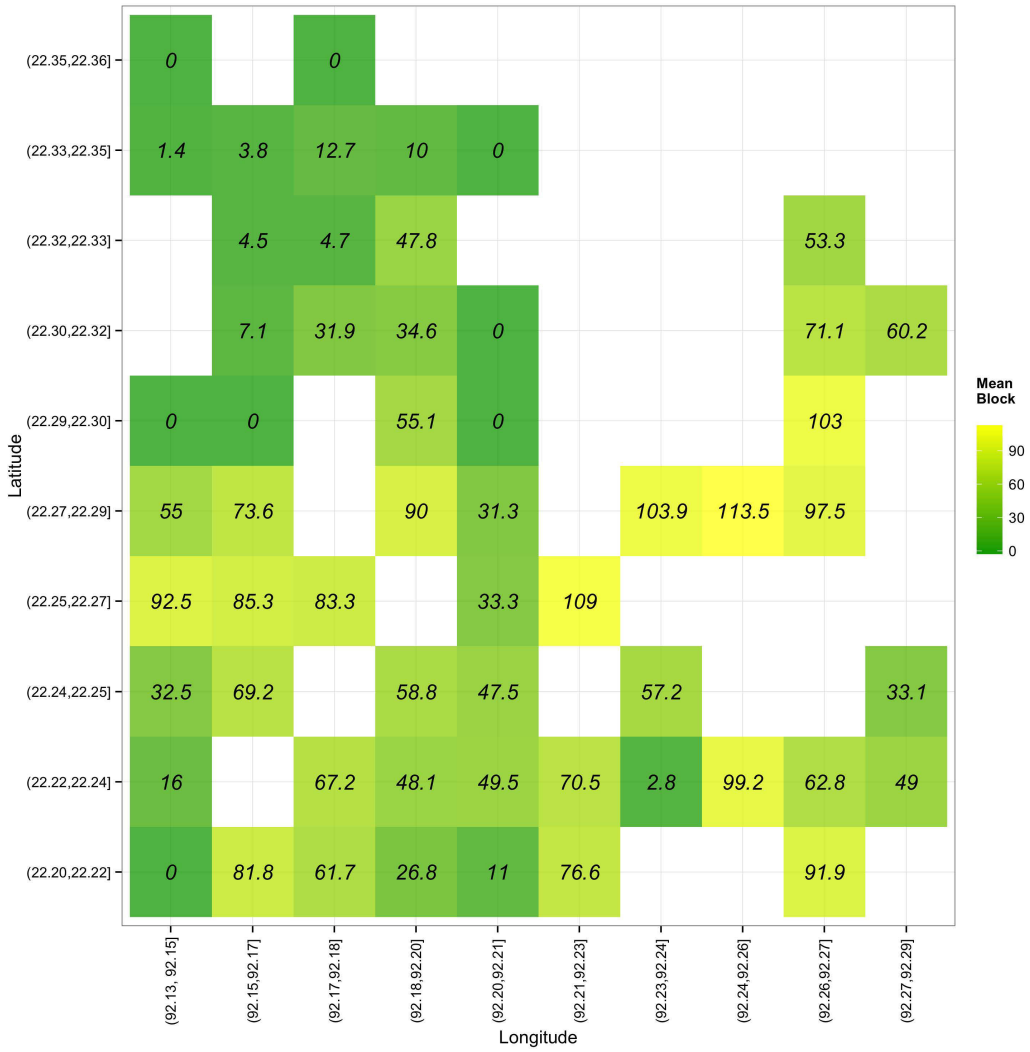


Figure A5.22: Summary of Average Number of *Anopheles* per Visit per Household for Households without Mud as Part of Building Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

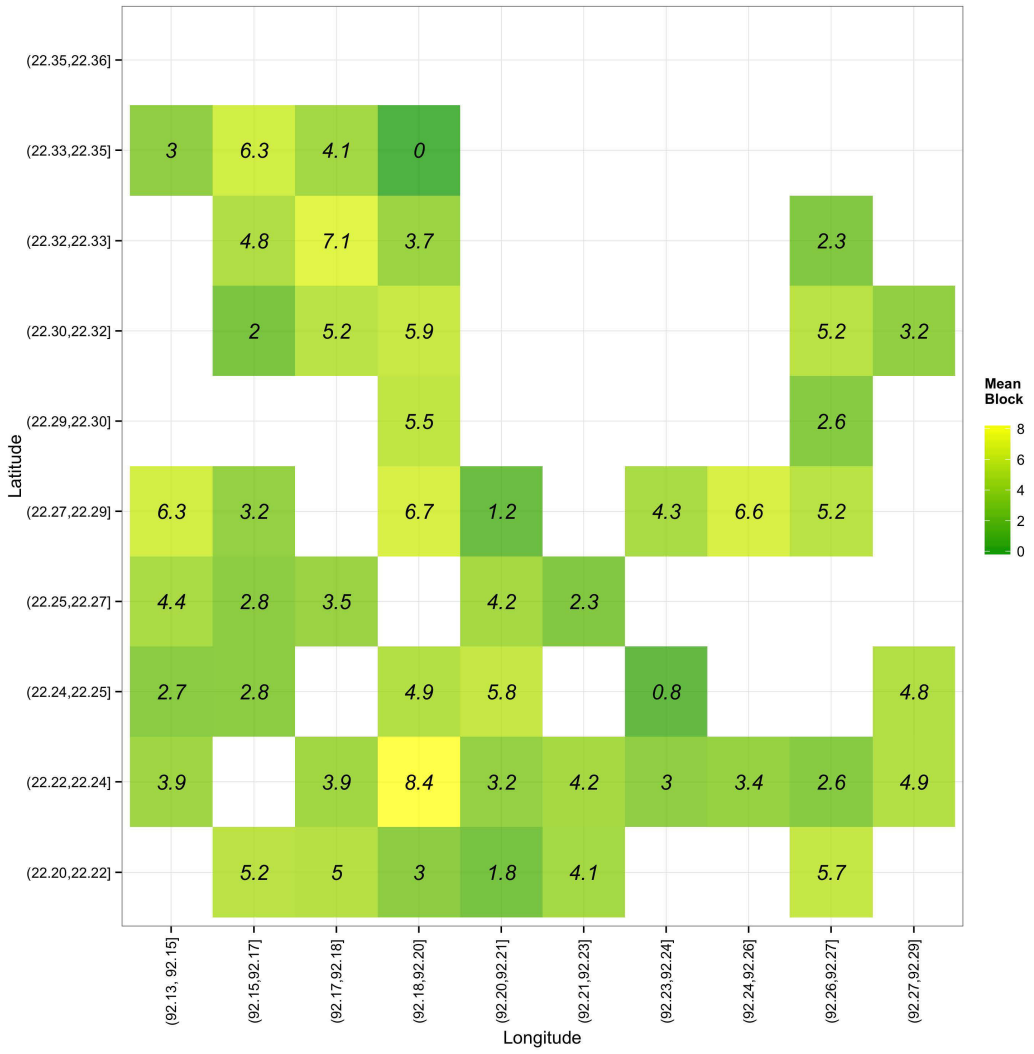


Figure A5.23: Summary of Average Number of *Anopheles* per Visit per Household for Households without Bamboo as Part of Building Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

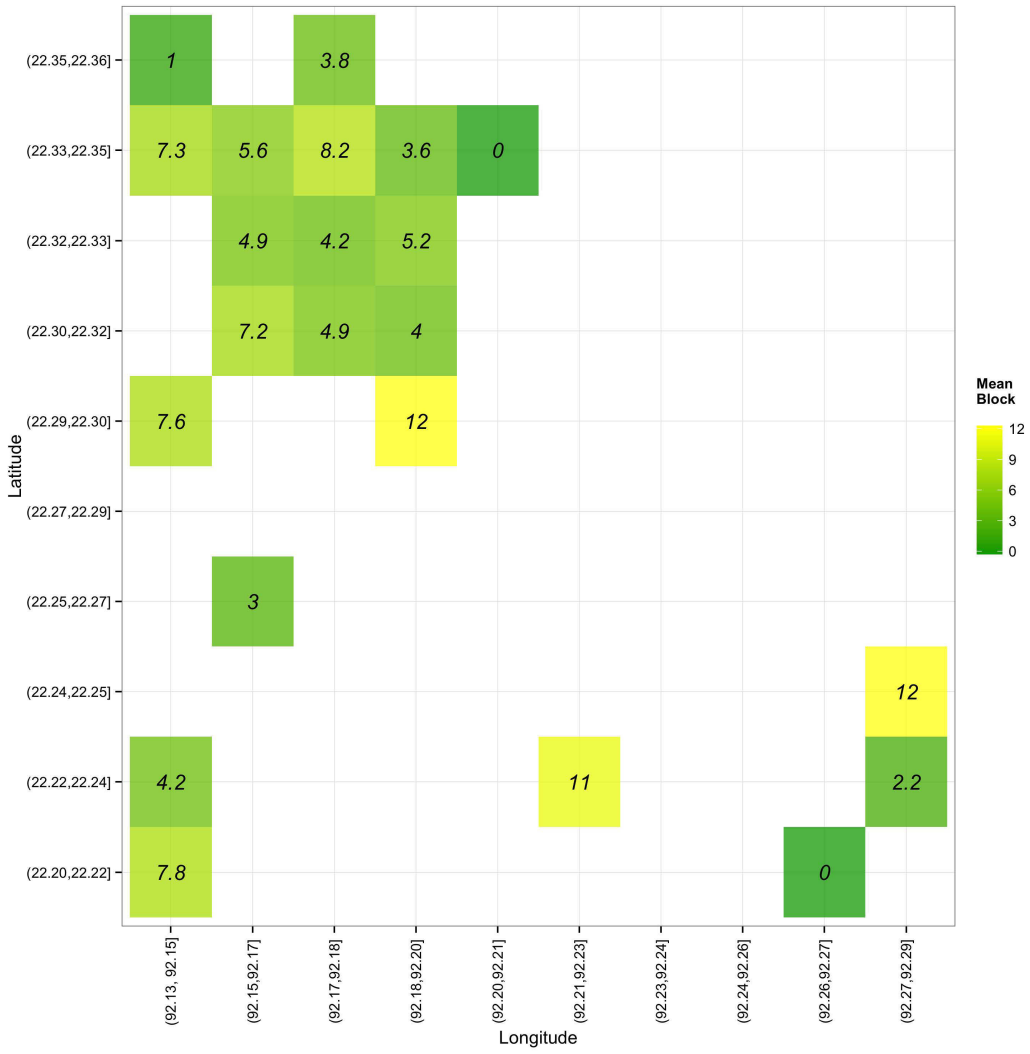


Figure A5.24: Summary of Average Number of *Anopheles* per Visit per Household for Households with Bamboo as Wall Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

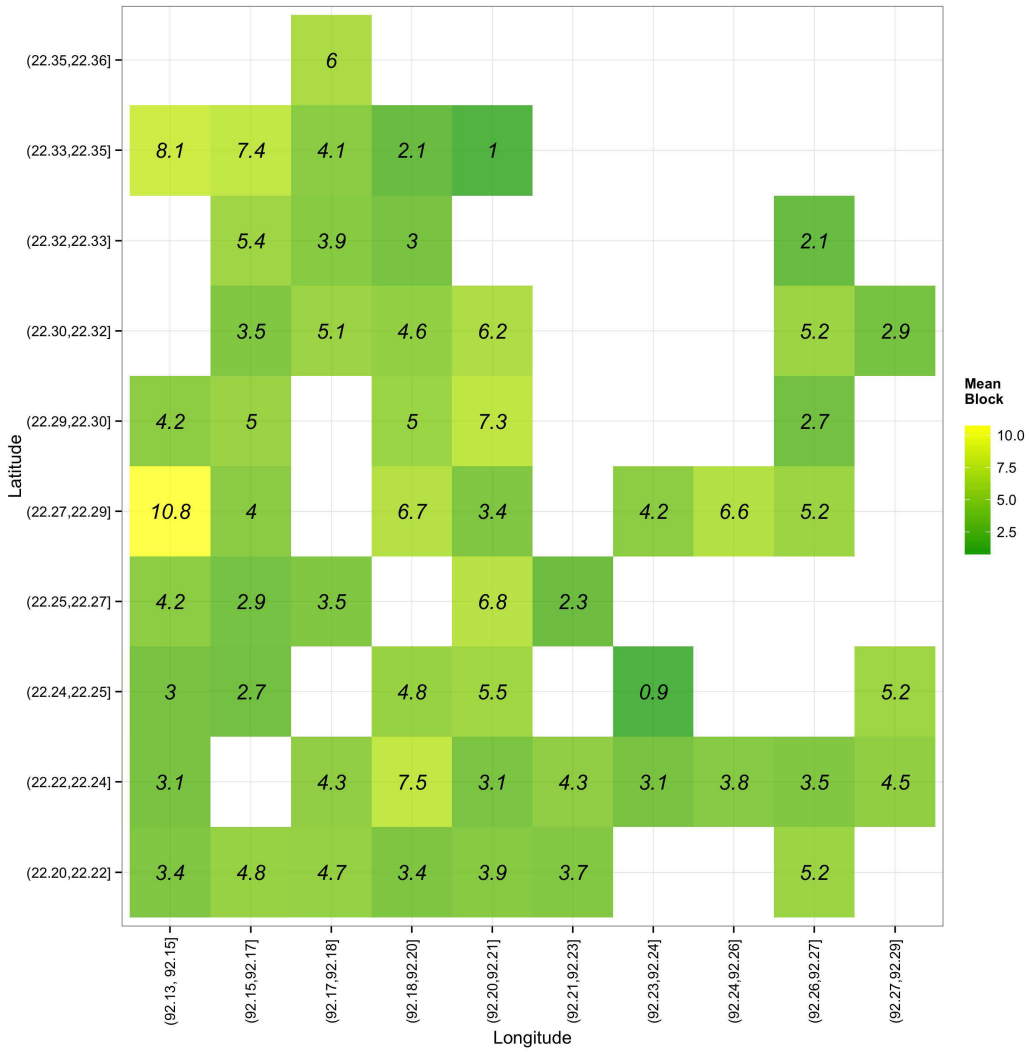


Figure A5.25: Summary of Average Number of *Anopheles* per Visit per Household for Households with Bamboo as Partition Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

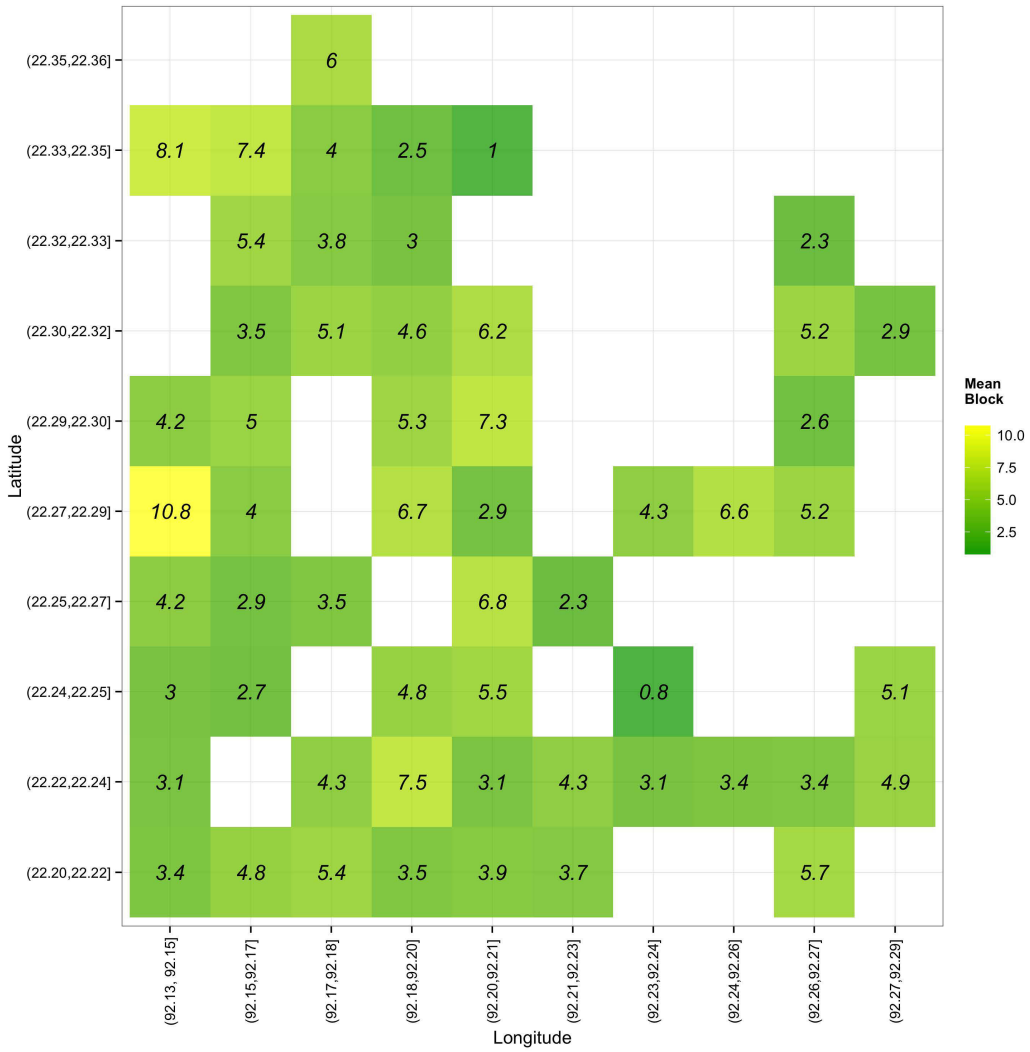


Figure A5.26: Summary of Average Number of *Anopheles* per Visit per Household for Households with Bamboo as Floor Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

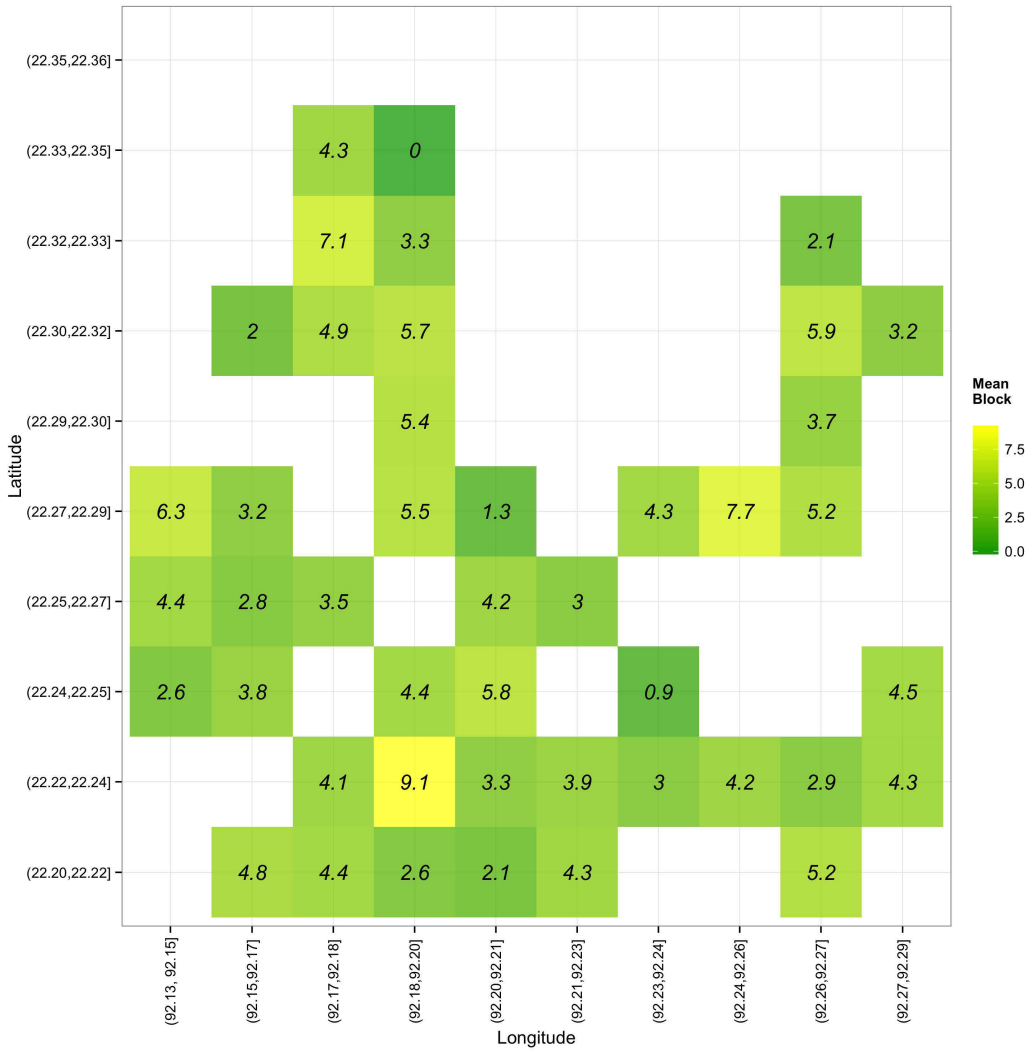


Figure A5.27: Summary of Average Number of *Anopheles* per Visit per Household for Households with Bamboo as part of the Common Combination of Building Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

