DETERMINANTS OF MALARIA IN THE CHITTAGONG HILL DISTRICTS OF BANGLADESH

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Abstract

Objective: This dissertation focuses on understanding the epidemiology of malaria in the Chittagong Hill Districts of Bangladesh, with particular focus on the genetic, seasonal, geographic, demographic and behavioral factors that influence symptomatic and asymptomatic malaria.

Methods: The data for this analysis comes from a longitudinal cohort study of approximately 24,000 people that began in October 2009 in an area of Bandarban District of Bangladesh covering an area of 179 km^2. A series of detailed surveys were conducted encompassing symptomatic, uncomplicated or asymptomatic malaria; demographics of the population; knowledge and practices related to malaria, hemoglobin E, geographic locations and environmental factors.

Results: This dissertation demonstrates a number of risk factors for malaria. Homozygous, but not heterozygous, Hemoglobin E was shown to be associated with an increase in mild clinical malaria infection. A detailed exploration of the epidemiology of sub-clinical *P. falciparum* infections demonstrated that they occur year-round, and account for the overwhelming majority of infections at any given time. An adjusted prevalence of sub-clinical infection based on active case finding and diagnosis by RDT and or microscopy was found to be 1.0% in the overall population and 3.2% in pregnant woman. The adjusted incidence was 32.9 (19.7-54.9) per 1,000 person-years. When examining incidence during pregnancy we found 19.4 (4.9-76.6) and 19.5 (8.8-43.11) per 1,000 person-years incidence among 15- to 39-year-old pregnant and recently pregnant women respectively compared to zero cases among non-pregnant 15- to 39-year-old women. Risk factors for asymptomatic malaria include pregnant women, males, jhum cultivators, those living closer to forests and at higher elevations, and marginally

among 5- to 14-year-olds and day laborers. When comparing sub-clinical and symptomatic clinical *P. falciparum* infections we found that hotspots overlapped, but there were substantial differences.

Conclusions: This study demonstrates a number of risk factors for both clinical and sub-clinical *P. falciparum* infections and describes when and where these infections are most likely to occur. This information can help to inform prevention and control programs, and to focus resources in areas and populations that are of high risk.

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ACT	Artemisinin Combination Therapy
GFATM	Global Fund to fight AIDS, TB and Malaria
HRP2	Histadine-rich Protein 2
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
IRS	Indoor Residual Spraying
IPT	Intermittent Preventive Treatment
IPTp	Intermittent Preventive Treatment during Pregnancy
ITN	Insecticide Treated Nets
LLIN	Long-lasting Insecticidal Net
MEP	Malaria Eradication Program
NGO	Non-Governmental Organization
pLDH	Parasite Lactase Dehydrogenase
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Tests
WHO	World Health Organization
WWI	World War One
WWII	World War Two

Chapter One: Introduction and Literature Review

1.1 Overview of Malaria

1.1.1 Global Disease Burden

The World Health Organization (WHO) estimates that 3.3 billion people globally are at risk for malaria, including 1.2 billion people at high risk, with 97 countries and territories having ongoing transmission of malaria in 2014.[1] There were an estimated 214 million cases (149-303 million) and 438,000 deaths (236,000-635,000) in 2015 from malaria.[2]

The 2015 morbidity and mortality represent a dramatic step forward in malaria control. From 2000 to 2015 the global incidence of malaria decreased by 37%, and global mortality decreased by 60%.[2] The population at risk has also dramatically decreased from 90% of the global population in the mid-19th century to under half at the present. Figure 1.1 maps the locations of areas at risk from the mid-19th century to 2010.[5] Despite these improvements, there are still large areas of the world at risk of malaria.

1.1.2 Clinical Presentation

The symptoms of malaria include periodic bouts of fever, chills, sweating and rigors, which occur every 2 to 3 days depending on the *Plasmodium* species. The classic malaria triad is fever, splenomegaly and anemia. Patients often have constitutional symptoms of headaches, nausea, body aches and weakness. These common 'flu-like' symptoms of malaria are likely caused by the release of cytokines such as TNFα from macrophages responding to cell debris and toxins released after the rupturing of erythrocytes during the erythrocytic cycle. *P. malariae* can result in nephrotic syndrome, which can also be fatal.

P. falciparum, the most severe form of malaria, is associated with numerous additional clinical symptoms including pulmonary edema, renal failure, coma ('cerebral malaria'), lactic acidosis, hypoglycemia, shock and death. *P. falciparum* has the ability to alter the membrane of the erythrocytes, allowing it to adhere to the endothelial surface of the lumen of blood vessels. This cytoadherence leads to sequestration of erythrocytes in blood vessels, which can slow blood flow and reduce oxygen to vital organs. This is the leading theory on the cause of cerebral malaria and certain other organ damage. The pathophysiology of renal failure is unclear but may relate to pre-renal factors such as dehydration, which often can be reversed with proper rehydration. Tissue dysfunction, hypoxia and impaired renal function can lead to lactic acidosis.

P. vivax and *P. ovale* are associated with milder symptoms including fevers, mild anemia, and splenic rupture. In the cases of *P. ovale* and *P. vivax*, relapse can occur as some of the sporozoites entering the hepatocytes remain inactive in the liver rather than immediately undergoing asexual reproduction, only to be reactivated later.[3-5]

It is challenging to separate age-independent effects and cumulative exposure, particularly in areas with high exposure. However, studies on migrants moving into endemic areas, who are naive to exposure, can help to parse out these distinctions. Protective immunity against *P. falciparum* malaria developed in a population of migrants in 18-24 months in a hyperendemic setting and after this exposure, these migrants then had symptoms similar to others of their age in the local population, which is dependent on the acquired immune response that changes with age. Young children often suffer severe malaria anemia, while older children are more likely to suffer from cerebral malaria and adults who are symptomatic are more likely than children to suffer renal, hepatic, and pulmonary complications of disease.[3,6,7]

For all forms of malaria, repeated symptomatic attacks can be very debilitating, shortening the lifespan, causing cachexia and splenic enlargement, and leading to loss of energy, weakness, a large swollen abdomen, neurologic sequelae, and decreased development of cognitive and behavioral capacity. It also has been shown to impair weight gain in children and cause anemia in children living in holoendemic settings.[8] However, the extent to which a person's immune system can fight illness and the access a person has to antimalarial medication can have a significant influence on the extent to which they suffer these debilitating effects.[3,9]

Malaria is a particular issue in pregnancy. It is associated with maternal anemia and death, as well as higher risk of miscarriage, stillbirth, infant mortality, lower birth weights and mother-to-child transmission of HIV.[3,10,11] Risks of infection peak at 13-16 weeks gestation.[10] These risks are higher among those who have less immunity, which is more likely in areas where there are lower rates of infection. However, even in endemic areas pregnancy is associated with some level of immunosuppression.[3,10] Reductions in malaria in pregnancy can result in a healthier infant population with higher birth weights and reduced congenital abnormalities, and thus an overall greater resilience to disease.[12]

1.1.3 The Life Cycle of Malaria

The life cycle of malaria includes the *Plasmodium* parasite, the mosquito vector and the human host.

The Plasmodium Parasite

Malaria is a group of related infections caused by several different parasite species of the genus *Plasmodium*. There are five species that are known to infect humans, *P. falciparum* and *P. vivax*, *P. malariae*, *P. ovale and P. knowlesi*. Today, *P. falciparum* and *P. vivax* are responsible for the largest burden of malaria. *P. vivax* is found in both temperate and tropical climates, while

P. falciparum is generally only found in tropical and subtropical areas, as it requires higher temperatures.

The Anopheles Mosquito Vector

Mosquitoes of the genus *Anopheles* can transmit malaria through the bite of a female mosquito. There are over 450 species of *Anopheles*; about 60 are considered vectors for *Plasmodium* species.[13] These species range from very major to minor roles in malaria transmission, with dominant species varying in different regions of the world.[14] Their ability to transmit is defined by vectorial competence and capacity, which vary between species, within species and with features of the local environment and climate.[13] Vectorial competence is a measure of the mosquitoes' capability to transmit malaria, while vectorial capacity is a measure of the efficiency of transmission.

Adult female *Anopheles* mosquitoes mainly feed on blood, the rich content of which is required for producing eggs. *Anopheles* also vary in how much they feed on humans or other animals, and at what times of day and in which environments they prefer to do so. Zoophilic mosquitoes prefer animals and anthropophilic prefer humans. Endophagic mosquitoes prefer to feed indoors, while exophagic prefer to feed outside. Exophilic mosquitoes prefer to rest outside and endophilic mosquitoes prefer to rest inside.[15]

After the female mosquito has her blood meal, her ovaries enlarge as the blood is digested and the eggs mature. During a single oviposition, the female *Anopheles* mosquito can lay 50-200 eggs. These eggs cannot withstand desiccation and thus require a wet environment. They usually hatch within 2-3 days, but can survive on wet mud for 16 days and hatch immediately when flooded. The type of water ranges by type of *Anopheline* mosquito, some preferring a range of different environments, including still water in puddles or pots, in ponds,

marshes or the edges of streams. Others prefer saltwater.[15] After oviposition, the eggs develop into larvae and then pupae, with the larval period lasting 7-11 days and the pupal stage 2-4 days, although in cooler climates these periods can be extended to several weeks or even months.[16]

The Life Cycle of Malaria

Figure 1.2 gives an overview of the life cycle of the *Plasmodium* parasite in humans and mosquitoes. An uninfected female mosquito of the genus *Anopheles* bites an infected human, taking up red blood cells containing gametocytes from the blood. Gametocytes (spermatocytes and oocytes) are the precursors to the male and female gametes, which are the germ cells. Within the mosquito gut, the red blood cell ruptures, releasing the gametocytes, which then divide by meiosis to develop into haploid gametes. These male and female gametes merge to form diploid zygotes, which develop into ookinocytes, which migrate into the midgut wall and form oocysts. The oocyst then balloons, produces thousands of haploid sporozoites and ruptures, releasing the sporozoites, which then travel in the insect hemocoel to the salivary glands. The incubation period in the mosquito, before it becomes infectious, depends on temperature as well as parasite species. At 28 °C, the duration of sporogony is 9-10 days for *P. falciparum*, 8-10 days for *P. vivax*, 14-16 days for *P. malariae* and 12-14 days for *P. ovale*.[13,16]

If the mosquito survives long enough after biting an infected person for sporogony to finish, it can then infect a new person when biting by releasing sporozoites into skin or subcutaneous tissue or directly into blood.[7,17,18] These sporozoites may go into the lymphatic system, but a proportion enter the bloodstream and then the liver, travel through Kupffer cells and then invade hepatocytes, where they undergo asexual development called schizogony and transform into thousands of merozoites.[17] The exoerythrocytic schizonts rupture, the merozoites

are released into the blood stream and invade erythrocytes, thus commencing the erythrocytic cycle.[7]

P. vivax parasites can only invade reticulocytes, while *P. falciparum* can attack all red blood cells. The merozoites enter through endocytosis, thus surrounding themselves by the host cell membrane within the cell. In this vacuole each merozoite commences further asexual development. The next form in *P. falciparum* infections is the immature trophozoites, which appears as a ring. These develop into trophozoites and then schizonts. Within each schizont, parasites replicate, creating 6 to 36 merozoites. The schizont then ruptures and the erythrocyte bursts, releasing merozoites into the bloodstream to infect other red blood cells. This cycle occurs about every 48 hours (tertian) for *P. vivax*, *P. falciparum* and *P. ovale,* and about every 72 hours for *P. malariae*.[17] These cycles of schizont rupture and red blood cell destruction are accompanied by cycles of fevers and other symptoms, which generally for *P. falciparum* begin 6-14 days after infection, although this period can be prolonged by acquired immunity, partial treatment or prophylaxis.[5] Gametocytes (male and female) generally appear 7-15 days after erythrocyte invasion for *P. falciparum* infections, but are often present before symptoms occur for *P. vivax* infections.[7]

P. falciparum in particular is able to form knob-like proteins on the surface of red blood cells, helping them to adhere to the endothelial cells which make up the interior surface of blood vessels, thus preventing them from being rapidly removed by the spleen and allowing it to spread merozoites to new erythrocytes. This sequestering of red blood cells occurs in many organs including the placenta, brain, lungs, and heart among others. They also block vessels and blood supply to vital organs, causing some of the symptoms malaria is known for, through lack of oxygen.[17]

The incubation period in humans ranges dramatically. For *P. falciparum* it is generally about 6-14 days, for *P. ovale* about 16-18 days, *P. malariae* 18-40 days or longer and *P. vivax* on average 12-17 days but can occur up to 6-12 months.[16]

Ecology

A number of ecological factors have been associated with malaria both globally and in hotspots in local areas. Ecology impacts malaria by impacting the preferred geographic locations and life span of *Anopheles* mosquitoes and in some cases, the locations and behavior of people.

Climate plays a particular role in defining the seasonality and geographic distribution of malaria. Malaria has been found to be associated with temperature, rainfall, humidity, water quality, elevation, deforestation, and agriculture, among other environmental factors.[19] Rainfall is important to provide microenvironments for oviposition and larval and pupal development. Without adequate rainfall these sites may not be available and with too much, they may be destroyed. Temperature is also of utmost importance as warmer temperatures are associated with mosquito survival more broadly, but particularly with a faster sporogenic cycle, or growth of parasites within the mosquito.[20] If the mosquito does not survive to the end of this cycle, malaria cannot be transmitted. Mosquitoes seek out microclimates that reflect their preferential habitats, such as areas of lower temperature if areal temperatures exceed their preferred levels. High transmission generally occurs closer to the equator, although transmission remains low in areas like the Sahara where little water is available. *P. vivax* appears to be able to withstand cooler temperatures than *P. falciparum*, and is thus the predominant species in the more moderate-temperature countries that are still battling malaria. The minimum temperature for development of the parasite is estimated to be between 14.5 and 15°C for *P. vivax* and 16 to 19°C for *P. falciparum*.[21]

Climate also influences human behavior such as sleeping locations and time spent outdoors. During harvest times in particular, there may be an increase the number of people working and sleeping outdoors.[20]

1.1.4 Human Immunity

The experience people have with malaria is dramatically impacted both by the *Plasmodium* species with which they are infected, as well as their own immune systems and genetic makeup. Stronger immunity can reduce malaria symptoms and mortality. Some aspects of immunity are acquired following exposure to disease, while others are related to the genetics of the host.

Acquired Immunity

If one does not succumb to disease, repeated infections with malaria are associated with varying levels of immunity to the symptoms of malaria upon repeated infections. In stable endemic areas, higher levels of acquired immunity dramatically reduce severe disease and death in the population that has survived earlier infections. Immunity to *P. falciparum* malaria is acquired relatively quickly compared to *P. vivax*.[7] At least partially a result of the change in immunity with age, young children suffer the most severe symptoms of malaria and are most likely to suffer anemia and die. Immunity, however, does not remain for long, as it can be lost after a period of about 6 months to a year of no exposure to infection.[3,7] So travelers who have left an endemic area to reside in a non-endemic area for several years and then return home are also at high risk for symptomatic infections. This loss of immunity is also of importance when considering the consequences of failed malaria control programs. If an area is successful in reducing vector populations or in controlling malaria by other means for a few years, but fails to

eliminate the disease entirely and resurgence occurs, the consequences of reinfection may be more severe in those who have lost immunity.

Those who live in unstable endemic areas, which have large fluctuations in exposure, or in hypoendemic areas where the chance of infection is lower and the time between infective bites longer, symptomatic malaria is seen across all ages, as much less of the population has developed immunity. This is also the case in the context of epidemic outbreaks in areas where there is very low exposure to infection before the time of the outbreak.[3,22-24]

Genetic Immunity

Given the tremendous impact malaria has had on humans throughout history, it is not surprising that genetic traits that are protective for malaria would be selected for over the course of human history. Haldane first proposed the hypothesis in 1948 in reference to thalassemias around the Mediterranean.[25]

Thalassemia is, however, only one of several red cell polymorphisms that have been shown to give a selective advantage for survival from malaria and thus have high allele frequencies in populations who live or whose ancestors lived in areas with high rates of malaria. Some of these protective polymorphisms include hemoglobin S[26-30] and C,[31-34] Thalassemias,[28,30,35-38] G6PD deficiency,[39-41] ovalocytosis,[28,42-46] lack of the Duffy antigen specifically for *P. vixax*,[28,47-50] and complement receptor protein polymorphisms.[28,51-54] These red blood cell polymorphisms protect against malaria through a variety of mechanisms including parasites not surviving lack of oxygen, RBC's resisting invasion of the parasite and the spleen clearing deformed cells. The amount and nature of this protection, like anything with malaria, can evolve. For instance, there are already several papers documenting *P. vivax* malaria in Duffy negative groups, which until recently were thought to be completely protected.[55,56]

1.1.5 Diagnosis

Giemsa-stain microscopy is currently the gold standard for malaria diagnosis. However, it requires a trained microscopist and laboratory equipment. As such, the quality of the results varies dramatically.[57-59] The threshold for diagnosis of malaria through microscopy is on the order of 4-20 parasites/μL in ideal conditions, and 50-100 parasites/μL in field conditions.[57]

The rapid diagnostic test (RDT) has been a game changer for malaria diagnosis in areas where a laboratory is not easily available. It has the advantage of immediate testing to inform point-of-care clinical decisions in the field. The RDT is based on detecting malaria antigens in the blood of the person being tested by using monoclonal antibodies against the parasite antigen through an immunochromatographic assay. The targeted antigens are ideally abundant during both the sexual and asexual phases of the parasite. Antigens used in tests include Histidine-Rich Protein 2 (HRP2) for detecting *P. falciparum* infections and genus specific *Plasmodium* aldolase and lactate dehydrogenase (pLDH) for detecting all malaria species. pLDH is better for monitoring clinical infections as it does not persist in the same way as HRP2, which often remains positive for 1-2 weeks following infection.[57,60,61] The sensitivity of RDTs is based on parasite density. RDT sensitivity goes down if parasite densities are less than 100/μL for *P. falciparum* and less than 5,000/μL for *P. vivax*.[62,63] In Thailand one study showed 100% sensitivity for parasite density above 500/μL and 83% for densities less than this.[63] Generally, sensitivity for non-*P. falciparum* tests is lower for HRP2-aldolase systems, while sensitivity is higher for the pLDH system for *P. vivax* infections. False positives can also be an issue with a few percent of tests, as the RDT can cross-react with rheumatoid factor and heterophile antibodies, although some improvements with respect to rheumatoid factor have been made in more recent tests.[57,64-66]

Molecular techniques such as DNA probes and polymerase chain reaction (PCR) are generally used for research rather than clinical purposes at this time. They can be useful for identifying submicroscopic infections.

Despite the availability of RDTs and microscopy, many people are diagnosed on clinical symptoms alone and treated presumptively for malaria without any formal testing. This strategy is not recommended by the WHO as many of the clinical symptoms of malaria overlap with other infections, thus leading overuse of antimalarials, and underdiagnosis of other potentially serious conditions.[67,68] As such, it is advisable to use confirmatory testing measures.

1.1.6 Treatment

Although there are a number of treatment options for malaria that have been developed over the years, resistance has been a problem. *P. falciparum* resistance to chloroquine was a particularly large blow as this drug was safe and very inexpensive. There is documented *P. vivax* and *P. malariae* resistance to chloroquine, although they are still sensitive to chloroquine in much of the world and *P. ovale* and *P. knowlesi* are considered sensitive globally.[68-70]

Currently, three days of one of the approved Artemisinin Combination Therapy (ACT) regimens is the gold standard for treatment of *P. falciparum* malaria for all but pregnant women in their first trimester.[70] ACTs currently result in more rapid clinical recovery than other drugs, and as they reduce gametocytes, they can also reduce transmission.[68,71,72]Artemesinin is not recommended during first trimester pregnancy, thus pregnant women in their first trimester are to receive 7 days of quinine and clindamycin.[70]

For severe malaria in children, in settings where full treatment is not available, an intramuscular injection of artesunate followed by referral is appropriate. If intramuscular artesunate is not available, intramuscular artemether can be used, and in the absence of

Artemether and artusunate, quinine can be used.[70] Injected/IV drugs may be necessary for more severe patients, particularly if they are not able to take anything orally.[68] If this is not available, rectal artesunate has been shown to reduce symptoms, disability and mortality in cases where there is at least a few-hour delay in reaching hospital services and can be used for children less than 6 years of age.[70]

ACT, however, is more expensive, making it out of range for some individuals to seek care if it is not covered by some other entity. For this reason a number of countries still use quinine as a first-line treatment, even though it has longer parasite clearance times, a higher case-fatality rate and a narrow therapeutic window.[68,73-75] It is clear, however, that despite high costs of drug, the decreased mortality associated with artemisinin drugs makes it more cost effective and the treatment of choice.[74,75] For this reason, the Affordable Medicines Facility-malaria was created to help subsidize ACT prices to below that of artemisinin monotherapy and similar to that of chloroquine or sulfadoxine-pyrimethamine.[76]

The use of combination therapy with artemisinins is critical as it can help slow down resistance developing against artemisinins. This is of particular concern as there is already evidence of increased parasite clearance time in several countries in Southeast Asia. There are several different combinations in use including artemether and lumefantrine, artesunate and amodiaquine, artesunate and mefloquine, artesunate and sulphadoxine-pyrimethamine and dihydroxyartemsinin and piperaquine. These treatments are recommended by WHO for different regions depending on strains of malaria and local resistance patterns.[68,70]

Treatment of *P. vivax, P. ovale, P. malariae and P. knowlesi* includes either treating with ACT or in areas where strains are still susceptible, chloroquine. For women in their first trimester, who have a chloroquine resistant strain, treat with quinine. *P. vivax* and *P. ovale*

require primaquine to treat the liver phase of the life cycle and prevent recrudescence. A 14-day course of primaquine is recommended for all but pregnant women, infants less than 6 months, women breastfeeding infants less than 6 months and women breastfeeding older infants unless it is established that there is no G6PD deficiency.[70] If close monitoring is feasible, G6PD deficient patients can receive 0.75 mg/kg primaquine once a week for 8 weeks while being monitored for evidence of hemolysis. It is not necessary to test for G6PD for the single dose 0.25/mg/kg version of primaquine, which is used to reduce transmissibility of *P. falciparum* infections.[70]

For pregnant women living in areas with endemic and seasonal malaria, intermittent preventive treatment (IPT) is often used to help reduce morbidity and mortality of pregnant woman and infants.[68,77-79] IPT has also been shown to be useful for school-aged children in the setting of unstable malaria.[80-82] WHO recommends all pregnant, non-HIV positive, women in areas with *P. falciparum* malaria have at least three treatments at least a month apart of sulfadoxine-pyrimethamine, for HIV positive women more frequent treatment is necessary.[68,78] They recommend infants less than 1 year receive two doses of sulfadoxine-pyrimethamine along with the second and their round of diphtheria, tetanus and pertussis (DTP) as well as their measles immunizations. For seasonal chemoprevention, children less than 6 years should be given amodiaquine and sulfadoxine-pyramithamine monthly during the transmission season.[70]

1.1.7 Prevention, Control & Elimination

In the past decade focus on malaria control and elimination has increased dramatically, with major efforts such as the Millennium Development Goals and the Roll Back Malaria Partnership, both with significant support from public and private investments. There has been a substantial increase in funding for malaria control and elimination, with the Global Fund to Fight AIDS, TB and Malaria (GFATM), the US Presidents Malaria Initiative, the World Bank's

Booster program and others.[1,83] With these new investments, we are again focusing on elimination of malaria as a goal. The current main prevention and control measures can be divided into vector control and human host pharmacologic control. Future strategies that are being developed focus on vaccines as a promising intervention.

Vector Control

As discussed above, there is a long history in the use of various vector control measures. Some aim to reduce breeding by draining still water or adding fish to containers, puddles, swaps, foods and other locations. Others focus on killing larvae or mosquitoes with larvicides, adding fish to containers, indoor residual spraying (IRS), insecticide treated nets (ITNs), personal insect repellants, and improving building structures by adding screens and other means to prevent mosquitoes from entering.

The two vector control strategies used most often include IRS and long lasting insecticidal nets (LLINs). Unlike ITNs, LLINs do not require retreatment as they maintain effective insecticide levels for at least three years. The money being invested in these areas has resulted in substantial progress in coverage of those at risk. According to WHO, about half of the population at risk in sub-Saharan Africa had access to ITN in 2013, a vast increase from the 3% having access in 2004. The use of IRS has actually decreased, with only 4% of the global population at risk being protected with IRS.[1]

LLINs and IRS require up-to-date understanding of the local disease vectors and their susceptibility to different insecticides. Both methods work through shortening the life span of the mosquito, making it less likely they will live long enough to transmit disease. LLINs also provide barrier protection if there are no holes and the nets are tucked in properly. As with pharmacologic approaches, issues of resistance have also developed in relation to insecticides for

nets and IRS. In particular, there has been increased resistance to pyrethroids, which are the most commonly used insecticides.[1,84-86] 53 of the 65 reporting countries reported resistance to at least 1 insecticide and 41 to more than one in 2010.[1] The WHO recommends 15 different compounds in four classes for IRS. In areas with high LLINs coverage, they recommend using a different chemical for IRS (i.e. non-pyrethryoids) applied in rotation with LLINs to help manage resistance.[87]

Behavioral resistance has also been seen in numerous species adapting to the control measures put in place. For instance, when large areas of the southwest Pacific used DDT for IRS, the three main species *An. faruti*, *An. koliensis* and *An. punctulatus* were initially controlled. However, *An. faruati*, which feeds from early evening throughout the night, replaced *An. koliensis* and *An. punctulatus*, which were late-night indoor feeders and did not change behavior following IRS campaign. *An. faruati* changed its behavior to feed outdoors, and adjusting to a higher proportion feeding in the early evening, thus protecting itself from the IRS.[88]

New research has focused on genetic modification of mosquitoes or mosquito symbionts as a means of making them resistant to or less likely to transmit *Plasmodium*.[89-91]

Human Host Strategies

As discussed in the treatment section above, several pharmacologic strategies exist to kill the parasite within the human host, both as prophylaxis and treatment.

These pharmacologic strategies require an extensive health infrastructure to provide prophylactic drugs to both travelers and those in endemic areas, and to provide testing and treatment services for those who are symptomatic, ideally catching disease early to lower the probability of transmission and likelihood of complications and death. These programs often

involve several levels of government, including national and regional programs, local health clinics and community health workers, among others.

The provision of pharmacologic interventions has also been improving at a global level. With respect to intermittent preventive treatment in pregnancy (IPTp), 35 countries have programs in this area, with 57% of pregnant woman in these countries receiving at least one dose in 2013. However, there is still a much higher rate of attendance at antenatal visits than of IPT administration, suggesting more could be done in this area.

Intermittent preventive treatment in children (IPTc) has also been considered for children living in places with seasonal malaria. In this strategy all children of a certain age, usually less than 5 years, are given prophylactic antimalarials. Amodiaquine plus sulphadoxine-pyrimethamine is the most common drug used for IPTc. According to a systematic review, including seven trials in West Africa, IPTc can prevent about three-quarters of clinical malaria cases and a similar proportion of severe malaria cases. It likely also reduces anemia and all-cause mortality. [92] The capacity for preventing morbidity and mortality, at least in the short term, is undeniable. However, there are several concerns with this approach. The biggest concern relates to the possibility of more rapid development of drug resistance following implementation of these programs. There is also some concern about the impact of decreased immunity on children who have aged out of the program.[93] Other concerns relate to the logistics of implementing such large-scale programs and convincing people to take medicine when they are not sick. Only six of the countries recommended by WHO to adopt seasonal IPTc programs have done so and only one has adopted the program for infants, although this is not yet implemented.[1]

Another strategy used more commonly in the middle of the last century was mass drug administration (MDA), often incorporated with IRS.[94,95] This strategy aims to stop transmission

by treating everyone in a given population at the same time, regardless of age or other factors. Similar to IPTc, there are concerns about this strategy accelerating drug resistance. There are also concerns about the efficacy, sustainability and feasibility of these strategies. A review that analyzed 182 published accounts of MDA noted the majority of reports did not follow up for more than 6 months, making the long-term success of these programs hard to assess.[96] Another review showed a short-term decrease in parasitemia following these programs, but again no long-term follow-up past 6 months. [97] Although these strategies are not necessarily widespread, this study showed that programs were more likely to be successful, at least in the short term, if they included directly observed therapy in the community rather than at a central point, the use of 8-aminoquinoline-based drugs to control *P. vivax*, high community engagement, and co-interventions such as IRS and ITNs. Also when island locations or other isolated areas use these strategies, they are more likely to be successful. The review noted no deaths and few adverse events related to hemolysis from G6PD deficiency, but noted the necessity for careful follow-up if using a 14-day course of primaquine.[96]

The use of diagnostic tests has increased in the past decade. In the WHO African region, the number of people suspected of malaria in public facilities who received diagnostic tests went from 40% in 2010 to 62% in 2013. The number of RDTs distributed globally by national malaria control programs went from less than 200,000 to 160 million from 2005 to 2013. The number receiving microscopic blood tests has not changed in the past year, remaining at 197 million globally. There are still large gaps in services provided to those who have symptomatic malaria. It is estimated that 9-26% of children with malaria received ACTs. The reasons for this low coverage relate to low access and care-seeking behavior among patients and failure of clinics to provide ACTs to presenting patients.[1]

Vaccines are another potentially promising area of research, which could change the trajectory of malaria control and elimination in the event they become operational.

Elimination

The WHO defines elimination as a country having zero locally acquired cases for three consecutive years. Many countries have decreased the number of cases dramatically from epidemic levels, shifting their focus from simply controlling malaria to elimination, and some have succeeded in eliminating malaria. In countries shifting focus to malaria elimination, the proportion of cases that are symptomatic may increase and the ages of infection may rise due to lack of immunity.[7]

Recently the Roll Back Malaria partnership's Global Malaria Action plan outlines a program to move toward the elimination of malaria, using the key tools of long-lasting insecticidal nets (LLIN's), IRS, IPTp and other vectorial control measures, including larvicide, rapid diagnosis using RDTs and/or microscopy and treatment using ACTs for *P. falciparum* and chloroquine and primaquine for *P. vivax*.[98]

1.2 Malaria in Bangladesh and the Chittagong Hill Districts

Bangladesh, a country of 159 million people in a landmass of 130,170 square km is one of the most densely populated countries on our planet, with 1,218 people per square km.[99,100] It is bordered by India to its west, north and east, Myanmar (Burma) to the southeast and the Bay of Bengal to the south. It lies at the mouth of the Ganges, Brahmaputra and Meghna Rivers, whose ever-changing flow and seasonal flooding defines the landscape of this low-lying country.

In order to gain control of the annual inundation of water during the monsoon season, the British in the second half of the nineteenth century built embankments and dams for roads and rail systems, irrigation and protection from severe flooding. While these developments did

achieve many of their aims, they also transformed much of the landscape, preventing water from flowing off lands and often creating waterlogged areas. This lack of flow also resulted in much less silt being deposited on farmlands, decreasing the soil fertility.[101,102] Prior to this time, malaria was under reasonable control, with much of the mosquito breeding grounds being inundated with the high flows of the seasonal floods. However, after these new developments, the waterlogged, poorly drained land provided a breeding ground for *Anopheles* mosquitoes, and Bangladesh saw a steep increase in morbidity and mortality from malaria. It was estimated, for instance, that in the 1870s some villages in West Bengal had 75% of their population infected, with a mortality of 25%.[101]

A high burden of disease plagued the country until the government instituted the Malaria Eradication Program (MEP) starting in 1961. The decline in malaria was particularly notable in the late 1960s to early 1970s. As can be seen in Figure 1.3, from 1968 to 1971 the incidence of malaria dropped from 10.8 to 4.22 per 100,000 population.[103] The incidence of malaria by district from 1968 to 1971 and from 1972 to 1977 can be seen in Figure 1.4. The eastern districts of the country including Sylhet, Chittagong and the Chittagong Hill Districts had the highest incidence of malaria at this time. The southern district of Patuakhali was also among the higher incidence districts during this first period, but unlike the rest of the country, decreased its malaria incidence during the 1970s. The authors theorize this may be related to a dramatic drop in the number of cattle in the area as a consequence of a tropical cyclone. They cite the climate as well as the Kaptai Dam built in the early 1960s as contributing factors to malaria. They also note that the introduction of high-yielding rice varieties in 1968 and large increases in the amount of land devoted to farming these high-yield varieties in the 1970s, along with the associated high levels of irrigation and standing water, provided an ideal breeding site for *Anopheles* species and was

associated with an increase in malaria.[103] Other factors that were positively associated with malaria during the waning period were forested areas, rainfall, temperature, livestock per rural household, percent of the area covered in water bodies, percent of the area with the high-yield rice varieties, and overall irrigated rice area. The western districts may have had particularly low rates as the MEP started there. This program included a household census to identify hotspots, household insecticide spraying, and case detection and treatment.[103]

In the early 1970s there was a resurgence of malaria. In a single year from 1971 to 1972 the annual incidence of malaria increased from 4.22 to 25.4 per 100,000 population and then remained high over the next 5 years. There are a number of factors that likely influenced this change. After decreases in malaria incidence seen in the late 1960s, focus on the MEP program dropped, with household spraying stopping and responsibility for the program going to local health authorities. At the same time, the Liberation War with Pakistan both directly impacted the MEP program and also created an increase in refugees from India, mass destruction of homes with associated increased exposure to mosquitoes, large reductions in the number of livestock that may have been bitten instead of humans, and generally a reduction in the standard of living and an increase in income inequality. These war-related factors were much more severe in the eastern areas of the country bordering with India, and less of a concern in the highest malaria prevalence areas in the west.[103]

A 21-month study in Sylhet district from 1975-1976 found that 94% of those tested had at least one positive blood smear, 88% positive for *P. falciparum,* and 70% positive with *P. vivax*, with 2.1% with coinfections. Both *P. falciparum* and *P. vivax* had the highest prevalence in the 5-14 age group. They found that the prevalence of *P. falciparum* was particularly high following the monsoon season, while *P. vivax* appeared more evenly during the year.[104] In

another study Rosenberg and colleagues noted that mosquito feeding patterns appeared to be influenced by the phases of the moon.[105]

Since the 1970s malaria has continued to be a problem in Bangladesh. Figure 1.5 summarizes statistics related to malaria from 1970 until 2013. The number of cases rose from the beginning of this period, peaking in 1994, with the number of deaths peaking in 1995. The number of blood smears examined decreased during this time, despite the increasing number of cases and the population at risk increasing. Of note, this is the reported confirmed number of malaria cases, which likely substantially underestimates the burden of malaria, as many cases are not confirmed and reported, and much of the population does not seek care for malaria. Another factor that may be influencing the rise of malaria is the Pakistan government's frequent use of DDT for malaria control prior to 1971. DDT was officially banned in 1985.[106] This along with a lack of attention and funding to malaria control programs was accompanied by the increasing number of cases.

Currently, malaria is endemic in 13 of 64 districts of Bangladesh, with over 14 million people at risk for infection, with the highest rates in the Chittagong Hill Districts, where *Plasmodium falciparum* is the predominant species and *Plasmodium vivax* occurs to a lesser extent.[107,108] Clinical infections tend to peak from June-August during the height of the rainy season.[109,110]

Bangladesh has a wide variety of species of *Anopheles* mosquitos. Several different studies have been conducted, identifying anywhere between 17 and 21 different species of *Anopheles* mosquitoes in Bangladesh, the concentration of which varied dramatically by location of the study.[111-114] These species have a wide range of preferences with respect to biting behavior and preferred habitats. In the Hill Districts region important *Anopheles* species with respect to

human malaria have varied depending on the study, but the following have been identified as potentially important in at least one study: *An. dirus*, *An. philippinensis*, *An. baimai*, *An. minimus*, *An annularis*, *An. varuna*, *An. barbirostris*, *An. subpictus*, *An. vagus*, *An. nigerrimus*, *An. maculatus*, *An. jeyporiensis*, and *An. nivipes*.[111-115]

In 2006 the GFATM gave Bangladesh 39.6 million U.S. dollars for malaria control, which were used to revamp the National Malaria Control Program implemented by the NGO BRAC and the Ministry of Health. The program focused on community advocacy, community-based testing and treatment using RDTs, ACT and LLINs, treating existing nets with deltamethrin and overall strengthening of the surveillance and control programs.[106,116]

The focus on ACT was important, as drug resistance and treatment failure had been documented for other therapies, including 56% of those taking 3 days of chloroquine, and 8-14% of those taking several other treatment regimens such as quinine for 7 days, or mefloquine and quinine in combo with sulfadoxine-pyrimathamine.[117]

From September-November 2007 BRAC, a large health-related Non-Governmental Organization (NGO), and the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), a large health research institution and hospital, collaborated to conduct a cross-sectional survey of the 13 endemic districts of the country in order to help guide distribution and use of GFATM funds, and provide baseline data to understand the effectiveness of these programs.[106] This study found in these 13 districts the prevalence of malaria as detected by RDT was 3.97%, with a *P. falciparum* only prevalence of 3.58%, a *P. vivax* only prevalence of 0.21% and a coinfection prevalence of 0.18%. They found the prevalence of *P. falciparum* to be 8.5% in 0- to 4-year-olds and 6.6% in 5- to 14-year-olds. The highest malaria prevalence regions, as can be seen in Figure 1.6, was in the Chittagong Hill Districts with 11.7% infected. The comparison

of this to Figure 1.4 shows that the areas of high prevalence are similar to what was documented in the 1960s and 1970s.

Following the increased efforts of the malaria control programs, there was a 65% reduction in malaria prevalence and a 91% decrease in malaria mortality observed between 2008 and 2012, likely the result of this focused effort.[108] Another study documented the cases in the main tertiary care center in the Chittagong Hill Districts also documenting a decrease in cases following interventions.[110] But without a consistent effort at elimination, these gains are tenuous.

1.3 Malaria Epidemiology Cohort Study

1.3.1 History and Rationale

In 2009, in the context of high rates of malaria documented in the Chittagong Hill Districts and the recent emphasis on malaria control in Bangladesh, the Johns Hopkins Malaria Institute in collaboration with icddr,b (International Centre For Diarrheal Disease Research, Bangladesh) started a cohort study to better understand the epidemiology of malaria in the Hill Districts district of Bandarban.

The study area of forested small hills with intermittent small rice fields is typical of the Chittagong Hill Districts and similar to the Southeast Asia Eco-Zone more broadly. Furthermore, in the context of the recent commitment to malaria control in the country, particularly in this region, this study can help document changes in rates of malaria, risk factors associated with malaria, and the success or failure of various interventions.

The goal of this study was to create an in-depth monitoring of malaria through detailed surveys on the population demographics, symptomatic cases, asymptomatic cases, behaviors, knowledge and practices related to malaria, and entomology in the study area. Each household in the study was mapped using GIS coordinates, and a detailed remote sensing map of the

environment was purchased to help inform a better understanding of the geography. Data from these surveys are being used to explore a number of different questions related to malaria in the region. The cohort study is still actively collecting data.

1.3.2 Methodology

The methodology of this study has been recorded in detail in a prior publication,[118] but will be summarized below. The study area consisted of two unions (the smallest rural administrative unit in Bangladesh) of Bandarban district in the Chittagong Hill Districts of Bangladesh, with a very diverse population consisting of more than a dozen resident ethnic groups and a minority of ethnic Bengalis. The area is known to be hypoendemic for *P. falciparum* and *P. vivax*.

The two unions consisted of about 24,000 people from 4,500 households over an area of 179 km^2 divided into 24 geographic clusters of about 1,000 persons.[118,119] The altitude of the area ranged from about 7 to 152 meters above sea level. The forests are thick and interspersed with clusters of households and rice paddies.

Any person who tested positive by RDT and or microscopy during any aspect of the study was treated with Artemether-lumefantrine (Coartam), and tested again by RDT and microscopy on days 2, 7 and 28 following treatment to ensure clearance of infection. For pregnant women in their first trimester, artemether/lumefantrine is not recommended, and this group was treated with quinine dihydrochloride. *P. vivax* infections were treated with chloroquine and primaquine, with pregnant women only receiving chloroquine.

Informed consent was obtained from all adult participants and guardians of child participants. This study has been approved by the Johns Hopkins Bloomberg School of Public Health and icddr,b IRB committees.

The populations were monitored by locally hired field staff that understood the local culture and had language skills matching the local dialects of the variety of ethnic groups in the area. The following surveys were included as part of the original study design:

1) A year-round demographic survey conducted on every household every 3-4 months including household members, age, sex, pregnancy and relationships.

2) An active surveillance survey consisted of 12 people from each union selected by random sampling every week (from October 2009 - October 2012), divided evenly between three age groups (0-<5 years, 5- <15 years, 15+ years). All participants in this survey were tested by RDT (FalciVax), microscopy and PCR. They also answered a series of questions related to symptoms of malaria and had their temperature measured. All pregnant women were also invited to participate in this survey.

3) A longitudinal study in which two of the 12 people from each union sampled from the active surveillance survey were randomly selected to participate and given three additional active survey visits over a period of 9 months. All pregnant women were also invited to participate in the longitudinal survey.

4) A passive surveillance survey documented all cases reporting symptoms of malaria and seeking care, either through contacting the study medical officer or field staff directly, or reporting to health workers from the NGO BRAC, who were also providing services in the area. After hearing of a possible case, field staff would visit, obtain blood for RDT, blood smear and PCR, as well as answer a series of questions related to symptoms. All study participants also had their temperature measured.

5) A series of surveys on knowledge, attitudes and practices related to malaria were conducted, including an assessment of socioeconomic status, health care seeking behavior, as well as the population's understanding of malaria causes, and control practices.

6) The entomological survey included collection of mosquitoes using a CDC light trap. The survey included five houses from each of 12 clusters in Kuhalong Union. These houses had collection done for five nights in a row each month. Mosquitoes were killed, counted, and their species was identified in the laboratory facility in Bandarban. They were then sent to the parasitology laboratory at icddr,b, which used ELISA to assess the presence of sporozoites and confirm species.

7) Weather data were acquired through collaboration with the Ministry of Agriculture, Soil Resources Development Institute that had been measuring basic climate indicators in Bandarban.

8) GIS coordinates were collected on all households for use in spatial analysis.

Additional surveys were added to the core surveys to examine specific questions that developed after the study began, such as the impact of a traditional agricultural practice called jhum cultivation, the use of cell phones, and the relationship of hemoglobin E to malaria. The surveys included in the specific analysis of this dissertation and methods for analysis are described within each section in more detail below.

1.3.3 Recent Findings

There have already been a number of papers that have been produced from data collected at this field site. The following summarizes the main findings.

An assessment of symptomatic malaria of this cohort from October 2009 to May 2012 demonstrated a seasonal epidemic dominated by *P. falciparum* malaria, with about 5% of

infections from *P. vivax* infections, with specific hotspots maintaining transmission and a number of associated risk factors.[119] The incidence of symptomatic clinically presenting *P. falciparum* malaria was 1.62 cases and 0.27 cases per 1,000 per month during the high- and low-transmission seasons respectively. They found reported bednet use was high (around 90%), and about 70-80% lived close to an animal shelter and or forested areas. Risk factors for clinically presenting *P. falciparum* infections included demographic factors such as age 5-14, members of specific ethnic groups, lower education, jhum cultivation, and other agricultural work and environmental factors such as being more distant to ponds and closer to forests.[119]

After finding a 1.7 times higher odds of malaria among those practicing jhum cultivation compared to other occupations,[119] a separate mixed methods survey was conducted to examine this practice and understand its possible relationship to malaria in more depth. Jhum cultivation is a traditional farming method practiced on the remote hillsides of this region, which involves planting a mix of rice, vegetable and other seeds. It includes the clearing and burning of plots, planting, weeding and harvesting products. Farmers often stay at small houses by the jhum plot for the summer, when the workload was more intense. Of the 24,074 individuals documented through demographic surveys from May 2010 to August 2012, 2,631 (11.3%) were jhum cultivators and another 2,537 (10.9%) lived in the same household as jhum cultivators. Over 99.5% of these populations were of tribal ethnicity. Compared to non-jhum cultivators, jhum cultivators were more likely to be male, older, and of tribal ethnicity. With respect to bednet use, the jhum cultivators (86.7) and jhum cohabitants (83.1) were less likely to use bednets than the non-jhum cultivators (90.4%). With respect to malaria, jhum cultivators and those living with jhum cultivators had 1.6 times higher odds of symptomatic malaria compared to non-jhum cultivators when controlling for sex, age and geographic cluster. The authors theorize that the

reasons for this higher risk include this population's likely higher exposure to mosquitoes in the areas where they practice cultivation, lower bednet use, and less access to treatment when in these remote areas.[120]

Another analysis looked at the role cell phones played in the detection and management of malaria in the population. Cell phones were introduced to the area very recently and ownership of phones has been expanding, with 20.9% of households having at least one phone. These households were distributed throughout the study area and even in the more remote areas, so that almost everyone lives near someone who has a phone and thus could have access to one in case of an emergency. Based on cell phone calls to the chief medical officer or other staff, 1,046 people were tested, 265 (25%) of which tested positive for malaria from June 2010-June 2012. This accounted for 52% of all clinical malaria infections documented through our study over the same period. This study emphasized the possibility such technology offers for research, public health messaging and case management in the area, particularly when combined with a field team that is well connected to and knowledgeable about the community.[121]

Another study in Kuhalong looked at proportions positive in the active surveillance system by RDT, microscopy and PCR. They found that 2% were positive by RDT and/or microscopy, while 6% were positive by PCR, suggesting a large number with submicroscopic infections. All the PCR isolates were chloroquine resistant and atovoquone sensitive using florescent TAQman probe analysis.[122]

Another assessment demonstrated the particularly high risk of infection among pregnant women, with asymptomatic infections occurring year-round.[123] This paper found that from 2010-2013 the prevalence of *P. falciparum* infection among pregnant women tested, regardless of symptoms, was 2.3% compared to 0.5% of non-pregnant women. Authors also found that these

infections were much less clustered in space and time than what was found in the symptomatic malaria cases for the population as a whole.[9]

An analysis of the data from the entomological survey found a diverse group of anopheline species in the area, a number of which were found to carry *Plasmodium*. Specifically they collected 2,837 anopholine mosquitoes representing 20 different species. The most populous species among the female *Anopheles* were *An. jeyporiensis* (18.9%), *An. vagus* (16.8%), *An. kochi* (14.4%), A*n. nivipes* (10.8%) and *An. barbirostris* (7.4%), with another 15 species making up 31.7% of those tested. CSP-ELISA analysis found that 11 (0.4%) of the 2,467 female mosquitoes tested were positive for *P. falciparum* including *An. barbirostris*, *An. jeyporiensis*, *An. kochi*, *An. maculatus*, *An. nigerrimus*, and *An. nivipes*. Four (0.2%) of the mosquitoes that underwent ELISA analysis were positive for *P. vivax*-210, including *An. nivipes*, *An. umbrosus* and *An. vagus*. There are several important points to these findings. There is a high diversity of *Anopheles* vectors in the area. This is the first time *An. jeyporiensis* and *An. kochi* have been documented to carry *Plasmodium* species. It is likely that *An. jeyporiensis*, and *An. nivipes* play a significant role in malaria in the area, given its larger population and confirmed infection. Although *An. maculatus* only accounted for 4.3% of the female mosquitoes collected, they had the highest proportion infected (2.1%) and thus also play a significant role. The authors note the importance of examining this wide variety of species for their ecological preferences for breeding, biting habits and insecticide resistance in order to better understand the ecology of malaria in the region.[113]

1.4 Specific aims of this study

This dissertation focuses understanding the variety of risk factors for malaria in this area of Bangladesh. The specific aims of each aspect of the study are as follows.

Specific aim 1:

To determine the prevalence of G6PD deficiency and hemoglobin E in the study region among the most populous ethnic groups. Then to examine the relationship between heterozygous and homozygous hemoglobin E and mild clinical *P. falciparum* malaria. Secondarily, examine the relationship of hemoglobin E and initial parasite density for those who have *P. falciparum* malaria and clearance of malaria by two days after initiation of treatment.

Specific aim 2:

To determine the prevalence and incidence of sub-clinical *P. falciparum* and *P. vivax* infections among those who live in the study area through the use of our active surveillance survey. To examine temporal and seasonal patterns of incidence and prevalence. Then to examine the associations of sub-clinical infections with geographic, demographic and behavioral risk factors.

Specific aim 3:

To determine the spatial and temporal relationships between clinically presenting symptomatic *P. falciparum* cases detected through passive surveillance, and sub-clinical infections found through active detection.

1.5 Tables and Figures

Figure 1.1: World distribution of malaria mid-19th century to 2010

Reprinted from: World Health Organization. Eliminating malaria: Learning from the past, looking ahead. *Progress & Impact Series*. 2011; 8:43-46.[124]

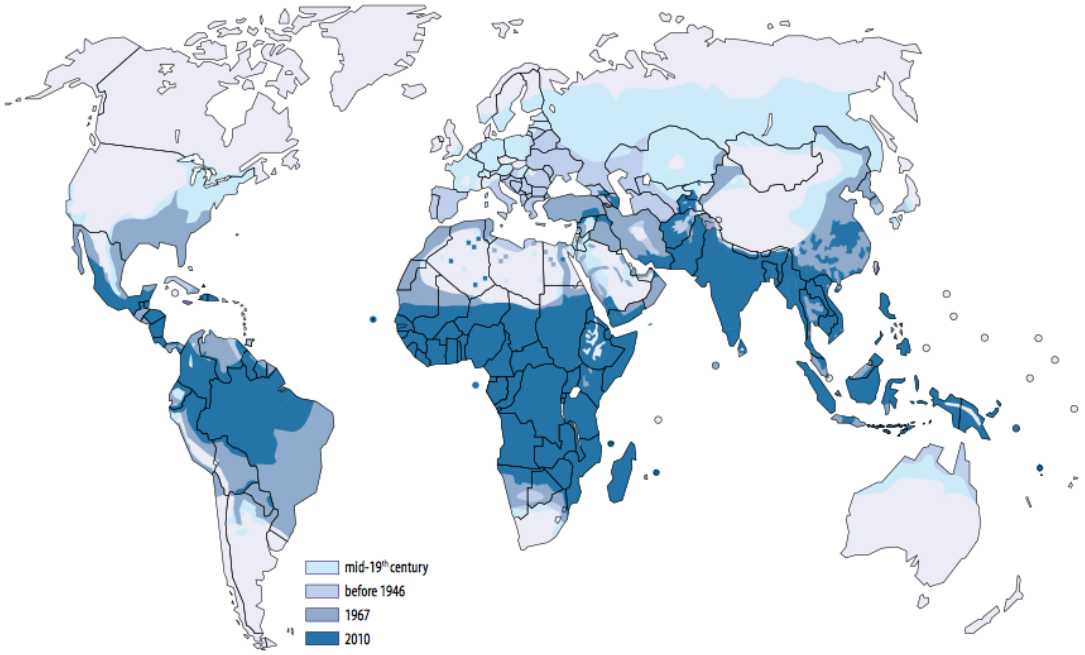


Figure 1.2: The life cycle of *Plasmodium falciparum*

Reproduced from: Winzeler, EA. Malaria research in the post-genomic era. *Nature*. 2008; 455 (7214): 751-769.[125]

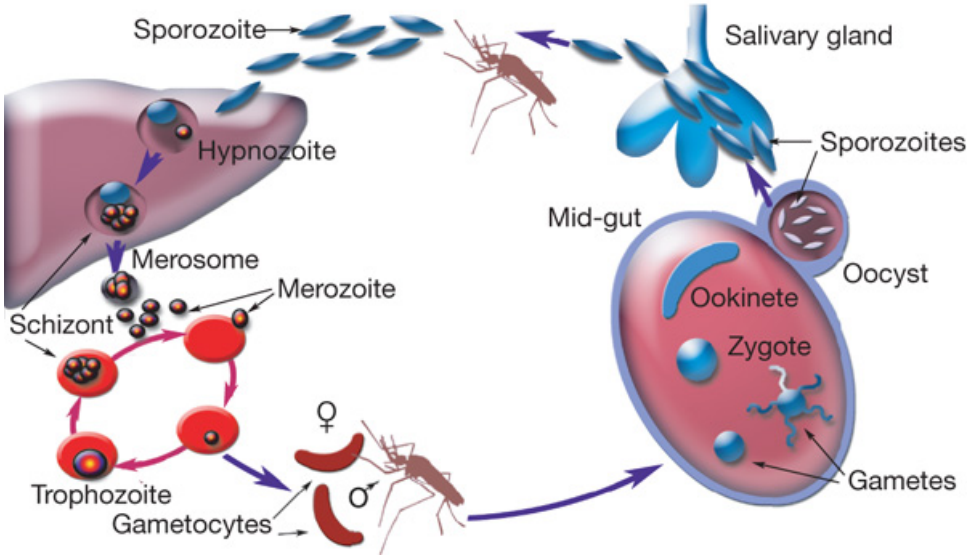


Figure 1.3: Incidence of malaria in Bangladesh 1968-1977

Modified from: Paul BK. Malaria in Bangladesh. *Geographical Review* 1984; 74(1): 63-75.[103]

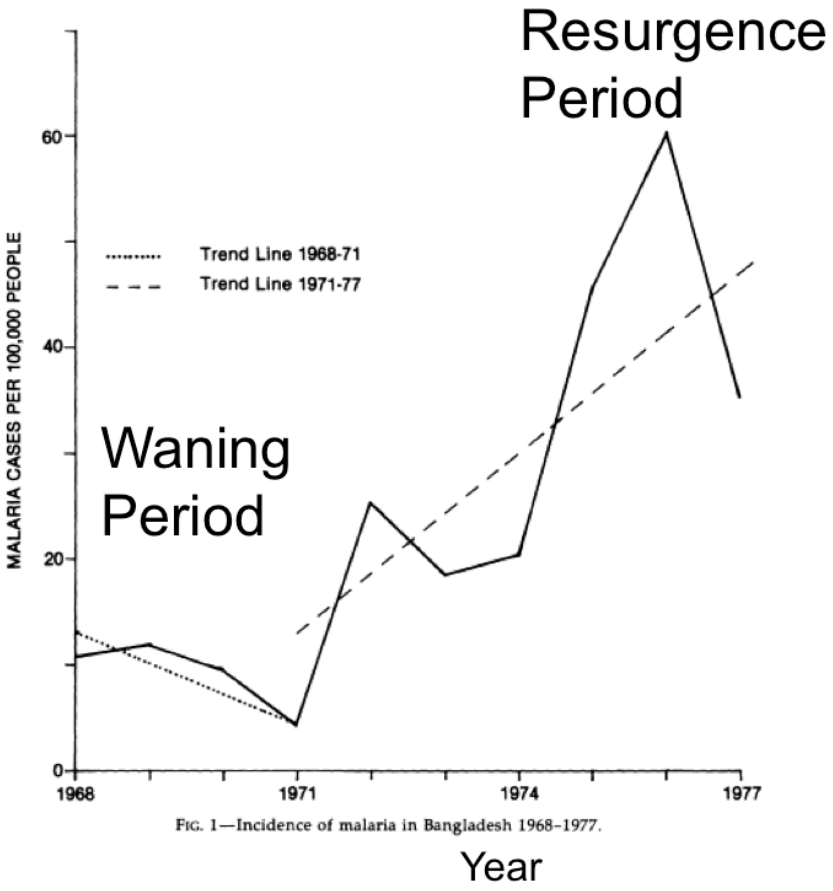


FIG. 1—Incidence of malaria in Bangladesh 1968-1977.

Figure 1.4: Incidence of malaria by district in period of (a) 1968 to 1971 and (b) 1972 to 1977

Reproduced from: Paul BK. Malaria in Bangladesh. *Geographical Review* 1984; 74(1): 63-75.[103]

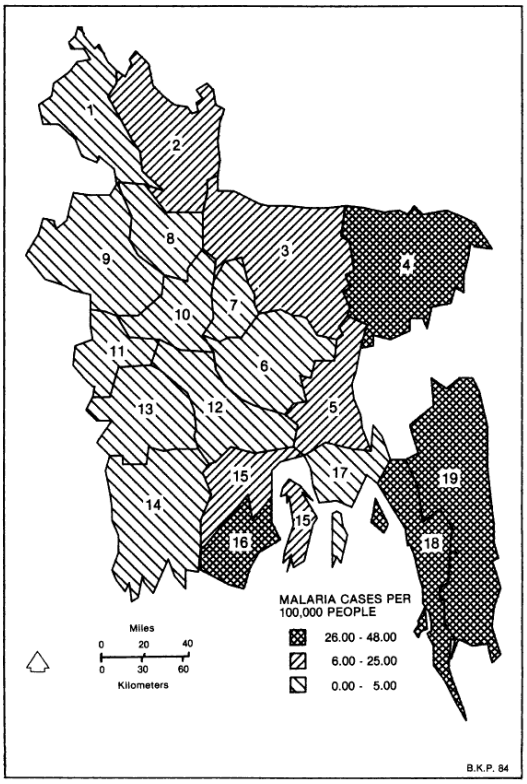


FIG. 2—Incidence of malaria by district 1968–1971.

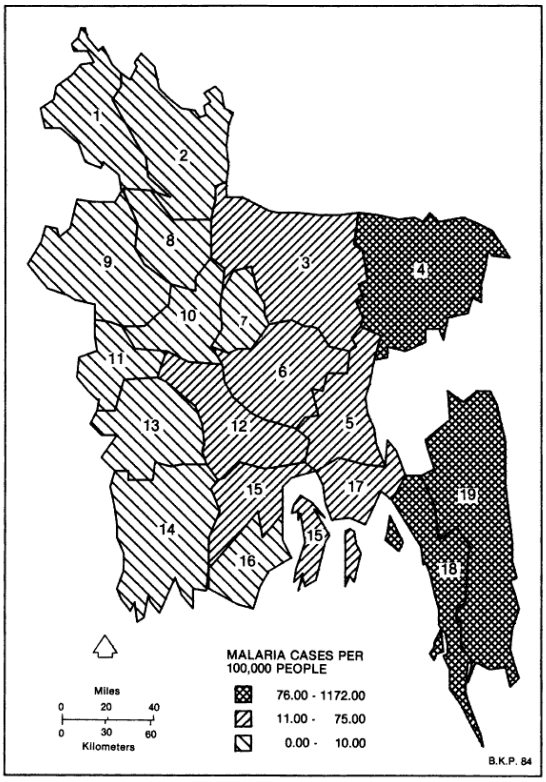


FIG. 3—Incidence of malaria by district 1972–1977.