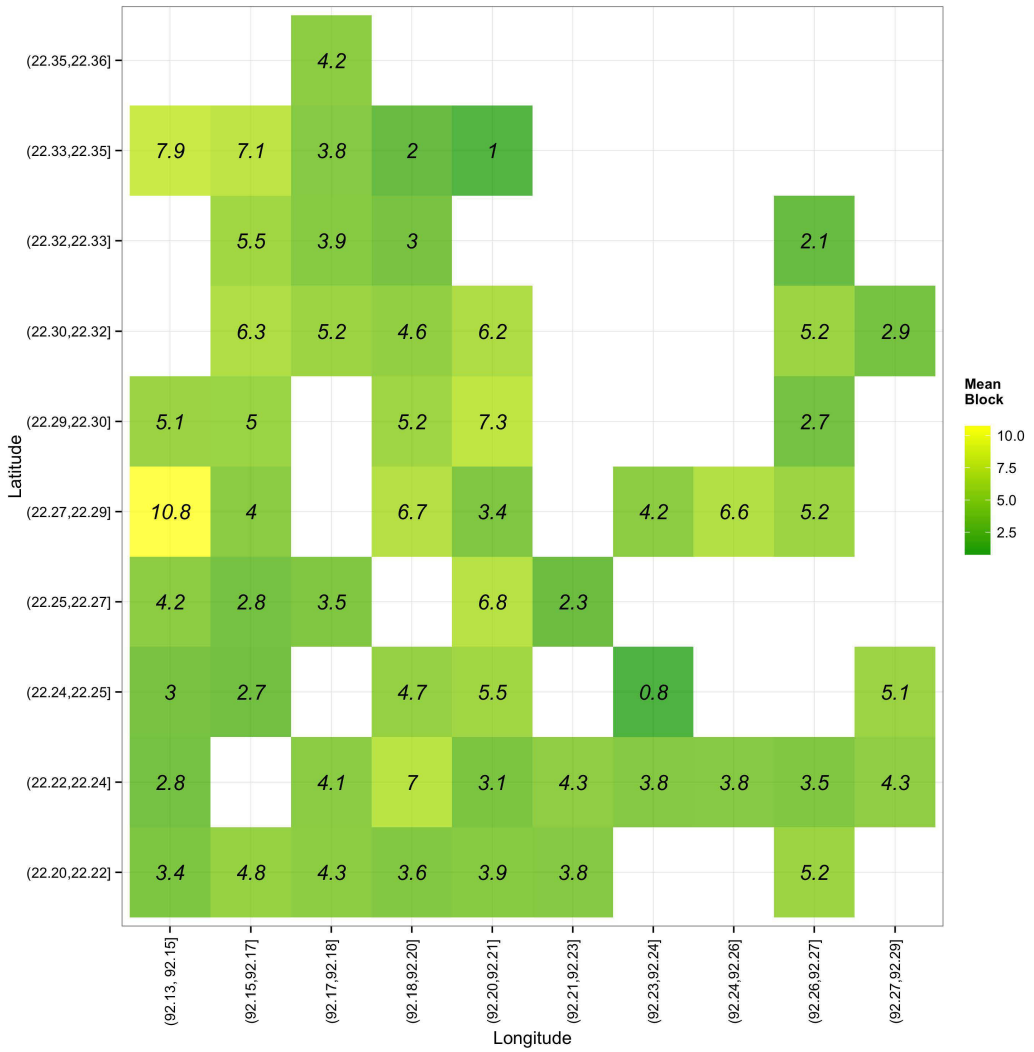


Figure A5.28: Summary of Difference in between Average Number of *Anopheles* per Visit per Household for Households without Bamboo as Wall Materials and Gridded Average Number of *Anopheles* per Visit per Household with Bamboo as Wall Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

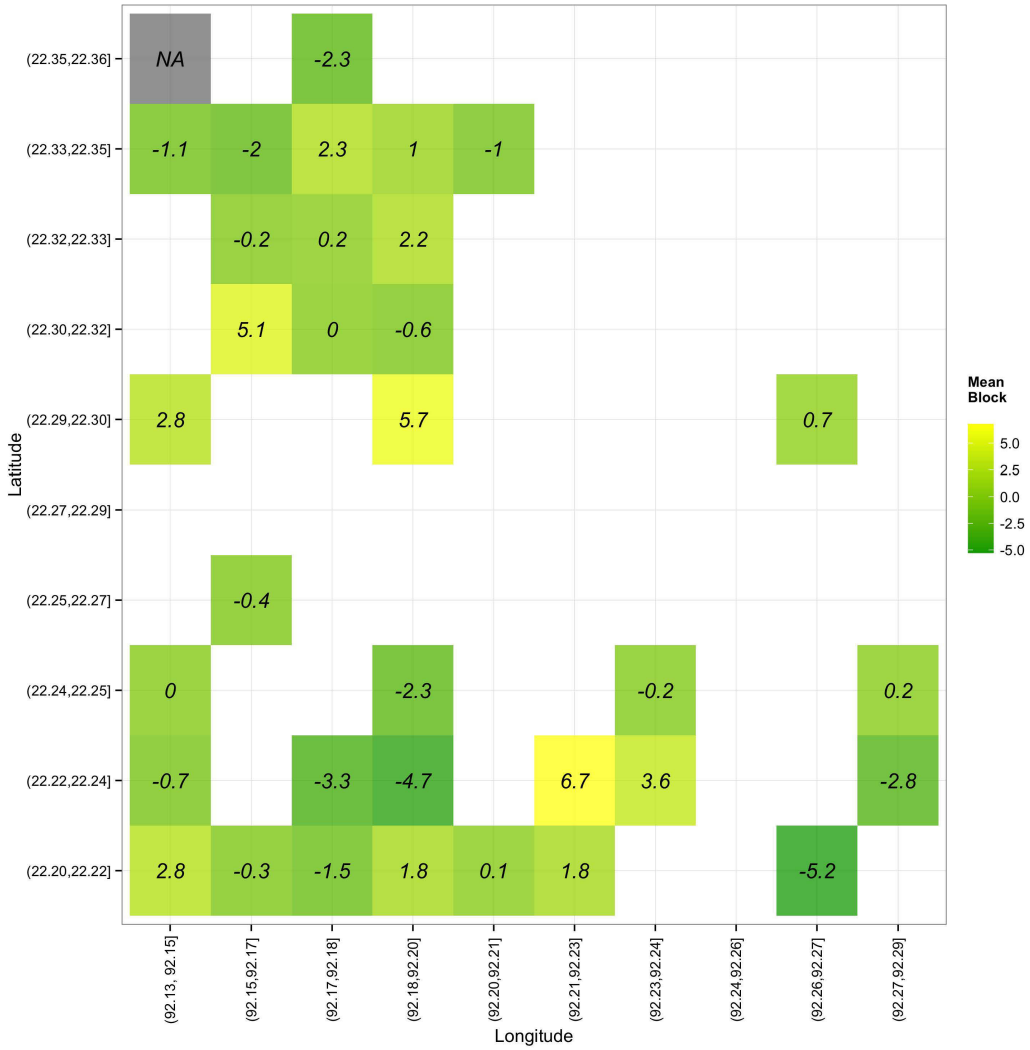


Figure A5.29: Summary of Difference in between Average Number of *Anopheles* per Visit per Household for Households without Bamboo as Floor Materials and Gridded Average Number of *Anopheles* per Visit per Household with Bamboo as Floor Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

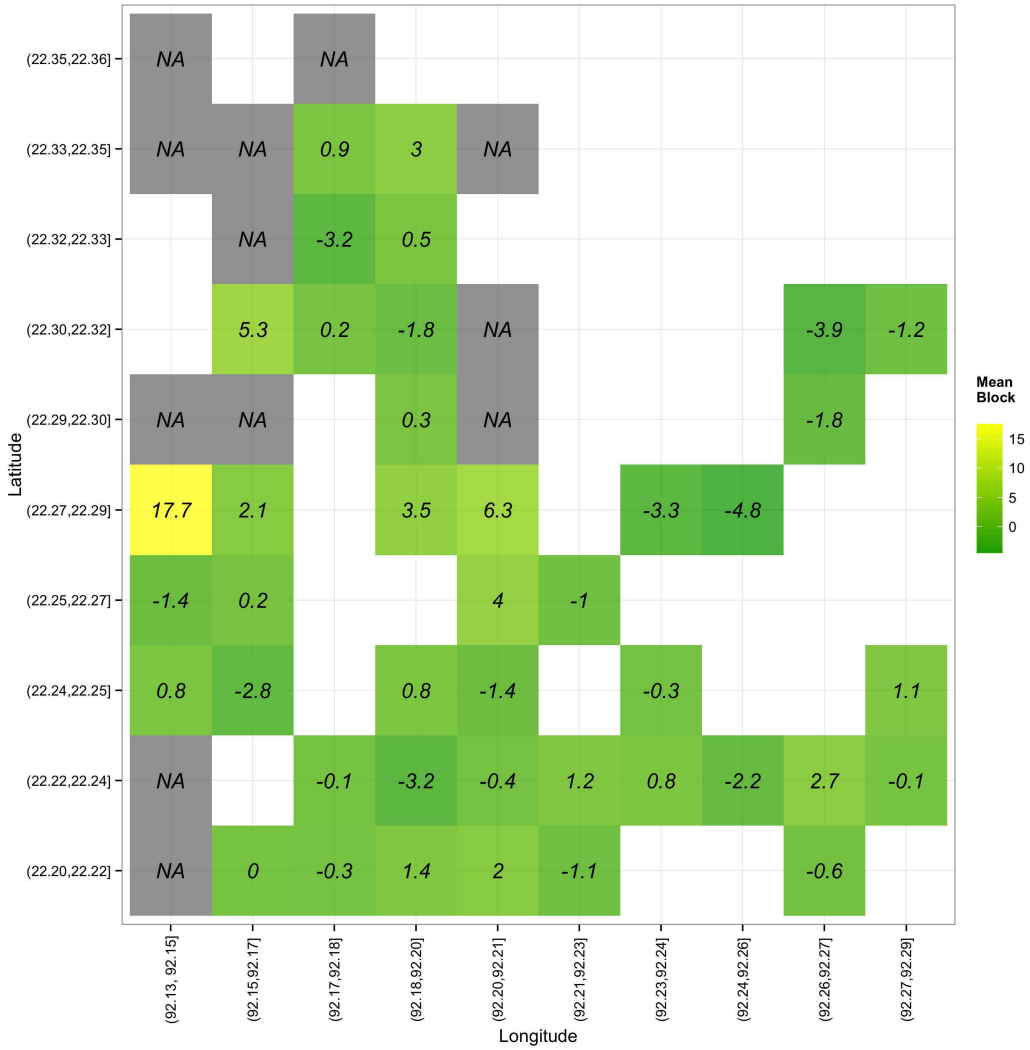


Figure A5.30: Summary of Difference in between Average Number of *Anopheles* per Visit per Household for Households without Bamboo as Partition Materials and Gridded Average Number of *Anopheles* per Visit per Household with Bamboo as Partition Materials, by Latitude and Longitude Grids (Extreme Outliers were Removed)

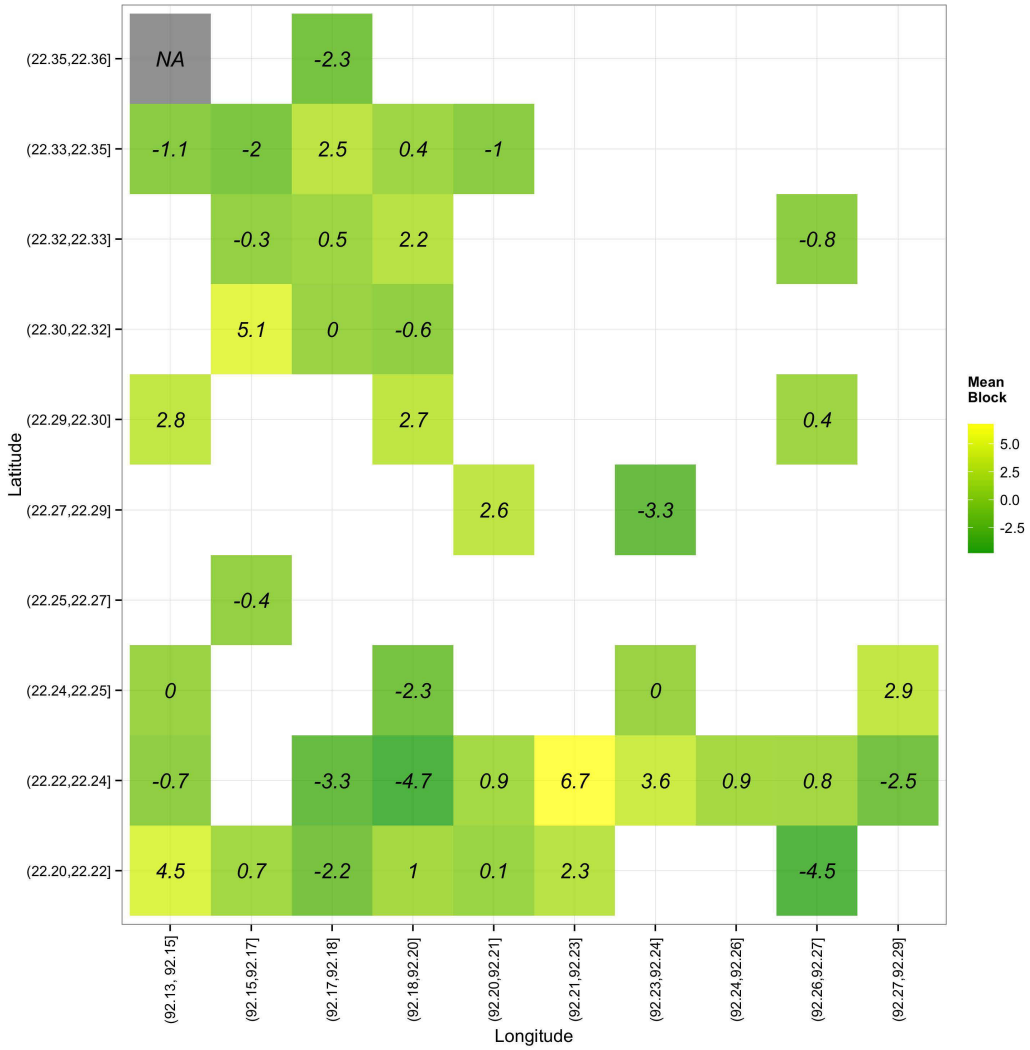


Figure A5.31: Summary of Difference in between Average Number of *Anopheles* per Visit per Household for Households without Bamboo as part of the Common Combination of Building Materials and Gridded Average Number of *Anopheles* per Visit per Household with Bamboo as part of the Common Combination of Building, by Latitude and Longitude Grids (Extreme Outliers were Removed)

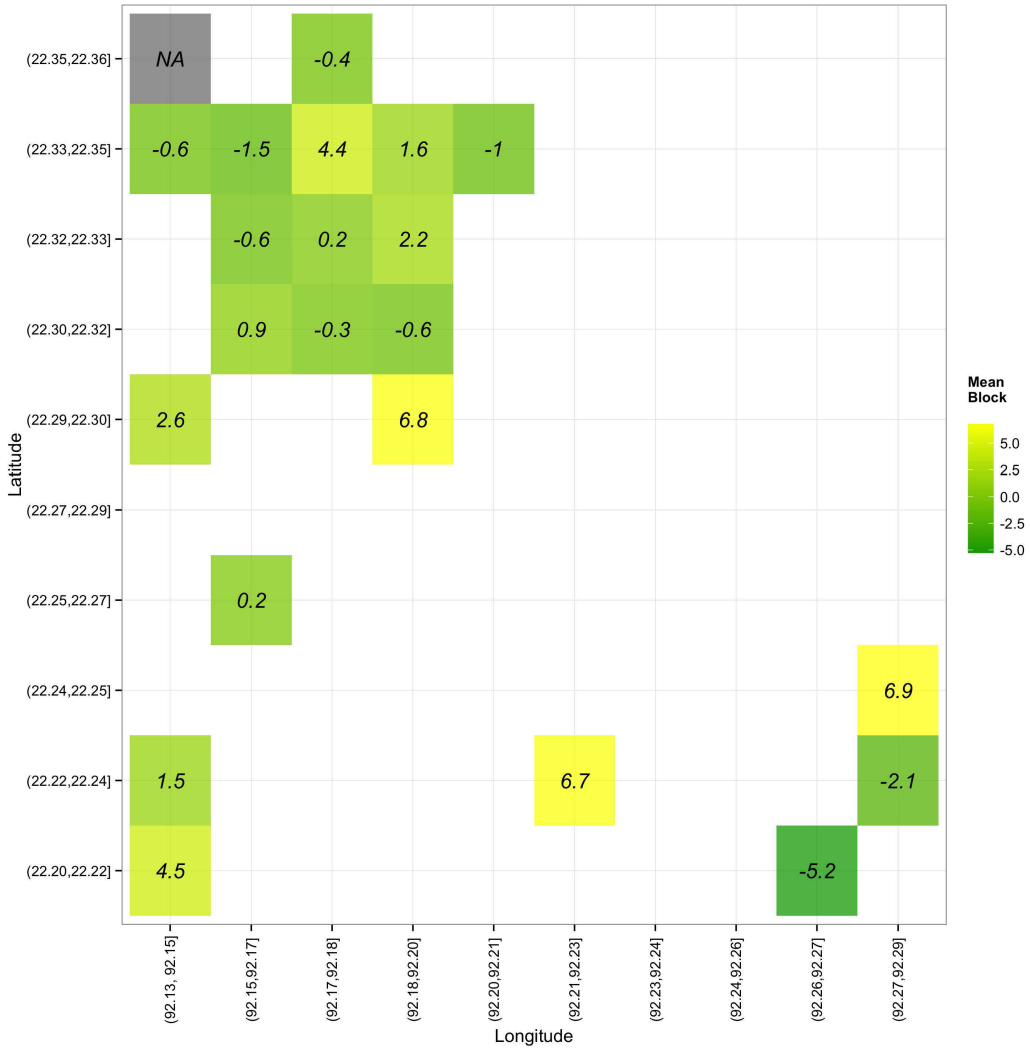
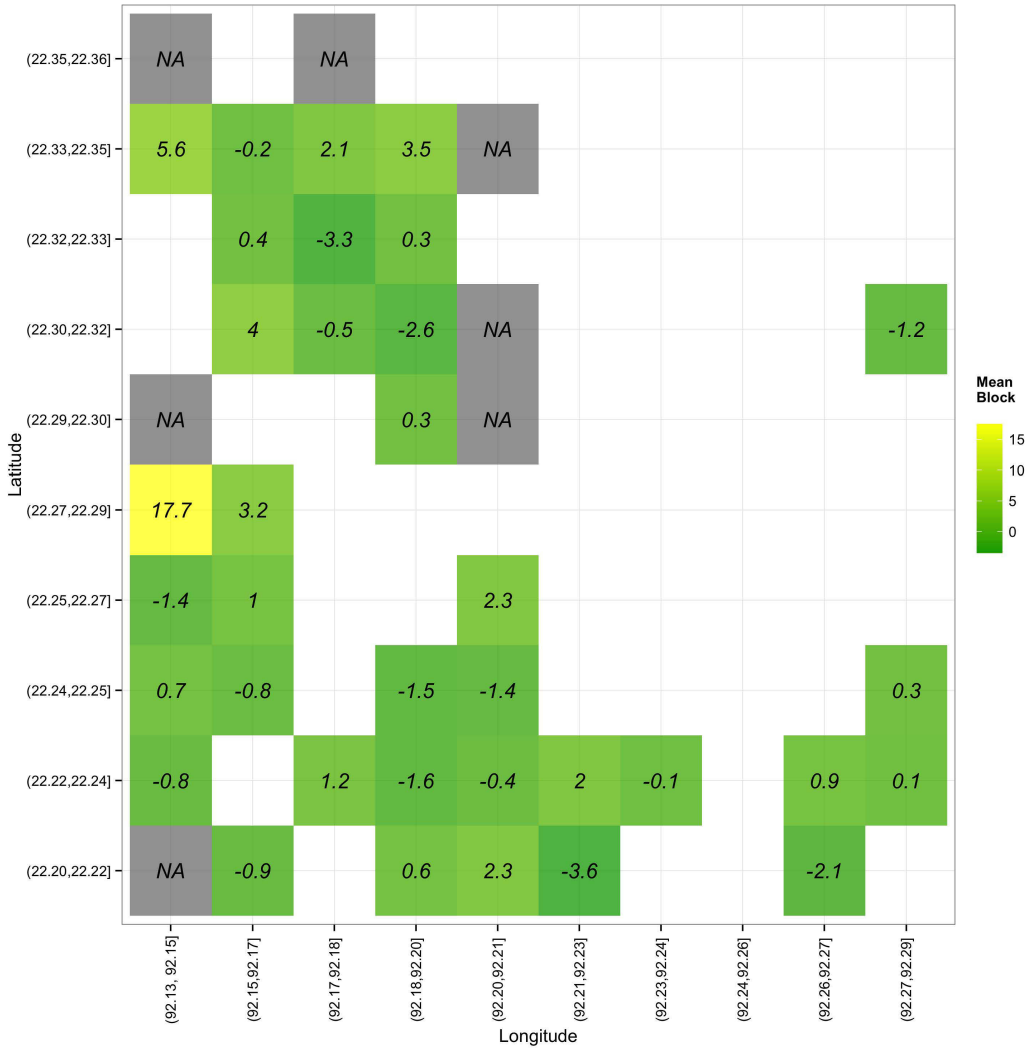


Figure A5.32: Summary of Difference in between Average Number of *Anopheles* per Visit per Household for Households with Mud as part of the Common Combination of Building Materials and Gridded Average Number of *Anopheles* per Visit per Household without Mud as part of the Common Combination of Building, by Latitude and Longitude Grids (Extreme Outliers were Removed)



Chapter 6

Paper 4: Association between Living Standards and Incidence of Human Malaria in southeastern Bangladesh

6.1 Abstract

6.1.1 Background

Southeastern Bangladesh is endemic with Malaria. Among all endemic districts, Bandarban District had one of the highest malaria prevalence. Information on how living standard—including household construction materials and social economic status—is related to malaria incidence is limited in the area.

6.1.2 Methods

A population based surveillance in Bandarban Study Area in Bandarban, Chittagong Hill Tracts, Bangladesh was conducted. The study site consisted of approximately 5,000 households and 22,000 residents from October 2009 to September 2013. We used standard questionnaires to survey of each household's (1) construction materials of its wall, roof, partition, floor and ground elevation, (2) ownership of 33 types of durable assets and (3) its household members' demographic characteristics. Malaria patients were identified through passive surveillance. FalciVax^{TM} Rapid Diagnostic Test (RDT) and Giemsa-Stain Microscopy were collected at home visits. Principal component analysis (PCA) and generalized estimating equation (GEE) Poisson regression were done to study living standards of the study population and risk factors associated with malaria incidence, respectively.

6.1.3 Results

There were 529 individuals tested positive with *Plasmodium falciparum* malaria, and 22,450 local residents surveyed for individual level and household level factors. We found (1) being female, (2) being older in age, (3) being married, (4) being educated, (5) having income from agricultural work not on own land, (6) not having uncultivated land, (7) having own lands cultivated by other, (8) having house on own land, (9) using covered ring well, tube well and private well/pump, (10) using pit latrine and slab toilet with boundary, (11) using electricity as main source of light at home, (11) owning electricity, a TV, a radio, a clock, a fan, a chair, a rickshaw, a tube well, a khat, and a variety store, and (12) not owning a blanket or dheki were some of the factors associated with reduced malaria incidence. (1) Having bamboo as a wall, partition and flooring material, (2) having corrugated tin or iron sheet as a roofing material, as well as (3) having elevated ground floor at home were related to elevated malaria incidence comparing to other types of building materials. Having higher social economic status also lowered the risk of malaria—even after adjusting for household building materials.

6.1.4 Discussion

Although many factors were identified as risk factors for malaria incidence, some of them were related. For example, many residents were married by 15 years old. Factors such as occupation were associated with malaria incidence. However, after limiting the finding to individuals older than 15, the difference across

occupations was not significant. Some associations—such as elevated malaria risk with the use of river/stream as main source of water—could be explained or hypothesized by the suitability of mosquito habitats. Others, like the link among building materials, mosquitoes and malaria incidence, should be further explored.

6.2 Background

Malaria has remained a serious problem worldwide. In 2013, 198 million cases were reported and 584,000 people died of malaria [48]. In Bangladesh, malaria is most prevalent in 13 out of 64 districts. These 13 districts are considered as malaria endemic [66]. Approximately 16.3 million people (11% of the nationwide population) lived in the endemic area [48]. Since a nationwide survey in 2007 [66], prevalence of malaria has gone down [101]. Nonetheless, Bangladesh has prevailed in the control phase—the first of four programmatic phases (i.e. control, pre-elimination, elimination, and prevention) in malaria elimination [3].

To transition from control phase to pre-elimination phase in an endemic area, it is important to examine all aspects of factors attributable to local malaria incidence. These include individual and household level risk factors such as housing construction materials, durable assets and domesticated animals owned by household members. As *Plasmodium falciparum* parasites need *Anopheles* mosquitoes as a vehicle to infect susceptible human subjects, the type of household living environment that attracts *Anopheles* mosquitoes could not be

overlooked. Ownership of durable assets as well as domesticated animals is an indication of living standards of a household. Different living standards not only affect residents' way of life, but could also serve as an indicator of disease risks . Studies focusing on these areas are limited in Bangladesh.

Previous studies in Bangladesh has touched on the variety of *Anopheles* mosquito species presented [139, 142], demographic risk factors [74, 75, 143, 144], weather fluctuation [143, 145] in relation to prevalence or odds of malaria in Chittagong Hill Tracts. Very few prior studies had briefly expressed factors such as mud walls and socioeconomic conditions [76, 144] in regarding to human malaria in endemic areas in Bangladesh. The goal of this paper is to bridge the gap in knowledge regarding housing materials, living standards and the incidence of malaria.

6.3 Methods

6.3.1 Study Location

This study was part of the Mapping Malaria Epidemiology Project in Bangladesh [87]. The study site was composed of Kuhalong Union and Rajbila Union—the smallest government and administrative unit in Bangladesh—in Bandarban District, Chittagong Hill Tracts in southeastern Bangladesh. The study site was approximately 17 kilometers by 17 kilometers in size. It was located in a rural, hilly area where agricultural work was a common practice. The study site housed roughly 5,000 households and 22,000 residence over the study period.

6.3.2 Study Time Frame

Overall, the study was conducted from 2009 to 2013. Starting out in Kuhalong Union in October 2009, the study team expanded its study site to its neighboring Rajbila Union in April 2010. The study continued in both Unions until September 2013.

6.3.3 Study Design

This study combined three sources of data. One was the use of population based surveillance system to detect human malaria. Another was the acquisition of demographic information on all participants. The other was the collection of household surveys on household construction materials, ownership of durable assets and ownership of domesticated animals. All surveys were executed using standardized questionnaires.

Human Malaria

A population-based passive surveillance system was used to detect *Plasmodium falciparum* (*Pf*) malaria among residents in Bandarban Study Area. Residents were told to contact one of the twenty field workers, the medical officer or the project manager if they have fever or other potential symptoms for malaria. Once a member of the study team was informed of an ill resident, it was reported to the project manager. The project manager, then, assigned a field

worker in the area to conduct a home visit. A rapid diagnostic test (RDT), as well as blood samples were collected during the visit. We used FalciVax^{TM} RDT kits at the study site. They provided immediate feedback on malaria test results. Blood samples were used to create thin and thick blood films for Giemsa-stained microscopy, the gold standard method in malaria diagnosis.

Some residents in the Bandarban Study Area entered our passive surveillance system via BRAC, a local non-government organization. When residents contacted BRAC, our project manager would be informed. The project manager followed the same protocol of assigning a field worker to conduct home visits. With informed consents, rapid diagnostic tests and blood samples were collected and examined.

If an individual was tested *Pf* positive by a FalciVax^{TM} RDT or Giemsa-stained microscopy, treatments were provided immediately according to the national guideline [146]. Follow-up visits of malaria positive individuals were done on Day 2, Day 7, and Day 28.

Demographic Survey

At baseline demographic survey, all households in the study area were visited by the study team. We used a standard questionnaire to record all household members' date of birth, gender, marital status, education, occupation, place of employment and full-time employment status. Each individual was assigned a unique identification number during the baseline demographic survey. During

home visits, the unique identification number was confirmed with the tested subject. In addition, date of visit was recorded at the time of malaria testing. Date of visit in conjunction with date of birth of study participants were used to calculated age of participants on the date of malaria diagnostic tests.

Household Construction Materials

All households were enumerated in the Bandarban Study Area. Each household was surveyed once on its housing construction materials during the study period. Head of Households was asked the type of materials used for wall, roof, floor and partition at the house. Types of wall materials included (1) Corrugated tin / iron sheet, (2) fired brick / cement, (3) tins, (4) pole and mud, (5) wood, (6) pole and grass, (7) stone, (8) unfired bricks, and (9) bamboo. Types of roofing materials included (1) straw / thatch, (2) asbestos, (3) pole and grass, (4) pole and mud, (5) bamboo, (6) mud tins / house of tins, (7) corrugated tin / iron sheet, (8) fired brick / cement, and (9) concrete / cement. Types of flooring materials included: (1) Mud, (2) bamboo, (3) semi-cemented, (4) vinyl, (5) cemented, and (6) wood. Types of partition materials included (1) jute stick, (2) wood, (3) concrete / cement, (4) mud, (5) tin, and (6) bamboo. All materials used by fewer than 5 households were combined into "Other" category.

Living Standards

Head of households were interviewed with pre-determined questions on ownership of durable assets in the house. There were 33 items on the list, including

(1) electricity, (2) television, (3) radio, (4) almirah, (5) bad, (6) clock, (7) refrigerator, (8) fan, (9) dining table, (10) telephone, (11) sofa set, (12) chair, (13) sewing machine, (14) blanket, (15) bednet, (16) power tiller, (17) rick mill, (18) rickshaw, (19) bicycle, (20) fishing boat, (21) modern agriculture machines, (22) shallow machine, (23) vehicles to rent out, (24) tube well, (25) crushing mill, (26) khat, (27) reserved clothes, (28) dheki, (29) variety store, (30) fish hatchery, (31) fishing net, (32) live stocks, and (33) poultry.

Additionally, annual income of the household, main source of income, number of dwelling units, style of land cultivation, ownership of a pond, source of water/light/fuel, and type of toilet and cooking location were also documented.

6.3.4 Study Population

Inclusion Criteria of Malaria Cases All individuals lived in Bandarban Study Area (i.e. Kuhalong Union and Rajbila Union in Bandarban, Chittagong Hill Tracts in Bangladesh) during October 2009 and September 2013 were eligible to be part of the study. To be included in the passive surveillance, an individual contacted directly with the study team or indirectly via BRAC.

Exclusion Criteria of Malaria Cases Individuals with only one *Pf* malaria test result (i.e. either from FalciVax^{TM} RDT or Giemsa stained microscopy) were excluded from the analysis. To avoid effects from malaria medication, follow-up examinations done on Day 2, Day 7 and Day 28 were not included in

the analysis. Only test results done on Day 0 were included in the final statistical analysis.

Final Study Population There were 22,325 participants in the study area at baseline. From October 2009 to September 2013, 616 individuals at the Bandarban Study Area were tested with both RDT and microscopy for *Pf* malaria on Day 0 of the passive surveillance.

6.3.5 Malaria Definition

There are many brands of rapid diagnostic devices on the market. Due to potential variations in RDT field performance, we chose to use Giemsa-stained microscopy as the sole standard for identifying malaria positive individuals in the statistical analysis of this paper—with the aim to make results of this study more applicable to areas without FalciVax^{TM} RDT.

6.3.6 Statistical Analysis

Variables of Interest

Exposure Exposure of interests was analyzed at individual level. Therefore, non-demographic risk factors measured at household level (i.e. household construction materials) or answered by household representatives (i.e. durable assets) were assumed to be identical across all members of the same family.

Thirty-three (33) types of durable assets were recorded by the questionnaire. We were interested in not only the impact of individual items but also their combined effect on malaria incidence. Therefore, for the combined effect, we utilized principal component analysis (PCA) [147, 148] to create a score as an indicator of the living standards of study participants.

Outcome We were interested in the effect of exposure on overall malaria incidence, as well as its impact on severe malaria cases. Therefore, we used the median parasite density examined by microscopy as a cut-off. For *Pf* malaria positive individuals with parasite density higher than the median, they were labeled as severe cases. The rest of the *Pf* malaria positive individuals were labeled as non-severe cases.

Regression Analysis

We used Generalized Estimating Equation (GEE) Poisson regression to estimate the log incidence rate of *Pf* malaria at the Bandarban Study Area. Poisson regression [149, 150] enabled the authors to analyze malaria cases as count data, with their person-time exposure calculated by number of days a person was in the study till the day of malaria examination. It was possible for an individual to have multiple malaria episodes (e.g. in different malaria seasons). There could also be unmeasured correlation between household members. Therefore, we applied Generalized Estimating Equation [151] to account for potential correlation among members from the same household and within the same individuals.

Software

Collected data were entered into and maintained in Microsoft Access 2007 (Redmond, WA). Data cleaning and analyses in this paper were performed in R version 3.0.2 [89].

6.4 Results

During the study period, 616 individual records were recorded through passive surveillance and were tested by both FalciVax^{TM} RDT and Giemsa stained microscopy. Out of 616 records, 529 of them (86%) were tested *Pf* positive by microscopy, the gold standard. However, not all records came from unique individuals. These 616 records were contributed by 558 unique participants at Bandarban Study Area. Fifty-one of the 558 participants (9%) had malaria tested for more than once, and recorded as *Day 0* in the surveillance system. Among these 51 individuals, 46 (90%), 4 (8%) and 1 (2%) of them had 2, 3, and 5 *Day 0* visits, respectively, from 2009 to 2013. As all records from the same participants were tested at least a month and a half apart, we considered these cases as separate episodes. Laboratory data and all surveys mentioned in the Methods Section were merged based on each participant's identification number. There were 22,450 rows of individual records used for analyses. To accommodate the non-uniqueness of malaria tested subjects, we incorporated GEE as a way to control for unmeasured correlation among members from the same household and within same participants.

6.4.1 Human Demographic Factors

The male to female ratio at the Bandarban Study Area was 0.99 : 1 (Male: N = 11,143; Female: N = 11,307). We have found females were 18% less likely to have *Pf* malaria than males (IRR= 0.82, 95% CI: [0.68, 0.99]) (Table 6.1). Other than being a female, being older in age, being married and having higher education were also protective against malaria (Tables 6.1 and 6.2). When age was viewed as a continuous variable, the chance of acquiring malaria is lower year after year as one aged (IRR = 0.99, 95% CI: [0.98, 0.99]). When looking into malaria incidence by age group, people who were 15 to 25 years old had 0.56 times the malaria incidence than individuals aged 5 to 15 years old (IRR = 0.56, 95% CI: [0.42, 0.76]). However, malaria incidence rate ratio comparing children under 5 and individuals aged 5 to 15 years old were not statistically significant (Table 6.1). Majority of the resident were married by age 15. During the study period, 10,682 people (48% of all population) were married and 10,485 were single (47%). People who were single had 53% more chance of acquiring malaria than people who were married (IRR = 1.53, 95% CI: [1.27, 1.85]) (Table 6.1). Residents at the study area had limited education. The median and mean years of education were 0 and 2.8 years, respectively, for people aged 15 and above. Top 25% of the 15 years and older population had more than 5 years of education. If we looked at the entire study population, having a secondary education or above (6-12 years of education, N = 3,163) was significantly beneficial in reducing malaria incidence compared to residents with no education (N = 11,476) (Table 6.2).

In terms of occupation, twenty percent of the population were unemployed at the time of the study (N = 4,554). Individuals who were unemployed had higher incidence of having malaria than housewives (N = 2,061) (IRR = 2.00, 95% CI: [1.37, 2.94]) and people who farmed at their own lands (N = 4,126) (IRR = 1.67, 95% CI: [1.23, 2.27]) (Table 6.2). For people who performed agriculture work on other people's land as the main source of income had 40% reduction in malaria incidence than those who did agriculture work on their own land (IRR = 0.61, 95% CI: [0.39, 0.94]) (Table 6.3). However, if a privately owned land is cultivated by others, the owners of the land were 60% less likely to have newly diagnosed malaria per person-time than people who own lands but not cultivated by others (IRR = 0.40, 95% CI: [0.24, 0.67]). If a privately owned land was left abandoned, the owners are 46% more likely to have malaria than those who cultivate their lands (logIRR = 1.46, 95% CI: [1.13, 1.89]) (Table 6.4).

Participants at the study area that didn't have a house on their owned land (N = 824) had almost twice the malaria incidence as those who had a house on their owned land (N = 21,457) (IRR = 1.98, 95% CI: [1.33, 2.95]) (Table 6.4).

Having a covered ring well (IRR = 0.49, 95% CI: [0.35, 0.69]), tube well (IRR = 0.15, 95% CI: [0.11, 0.21]) or a private well / pump (IRR= 0.23, 95% CI: [0.09, 0.63]) had reduced malaria incidence up to 85 percent comparing to residents used river and stream as their main source of water. Having pit latrine (IRR = 0.42, 95% CI: [0.30, 0.59]) and slab toilet with boundary (IRR = 0.39, 95% CI: [0.28, 0.54]) was associated with lower malaria incidence as compared to open defecation. Last but not least, houses that were powered by electricity

(number of residents: N = 2,396) comparing to the ones lit by oil lamps (number of residents: N = 17,797) as main source of light has shown to help decrease their residents' likelihood of having malaria (IRR = 0.22, 95% CI: [0.12, 0.44]). (Table 6.5)

6.4.2 Household Construction Material

Individuals lived in a house built with bamboo walls (N = 18,339) were 10 times more likely to have malaria than people who had a fired brick / cemented wall (N = 725) (IRR = 10.00, 95% CI: [2.38, 50.00]), and nearly twice as likely to those who live in houses with pole and mud walls (N = 3,075) (IRR = 1.85, 95% CI: [1.16, 2.94]). Similar conclusion can be drawn comparing partition materials used in the house: Having a concrete / cement (number of residents: N = 687) (IRR = 0.11, 95% CI: [0.03, 0.46]) or mud (number of residents: N = 3,015) (IRR = 0.60, 95% CI: [0.38, 0.94]) built partition in the house statistically significantly lowered the malaria incidence for individuals than those lived in houses with bamboo built partitions (N = 17,640). (Table 6.6)

As for flooring materials, individuals living in houses with bamboo floors (N = 7,725) had 111% increase in malaria risk (IRR = 2.11, 95% CI: [1.62, 2.75]) compared to those lived in mud flooring houses (N = 11,250). Participants who lived in cemented flooring houses (N = 991) had shown a statistically significantly lower incidence among the group than those who lived in a mud flooring households (IRR = 0.35, 95% CI: [0.12, 0.99]). Among all roofing materials used in the study site, corrugated tin and iron sheet roofs were the most popular kind

(number of residents: N = 14,904), followed by straw and thatch roofs (number of residents: N = 6,766). The study team has recognized houses with straw / thatch roofs were associated with 1.34 times the malaria incidence among residents than those lived under corrugated tin / iron sheet roofs (IRR = 1.34, 95% CI: [1.04, 1.73]). (Table 6.6)

Approximately half of the study population (N = 9,577 (43%)) lived in houses with elevated ground foundation. This was to prevent flooding during monsoon season and/or to keep domesticated animals underneath elevated houses. The other half of the study population (N = 12,873 (57%)) lived in houses built directly on ground surface. Having lived in houses with elevated ground floor was associated with 2.14 times the malaria incidence among residents than those who lived in houses that were built on the ground (IRR = 2.14, 95% CI: [1.67, 2.75]). (Table 6.7)

6.4.3 Living Standards

Descriptive

Out of 22,450 records in the study area, 22,300 of them (99.33%) had access to a bednet in the household. More than 50% of the residents in the study area have a clock (N = 13,044 (58.10%)) or a blanket (N = 18,380 (81.87%)) at home. Thirty to forty percent of the individuals at the study area have access to a bed (N = 10,536 (46.93%)), a telephone (N = 7,702 (34.31%)), a chair (N = 8,553 (38.10%)), a khat (N = 7,191 (32.03%)), reserved clothes (N = 8,719 (38.84%)), live stocks (N = 9,957 (44.35%)) and poultry (N = 10,781 (48.02%))

at home. Approximately 10 to 20 percent of the study population has access to electricity (N = 2,556 (11.39%)), a television (N = 3,125 (13.92%)), a radio (N = 4,123 (18.37%)), an almirah (N = 4,715 (21.00%)), a fan (N = 2,046 (9.11%)). Items such as bicycles (N = 646 (2.88%)), dining tables (N = 893 (3.98%)) and refrigerators (N = 157 (0.70%)) are rarities in the area (Table 6.8).

Individual Durable Asset

Among items owned by more than 5% of the residents, having electricity (IRR = 0.25, 95% CI: [0.13, 0.45]), a TV (IRR = 0.50, 95% CI: [0.33, 0.78]), a radio (IRR = 0.64, 95% CI: [0.45, 0.91]), a clock (IRR = 0.65, 95% CI: [0.51, 0.83]), a fan (IRR = 0.30, 95% CI: [0.16, 0.57]), a chair (IRR = 0.38, 95% CI: [0.28, 0.52]), a tube well (IRR = 0.12, 95% CI: [0.04, 0.32]) and a khat (IRR = 0.40, 95% CI: [0.29, 0.55]) were associated with having lower risk of having malaria comparing to those who didn't own these items. On the other hand, not having a blanket (IRR = 0.63, 95% CI: [0.42 , 0.94]) was more protective from malaria than having a blanket. (Tables 6.9 to 6.12)

Combined Effect of Durable Assets: PCA

We focused on the 16 items that were owned by more than 5% of the study population and ran the Principal Component Analysis. The only exception was having access to a bednet at home. We excluded bednets as one of the PCA items due to 99.33% of individuals had access to one bednet at home, which provided very little variation (standard deviation of 0.08). (Table 6.8)

If we call the ownership of having the 16 durable assets an indicator of living standards, the first principal component explained 30% of its variability (Table 6.13, Figure 6.1). Negative loading of principal components showed the scores calculated by PCA was indicating the degree of "lack of wealth" (Table 6.8). In other words, the higher a participant's score was, the worse the person's living condition was. The overall distribution of PC 1 was skewed to the left. That is, many more individuals lived in worse living conditions (higher *PC 1* scores) than in better living conditions (lower *PC 1* scores).

From GEE Poisson regressions, we saw people with lower living standards (higher *PC 1* scores) had 1.39 times the malaria incidence rate than those who lived in a higher living standards (lower *PC 1* scores) (IRR = 1.39, 95% CI: [1.21, 1.59]) (Table 6.14). If we used individuals at the highest 20 percentile in living standards (i.e. lowest 20% in *PC 1* scores) as a reference, we could find statistically significantly lower malaria incidence in participants living in those conditions than the ones lived in lower standards (i.e. higher *PC 1* scores). This is true for participants who had a living condition at the middle 40 to 60 percentile (IRR = 2.35, 95% CI: [1.51, 3.65]), lower 20-40th percentile (IRR = 2.37, 95% CI: [1.53, 3.68]) and the bottom 20 percentile in living standards (IRR = 2.28, 95% CI: [1.46, 3.56]). However, difference in malaria incidence was not apparent between participants living in the top 20% and 60-80th percentile of living conditions (i.e. between bottom 20% and 20-40th percentile of *PC1*) (IRR = 1.42, 95% CI: [0.87, 2.30]). (Table 6.15)

6.4.4 Living Standards and Household Construction Materials

After looking at the living standards and household construction materials separately, the authors combined the indicators and looked at their joint impact on study participants' malaria incidence.

Comparing to people who live in houses with the same type of wall, roof, partition and floor, participants with a lower living standards (ie. higher *PC 1* scores) had a statistically significantly higher malaria incidence by 1.19 (IRR = 0.19, 95% CI: [1.00, 1.42]) to 1.35 (IRR = 1.35, 95% CI: [1.17, 1.56]) times than those with a higher living standards (i.e. lower *PC 1* scores). Among individuals with the same living standards (i.e. same *PC 1* scores), living in a bamboo walled house is still associated with 6.6 times the malaria incidence comparing to individuals living in a house with fired brick/cemented walls (IRR = 6.67, 95% CI: [1.59, 25.00]). Floors and partitions made out of bamboos remained a risk factor in introducing higher malaria incidence among residents comparing to those resided in houses with mud floors (IRR = 1.91, 95% CI: [1.41, 2.59]) and concrete / cemented partitions (IRR = 6.25, 95% CI: [1.49, 25.00]), after controlling for individuals' living standards. Straw and thatch roof, on the other hand, is no longer statistically significantly different from corrugated tin and iron sheet roof in posing higher malaria risks for residents, comparing those with the same living standards (Tables 6.16 and 6.17). Finally, in the presence of the same living standards, malaria incidence remained statistically significantly higher among those lived in houses with elevated ground to those

without (IRR = 1.91, 95% CI: [1.44, 2.52]) (Table 6.18).