Figure 1.5: Malaria Statistics Bangladesh 1970-2013

Data from SEARO statistics for Bangladesh and the 2013 and 2014 World Malaria Reports, courtesy of David Sullivan, Johns Hopkins Bloomberg School of Public Health.[1,126,127]

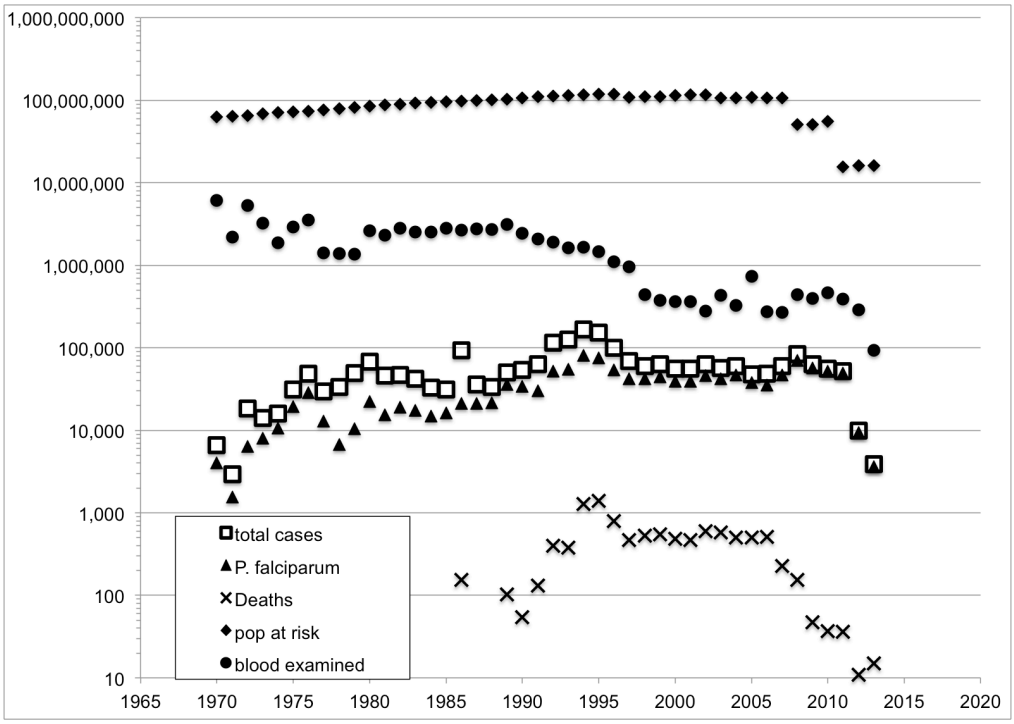
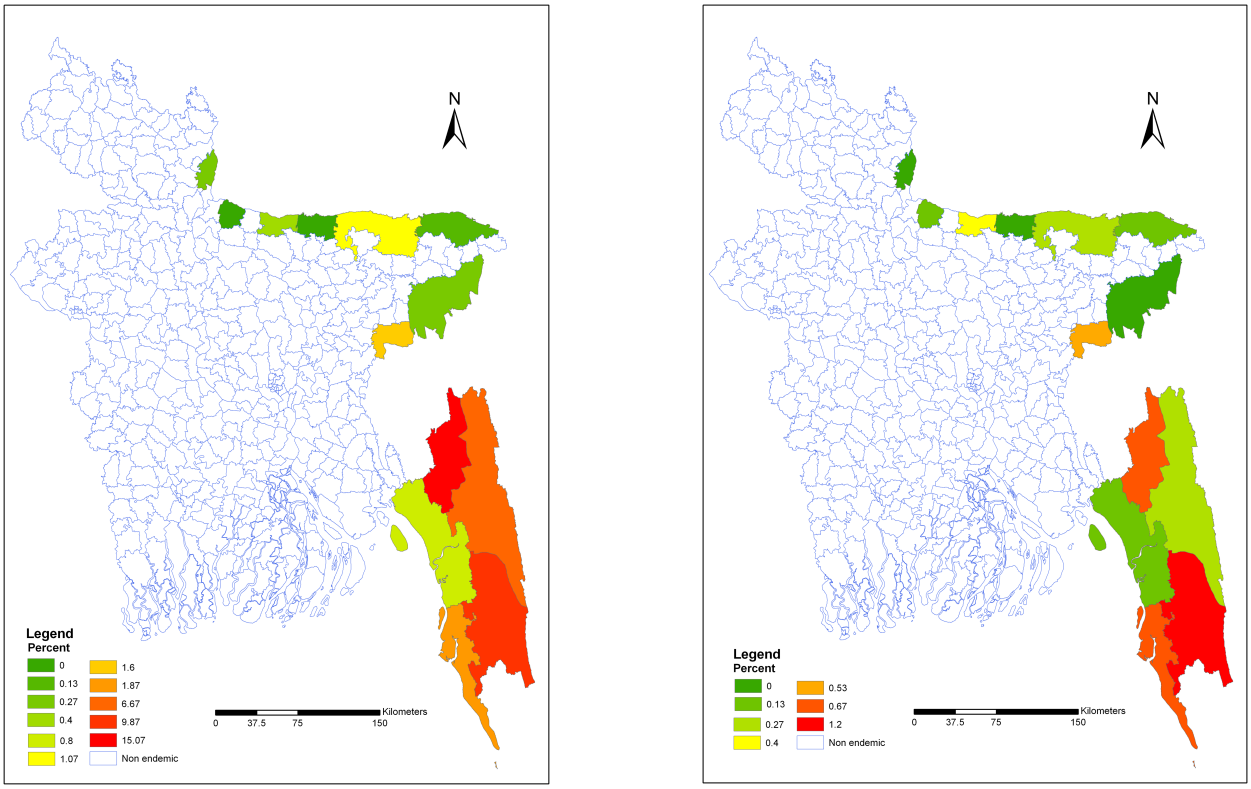


Figure 1.6: Prevalence of (a) *P. falciparum* and (b) *P. vivax* malaria in the 13 endemic districts of Bangladesh

A cross-sectional study conducted by BRAC and ICDDR,B from September-November 2007. Reprinted from: Haque U, Ahmed SM, Hossain S, et al. Malaria prevalence in endemic districts of Bangladesh. *PLoS One*. 2009; 4(8): e6737.[106]



Chapter Two: Hemoglobin E and Glucose-6-Phosphate Dehydrogenase Deficiency and *Plasmodium falciparum* Malaria in the Chittagong Hill Districts of Bangladesh

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This chapter has been published in *AJTMH* 2015 vol. 93, no. 2, 281-286.[128]

2.1 Abstract

Hemoglobin E is largely confined to south and southeast Asia. The association between hemoglobin E (HbE) and malaria is less clear than that of hemoglobin S and C. As part of a malaria study in the Chittagong Hill Districts of Bangladesh, an initial random sample of 202 individuals showed that 39% and 49% of Marma and Khyang ethnic groups, respectively, were positive for either heterozygous or homozygous hemoglobin E. In this group, 6.4% were also found to be severely deficient and 35% mildly deficient for glucose-6-phosphate dehydrogenase (G6PD). In a separate *Plasmodium falciparum* malaria case–uninfected control study, the odds of having homozygous hemoglobin E (HbEE) compared with normal hemoglobin (HbAA) were higher among malaria cases detected by passive surveillance than age and location matched uninfected controls (odds ratio [OR] = 5.0, 95% confidence interval [CI] = 1.07–46.93). The odds of heterozygous hemoglobin E (HbAE) compared with HbAA were similar between malaria cases and uninfected controls (OR = 0.71, 95% CI = 0.42–1.19). No association by hemoglobin type was found in the initial parasite density or the proportion parasite negative after 2 days of artemether/lumefantrine treatment. HbEE, but not HbAE, status was associated with increased passive case detection of malaria.

2.2 Introduction

There is strong epidemiologic and biomolecular evidence supporting the protective effects of hemoglobin S and C on severe malaria, but their protective effects on uncomplicated and asymptomatic malaria are more contested.[27,31-34] In southeast Asia hemoglobin E (HbE; β 26Glu→Lys) predominates, with reported prevalence of 30‑45% (as high as 74% documented among an ethnic minority group in northeastern Thailand).[129,130] Those with homozygous hemoglobin E (HbEE) have mild disease with anemia, splenomegaly, and hemolysis, while the heterozygous state is generally asymptomatic.[28] A study suggested a selective population advantage of high HbE allele frequencies,[131] consistent with higher rates of hemoglobinopathies in malaria-infected areas, a theory proposed by Haldane over 60 years ago.[25]

However, there is a general paucity of evidence on the role of HbE in malaria pathogenicity. One study showed the rate of development of severe, acute malaria was only 2.4% for heterozygous hemoglobin E (HbAE) patients compared with 18% for patients without HbE.[132] In Myanmar, patients with HbAE and normal hemoglobin (HbAA) showed similar parasitemia rates and clinical severity of malaria. However, the number of HbEE patients was small and none had severe disease.[133] Another study with acute *falciparum* malaria patients being treated with artemisinin derivatives showed that patients with HbAE had significantly faster parasite clearance compared with controls with no hemoglobinopathies, a difference not demonstrated with the use of other antimalarial drugs.[134] The authors hypothesize that this result might be related to potentiating effects of the oxidative reactions of artemisinins. One study in Thailand showed that there was no significant difference in allele frequency of HbE between patients with mild malaria and those with cerebral malaria.[130] This study only had three HbEE patients, none of which developed cerebral malaria.

During a 3-year malaria epidemiologic project in the Chittagong Hill Districts of Bangladesh, an area hypoendemic for *P. falciparum* and *P. vivax*, we first investigated the prevalence of HbE and glucose-6-phosphate dehydrogenase (G6PD) deficiency among the two most populous ethnic minority groups. A separate subsequent malaria case–uninfected control study assessed the association of HbE in malaria cases detected principally by passive means compared with age and location matched uninfected controls, to address the contribution of HbE to malaria hotspots. Additional questions involved the relationship of HbE and initial parasite density as well as the clearance of parasites 2 days after treatment initiation.

2.3 Methods

The study area consisted of two unions in the Chittagong Hill Districts of Bangladesh, an area now known as hypoendemic for *P. falciparum* and *P. vivax*, with more than a dozen resident ethnic groups. As part of an epidemiology cohort study, we monitored populations year-round in all age groups, performing demographic surveys, active and passive surveillance, entomological sampling and mapping. The unions consisted of about 24,000 people, divided into 24 geographic clusters of about 2.5 km^2 and similar population sizes of about 1,000 persons.[118,120] Informed consent was obtained from all adult participants and guardians of child participants. This study has been approved by the Johns Hopkins Bloomberg School of Public Health and International Center For Diarrheal Disease Research, Bangladesh (icddr,b) Institutional Review Board (IRB) committees.

There were three aspects to the analysis in this study: 1) determining the cross-sectional prevalences of HbE and G6PD deficiency in two of the more populous local ethnic groups in which blood sampling took place in the dry season (December 2010 and April–May 2011), 2) examining the associations between HbE and *P. falciparum* malaria cases (dates of malaria range

from May 29, 2010 to February 16, 2012) compared with uninfected controls, with HbE sampling performed from May 2012 to August 2013 (3 months to several years after malaria episode, with all but a few over a year later), and 3) assessing the association of HbE on both initial parasite density and absence of parasites 2 days after initiation of treatment of *P. falciparum*–infected cases, as a proxy for clearance.

2.3.1 Estimation of prevalences of HbE and G6PD deficiency

A total of 112 Marma and 90 Khyang individuals, aged 19–31 years, already part of the larger epidemiologic study, were randomly selected using a random number generator with blood analysis done in December 2010 and March–April 2011. In the Marma tribe, there were 73 females and 39 males. In the Khyang tribe, there were 50 females and 40 males. Five women were pregnant at the time of blood draw based on documented pregnancy and birth records from our larger cohort study.

The blood samples were analyzed for malaria by rapid diagnostic test (RDT, FalciVax, Zephyr Biomedical Systems, Verna, Goa, India) and microscopy. Analysis of hemoglobin amounts and types was done using an automated hemoglobin testing system (VARIANT™ II Hemoglobin Testing System, Biorad Incorporated, Hercules, CA). Pregnant woman were excluded from the hemoglobin analysis. Laboratory analysis for G6PD deficiency was performed on the Hitachi 902 instrument (Roche Diagnostics, Basel, Switzerland) using Randox reagents (Randox Laboratories, Crumlin, United Kingdom). The G6PD level was categorized by the WHO classification using a population-defined mean of 11.8 U/g Hb, from Cambodia,[135] since we lack a local population-defined mean for Bangladesh. Using 11.8 U/g Hb, we established five classes: Class I: very severely deficient with < 1% residual activity (≤ 0.12 U/g Hb); Class II: severely deficient, > 1–10% residual activity (> 0.12–1.2 U/g Hb); Class III: mildly deficient, >

10–60% residual activity (> 1.2–7.1 U/g Hb); Class IV: normal activity, > 60–150% residual activity (> 7.1–17.7 U/g Hb); and Class V: increased activity, > 150% residual activity (> 17.7 U/g Hb).

2.3.2 Examining the associations between HbE and P. falciparum malaria

A second and separate aspect of the study was a nested *P. falciparum* malaria case–uninfected control analysis for HbE status and RDT- or microscopy-positive *P. falciparum* malaria among the most populous Marma ethnic group. Malaria was detected by both passive and active means from our cohort study data. The malaria case definition required a positive RDT or microscopy and was divided between 1) passive case detection when individuals had sought treatment of fever, history of fever, or other symptoms, and were found to have a positive RDT and/or microscopy and 2) active case detection in which individuals tested positive after being randomly selected for RDT/microscopy testing within a predetermined sampling structure according to cluster and age, as used for the larger cohort study.[118,119] From the larger epidemiologic cohort study, 170 passive malaria cases were randomly selected from 472 using a random number generator. [119] All cases detected by active surveillance meeting this criteria from our cohort study were also included in this analysis. Uninfected controls were matched by age (6 months–4 years; 5 years–14 years; 15+ years), cluster (by 24 geographic areas), and ethnic group (all Marma). Uninfected controls were chosen initially on the basis of never having had documented positive malaria by RDT/microscopy during the cohort study that began in October 2009. They also had to have been selected for random active surveillance and to have specifically tested negative for malaria within 12 weeks of the time the case tested positive, with preference to those tested closer in time to the case. Participants with HbEE and HbAE were compared with those with HbAA, including those with low A2 (excluding those with β-

thalassemia trait and the two cases of *P. vivax* malaria). The two cases that had malaria twice were only included one time for this analysis. Analysis was done using McNemar's test on matched pairs with a sensitivity analysis using conditional logistic regression, including those without matched pairs.

Using McNemar's test for the passive case–control analysis, 142 matched pairs were available after eliminating those with β-thalassemia. For the 142 eligible pairs, when comparing any HbE versus normal, if we assume a 50% probability of exposure, a power of 80%, a probability of an exposure discordant pair of 50%, and an alpha of 5%, we would at a minimum be able to detect an odds ratio (OR) of 2. When comparing HbEE versus normal, the minimum detectable OR (after reducing sample size by 36%) is 2.8. For the active sample (21 matched pairs), when similar comparisons are made using the same assumptions, we did not have the power to detect anything but the most extreme relationships. For any HbE versus normal, there is a minimum detectable OR of 7.8 and for HbEE versus normal, the minimum detectable OR would be 12.5 or greater.

2.3.3 Assessment of initial parasite density and absence of parasites 2 days after initiation of treatment

Some of the malaria cases had only an initial RDT available, rather than a blood film for quantification. The geometric means and 95% confidence intervals (CIs) were calculated on the log-normalized parasite density using Student t tests. The means were compared through analysis of variance (ANOVA) tests. We also conducted a case–control analysis comparing the HbE status of subjects from any ethnic group who had persistent parasitemia 2 days after the initiation of treatment with artemether/lumefantrine (N = 48), with those *P. falciparum* malaria patients with absent parasites 2 days later (N = 288). The controls were a convenience sample selected

from those *P. falciparum* malaria patients who were not positive on Day 2 in our active and passive studies described above.

All analyses were conducted using R statistical software (Vienna, Austria).[136]

2.4 Results

2.4.1 Population hemoglobin analysis

Initially, blood samples were taken for hemoglobin analysis on 202 study participants, 19–31 years of age, from the two most populous ethnic groups, in the dry season (December 2010 and late April to early May 2011). None of the participants tested positive for malaria by RDT or microscopy at this time. In total, 36% were heterozygote for HbE and 8% were homozygous, as shown in Table 2.1. The Khyang ethnic group had 11% (N = 10) homozygous for HbE and 38% (N = 34) heterozygous, while the Marma ethnic group had 5% (N = 6) homozygous and 34% (N = 38) heterozygous. These differences were not significant by Fisher's exact test (two-sided P value of 0.12). Hemoglobin Hope, a beta chain Gly136(H14)Asp mutation, was found in 1.5% of those sampled (N = 3). Low hemoglobin A2 was found in 5% (N = 10). Hemoglobin A2, a normal variant consisting of two alpha and two delta chains, may be elevated in people with β-thalassemias and has a lowered value in people with iron-deficient anemia or α-thalassemias, persistence of fetal hemoglobin, or hemoglobin H (thalassemia intermedia).[137]

The overall mean hemoglobin among the 196 (6 removed because of pregnancy at the time of blood draw) malaria-negative Marma and Khyang people (aged 19–31 years) tested in the initial prevalence survey was 12.6 g/dL (13.6 g/dL male, 12.0 g/dL female). We did not find a significant difference in hemoglobin levels by race or by HbE type in this population when controlling for age, sex, and pregnancy status (P value = 0.43).

2.4.2 G6PD deficiency

Of the 202 participants tested for G6PD deficiency, the mean was 7.42 U/g Hb, with 58.9% having normal G6PD activity, 34.7% having mild deficiency, and 6.4% having severe deficiency (Figure 2.1). Unlike with hemoglobin levels, where no significant difference was found by ethnic group, we found that there was a significantly higher proportion of cases that were G6PD deficient among the Marma, with a mean 6.68 U/g Hb, compared with that of Khyang who had a mean G6PD activity of 8.35 U/g Hb, a difference of 1.68 (95% CI = −2.57 to −0.78), and a P value of 0.0003 using Welch two-sample t test. Thus, half the Marma population had at least a mild deficiency compared with 30% of the Khyang population.

2.4.3 Case–control analysis of HbE and malaria

From the larger epidemiologic cohort, 170 passive malaria cases were randomly selected from a total of 472 cases appearing over the 2-year period. A total of 146 uninfected controls matched by age, cluster, and ethnic group were identified, located, and tested for hemoglobin amount and type. Of these 146 matched pairs, three were eliminated with β-thalassemia trait, one was eliminated as the case had *P. vivax* malaria, leaving 142 *P. falciparum* cases and matched uninfected controls (Table 2.2). We found significantly higher odds of malaria among patients with HbEE compared with those with HbAA (including those with low A2), based on our sample of age, location and ethnic group matched pairs using McNemar's exact test (OR = 5.0, 95% CI = 1.07–46.93; P value = 0.039). If the matched design is ignored, the unpaired analysis has similar prevalence of HbEE among cases and controls (10 and 12 out of the 142, respectively), while the paired analysis reached significance in the HbEE matched comparison of 10 to 2 normal Hb. Although not statistically significant, we found the opposite trend when comparing those with HbAE to HbAA (OR = 0.71, 95% CI = 0.42–1.19; P value = 0.22). A sensitivity analysis using

conditional logistic regression, including the cases or controls that were collected without a match for both the active and passive analysis, resulted in similar ORs and no change in the conclusions of the study.

To examine malaria cases identified from the active surveillance, 24 randomly selected subjects who tested positive for malaria during the active surveillance were compared with age, cluster, and ethnic group matched uninfected controls. Of the 24 infected subjects, 23 had participating matched pairs. Of these, one case and one control had β-thalassemia trait, leaving 21 matched pairs for analysis. With this small sample size, we did not see any significant difference in proportion having HbEE and HbAE among matched malaria cases and controls. The conditional logistic regression analysis did not change this finding.

Next we examined if the initial parasite density of malaria cases might be different by hemoglobin status. Of the 24 cases from active surveillance (some were RDT only), we found an initial parasite density on 20, with a geometric mean of 1,320 (95% CI = 694–2,511). Of the 170 passive *P. falciparum* malaria cases, 122 had available parasite density, with a geometric mean of 4,254 (95% CI = 332–5,455). The mean parasite density did not significantly differ by hemoglobin type in either the active (F = 0.25, P value = 0.78) or passive studies (F = 1.13, P value = 0.34) (Table 2.3).

The last part of the analysis related to an examination of the hemoglobin status of those patients who had positive *P. falciparum* parasitemia (possible delayed clearance) 2 days after initiating treatment with artemether/lumefantrine (N = 47). This group of 47 patients, parasite positive on day 2, was made up of seven malaria case found positive through our active surveillance and 40 patients with malaria detected by the passive surveillance, representing a range of ethnicities including 31 Marma, four Chakma, one Tripura, two Khyang, seven Bengali,

and two from other ethnic groups. The matched infected controls in this analysis were all the *P. falciparum* cases for which we had conducted hemoglobin type analysis and had cleared parasites by day 2 (20 active and 165 passive). We also compared the Marma cases that had persistent parasitemia to controls, as all controls were Marma. Although the point estimates of the ORs were below 1 (suggestive of a possible faster clearance with HbE), we did not see a significant difference of absence of parasites by day 2 stratified by HbE status (Table 2.4). This result did not change if we separated active and passive malaria cases.

2.5 Discussion

In southeast Asia, an area that was hyperendemic for malaria, HbE has high prevalences.

In the Chittagong Hill Districts, malaria has persisted in this forested, hilly environment with seasonal heavy rains. In this study, a little under half of the randomly sampled Marma and Khyang populations had some variant of HbE. Among mild clinical malaria cases collected through passive sampling, there is some evidence of an increased proportion of homozygous HbEE compared with HbAA among those with malaria compared with matched uninfected controls. There was no significant difference in HbAE levels compared with normal levels among cases and controls. With our asymptomatic malaria case–control study collected through active sampling, we had too small a sample to define the relationship between malaria and HbE.

Differences have been noted in hospitalized, severe malaria cases in regard to HbE status, with some evidence of a protective effect of HbEE[133] and HbAE[132] from severe complications of malaria in a hospital setting. In this study, we did not have enough severe malaria cases to analyze the association between severe disease and HbE status, but rather we compared those without infection to those who presented with mild clinical infection in a community setting. It is possible that the protective effects may only apply to reducing severe symptoms, a result found

by Hutagalung and others,[132] rather than preventing infection or onset of mild symptoms of infection. As we lacked sufficient power to compare asymptomatic cases found through active surveillance, it is not possible to parse out this difference with this analysis. However, as Billo and others[29] have suggested, it is possible that the cross-sectional analyses used were not robust enough to identify these differences and that a longitudinal approach was needed. The small sample size and lack of significance when comparing the marginal frequencies of hemoglobin status by malaria suggest that further research on this question is needed to fully understand the relationship of HbE and malaria. With increased power, factors such as age and geographic clusters could be further explored. Furthermore, a study including populations with asymptomatic/submicroscopic infections, clinical mild malaria infections, as well as severe malaria may help to parse out whether the associations we found relate to HbEE or HbAE being associated with the initial malaria infection, becoming clinically symptomatic, and/or having severe symptoms and complications.

This study did not provide evidence that hemoglobin type is associated with either geometric mean parasite density on the day of diagnosis or clearance of parasitemia by day 2 after beginning treatment. The day 2 estimates were approximate, as the day 2 sample was collected at any time during day 2 and could have ranged by a number of hours, possibly impacting our results. Bangladesh, despite repeated measurements, has yet to show delayed clearance with artemisinin treatment as seen in Cambodia.[138-141]

With respect to G6PD deficiency, we found 6% with severe deficiency in this population. This suggests one should screen for deficiency with a point-of-care test in this population before use of a 14-day course of primaquine used to eliminate the liver stage in *P. vivax* infections or long half-life tafenoquine.[142,143] Single dose primaquine at 0.25 mg base/kg is well tolerated even

in those severely deficient for G6PD, and is recommended by the WHO in addition to artemisinin combination therapy, for all but pregnant woman and infants under 1 year as a gametocytocidal drug for those with *P. falciparum* infections as part of an elimination strategy. [144] The high levels of G6PD deficiency in this area should thus not be a reason to stop the implementation of these recommendations.

Limitations of this study include inability to locate all of the controls that had been selected for our cases. The population that did not have controls was slightly (although not statistically significantly) younger. As age and location may be related to our outcome, this was a limiting factor of our analysis. The analysis, particularly for the malaria cases detected through active surveillance, was limited by sample size. For the elimination studies, the number of hours defined as '2 days' varied depending on when during the day the patients were originally tested/treated and when field workers were able to return to their villages. Thus the timing is not as controlled nor measured as often as some hospital-based parasite elimination studies. With respect to G6PD measurements, reticulocyte count was not taken at the time of blood draw. As patients were not ill at the time of blood draw, this is unlikely to substantially impact results. However, we would underestimate true rates of deficiency in the rare cases of high reticulocytosis elevating levels of G6PD.

In summary, we found high levels of G6PD deficiency, HbE, and anemia in this population. Uncomplicated malaria was more common among patients with homozygous HbE compared with controls, while no difference was found in the prevalence of malaria in patients with HbAE compared with normal patients. Focal populations of certain ethnic groups with remnant high prevalence of HbEE may provide an increased risk of uncomplicated malaria

secondary to either longer duration of infection or increased risk of infection, and thus malaria control in these populations is necessary for elimination programs to be successful.

2.6 Acknowledgements

We acknowledge the surveillance staff at the icddr,b field office in Bandarban for helping to arrange this study. We express our gratitude to the late Ashish Chowdhury who helped with the G6PD analysis. We also thank Ciprian M. Crainiceanu for his statistical support and express gratitude to the local population for their participation in the study and their enthusiasm.

2.7 Tables and Figures

Table 2.1: Hemoglobin type and quantity analysis for random sampling of the Marma and Khyang ethnic groups excluding six pregnant women (N = 196)

Frequency distribution	Number	Percentage	Hemoglobin mean (g/dL)	Hemoglobin quartiles 25%; 75%	Hemoglobin minimum; maximum
HbEE	16	8.2	12.2	10.9; 13.1	9.8; 14.8
HbAE	69	35.2	12.5	11.4; 13.7	7.1; 16.0
Normal study	98	50.0	12.9	11.7; 14.1	6.9; 18.1
Hb Hope	3	1.5	12.5	11.4; 14.3	8.9; 14.6
Low Hb A2 level	10	5.1	11.45	10.8; 12.8	7.9; 13.2
Total	196	100.0	12.6	11.5; 13.8	6.9; 18.1

HbAE = heterozygous hemoglobin E; HbEE = homozygous hemoglobin E; Hb Hope = a beta chain Gly136(H14)Asp mutation.

Figure 2.1: Frequency of glucose-6-phosphate dehydrogenase (G6PD) deficiency and World Health Organization classification categories

The frequency of 202 G6PD enzyme levels from the random sampling of Marma and Khyang tribal members.

Class I: very severely deficient with < 1% residual activity (≤ 0.12 U/g Hb);
Class II: severely deficient, > 1–10% residual activity (> 0.12–1.2 U/g Hb);
Class III: mildly deficient, > 10–60% residual activity (> 1.2–7.1 U/g Hb);
Class IV: normal activity, 60–150% residual activity (7.1–17.7 U/g Hb);
Class V: increased activity, > 150% residual activity (> 17.7 U/g Hb).

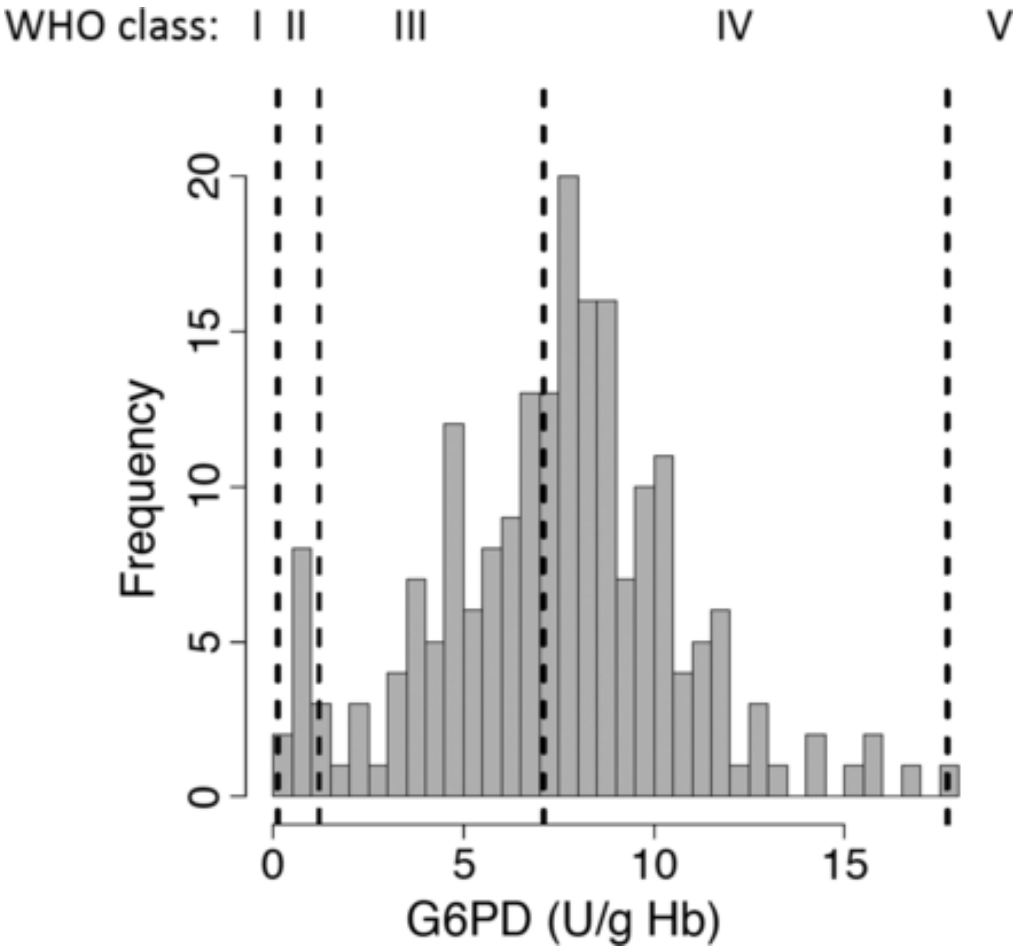


Table 2.2: Hemoglobin type and malaria in matched pairs for passive *Plasmodium falciparum* malaria case—uninfected control analysis

Uninfected controls	Cases			Total
	HbEE	HbAE	Normal	
HbEE	1	7	2*†	10
HbAE	1	26	38†‡	65
Normal	10*†	27†‡	30	67
Total	12	60	70	142

HbAE = heterozygous hemoglobin E; HbEE = homozygous hemoglobin E.
McNemar's test results:
* For HbEE vs. HbAA analysis: Odds ratio (OR) = 5.0, 95% confidence interval (CI) = 1.07–46.93; McNemar's exact two-tailed P value = 0.039.
† For any HbE vs. HbAA analysis: OR = 0.93, 95% CI = 0.58–1.48; McNemar's exact two-tailed P value = 0.82.
‡ For HbAE vs. HbAA analysis: OR = 0.71, 95% CI = 0.42–1.19; McNemar's exact two-tailed P value = 0.22.
Conditional logistic regression results:
HbEE vs. HbAA: OR = 5.0, 95% CI = 1.1–22.8; P value = 0.038.
Any HbE vs. HbAA: OR = 0.92, 95% CI = 0.59–1.45; P value = 0.73.
HbAE vs. HbAA: OR = 0.71, 95% CI = 0.43–1.16; P value = 0.18.

Table 2.3: **Hemoglobin type and initial parasite density**

	HbEE	**HbAE**	**β-Thalassemia trait**	**HbAA**
Active cases (N = 23)*				
Absent parasitemia N (%)	–	2 (18)	0 (0)	1 (9)
Positive parasitemia N (%)	–	9 (82)	1 (100)	10 (91)
Range (parasites/μL)†	–	200–17,320	–	200–11,200
Geometric mean parasite density (parasites/μL)†‡ (95% CI)	–	1,694 (561–5,113)	1,000	1,084 (391–3,009)
Passive cases (N = 147)§				
Absent parasitemia N (%)	1 (8)	9 (14)	0 (0)	15 (22)
Positive parasitemia N (%)	12 (92)	54 (86)	3 (100)	53 (78)
Range (parasites/μL)†	480–31,400	240–112,000	3,280–14,240	480–60,000
Geometric mean parasite density (parasites/ μ L) † // (95% CI)	2,232 (845–5,900)	4,175 (2,815–6,193)	6,175 (947–40,271)	4,912 (3,425–7,044)

CI = confidence interval; HbAA = normal hemoglobin; HbAE = heterozygous hemoglobin E; HbEE = homozygous hemoglobin E.

* Blood samples not collected for one of 24 active cases (1 HbEE).

† Range and geometric mean parasite density are calculated including all positive values.

‡ ANOVA comparison of geomeans F = 0.25, P value = 0.78.

§ Blood films not available for 22 of 169 passive cases (1 β–thalassemia trait, 8 HbAE, 1 HbEE, 12 HbAA).

// ANOVA comparison of geomeans F = 1.13, P value = 0.34.

Table 2.4: Hemoglobin type in parasite clearance analysis

	HbEE *N* (%)	**HbAE *N* (%)**	**Any HbE *N* (%)**	**β-Thalassemia trait *N* (%)**	**HbAA *N* (%)**	***N***
All day 2 positive cases	3* (6)	16 (34)	19‡ (40)	3 (6)	25 (53)*‡	47
Day 2 positive Marma cases	2† (6)	11 (35)	13§ (42)	1 (3)	17 (55)†§	31
Day 2 negative Marma cases	13*† (7)	80 (43)	93‡§ (50)	5 (3)	87 (47)*†‡§	185

HbAA = normal hemoglobin; HbAE = heterozygous hemoglobin E; HbE = hemoglobin E; HbEE = homozygous hemoglobin E.

* For HbEE vs. HbAA analysis using all day 2 + cases: OR = 0.80, 95% CI = 0.14–3.26; Fisher's exact two-tailed P value = 1.

† For HbEE vs. HbAA analysis using only Marma day 2 + cases: OR = 0.79, 95% CI = 0.08–4.01; Fisher's exact two-tailed P value = 1.

‡ For any HBE vs. HbAA analysis: OR = 0.71, 95% CI = 0.34–1.45; Fisher's exact two-tailed P value = 0.40.

§ For any HBE vs. HbAA analysis using only Marma day 2 + cases: OR = 0.72, 95% CI = 0.30–1.67; Fisher's exact two-tailed P value = 0.44.

Chapter Three: Sub-clinical *P. falciparum* infections act as year-round reservoir for malaria in the Chittagong Hill Districts of Bangladesh

3.1 Abstract

Malaria is endemic in 13 of 64 districts in Bangladesh, with the highest rates in the Chittagong Hill Districts, where *Plasmodium falciparum* is the predominant species. This study describes the epidemiology of sub-clinical *P. falciparum* and *P. vivax* infections in two unions of Bandarban, an area with a population of about 24,000. Active surveillance for malaria infection and laboratory analysis was conducted by testing 3,971 people from October 2009 to October 2012 without regard to symptoms, 3,382 from a population random sample and 589 were selected due to pregnancy. Of these samples, 35 and 18 tested positive for *P. falciparum* infection through RDT and/or microscopy respectively, and one from the random sample tested positive for *P. vivax* infection via microscopy. Thus the estimated point prevalence of *P. falciparum* infection was 1.0% of the overall population and 3.2% of pregnant woman. There was no significant increase in the prevalence of sub-clinical *P. falciparum* when comparing high to low seasons (OR 1.89, 95%CI: 0.89-4.30, p-value 0.09). 69% of those with sub-clinical *P. falciparum* infections reported at least one symptom commonly associated with malaria (fever, muscle aches, fatigue and headache) in the prior two weeks compared to only 18% of those without a detected *P. falciparum* infection (OR (95% CI) = 10.3 (4.8-23.4). We found a higher prevalence of these sub-clinical infections among males, pregnant woman, jhum cultivators, those living closer to forests and at higher elevations, and marginally among 5-14 year olds and day laborers.

A subset of 1,253 people from the active surveillance study (664 from random sample, 589 due to pregnancy) was selected to be followed every three months for 9 months to estimate malaria incidence. *P. falciparum* incidence was estimated to be 39.9 per 1,000 person-years

(19.7-54.9) in the randomly selected population. Among those who were pregnant or recently pregnant, the incidence rates were 19.43 (4.9-76.6) and 19.52 (8.8-43.11) respectively, while no infections were detected among 15- to 39-year-old women in the longitudinal study who were not pregnant or recently pregnant. Extrapolating the overall incidence rate to the population as a whole, we estimate 790 people (473-1,318) a year acquire sub-clinical *P. falciparum* infections detectable by RDT and or microscopy, compared to an average of 189 passively detected symptomatic cases a year that presented to clinic over this period (mid-October 2009 to mid-October 2012.).

We conclude that hypoendemic sub-clinical and uncomplicated *P. falciparum* malaria continues in the Chittagong Hill Districts. The sub-clinical infections were associated with a number of household and demographic factors, similar to those found for symptomatic cases. Unlike clinical symptomatic malaria, which is highly seasonal, these actively detected infections were present year-round, make up the vast majority of infections at any given time and likely act as reservoirs for continued transmission.

3.2 Introduction

The World Health Organization estimates that 3.3 billion people globally are at risk for malaria, with an estimated 214 cases and 438,000 deaths in 2015 from malaria.[2] Ninety-seven countries and territories had ongoing transmission of malaria in 2014.[1] In the past decade focus on malaria control and elimination has increased dramatically with major efforts such as the Millennium Development Goals and the Roll Back Malaria Partnership, both with significant public and private investments. Many countries have reported a decrease in the number of cases from epidemic levels and have shifted their focus from simply controlling malaria to its elimination, with some successfully reaching this goal.[7] In this context, it is important to

understand the epidemiology of not just the symptomatic cases, but also that of people that may carry infection without ever presenting to a physician, as they can continue the epidemic chain, particularly in the low seasons when fewer symptomatic cases are present.

In Bangladesh, malaria is endemic in 13 of 64 districts, with over 14 million people at risk for infection, with the highest rates in the Chittagong Hill Districts, where *P. falciparum* is the predominant species and *P. vivax* occurs to a lesser extent.[107,108] A wide variety of mosquito species transmit malaria in the region.[107-109,113] Clinical infections tend to peak from June-August during the height of the rainy season.[109,110] The Bangladesh National Malaria Control Program implemented by the Ministry of Health and the non-profit group BRAC focused on the implementation of a variety of interventions starting in 2007, including providing community-based testing and treatment with artemisinin-based drugs, providing long-lasting insecticide treated bednets, and overall strengthening the malaria surveillance and control programs. A 65% reduction in malaria prevalence and a 91% decrease in malaria mortality was observed between 2008 and 2012, likely as a result of this focused effort. [108] But without a consistent effort at elimination these gains are tenuous.

A cohort study supported by The Johns Hopkins Malaria Institute in collaboration with the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) started in 2009 aimed to better understand the epidemiology of malaria in the Hill Districts district of Bandarban. An assessment of symptomatic malaria of this cohort demonstrated a seasonal epidemic with specific hotspots maintaining transmission and a number of associated risk factors.[119] Another assessment demonstrated the particularly high risk of infection among pregnant women, with sub-clinical infections occurring year-round.[123] This paper explores

aspects of sub-clinical infections in the Hill Districts of Bangladesh among all age groups and in pregnant and non-pregnant women in this cohort.

3.3 Methods

The study area consisted of two unions in the Chittagong Hill Districts of Bangladesh, an area now known as hypoendemic for *P. falciparum* and *P. vivax*, with more than a dozen resident ethnic groups. As part of an epidemiologic cohort study, we monitored populations year-round in all age groups, performing demographic surveys, active and passive surveillance, entomological sampling and mapping. The unions consisted of about 24,000 people from 4,500 households over an area of 179 km^2 divided into 24 geographic clusters of about 1,000 persons.[118,119] Informed consent was obtained from all adult participants and guardians of child participants. This study was approved by the IRB of the Johns Hopkins Bloomberg School of Public Health and the Ethical Review Committee of the icddr,b.

There were four aspects to this analysis: 1) estimating the prevalence of sub-clinical *P. falciparum* and *P. vivax* malaria and associated seasonal and geographic factors in all age groups and specifically in pregnant woman year-round, 2) determining the extent of symptoms among those with sub-clinical *P. falciparum* infections in this active screen 3) determining the association of sub-clinical *P. falciparum* infections with demographic, and behavioral risk factors, and 4) estimating the incidence of sub-clinical *P. falciparum* infections in the population and specifically in pregnant woman.

Data were analyzed using R statistical software (Vienna, Austria) including packages gmodels, stats, geoR, zoo, spatstat, maptools, maps and lubridate.[136,145-150]

3.3.1 Estimation of prevalence of sub-clinical P. falciparum and P. vivax infections and associated geographic and seasonal factors

The active surveillance system was created to determine the prevalence of malaria. It started in mid-October 2009 in Kuhalong Union, then was expanded to cover Rajbila Union as well in May 2010, with both being followed until mid-October 2012. The sampling structure was set up so that each of the 12 geographic clusters in each union had one person randomly selected for active sampling per week from one of three age groups (0-<5, 5-14, 15+). The age group of individuals selected for sampling in a given cluster would rotate each week, with 4 people from each age group in a given week selected using a random number generator (www.randomizer.org). If someone was not available, the person four numbers away within the selected age group and cluster was then sampled. All pregnant women in the study area were also selected into the active surveillance system for research and clinical follow-up. As sampling was done with replacement, there was the possibility for multiple selections of the same person over study period. 7.2% of the randomly selected population and 2.2% of the pregnancy selected populations were made up of people who were selected multiple times.

Those selected for sampling answered a detailed survey related to symptoms, a rapid diagnostic test for *P. falciparum* and *P. vivax* malaria was conducted, and blood was collected for microscopy. These samples were brought back to the field station in Bandarban for laboratory analysis.

Analysis was conducted on the random sample and separately for all pregnant women. A sensitivity analysis was conducted including all actively sampled populations (from pregnancy and randomly selected populations) for most aspects of the study. The prevalence by age group (<5, 5-14, 15-39, 40+ years) and sex was calculated, and an overall adjusted prevalence was

calculated using these rates weighted by the proportion of the age/sex group in the overall population using direct adjustment.

3.3.2 Determining the extent of symptoms present in those with sub-clinical P. falciparum infections

For each person included in the active surveillance study, a detailed survey was included, with a questionnaire on physical symptoms experienced in the last 48 hours and 2 weeks. Adult participants answered the survey directly. For children the survey was filled out by their caregiver with input from children when able. Surveyors also took temperatures of participants either by axillary or oral methods. We considered the participant to be febrile by the oral method at 38.3 °C and above, and by axillary method at 37.5 °C and above. We conducted both univariate and multivariate regression to assess associations of a variety of physical symptoms, and actively detected *P. falciparum* infection to determine if people with sub-clinical infections had more systemic symptoms than the general population or were truly asymptomatic. The odds ratio of *P. falciparum* infection was calculated for each symptom using univariate analysis, and then specifically selecting for reported fever, headache, fatigue and muscle aches using multivariate regression, as these symptoms are commonly associated with malaria.

As no substantial difference was found between symptoms in the prior 48 hours compared to the 48 hour- to 2-week periods, the data was combined to look at symptoms in the prior two weeks. This analysis was presented for the randomly selected population as well as for all those pregnant at the time of survey, including those in the random and pregnancy selected groups.

3.3.3 Determining the association of sub-clinical P. falciparum infection with demographic and behavioral risk factors

As part of the cohort study a demographic study of the population was conducted on households initially in 2009 and thereafter updated every 3 to 4 months for births, deaths and in/out migrations. Another survey conducted yearly addressed a number of questions related to malaria control practices, household behaviors and household proximity to certain environmental features such as water features and forest. Remote sensing was also used to calculate elevation and distance to the nearest stream or river. The results from this survey were compared with the results from malaria random sampling to determine which factors may be associated with sub-clinical *P. falciparum* infections. Univariate analyses are compared with previously published data for clinical symptomatic *P. falciparum* malaria in this cohort study.[119] Multivariate logistic regression was then conducted using the above sociodemographic and household risk factors using normalized covariates for all continuous factors, then a stepwise logistic regression using forward and backward selection was used to select the final model. Only those people with no missing data on the covariates considered were used for step-wise regression. This process was repeated with a larger dataset once several covariates with larger numbers of missing values were eliminated from the model.

3.3.4 Estimation of sub-clinical P. falciparum infection incidence

Within the population selected to our active surveillance study described above, a subset was selected for our nested longitudinal study. This population was tested by RDT and microscopy as per the active surveillance protocol described above on the initial visit and then at approximately 3, 6 and 9 months following the initial visit. The person-time under the nested longitudinal study and associated incident infections (not including those cases found on the

initial visit) were documented and used to calculate the incidence rate in this population. 1.5 months of person-time was deducted for all incident cases with the assumption that the case occurred on average halfway through the follow-up time for that period. If the period between visits exceeded 4 months, the person-time and associated cases for this period were eliminated from the analysis to reduce the possibility of missed infections.

The sampling scheme was set up so that two of twelve people from each union selected for the active survey each week were selected for longitudinal follow-up. The sampling setup repeated itself every 18 weeks, with each cluster having one person from each of three age groups selected in that period. All pregnant women were also entered into the study for longitudinal and clinical follow-up. If the woman randomly selected was pregnant, another woman from that cluster was also selected to enter the study. The data was analyzed using all randomly selected people (including those who were pregnant).

The incidence in the randomly selected population, as well as those entered as part of pregnancy follow-up, were analyzed and compared by age, sex and pregnancy status. The age group a person was assigned to was the age at the time of blood draw for laboratory analysis, even if they were selected into a younger age group at the beginning of the longitudinal follow-up, and thus several people will contribute to data from multiple age groups.

The overall incidence rate is adjusted by age and sex using direct standardization and the overall population age/sex distribution, to account for oversampling the younger age groups and the disparity of missing data by sex among the adult population.

To explore the changes in incidence in pregnancy, all 15- to 39-year-old women in both the random and pregnancy follow-up were divided into three groups: pregnant woman, recently pregnant and not pregnant. We defined these states on the basis of the pregnancy status at the

specific follow-up visit. Those who were 'recently pregnant' had been pregnant during earlier follow-up visits for this selection into the longitudinal cohort. 'Not pregnant', were not pregnant on the current or any of the prior follow-up visits for this selection.

Those testing positive for malaria by either RDT and/or microscopy were treated according to the National Malaria Treatment Guidelines of Bangladesh and then followed (repeat RDT/microscopy) on day 2, 7 and 28 following diagnosis to ensure clearing of infection. Standard *P. falciparum* malaria cases were treated with artemether/lumefantrine. For pregnant women in their first trimester, artemether/lumefantrine is not recommended, and this group was treated with quinine dihydrochloride. *P. vivax* infections were treated with chloroquine and primaquine, with pregnant women only receiving chloroquine.

One person had a documented infection two times during follow-up, but as they had documented clearance of parasites between these two visits, it was treated as a second incident case.

3.4 Results

3.4.1 Demographics

Of the 3,978 people surveyed for active screening, 3,971 have laboratory information available. Of these, 3,382 were selected through population random sampling, while 589 were selected due to positive pregnancy test and thus entered into the longitudinal survey. Table 3.1 describes the demographics of those randomly selected for active surveillance, the subset of these selected for the nested longitudinal study, as well as those who were selected due to pregnancy.

The longitudinal dataset has a disproportionate number of women. This may be due to the higher likelihood of women being present at the time of survey, and the replacement of non-

present people with either sex. Of note, most demographic covariates were not highly correlated, but religion and ethnic group were highly related (with only 3% of those tested not having the dominant religion of their ethnic group) and thus religion is not included in the rest of the analysis.

3.4.2 Estimation of prevalence of sub-clinical P. falciparum and P. vivax infections and associated geographic and seasonal factors

Of the 3,971 people with available laboratory information, 53 had documented sub-clinical *P. falciparum* infections, with 51 positive by RDT and 44 positive by blood smear for *P. falciparum*. The random sample accounted for 35 infections, while the pregnancy selected sample accounted for 18. There was 1 positive blood smear for *P. vivax*. The geometric mean parasite density of the 44 positive *P. falciparum* infections was 902 parasites/μL (range 40-14,600). The proportion of infections that were positive by sex and age group can be seen in Table 3.2. Based on the random sample, the age- and sex-adjusted point prevalence of *P. falciparum* infection is 10.43/1,000 population (95%CI: 7.00-13.85). Given the low number of *P. vivax* infections detected, the rest of the analysis will relate to sub-clinical *P. falciparum* infection. Among the 631 pregnant women tested, there were 20 infections of *P. falciparum* infection detected, equating to a point prevalence of 31.70/1,000 population (95%CI: 18.03-45.36).

The proportion testing positive for sub-clinical *P. falciparum* infections by month of the year can be seen in Figure 3.1. Compared to our passive surveillance system, in which we found that greater than 85% of infections occurred during the May-October rainy season,[119] we did not find a significant difference in sub-clinical *P. falciparum* infections by season, with a substantial

proportion of the infections found year-round (OR 1.89, 95%CI: 0.89-4.30, p-value 0.09) (Table 3.3).

The spatial intensity of all active survey participant household locations and those that tested positive for a *P. falciparum* infection can be seen in Figure 3.2. It is useful to note that sub-clinical *P. falciparum* infections are not simply a function of population density. In the southwest corner of the map (the southern area of Kuhalong Union) there are several areas with high population density with very few infections. On the other hand, the northeast corner of the map (an area of Rajbila Union) has a disproportional number of infections considering the lower population density in the area. The factors that might account for some of these differences will be explored further in this paper. We found higher actively detected malaria prevalence in Rajbila union compared to Kuhalong (Table 3.5), as was the case in our prior passive study of clinically presenting symptomatic malaria.[119] Sub-clinical infections appear to cluster in certain areas more than others, with 0-5% of those sampled from the 24 geographic clusters tested positive.

3.4.3 Determining the extent of symptoms present in persons with sub-clinical P. falciparum infections

As part of the active surveillance study, patients were asked whether they had a series of physical symptoms in the prior two weeks. The results of this survey and the crude relationship of each symptom to *P. falciparum* infection can be found in Table 3.4a for those in the random study and Table 3.4b for all pregnant women regardless of study type. Combining random sampled and pregnancy sampled populations, we found that 77% of those who tested positive for *P. falciparum* infection had at least one clinical symptom listed in Table 3.4, compared to only 29% of those not positive for *P. falciparum* infection (OR = 8.2; 95% CI=4.2-17.2). Generally

speaking, most of the symptoms surveyed were found in higher rates among those who had sub-clinical *P. falciparum* compared to those who did not. These differences in symptoms with and without *P. falciparum* infection were also present among pregnant women, but with lower odds ratios, as a higher proportion of women without malaria were experiencing constitutional symptoms (Table 3.4b).

When specifically examining the association of fever, muscle aches, fatigue, and headache with sub-clinical *P. falciparum* infection, 69% of those with *P. falciparum* infection had at least one of these symptoms compared to 18% of those without *P. falciparum* infection (OR = 9.9; 95% CI = 5.2-19.8). A multiple covariate regression analysis among the randomly detected population, including fever, muscle aches, fatigue and headache in the prior 2 weeks and actively detected *P. falciparum* infection, showed that when controlling for the other symptoms, fever (OR =10.3; 95% CI =4.2-24.9; p value: <0.001), and headaches (OR = 3.8; 95% CI = 1.5-9.4; P-value: 0.004) were positively associated with sub-clinical infection, while no association was found with fatigue (OR = 0.59; 95% CI = 0.23-1.5; p-value: 0.274) or muscle aches (OR = 1.0; 95% CI = 0.40-2.6; p-value: 0.944).

When analyzing measured fever at the time of survey, for the randomly selected population, 2 (5.7%) of the 35 positive *P. falciparum* infections and 20 (0.6%) of the 3,347 without infection tested had a fever (OR =10.1; 95% CI = 1.1-44.3; p-value = 0.02)

3.4.4 Determining the association of sub-clinical P. falciparum infection with demographic and behavioral risk factors

Demographics: The univariate relationship between demographic factors and sub-clinical *P. falciparum* infections from the population random survey can be found in Table 3.5. These factors are compared with the incidence rates of symptomatic infections by these same

demographic factors calculated in a prior study.[119] Factors that were significantly associated with sub-clinical malaria included union, ethnicity, and occupation, and marginally age group. Specifically of note, the farming practice of jhum cultivation provides an occupational risk factor for sub-clinical *P. falciparum* infection. Education level was not found to be significantly associated with sub-clinical infection.

Table 3.2 shows the prevalence sub-clinical infections by age and sex among the randomly selected population and the odds of infection for each age/sex category compared to female under 5-years-olds. Although there is too little power to define distinctions by age and sex category, trends suggest overall higher prevalence among males than females and higher prevalence in those 5-39 years old, than among young children or older adults. The highest prevalence age group category for women was 15-39 and for men was 5-14 years.

Household risk factors: Table 3.6 is a summary of the household risk survey and how it relates to sub-clinical *P. falciparum* infections. This is again compared to the findings for the relationship of these risk factors to clinically symptomatic malaria from a prior study.[119] In those randomly selected into the study, as with the symptomatic incidence study, there were no associations on crude analysis found for sleeping under a bednet the night before and owning animals. 91.5% claim to have slept under a bednet the prior night and 84.1% of those surveyed lived in a household that owned some type of animal. As with the symptomatic study, there appears to be a trend of increasing distance from ponds being a risk factor, although this trend was not statistically significant for the sub-clinical analysis. Living closer to forests is associated with higher risk of both sub-clinical infections and symptomatic malaria. The remote sensing data was just used for the active study. Here we did not find an association distance to streams.

Altitude in the study area ranges from 7-147 meters above sea level. A strong association between altitude and malaria was present, with those in the highest elevation group (125-147m above sea level) having 81.6 (8.2-810.5) times the odds of getting sub-clinical malaria compared to those in the lowest elevation group (7-25m above sea level).

Multivariate regression:

In this analysis we examined a selection of the above individual, household and other demographic factors and their association with malaria. Individual characteristics included union, sex, age group, race, education, occupation and current pregnancy status. Household factors included whether a bednet is located over the bed, whether animals were owned by members of that household, distance to the forests, distance to the closest river/stream, distance to the nearest pond and altitude.

Univariate logistic regression was performed on each factor. Next, stepwise logistic regression was performed using backward and forward elimination including only people without any missing values for any of the covariates tested (N=3,948, 99.4% of those surveyed through our active surveillance system). Once the use of bednets was eliminated as a factor, a second stepwise logistic regression was conducted with a larger dataset, as much of the missing data was in this factor. This process was conducted for a combined group of the randomly selected and pregnancy selected populations, as well as for the randomly selected group alone.

Both strategies selected for the same set of covariates as important to explaining asymptomatic malaria. These include sex, age, occupation, distance to forest, elevation and pregnancy as can be seen in Table 3.7. Results suggest that higher risk groups include pregnant woman, males, jhum cultivators, those living at higher elevations and closer to forests and marginally those aged 5-14 years and day laborers. Although not statistically significant, adults

over forty and children under 5 were at less risk of malaria than those between ages 5-39. Although similar trends were seen with both methods, when all those in the pregnancy study were included in the model, the 15-39 age group had a higher estimate for odds of malaria when compared to those under 5 years old. The odds ratios for the three occupational categories considered compared to everyone else had increased odds ratios when the pregnancy selected individuals were not present, likely because the large number of pregnant woman were mostly housewives, which were put into the 'other' category.

Of note, ethnicity remained an important contributor to explaining sub-clinical infections until elevation was added to the model. The Tripura in particular were higher risk and tend to live much higher up than other populations. On the other hand, the Bengalis appeared lower risk and lived mostly at lower elevations.

There was mild remaining residual spatial variation not explained by the models.

3.4.5 Estimation of sub-clinical P. falciparum infection incidence

The randomly selected nested longitudinal study selected 655 unique people (664 including multiple selections of the same individuals), 629 of which have at least one follow-up visit and 620 of which had at least one follow-up visit that was no greater than 4 months after the first. The follow-up time for these 620 individuals was 431.55 years (430.5 when adjusted for assumption that people become infected half way through follow-up interval).

Table 3.8 summarizes the incidence rates in each age/sex group among the randomly selected population. Although the sample size is too small for definitive comparisons, the age/sex group with the highest estimate of *P. falciparum* infection incidence was adult males (15-39 and 40+) and adult females were estimated to be the lowest with an incidence of 0

infections/1,000 person-years. The overall age and sex adjusted incidence rates is estimated to be rate was 39.9/1,000 person-years (19.7-54.9).

Table 3.9 compares adult women (ages 15-39) by pregnancy status. Women from both random- and pregnancy-selected populations were split into three groups: pregnant at time of follow-up visit, recently pregnant (i.e. not pregnant but had been pregnant earlier in one of the prior visits) and not pregnant at any point during the longitudinal follow-up. The estimated rates for both currently pregnant and recently pregnant were both around 19.5 incident infections/1000 person years. There were no infections among the population that was not pregnant nor recently pregnant in the longitudinal study.

3.5 Discussion

This study demonstrates that sub-clinical malaria infections make up the majority of infections in the Hill Districts of Bangladesh. A prior study estimated symptomatic malaria incidence rate to be 11.4 incident infections/1,000 person-years in this population. [119] This is substantially lower than the sub-clinical *P. falciparum* incidence rate found by random sampling of 32.9 incident infections/1,000 person-years. We also calculated an adjusted prevalence of 10.43 sub-clinical *P. falciparum* infections/1,000 population.

If we apply these rates to the population of about 24,000 in Kuhalong and Rajbila Unions, we estimate 790 (473-1,318) incident sub-clinical infections per year and an average of 250 (168-332) prevalent sub-clinical infections at any one time. As there was an average of 189 clinical symptomatic incident infections/year detected during this period, we can estimate approximately 979 total infections per year, 81% of which were sub-clinical. And since symptomatic cases are generally treated promptly, these infections have short durations; thus, we can conclude that the vast majority of infections at a given time are sub-clinical.

Furthermore, as this study used RDT and microscopy for detection of sub-clinical infections and does not include PCR, it likely underestimates the true burden of sub-clinical infections. Lucy Okell and colleagues have created a formula to estimate the sub-clinical infectious prevalence based on the microscopy positive prevalence in an area. [151] Using this formula, our study area is likely to have an infection prevalence of about 4.0% for *P. falciparum*, compared to our estimates of just over 1.0% based on RDT and microscopy. Similar estimates would be reached extrapolating from Mallika Imwong and colleagues, who found that the number of infections that are PCR positive is about four times higher than that based on microscopy and RDT in several countries in Southeast Asia.[152] If these rates are reflective of the area, an even higher proportion of the infections would be sub-clinical than estimated above.

The extent to which sub-microscopic infections transmit malaria is still being explored. However, it has been estimated that in very low transmission settings, submicroscopic infections account for 70-80% of all those infected and 20-50% of all human-to-mosquito transmissions come from submicroscopic carriers of infection.[151,153] It has been shown that almost all patients with infections have mature gametocytes in their blood, including asymptomatic and sub-microscopic infections, and modeling experiments show that transmission to mosquitoes depends on a number of factors including gametocytes density, a relationship which is non-linear and varies by setting.[153-156] In some settings sub-microscopic gametocyte densities have resulted in frequent mosquito infection, while in others they have not.[151,153,157] However, it has been estimated that the proportion of mosquitoes that become infected is on the order of twofold to fivefold lower when feeding on an individual with a submicroscopic infection compared to one with a microscopy positive infection.[153]

Thus although the specific nature and extent of infectiousness of sub-clinical infections needs to be better characterized, these infections are likely contributing substantially to transmission, particularly during the low season when symptomatic infection rates are very low. Although the majority of those that tested positive for *P. falciparum* infections in this study had mild clinical symptoms in the two weeks prior to infection, they were not disruptive enough to their lives to seek clinical care, even in the context of a study that made such care more accessible than in many of the surrounding areas. Given the population without malaria also reported substantial (although lower) levels of these symptoms, it is not surprising that they do not always seek care for mild systemic symptoms. Thus without active surveillance, these mild and asymptomatic people would not have been detected unless symptoms worsened and they chose to seek care.

Understanding the specifics of spatial and temporal distribution and risk factors for these mild and asymptomatic infections thus becomes critical for malaria control and elimination programs. Without addressing these infections, it will likely not be possible to eliminate malaria from an area.

The many risk factors for mild clinical infection are similar to those seen for the symptomatic clinically presenting malaria patients as demonstrated from our prior analysis, [119] although we expanded this analysis to include several more environmental factors. The biggest risk factors for sub-clinical *P. falciparum* infection appear to be pregnancy, living at high elevation and close to forests, being involved in jhum cultivation or other higher risk occupations, being an older child or younger adult, and being male. A prior study demonstrated that this region has a large variety of *P. falciparum* carrying mosquitos that show a variation in

biting behaviors, some of which bite more in the daytime and outside of the home, as a result although bednets will be helpful, they will not prevent all infections.[113]

As would be expected due to increasing immunity in the population, the prevalence of mild/asymptomatic infection appears in older age groups than do the symptomatic cases. In particular, it appears that the highest risk groups were males between 5-14 and females between 15-39 years old. The under-five and over-40 age groups had lower prevalence. Interestingly, when examining malaria incidence, the highest risk age group were adult males, whose point estimate for incidence was more than double any other age/sex group. Much of the adult male population is involved in activities such as jhum cultivation (a type of farming on the hillsides) and rubber plantation farming among other activities that may lead to increased risk, but one would expect to see this relationship in symptomatic prevalence as well.

The comparison of the pregnant women to the adult women who were not pregnant showed a stark increase in risk for malaria for pregnant women and recently pregnant women in this area, reinforcing that found by prior studies of prevalence.[123] This increased risk, combined with the potential complications of malaria during pregnancy emphasizes the need for continued testing and follow-up of all pregnant individuals.

Limitations: With only 53 infections of mild/asymptomatic malaria detected during this period, there was a lack of power for many of the subgroup analyses. We also saw patterns in age and sex difference in malaria incidence and prevalence, which should be confirmed with higher powered studies. Similarly, the lack of a large number of infections, makes large multivariate analysis challenging.

A second limitation relates to the symptoms associated with actively detected malaria. This survey was conducted at the same time as RDT was completed. In some cases, respondents

learned the results of the RDT before they had fully completed the survey. This could result in recall bias, which if it occurred, would likely mean those who had a positive RDT would recall being symptomatic more than they would have had they not just learned they had malaria. And thus if the results were biased they would tend towards over-reporting of symptoms among those with malaria.

As noted above, this study only used RDT and microscopy for identification of infections. The use of PCR would help to clarify the proportion of infections that are sub-microscopic.

Lastly, due to the treatment of all detected infections, we are unable to establish the natural history of the sub-clinical infections and thus do not know the average length of infections or the proportion of infections that would have developed into symptomatic cases. However, we can say that the latter is likely only a small proportion, given the substantially lower incidence of symptomatic cases more broadly.

3.7 Conclusions

The Hill Districts of Bangladesh have hypoendemic malaria in which sub-clinical infections make up the majority of infections at a given time. Unlike those symptomatic cases leading people to seek treatment, which have a strong seasonal pattern peaking in the rainy season, these sub-clinical infections occur year-round. Any attempts at elimination of malaria in this region will thus require more than finding and treating symptomatic patients, as sub-clinical infections likely act as a reservoir for continued transmission.