6.5 Discussion

We followed residents in the Bandarban Study Area from October 2009 to September 2013. We enumerated all households and residents and provided them with unique IDs. We surveyed all residents on their demographic characteristics, household building materials and household assets. Individuals who felt ill were visited by the study team. Malaria was then tested. With the presence of malaria, treatment was provided. This method has provided us with strength in population based surveillance, high accessibility via home visits, immediate diagnosis, treatment and follow-ups (if $Pf(+)$). However, the reporting of malaria relied on individuals to be health conscious and be aware of malaria to contact the study team. Our long term presence at the study site, having field workers on the ground every day and our collaboration with BRAC, hopefully, helped reduce the possibility of under reporting of unwell residents.

With the population based surveillance under Mapping Malaria Epidemiology Project [87], we were able to incorporate all residents in the study area to calculate the malaria incidence. We used their time in the study to calculate exposure time. However, not all residents were enumerated on day 1 of the study. Therefore, exposure time of each resident was depend upon their "entry" into the study—that is, the first day a household and its members were surveyed with baseline characteristics. The unmeasured exposure dates prior to the start of

the study and prior to the initial household survey were truncated. Using the first day of baseline survey as the starting date of each household ensured all members of the household were informed about the study and had provided consent during the initial visit.

We could had applied the same analysis from this paper to individuals in the active and nested longitudinal surveillance. Active and nested longitudinal surveillance had a much smaller selected study population from 2009 to 2013. The selection process was described by Khan and colleagues [87]. With the same analysis across passive, active and nested longitudinal surveillance, we could discuss the meaning of household assets, building materials and individual characteristics in relation to malaria in each scenarios. A concern in doing so would be the sample size. In this paper, we utilized the information gathered from then entire population, which consisted of more than twenty-two thousand people. From passive surveillance, we identified 529 malaria positive individuals during home visits. During the same study time frame, our active surveillance project diagnosed 28 out of 2737 selected residents with malaria. With 28 malaria positive individuals, informative estimation could be harder to come by when studying the association between social economic status, living standards and the presence of malaria. Hence, the incidence calculated from this paper was viewed as a proxy of "true" malaria incidence. Results provided from this paper, thus, could only serve as an initial guide. More study is still needed. The association between risk factors and malaria incidence could not and should not be deemed as causation.

One advantage of this paper was the use of both household level and individual level factors. Individual level factors included not only gender and age, but also year of education, material status and more. Household level factors incorporated household building materials, assets, source of water, type of toilet and many others that could affect living standards. Factors from both levels have broadened our spectrum in analyzing how living standards could associate with risk of malaria. We applied each household level factor to every member of the household for analysis. This was done based on the assumption that every member in the same household shared the same resources. For example, each member of a household was exposed to the same household building materials and shared the same source of water, etc.. We used generalized estimating equation (GEE) to take into account the unmeasured correlation between household members.

For household level factors, we chose to use GEE [151] instead of multilevel models [152], since GEE models are more stable with relatively smaller cluster sizes. The two Unions—Kuhalong Union and Rajbila Union—in the study site were adjacent to each other. Both had similar population composition and living conditions. Therefore, analyses were not performed by Union. One limitation, however, was the frequency these independent factors were surveyed. Baseline demographic factors, household building materials and social economic factors were surveyed once. The change in ownership of household assets, if any, for example, would not be reflected in the analysis. Although this one-time baseline survey prevented us from understanding the change in relation between social economic factors and malaria incidence over time, it was also not likely living

standards would change over night in Bandarban Study Area, a rural and resource poor setting. Therefore, the individual and household level independent factors were assumed to be constant during the study period.

During data collection, we also noticed some (51) individuals were tested with malaria more than once. Since the tests were performed at least 1.5 months apart, we took these cases to be separate episodes. From 2009 to 2013, 90% (N = 46) of them had two records. The other 5 were tested 3 (N = 4) or 5 (N = 1) times. As the number of additional 58 records from non-unique individuals only encompassed a small proportion (< 1%) of 22,450 records from the entire study population. We believe it was sufficient to simply rely on GEE to take into account the within person and within household variability. Nonetheless, it would be interesting to understand why some individuals were more likely to have multiple malaria testing records than others. Was it because these 51 individuals were more health conscious? Or, was there another factor that triggered the malaria testing? These questions could be answer by future studies.

From GEE Poisson regressions, we found age, gender, marital status and year of education were related to risk of malaria. To be more specific, older in age, being a female, married and having more than elementary school level education were associated with lower malaria incidence. Although the gender analysis indicated females were 2% less likely to have malaria than males, the low malaria prevalence (1-2%) in Bandarban Study Area made the actual difference in number negligible. As for age, it's not surprising elder individuals are less prone to malaria. Immunity and constant environmental exposure to malaria could make

local residents less susceptible to the disease. On the other hand, asymptomatic malaria cases could be more likely hidden within elderly population. This assumption could be further tested using our active surveillance system. From the analysis, we found single residents were associated with higher malaria incidence. In fact, most residents in Bandarban Study Area were married by 15 years old. Therefore, younger in age and being single were correlated. As mentioned above, age was also a risk factor associated with malaria incidence. Hence, the association between marital status and malaria incidence could not be lightly taken by its face value.

Bandarban Study Area was a community were education was limited. Mean and median years of education were less than 3 years. We found individuals with no education and individuals having 6 years or fewer years of education did not have significant difference in malaria incidence. These two groups of individuals also had the highest malaria incidence comparing to others with more than elementary school level education. Having equivalent of middle and high school education was significantly associated with lower incidence. In this paper, we did not discuss whether malaria prevention, treatment and control were taught during higher education process. However, we would like to think individuals with higher education could comprehend malaria-related information better, if informed. Therefore, they know how to prevent and seek treatment at the first sign of malaria. There were 166 individuals ($< 1\%$ of population in Bandarban Study Area) had more than 12 years of education. There malaria incidence was also lower than individuals with limited education. Significance was, nonetheless, not found. This could be resulted from limited sample with only 1 person

in this group acquired malaria during study period.

We followed the analysis by looking at the association between malaria incidence and occupation, as well as location of resident's workplace. With occupation, we found being unemployed and being students were not significantly different in attributing to malaria risk. Being unemployed had significantly higher malaria incidence than being housewives, farming on own lands, having day labor jobs (severe only) and other occupations (severe only). One of the reasons behind it could be its association with age. Both unemployed population and students were trended younger than other occupation. Fifty-seven percent (79%) of the unemployed and eighty-three percent (83%) of the students were under 15 years of age. This was in contrast to having less than 1% of housewives, 1.4% of farming on own lands, 2.9% of day labor and 1.9% of other occupation at the same age range. If we focused the GEE Poisson regression on individuals older than 15 years of age, having *Other* occupation had significantly higher malaria incidence than individuals farming on their own lands (IRR = 1.67, 95% CI: [1.14, 2.44]). Otherwise, the difference in malaria incidence across occupations were no longer seen (data not shown). Higher malaria incidence in the *Other* occupation group could potentially be attributed to individuals who were Jhum cultivators. Jhum cultivation is a mean of practice in agriculture. Cultivators slash-and-burn the land to create vegetation field. Migration and shifting between lands were seen among Jhum cultivators.

With place of employment, we found individuals worked at *Union of Residence* or *Other Union* had significant lower malaria incidence than those identified

their workplace as *Not Applicable*. *Not Applicable* included individuals who were unemployed (N = 4554), housewives (N = 2061), students (N = 5156), disabled (N = 11), retired (N = 7) and with other occupation (N = 16). Similar to above, if we narrowed the analysis down to individuals who were 15 years and older, this overall association between place of employment and malaria incidence changed. In this case, we found working at *Union of Residence* (N = 10044) had 1.43 times the malaria incidence than *Not Applicable* (N = 3957) (IRR = 1.43, 95% CI: [1.06, 1.93]) (data not shown). The overall association between place of employment and malaria incidence across all age groups could had been driven by the high proportion of unemployment individuals and students who were younger in age. The reverse in the association among individuals 15 and older could be related but not limited to (1) having more educated individuals (6+ years of education) in *Not Applicable* group (30%) than in working at *Union of Residence* group (14%) and (2) having majority of Jhum cultivators working in the *Union of Residence*. That is, 99% of Jhum cultivators identified themselves as working at *Union of Residence* and none in the *Not Applicable* group. Among 1019 Jhum cultivators working at the *Union of Residence*, 47 of whom (5%) had positive *Plasmodium falciparum* malaria results. This was higher than our overall prevalence in the study area. Jhum was later hypothesized to be a risk factor for acquiring malaria, and were studied by the study team—under the overall Mapping Malaria Epidemiology Project [87].

In this paper, we also discussed the main source of income, agricultural work on land, household location and the ownership of pond. Interesting, local residents relied on doing agricultural work on their own lands as the main source of

income had significantly higher malaria incidence than those doing agricultural work on others land as the main income. However, for residents with privately owned land, *not having house on own land, not cultivated by others* as well as *not cultivated at all (abandoned* were associated with increased malaria risk. We cross-tabulated the relationship between personally cultivated land, land cultivated by others and non-cultivated land. These three types of agricultural work were not mutually exclusive. For example, 11,166 local residents personally cultivated their own land without others help. Among them, 3,604 individuals also had non-cultivated land. Another example: 1,277 local residents had lands that were both personally cultivated and with help by others. Three hundred and seventy (370) of whom also own lands that were not cultivated. The interpretation of this could be more complex. Nonetheless, we could hypothesize individuals doing agricultural work on their own land as a main source of income (N = 9,020) were less likely to have their land cultivated by others. In fact, 7,854 out of 9,020 residents (87%) did not hire others to cultivate their land. If agriculture field was a key reservoir for *Anopheles* mosquitoes in Bandarban Study Area, spending more time on the field could increase the chance of being bitten by malaria-infected mosquitoes. This increased risk reflected on our finding in increased malaria incidence associated with *own land, not cultivated by others* status. Having abandoned or not cultivated field could create uninterrupted larvae/mosquito habitats. Therefore, having uncultivated own land could increase the risk of environmental exposure to malaria. Similarly, no house on own land could mean having less attentive land. With potentially more mosquitoes survived on own land, risk of having malaria increased. As for the ownership of pond, we found sharing the ownership with others (N =

15) could significantly increase malaria incidence 5.7 times comparing to those without a pond (N = 21,906). We could hypothesize the shared pond is a more desirable environment for mosquitoes. However, as majority of the residents did not own a pond (98%). This hypothesis should be further studied before making any inference.

When looking at the association between malaria incidence and the main source of water at household, we found using uncovered water storage containers at home was not significantly different from using river or stream as the source of water. Using covered ring well, tube well, private well and pump, on the other hand, reduced risk of malaria than having river or stream as main source of water. Covered wells and pump were less likely to serve as mosquito habitats. On the contrary, river and stream could be viable habitats for mosquitoes. Hence, residents used river or stream as their main source of water were more likely to have additional exposure to malaria-infected mosquitoes than residents using covered wells and pumps as the source of water. Similar to the idea of using covered water source to reduce the risk of malaria, we found having covered toilet also provided protection in acquiring malaria. Residents using pit latrine (N = 5,254) and slab toilet with boundary (N = 6,503) had significantly lower malaria incidence than those using bushes. Since *pit latrine* and *slab toilet with boundary* were covered or had an outhouse, they were less likely to serve as a reservoir for mosquitoes. Using bushes (N = 8,425) as the main type of toilet was no different than using hanging latrine (N = 1,377) or using slab toilet without boundary (N = 635). Hanging latrines were usually built on water source like river and stream. *Bushes*, *hanging latrines* and *slab toilets without*

boundary were more likely to have access to sources like rain water, rivers, soils in the open field. These types of toilets were not only prone to be locations for mosquito habitats, but also increased risk of other health issues. To note, although results did not show difference in using *bushes* versus *modern, flush toilet* as the main type of toilet at household, there were too few people in the study area use modern flush toilet (N = 256, with 3 *Pf* positive individuals) to make a notifiable difference in incidence comparing to the ones using bushes (N = 8,425, with 300 *Pf* positive individuals) (IRR = 0.43, 95% CI: [0.10, 0.56]).

Individuals using electricity as the source of light at home (N = 240) was associated with lower malaria incidence comparing to the ones using oil lamps as the light source (N = 17,797). This could be resulted from family with electricity are likely in a better financial situation. Houses with electricity could be more enclosed. Residents at houses with electricity could have more up-to-date knowledge, attitude and practice toward malaria. The scent and dim lighting generated from oil lamp could also channel behavior of *Anopheles* mosquitoes differently. These hypotheses are awaited to be tested. When interpreting the result between source of light and its association with malaria incidence, the small number of residents living with electricity should be interpret with caution. With the mention of financial and social economic status, we also measured the annual income and ownership of 33 different types of household assets. In regard to individual household asset in relation to malaria incidence, we have identified household items that were associated with reduced malaria risk: Electricity, TV, radio, bed, clock, fan, chair, tube well and khat. As less than half of the population owned each of the items (except for *Clock*, at 58%),

individuals with access to these commodities were considered in better living situation. Having blankets at home, which owned by 82% of the local residents, was associated with elevated malaria incidence. To better understand the relationship between household assets and malaria incidence, we applied principal component analysis (PCA) to sum up the effect of owning various type of household assets as an indicator. PCA had shown the study area was lack of wealth. Household annual income and PCA did not have linear relationship. For example, some claimed the main source of income came from pension or money received domestically or abroad. This amount might not reflect on commodities purchased for household. Therefore, as an indicator of social economic status, we focused on household assets that an individual owned—instead of gauging based on annual income. The regression analysis of malaria incidence on the first principal component (PC) affirmed the worse the social economic status (i.e. the higher the negative PC), the higher the malaria incidence.

Principal components and household building materials were later analyzed together as indices of living standards. We first started out by looking at household building materials by sections individually. Sections included, but not limited to, wall, partition and floor. We found living in houses with bamboo floor, bamboo partition, bamboo wall provided residents with higher risk in malaria than living in houses with mud floor, mud partition and mud wall, respectively. Interestingly, in our previous paper, houses built with mud were associated with having more mosquitoes indoor than houses built with bamboos. How did more *Anopheles* mosquitoes in a mud house translated to relatively lower malaria incidence than being in a bamboo house should be further studied. Previously,

when we looked at the relationship between household building materials and mosquito abundance, we focused on the number of mosquitoes per house. Each household is considered as one unit. Now, as our current focus was on building materials in relation to individual human, we assigned building materials used at each house to its residents. Having 5 members in one household, for example, meant having the same housing structures weighted 5 times in the analysis. With GEE adjustment, we took into account the unmeasured correlation among residents within the same household. Similarly, we also found higher malaria incidence was associated with elevated ground floor by using the GEE Poisson regression. Elevated ground floor, on the other hand, was associated with fewer mosquitoes in the same household. This indicated the three way relationship among number of mosquitoes, building materials and malaria incidence was not simple. Association is not synonymous with causation. Malaria incidence or the abundance of *Anopheles* mosquitoes will not necessarily be lowered by blindly tearing down bamboo houses or mud houses.

In our previous study, we counted *Anopheles* mosquitoes found in the standard CDC mosquito light traps. Light traps were set indoor for 12 hours (over night) in selected household. In this study, we associated malaria incidence with housing materials based on the person's home location. However, in both studies, we couldn't take into account where the *Anopheles* mosquitoes had been prior to being captured by the light traps. We did not measure the mosquito bite rate at households where we conducted entomological surveillance. We also did not track all residents activities 24/7. It was not known to us if a malaria positive person was bitten by malaria infected *Anopheles* mosquitoes at home

or elsewhere. Although the two studies were conducted at the same study site (i.e. Bandarban Study Area) during the same time frame (2009-2013), we've only scratched the surface to understand the relationship between household building materials and malaria incidence and its connection to the association between household building materials and abundance of *Anopheles* mosquitoes. If funding and resources were plentiful, a randomized control trial in Bandarban with factorial design of key housing materials and structure, along with active entomological and human surveillance of malaria could be beneficial in understanding the connection among them.

For now, we further looked at the living standard and malaria incidence by incorporating both household building materials and individual's social economic status (represented by the first principal component). The findings showed given the same household building materials or ground elevation status of a house, individuals with a lower social economic status (SES) had a statistically significant higher malaria incidence than those with a higher SES. Meanwhile, given the same SES status, having bamboo wall, bamboo floor and bamboo partition remained significantly associated with higher malaria incidence than fired brick/cemented walls, mud floors and concrete/cemented partitions, respectively. Given the same living standards, what makes individuals lived in bamboo houses in Bandarban Study Area more vulnerable to malaria remain unanswered. Was it the knowledge, attitude and practice of malaria different among residents in these houses? Was it due to other factors? Future studies needs to be made to answer these questions.

In closing, the strength of this study lied within its multi-spectrum population based surveillance. In analyzing the association between living standards and malaria incidence, we not only gathered individual-level factors (e.g. gender, age, education, etc.), household-level factors (e.g. source of water, type of toilet, household assets, building materials, etc.), but also conducted malaria diagnoses via home visits. We confirmed individual's malaria status by using the gold standard, Giemsa-stain microscopy. Factors related to malaria incidence across all cases and among severe cases were also compared—although factors related to both were very comparable. We converted individual household assets as an index to gauge social economic status among local residents; we further incorporated household assets and household building materials as indicators of living standards. We applied generalized estimating equation (GEE) Poisson regression to look at malaria incidence on all factors, with the understanding that unmeasured correlation within household members could exist. Limitations included the frequency on individual and household factors were surveyed (1), and the assumption of household level factors were consensus across all members in the same household. In addition, malaria cases were recruited from passive surveillance. To apply passive surveillance to this paper, assumptions needed to be made that all individuals were equally conscious about malaria and would notify the study team at the first sign of symptoms. Throughout the study from 2009 to 2013, there might be malaria cases that we were unaware of. There were also individuals tested with malaria for more than once (in different seasons). Our long term presence in the study site as well as locally hired and trained personnel, however, provided immense advantage for the project. Their

understanding of local culture and local tribal languages had helped us communicate the project with residents in Bandarban Study Area on the daily basis. This study—looking at the association between living standards and malaria incidence—was a good starting point. Future studies are needed to further dig into the *WHY* behind the relationship between *bamboo* materials, agricultural choices, etc. and the elevated malaria incidence.

Table 6.1: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Individual Level Human Factors, I

Factor	Sample Size (Mean ± SD)			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Gender								
Male	11143	10848	295	Ref	—	—		—
Female	11307	11073	234	0.82	1.10	0.68	0.99	0.038
Age (year)								
Age	26.1 ± 19.1	26.2 ± 19.2	20.6 ± 16.3	0.99	1.00	0.98	0.99	< 0.001
Age Group (year)								
[0,5)	2780	2707	73	0.89	1.16	0.67	1.18	0.403
[5,15)	5257	5096	161	Ref	—	—		—
[15,25)	3924	3861	63	0.56	1.16	0.42	0.76	< 0.001
[25,35)	3863	3783	80	0.73	1.16	0.55	0.97	0.031
[35,45)	2617	2574	43	0.58	1.19	0.42	0.81	0.002
[45,55)	1903	1867	36	0.68	1.20	0.48	0.98	0.038
55+	2037	2022	15	0.31	1.27	0.19	0.49	< 0.001
Marital Status								
Married	10682	10507	175	Ref	—	—		—
Single	10485	10200	285	1.53	1.10	1.27	1.85	< 0.001
Other	1283	1214	69	0.61	1.31	0.36	1.04	0.068

Table 6.2: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Individual Level Human Factors, II

Factor	Sample Size (Mean ± SD)			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Year of Education								
Education (year)	2.40 ± 3.22	2.42 ± 3.23	1.77 ± 2.65	0.97	1.02	0.94	0.99	0.021
Year of Education Category								
0	11476	11211	265	Ref	—	—	—	—
(0,6]	7679	7505	174	1.10	1.10	0.91	1.33	0.307
(6,9]	2440	2412	28	0.68	1.20	0.47	0.97	0.034
(9,12]	723	720	3	0.41	1.35	0.23	0.74	0.003
(12,20]	64	63	1	0.73	2.78	0.10	5.41	0.757
Occupation								
Unemployed	4554	4431	123	Ref	—	—	—	—
Housewife	2061	2041	20	0.50	1.21	0.34	0.73	< 0.001
Student	5156	5022	134	1.01	1.14	0.78	1.31	0.948
Farming (Own)	4126	4067	59	0.60	1.17	0.44	0.81	0.001
Farming (Lend)	1420	1400	20	0.66	1.25	0.42	1.02	0.061
Daily labour	2337	2291	46	0.81	1.21	0.55	1.18	0.264
Other	2796	2669	127	0.80	1.21	0.55	1.16	0.23
Place of Employment								
Union of Residence	10229	10037	192	0.78	1.11	0.64	0.95	0.012
Other Union	348	346	2	0.37	1.57	0.15	0.89	0.027
Not Applicable	11805	11528	277	Ref	—	—	—	—
Full Time Employment Status								
Yes	10247	10058	189	0.76	1.11	0.63	0.93	0.007
No	330	325	5	0.82	1.41	0.42	1.60	0.556
Not Applicable	11805	11528	277	Ref	—	—	—	—

Table 6.3: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Factors, I

Factor	Sample Size (Mean ± SD)			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Annual Income								
Income	65387 ± 51247	65458 ± 51155	62424 ± 54911	1.00	1.00	1.00	1.00	0.001
Main Source of Income								
Agriculture, Own Land	9020	8814	206	Ref	—	—	—	—
Agriculture, Not Own Land	2885	2845	40	0.61	1.25	0.39	0.94	0.024
Day Labor	4558	4455	103	0.97	1.19	0.69	1.36	0.855
Business	1634	1613	21	0.60	1.37	0.33	1.12	0.107
Service	1194	1176	18	0.57	1.42	0.29	1.14	0.115
Mortgaged Land	450	438	12	1.19	1.49	0.54	2.61	0.666
Fishing	170	167	3	0.98	2.12	0.22	4.28	0.977
Pension	55	53	2	2.30	2.08	0.55	9.63	0.255
Money from Inside Country	17	17	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
Money from Abroad	42	42	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
Food for Work	40	40	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
Rent from House/Shop	95	95	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
Others	2290	2166	124	2.11	1.18	1.52	2.92	< 0.001

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.4: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Factors, II