3.8 Tables and Figures

Table 3.1: Basic demographics of active surveillance study population

Demographic and household factors		Active malaria survey N (%) (random selection)	Nested longitudinal study N (%) (random selection)	Pregnancy selected longitudinal study N (%)
Union	Rajbila	1,509 (44.6)	275 (41.4)	294 (49.9)
	Kuhalong	1,873 (55.4)	389 (58.6)	295 (50.1)
	Total	**3,382(100.0)**	**664 (100.0)**	**589 (100.0)**
Sex/pregnancy (on first visit)	Males	1,615 (47.8)	301 (45.3)	0 (0)
	Females	1,767 (52.2)	363 (54.7)	589 (100.0)
	Pregnant	42 (2.4)	32 (8.8)	589(100.0)
	Not pregnant	1725 (97.6)	331 (91.2)	0 (0)
	Total	**3,382 (100.0)**	**664 (100.0)**	**589 (100.0)**
Age	6 mo- < 5 years	862 (25.5)	161 (24.2)	0 (0)
	5-14 years	1,277 (37.8)	249 (37.5)	5 (0.8)
	15-39 years	761 (22.5)	161 (24.2)	579(98.3)
	≥40 years	482 (14.3)	93 (14.0)	5 (0.8)
	Total	**3,382 (100.0)**	**664 (100.0)**	**589 (100.0)**
Ethnicity	Bengali	707 (20.9)	143 (21.5)	111 (18.8)
	Tribal	2,675 (79.1)	521 (78.5)	478 (81.2)
	Marma (1)	1995 (74.6)	379 (72.7)	370 (77.4)
	Tanchanga(4)	308 (11.5)	62 (11.9)	48 (10.0)
	Khyang (5)	177 (6.6)	37 (7.1)	31 (6.5)
	Chakma (2)	115 (4.3)	28 (5.4)	18 (3.8)
	Tripura (3)	57 (2.1)	8 (1.5)	7 (1.5)
	Bawn (6)	20 (0.7)	7 (1.3)	3 (0.6)
	Mro (8)	2 (0.1)	0 (0)	0 (0)
	Rkhaine (7)	1 (0.0)	0 (0)	1 (0.2)
	Total	**3,382 (100.0)**	**664 (100.0)**	**589 (100.0)**
Religion	Buddhist	2636 (77.9)	515 (77.6)	475 (80.6)
	Muslim	607 (17.9)	125 (18.8)	94 (16.0)
	Christian	74 (2.2)	14 (2.1)	10 (1.7)
	Hindu	65 (1.9)	10 (1.5)	10 (1.7)
	Total	**3,382 (100.0)**	**664 (100.0)**	**589 (100.0)**
Education level (age ≥15 years)	0-2 years	743 (59.8)	155 (61.0)	305 (52.2)
	3-5 years	233 (18.7)	55 (21.7)	121 (20.7)
	≥6 years	267 (21.5)	44 (17.3)	158 (27.1)
	Total	**1,243 (100.0)**	**254 (100.0)**	**584 (100.0)**
Occupation (age ≥15 years)	Farmer	465 (37.4)	85 (33.5)	128 (21.9)
	Housewife	173 (13.9)	51 (20.1)	308 (52.7)
	Day laborer	173 (13.9)	41 (16.1)	56 (9.6)
	Student	130 (10.5)	19 (7.5)	19 (3.3)
	Jhum cultivator	95 (7.6)	17 (6.7)	22 (3.8)
	Unemployed	92 (7.4)	21 (8.3)	26 (4.5)
	Other	115 (9.3)	20 (7.9)	25 (4.3)
	Total	**1,243 (100.0)**	**254 (100.0)**	**584 (100.0)**

Table 3.2: Sub-clinical *P. falciparum* infections by sex and age

SEX/AGE Category		Total (%)	Total without *P. falciparum* infection (%)	Total with *P. falciparum* infection (%)	Odds ratio (95% CI)	p-value
Female	<5	426 (12.6)	424 (12.7)	2 (5.7)	1.0	-
Female	5-14 years	664 (19.6)	660 (19.7)	4 (11.4)	1.28 (0.23-7.05)	0.773
Female	15-39 years	440 (13.0)	435 (13.0)	5 (14.3)	2.43 (0.47-12.63)	0.289
Female	40+ years	237 (7.0)	236 (7.1)	1 (2.9)	0.90 (0.08-9.97)	0.930
Male	<5	436 (12.9)	433 (12.9)	3 (8.6)	1.47 (0.24-8.84)	0.675
Male	5-14 years	613 (18.1)	599 (17.9)	14 (40.0)	4.95 (1.12-21.93)	0.035*
Male	15-39 years	321 (9.5)	317 (9.5)	4 (11.4)	2.68 (0.49-14.71)	0.258
Male	40+ years	245 (7.2)	243 (7.3)	2 (5.7)	1.74 (0.24-12.47)	0.579
Total		3,382 (100)	3,347(100)	35 (100)		

Figure 3.1: Proportion testing positive for *P. falciparum* infection by RDT and/or microscopy during active surveillance by month, age and study type

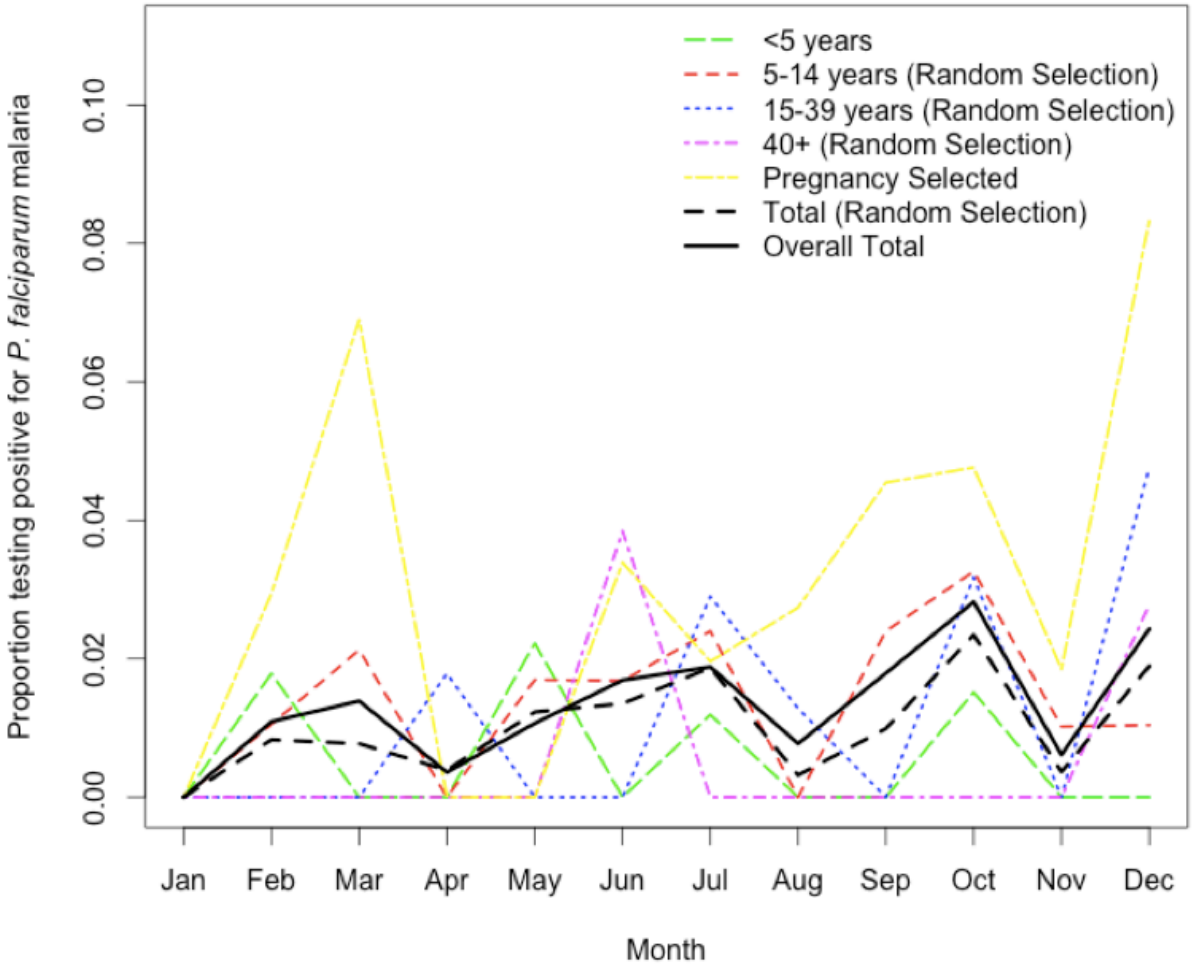


Table 3.3: Seasonal variation in detection of sub-clinical *P. falciparum* infections by active surveillance

Season	Number of positive *P. falciparum* infections (% of those tested)	Number tested	OR (95% CI) low season: high season	Fisher's exact test p-value
Low season (November-April) Random Selection Pregnancy Selection	11 (0.7)	1566	1.89 (0.89-4.30)	0.089
High season (May-October) Random selection Pregnancy selection	24 (1.3)	1816		
Year-round	35 (1.0)	3382		

Figure 3.2: Spatial Intensity of household locations by active surveillance study type

a) All active survey study participants b) Sub-clinical *P. falciparum* infections

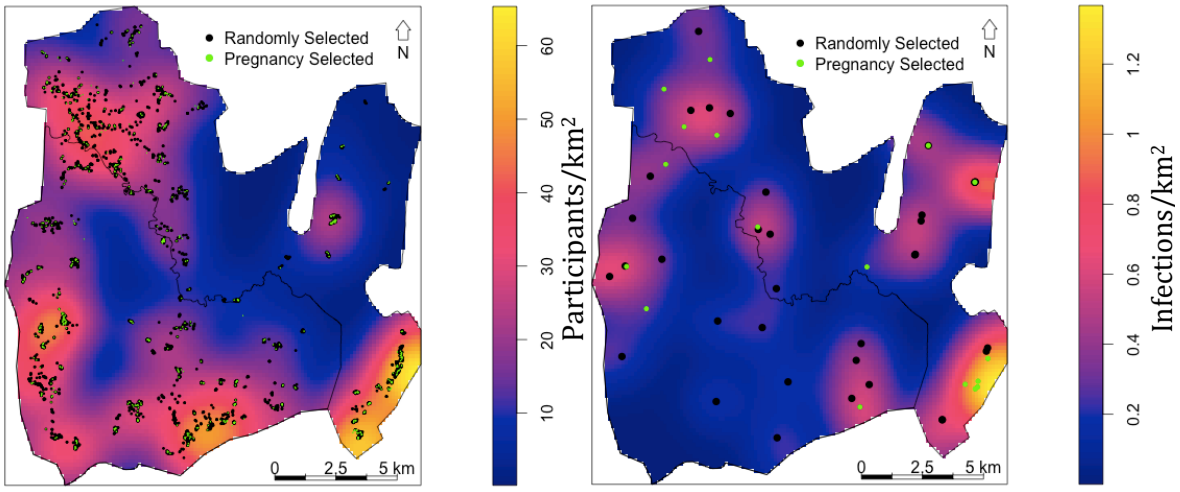


Table 3.4: Association of sub-clinical *P. falciparum* infections with reported symptoms in prior 2 weeks

a) Randomly selected active survey participants

Symptoms		Negative for *P. falciparum* infection N (%)	Positive for *P. falciparum* infection N (%)	OR (95% CI)	Fisher's exact test p-value
Single Symptom Associations					
Fever	No Yes	3137 (93.8) 208 (6.2)	16 (45.7) 19 (54.3)	17.9 (8.6-37.7)	<0.001
Headache	No Yes	3093 (92.5) 252 (7.5)	18 (51.4) 17 (48.6)	11.5 (5.5-24.1)	<0.001
Chills	No Yes	3241 (96.9) 104 (3.1)	24 (68.6) 11 (31.4)	14.2 (6.1-31.2)	<0.001
Nausea	No Yes	3265 (97.6) 80 (2.4)	26 (74.3) 9 (25.7)	14.1 (5.6-32.3)	<0.001
Vomiting	No Yes	3293 (98.4) 52 (1.6)	29 (82.9) 6 (17.1)	13.1 (4.3-33.9)	<0.001
Diarrhea	No Yes	3295 (98.5) 50 (1.5)	32 (91.4) 3 (8.6)	6.2 (1.2-20.8)	0.017
Cough	No Yes	2859 (85.5) 486 (14.5)	25 (71.4) 10 (28.6)	2.4 (1.0-5.1)	0.029
Fatigue	No Yes	3147 (94.1) 198 (5.9)	28 (80.0) 7 (20.0)	4.0 (1.4-9.5)	0.004
Muscle ache	No Yes	3085 (92.2) 260 (7.8)	28 (80.0) 7 (20.0)	3.0 (1.1-7.0)	0.017
Muscle weakness	No Yes	3048 (91.1) 297 (8.9)	27 (77.1) 8 (22.9)	3.0 (1.2-7.0)	<0.001
Convulsions/ seizure	No Yes	3340 (99.9) 5 (0.1)	35 (100.0) 0 (0)	0 (0-107.3)	1
Summary Measures					
Any of the symptoms listed above	No Yes	2492 (74.5) 853 (25.5)	9 (25.7) 26 (74.3)	8.4 (3.8-20.53)	<0.001
Fever, headache, fatigue, muscle aches	No Yes	2760 (82.5) 585 (17.5)	11 (31.4) 24 (68.6)	10.3 (4.8-23.4)	<0.001
TOTAL (N)		3345	35		

b: All pregnant women from active survey

Symptoms		Negative for *P. falciparum* infection N (%)	Positive for *P. falciparum* infection N (%)	OR (95% CI)	Fisher's exact test p-value
Single symptom associations					
Fever	No Yes	543 (88.9) 68 (11.1)	8 (40.0) 12 (60.0)	11.9 (4.3-34.8)	<0.001
Headache	No Yes	420 (68.7) 191 (31.3)	8 (40.0) 12 (60.0)	3.3 (1.2-9.4)	0.013
Chills	No Yes	575 (94.1) 36 (5.9)	14 (7.0) 6 (30.0)	6.8 (2.0-20.3)	0.001
Nausea	No Yes	518 (84.8) 93 (15.2)	11 (55.0) 9 (45.0)	4.5 (1.6-12.4)	0.002
Vomiting	No Yes	536 (87.7) 75 (12.3)	15 (75.0) 5 (25.0)	2.4 (0.66-7.1)	0.160
Diarrhea	No Yes	604 (98.9) 7 (1.1)	20 (100.0) 0 (0.0)	0 (0.0 -22.4)	1
Cough	No Yes	538 (88.1) 73 (11.9)	15 (75.0) 5 (25.0)	2.5 (0.68-7.4)	0.015
Fatigue	No Yes	509 (83.3) 102 (16.7)	16 (80.0) 4 (20.0)	1.3 (0.30-4.0)	0.760
Muscle ache	No Yes	485 (79.4) 126 (20.6)	9 (45.0) 11 (55.0)	4.7 (1.7-13.1)	<0.001
Muscle weakness	No Yes	444 (72.7) 167 (27.3)	10 (50.0) 10 (50.0)	2.7 (0.97-7.2)	0.040
Convulsions/ seizure	No Yes	604 (98.9) 7 (1.1)	18 (90.0) 2 (10.0)	9.5 (0.90-54.9)	0.030
Summary measures					
Any of the symptoms listed above	No Yes	286 (46.8) 325 (53.2)	3 (15.0) 17 (85.0)	5.0 (1.4-26.8)	0.005
Fever, headache, fatigue, muscle aches	No Yes	313 (51.2) 298 (48.8)	3 (15.0) 17 (85.0)	5.9 (1.69-31.9)	0.001
TOTAL (N)		611	20		

Table 3.5: Comparison of sociodemographic risk factors for actively detected sub-clinical *P. falciparum* infections with passive incident clinical malaria infections during high- and low-transmission seasons

	Actively sampled mild/asymptomatic infections				Passive symptomatic infections*						
	Year-round				High transmission season				Low transmission season		
Household factors	N (%) Negative	N (%) Positive	OR (95% CI)	p-value	Total Population	Cases	Incidence per 1,000/ month	P-value	Cases	Incidence per 1,000/ month	P-value
Union											
Rajbila	1489 (98.7)	20 (1.3)	1	-	10,498	234	2.05	<0.001	34	0.30	0.413
Kuhalong	1858 (99.2)	15 (0.8)	0.60 (0.31-1.2)	0.138	12,874	171	1.26		33	0.24	
Sex											
Males	1592 (98.6)	23 (1.4)	1		11,456	217	1.78	0.053	35	0.29	0.570
Females	1755 (99.3)	12 (0.7)	0.47 (0.23-0.95)	0.037*	11,916	188	1.47		32	0.25	
Age											
<6 months	0	0			985	0	0	<0.001	0	0	<0.001
6-59 months	857 (99.4)	5 (0.6)	1	-	2,621	43	1.48		16	0.55	
5-14 years	1259 (98.6)	18 (1.4)	2.5 (0.91-6.6)	0.077	5,200	149	2.75		23	0.42	
≥15 years	1231 (99.0)	12 (1.0)	1.7 (0.59-4.8)	0.336	14,563	213	1.34		28	0.18	
15-39 years	752 (98.8)	9 (1.2)	2.1 (0.68-6.1)	0.199							
≥40 years	479 (99.4)	3 (0.6)	1.1 (0.26-4.5)	0.923							
Ethnicity											
Bengali	704 (99.6)	3 (0.4)	1	-	4,821	33	0.64	<0.001	6	0.12	<0.001
Total tribal	2643 (98.8)	32 (1.2)	2.8 (0.87-9.3)	0.085	18,551	372	1.88		61	0.31	
Marma	1978 (99.1)	17 (0.9)	2.0 (0.59-6.9)	0.264	14,035	275	1.84		34	0.23	
Tanchangya	300 (97.4)	8 (2.6)	6.3 (1.6-23.8)	0.007*	2,085	39	1.74		9	0.40	
Khyang	174 (98.3)	3 (1.7)	4.0 (0.81-20.2)	0.089	1,134	3	0.24		5	0.40	
Chakma	114 (99.1)	1 (0.9)	2.1 (0.21-20.0)	0.533	802	15	1.74		7	0.81	
Tripura	54 (94.7)	3 (5.3)	13.0 (2.6-66.2)	0.002*	391	20	5.42		4	1.08	
Other tribal	2	0	0 (0-Inf)	0.997	104	20	20.70		2	2.07	
Education level, (age ≥ 15 years, n=1243)											
0-2 years	735 (98.9)	8 (1.1)	1	-	8,598	136	1.40	0.604	16	0.17	0.338
3-5 years	229 (98.3)	4 (1.7)	1.6 (0.48-5.38)	0.443	2,668	38	1.32		8	0.28	
≥6 years	267 (100.0)	0 (0.0)	0 (0-Inf)	0.988	3,297	39	1.18		4	0.12	
Occupation (age ≥ 15 years, n=1243)											
Agricultural	461 (99.1)	4 (0.9)	1		4,609	48	0.93	<0.001	11	0.21	0.643
Day labor	170 (98.3)	3 (1.7)	2.0 (0.45-9.2)	0.356	1,996	42	1.91		5	0.23	
Jhum cultivation	91 (95.8)	4 (4.2)	5.1 (1.2-20.7)	0.024*	2,544	71	2.42		5	0.17	
Other	509 (99.8)	1 (0.2)	0.23 (0.03-2.0)	0.185	5,414	52	0.92		7	0.12	
Unemployed	92 (100.0)	0 (0)	0 (0-Inf)	0.996							
Housewife	173 (100.0)	0 (0)	0 (0-Inf)	0.994							
Student	149 (100.0)	0 (0)	0 (0-Inf)	0.995							
Other	114 (99.1)	1 (0.9)	1.0 (0.11-9.2)	0.992							
Total					**23,372**	**405**	**1.62**		**67**	**0.27**	

*Passive data taken from prior survey in this cohort[119]

Table 3.6: Comparison of household risk factors for actively detected sub-clinical *P. falciparum* infections with passive incident clinical malaria cases during high- and low-transmission seasons

	Active mild/asymptomatic infections				Passive symptomatic infections						
	Year-round				High transmission season				Low transmission season		
Household factors	N (%) negative	N (%) positive	OR (95% CI)	p-value	Total population	Cases	Incidence per 1,000/ month	P-value	Cases	Incidence per 1,000/ month	P-value
Bednet use (slept under net night before survey), n=3,257											
Yes	2950 (99.0)	31 (1.0)	1.0	-	18,869	306	1.48	<0.001	54	0.26	0.611
No	272 (98.6)	4 (1.4)	1.4 (0.49-4.0)	0.530	2,255	58	2.66		7	0.32	
Own animals, n=3,382											
Yes	2817 (99.0)	28 (1.0)	1.0	-	19,087	337	1.65	0.407	126	0.26	0.923
No	530 (98.7)	7 (1.3)	1.3 (.58-3.1)	0.504	4,285	68	1.48		30	0.27	
Distance from house to pond (meters), n=3381 (Two "don't knows" were excluded)											
0-50	654 (99.7)	2 (0.3)	1.0	-	4,312	31	0.68	<0.001	7	0.015	0.015
51-100	262 (99.2)	2 (0.8)	2.5 (0.40-17.8)	0.362	1,897	13	0.64		1	0.05	
>100	167 (98.8)	2 (1.2)	3.9 (.55-28.0)	0.174	1,022	13	1.19		5	0.46	
No pond	2263 (98.7)	29 (1.3)	4.2 (1.0-17.6)	0.050	16,141	345	1.99		54	0.31	
Distance from house to forest (meters), n=3,382											
0-25	1134 (98.6)	16 (1.4)	1.0	-	7,869	183	2.16	<0.001	34	0.40	0.013
26-50	1343 (98.8)	17 (1.3)	0.90 (0.45-1.78)	0.756	9,187	126	1.29		22	0.23	
>50	870 (99.8)	2 (0.2)	0.16 (0.04-0.72)	0.016*	6,316	96	1.43		11	0.16	
Distance from river or stream (meters), n=3,971											
0-50	569 (99.5)	3 (0.5)	1.0	-							
51-100	587 (98.8)	7 (1.2)	2.3 (0.58-8.8)	0.239							
100-250	1137 (99.0)	11 (1.0)	1.8 (0.51-6.6)	0.353							
250+	1054 (98.7)	14 (1.3)	2.5 (0.72-8.8)	0.148							
Altitude (meters above sea level), n=3,968											
7-25	734 (99.9)	1 (0.1)	1.0	-							
25-50	1545 (99.1)	14 (0.9)	6.7 (0.87-50.7)	0.067							
50-75	688 (98.7)	9 (1.3)	9.6 (1.2-76.0)	0.032*							
75-125	353 (10.5)	8 (2.2)	16.6 (2.1-133.6)	0.008*							
125-147	27 (90.0)	3 (10.0)	81.6 (8.2-810.5)	<0.001 *							
Total	**3,347**	**35**			**23,372**	**405**	**1.62**		**67**	**0.27**	

Table 3.7: Regression model of sub-clinical *P. falciparum* infection with household and demographic factors

		Unadjusted (N=4,010)		**Adjusted (N=3,986)**	
Demographic and household factors		OR (95% CI)	p-value	OR (95%CI)	p-value
Sex	Male Female	1.0 0.47 (0.23-0.95)	- 0.037*	1.0 0.43 (0.20-0.92)	- 0.030*
Age	<5 years 5-14 years 15-39 years 40+ years	1.0 2.5 (0.91-6.6) 2.1 (0.68-6.1) 1.1 (0.26-4.5)	- 0.077 0.199 0.923	1.0 2.4 (0.88-6.5) 0.59 (0.11-3.0) 0.34 (0.05-2.23)	- 0.087 0.521 0.258
Occupation (all age groups)	Other Agricultural Day labor Jhum Cultivation	1.0 0.93 (0.32-2.7) 1.8 (0.55-6.2) 4.7 (1.6-13.9)	- 0.896 0.322 0.005*	1.0 3.0 (0.62-14.5) 4.1 (0.87-19.9) 8.9 (1.7-45.9)	- 0.170 0.075 0.009*
Distance from house to forest	Per increase in Z-score	0.28 (0.11-0.76)	0.012*	0.42 (0.17-1.1)	0.074
Elevation	Per increase in Z-score	1.8 (1.4-2.3)	<0.001*	1.6 (1.2-2.0)	<0.001*
Pregnancy	No Yes	1.0 5.0 (1.2-21.6)	- <0.031*	1.0 13.3 (2.3-77.2)	- 0.004*

Table 3.8: **Incidence rate of sub-clinical *P. falciparum* infections by age and gender among randomly selected longitudinal survey participants**

AGE	Incident infections	People followed	Person years	Incidence rate (per 1,000 person-years)	95% CI
<5 male	2	77	50.41	39.7	10.2-154.3
<5 female	1	64	40.65	24.6	3.6-170.5
5-14 male	1	127	82.19	12.2	1.7-85.4
5-14 female	3	133	86.22	34.8	11.4-105.8
15-39 male	2	53	34.77	57.5	15.0-220.9
15-39 female	0	109	76.50	0	0-Inf
40+ male	3	45	29.66	101.2	34.6-295.8
40+ female	0	46	29.67	0	0-Inf
Total male (crude)	8	285	197.02	40.6	20.6-80.0
Total male (adjusted)	10.9*	285	197.02	55.2	31.0-98.4
Total female (crude)	4	335	233.03	17.2	6.5-45.4
Total female (adjusted)	2.6*	335	233.03	11.04	3.3-37.2
Total (crude)	12	620	430.05	27.9	16.0-48.7
Total (adjusted)	14.1*	620	430.05	32.9	19.7-54.9

*Using direct adjustment, applying rates to age/sex structure of overall population

Table 3.9: **Incidence rate of sub-clinical *P. falciparum* infections by pregnancy status in woman age 15-39 years**

AGE	Incident infections	Number of people	Person-years	Incidence rate (per 1,000 person-years)	95% CI
Pregnant at this visit	2	339	102.95	19.43	4.9-76.6
Recently pregnant	6	565	307.37	19.52	8.8-43.11
Not Pregnant	0	83	56.47	0	0-Inf

3.9 Chapter Three Appendix

All analyses in this chapter were completed with just the randomly selected population. However, a sensitivity analysis was conducted using a combination of the random and pregnancy-selected population. Tables for this when not presented above are listed below:

TABLE 3.2b: Malaria by sex and age category

SEX/AGE Category		Total (%)	Total without *P. falciparum* malaria (%)	Total with *P. falciparum* malaria (%)	Odds ratio (95% CI)	p-value
Female	<5	426 (10.7)	424 (10.8)	2 (3.8)	1.0	-
Female	5-14 years	669 (16.8)	664 (16.9)	5 (9.4)	1.60 (0.31-8.27)	0.577
Female	15-39 years	1019 (25.7)	997 (25.4)	22 (41.5)	4.68 (1.09-19.99)	0.037*
Female	40+ years	242 (6.1)	241 (6.2)	1 (1.9)	0.88 (0.08-9.76)	0.917
Male	<5	436 (11.0)	433 (11.1)	3 (5.7)	1.47 (0.25-8.84)	0.675
Male	5-14 years	613 (15.4)	599 (15.3)	14 (26.4)	4.95 (1.12-21.93)	0.035*
Male	15-39 years	321 (8.1)	317 (8.1)	4 (7.5)	2.68 (0.49-14.70)	0.258
Male	40+ years	245 (6.2)	243 (6.2)	2 (3.8)	1.75 (0.24-12.47)	0.579
Total		3971 (100)	3918(100)	53 (100)		

Table 3.3b: Seasonal variation in detection of sub-clinical *P. falciparum* infections by active surveillance

Season	Number of positive *P. falciparum* infections (% of those tested)	Number tested	OR (95% CI) low season: high season	Fisher's exact test p-value
Low season (November-April) Random selection Pregnancy selection	17 (1.0)	1771	1.72 (0.94-3.27)	0.071
High season (May-October) Random selection Pregnancy selection	36 (1.6)	2200		
Year-round	53 (1.3)	3918		

Table 3.4b: Association of sub-clinical *P. falciparum* infections with reported symptoms in prior 2 weeks

Symptoms		Negative for *P. falciparum* malaria N (%)	Positive for *P. falciparum* malaria N (%)	OR (95% CI)	Fisher's exact test p-value
Single symptom associations					
Fever	No Yes	3651 (93.2) 265 (6.8)	24 (45.3) 29 (54.7)	16.6 (9.2-30.3)	<0.001
Headache	No Yes	3490 (89.1) 426 (10.9)	26 (49.1) 27 (50.9)	8.5 (4.7-15.3)	<0.001
Chills	No Yes	3781 (96.6) 135 (3.4)	37 (69.8) 16 (30.2)	12.1 (6.1-22.9)	<0.001
Nausea	No Yes	3750 (95.8) 166 (4.2)	35 (66.0) 18 (34.0)	11.6 (6.0-21.5)	<0.001
Vomiting	No Yes	3795 (96.9) 121 (3.1)	43 (81.1) 10 (18.9)	7.2 (3.2-15.2)	<0.001
Diarrhea	No Yes	3859 (98.5) 57 (1.5)	50 (94.3) 3 (5.7)	4.1 (0.79-13.2)	0.045
Cough	No Yes	3372 (86.1) 544 (13.9)	39 (73.6) 14 (26.4)	2.2 (1.1-4.2)	0.015
Fatigue	No Yes	3626 (92.6) 290 (7.4)	42 (79.2) 11 (20.8)	3.3 (1.5-6.6)	0.002
Muscle ache	No Yes	3538 (90.3) 378 (9.7)	35 (66.0) 18 (34.0)	4.8 (2.5-8.8)	<0.001
Muscle weakness	No Yes	3461 (88.4) 455 (11.6)	35 (66.0) 18 (34.0)	3.9 (2.1-7.2)	<0.001
Convulsions/ seizure	No Yes	3904 (99.7) 12 (0.3)	51 (96.2) 2 (3.8)	12.7 (1.4-59.4)	0.014
Summary measures					
Any of the symptoms listed above	No Yes	2766 (70.6) 1150 (29.4)	12 (22.6) 41 (77.4)	8.2 (4.2-17.2)	<0.001
Fever, headache, fatigue, muscle aches	No Yes	3057 (78.1) 859 (21.9)	14 (26.4) 39 (73.6)	9.9 (5.2-19.8)	<0.001
TOTAL (N)		3918	53		

Table 3.5b: Comparison of sociodemographic risk factors for actively detected sub-clinical *P. falciparum* infections with passive incident malaria cases during high- and low-transmission season

	Actively sampled mild/asymptomatic infections				Passive symptomatic infections						
	Year-round				High transmission season				Low transmission season		
Household factors	N (%) Negative	N (%) Positive	OR (95% CI)	p-value	Total Population	Cases	Incidence per 1,000/ month	P-value	Cases	Incidence per 1,000/ month	P-value
Union											
Rajbila	1769 (98.1)	34 (1.9)	1		10,498	234	2.05	<0.001	34	0.30	0.413
Kuhalong	2149 (99.1)	19 (0.9)	0.47 (0.27-0.84)	0.010*	12,874	171	1.26		33	0.24	
Sex											
Males	1592 (98.6)	23 (1.4)	1.0	-	11,456	217	1.78	0.053	35	0.29	0.570
Females	2326 (98.7)	30 (1.3)	0.89 (0.52-1.5)	0.684	11,916	188	1.47		32	0.25	
Age											
<6 months	0	0			985	0	0	<0.001	0	0	<0.001
6-59 months	857 (99.4)	5 (0.6)	1	-	2,621	43	1.48		16	0.55	
5-14 years	1263 (98.5)	19 (1.5)	2.6 (0.96-6.9)	0.060	5,200	149	2.75		23	0.42	
≥15 years	1798 (98.4)	29 (1.6)	2.8 (1.1-7.2)	0.036*	14,563	213	1.34		28	0.18	
15-39 years	1314 (98.1)	26 (1.9)	3.4 (1.3-8.9)	0.013*							
≥40 years	484 (99.4)	3 (0.6)	1.1 (0.25-4.5)	0.934							
Ethnicity											
Bengali	814 (99.5)	4 (0.5)	1	-	4,821	33	0.64	<0.001	6	0.12	<0.001
Total tribal	3104 (98.4)	49 (1.6)	3.2 (1.2-8.9)	0.025*	18,551	372	1.88		61	0.31	
Marma	2342 (99.0)	23 (1.0)	2.0 (0.69-5.8)	0.202	14,035	275	1.84		34	0.23	
Tanchangya	341 (95.8)	15 (0.4)	9.0 2.9-27.2)	<0.001 *	2,085	39	1.74		9	0.40	
Khyang	204 (98.1)	4 (1.9)	4.0 (0.99-16.1)	0.052	1,134	3	0.24		5	0.40	
Chakma	130 (97.7)	3 (6.1)	4.7 (1.0-21.2)	0.044*	802	15	1.74		7	0.81	
Tripura	60 (93.8)	4 (6.2)	13.6 (3.3-55.6)	<0.001 *	391	20	5.42		4	1.08	
Other tribal	27 (100.0)	0 (0)	0 (0-Inf)	0.987	104	20	20.70		2	2.07	
Education level (age ≥ 15 years, n=1827)											
0-2 years	1027 (98.0)	21 (2.0)	1	-	8,598	136	1.40	0.604	16	0.17	0.338
3-5 years	350 (98.9)	4 (1.1)	0.56 (0.19-1.6)	0.289	2,668	38	1.32		8	0.28	
≥6 years	421 (99.1)	4 (0.9)	0.46 (0.16-1.4)	0.162	3,297	39	1.18		4	0.12	
Occupation (age ≥ 15 years, n=1827)											
Agricultural	585 (98.7)	8 (1.3)	1	-	4,609	48	0.93	<0.001	11	0.21	0.643
Day labor	226 (98.7)	3 (1.3)	0.97 (0.26-3.7)	0.965	1,996	42	1.91		5	0.23	
Jhum cultivation	112 (95.7)	5 (4.3)	3.3 (1.0-10.2)	0.0411 *	2,544	71	2.42		5	0.17	
Other	875 (98.5)	13 (1.5)	1.1 (0.45-1.6)	0.855	5,414	52	0.92		7	0.12	
Unemployed	115 (97.5)	3 (2.5)	1.9 (0.50-7.3)	0.346							
Housewife	473 (98.3)	8 (1.7)	1.2 (0.46-3.3)	0.673							
Student	149 (100.0)	0 (0)	0 (0-Inf)	0.986							
Other	138 (98.6)	2 (1.4)	1.1 (0.22-5.1)	0.942							
Total	**3,918**	**53**			**23,372**	**405**	**1.62**		**67**	**0.27**	

Table 3.6b: Comparison of household risk factors for actively detected sub-clinical *P. falciparum* infections with passive incident clinical malaria cases during high- and low-transmission seasons

	Active mild/asymptomatic infections				Passive symptomatic infections						
	Year-round					High transmission season			Low transmission season		
House hold factors	N (%) negative	N (%) positive	OR (95% CI)	p-value	Total population	Cases	Incidence per 1,000/ month	P-value	Cases	Incidence 1,000/ month	P-value
Bednet use (slept under bednet night before survey), n=3,719											
Yes	3332 (98.9)	38 (1.1)	1.0	-	18,869	306	1.48	<0.001	54	0.26	0.611
No	344 (98.6)	5 (1.4)	1.27 (0.50-3.3)	0.613	2,255	58	2.66		7	0.32	
Own animals, n=3,971											
Yes	3,273 (98.8)	40 (1.2)	1.0	-	19,087	337	1.65	0.407	126	0.26	0.923
No	645 (98.0)	13 (2.0)	1.65 (0.88-3.1)	0.120	4,285	68	1.48		30	0.27	
Distance from house to pond (meters), n=3,969											
0-50	755 (99.7)	2 (0.3)	1.0	-	4,312	31	0.68	<0.001	7	0.015	0.015
51-100	297 (98.3)	5 (1.7)	6.4 (1.2-32.9)	0.026*	1,897	13	0.64		1	0.05	
>100	193 (97.5)	5 (2.5)	9.8 (1.9-50.8)	0.007*	1,022	13	1.19		5	0.46	
No pond	2672 (68.4)	40 (1.5)	5.7 (1.4-23.4)	0.017*	16,141	345	1.99		54	0.31	
Distance from house to forest (meters), n=3,971											
0-25	1328 (98.1)	26 (1.9)	1.0	-	7,869	183	2.16	<0.001	34	0.40	0.013
26-50	1553 (98.7)	21 (1.3)	0.69 (0.39-1.2)	0.211	9,187	126	1.29		22	0.23	
>50	1037 (99.4)	6 (0.6)	0.30 (0.12-0.72)	0.007*	6,316	96	1.43		11	0.16	
Distance from river or stream (meters), n=3,971											
0-50	672 (99.4)	4 (0.6)	1.0	-							
51-100	710 (98.3)	12 (1.7)	2.8 (0.91-8.8)	0.072							
100-250	1311 (98.9)	15 (1.1)	1.9 (0.64-5.8)	0.247							
250+	1223 (98.3)	21 (1.7)	2.9 (0.99-8.4)	0.053							
Altitude (meters above sea level), n = 3,968											
7-25	846 (99.9)	1 (0.1)	1.0	-							
25-50	1820 (98.8)	22 (1.2)	10.2 (1.4-7.6)	0.023*							
50-75	814 (98.2)	15 (1.8)	15.6 (2.1-10.8)	0.008*							
75-125	408 (98.8)	9 (2.2)	18.7 (2.4-14.8)	0.006							
125-147	28 (84.8)	5 (15.2)	151.1 (17.1-13.4)	<0.001*							
Total					**23,372**	**405**	**1.62**		**67**	**0.27**	

Table 3.7b: Regression model of sub-clinical *P. falciparum* infection with household and demographic factors

			Unadjusted (N=4,010)		Adjusted (N=3,986)	
Demographic and household factors		Missing N	OR (95% CI)	p-value	OR (95%CI)	p-value
Sex	Male Female	0	1.0 0.89(0.52-1.5)	- 0.684	1.00 0.42 (0.20-0.88)	- 0.021*
Age	<5 years 5-14 years 15-39 years 40+ years	0	1.0 2.6 (0.96-6.9) 3.4 (1.3-8.9) 1.1 (0.25-4.5)	- 0.060 0.036 0.934	1.00 2.6 (0.94-6.9) 1.2 (0.32-4.2) 0.75 (0.15-3.6)	- 0.065 0.819 0.721
Occupation (all age groups)	Other Agricultural Day labor Jhum cultivation	0	1.0 1.1 (0.51-2.6) 1.1 (0.32-3.4) 3.6 (1.4-9.2)	- 0.825 0.952 <0.001*	1.0 1.3 (0.54-3.35) 1.1 (0.32-4.1) 3.1 (1.0-9.7)	- 0.530 0.831 0.049*
Distance from house to forest	Per increase in Z-score	0	0.54 (0.29-1.0)	0.0494	0.54 (0.27-1.1)	0.088
Elevation	Per increase in Z-score	3	1.8 (1.4-2.1)	<0.001*	1.6 (1.3-2.0)	<0.001*
Pregnancy	No Yes	0	1.0 3.3 (1.9-5.8)	- <0.001*	7.0 (2.5-19.8)	- <0.001*

Chapter Four: Temporal and spatial differences between sub-clinical and clinical *P. falciparum* malaria infections in the Chittagong Hill Districts, Bangladesh

4.1 Abstract:

In this analysis we compared factors of those testing positive for sub-clinical *P. falciparum* infections in our active survey to those presenting with clinical *P. falciparum* malaria in our passive survey. The analysis demonstrated that sub-clinical infections are distributed relatively evenly year-round, while clinical infections peak in incidence in June and July. A regression between clinical and sub-clinical infections demonstrated that parasite count was the factor most associated with symptomatic infection. We also demonstrated that although the hotspots overlap, there were substantial differences in the areas of higher risk.

4.2 Introduction:

In Bangladesh, malaria is endemic in 13 of 64 districts, with over 14 million people at risk for infection, with the highest rates in the Chittagong Hill Districts, where *Plasmodium falciparum* is the predominant species and *P. vivax* occurs to a lesser extent.[107,108] A wide variety of mosquito species transmit malaria in the region.[107-109,113] Clinical infections tend to peak from June-August during the height of the rainy season.[109,110] The Bangladesh National Malaria Control Program implemented by the Ministry of Health and the non-profit group BRAC focused on a variety of interventions starting in 2007, including providing community-based testing and treatment with artemisinin-based drugs, supplying long-lasting insecticide treated bednets, and an overall strengthening of the malaria surveillance and control programs. A 65% reduction in malaria prevalence and a 91% decrease in malaria mortality was observed between 2008 and 2012, likely as a result of this focused effort.[108] But without a consistent focus on elimination, these gains are tenuous.

A cohort study supported by The Johns Hopkins Malaria Institute, in collaboration with icddr,b, began in 2009, to better understand the epidemiology of malaria in the Chittagong Hill Districts near Bandarban. An assessment of symptomatic malaria of this cohort demonstrated a seasonal epidemic, with specific hotspots maintaining transmission and a number of associated risk factors.[119] Another assessment demonstrated the particularly high risk of infection among pregnant women with asymptomatic sub-clinical infections occurring year-round.[123] As more countries shift from highly endemic to hypoendemic settings, finding the remaining hotspots for malaria and responding appropriately will be imperative to the success of control and elimination programs. Most of the current strategies are based on locating hotspots associated with symptomatic, clinically presenting cases. In this paper, we seek to understand and explore the extent to which clinical and sub-clinical infections overlap in space and time, to determine whether basing hotspot location on symptomatic clinical cases is an adequate strategy.

4.3 Methods:

This part of the study was conducted in the Chittagong Hill Districts study site described in prior chapters. There were three aspects to the analysis in this chapter: 1) estimating and mapping the spatial intensities of sub-clinical and clinical *P. falciparum* infections, overall and by season, 2) using K-function methodology, explore the extent that *P. falciparum* infections cluster spatially and by season and examine the extent to which sub-clinical infections cluster around clinical cases, 3) using a regression analysis to identify potential demographic, behavioral and environmental risk factors that differ between people with sub-clinical infections and those that presented with symptomatic malaria. Due to the existence of only a single *P. vivax* infection through active surveillance, this paper will be limited to the analysis of *P. falciparum* malaria.

Data was analyzed using R statistical software (Vienna, Austria) and ArcMAP (Redlands, California).[136,158] R packages included spatstat, maptools, splancs, maps, SDMTools and GISTools.[146,149,159-162]

4.3.1 Estimation of spatial intensities of P. falciparum and P. vivax malaria and associated geographic and seasonal factors

The active surveillance system began in mid-October 2009 in Kuhalong Union, and in May 2010 was expanded to include Rajbila Union, with initial prevalence sampling continuing through mid-October 2012. The sampling structure was described above in Chapter 2. For this analysis we combined all actively sampled persons, including the random and pregnancy selected groups. Due to potential biases introduced from oversampling pregnant women, a sensitivity analysis was conducted with just those people who were randomly selected into our active survey. Results of the sensitivity analysis can be found in the Chapter Four Appendix.

The passive surveillance system also began in October 2009 and continues to the present (November 2015). Infections are included if they were diagnosed as having malaria based on an RDT or microscopy when they sought care for suspected malaria, either through the local BRAC clinic or directly through the study doctor or field workers. Patients contacted our team either by cell phone or through talking to a field worker who made regular visits to each village. We had agreements with BRAC and the local hospital to share data on patients from our study area. In this analysis, we limited the follow-up to the same period as the active surveillance (i.e. mid-October 2009 to mid-October 2012).

In this part of the analysis, the latitude and longitude of household locations of both sub-clinical and clinical infections were identified using a GPS device. These locations were reprojected using ARCMap to UTM 46N, a planar projection.

Spatial intensity, defined as the expected number of events per unit area, was estimated using the non-parametric kernel density approach and mapped to highlight spatial variation in the concentration for both the sub-clinical and clinical locations.[163] The ratio of these respective spatial intensities was then taken and mapped to characterize the spatial variation (spatial odds) in expected numbers of sub-clinical infections relative to clinical infections. This intensity analysis was performed for all infections and then stratified by season (High Season: May-October, Lowe Season: November-April).

4.3.2 Determining the extent of spatial clustering in sub-clinical and clinical P. falciparum infections and the extent of clustering of sub-clinical infections around clinical cases

Spatial clustering is the property that describes how dispersed or tightly compact a set of mapped events are, complimentary to spatial intensity, which characterizes spatial variation in the location of events. The K-function, which estimates the expected number of other events within a range of distances of each event, was used to assess spatial clustering.[163] K-functions for both the sub-clinical and clinical infection locations were estimated separately and then the difference in K-functions (K sub-clinical - K clinical) was used to assess the extent to which their level of spatial clustering differed and at what range of distances do these difference occur. The K-function clustering analysis was performed for all *P. falciparum* infections and then stratified by season. Significant differences in spatial clustering were assessed using the Monte Carlo random labeling approach.[164]

The Cross-K function is similar to the K-function but assesses spatial interaction between two sets of event locations by estimating for a range of distances the expected number of one type of event around the second type. A Cross-K function was estimated and used to assess the extent of clustering of sub-clinical infections around clinical cases. The results were compared

to the theoretical value of the cross K-function under the null hypothesis that the two types of event locations (sub-clinical and clinical infections) are spatially independent.[165] For this analysis, the number of sub-clinical infections was multiplied by the inverse of the proportion of the population sampled, to estimate the number of sub-clinical infections likely present in the entire population rather than just those sampled.

4.3.3 Regression analysis comparing sub-clinical to clinical infections

As part of the cohort study, a demographic study of the population was conducted on households initially in 2009 and was updated every 3 to 4 months for births, deaths and in/out migrations. Another survey conducted yearly addressed a number of questions related to malaria control practices, household behaviors and distances to certain environmental features such as water features and forest. Remote sensing was also used to calculate elevation and distance to rivers. These factors were assessed using univariate and multivariate logistic regression analysis as to their strength in discriminating the binary outcome of the person having a sub-clinical or clinical infection. A final model was selected using a compromise between forward selection and backward elimination. Age group was included in the final model regardless of selection to control for sampling design. A semivariogram of the standardized residuals from the final regression model was used to assess residual spatial variation and inference adjusted accordingly.[166]

4.4 Results:

4.4.1 Estimation of spatial intensities of P. falciparum and P. vivax malaria and associated geographic and seasonal factors

As discussed in Chapter 2 above, of the 3,978 people surveyed for active screening, 3,971 had laboratory information available. Of these, 53 tested positive by RDT and/or microscopy for

P. falciparum infections and one tested positive for *P. vivax* infection by microscopy; 35 of these sub-clinical infections were through the random sampling scheme and 18 were selected due to pregnancy. The geometric mean parasite density of the 44 microscopy positive infections was 902 parasites/μL (range 40-14,600 parasites/μL).

In the passive survey, 572 *P. falciparum* clinical infections were found to be positive by RDT and/or microscopy after seeking care for symptoms of malaria between mid-October 2009 and mid-October 2012. 570 (99.7%) were positive by RDT, while 456 (79.7%) were positive by microscopy, while 63 (13.8%) did not have completed blood films. The geometric mean parasite density of the 456 clinical microscopy positive infections was 4,740 parasites/μL (range 100-144,000 parasites/μL).

One of the 53 *P. falciparum* infections, two of the *P. falciparum* negative participants from the active survey, and six of the 572 from the passive survey during this period, did not have available spatial information and were dropped from the spatial aspect of the analysis, although they were still included in seasonality statistics and the regression analysis.

Figure 4.1a and 4.1b shows the overall intensities mapped through a kernel density estimator with a 1 km bandwidth radius for both study types. There is considerable overlap in areas of elevated intensity between the passively detected symptomatic cases and the actively detected sub-clinical infections. However, there are some substantial differences. For instance, there is a substantial cluster of clinical cases at the center of the overall map in the northern part of Kuhalong Union, but not in the map of the sub-clinical infections. Figure 4.1c maps the spatial odds of sub-clinical infections to clinical cases. Areas in darker blue are those with several symptomatic cases and few asymptomatic infections. Areas in yellow and red were those with a higher ratio of sub-clinical infections compared to clinical cases. These red/yellow areas would

be areas where sub-clinical infections are more likely to be missed if detection was based on symptomatic clustering alone.

Figure 4.2 compares the number and proportion of infections testing positive in our active study with the number of positive cases detected through our passive study, by month of the year. It is clear that the clinical infections have much more seasonality, with an extensive peak during the high season, particularly in June and July, while the sub-clinical infections appear year-round. For the passive study, of the 572 positive clinical cases, 495 (86.5%) occurred during the May-October high season and 77 (13.5%) during the November-April low season. Similar percentages were seen when looking over the whole course of the passive study. For the active study, 36 (68%) were detected during high season, making up 1.6% of the 2,200 people tested during this period, and 17 (32%) were detected during low season, accounting for 1.0% of the 1,771 people surveyed during this season.

Figure 4.3 shows the spatial intensity maps by season. For both studies, there are fewer infections during the low season, although as noted above for the sub-clinical infections, this finding is partially a result of fewer people being surveyed during this time. The areas of higher risk by season appear to differ among the sub-clinical clinical infections. The clinical cases in the low season for the most part appear in the higher intensity areas from the high season, with few cases outside these areas. This pattern was not observed with the sub-clinical infections.

4.4.2 Determining the extent of spatial clustering in sub-clinical and clinical P. falciparum infections and the extent of clustering of sub-clinical infections around clinical cases

The K-function demonstrates spatial clustering. Figure 4.4 demonstrates that the passively detected clinical infections consistently cluster more than would be predicted by complete spatial randomness, while the sub-clinical infections appear to cluster more than

complete randomness during the low season, but during the high season are relatively randomly placed. When comparing the clinical and sub-clinical infections in the high season, the clinical cases tend to cluster more than the sub-clinical infections. For the low season, there does not appear to be much of a difference between them, although there is perhaps more clustering for the clinical cases at less than 1 km. This relationship can be further gleaned by the difference in K-functions. The difference in K-functions again confirms that in the high season, clinical cases appear to cluster more than the sub-clinical infections, bordering on significance up to about 1.5 km. During the low season, again there appears to be more clustering of clinical cases at shorter than 1km and above 4km.

The cross-K function seen in Figure 4.5 demonstrates the extent of clustering of an actively detected sub-clinical infection around a clinical case. Extrapolating the number of actively sampled infections to the number of expected sub-clinical infections in the entire population, there may be a slight increase in the number of sub-clinical infections near clinical cases up to about 2.5-3 km. However, the increase is small, with an average of only 4-5 more infections than would be expected if they were not related.

4.4.3 Regression analysis comparing sub-clinical to clinical infections

In this part of the analysis, certain demographic, behavioral and environmental factors appear to be associated with clinical and sub-clinical *P. falciparum* infections. This is not an exploration of the factors that are associated with a higher risk for *P. falciparum* infections, which has been done in other studies for the passive survey[119] and in chapter two of this thesis for the active survey. Rather, these are the factors associated with a higher risk for clinically presenting symptomatic *P. falciparum* malaria over sub-clinical *P. falciparum* infections.

The results of the univariate analysis can be seen in Tables 4.1 and 4.2. Certain ethnic groups appear to have a lower risk of presenting clinically when infected. Housewives had a higher proportion of sub-clinical infections compared to clinical, although this result was likely due to the over-selection of pregnant women in the sample design, as the association was not found in the sensitivity analysis when only randomly selected people were included. Although no significant association was found, there was a trend toward more infections likely to be sub-clinical in the 5-39 year-olds compared to people under 5 and over 40. When examining just the randomly sampled people in the sensitivity analysis, this relationship only held for 5-14 year-olds.

From the household survey in Table 4.2, we see some associations at certain distances from streams and ponds, although the associations do not change consistently as distance increases. There does not appear to be an association with sleeping under a bednet, owning animals, distance to forests, and elevation in a univariate analysis.

The final model presented in Table 4.3. The most significant factor distinguishing clinical and sub-clinical infections was parasite density. Increasing distance from streams appeared to be associated with more clinical than sub-clinical infections. There were possible trends of differing rates among certain ethnic groups, although none reached statistical significance. There was also a trend of a possible increase in clinical infection at greater distances from forests.

4.5 Discussion:

It is clear from this analysis that clinical symptomatic infections appear mostly during the rainy season, while sub-clinical infections occur year-round. Although there is some overlap in the hotspots, there are some areas in which they do not. It does appear that the presence of a clinical case is associated with a slight increase in the risk of finding a sub-clinical infection up

to about 2.5-3 km, meaning the hotspots overlap more than not. However, using clinical cases to identify sub-clinical hotspots, may result in some sub-clinical hotspots being overlooked.

The factors that contribute to an infection being clinical rather than sub-clinical are complex. First, there are the factors that impact getting infected in the first place. These include a variety of environmental, behavioral and genetic factors, which have been discussed in prior chapters. But once people are infected, other factors impact whether the person develops symptoms concerning enough for them to seek care. Their level of symptoms may be impacted by prior infections and the development of immunity, which could be related to age, as well as behavioral, environmental and occupational factors related to exposure; there are also genetic differences that may make individual more susceptible to symptomatic infection.

Even if symptoms do develop, a clinical infection requires that the person has access and choses to seek care. Although everyone in this study had access to free care by speaking with a field staff in the village or calling the study doctor, it is quite possible that those working at distant jhum cultivation sites did not. It is also easier for those who live on the road to get to a clinic, while those a four-hour walk from the nearest road may have less access. We suspect for this study, the general ease of access and availability of the study clinician to make house calls means that these barriers likely did not substantially decrease the number of people who sought care.

Although much more research needs to be done to parse out these varying risk factors, as most did not reach statistical significance in this analysis, some trends can be seen. We found more symptomatic infections with increasing distance from rivers and streams and a trend of increasing symptoms with increasing distance to forests. It is possible that these factors reflect the likelihood of prior infections, thus those living closer to streams and forests may have higher

immunity due to higher levels of prior infections. It may be that those living in areas where they are more likely to be exposed, are more likely to remain less symptomatic due to development of immunity, while those living in areas with lower-risk of exposure that do get bitten are more likely to be symptomatic due to lower levels of immunity. Although our study did not examine sub-microscopic infection, a similar trend was found to be the case in a study in Tanzania that showed that 80% of infections in high-exposure households were submicroscopic compared with only 31% in low-exposure households.[167] Several studies have also found adults are more likely than children to have submicroscopic infection. We saw this trend with respect to older children but interestingly, adults over 40 again appeared to have a trend towards more symptomatic infections.

The trend with housewives being more likely to have a sub-clinical infection appeared to relate to pregnant women being more frequently sampled in our active survey. In the sensitivity analysis with only randomly sampled people we did not find this association and further found a trend of women being more likely symptomatic than men. It also may relate to these infections being caught during pregnancy before they become symptomatic as part of regular follow-up during pregnancy. We have shown in chapter two that pregnant women are at substantially higher risk of developing infections in the first place.

There are several limitations to this analysis. First, the lack of PCR means that substantial numbers of sub-microscopic infections were likely missed in the active survey that could have contributed to the analysis. Second, in comparing just the sub-clinical infections and clinical cases, we are assuming that the sampling in the active study is close to reflective of the population in general. As participants were randomly selected by age group and all pregnant women were included, this assumption was generally accurate, with a few notable exceptions.

Pregnant women were oversampled, as were women in general. This issue was dealt with by the sensitivity analysis excluding this subset of the active survey.

Due to the nature of the asymptomatic data collection, only one person a week was surveyed in each of the 24 geographic clusters for our prevalence survey. As a result, clusters in the same space and time may be missed. This issue is partially mitigated by the long period of infection likely for most of those in the active survey. Secondly, due to the overall low number of infections detected through active surveillance, we included all pregnant woman as well as those sampled through our random selection scheme. As a result, those in the active dataset over-represent pregnant women and younger children. Age was included in the regression analysis to control for the sampling setup, but pregnancy data was not available for all symptomatic cases. However, the sub-clinical hotspot seen in the lower right area of the intensity maps was mostly made up of pregnant women, a result that could be further explored. It was also challenging to define spatial relationships on the basis of only 53 positive sub-clinical infections and 35 in the sensitivity analysis, particularly when broken down by season or any other factors, and thus a larger sample size would help to further delineate these relationships. Lastly, the sub-clinical infections, rather than the proportion testing positive, were compared during this analysis. It is likely that the infection intensity is reasonably reflective of the intensity of the proportion positive, as the population was randomly sampled in the active survey and all pregnant women regardless of location were included; nevertheless, it is possible the sampling design introduced some bias.

For both passive and active surveillance, the coverage from October 2009-April 2010 was for only one (Kuhalong) of the two unions. Thus the number of infections picked up is less than during the same periods in the following years. This fact could potentially influence the

seasonality assessments, but as it impacted both the active and passive surveys, its effect may not have been that substantial. For the passive data, from May 1, 2010-May 1, 2014, 85% of cases were seen during high season and 15% during low, which are very similar to the percentages seen in our shortened symptomatic dataset.

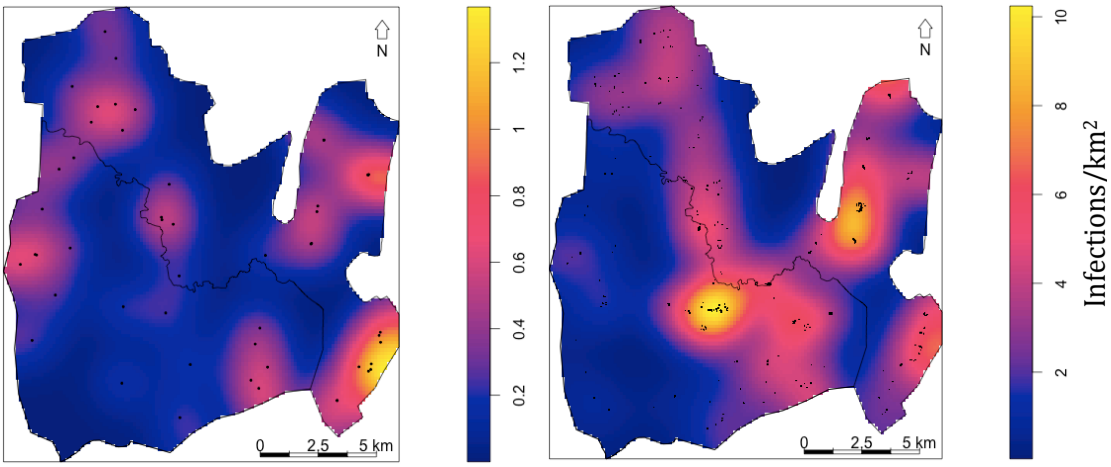
4.6 Conclusion:

The majority of *P. falciparum* infections in the area are sub-clinical and appear year-round. Although the clinical and sub-clinical hotspots do overlap, clinical symptomatic hotspots may not fully predict areas of ongoing asymptomatic transmission, particularly during the low season. It is possible that this is because areas that are at higher risk of infection may also have higher levels of population immunity.

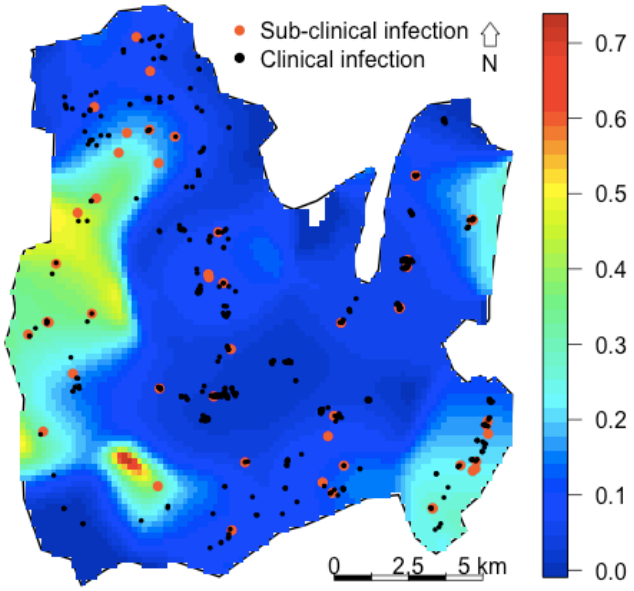
4.7 Tables and Figures

Figure 4.1:*P. falciparum* case intensity from October 2009-2012 for a) active and b) passive surveillance and c) the spatial odds for sub-clinical to clinical cases

a) *P. falciparum* sub-clinical infections* b) *P. falciparum* clinical infections**



c) Spatial odds for sub-clinical to clinical infections***



*Intensity is the intensity of sub-clinical infections in the active survey per square km. The population intensity is about 6 times the intensity of those positive in the survey on average at a given time.
**The clinical cases reflect the cumulative cases over a three-year period in the passive survey
***The value of the odds ratio thus is a comparison of these cases and not reflective of population values in general. However, the relative intensities should be representative.