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Household Member Works at NGO								
Yes	9616	9376	240	1.11	1.13	0.87	1.41	0.417
No	12829	12540	289	Ref	—	—	—	—
No. of Dwelling Units								
0 to 1	4235	4116	119	1.27	1.17	0.93	1.72	0.129
2	8414	8224	190	Ref	—	—	—	—
3	6717	6563	154	0.93	1.17	0.68	1.26	0.638
4+	3084	3018	66	0.84	1.25	0.54	1.30	0.432
Own land, Personally Cultivated								
Yes	12454	12151	303	Ref	—	—	—	—
No	9776	9555	221	0.88	1.13	0.69	1.12	0.306
Shared	220	215	5	1.13	1.83	0.34	3.69	0.844
Own land, Cultivated by Others								
Yes	2205	2180	25	0.40	1.30	0.24	0.67	< 0.001
No	20223	19719	504	Ref	—	—	—	—
Shared	22	22	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
Own land, Not Cultivated								
Yes	5741	5564	177	1.46	1.14	1.13	1.89	0.004
No	16705	16353	352	Ref	—	—	—	—
Shared	4	4	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
House on Own Land								
Yes	21457	20976	481	Ref	—	—	—	—
No	824	781	43	1.98	1.22	1.33	2.95	0.001
Shared	169	164	5	2.53	1.81	0.79	8.12	0.118
Own Pond								
Yes	529	524	5	0.39	1.66	0.14	1.04	0.06
No	21906	21384	522	Ref	—	—	—	—
Shared	15	13	2	5.68	2.34	1.07	30.20	0.041

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.5: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Factors, III

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Source of Water								
River, Stream	6930	6619	311	Ref	—	—	—	—
Piped into Dwelling	270	258	12	0.77	1.49	0.35	1.67	0.505
Uncovered House Water Storage Container	445	424	21	1.09	1.41	0.56	2.12	0.806
Covered Ring Well	4054	3954	100	0.49	1.19	0.35	0.69	< 0.001
Tube Well	9892	9818	74	0.15	1.18	0.11	0.21	< 0.001
Private Well or Pump	613	605	8	0.23	1.66	0.09	0.63	0.004
Irrigation Channel	90	89	1	0.48	2.67	0.07	3.29	0.453
Rainwater	46	46	0	$-\infty$	(Did Not Converge [α])			
Dam	75	75	0	$-\infty$	(Did Not Converge [α])			
Others	35	33	2	1.03	2.60	0.16	6.67	0.976
Type of Toilet								
Bush, Field	8425	8125	300	Ref	—	—	—	—
Modern, Flush	256	253	3	0.43	2.07	0.10	1.78	0.241
Pit Latrine	5254	5175	79	0.42	1.19	0.30	0.59	< 0.001
Hanging Latrine	1377	1338	39	0.78	1.28	0.48	1.26	0.308
Slab toilet with Boundary	6503	6406	97	0.39	1.18	0.28	0.54	< 0.001
Slab toilet without Boundary	635	624	11	0.49	1.47	0.23	1.05	0.065
Source of Light								
Oil Lamp	17797	17334	463	Ref	—	—	—	—
Electricity	2396	2379	17	0.22	1.40	0.12	0.44	< 0.001
Lantern	240	239	1	0.19	2.73	0.03	1.36	0.098
Solar Energy	2017	1969	48	0.83	1.28	0.51	1.35	0.446
Type of Fuel								
Firewood, Straw	22432	21903	529	Ref	—	—	—	—
Others	18	18	0	$-\infty$	(Did Not Converge [α])			
Cooking Location at Home								
Indoor	21721	21204	517	Ref	—	—	—	—
Outdoor	572	563	9	0.83	1.47	0.39	1.76	0.628
Both Indoor and Outdoor	157	154	3	1.11	1.77	0.36	3.42	0.851

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.6: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Building Materials

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Wall								
Bamboo	18339	17871	468	Ref	—	—	—	—
Fired Brick, Cement	725	712	13	0.10	2.06	0.02	0.42	0.002
Pole and Mud	3075	3035	40	0.54	1.27	0.34	0.86	0.009
Wood	174	168	6	1.50	1.82	0.47	4.83	0.496
Others	137	135	2	0.88	2.07	0.21	3.65	0.862
Roof								
Corrugated Tin, Iron Sheet	14904	14573	331	Ref	—	—	—	—
Straw, Thatch	6766	6580	186	1.34	1.14	1.04	1.73	0.024
Bamboo	45	45	0	$-\infty$	(Did Not Converge [α])			
Mud Tins, House of Tins	525	517	8	0.93	1.53	0.40	2.13	0.86
Fired Brick, Cement	83	82	1	0.56	2.62	0.09	3.73	0.551
Concrete, Cement	81	80	1	$-\infty$	(Did Not Converge [α])			
Others	46	44	2	3.00	2.05	0.73	12.26	0.126
Partition								
Bamboo	17640	17208	432	Ref	—	—	—	—
Wood	210	204	6	1.20	1.83	0.37	3.92	0.758
Concrete, Cement	687	674	13	0.11	2.07	0.03	0.46	0.002
Mud	3015	2974	41	0.60	1.26	0.38	0.94	0.027
Others	898	861	37	1.98	1.23	1.32	2.96	0.001
Floor								
Mud	11250	11069	181	Ref	—	—	—	—
Bamboo	7725	7452	273	2.11	1.14	1.62	2.75	< 0.001
Semi-Cemented	543	525	18	1.06	1.47	0.50	2.25	0.87
Cement	991	982	9	0.35	1.71	0.12	0.99	0.048
Wood	1936	1888	48	1.50	1.26	0.96	2.34	0.076
Others	5	5	0	$-\infty$	(Did Not Converge [α])			

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.7: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Ground Elevation

Factor	Sample Size (Mean ± SD)			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Ground Elevation								
Yes	9577	9255	322	2.14	1.14	1.67	2.75	< 0.001
No	12873	12666	207	Ref	—	—	—	—
Ground Elevation Height								
Height	36.6 ± 47.8	36.3 ± 47.8	48.2 ± 46.8	1.00	1.00	1.00	1.01	0.002

Table 6.8: Summary of Personally Own Assets and Their Loadings as Principal Components [α]

	Owning the Asset? (Yes/No)			Loadings					
Asset	Yes Freq.	Yes Pct.	Std. Dev.	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Electricity	2556	11.39	0.318	-0.11	0.17	-0.08	0.29	-0.26	-0.18
TV	3125	13.92	0.346	-0.22	0.12	-0.03	0.32	-0.18	-0.08
Radio	4123	18.37	0.387	-0.20	0.20	0.01	0.33	0.12	-0.02
Almirah	4715	21.00	0.407	-0.28	0.16	-0.01	0.27	-0.15	-0.11
Bed	10536	46.93	0.499	-0.31	-0.30	0.51	0.05	-0.13	-0.36
Clock	13044	58.10	0.493	-0.33	0.22	-0.11	-0.11	0.78	-0.37
Fan	2046	9.11	0.288	-0.12	0.14	-0.08	0.28	-0.23	-0.13
Telephone	7702	34.31	0.475	-0.37	0.15	-0.11	0.15	0.10	0.57
Chair	8553	38.10	0.486	-0.40	0.19	-0.12	-0.41	-0.20	0.08
Blanket	18380	81.87	0.385	-0.15	-0.22	0.00	0.14	0.13	0.52
Tube Well	1304	5.81	0.234	-0.06	0.07	0.02	0.00	0.05	0.10
Khat	7191	32.03	0.467	-0.35	0.11	-0.02	-0.56	-0.32	-0.02
Reserved Clothes	8719	38.84	0.487	-0.28	-0.30	0.56	0.03	0.12	0.16
Fishing Net	1138	5.07	0.219	-0.05	0.04	-0.04	0.03	-0.03	-0.04
Live Stocks	9957	44.35	0.497	-0.20	-0.46	-0.49	0.08	-0.06	-0.11
Poultry	10781	48.02	0.500	-0.18	-0.54	-0.36	0.01	0.04	-0.11

[α] Only assets with more than 5 percent of the ownership in the study population were included in the Principal Component Analysis. The only exception is "Bednet". Bednet was excluded from the analysis due to less than 1 percent of the population (0.67%) did not claim to have bednet during the study period (Standard Deviation = 0.08).

Table 6.9: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Assets, I

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Electricity								
Yes	2556	2537	19	0.25	1.36	0.13	0.45	< 0.001
No	19894	19384	510	Ref	—	—	—	—
TV								
Yes	3125	3083	42	0.50	1.25	0.33	0.78	0.002
No	19325	18838	487	Ref	—	—	—	—
Radio								
Yes	4123	4055	68	0.64	1.20	0.45	0.91	0.013
No	18327	17866	461	Ref	—	—	—	—
Almirah								
Yes	4715	4601	114	0.87	1.18	0.63	1.21	0.414
No	17735	17320	415	Ref	—	—	—	—
Bed								
Yes	10536	10322	214	0.80	1.13	0.63	1.03	0.082
No	11914	11599	315	Ref	—	—	—	—
Clock								
Yes	13044	12786	258	Ref	—	—	—	—
No	9406	9135	271	1.54	1.13	1.21	1.96	< 0.001
Refrigerator								
Yes	157	155	2	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22293	21766	527	Ref	—	—	—	—
Fan								
Yes	2046	2028	18	0.30	1.38	0.16	0.57	< 0.001
No	20404	19893	511	Ref	—	—	—	—
Dining Table								
Yes	893	880	13	0.63	1.51	0.28	1.42	0.267
No	21557	21041	516	Ref	—	—	—	—

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.10: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Assets, II

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Telephone								
Yes	7702	7540	162	0.86	1.15	0.66	1.13	0.276
No	14748	14381	367	Ref	—	—	—	—
Sofa								
Yes	737	730	7	0.46	1.86	0.14	1.53	0.204
No	21713	21191	522	Ref	—	—	—	—
Chair								
Yes	8553	8441	112	0.38	1.17	0.28	0.52	< 0.001
No	13897	13480	417	Ref	—	—	—	—
Sewing Machine								
Yes	1072	1057	15	0.62	1.42	0.31	1.23	0.172
No	21378	20864	514	Ref	—	—	—	—
Blanket								
Yes	18380	17923	457	Ref	—	—	—	—
No	4070	3998	72	0.63	1.23	0.42	0.94	0.024
Bednet								
Yes	22300	21772	528	Ref	—	—	—	—
No	150	149	1	0.29	2.68	0.04	2.01	0.211
Power Tiller								
Yes	304	296	8	1.10	1.82	0.34	3.54	0.875
No	22146	21625	521	Ref	—	—	—	—
Rice Mill								
Yes	202	197	5	0.86	2.12	0.20	3.73	0.838
No	22248	21724	524	Ref	—	—	—	—
Crushing Mill								
Yes	39	39	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22411	21882	529	Ref	—	—	—	—

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.11: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Assets, III

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Rickshaw								
Yes	519	516	3	0.28	1.78	0.09	0.87	0.028
No	21931	21405	526	Ref	—	—	—	—
Bicycle								
Yes	646	635	11	0.72	1.49	0.33	1.59	0.418
No	21804	21286	518	Ref	—	—	—	—
Fishing Boat								
Yes	16	16	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22434	21905	529	Ref	—	—	—	—
Modern Agriculture Machine								
Yes	444	441	3	0.36	1.78	0.12	1.13	0.081
No	22006	21480	526	Ref	—	—	—	—
Shallow Machine								
Yes	510	501	9	0.57	1.88	0.17	1.97	0.378
No	21940	21420	520	Ref	—	—	—	—
Vehicles to Rent Out								
Yes	62	61	1	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22388	21860	528	Ref	—	—	—	—
Tube Well								
Yes	1304	1290	14	0.12	1.66	0.04	0.32	< 0.001
No	21146	20631	515	Ref	—	—	—	—
Khat								
Yes	7191	7099	92	0.40	1.18	0.29	0.55	< 0.001
No	15259	14822	437	Ref	—	—	—	—
Reserved Clothes								
Yes	8719	8525	194	0.96	1.14	0.74	1.23	0.731
No	13731	13396	335	Ref	—	—	—	—

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.12: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Household Assets, IV

Factor	Sample Size			*Pf* Positive—All				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Dheki								
Yes	126	116	10	3.82	1.61	1.51	9.69	0.005
No	22324	21805	519	Ref	—	—	—	—
Variety Store								
Yes	656	652	4	0.14	2.02	0.04	0.55	0.005
No	21794	21269	525	Ref	—	—	—	—
Fish Hatchery								
Yes	153	152	1	0.32	2.68	0.05	2.23	0.253
No	22297	21769	528	Ref	—	—	—	—
Fishing Net								
Yes	1138	1122	16	0.59	1.46	0.28	1.25	0.17
No	21312	20799	513	Ref	—	—	—	—
Poultry								
Yes	10781	10530	251	1.02	1.13	0.81	1.30	0.843
No	11669	11391	278	Ref	—	—	—	—
Live Stocks								
Yes	9957	9748	209	0.86	1.13	0.67	1.09	0.203
No	12493	12173	320	Ref	—	—	—	—

Table 6.13: Standard Deviation, Proportion of Variance and Cumulative Proportion of Principal Components Generated from 16 Personally Owned Assets

	PC1	PC2	PC3	PC4	PC5	PC6
Standard Deviation	0.909	0.652	0.527	0.429	0.403	0.390
Proportion of Variance	0.298	0.153	0.100	0.066	0.059	0.055
Cumulative Proportion	0.298	0.451	0.551	0.617	0.676	0.731
	PC7	PC8	PC9	PC10	PC11	PC12
Standard Deviation	0.345	0.331	0.313	0.304	0.291	0.279
Proportion of Variance	0.043	0.039	0.035	0.033	0.030	0.028
Cumulative Proportion	0.773	0.813	0.848	0.881	0.912	0.940
	PC13	PC14	PC15	PC16		
Standard Deviation	0.240	0.219	0.205	0.140		
Proportion of Variance	0.021	0.017	0.015	0.007		
Cumulative Proportion	0.961	0.978	0.993	1.000		

Table 6.14: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Principal Component 1 of Assets

Factor	*Pf* Positive—All				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets					
Principal Component 1	1.39	1.07	1.21	1.59	< 0.001

Table 6.15: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Principal Component 1 of Assets in Quintile

Factor	*Pf* Positive—All				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets—Principal Component 1					
Lowest Quintile 0-20th	Ref	—	—	—	—
20-40th	1.42	1.28	0.87	2.30	0.161
40-60th	2.35	1.25	1.51	3.65	< 0.001
60-80th	2.37	1.25	1.53	3.68	< 0.001
Highest Quintile 80-100th	2.28	1.26	1.46	3.56	< 0.001
Assets—Living Standard [α]					
Highest Quintile 80-100th	Ref	—	—	—	—
60-80th	1.42	1.28	0.87	2.30	0.161
40-60th	2.35	1.25	1.51	3.65	< 0.001
20-40th	2.37	1.25	1.53	3.68	< 0.001
Lowest Quintile 0-20th	2.28	1.26	1.46	3.56	< 0.001

[α] *Principal Component 1 was used to represent Living Standard. The higher the component value, the lower the living standard.*

Table 6.16: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of All *Plasmodium falciparum* Malaria on Principal Component 1 of Assets and Household Building Materials, I

Factor	*Pf* Positive—All				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets—Principal Component 1	1.29	1.08	1.12	1.49	< 0.001
Building Material: Wall					
Bamboo	Ref	—	—	—	—
Fired Brick, Cement	0.15	2.08	0.04	0.63	0.010
Pole and Mud	0.69	1.28	0.43	1.10	0.121
Wood	1.77	1.83	0.54	5.77	0.343
Others	1.10	2.07	0.26	4.55	0.899
Assets—Principal Component 1	1.35	1.07	1.17	1.56	< 0.001
Building Material: Roof					
Corrugated Tin, Iron Sheet	Ref	—	—	—	—
Straw, Thatch	1.12	1.14	0.86	1.46	0.390
Bamboo	$-\infty$	(Did Not Converge $^{\alpha}$)			
Mud Tins, House of Tins	0.91	1.53	0.40	2.08	0.820
Fired Brick, Cement	0.70	2.67	0.10	4.81	0.720
Concrete, Cement	$-\infty$	(Did Not Converge $^{\alpha}$)			
Others	2.87	2.01	0.73	11.30	0.130

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.17: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of *Plasmodium falciparum* Malaria on Principal Component 1 of Assets and Household Building Materials, II

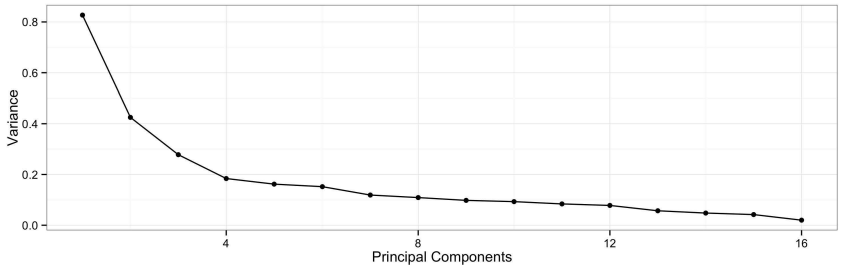
Factor	*Pf* Positive—All				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets—Principal Component 1	1.27	1.08	1.10	1.47	0.001
Building Material: Partition					
Bamboo	Ref	—	—	—	—
Wood	1.39	1.83	0.42	4.55	0.589
Concrete, Cement	0.16	2.08	0.04	0.67	0.012
Mud	0.75	1.27	0.46	1.20	0.223
Others	1.80	1.23	1.20	2.69	0.005
Assets—Principal Component 1	1.19	1.09	1.00	1.42	0.044
Building Material: Floor					
Mud	Ref	—	—	—	—
Bamboo	1.91	1.17	1.41	2.59	< 0.001
Semi-Cemented	1.23	1.47	0.58	2.61	0.588
Cement	0.43	1.74	0.14	1.27	0.127
Wood	1.53	1.25	0.98	2.38	0.060
Others	$-\infty$	(Did Not Converge $^{\alpha}$)			

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table 6.18: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of *Plasmodium falciparum* Malaria on Principal Component 1 of Assets and Household Ground Elevation Status

Factor	*Pf* Positive—All				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets—Principal Component 1	1.24	1.08	1.06	1.45	0.007
Ground Elevation					
Yes	1.91	1.15	1.44	2.52	< 0.001
No	Ref	—	—	—	—

Figure 6.1: Scree Plot of Principal Components, based on Personally Owned Assets



Appendix

Table A6.1: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Individual Level Human Factors, I

Factor	Sample Size (Mean ± SD)			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Gender								
Male	11143	10970	173	Ref	—	—	—	—
Female	11307	11176	131	0.78	1.14	0.60	1.00	0.046
Age (year)								
Age	26.1 ± 19.1	26.2 ± 19.2	18 ± 15.5	0.97	1.00	0.97	0.98	< 0.001
Age Group (year)								
[0,5)	2780	2730	50	0.89	1.18	0.64	1.23	0.487
[5,15)	5257	5150	107	Ref	—	—	—	—
[15,25)	3924	3893	31	0.40	1.23	0.27	0.60	< 0.001
[25,35)	3863	3826	37	0.48	1.23	0.32	0.73	0.001
[35,45)	2617	2602	15	0.30	1.29	0.18	0.50	< 0.001
[45,55)	1903	1885	18	0.47	1.30	0.28	0.79	0.004
55+	2037	2029	8	0.22	1.40	0.11	0.42	< 0.001
Marital Status								
Married	10682	10603	79	Ref	—	—	—	—
Single	10485	10301	184	2.28	1.15	1.72	3.02	< 0.001
Other	1283	1242	41	0.35	1.70	0.12	0.99	0.049

Table A6.2: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Individual Level Human Factors, II

Factor	Sample Size (Mean ± SD)			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Year of Education								
Education (year)	2.4 ± 3.22	2.41 ± 3.22	1.86 ± 2.6	0.97	1.02	0.93	1.00	0.083
Year of Education Category								
0	11476	11341	135	Ref	—	—	—	—
(0,6]	7679	7563	116	1.43	1.13	1.11	1.83	0.005
(6,9]	2440	2427	13	0.59	1.31	0.35	1.00	0.050
(9,12]	723	722	1	0.26	1.67	0.10	0.73	0.010
(12,20]	64	63	1	1.43	2.67	0.21	9.82	0.714
Occupation								
Unemployed	4554	4480	74	Ref	—	—	—	—
Housewife	2061	2051	10	0.37	1.36	0.20	0.68	0.001
Student	5156	5063	93	1.16	1.19	0.82	1.63	0.413
Farming (Own)	4126	4097	29	0.46	1.25	0.30	0.72	0.001
Farming (Lend)	1420	1408	12	0.56	1.36	0.30	1.02	0.059
Daily labour	2337	2316	21	0.56	1.32	0.33	0.97	0.037
Other	2796	2731	65	0.55	1.31	0.32	0.94	0.028
Place of Employment								
Union of Residence	10229	10142	87	0.54	1.15	0.41	0.72	< 0.001
Other Union	348	346	2	0.45	1.77	0.15	1.37	0.159
Not Applicable	11805	11628	177	Ref	—	—	—	—
Full Time Employment Status								
Yes	10247	10162	85	0.53	1.15	0.40	0.70	< 0.001
No	330	326	4	0.89	1.54	0.38	2.07	0.789
Not Applicable	11805	11628	177	Ref	—	—	—	—

Table A6.3: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Factors, I

Factor	Sample Size (Mean ± SD)			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Annual Income								
Income	65387 ± 51247	65402 ± 51112	64298 ± 60398	1.00	1.00	1.00	1.00	0.013
Main Source of Income								
Agriculture, Own Land	9020	8905	115	Ref	—	—	—	—
Agriculture, Not Own Land	2885	2855	30	0.81	1.29	0.49	1.33	0.398
Day Labor	4558	4492	66	1.19	1.23	0.80	1.78	0.394
Business	1634	1621	13	0.65	1.47	0.30	1.37	0.255
Service	1194	1184	10	0.49	1.53	0.21	1.12	0.090
Mortgaged Land	450	447	3	0.52	2.07	0.12	2.14	0.362
Fishing	170	170	0	$-\infty$	(Did Not Converge [α])			
Pension	55	55	0	$-\infty$	(Did Not Converge [α])			
Money from Inside Country	17	17	0	$-\infty$	(Did Not Converge [α])			
Money from Abroad	42	42	0	$-\infty$	(Did Not Converge [α])			
Food for Work	40	40	0	$-\infty$	(Did Not Converge [α])			
Rent from House/Shop	95	95	0	$-\infty$	(Did Not Converge [α])			
Others	2290	2223	67	2.03	1.24	1.33	3.10	0.001