Figure 4.2: The number of clinical and sub-clinical cases detected by month through the active and passive surveillance systems and the proportion testing positive in the active surveillance system

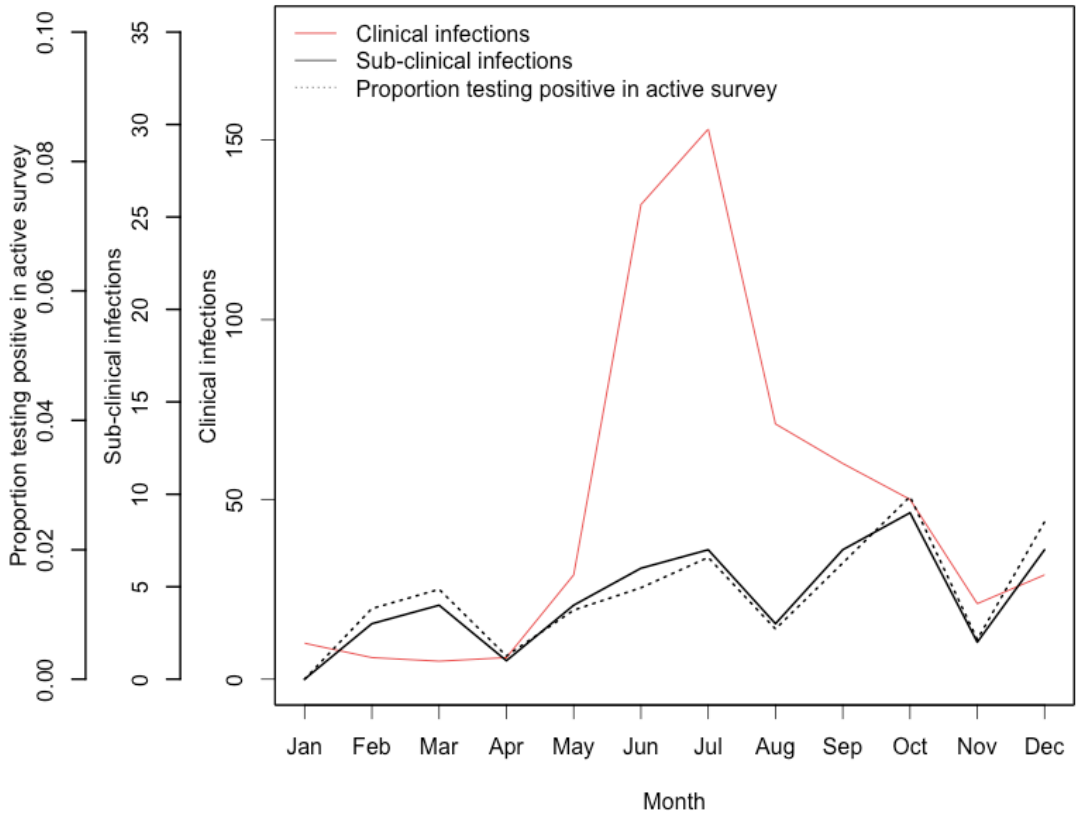
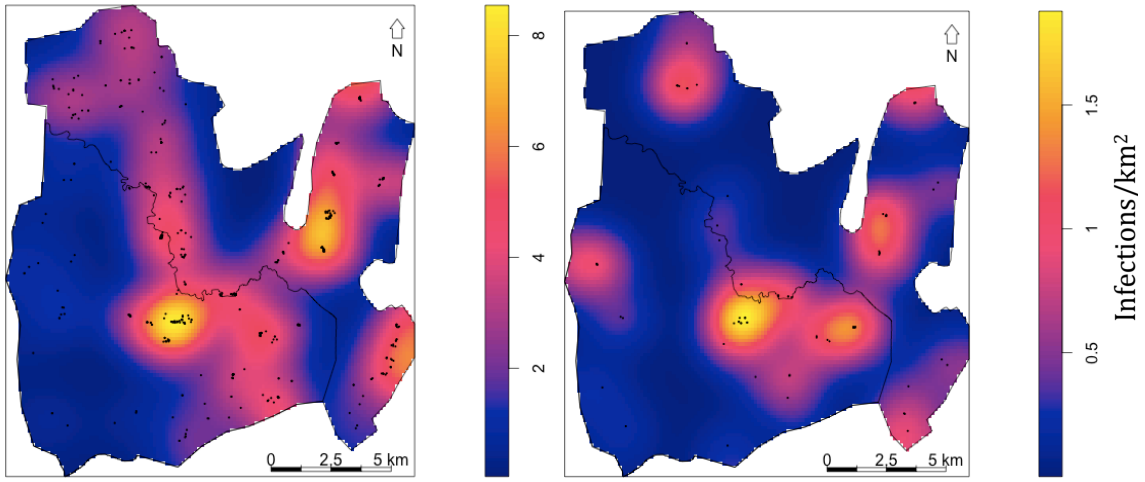
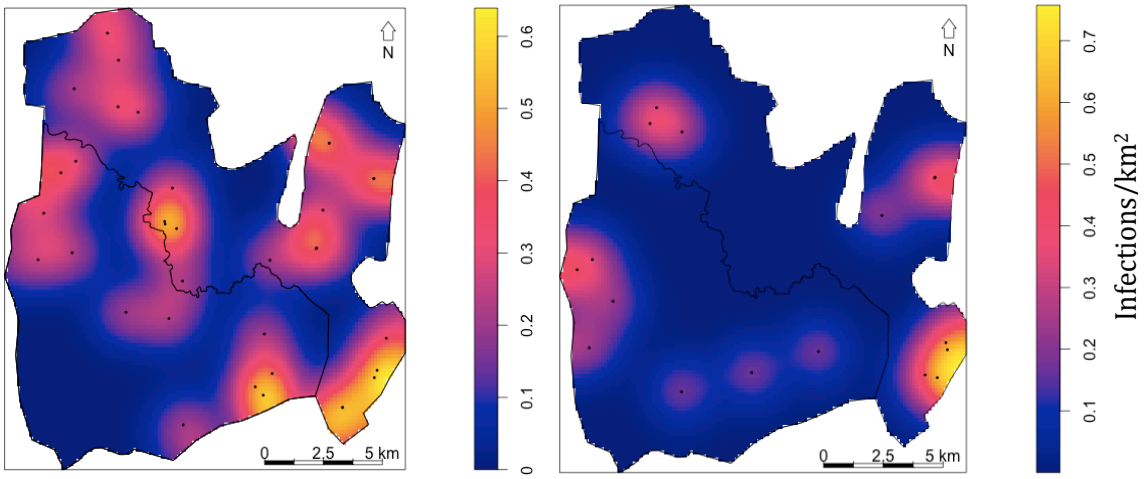


Figure 4.3: Case intensity for clinical and sub-clinical *P. falciparum* infections by season

a) High season clinical infections* b) Low season clinical infections*



a) High season sub-clinical infections** b) Low season sub-clinical infections**

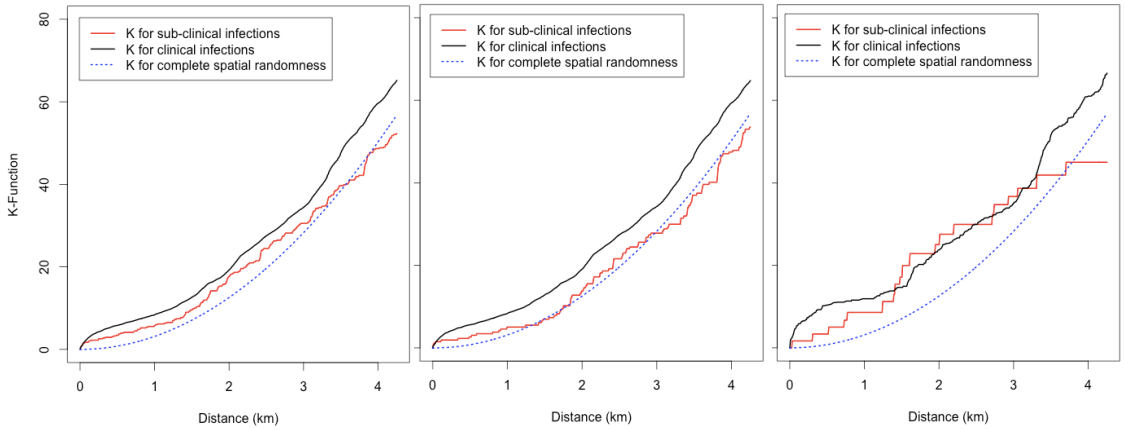


*Intensity of the clinical cases is the cumulative intensity of cases over this three-year period of the study.
**Intensity is the intensity of sub-clinical cases in the survey per square km. The population intensity is about 6 times the intensity in these figures at any given time.

Figure 4.4: Clustering of sub-clinical and clinical infections by K-functions (a-c) and difference in K-functions (d-e)

K-functions:

(a) Year-round (b) High season (c) Low season



Difference in K-functions:

(a) Year-round (b) High season (c) Low season

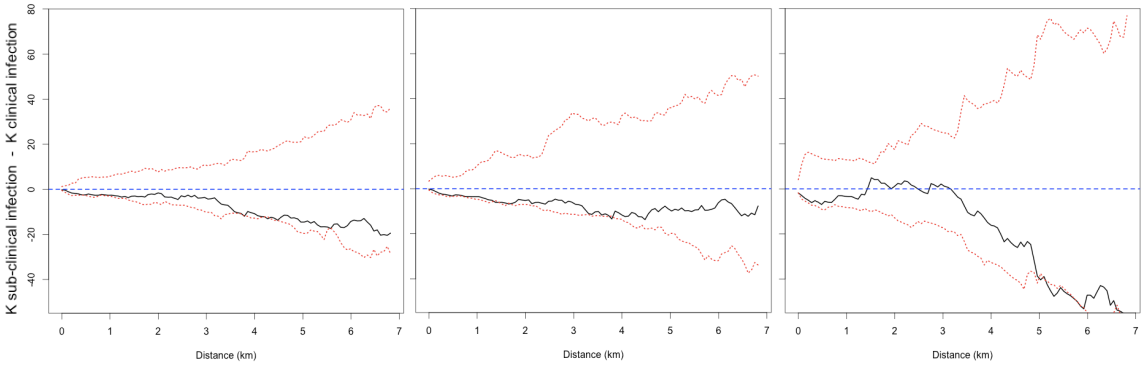


Figure 4.5: Cross-K function showing the average number of sub-clinical infections in the population expected within varying distances from a clinical case

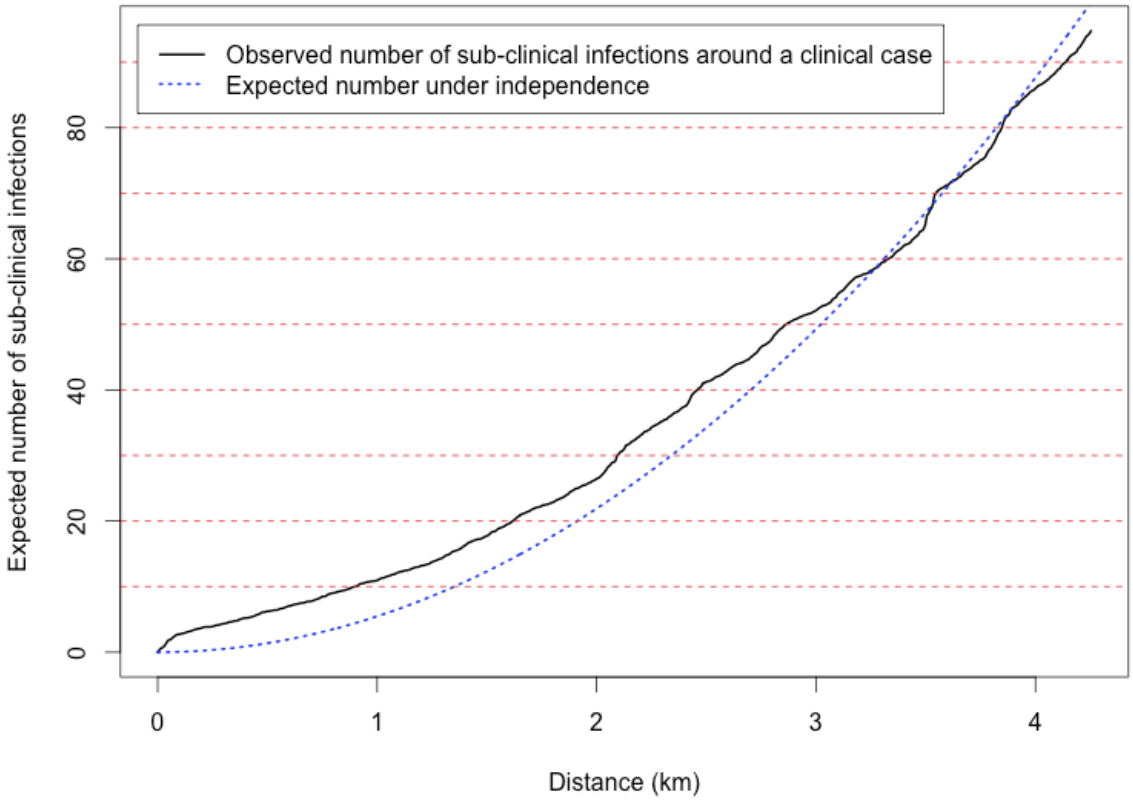


Table 4.1: Demographic features and relationship to clinical vs. sub-clinical *P. falciparum* infection

Household factors	N (%) clinical infections	N (%) sub-clinical infections	OR of clinical compared to Sub-clinical (95% CI)	p-value
Union (N=625)				
Rajbila	329 (57.5)	34 (64.2)	1.0	-
Kuhalong	243 (45.2)	19 (35.8)	1.32 (0.73-2.38)	0.350
Sex (N=573)				
Males	283 (54.4)	23 (43.4)	1.0	
Females	237 (45.6)	30 (56.6)	0.64 (0.36-1.14)	0.128
Age (N=573)				
0-59 months	65 (12.5)	5 (9.4)	1.0	-
5-14 years	183 (35.2)	19 (35.8)	0.74 (0.27-2.07)	0.566
15-39 years	187 (36.0)	26 (49.1)	0.55 (0.20-1.5)	0.245
≥40 years	85 (16.3)	3 (5.7)	2.18 (0.50-9.48)	0.298
Ethnicity (N=573)				
Bengali	48 (9.2)	4 (7.5)	1.0	-
Total tribal	472 (90.8)	49 (92.5)	0.80 (0.28-2.33)	0.685
Marma	335 (64.4)	23 (43.4)	1.21 (0.40-3.67)	0.731
Tanchangya	56 (10.8)	15 (28.3)	0.31 (0.10-1.00)	**0.050***
Khyang	15 (2.8)	4 (7.5)	0.19 (0.04-0.89)	**0.035***
Chakma	22 (4.2)	3 (5.7)	0.61 (0.13-2.98)	0.541
Tripura	26 (5.0)	4 (7.5)	0.54 (0.12-2.35)	0.412
Other tribal	24 (4.6)	0 (0)	$3.54x10^6$ (0-Inf)	0.985
Education level (age ≥ 15 years, n=301)				
0-2 years	169 (62.1)	21 (72.4)	1.0	-
3-5 years	53 (19.5)	4 (13.8)	1.65 (0.53 -5.03)	0.380
≥6 years	50 (18.4)	4 (13.8)	1.55 (0.51-4.76)	0.439
Occupation (age ≥ 15 years, n=301)				
Agricultural	92 (33.8)	8 (27.6)	1.0	-
Day labor	59 (21.7)	3 (10.3)	1.71 (0.43-6.75)	0.442
Jhum cultivation	47 (17.3)	5 (17.2)	0.82 (0.25-2.65)	0.734
Unemployed	14 (5.1)	3 (10.3)	0.41 (0.10-1.72)	0.220
Housewife	17 (6.2)	8 (27.6)	0.18 (0.06-0.56)	**0.003***
Student	23 (8.5)	0 (0)	$1.0x10^7$ (0-Inf)	0.991
Other	20 (7.5)	2 (6.9)	0.87 (0.17-4.44)	0.866

Table 4.2: Association of household risk factors with clinical vs. sub-clinical *P. falciparum* infections

Household factors	N (%) clinical infection	N (%) sub-clinical infection	OR (95% CI)	p-value
Slept under bednet night before, n=555				
Yes	439 (85.7)	38 (88.4)	1.0	-
No	73 (14.3)	5 (11.6))	1.26 (0.48-3.32)	0.634
Own Animals (n=573)				
Yes	429 (82.5	40 (75.5)	1.0	-
No	91 (17.5)	13 (24.5)	0.65 (0.34-1.27)	0.21
Distance from house to pond (meters), n=572				
0-50	48 (9.2)	2 (3.8)	1.0	-
51-100	14 (2.7)	5 (9.6)	0.12 (0.02-0.67)	**0.016***
>100	19 (3.7)	5 (9.6)	0.16 (0.03-0.89)	**0.036***
No pond	439 (84.4)	40 (76.9)	0.46 (0.11-1.96)	0.291
Distance from house to forest (meters), n=573				
0-25	245 (47.1)	26 (49.1)	1.0	-
26-50	152 (29.2)	21 (39.6)	0.77 (0.42-1.41)	0.397
>50	123 (23.7)	6 (11.3)	2.18 (0.87-5.44)	0.095
Distance from river or stream all sizes (meters), n=618				
0-50	72 (12.7)	4 (7.7)	1.0	1
51-100	64 (11.3)	12 (23.1)	0.30 (0.09-0.97)	**0.043***
100-250	200 (35.3)	15 (28.8)	0.74 (0.24-2.31)	0.604
250+	230 (40.6)	21 (40.4)	0.61 (0.20-1.83)	0.377
Altitude (meters above sea level), n=618				
10-25	18 (3.2)	1 (1.9)	1.0	-
25-50	163 (28.8)	22 (42.3)	0.41 (0.05-3.25)	0.399
50-75	169 (29.9)	15 (28.8)	0.62 (0.08-5.04)	0.659
75-125	188 (33.2)	9 (17.3)	1.16 (0.14-9.73)	0.891
125-147	28 (4.9)	5 (9.6)	0.31 (0.03-2.90)	0.304

Table 4.3: Regression model of clinical vs. sub-clinical *P. falciparum* infection with household and demographic factors

		Unadjusted (N=547)		**Adjusted (N=547)**	
Demographic and household factors		OR (95% CI)	p-value	OR (95%CI)	p-value
Age	<5 years	1.0	-	1.0	-
	5-14 years	0.82 (0.29-2.29)	0.699	0.83 (0.28-2.42)	0.725
	15-39 years	1.10-0.38-3.18)	0.866	0.97 (0.32-2.93)	0.950
	40+ years	2.34 (0.54-10.23)	0.256	2.54 (0.56-11.59)	0.227
Ethnicity	Bengali	1.0	-	1.0	-
	Marma	1.39 (0.46-4.25)	0.560	1.65 (0.50-5.53)	0.412
	Tanchangya	0.60 (0.17-2.13)	0.428	0.38 (0.10-1.51)	0.170
	Khyang	0.24 (0.04-1.27)	0.092	0.28 (0.04-1.74)	0.171
	Chakma	1.87 (0.20-17.8)	0.587	2.34 (0.23-24.12)	0.474
	Tripura	0.58 (0.13-2.52)	0.464	0.47 (0.10-2.22)	0.338
	Other tribal	$3.78x10^6$ (0-Inf)	0.985	$2.78x10^6$ (0-Inf)	0.985
Distance from house to forest	Per increase in Z-score	1.59 (0.91-2.80)	0.105	1.64 (0.87-3.08)	0.124
Distance from house to closest stream	Per increase in Z-score	1.07 (0.77-1.49)	0.686	1.49 (1.00-2.23)	**0.050***
Log parasite count	Per increase in Z-score	1.62 (1.2-2.18)	**0.001***	1.79 (1.23-2.46)	**<0.001***

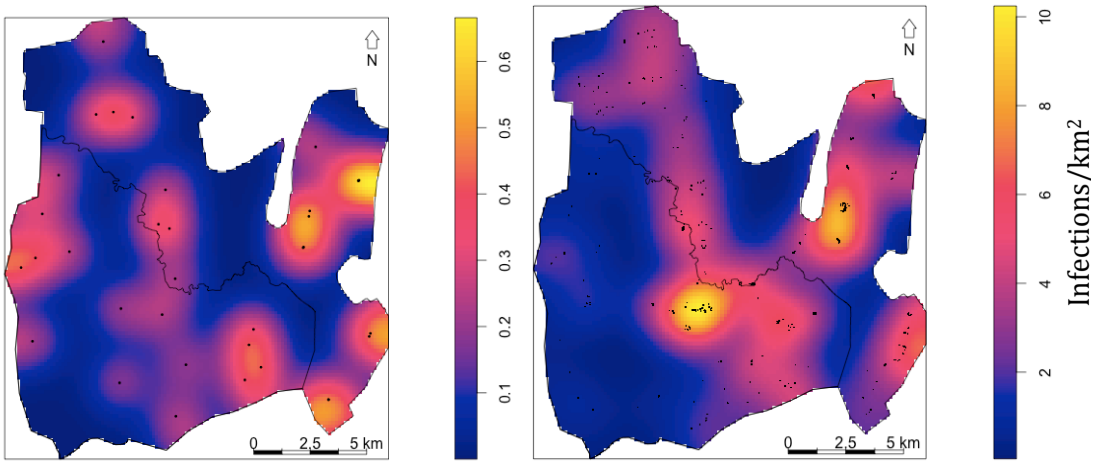
4.8 Chapter Four Appendix

The figures below show the sensitivity analysis including the randomly sampled sub-clinical infections, excluding those selected due to pregnancy.

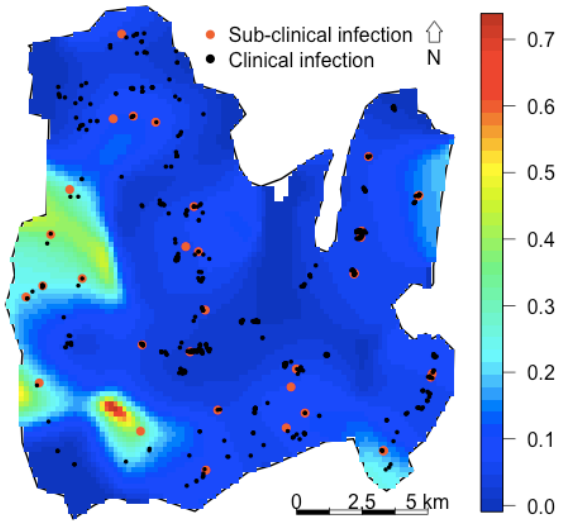
Figure 4.1b: *P. falciparum* case intensity from October 2009-2012 for a) active and b) passive surveillance and c) the spatial odds for sub-clinical infections to clinical cases

a) *P. falciparum* sub-clinical infections*

b) *P. falciparum* clinical infections*



c) Spatial odds for sub-clinical infections to clinical cases***



*Intensity of the sub-clinical cases is the intensity of cases in the active survey per square km. The population intensity is about 6 times the intensity of those positive in the survey on average at a given time.
**The clinical cases reflect the cumulative cases over a three-year period in the passive survey
***The value of the odds ratio thus is a comparison of these cases and not reflective of population values in general. However, the relative intensities should be representative.

Figure 4.2b: The number of clinical and sub-clinical cases detected by month through the active and passive surveillance systems and the proportion testing positive in the active surveillance system

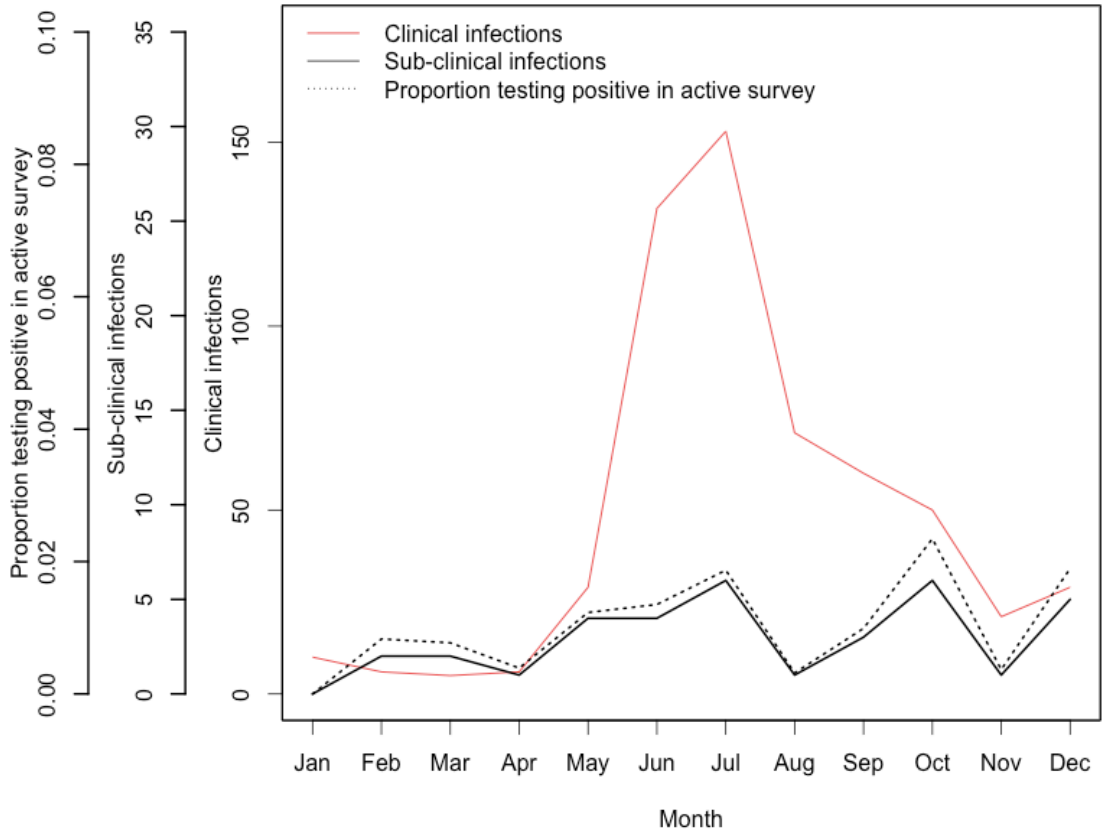
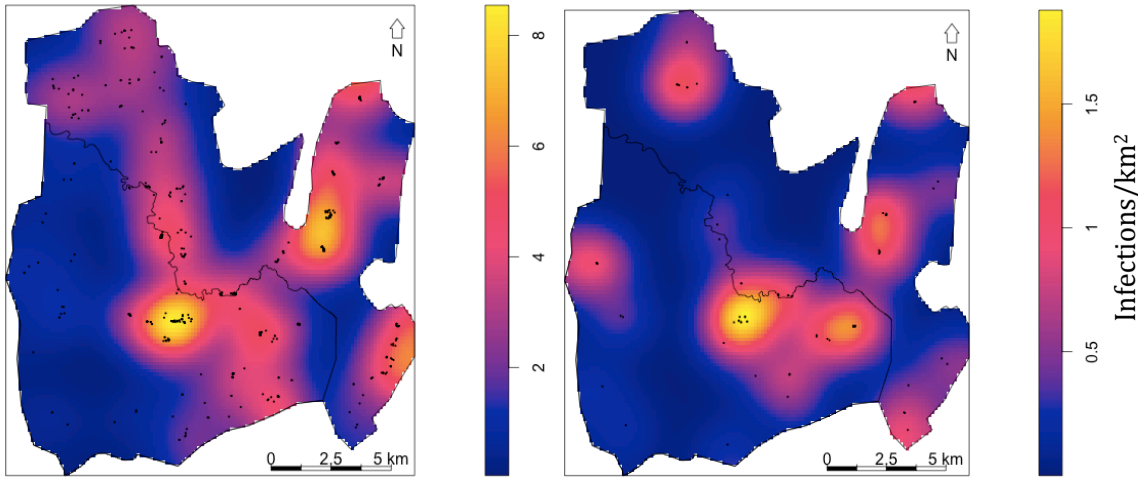
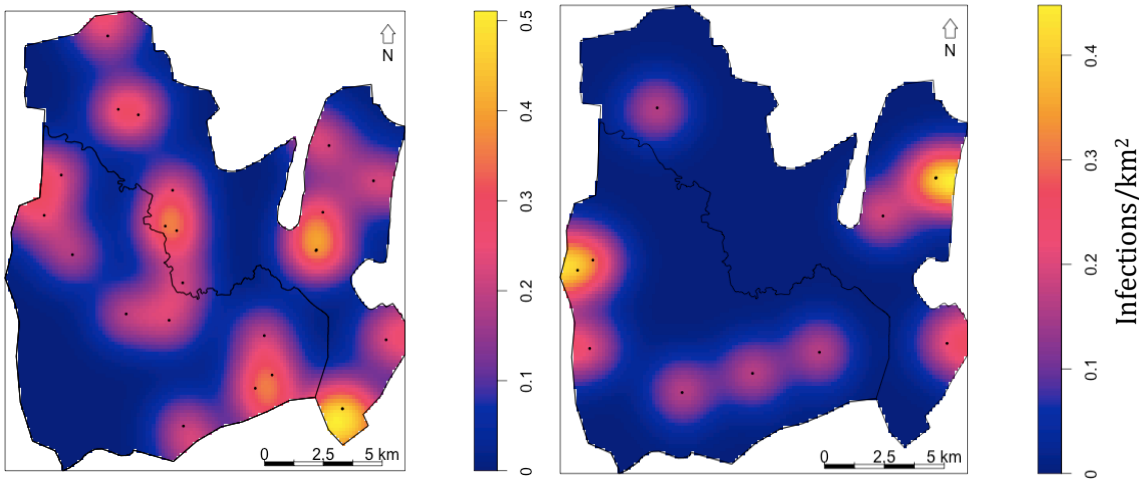


Figure 4.3b: Case intensity for clinical and sub-clinical *P. falciparum* infections by season

a) High season clinical infections* b) Low season clinical infections*



a) High season sub-clinical random infections* b) Low season sub-clinical Random random Infections**

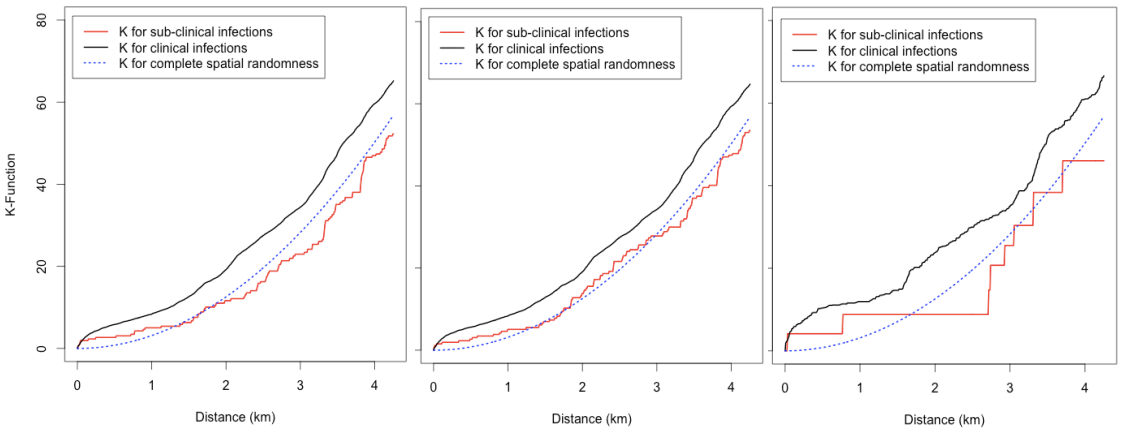


*Intensity of the clinical infections is the cumulative intensity of cases over this three-year period of the study.
**Intensity of the sub-clinical infections is the intensity of positive cases in the active survey per square km. The population intensity is about 7 times the intensity in these figures at any given time.