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.4: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Factors, II

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Household Member Works at NGO								
Yes	9616	9473	143	1.16	1.17	0.86	1.56	0.337
No	12829	12668	161	Ref	—	—	—	—
No. of Dwelling Units								
0 to 1	4235	4162	73	1.46	1.22	0.98	2.16	0.060
2	8414	8315	99	Ref	—	—	—	—
3	6717	6622	95	1.02	1.21	0.70	1.48	0.936
4+	3084	3047	37	0.84	1.29	0.51	1.38	0.483
Own land, Personally Cultivated								
Yes	12454	12298	156	Ref	—	—	—	—
No	9776	9631	145	1.17	1.17	0.87	1.59	0.292
Shared	220	217	3	1.18	2.74	0.16	8.52	0.868
Own land, Cultivated by Others								
Yes	2205	2192	13	0.28	1.44	0.14	0.57	< 0.001
No	20223	19932	291	Ref	—	—	—	—
Shared	22	22	0	$-\infty$	(Did Not Converge [α])			
Own land, Not Cultivated								
Yes	5741	5639	102	1.45	1.17	1.06	1.99	0.019
No	16705	16503	202	Ref	—	—	—	—
Shared	4	4	0	$-\infty$	(Did Not Converge [α])			
House on Own Land								
Yes	21457	21185	272	Ref	—	—	—	—
No	824	797	27	2.19	1.30	1.30	3.68	0.003
Shared	169	164	5	4.73	1.84	1.44	15.55	0.010
Own Pond								
Yes	529	524	5	0.67	1.66	0.25	1.81	0.434
No	21906	21609	297	Ref	—	—	—	—
Shared	15	13	2	9.48	2.42	1.67	53.77	0.011

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.5: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Factors, III

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Source of Water								
River, Stream	6930	6750	180	Ref	—	—		—
Piped into Dwelling	270	262	8	0.78	1.55	0.33	1.83	0.563
Uncovered House Water Storage Container	445	438	7	0.67	1.64	0.25	1.78	0.425
Covered Ring Well	4054	3996	58	0.50	1.25	0.33	0.77	0.002
Tube Well	9892	9849	43	0.15	1.24	0.10	0.22	< 0.001
Private Well or Pump	613	608	5	0.22	2.17	0.05	1.01	0.051
Irrigation Channel	90	89	1	0.89	2.67	0.13	6.09	0.905
Rainwater	46	46	0	$-\infty$	(Did Not Converge [α])			
Dam	75	75	0	$-\infty$	(Did Not Converge [α])			
Others	35	33	2	1.69	2.62	0.26	11.12	0.587
Type of Toilet								
Bush, Field	8425	8260	165	Ref	—	—	—	—
Modern, Flush	256	253	3	0.76	2.08	0.18	3.22	0.712
Pit Latrine	5254	5205	49	0.48	1.23	0.32	0.73	0.001
Hanging Latrine	1377	1352	25	0.92	1.34	0.52	1.63	0.780
Slab toilet with Boundary	6503	6448	55	0.39	1.22	0.26	0.58	< 0.001
Slab toilet without Boundary	635	628	7	0.51	1.78	0.17	1.58	0.244
Source of Light								
Oil Lamp	17797	17535	262	Ref	—	—	—	—
Electricity	2396	2387	9	0.17	1.58	0.07	0.43	< 0.001
Lantern	240	240	0	$-\infty$	(Did Not Converge [α])			
Solar Energy	2017	1984	33	1.08	1.31	0.63	1.82	0.787
Type of Fuel								
Firewood, Straw	22432	22128	304	Ref	—	—	—	—
Others	18	18	0	$-\infty$	(Did Not Converge [α])			
Cooking Location at Home								
Indoor	21721	21422	299	Ref	—	—	—	—
Outdoor	572	568	4	0.66	1.67	0.24	1.81	0.419
Both Indoor and Outdoor	157	156	1	0.66	2.73	0.09	4.73	0.680

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.6: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Building Materials

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Wall								
Bamboo	18339	18074	265	Ref	—	—	—	—
Fired Brick, Cement	725	716	9	0.17	2.15	0.04	0.74	0.018
Pole and Mud	3075	3051	24	0.59	1.27	0.37	0.94	0.028
Wood	174	169	5	2.13	2.07	0.51	8.88	0.301
Others	137	136	1	0.70	2.73	0.10	5.03	0.726
Roof								
Corrugated Tin, Iron Sheet	14904	14715	189	Ref	—	—	—	—
Straw, Thatch	6766	6659	107	1.38	1.17	1.00	1.88	0.047
Bamboo	45	45	0	$-\infty$	(Did Not Converge [α])			
Mud Tins, House of Tins	525	519	6	1.24	1.60	0.50	3.12	0.642
Fired Brick, Cement	83	82	1	1.03	2.62	0.16	6.84	0.973
Concrete, Cement	81	81	0	$-\infty$	(Did Not Converge [α])			
Others	46	45	1	3.18	2.81	0.42	24.13	0.263
Partition								
Bamboo	17640	17393	247	Ref	—	—	—	—
Wood	210	205	5	1.69	2.08	0.40	7.11	0.477
Concrete, Cement	687	678	9	0.18	2.15	0.04	0.81	0.025
Mud	3015	2991	24	0.63	1.28	0.39	1.01	0.056
Others	898	879	19	1.80	1.30	1.07	3.03	0.027
Floor								
Mud	11250	11146	104	Ref	—	—	—	—
Bamboo	7725	7564	161	2.13	1.18	1.54	2.93	< 0.001
Semi-Cemented	543	532	11	0.68	1.86	0.20	2.30	0.533
Cement	991	987	4	0.23	2.04	0.06	0.93	0.039
Wood	1936	1912	24	1.27	1.33	0.73	2.21	0.406
Others	5	5	0	$-\infty$	(Did Not Converge [α])			

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.7: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Ground Elevation

Factor	Sample Size (Mean ± SD)			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf(−)*	*Pf(+)*	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Ground Elevation								
Yes	9577	9391	186	2.18	1.17	1.60	2.96	< 0.001
No	12873	12755	118	Ref	—	—	—	—
Ground Elevation Height								
Height	36.6 ± 47.8	36.5 ± 47.8	46.1 ± 45.6	1.00	1.00	1.00	1.01	0.002

Table A6.8: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Assets, I

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Electricity								
Yes	2556	2547	9	0.16	1.58	0.07	0.40	< 0.001
No	19894	19599	295	Ref	—	—	—	—
TV								
Yes	3125	3097	28	0.61	1.30	0.36	1.03	0.062
No	19325	19049	276	Ref	—	—	—	—
Radio								
Yes	4123	4080	43	0.72	1.24	0.47	1.10	0.128
No	18327	18066	261	Ref	—	—	—	—
Almirah								
Yes	4715	4649	66	0.83	1.22	0.56	1.24	0.368
No	17735	17497	238	Ref	—	—	—	—
Bed								
Yes	10536	10412	124	0.85	1.17	0.62	1.15	0.284
No	11914	11734	180	Ref	—	—	—	—
Clock								
Yes	13044	12903	141	Ref	—	—	—	—
No	9406	9243	163	1.69	1.16	1.26	2.28	0.001
Refrigerator								
Yes	157	156	1	$-\infty$	(Did Not Converge [α])			
No	22293	21990	303	Ref	—	—	—	—
Fan								
Yes	2046	2035	11	0.29	1.47	0.14	0.63	0.002
No	20404	20111	293	Ref	—	—	—	—
Dining Table								
Yes	893	887	6	0.55	1.58	0.22	1.35	0.193
No	21557	21259	298	Ref	—	—	—	—

[α] These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.9: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Assets, II

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Telephone								
Yes	7702	7609	93	0.86	1.18	0.62	1.18	0.347
No	14748	14537	211	Ref	—	—	—	—
Sofa								
Yes	737	733	4	0.48	1.83	0.15	1.58	0.227
No	21713	21413	300	Ref	—	—	—	—
Chair								
Yes	8553	8487	66	0.39	1.21	0.27	0.57	< 0.001
No	13897	13659	238	Ref	—	—	—	—
Sewing Machine								
Yes	1072	1065	7	0.52	1.53	0.23	1.20	0.124
No	21378	21081	297	Ref	—	—	—	—
Blanket								
Yes	18380	18117	263	Ref	—	—	—	—
No	4070	4029	41	0.66	1.28	0.41	1.06	0.087
Bednet								
Yes	22300	21997	303	Ref	—	—	—	—
No	150	149	1	0.53	2.69	0.08	3.67	0.520
Power Tiller								
Yes	304	299	5	1.41	1.67	0.51	3.87	0.503
No	22146	21847	299	Ref	—	—	—	—
Rice Mill								
Yes	202	198	4	1.18	2.04	0.29	4.79	0.815
No	22248	21948	300	Ref	—	—	—	—
Crushing Mill								
Yes	39	39	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22411	22107	304	Ref	—	—	—	—

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.10: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Assets, III

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Rickshaw								
Yes	519	517	2	0.31	2.03	0.08	1.24	0.097
No	21931	21629	302	Ref	—	—	—	—
Bicycle								
Yes	646	640	6	0.77	1.75	0.26	2.31	0.641
No	21804	21506	298	Ref	—	—	—	—
Fishing Boat								
Yes	16	16	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22434	22130	304	Ref	—	—	—	—
Modern Agriculture Machine								
Yes	444	442	2	0.42	2.04	0.10	1.69	0.220
No	22006	21704	302	Ref	—	—	—	—
Shallow Machine								
Yes	510	506	4	0.29	2.71	0.04	2.05	0.215
No	21940	21640	300	Ref	—	—	—	—
Vehicles to Rent Out								
Yes	62	62	0	$-\infty$	(Did Not Converge $^{\alpha}$)			
No	22388	22084	304	Ref	—	—	—	—
Tube Well								
Yes	1304	1293	11	0.20	1.69	0.07	0.57	0.002
No	21146	20853	293	Ref	—	—	—	—
Khat								
Yes	7191	7140	51	0.35	1.24	0.23	0.54	< 0.001
No	15259	15006	253	Ref	—	—	—	—
Reserved Clothes								
Yes	8719	8605	114	1.00	1.17	0.73	1.36	0.989
No	13731	13541	190	Ref	—	—	—	—

$^{\alpha}$ These variables did not converge. For these variables, after controlling for the others, were associated with complete protection. However, due to insufficient sample size, statistical evaluation could not be made.

Table A6.11: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Household Assets, IV

Factor	Sample Size			*Pf* Positive—Severe				
						95% CI		
Variable	All	*Pf*(−)	*Pf*(+)	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Dheki								
Yes	126	121	5	3.40	1.83	1.04	11.10	0.043
No	22324	22025	299	Ref	—	—	—	—
Variety Store								
Yes	656	654	2	0.12	2.71	0.02	0.87	0.036
No	21794	21492	302	Ref	—	—	—	—
Fish Hatchery								
Yes	153	152	1	0.56	2.68	0.08	3.89	0.561
No	22297	21994	303	Ref	—	—	—	—
Fishing Net								
Yes	1138	1126	12	0.78	1.46	0.37	1.65	0.522
No	21312	21020	292	Ref	—	—	—	—
Poultry								
Yes	10781	10639	142	0.97	1.16	0.72	1.31	0.836
No	11669	11507	162	Ref	—	—	—	—
Live Stocks								
Yes	9957	9834	123	0.93	1.17	0.69	1.26	0.647
No	12493	12312	181	Ref	—	—	—	—

Table A6.12: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Principal Component 1 of Assets

Factor	*Pf* Positive—Severe				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets					
Principal Component 1	1.39	1.09	1.18	1.64	< 0.001

Table A6.13: Individual Level Generalized Estimating Equation (GEE) Poisson Regressions: Log Incidence Rate of Severe *Plasmodium falciparum* Malaria on Principal Component 1 of Assets in Quintile

Factor	*Pf* Positive—Severe				
			95% CI		
Variable	$e^{Coef.}$	e^{SE}	e^{LCL}	e^{UCL}	P-Value
Assets—Principal Component 1					
Lowest Quintile 0-20th	Ref	—	—	—	—
20-40th	1.39	1.34	0.78	2.48	0.261
40-60th	2.23	1.32	1.30	3.84	0.004
60-80th	2.56	1.31	1.51	4.32	< 0.001
Highest Quintile 80-100th	2.16	1.31	1.27	3.69	0.005
Assets—Living Standard					
Highest Quintile 80-100th	Ref	—	—	—	—
60-80th	1.39	1.34	0.78	2.48	0.261
40-60th	2.23	1.32	1.30	3.84	0.004
20-40th	2.56	1.31	1.51	4.32	< 0.001
Lowest Quintile 0-20th	2.16	1.31	1.27	3.69	0.005

Chapter 7

Summary, Implications and Future Directions

7.1 Summary

This dissertation was conducted under a 4-year population based surveillance project—"*Mapping Malaria Epidemiology in Bangladesh*"—in rural Bangladesh. *Mapping Malaria Epidemiology in Bangladesh* was funded by *Johns Hopkins Malaria Research Institute* (JHMRI) in 2009, in collaboration with *International Center for Diarrheal Diseases in Bangladesh* (icddr,b). The study site included Kuhalong Union and Rajbila Union—two smallest rural administrative units located in northern Bandarban District within Chittagong Hill Tracts in southeastern Bangladesh.

The main goals of the dissertation were

1. to identify risk factors associated with presence of *Plasmodium falciparum* malaria and

2. to help facilitate the country move toward malaria elimination.

To achieve these two goals, we started out by examining the tool used for malaria diagnosis. We then moved on to examine ways to help broaden surveillance efforts. Finally, we concluded the dissertation by exploring risk factors associated with malaria. That is, we studied the relationship between abundance of *Anopheles* mosquitoes and its housing environment as well as between living standard and malaria incidence.

To be more specific, these efforts were documented in following aspects:

1. to evaluate field performance of FalciVax^{TM} Rapid Diagnostic Tests, a

malaria diagnostic device used in Kuhalong and Rajbila Unions from 2009 to 2013 (Paper 1),

2. to identify clinical malaria symptoms in association with levels of *Plasmodium falciparum* density, with an aim to provide insight on building a reactive surveillance system (Paper 2),

3. to analyze choices of household building materials in association with population size of *Anopheles* species identified inside households (Paper 3), and

4. to study association between living standard and malaria incidence (Paper 4).

With 5,006 households and 22,325 residents located at the study site, we collected demographic information, socioeconomic information and information on household building materials from all residents and households in the study area. Within the study population, we tested 616 individuals from passive surveillance for *Plasmodium falciparum* malaria infection. Of whom, 529 tested subjects were *Plasmodium falciparum* malaria positive. We selected 1079 households for entomological survey. Of which, one thousand and sixty three (1,063) households were included in analysis. Meanwhile, more than twenty-two thousand *Anopheles* mosquitoes (22,214) were collected through forty-three hundred household visits (4,363).

Key findings of the dissertation were:

- **Paper 1-a**. High sensitivity and low specificity of FalciVax^{TM} Rapid

Diagnostic Tests

- **Paper 1-b**. Estimates of sensitivity and specificity could benefit from modeling when sample size is small

- **Paper 2**. Having fever at the time of malaria diagnostic tests, or having shorter symptom duration were associated with higher odds of having parasite density above median. Having self-reported fever at night, and being older in age were associated with lower odds of having parasite density above median.

- **Paper 3**. Using mud as a household building material was associated with having higher number of *Anopheles* species inside households than using bamboo as a household building material. With the adjustment of information on areal level building materials, the effect of mud was not significantly different than the effect of bamboo on the numbers of *Anopheles* species inside households

- **Paper 4-a**. Living in households built with bamboo was associated with higher malaria incidence than living in household built with mud. The relationship remained significant even after adjusting for living standards.

- **Paper 4-b**. Having a higher living standard, having using covered ring well, tube well and private well/pump, and using pit latrine and slab toilet with boundary were associated with reduced risk in malaria incidence

7.1.1 Brief Summary by Key Findings

Paper 1-a: High sensitivity and low specificity of FalciVax™ Rapid Diagnostic Tests

FalciVax Pv/Pf™ (Zephyr Biomedical Systems, India) was a malaria diagnostic device used in Bandarban Study Area from 2009 to 2013. Its World Health Organization (WHO) product performance against clinical samples containing *Plasmodium falciparum* showed a panel score of 98 for low parasite density (200 parasites/mu l) and a panel score of 100 for high parasite density (2000 parasites/mu l). The scores were recognized by WHO as being at good standing.

In Bandarban Study Area, we compared FalciVax™ against microscopy. The reporting sensitivity was high (99.6%). However, the reporting specificity was low (33.3%). Field performance did not vary by season or participants' febrile status (which was measured at the time of conducting malaria diagnostic tests). However, field performance of FalciVax™ had a statistically significant difference between 2011 and 2012.

The high sensitivity and low specificity could be related to high reporting prevalence (86%). Study participants were enrolled through passive surveillance. Local residents who felt ill or had fever contacted the study team for malaria diagnosis. Therefore, tested study participants were more inclined to be malaria positive than the general population. A rapid diagnostic test is in general more

sensitive in malaria diagnosis when testing individuals with high parasite density than the ones with low parasite density. FalciVax^{TM} had shown a false positive percentage of 0.8% in product performance tests done by WHO laboratory. Percent false positive seemed to increase in the field.

Detection limit of rapid diagnostic tests also suggest the potential presence of spectrum bias. Spectrum bias indicates sensitivity and specificity of a screening test is subjected to prevalence of a disease. With a higher prevalence, spectrum bias usually suggests sensitivity of a screening tool would go up and specificity of the screening tool would go down. If spectrum bias truly existed in this scenario, we could expect two things:

1. Overall prevalence of *Plasmodium falciparum* malaria was 1-2% in Bandarban Study Area (according to Active Surveillance that was not included in this dissertation). The overall prevalence was significantly lower than the reporting prevalence done through passive surveillance. If the same field performance test was conducted among all residents throughout the study area, FalciVax^{TM} Rapid Diagnostic Test would be expected to have a lower sensitivity (than 99.6%) and a higher sensitivity (than 33.3%).

2. According to WHO, a rapid diagnostic test device is at good standing if its sensitivity is greater than or equal to 95%, and if its specificity is greater than or equal to 90%. Due to the varying prevalence of *Plasmodium falciparum* malaria across settings, how to apply WHO's good standing standard in the lab to the good standing standard in the field becomes an important matter.

Paper 1-b. Estimates of sensitivity and specificity could benefit from modeling when sample size is small

In paper 1, we used the modeled sensitivity and specificity to analyze the field performance of FalciVax^{TM} Rapid Diagnostic Test by category of interests. The sample size in each subcategory was often small. We found using logistic regressions to model sensitivity, specificity, as well as positive and negative predictive values could help stabilize the estimates.

Paper 2. Having fever at the time of malaria diagnostic tests, or having shorter symptom duration were associated with higher odds of having parasite density above median. Having self-reported fever at night, and being older in age were associated with lower odds of having parasite density above median

In paper 2, we enrolled 616 Bandarban Study Area residents through passive surveillance. Home visits were made to their residences. During home visits, malaria diagnostic tests were done (i.e. FalciVax^{TM} Rapid Diagnostic Test and microscopy), body temperatures were measured, self-reported symptoms and symptom duration were asked using standard questionnaires.

In this paper, *Plasmodium falciparum* malaria status was determined by microscopy. A trained microscopist recorded parasite density identified from each

participant's blood slides. Based on the distribution of study participants' *Plasmodium falciparum* density, a median cutoff was used to create levels of parasite density. Having *high parasite density* meant a person had *Plasmodium falciparum* density greater than median. Having *low parasite density* meant a person had *Plasmodium falciparum* density less than or equal to median.

We divided the study site into a 10-by-10 grid. Based on tested individuals' household location, we calculated average and median log parasite density of the area. Parasite density of tested individuals did not show any discernible spatial pattern. We recognized parasite density varies over time. Here, parasite density was measured once. To make data comparable, we chose to conduct malaria diagnosis, create blood films for parasite density measurement, measure body temperature and survey self-reported symptoms all during the same sitting at home visits. Instead of using the exact measurement of parasite density, density was also converted into levels.

We used the body temperature measured at home visit to determine if a participant had fever at the time of malaria diagnosis. If an individual's oral temperature was greater than or equal to 37.5°C or if an individual's axillary temperature was greater than or equal to 37.2°C, the individual was considered as being febrile.

Seventeen self-reported symptoms were recorded during home visits. Symptoms included in the questionnaire were "*fever with shivering*", "*fever at day*

time", "*fever at night*", "*fever with sweating*", "*intermittent fever*", "*remission of fever with sweating*", "*headache*", "*chills*", "*nausea*", "*vomiting*", "*diarrhea*", "*cough*", "*fatigue*", "*muscle ache*", "*muscle weakness*", "*convulsions, seizure*" and "*anemia*".

This paper showed having *fever* at the time of malaria diagnostic tests, or having shorter *symptom duration* were associated with higher odds of having parasite density above median. Having self-reported *fever at night*, and being older in *age* were associated with lower odds of having parasite density above median. On the other hand, *number of self-reported symptoms* and individual symptoms other than *fever at night* were not associated with changing levels of parasite density.

This information would be beneficial when designing a reactive surveillance system in Bandarban Study Area. This information could also be used in promoting case awareness in the area. However, while *fever* can be used as an additional criterion while broadening the case search, it should not be used as sole criteria for malaria diagnosis—as fever is not a malaria-specific symptom.

Paper 3. Using mud as a household building material was associated with having higher number of Anopheles species inside households than using bamboo as a household building material. With the adjustment of information on areal level building materials, the effect of mud was not significantly different than the effect of bamboo on the numbers of Anopheles species inside households

In paper 3, 1079 households were selected for entomological survey. Among those, 1063 households were included in the final analysis. In the entomological survey, study team set up CDC light traps inside studied households. Each light trap was hung from 6-7 pm at night to 6-7 am the following morning. After a 12-hour mosquito collection, light traps were brought back to field laboratory for examination.

Number of Anopheles mosquitoes by species were counted and documented by a trained entomologist. Meanwhile, the study team collected information on building materials used at each participated household. Building materials were recorded in four sections: "*wall*", "*roof*", "*partition*", and "*floor*". In addition, whether a house had "*elevated ground floor*" was also recorded. The most commonly used materials for "*wall*", "*partition*", and "*floor*" in Bandarban Study Area were "*bamboo*" and "*mud*". The most commonly used materials for "*roof*", on the other hand, were "*corrugated tin / iron sheet*" and "*straw / thatch*".

We found using *mud* as a household building associated with having higher number of *Anopheles* species inside households than using *bamboo* as a household building material. With the adjustment of information on areal level building materials, the effect of *mud* was not significantly different than the effect of *bamboo* on the numbers of *Anopheles* species inside households.

We used average number of *Anopheles* per household per night as the unit for

analysis. This was to ensure building materials used at each household were weighed equally. A grid-based areal adjustment on number of *Anopheles* per household per night was later done to single out the effect of one building material on size of *Anopheles* population. Using a grid-based areal level adjustment provided least biased results.

As average numbers of *Anopheles* per household per night were small, the analysis wasn't separated by *Anopheles* species. Different *Anopheles* species could have preferential association on different building materials. Although difference between *mud* and *bamboo* in association with number of *Anopheles* mosquitoes as a whole diminished after areal adjustment, more detailed studies on preference of building materials among individual *Anopheles* species should be done.

With the knowledge of *Anopheles* species that are more important to human malaria in Bandarban Study Area, and with the knowledge of preferred building materials of these *Anopheles* species, an area-specific vector control method could be designed and implemented.

Paper 4-a. Living in household built with bamboo material was associated with higher malaria incidence than using mud as a household building material. The relationship remained significant even after adjusting for living standards

Paper 4-b. Having a higher living standard, having using covered ring well, tube well and private well/pump, and using pit latrine and slab

toilet with boundary were associated with reduced risk in malaria incidence

In Paper 4, we examined socioeconomic status and living standard related risk factors in association with malaria incidence. We calculated the exposure person-time among all residents in the study area. Malaria status was based on the microscopy results from passive surveillance. Basic demographic characteristics (e.g. education, occupation, etc.) and socioeconomic indicators (e.g. household assets, etc.) were surveyed across the study site. Poisson regression where analyzed on an individual level.

We found being *female*, being older in *age*, being *married*, being *educated*, having income from *agricultural work not on own land*, not *having uncultivated land*, having *own lands cultivated by other*, having *house on own land*, using *covered ring well*, *tube well* and *private well/pump*, using *pit latrine* and *slab toilet with boundary*, and using *electricity* as main source of light at home were factors associated with reduced malaria incidence.

In addition, we used the first principal component—generated with household assets—to represent residents' living standard. We found having higher *living standard* was related to lower malaria incidence. Assets included in the analysis were represented by *electricity*, a *TV*, a *radio*, a *clock*, a *fan*, a *chair*, a *rickshaw*, a *tube well*, a *khat*, a *variety store*, *blanket*, *dheki* and more.

Household building materials could also represent residents' living standard.

Having *bamboo* as a wall, partition and flooring material, having *corrugated tin or iron sheet* as a roofing material, as well as having *elevated ground floor* at home were related to elevated malaria incidence comparing to other types of building materials.

Here, we used reported incidence to analyze individual-level and household-level risk factors in association with malaria incidence. We found having better infrastructure, for example, having a *slab toilet with boundary* (versus using *bushes* as toilets) or having a *covered ring well* (versus using *rivers* as source of water), could help reduce malaria incidence. However, even with the same housing structure (i.e. household building materials), having a higher living standard was related to a lower malaria incidence. Although living standard was represented by ownership of household assets in this paper, to truly improve residents' living standard, factors such as education could not be overlooked.

7.1.2 Strengths and Opportunities

This dissertation took place under the *Mapping Malaria Epidemiology in Bangladesh* project. As a population-based surveillance project, the study team not only enumerated all members of the study area, but also enrolled residents from all age groups for malaria diagnoses. This dissertation incorporated multiple surveillance systems and surveys from the overall project. These included "*passive surveillance*", "*entomological surveillance*", "*household building material survey*" and "*socioeconomic survey*". With multiple surveillance and survey

projects being carried out everyday in the field, our field team familiarized themselves with all residents in the study area. In addition, all (20) field workers, the field assistant, the field manager and the field physician were all locally hired. With their roots in our studied communities and the long term presence (2009-2013) of the study team, these had helped the project proceed more smoothly.

In this study, we provided home visits to all residents who felt ill and contacted the study team. We used both rapid diagnostic tests and microscopy as the methods for malaria diagnoses. With the availability of rapid diagnostic tests, residents could know their malaria infection status during the visits. Treatments were also provided immediately if an individual was *Plasmodium falciparum* positive. This timely feedback system not only encouraged local residents to contact the study team when ill, but also helped reducing further malaria transmission among the study population.

Microscopy was chosen as a comparative standard for malaria diagnosis. Thin and thick blood films were collected at home visits for microscopy tests in a field laboratory. A trained microscopist was in charge of examining blood films. The microscopist would then document the infected *Plasmodium* species and quantify parasite density (if any). Although microscopy is more sensitive in malaria diagnosis than rapid diagnostic tests, microscopy required an experienced microscopist and laboratory equipment. Therefore, results from reading blood films could not be completed at home visits. Hence, having rapid diagnostic tests as a front line diagnostic method and having microscopy as a confirmation

method benefited both the study team and local residents.

Another strength of the study was the ability to look at multiple aspects of risk factors in association with malaria. We collected individual level factors and household level factors. Individual factors included age, gender, years of education, marital status, occupation, *Plasmodium falciparum* malaria status, *Plasmodium falciparum* density and more. Household factors included, but were not limited to, source of income, source of water, durable assets at households, household building materials, and number of *Anopheles* vector mosquitoes identified within a household. The wide variety of information collected through the project broadened our horizon in understanding the association between potential risk factors and *Plasmodium falciparum* malaria.

To ensure quality of the surveillance project, oral and written exams were held to examine field workers' understanding of English and Bengali. In addition, training on data collection and questionnaire comprehension was provided to all field workers. For quality assurance, home visits and data collection were conducted in a team of two. Every field worker was paired with another field worker. This team pairing process was done every few months to ensure checks and balances between field workers. This team training and pairing process enabled the data collection process to uphold a higher standard.

An additional strength of the study was a form processing system used for data entry. All questionnaires used in this dissertation were pre-coded. Once a

survey was done, questionnaires used for the survey were scanned through ABBYY FlexiCapture 8.0. ABBYY FlexiCapture 8.0 was designed to recognize field workers' handwriting. Once recognized by the software, the corresponding numbers or letters would be entered into Microsoft Access. A preliminary data entry was done. The form processing software allowed the data entry process to be more streamlined. It also saved time and reduced potential human errors in data entry. The field assistant further verified entered data, which was an added layer of precaution and strength of the study.

Last but not least, we modeled sensitivity, specificity, positive and negative predictive values to stabilize field performance estimates within each subcategory (Paper 1); we used levels of parasite density—instead of the absolute value of *Plasmodium falciparum* density—to acknowledge the non-stationary nature of the density estimates (Paper 2); we created a 10-by-10 grid at the study site as the least biased method to take into account the areal effect of household building materials on number of Anopheles species (Paper 3); we used generalized estimating equation to take into account unmeasured correlation within household members (Paper 4). These analytic choices also helped strengthen the study.

7.1.3 Limitations and Threats

Plasmodium falciparum malaria cases were enrolled through passive surveillance. Residents at the study area were told to contact the study team if they

felt ill or had fever. Once contacted, the study team will conduct home visits for malaria diagnosis. This case detection method relied on the health consciousness to be enrolled in the program: Residents had to be aware of the program and be aware of their symptoms. Therefore, prevalence and incidence calculated were reported prevalence and reported incidence, respectively. Therefore, results related to reported prevalence and reported incidence represented a phenomenon at the study area. Whether these results can be generalized to the entire study population should be further studied.

In this dissertation, we conducted surveys on household building materials, durable assets and socioeconomic status. These surveys were only conducted once. We assumed all these variables collected through the surveys did not vary from 2009 to 2013. In addition, all household members were assumed to live under the same house (i.e. with the exposure to the same household building materials), share the same household assets and living conditions. Although household building materials and living conditions were less likely to change in a rural setting like Bandarban Study Area in four years, the assumptions on stationary household conditions could be a threat as years of the study went up.

In the entomological study, we surveyed more than 1000 households. We set up a CDC light trap inside each selected household for 12 hours (from 6-7 PM to 6-7 AM). However, the study was not designed to have study team members staying overnight at surveyed houses. It was unclear to the study team whether residents at selected households slept by the light trap with the proper use of

bed nets. This was a limitation of the study. It was also a threat to have potentially underestimated the number of *Anopheles* vector mosquitoes in the study area.

Another limitation regarding to the entomological study was the variety of Anopheles species in the study area. More than 20 *Anopheles* species were identified in the study area. However, due to the sample size, we didn't analyze the association between average number of Anopheles mosquitoes per household per night and household building materials by *Anopheles* species. We studied an overall association. However, different *Anopheles* species could have different preference in household building materials. Not all *Anopheles* species were equally important in attribution to human malaria. Therefore, the thread of unable to study species-specific relationship between *Anopheles* and building materials in this dissertation could be an opportunity for future directions.

In the socioeconomic survey, we collected information on household ownership of 33 durable assets. We also asked study participants about their source of income, employment status, source of water and type of toilet, etc.. This questionnaire used throughout the study period was designed based on the knowledge about the study site. It has its similarities with the household questionnaire used under the *United States Agency for International Development* (USAID) *Demographic and Health Surveys* (DHS) program. However, they were not identical. This was a limitation in generalizability. In the analysis, we used principal component to look at living standard in quintile in association with reported *Plasmodium falciparum* malaria incidence.

For the analysis, we first examined the association between each socioeconomic status-related item and reported *Plasmodium falciparum* malaria incidence. We further used principal component to look at the living standard in association with *Plasmodium falciparum* malaria incidence by quintile. In the former analysis, a potential threat of multiplicative effect could exist. Having examined 33 assets one-by-one, it was more likely the association was statistically significant by chance. Therefore, how to use the provided information to create a meaningful index representing the living standard could not be overlooked.

7.2 Implications

Over the past decade, the numbers of reported malaria cases in Bangladesh have gone down. The country had 84,690 reported malaria cases in 2008. In 2013, this case number declined to 26,891. The decrease in malaria in Bangladesh could be attributed to execution of the *National Malaria Strategic Plan* (NMSP), led by the Government of Bangladesh. NMSP was a collaborative effort done by local non-government organizations (e.g. BRAC), research institutes (e.g. *International Centre for Diarrhoeal Disease Research, Bangladesh* and *Johns Hopkins Malaria Research Institute*), and international organizations (e.g. *World Health Organizations* and *The Global Fund to Fight AIDS, Tuberculosis and Malaria*). In addition to Bangladesh's *National Malaria Strategic Plan*, The *Asia Pacific*

Malaria Elimination Network (APMEN) and *Asia Pacific Leaders Malaria Alliance* (APLMA) were also two key forces in pushing malaria elimination in the Asia Pacific region. With the help domestically and internationally, the goal of country is to reach malaria elimination in Bangladesh by 2020.

To date, more than 80% of the cases in Bangladesh come from Chittagong Hill Tracts. In 2013, 21,531 out of 26,891 reported malaria cases were recorded in Chittagong Hill Tracts. Among the three districts in Chittagong Hill Tracts (i.e. Bandarban District, Rangamati District and Khagrachari District), Bandarban District remained the most malaria prevalent. That is, 9,459 out of 21,531 malaria cases reported in Chittagong Hill Tracts in 2013 were from Bandarban District. To help achieve the overall goal of malaria elimination in Bangladesh, we set a surveillance project in Bandarban District. We aimed to provide information on malaria epidemiology in the Bandarban Study Area. We also aimed to identify malaria risk factors that were associated with residents in the Bandarban Study Area.

This dissertation was conducted under the *Mapping Malaria Epidemiology Project in Bangladesh*. The project was funded in 2009 under the Pilot Grant at *Johns Hopkins Malaria Research Institute*. The principal investigator of the project was Dr. David Sullivan. The study site was located in Kuhalong Union and Rajbila Union in northern Bandarban District. It included 5,006 households and 22,325 residents from 2009 to 2013.

Public health implications concluded from this dissertation were discussed in

three main directions: early diagnosis, infrastructure, and education. In early diagnosis, we focused on rapid diagnostic tests, hot spot and reactive case detection. In infrastructure, we looked at living environment and household building materials. Discussion of the implications can be found below.

7.2.1 Early diagnosis

Rapid Diagnostic Tests

In order to stop the malaria transmission, it is important to identify and treat malaria cases at a timely manner. In the field, we used FalciVax^{TM} rapid diagnostic tests as one of our front line malaria diagnostic tools. For treatment purposes—If an individual showed a positive test result from either a rapid diagnostic test or a microscopy test, we considered the person was malaria positive. This definition was to ensure all cases was treated before the disease continued to progress.

In the field performance study, we compared the test results collected prior to administering the treatment (if positive). We found the field performance of FalciVax^{TM} rapid diagnostic test had high sensitivity and low specificity, when compared against its microscopy results. From the laboratory tests performed by the *World Health Organization*, FalciVax^{TM} is one of the commercial devices listed at good standing (i.e. sensitivity ¿= 95% and specificity ¿= 90%). This study showed the laboratory performance and field performance may not always be in line with each other.

However, whether the high sensitivity and low specificity of FalciVax^{TM} rapid diagnostic test device was related to the high reported prevalence—as suggested by spectrum bias—remains to be verified. Spectrum bias suggested sensitivity and specificity of a diagnostic test is depending upon prevalence of a disease. In this dissertation, we used microscopy as our comparative standard. It relied on a skilled microscopist to detect malaria parasites in the thin and thick blood films. Although microscopy is known to be more sensitive than rapid diagnostic tests, both tests still have their detection limits. The detection limit for a rapid diagnostic test is approximately 100 parasites/μl. The detection limit is > 5 parasites/μl for an experienced and skilled microscopist. The detection limit of the comparative standard affects the degree of spectrum bias.

To better verify the field performance of FalciVax^{TM} rapid diagnostic tests (or other commercial brands), there are two ways to do it. First, choose a comparative standard that have lower detection limit for malaria parasite. Second, test the field and laboratory performance of a rapid diagnostic test in a high malaria prevalence setting and in a low malaria prevalence setting. Nonetheless, there are pros and cons of both methods.

In the first method, we suggested using a more precise malaria diagnostic method as our comparative standard (e.g. real-time polymerase chain reaction (PCR) or nested PCR) to test the field performance of a rapid diagnostic test. The advantage of using PCR is its low detection limit. It can detect the presence of malaria parasite at > 1 parasite/μl. The disadvantage of using PCR is its expensive cost and the requirement of advanced laboratory equipment. In

the second method, we suggested to test field performance of a rapid diagnostic test to at both high and low malaria prevalence settings, and compared the results with its laboratory performance. The advantages of using the second method against microscopy are (1) its accessibility to have both microscopy and rapid diagnostic tests in rural area, (2) its moderate cost to conduct both microscopy and rapid diagnostic tests, and (3) its ability to test the existence of spectrum bias. The disadvantage of using the second method is the potential non-comparability of study populations between in a high malaria prevalence setting and in a low malaria prevalence setting.

As detection limits exist in all brands of rapid diagnostic tests, false positive and false negative is unavoidable. Whilst the effort in lowering the false positive and false negative percentage in malaria diagnosis, it is also important to discuss how to apply the good standing standard of a rapid diagnostic test, as provided by the World Health Organization, from a laboratory standpoint to the field.

Hot spot and reactive case detection

In the efforts in malaria elimination, it is crucial to broaden malaria searches. The earlier a malaria-infected person is diagnosed and treated, the better the chance to stop the malaria transmission. Passive surveillance and active surveillance are two main methods for case detection. Passive surveillance relies on study participants to notify the study team. However, it is too costly to examine every participant, as in active surveillance.

To help broaden the malaria search in Bandarban study area, we laid the base by studying the spatial distribution of parasite density, as well as by analyzing the measured fever status and self-reported symptoms in association with levels of parasite density. The goal was to find symptoms that were associated with (relatively) more severed malaria infected individuals. Health professionals could use identified symptoms at higher risk as additional case detection variables. The identified symptoms could also be a focus for future case awareness among local residents.

We found no discernible spatial patterns of parasite density. However, we found having fever at the time of malaria diagnostic tests, having self-reported fever at night, having shorter symptom duration, and being older in age were key factors associated with change in relative severity of malaria (i.e. significantly higher or lower parasite density above median.)

In recent years, reactive case detection and hot spot analysis have been used as measures to strengthen case findings. Reactive case detection examines family members or neighbors of a positive case for malaria infection. Hot spot analysis identifies spatial clustering of malaria or spatial clustering of potential risk factors.

Our study provided a means to connect reactive case detection and hot spot analysis. To broaden the case search and case awareness of malaria in Bandarban Study Area, weekly maps of fever cases—for example—can be mapped

and updated. It could be reported through community health workers or local sentinel stations (e.g. local drug stores). Once a map is generated, malaria diagnosis could begin from areas with highest cluster of febrile individuals (i.e. hot spots). This could be done in addition to residents who voluntarily contact health facilities for malaria testing. As malaria infected individuals are diagnosed through clusters or voluntarily testing, subsequent reactive case detection method could be applied to their family members and neighbors.

With the knowledge on factors associated with more severed malaria, hot spot analysis and reactive case detection, more malaria-infected individuals could be diagnosed and treated at a timely manner. Meanwhile, using symptom-related risk factors to promote malaria case awareness could help reinforce the importance of malaria diagnosis among local residents. With broadened case search, diagnosis and treatment, malaria elimination could be more in reach.

7.2.2 Infrastructure

Living environment

To examine risk factors associated with malaria incidence, we used a series of socioeconomic related questions to identify an individual's living standard. Questions included in the questionnaire were on source of income, type of occupation, agricultural land use, durable assets, housing situation (e.g. source of water and type of toilet), household building materials and more.

There are many ways to measure living standards. Here we created an indicator

by using durable assets in the household (e.g. a TV, a radio, a clock, a fan, a chair and a rickshaw). From the analysis, we found individuals with higher living standards had lower malaria incidence. In addition, using covered ring well, tube well and private well/pump, using pit latrine and slab toilet with boundary, using electricity as main source of light at home were associated with reduced malaria incidence.

In Bandarban study area, it is still common to shower in the river, use stream and river as source of water, use bushes as toilets and use oil lamps as source of light. The results of the analyses raised a more fundamental issue. The living environment and mosquito habitats shared common grounds. Therefore, vector control is an important matter. It will be a long process culturally and economically to fully change the environmental infrastructure of the households and the communities.

However, the environmental modification process could start by building more pit latrines in the local communities. Having running water in the households with better water supply system could be a more mid to long-term approach. With a better water infrastructure design, it could incentivize local residents from using rivers and streams as main source of water.

Prior to the completion of upgrading environmental infrastructures, vector control and management (e.g. removal of aquatic weeds and riverine vegetation) as well as reducing contacts with *Anopheles* vector species (e.g. using long lasting insecticide nets) are key to help reduce onset of malaria.

Household building materials

In the dissertation, we examined how household building materials were associated with average numbers of *Anopheles* per household per night. We also analyzed the association between household building materials and malaria incidence.

Mud and *bamboo* were the two common wall, partition and flooring materials. We found using *mud* as building materials were associated with higher number of *Anopheles* in the household comparing using *bamboo* as building materials. After adjusting for areal variability, having some *mud* or *bamboo* as part of building materials did not provide significant difference in numbers of Anopheles found at houses. Having *bamboo*, on the other hand, as a building material was related to elevated malaria incidence comparing to other types of building materials.

The relationship between *Anopheles* vector mosquitoes, housing environment and human malaria are complicated. Here, we cannot pin point one specific housing material that was significantly associated with both higher number of *Anopheles* species and increased malaria incidence. However, we found structurally, many *bamboo* houses were built without windows and doors. The areas designated for windows and doors would have cutouts from the *bamboo* structure. Having open access of the house could mean having a higher chance of exposing to *Anopheles* indoor.

In our analyses, all *Anopheles* mosquitoes identified through entomological surveillance were combined as one *Anopheles* count. More studies are needed to understand how various building materials are associated with population size of individual *Anopheles* species.

Anopheles species could be zoophilic, anthropophilic or both. Many bamboo houses were also built with an elevated ground floor. Households with an elevated ground floor could utilize the space beneath the ground floor as an area for domesticated animals. By understanding how *Anopheles* species choose their hosts and their Entomological Inoculation Rate in Bandarban Study Area would help understand the relationship between *Anopheles* vector mosquitoes, housing environment and human malaria.

From a research standpoint, a more detailed study should be done on a three-way relationship among *Anopheles* vector mosquitoes, housing environment (i.e. mud versus bamboo) and human malaria in Bandarban Study Area. From a risk reduction standpoint, how to improve the infrastructure of a household (e.g. install windows, screens and doors) to better accommodate residents and domesticated animals in the same housing structure and how to execute malaria prevention methods (e.g. indoor residual spraying and using long lasting mosquito nets) are crucial regardless of the building materials used.

7.2.3 Education

When studying the relationship between living standards and malaria incidence, we also examined how years of education was associated with malaria incidence. We found more educated individuals is less prone to having onsets of malaria.

The average years of education was in between 2 to 3 years in Bandarban Study Area. With limited education among residents, this could affect how effective a health education and communication program would work in this rural setting. Although not mentioned in this dissertation, it would be important to study local residents' Knowledge, Attitude and Practice (KAP) on malaria. With the use of a conceptual framework, we can better understand perceived concepts of malaria among residents. We can also understand how family and peer affect residents' attitude and decisions toward malaria. Moreover, we can understand residents' actions toward malaria prevention, management and control methods.

To reduce malaria incidence, education is a step that cannot be overlooked. A long-term solution is to increase the level of education among residents. A short to mid-term solution is to understand the best health communication strategy for this community and devise a malaria program that is suitable for the population in Bandarban Study Area.

7.3 Future Directions

Future directions of the study include:

1. To incorporate hot spot and re-active surveillance methods in malaria case detection in rural Bangladesh.

2. To compare field performances of FalciVax^{TM} rapid diagnostic under different level of *Plasmodium falciparum* malaria prevalence.

3. To identify a different commercial device for rapid diagnostic test (RDT) under the guideline of National Malaria Strategic Plan.

4. To identify *Anopheles* vector species that are the most important to malaria infection in Chittagong Hill Tracts in rural Bangladesh.

5. To study the association between population dynamics of Anopheles species and weather in Chittagong Hill Tracts in rural Bangladesh.

6. To study the socioeconomic status and living standard of residents in Bandarban Study Area in relation to malaria by using the *United States Agency for International Development Demographic and Health Surveys* (USAID-DHS) program standard.

7. To study the association between knowledge, attitude and practice (KAP) on malaria and malaria incidence.

The rationale for each future direction is discussed below.

Direction 1. To incorporate hot spot and re-active surveillance methods in malaria case detection in rural Bangladesh

In this dissertation, we used passive surveillance with home visits as our case detection method. To help the country reach malaria elimination, it is important to broaden the malaria case search. How to expand passive surveillance and incorporate clinical malaria symptoms as an effective hot spot and re-active surveillance method will be an important topic to look at.

Direction 2. To compare field performances of FalciVax^{TM} rapid diagnostic under different level of *Plasmodium falciparum* malaria prevalence.

At our study, we examined field performance of FalciVax^{TM} Rapid Diagnostic Test using passive surveillance. The reported prevalence was high. The results suggested the potential existence of spectrum bias. To test the hypothesis, this result could have been compared with the same study population with a low prevalence (i.e. through active surveillance). Our study conducted active surveillance by selecting random sample of residents in the study area for malaria diagnosis. However, due to having prevalence of 1-2% among selected individuals, only fewer than 30 individuals were tested positive over a four-year study period. The sample size was too small to be compared with the field performance of FalciVax^{TM} Rapid Diagnostic Test through passive surveillance. If comparable, we can examine whether sensitivity decreases and specificity increases with a lowered prevalence of *Plasmodium falciparum* malaria.

Direction 3. To identify a different commercial device for rapid diagnostic test (RDT) under the guideline of National Malaria Strategic Plan.

There are more than 200 commercial RDT devices worldwide. To help the country reach malaria elimination, it is important to identify an effective malaria detection method in rural areas. One approach is to research an alternative rapid diagnostic test device to FalciVax^{TM} RDT. Although this dissertation focused on *Plasmodium falciparum* (*Pf*) malaria in Bandarban Study Area, *Plasmodium vivax* (*Pv*) malaria could also be found. How to balance the field performance of a *Pf*/*Pv* RDT device and its cost would be an important issue to look into.

Direction 4. To identify *Anopheles* vector species that are the most important to malaria infection in Chittagong Hill Tracts in rural Bangladesh.

In this dissertation, we analyzed the relationship between Anopheles mosquitoes and household building materials as a whole. We did not analyze the association by *Anopheles* vector species. With more than 20 *Anopheles* species presented at the study area, it is important to identify the species that are most important to human malaria. Once identified, future studies could focus resources on these *Anopheles* vector species.

Direction 5. To study the association between population dynamics of *Anopheles* species and weather in Chittagong Hill Tracts in rural Bangladesh.

In the dissertation, we only examined the association between numbers of *Anopheles* mosquitoes and types of household building materials used inside a household. However, population dynamics of *Anopheles* mosquitoes could also be affected by the fluctuation of weather. To study the association between numbers of *Anopheles* vector mosquitoes and different weather indicators could help understand the seasonal distribution of *Anopheles* vector mosquitoes in Bandarban Study Area.

Direction 6. To study the socioeconomic status and living standard of residents in Bandarban Study Area in relation to malaria by using the *United States Agency for International Development Demographic and Health Surveys* (USAID-DHS) program standard.

In this dissertation, we surveyed assets and resources commonly used in Bandarban Study Area as our base to identify the living standards of the residents. We used principal component analysis to create an index for living standards. If our surveyed questions and answers could be compared and incorporated into the USAID-DHS format, a more widely recognized socioeconomic index could be generated. If a DHS index is incorporated in the analysis, the comparability and generalizability of the study at Paper 4 could be greater.

Direction 7. To study the association between knowledge, attitude and practice on malaria and malaria incidence.

In this dissertation, we examined the methods in malaria detection; we used the association between level of parasite density and clinical malaria symptoms as a gateway to discuss its potential in broadening malaria case search; we looked how abundance of *Anopheles* vector mosquitoes could be related to household building materials used in Bandarban Study Area; we then examined how the living standard of study participants was associated with malaria incidence. There were external factors related to malaria infection. Internally, residents' knowledge, attitude and practice on malaria were equally important. How to use conceptual frameworks, health education and health communication to help understand the relationship between residents' knowledge, attitude and practice toward malaria and malaria infection will be crucial to facilitate the malaria elimination process in Bangladesh.

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Killeen, Stefan Dongus, Gregor J Devine, and Silas Majambere. Comprehensive sterilization of malaria vectors using pyriproxyfen: a step closer to malaria elimination. *Am J Trop Med Hyg*, 90(5):852–5, May 2014. doi: 10.4269/ajtmh.13-0550.

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region of bangladesh-india border. *Parasit Vectors*, 8:195, 2015. doi: 10.1186/s13071-015-0803-8.

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EDUCATION

Johns Hopkins Bloomberg School of Public Health (JHSPH) *Aug 2010—Present*
Ph.D Candidate in International Health

Dissertation: Risk Profiling of Malaria Epidemiology in Rural Bangladesh
Extensive Coursework in Biostatistics and Epidemiology (See Coursework at Page 8)
JHSPH Student Assembly President 2010—2013

Johns Hopkins Bloomberg School of Public Health (JHSPH) *Aug 2007—May 2009*
M.H.S in Epidemiology

Thesis: A Method to Geocode Rural Addresses and Post Office Boxes: Application to a Study of Drinking Water Nitrate Exposure and Cancer Incidence
Extensive Coursework in Biostatistics and Epidemiology (See Coursework at Page 8)
JHU Taiwanese Student Association Vice President 2008—2009

National Taiwan University School of Public Health (NTUSPH) *Sep 2003—Jun 2007*
B.S. in Public Health

Thesis: The Impacts of Meteorological Factors on the 2003-2004 and 2006-2007 Avian Influenza Epidemics in Japan and South Korea
NTUSPH Student Government Treasurer 2006—2007

TRAINING

University of Washington, Seattle, WA *2012*
Department of Biostatistics
Summer Institute in Statistics and Modeling in Infectious Diseases

University of California at Santa Barbara, Santa Barbara, CA *2010*
NIH-Supported Training Program in Advanced Spatial Analysis

Centers for Disease Control, Taipei, Taiwan *2006*
Contract Diagnostic Virology Laboratory, Virology Lab Trainee

Centers for Disease Control and **Academia Sinica**, Taipei, Taiwan *2005*
Pandemic Influenza Preparedness Program, Technical Epidemic Investigation Staff

TECHNICAL STRENGTHS

Statistical Software	R, STATA, SAS
Document Preparation	LaTeX, R/Knitr, Markdown, Microsoft Office Word
Document Presentation	Microsoft Office PowerPoint, R/Beamer
Reference Management	BibTeX, EndNote, RefWorks
Database	Microsoft Access
Mapping Tools	ESRI ArcGIS, QGIS, Google Earth Pro, R
Operating System	Macintosh, Windows
Other	Adobe Illustrator, Microsoft Office

HONORS AND AWARDS

Academic Scholarship *2011—2016*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of International Health

Academic Scholarship and Travel Scholarship *2012*
University of Washington, Seattle, WA
Department of Biostatistics
Summer Institute in Statistics and Modeling in Infectious Diseases

Study Abroad Scholarship *2010—2012*
Ministry of Education, Taiwan

Recognition Award for Outstanding Services as Student Assembly President *2012*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Global Health Established Field Placements Grant Recipient *2011*
Johns Hopkins Center for Global Health, Baltimore, MD

Student Recognition Award *2011*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Scholarship of NIH-Supported Training Program in Advanced Spatial Analysis *2010*
University of California at Santa Barbara, Santa Barbara, CA

Teaching Assistant (TA) Recognition Award (Nominated) *2010*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Academic Scholarship *2008—2009*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of Epidemiology

Fellowship of Student Research Training Program *2008*
National Institutes of Health, Rockville, MD
National Cancer Institute

Marilyn Menkes Book Award (Nominated) *2008*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of Epidemiology

Research Scholarship *2006—2007*
National Science Council, Taiwan

Dean's List *2004—2005*
National Taiwan University, Taiwan

Presidential Award *2004*
National Taiwan University, Taiwan

Asia-Pacific Economic Cooperation (APEC) Ambassador *2004*
National Youth Commission, Executive Yuan, Taiwan

FUNDED PROJECTS

Awarded Grant	The Impacts of Meteorological Factors on the 2003—2004 and 2006—2007 Avian Influenza Epidemics in Japan and South Korea. (08/01/2006—02/28/2007), National Science Council, Taiwan

SELECTED PUBLICATIONS

Book Chapter

TH Wen, NH Lin, **KCM Lin**, IC Fan, MD Su, and CC King. A Spatial-Temporal Approach to Differentiate Epidemic Risk Patterns. In *GIS for Health and the Environment*, pages 214227. Springer, 2007.

Journal Article

TH Wen, NH Lin, DY Chao, KP Hwang, CC Kan, **KCM Lin**, JTS Wu, SYJ Huang, IC Fan, and CC King. Spatial-Temporal Patterns of Dengue in Areas at Risk of Dengue Hemorrhagic Fever in Kaohsiung, Taiwan, 2002. *Int J Infect Dis*, 14(4):e33443, Apr 2010. ISSN 1878-3511 (Electronic); 1201-9712 (Linking). doi: 10.1016/j.ijid.2009.06.006.

CM Liu, SH Lin, YC Chen, **KCM Lin**, JTS Wu, and CC King. Temperature Drops and the Onset of Severe Avian Influenza A H5N1 Virus Outbreaks. *PLoS One*, 2(2):e191, 2007. doi: 10.1371/journal.pone.0000191.

Conference Poster and Presentation

KCM Lin, WA Khan, DA Sack, S Ahmed, CS Prue, MS Alam, J Khyang, M Ram, J Akter, MM Nyunt, D Norris, G Glass, T Shields, MZ Haq, DJ Sullivan Jr. (2012, April). *Mapping Malaria Epidemiology in Endemic Bangladesh: An Overview of the Summer Project.* Global Health Research Day, Baltimore, Maryland, USA

W Pan, M Kosek, P Yori, MP Olortegui, **KCM Lin**, R Gilman (2009, November). *Impact of Climate Variability on Diarrhea Pathogenicity.* GEOMED/SAMSI 2009 - Conference on Geomedical Systems, Charleston, South Carolina, USA

KCM Lin, SL Heltshe, JR Nuckols, P Riggs, M Airola, MH Ward (2008, August). *Thyroid Cancer Incidence and Drinking Water Nitrate Levels in Nebraska.* 2008 NIH Summer Poster Day, Bethesda, Maryland, USA

KCM Lin, Heltshe SL, Nuckols JR, Riggs P, Airola M, Ward MH (2008, August). *Thyroid Cancer Incidence and Drinking Water Nitrate Levels in Nebraska.* Tenth Annual Division of Cancer Epidemiology and Genetics Summer Recognition and Poster Event, National Cancer Institute, Bethesda, Maryland, USA

KCM Lin (2008, May). *How Modeled PM 2.5 Values Correlate with Asthma Inpatient Hospitalization in the Baltimore Area? A Time-Stratified Bidirectional Case-Crossover Study.* Baltimore Research Day, Baltimore, Maryland, USA

DR Chen, **KCM Lin**, TH Wen (2007, November). *Spatial Analysis in Social Capital Heterogeneity.* Annual Meeting of Taiwanese Sociological Association 2007: East Asian Comparative Research, Taipei, Taiwan

CC King, CM Liu, HP Lin, CC Lee, **KCM Lin**, HN Liu, CY Hsiao, PS Chiang, WR Chen, SH Lin, FC Hu (2007, June). *Integrated Global Surveillance for Avian Influenza A/H5 Virus Outbreaks Considering Meteorological Factors.* Options for the Control of Influenza VI Conference 2007, Toronto, Ontario, Canada

SH Lin, CM Liu, HF Wu, **KCM Lin** (2007, May). *Comparison of three statistical downscaling methods for simulating temperature and precipitation in Taiwan.* Taiwan Geosciences Assembly 2007, Taoyuan, Taiwan

KCM Lin, YY Fang, WL Huang (2006, April). *Hazardous recognition at work: Take staff of convenience store chains.* International Conference of Industrial Hygiene and Occupational Medicine 2006, Taipei, Taiwan

Invited Talk at Scientific Meetings

KCM Lin (July 2, 2008). *How Modeled PM 2.5 Values Correlate with Asthma Inpatient Hospitalization in the Baltimore Area? A Time-Stratified Bidirectional Case-Crossover Study.* ORD AMI PM2.5 Community of Practice Meeting (Meeting with scientists from Environmental Protection Agency (EPA) Region III and Maryland Department of Health and Mental Hygiene (DHMH) Environmental Public Health Tracking Program), Maryland, USA

KCM Lin (June 05, 2007). *GIS analysis in AIV Epidemics: A preliminary analysis in Indonesia.* Center for Geographic Information Sciences, Research Center for Humanities and Social Science, Academia Sinica, Taipei, Taiwan

KCM Lin (March 28, 2007). *The Impact of Meteorological Factors on Avian Influenza Epidemics, 2003-2007: A Case Study in Japan and S. Korea.* 228 M8450 Geographic Information Analysis and Modeling. Graduate Institute of Geography, National Taiwan University, Taipei, Taiwan.

Colloquia

KCM Lin (May 16, 2008). *How Modeled PM 2.5 Values Correlate with Asthma Inpatient Hospitalization in the Baltimore Area? A Time-Stratified Bidirectional Case-Crossover Study.* PHASE Symposium (Public Health Applications for Student Experience, PHASE, Johns Hopkins Bloomberg School of Public Health), Maryland Department of Health and Mental Hygiene, Maryland, USA

TC Fu, **KCM Lin**, SY Yang (May 14, 2008). *The Effect of Household Wealth and Neighborhood Violence on Depression: a Comparison between Multilevel versus Multiple Logistic Regression Models* 340.754 Methodologic Challenges in Epidemiologic Research (Practicum). Department of Epidemiology, Johns Hopkins University, Maryland, USA

RELEVANT RESEARCH EXPERIENCE

Student Investigator *2011/06—2011/08*
Johns Hopkins Malaria Research Institute and International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Statistical Programmer *2010/08—2011/08*
Global Disease Epidemiology and Control Program, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Research Associate *2009/11—2010/08*
Global Disease Epidemiology and Control Program, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Research Assistant *2009/07—2009/10*
Global Disease Epidemiology and Control Program, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Research Assistant *2009/04—2009/10*
Infectious Disease Epidemiology Program, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Summer Research Fellow *2008/06—2008/08*
Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, USA

Intern *2007/10—2008/05*
Environmental Public Health Tracking Program, Maryland Department of Health and Mental Hygiene, Baltimore, Maryland, USA

Research Fellow *2005/08—2007/06*
Infectious Disease Lab, Graduate Institute of Epidemiology, National Taiwan University, Taipei, Taiwan

Research Fellow *2007/01—2007/06*
Global Change Research Center, National Taiwan University, Taipei, Taiwan

Research Fellow *2006/09—2007/06*
Center for Geographic Information Science, Academia Sinica, Taipei, Taiwan

Intern *2006/08—2006/08*
Center for Geographic Information Science, Academia Sinica, Taipei, Taiwan

Intern *2006/07—2006/07*
Division of Surveillance, Centers for Disease Control (CDC), Taipei, Taiwan

Research Fellow *2006/01—2006/07*
Contract Diagnostic Virology Laboratory, Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan

Research Assistant *2004/10—2005/06*
Graduate Institute of Preventive Medicine, National Taiwan University, Taipei, Taiwan

Research Assistant *2004/01—2004/07*
National Taiwan University Hospital, Taipei, Taiwan

Research Assistant *2003/10—2004/01*
Graduate Institute of Preventive Medicine, National Taiwan University, Taipei, Taiwan

RELEVANT TEACHING EXPERIENCE

Biostatistics 2009 - 2012
Teaching Assistant and Tutor *Baltimore, Maryland*

- TA: Statistical Methods in Public Health I, II, III and IV at Johns Hopkins Bloomberg School of Public Health (AY 2009-2010, AY 2011-2012)
- Tutor: Statistical Methods in Public Health I, II, III and IV at Johns Hopkins Bloomberg School of Public Health (AY 2009-2012)
- Tutor: Advanced Data Analysis Workshop at Johns Hopkins Bloomberg School of Public Health (27th Annual Graduate Summer Institute of Epidemiology and Biostatistics, AY 2009-2010)

Epidemiology 2008 - 2010
Teaching Assistant and Tutor *Baltimore, Maryland*

- TA: Applied Epidemiology I at Johns Hopkins Bloomberg School of Public Health (AY 2008-2009)
- TA: Principle of Epidemiology at Johns Hopkins Bloomberg School of Public Health (AY 2009-2010)
- Tutor: Principle of Epidemiology at Johns Hopkins Bloomberg School of Public Health (AY 2008-2009)
- Tutor: Epidemiologic Methods I at Johns Hopkins Bloomberg School of Public Health (AY 2009-2010)
- Tutor: Foundations in Public Health: Epidemiology, Ethics and Health Care Systems at Johns Hopkins School of Medicine (AY 2009-2010)

Geographic Information Science 2007
Lecturer *Taipei, Taiwan*

- Class: GIS Application and Database System at Global Change Research Center, National Taiwan University (May 10, 2007)
- Class: GIS Lab - ArcView 3.3 at Infectious Disease Lab, Institute of Epidemiology, National Taiwan University (April 14, 2007)
- Class: GIS - From Innovation to Implementation at Global Change Research Center, National Taiwan University (January 31, 2007)

Mandarin Chinese 2007 - 2008
Teaching Assistant *Baltimore, Maryland*

- TA: Medical Chinese at Johns Hopkins Medical Institute

LEADERSHIP

Exchange Station Manager *2016/09*
Ragnar Relay Series, Washington, DC

Ex-Officio *2012/05—2013/05*
Student Assembly, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

MHS Student Representative *2012/03—2013/05*
Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

School of Public Health Representative *2012/01—2012/05*
The Welch 21st Century Committee, Johns Hopkins University, Baltimore, MD

President *2011/05—2012/05*
Student Assembly, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Vice President *2010/09—2011/05*
Asian and Pacific Islander Public Health Group, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Student Chair *2010/09—2013/05*
Deans for Student Network, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

President-Elect *2010/05—2011/05*
Student Assembly, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Faculty Advisor *2009/12—2010/08*
Taiwanese Student Association, Johns Hopkins University, Baltimore, MD

Co-Coordinator *2008/09—2009/02*
Japanese Public Health and Healthcare System Educational Trip, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Co-Coordinator *2008/08—2009/05*
Occupational and Environmental Journal Club, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Representative *2008/08—2009/08*
Taiwanese Student Association in Greater DC Area (DCTSA), Greater DC Area (DC, MD, DE, VA, WV, NC), USA

Co-Founder *2008/05*
Asian and Pacific Islander Public Health Group, Johns Hopkins Bloomberg School of Public Health, Maryland, USA

Vice President *2008/04—2009/05*
Taiwanese Student Association, Johns Hopkins University, Maryland, USA

Conference Breakout Session Moderator *2008/09—2008/10*
2008 ESRI Health GIS Conference GIS: Shaping Global Health, Washington, DC, USA

Delegate *2006/08*
Harvard College Asian Business Forum, Mumbai, India

Delegate *2006/08*
Harvard Project Asian and International Relations, Singapore, Singapore

Treasurer *2005/06—2006/06*
Student Government, Department of Public Health, National Taiwan University, Taipei, Taiwan

Interpreter and Receptionist of International Elite Runners *2004/12*
ING Taipei International Marathon 2004, Taipei, Taiwan

Ambassador *2004/01—2004/08*
Asia-Pacific Economic Cooperation (APEC) International Youth Camp, Taipei, Taiwan

GRADUATE LEVEL COURSEWORK

Biostatistics	Statistical Methods in Public Health I, II, III and IV (*16 Credits*)
	Methods in Biostatistics I, II, III and IV (*16 Credits*)
	Essentials of Probability and Statistical Inference I, II, III and IV (*16 Credits*)
	Survival Analysis I and II (*6 Credits*)
	Multilevel Statistical Models in Public Health (*4 Credits*)
	Spatial Analysis and GIS I and II (*7 Credits*)
	Advanced Special Topics in Biostatistics (*2 Credits*)
Epidemiology	Epidemiologic Methods I, II, III and IV (*16 Credits*)
	Epidemiology of Infectious Diseases (*4 Credits*)
	Environmental Epidemiology (*2 Credits*)
	Occupational Epidemiology (*4 Credits*)
	Epidemiology and Natural History of Human Viral Infections (*6 Credits*)
	Epidemiology and Public Health Impact of HIV and AIDS (*4 Credits*)
	Advanced Topics on Control and Prevention of HIV/AIDS (*4 Credits*, Audit)
	History of Epidemiology (*2 Credits*)
International	Design and Conduct of Community Trials (*4 Credits*)
Health	The Design and Analysis of Cluster Randomized Trials (*2 Credits*, Audit)
	Infectious Diseases and Child Survival (*3 Credits*)
	Assessment of Nutritional Status (*3 Credits*)
	Water and Sanitation Needs in Complex Humanitarian Emergencies (*2 Credits*)
	Health Behavior Change at the Individual, Household and Community Levels (*4 Credits*)
	Principles of Population Change (*4 Credits*)
	Global Disease Control Programs and Policies (*4 Credits*)
	Vaccine Development and Application (*3 Credits*)
	Vaccine Seminar (*1 Credit*)
	Global Disease Epidemiology and Control Program Seminar (*4 Credits*)
	Introduction to International Health (*4 Credits*)
Other	Malariology (*4 Credits*)
	Remote Sensing of the Environment (*6 Credits*, Audit)
	Public Health Perspectives and Research I and II (*2 Credits*)
	Research Ethics Related Courses (*4 Credits*)
	Introduction to the Biomedical Sciences (*4 Credits*)

ADDITIONAL INFORMATION

Languages	English, Mandarin Chinese, Taiwanese
Hobby	Programming, Reading Maps, Distance Running, Photography, Design