Figure 4.4b: Clustering of sub-clinical and clinical infections by K-functions (a-c) and difference in K-functions (d-e)

K-functions:

(a) Year-round (b) High season (c) Low season



Difference in K-functions:

(a) Year-round (b) High season (c) Low season

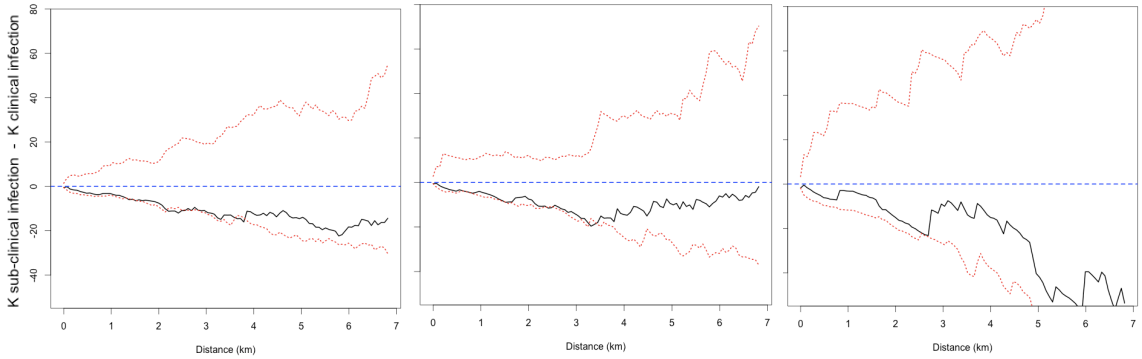


Figure 4.5b: Cross-K function showing the average number of sub-clinical infections in the population expected within varying distances from a clinical case

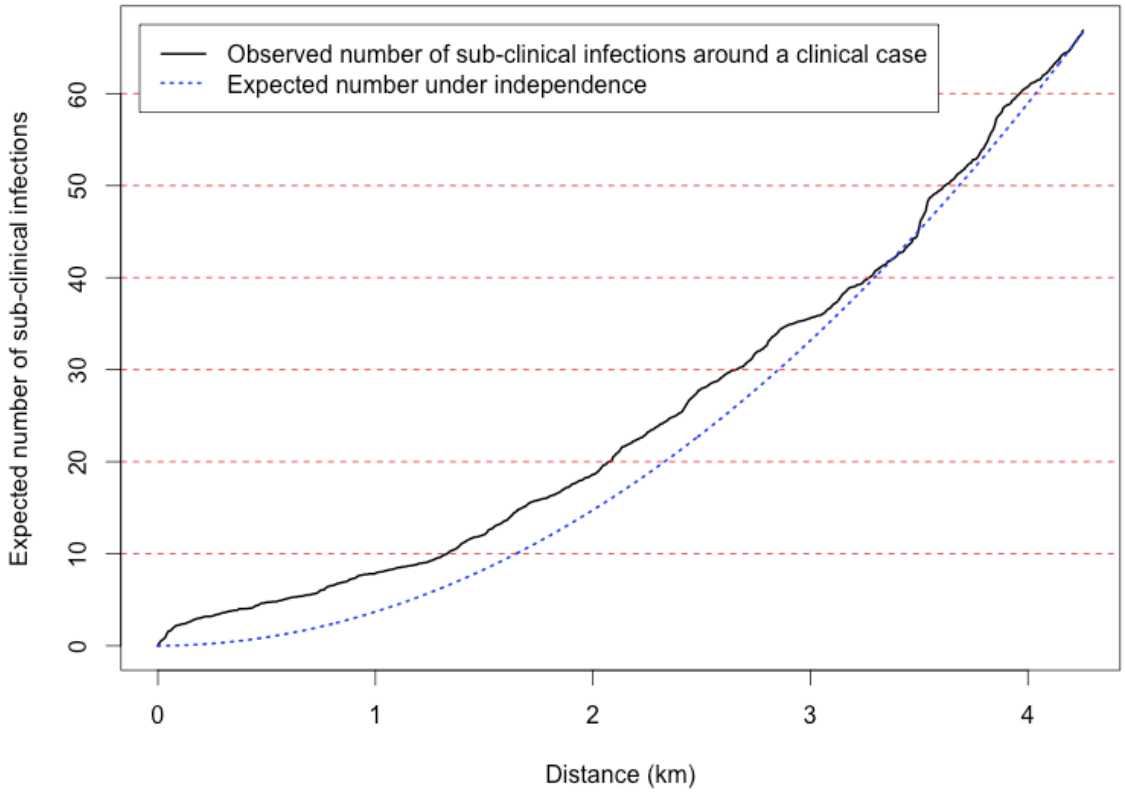


Table 4.1b: Demographic features and relationship to Clinical vs. Sub-clinical *P. falciparum* Infection

Household factors	N (%) clinical infections	N (%) sub-clinical infections	OR of clinical compared to Sub-clinical (95% CI)	p-value
Union (N=607)				
Rajbila	329 (57.5)	20 (57.1)	1.0	-
Kuhalong	243 (45.2)	15 (42.9)	0.98(0.49-1.97)	0.965
Sex (N=555)				
Males	283 (54.4)	23 (65.7)	1.0	-
Females	237 (45.6)	12 (34.3)	1.6 (0.78-3.30)	0.197
Age (N=555)				
0-59 months	65 (12.5)	5 (14.3)	1.0	-
5-14 years	183 (35.2)	18 (51.4)	0.78 (0.29-2.20)	0.640
15-39 years	187 (36.0)	9 (25.7)	1.60 (0.52-4.96)	0.416
≥40 years	85 (16.3)	3 (8.6)	2.18 (0.50-9.48)	0.298
Ethnicity (N=555)				
Bengali	48 (9.2)	3 (8.6)	1.0	-
Total tribal	472 (90.8)	32 (91.4)	0.92 (0.27-3.13)	0.896
Marma	335 (64.4)	17 (48.6)	1.23 (0.35-4.37)	0.747
Tanchangya	56 (10.8)	8 (22.9)	0.44 (0.11-1.75)	0.241
Khyang	15 (2.8)	3 (8.6)	0.19 (0.03-1.08)	0.061
Chakma	22 (4.2)	1 (2.9)	1.38 (0.13-14.05)	0.788
Tripura	26 (5.0)	3 (8.6)	0.54 (0.10-2.89)	0.472
Other tribal	24 (4.6)	0 (0)	$2.66x10^{6}$ (0-Inf)	0.985
Education level (age ≥ 15 years, n=284)				
0-2 years	169 (62.1)	8 (66.7)	1.0	-
3-5 years	53 (19.5)	4 (33.3)	0.63 (0.18-2.18)	0.461
≥6 years	50 (18.4)	0 (0)	$1.49x10^{6}$(0-Inf)	0.991
Occupation (age ≥ 15 years, n=284)				
Agricultural	92 (33.8)	4 (33.3)	1.0	-
Day labor	59 (21.7)	3 (25.0)	0.86 (0.18-3.98)	0.841
Jhum cultivation	47 (17.3)	4 (33.3)	0.51 (0.12-2.15)	0.357
Unemployed	14 (5.1)	0 (0)	$1.37x10^{7}$ (0-Inf)	0.995
Housewife	17 (6.2)	0 (0)	$1.37x10^{7}$ (0-Inf)	0.995
Student	23 (8.5)	0 (0)	$1.37x10^{7}$ (0-Inf)	0.994
Other	20 (7.5)	1 (8.3)	0.87 (0.09-8.28)	0.903

Table 4.2b: Association of household risk factors with clinical vs. sub-clinical *P. falciparum* infections

Household factors	N (%) clinical infection	N (%) sub-clinical infection	OR (95% CI)	p-value
Slept under bednet night before, n=547				
Yes	439 (85.7)	31 (88.6)	1.0	-
No	73 (14.3)	4 (11.4)	1.28 (0.44-3.77)	0.642
Own Animals (n=573)				
Yes	429 (82.5	28 (80.0)	1.0	-
No	91 (17.5)	7 (20.0)	0.85 (0.36-2.01)	0.708
Distance from house to pond (meters), n=555				
0-50	48 (9.2)	2 (5.7)	1.0	-
51-100	14 (2.7)	2 (5.7)	0.29 (0.04-2.27)	0.238
>100	19 (3.7)	2 (5.7)	0.40 (0.05-3.03)	0.371
No pond	439 (84.4)	29 (82.9)	0.63 (0.15-2.73)	0.537
Distance from house to forest (meters), n=555				
0-25	245 (47.1)	16 (45.7)	1.0	-
26-50	152 (29.2)	17 (48.6)	0.58 (0.29-1.19)	0.139
>50	123 (23.7)	2 (5.7)	4.0 (0.91-17.80)	0.067
Distance from river or stream all sizes (meters), n=555				
0-50	72 (12.7)	3 (8.6)	1.0	-
51-100	64 (11.3)	7 (20.0)	0.38 (0.09-1.54)	0.175
100-250	200 (35.3)	11 (31.4)	0.76 (0.21-2.80)	0.677
250+	230 (40.6)	14 (40.0)	0.68 (0.19-2.45)	0.560
Altitude (meters above sea level), n=618				
10-25	18 (3.2)	1 (2.9)	1.0	-
25-50	163 (28.8)	14 (40.0)	0.65 (0.08-5.23)	0.682
50-75	169 (29.9)	9 (25.7)	1.04 (0.12-8.79)	0.969
75-125	188 (33.2)	8 (22.9)	1.31 (0.15-11.08)	0.807
125-147	28 (4.9)	3 (8.6)	0.52 (0.05-5.40)	0.582

Table 4.3b: Regression model of clinical vs. sub-clinical *P. falciparum* infection with household and demographic factors

		Unadjusted (N=541)		Adjusted (N=541)	
Demographic and household factors		OR (95% CI)	p-value	OR (95%CI)	p-value
Sex	Male	1.0	-	1.0	-
	Female	1.62 (0.79-3.34)	0.187	2.01 (0.91-4.44)	0.082
Age	<5 years	1.0	-	1.0	-
	5-14 years	0.86 (0.31-2.43)	0.777	0.86 (0.28-2.65)	0.797
	15-39 years	1.70 (0.55-5.30)	0.356	1.54 (0.46-5.18)	0.486
	40+ years	2.34 (0.54-10.23)	0.256	2.75 (0.58-12.96)	0.200
Ethnicity	Bengali	1.0	-	1.0	-
	Marma	1.29 (0.36-4.59)	0.693	1.58 (0.38-6.62)	0.527
	Tanchangya	0.45 (0.11-1.80)	0.258	0.26 (0.05-1.25)	0.091
	Khyang	0.18 (0.03-1.04)	0.056	0.24 (0.03-1.83)	0.167
	Chakma	1.40 (0.14-14.35)	0.776	1.62 (0.14-18.87)	0.700
	Tripura	0.58 (0.11-3.09)	0.520	0.34 (0.05-2.12)	0.245
	Other tribal	$2.83x10^6$ (0-Inf)	0.986	$1.98x10^6$ (0-Inf)	0.985
Distance from house to forest	Per increase in Z-score	1.77 (0.93-3.36)	0.083	1.79 (0.86-3.70)	0.117
Distance from house to closest stream	Per increase in Z-score	1.05 (0.74-1.49)	0.780	1.60 (1.03-2.51)	**0.038***
Log parasite count	Per increase in Z-score	1.93 (1.41-2.66)	**<0.001***	2.10 (1.49-2.95)	**<0.001***

Chapter Five: Conclusions and Recommendations

5.1. Summary of Major Findings

5.1.1. Paper I: Hemoglobin E and Glucsoe-6-Phosphate Dehydrogenase Deficiency and Plasmodium falciparum malaria

Chapter two of this dissertation outlines the findings of our analysis on G6PD, Hemoglobin E and malaria. We found high levels of G6PD deficiency, HbE, and anemia among this population in Bandarban. Uncomplicated malaria, treated in the community, was more common among patients with homozygous HbE compared with controls, while no difference was found in the prevalence of malaria in patients with HbAE compared with normal patients. Focal populations of certain ethnic groups with remnant high prevalence of HbEE may provide an increased risk of uncomplicated malaria secondary to either longer duration of infection or increased risk of infection, and thus malaria control in these populations is necessary for elimination programs to be successful.

5.1.2. Paper II: Sub-clinical P. falciparum infections act as year-round reservoir for malaria in the Chittagong Hill Districts of Bangladesh

The Hill Districts region of Bangladesh has hypoendemic malaria in which sub-clinical infections make up the majority of infections and likely act as a reservoir for continued infections. Unlike those symptomatic cases leading people to seek treatment, which have a strong seasonal pattern peaking in the rainy season, these sub-clinical infections remain year-round. Any attempts at elimination of malaria in this region will thus require more than simply finding and treating symptomatic patients, as people with sub-clinical infections will continue to act as carriers for continued transmission.

5.1.3. Paper III: Temporal and spatial differences between sub-clinical and clinical P. falciparum malaria infections in the Chittagong Hill Districts, Bangladesh

The majority of *P. falciparum* infections in the area are sub-clinical and appear year-round. Although the clinical and sub-clinical hotspots do overlap, clinical symptomatic hotspots may not fully predict areas of ongoing asymptomatic transmission, particularly during the low season. This result may relate to recent evidence suggesting that areas at higher risk of infection may also have higher levels of population immunity.[153]

5.2. Study limitations

Specific limitations for each study were discussed in chapters two through four. However, several aspects of the cohort study more broadly are discussed here.

This study is a large cohort study with detailed and relatively frequent surveys of the population over several years. Some of the research is based on only a few years of collection and will be strengthened by more years of follow-up. In particular, when subsets of the population are explored, as was the case for our hemoglobin E study, increased sample sizes would help to further delineate relationships.

Although this is one small area of the Hill Districts, the area selected was similar environmentally and in the ethnic makeup to many areas of the Hill Districts region. It is thus expected that the results from this study should be applicable to this region as a whole. However, the Hill Districts are very different both environmentally and in ethnic makeup from the main part of Bangladesh, and thus these findings are not applicable to all areas of the country.

5.3. Recommendations for future research

This study points to a number of population groups that have higher risk for symptomatic and asymptomatic malaria. As with any study, it would be useful to determine if these risk factors are similar in other areas of Bangladesh and South and Southeast Asia.

It would also be useful to better understand local vector behavior and environmental preferences. As it was established that there are a large variety of mosquitoes that carry malaria, understanding their biting habits and preferences could be very important to planning which interventions are most likely to be successful.

More research is needed on the relationship of homozygous hemoglobin E and malaria. Further studies are needed to delineate if homozygous hemoglobin E populations are more likely to be infected, or more likely to carry the infection for a longer time. As other studies have noted the reduced likelihood of severe infection, it would not be surprising if infections were less symptomatic and thus took longer to present for clinical care. But the relationship of hemoglobin E to malaria at all levels of severity from submicroscopic, to mildly symptomatic and severe infection needs to be further clarified. This study did not have the power to examine the relationship of sub-clinical infections and hemoglobin E, something that would help to parse out these distinctions.

Further research on the relationship of clinical and sub-clinical infections over space and time could be very useful for focusing interventions. As the sampling structure of our active study only had one person selected from each area each week, it would be possible to have missed some sub-clinical infection hotspots. A study that specifically sampled the population at certain distances from a symptomatic case, soon after that case was detected, may be able to better address how much increased risk there may be for asymptomatic infection in areas near a

symptomatic case. This type of study would be especially important if one were to identify hot spot areas for targeting a mass drug treatment intervention.

Furthermore, it is important to sample for hotspots during the low season, when very few symptomatic cases are occurring, as these areas are of particular importance to stopping transmission.

5.4 Conclusions and Policy Implications

Malaria control in Bangladesh in the past decade has led to reductions in disease to hypoendemic levels in select areas, and the elimination of disease in others. As such, it presents a case study of a country that could possibly eliminate malaria. The hypoendemic areas of Bangladesh provide an ideal location to study transmission and develop focused interventions for control and elimination.

Because of successful malaria control in the past decade, compared to other national health priorities, malaria currently accounts for reasonably low levels of morbidity and mortality. This has the obvious implication of possible slackening in the national malaria program in favor of other priorities. However, as seen with numerous other countries, backing off on malaria control can easily lead to a resurgence of disease. This resurgence can often be worse than prior to the interventions, due to reductions in population immunity. Given this scenario, it is important to continue to focus on the endemic districts in Bangladesh to control disease.

With respect to elimination, Bangladesh faces several relatively large challenges. One limitation is its proximity to Myanmar (Burma), which until recently has put very little focus to controlling malaria or the health infrastructure in general, although recent political changes will hopefully lead to greater focus on public health and malaria control.[168] There has been some recent investment in malaria programs for Myanmar from the Three Millennium Development

Goal fund and the Global Fund's Regional Artemisinin Initiative.[169] If Myanmar is unable to sufficiently address malaria control, it will be altogether too easy for the disease to continue to reestablish itself in areas of Bangladesh near the border, even if areas in Bangladesh become (temporarily) malaria free.

Despite this challenge, control of malaria is becoming increasingly important in this region of the world. Although there was no increased time to clearance of parasites following treatment documented in this study and there is no current documentation of delayed clearance in Bangladesh, with the documentation of increased clearance of infection with artemisinins in several other Southeast Asian countries,[138-141] it will likely not be long until this problem reaches Bangladesh. As Bangladesh supplies numerous relief workers through the UN system, and the world population in general is increasingly transient, the possibility of resistance in this region is a global threat.

As documented by this cohort study, the vector populations in this region are quite diverse. As such, vector control is more complicated. Control strategies that work for one vector species, may not work for others. Further understanding of these different species and their ecological niches will be imperative to malaria control.

Although it is the common understanding that as the burden of malaria decreases in a country and the region moves from hyperendemic to endemic and then to hypoendemic, the proportion of infections that are symptomatic increases, this study documents that there remains a very large burden of infection in those who have sub-clinical infections.

There are particular populations that were documented in this study as higher risk for malaria, both asymptomatic and symptomatic. The active study in this area was able to identify several high-risk groups of import for sub-clinical infections. These include pregnant woman,

jhum cultivators, males, those living closer to forests and at higher elevations and marginally 5-14 year olds and day laborers. We also found substantial spatial variation in risk of infection.

For their own health and wellness, these populations thus need increased attention for malaria control programs, to reduce morbidity and mortality. Furthermore, those populations that may be more likely to have sub-clinical infections in particular, are more likely to act as carriers, particularly in the low season where they can maintain the transmission chain through to the next high season. Thus interventions that can focus on hotspots of low-season sub-clinical transmission may be very important in elimination of the disease.

There are already several programs focused on high-risk populations. Intermittent preventive treatment for pregnant woman helps to address their higher risk of infection and help to reduce the complications of malaria in pregnancy. All women in the Chittagong Hill Districts should be screened for malaria during prenatal visits.

The jhum cultivators have been documented as at a higher risk of asymptomatic malaria in this study, and a prior study showed that they and their families are more likely to have asymptomatic infections. [120] Programs to ensure that this population has access to bednets in their temporary shelters during the jhum cultivation season would be a good first step. Also encouraging mosquito repellant and other protective covering while farmers work outside and travel through wooded areas may be considered.

Similarly, further research into the day labor population and which subsets of day laborers may be at higher risk is needed. A number of day laborers work in the wooded rubber plantations, and it is possible that this is a higher-risk population. Similar programs for prevention could be focused on this population if they are shown to be of higher risk as anticipated.

Similarly, the school-aged children, particularly males, are at high risk, so taking advantage of the school system as a place to provide education on malaria control may be an effective strategy.

Certain ethnic groups such as the Tripura and Tanchangya were found to be at higher risk of infection. These differences appeared to be mainly explained by the locations where most of these populations lived, which were often at higher elevation and close to forests. Focused attention on control programs in these areas, may also help to reduce transmission and decrease morbidity.

The fourth chapter in this thesis focused on the spatial clustering of symptomatic and sub-clinical infections. As there were several areas of higher risk of asymptomatic infection that did not have high numbers of symptomatic cases, it will not be enough to focus our policies and programs on areas simply based off of those areas with higher numbers presenting to hospitals and clinics for treatment as we will miss hotspots for continuing transmission. It will thus be necessary to do research to identify these hotspots in areas trying to eliminate malaria.

In conclusion, this study emphasizes the need for continued emphasis on malaria control, particularly focused on active detection due to high proportions of sub-clinical infections. Further understanding of the unique risk factors and variety of vectors in this area will help to guide control and eventually elimination programs.

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November, 2015

CURRICULUM VITAE

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EDUCATION AND TRAINING

Degree	Year	Institution	Field
Dr.P.H.	2011-Present	Johns Hopkins Bloomberg School of Public Health	International Health
M.D.	2008-Present	Johns Hopkins School of Medicine	Medicine
M.P.H.	2006-2007	Johns Hopkins Bloomberg School of Public Health	Health & Human Rights/ Humanitarian Assistance
B.A.	2000-2004	Kenyon College	Chemistry & International Studies

PROFESSIONAL EXPERIENCE

Jun, 2012 - Present **DrPH Candidate, Student Investigator** - Johns Hopkins School of Public Health, Department of International Health, Baltimore, MD & Bangladesh:

- Doctoral student investigator in "mapping malaria epidemiology in Bangladesh" funded by the Johns Hopkins Malaria Institute, the World Bank, DFID and others.
- Thesis research focused on the epidemiology and control of asymptomatic malaria as well as demographic and environmental risk factors for malaria in the Hill Districts of Bangladesh.

Sep, 2013 - Present **Graduate Research Analyst** - Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health:

- Conducted research related to cholera outbreaks, with particular focus on refugee camp settings and modeling environmental/climatic influences on disease

Jun – Aug, 2009 & Oct, 2013 - Jan, 2015 **Graduate Research Assistant** - Johns Hopkins Center for a Livable Future:

- Conducted research and literature reviews related to water quality, food systems, public health and human rights

Jan, 2012 - Dec, 2013 **Graduate Research Assistant -** Department of Anesthesiology, Johns Hopkins School of Medicine:

- Conducted qualitative research exploring patients and clinicians lived experiences regarding palliative care in intensive care units

Aug, 2004 - Oct, 2005 **Fulbright Student Researcher -** Mahasarakham University Department of Environmental Science, Mahasarakham, Thailand:

- Researched community involvement in water resource management in northeastern Thailand

Jun - Aug, 2001-2003 **Student Researcher** - Princeton University Chemistry Department, Princeton, NJ:

- Researched the effects of intermolecular multiple quantum coherences on NMR microscopy imaging.
- Advised two incoming graduate students in their research

PROFESSIONAL ACTIVITIES

May - Jun, 2012 **Consultant -** Project Concern International, Kuhlna, Bangladesh:

- Assisted with redeveloping CRA methodology

Nov, 2007 - Jul, 2008 **Rotary Ambassadorial Scholar** - Mae Tao Clinic, Mae Sot, Thailand:

- Responsible for writing grant proposals and reports
- Conducted research related to barriers to accessing healthcare among the population of Burmese people seeking care at the clinic

Sep, 2006 - May, 2007 **Service-Learning Facilitator -** Students Sharing Coalition, Baltimore, MD:

- Designed lessons and organized service-learning trips for Baltimore teenagers

Feb - Jul, 2006 **Residential Counselor** - Carrier Clinic, Belle Mead, NJ:

- Provided a therapeutic milieu and implemented treatment plans for at-risk youth in a residential setting

HONORS AND AWARDS

Global Health Established Field Placement Award Recipient: Scholarship supporting work on a malaria mapping epidemiology study in Bangladesh (2012)
Medical Scientist Training Program Grant Recipient: NIH grant supporting medical and doctoral training. (2008-Present)
Delta Omega: Public health honor society, recognizing scholarship and achievement. (2007)
Department of Health and Human Services Public Health Training Grant Recipient: For graduate study, recognizing academic achievement, one of nine students selected. (2007)
M.P.H. Field Experience Fund Award: Awarded for research relating to international public health, recognizing academic excellence. (2007)
Rotary Ambassadorial Scholarship: For research related to health and human rights among the Burmese population seeking healthcare at the Mae Tao Clinic in Mae Sot, Thailand. Recognizing academic achievement and service (2007)
Women's Overseas Service League Scholarship Recipient: Recognizing public service. (2006)
Margaret Yardley Fellowship Recipient: Recognizing scholastic achievement and service. (2006)

Marshall Scholarship Finalist: Recognizing scholastic achievement. One of 22 students selected in the New York region. (2005)
Kenyon College Salutatorian: Graduated second in a class of approximately 400 students. (2004)
Fulbright Scholar: U.S. State Department grant for conducting international research (2004-2005)
Sigma Iota Rho: International studies honor society, recognizing scholarship and service in global studies. (2004)
Sigma Xi: Scientific honor society, recognizing scientific achievement and research. (2004)
Phi Beta Kappa: Academic honor society, recognizing distinction in the liberal arts and sciences. One of 11 students inducted on the basis of three years of study. (Inducted 2003; Club Historian Spring 2004)
Goldwater Scholar: U.S. Congressional scholarship, recognizing achievement in the sciences. (2002-2004)
Princeton Materials Institute REU Fellowship: For summer research in the sciences at Princeton University, the only first year student to be selected. (2001)
Kenyon College Distinguished Academic Scholar: Recognizing outstanding academic achievement, leadership potential and extracurricular accomplishments. (2000-2004)

PUBLICATIONS

Journal Articles – Published and peer reviewed

1. **Shannon KL**, Ahmed S, Rahman H, Prue CS, Khyang J, Ram M, Haq MZ, Akter J, Glass GE, Shields T, Nyunt MM, Khan WA, Sack DA, Sullivan DJ. Hemoglobin E and glucose-6-phosphate dehydrogenase deficiency and *Plasmodium falciparum* malaria in the Chittagong Hill Districts of Bangladesh. *Am J Trop Med Hyg*. 2015; 14-0623.
2. **Shannon KL**, Kim BF, McKenzie SE, Lawrence RS. Food system policy, public health, and human rights in the United States. *Annu Rev Public Health*. 2015; 36: 151-173.
3. Moore SM, **Shannon KL**, Zelaya CE, Azman AS, Lessler J. Epidemic risk from cholera introductions into Mexico. *PLoS Curr.*2014; 6:10.1371.
4. Prue CS, **Shannon KL**, Khyang J, Edwards LJ, Ahmed S, Ram M, Shields T, Hossain MS, Glass GE, Nyunt MM, Sack DA, Sullivan DJ, Khan WA. Mobile phones improve case detection and management of malaria in rural Bangladesh. *Malar J.* 2013; 12(48): 1475-2875.
5. **Shannon KL**, Branca RT, Galiana G, Cenzano S, Bouchard LS, Soboyevo W, and Warren WS. Simultaneous acquisition of multiple orders of intermolecular multiple-quantum coherence images in vivo. *Magn Reson Imaging.* 2004; 22(10): 1407-1412.
6. 7. Tang X, Ong H, **Shannon K**, Warren WS. Simultaneous acquisition of multiple orders of intermolecular multiple-quantum coherence images. *Magn Reson Imaging.* 2003; 21(10): 1141-1149.

Journal Articles and Editorials – Not peer reviewed

7. **Shannon K**. Tsunami: 9 Months from the Devastation. *Thailand-U.S. Educational Foundation (Fulbright) Newsletter*. 2005; 14: 6-7,12.
8. **Shannon K**. A Fear for Hope. *Perspectives*. 2002; 3: 44-45.

Book Chapters

9. **Shannon KL**, Lawrence RS. Anthropogenic sources of water pollution: Part 1. In: Selendy J,

ed. *Water and Sanitation-Related Diseases and the Environment: Challenges, Interventions, and Preventive Measures.* Hoboken, NJ: John Wiley & Sons; 2011: 289-302.

PRESENTATIONS

Scientific Meetings

1. **Shannon KL**, Ahmed S, Khyang J, Prue CS, Akter J, Alam MS, Ram M, Nyunt MM, Glass G, Shields T, Curriero F, Khan WA, Sack DA, Sullivan DJ. Asymptomatic malaria acts as year-round reservoir for transmission in the Chittagong Hill Districts of Bangladesh. Paper presented at: American Society of Tropical Medicine and Hygiene Conference; October 25-29, 2015; Philadelphia, PA.
2. **Shannon KL**, Bompangue D, Azman A, Moore S, Zaitchik B, Lessler J. Climatic influences on endemic cholera in Kalemie, Democratic Republic of the Congo. Paper presented at: American Society of Tropical Medicine and Hygiene Conference; October 25-29, 2015; Philadelphia, PA.
3. Aung W, Moldovan R, **Shannon K**, Peters J, Redstone L, An SJ, Duong J, Koegler E, Nadison M, Pronovost PJ, Aslakson R. Intensive Care Unit Nurse Identified Perceptions of Palliative Care. Paper presented at: American Academy of Hospice and Palliative Medicine Conference; March 13-16, 2013; New Orleans, LA.
4. **Shannon KL**, Aung W, Peters J, Redstone L, An SJ, Duong J, Koegler E, Nadison M, Pronovost PJ, Aslakson R. Perceptions of the terms 'palliative care' and 'palliative medicine' amongst surgical ICU nurses, surgeons, and critical care anesthesiologists. Paper presented at: American Society of Anesthesiologists Annual Meeting; October 13-17, 2012; Washington, DC.
5. Reardon J, Peters J, Aung W, **Shannon K**, Redstone L, An S, Duong J, Koegler E, Nadison M, Pronovost PJ, Aslakson R. Intensivist and Surgeon-Identified Themes Concerning Long-Stay Surgical ICU Patients. Paper presented at: Society of Critical Care Medicine (SCCM) 41st Critical Care Congress; February 4-8, 2012; Houston, TX.
6. **Shannon KL**. The Social and Environmental Impacts of the Hua Na Dam and Khong-Chi-Mun Project: The Necessity for More Research and Public Participation. Paper presented at: Water in Mainland Southeast Asia Conference; November 29-December 2, 2005; Siem Reap, Cambodia.
7. **Shannon KL**, Inmuong Y, and Srangsok S. Social and Environmental Impacts of the Hua Na Dam: The Need for a Participatory Approach. Paper presented at: The 25th International Association for Impact Assessment Conference: Ethics & Quality; May 31-June 3, 2005; Boston, MA.
8. **Shannon KL**, Galiana G, Branca RT, Tang X, Ong H, and Warren WS. Simultaneous Acquisition of Multiple-Quantum Coherence Images. Paper presented at: The 45th Experimental Nuclear Magnetic Resonance Conference; April 18-23, 2004; Pacific Grove, CA.

Invited Seminars

9. **Shannon KL**, Sullivan DJ. Oligosymptomatic malaria in the Chittagong Hill Tracts of Bangladesh. Lecture presented: Johns Hopkins Bloomberg School of Public Health, Malaria Research Institute; June 12, 2015.
10. **Shannon KL**. The Khong-Chi-Mun Project and Hua Na Dam: Reevaluating Large Water

Diversion Schemes in Northeastern Thailand. Lecture presented: Kasetsart University Graduate School; December 8, 2005; Bangkok, Thailand.

RESEARCH GRANT PARTICIPATION

Title of Grant, Dates and Sponsoring Agency: Mapping Malaria Epidemiology in the Hill Tracts of Bangladesh, April 2009-Present, Johns Hopkins Malaria Research Institute, DFID, World Bank, TOMs, MSHR, Australia
Principal Investigator and Funding Level: David Sullivan, $1,660,000 over 6 years
Main Grant Objective: This project will characterize the epidemiology of malaria disease and transmission, provide a framework for detailed risk factor analysis, as well develop and evaluate specific interventions in this region. The project will establish a collaboration between JHUBSPH MRI and the ICDDR,B to improve the effectiveness of the national malaria control program
Principal Responsibilities: Graduate student investigator, responsible for conducting analysis related to the genetic, environmental, demographic and geographic risk factors for asymptomatic and clinically presenting malaria

Title of Grant, Dates and Sponsoring Agency: Modeling cholera transmission to inform use of Oral Cholera Vaccine (OPP1089243), June 2013-September 2015, Bill and Melinda Gates Foundation
Principal Investigator and Funding Level: Justin Lessler, $662,094 over two years.
Main Grant Objective: This project aims to use mathematical modeling of cholera dynamics to guide decisions as to best practices for the use of oral cholera vaccines for prevention and control of cholera and to better understand the impacts of oral cholera vaccine use
Principal Responsibilities: Graduate student investigator, responsible for conducting analysis related to cholera outbreaks in refugee camps as well as non-refugee settings, with specific focus on environmental and climatic associations with cholera

Title of Grant, Dates and Sponsoring Agency*:* The Social and Environmental Impacts of the Hua Na Dam Project: The Role of Local Communities in Decision Making, August 2004-October 2005, The Fulbright Program under The Thailand-United States Educational Foundation
Principal Investigator and Funding Level: Kerry Lee Shannon, $10,000
Main Grant Objective: Increase understanding of water development projects in Northeastern Thailand, the impact these projects are having on the environment and local communities, and the role of local community organizations in decision making
Principal Responsibilities: Fulbright student investigator. Designed, conducted and presented research

Title of Grant, Dates and Sponsoring Agency: Palliative care-related experiences of surgical ICU patients, their family members, and clinicians, 2012-2014, Foundation for Anesthesia Education and Research – Mentored Research Training Grant – Clinical and Translational Sciences
Principal Investigator and Funding Level: Rebecca Aslakson (Mentor: Peter Pronovost), $175,000 over two years

Main Grant Objective: Complete a qualitative study exploring the palliative care-related experiences of surgical ICU patients, their family members, surgeons, surgical ICU nurses, and surgical ICU intensivists
Principal Responsibilities: Student Investigator to include qualitative research exploring patients and clinicians lived experiences regarding palliative care in intensive care units

Title of Grant, Dates and Sponsoring Agency: "Intermolecular Multiple-Quantum Coherences and their Applications" (R01EB02122), 1992-2015, NIH
Principal Investigator and Funding Level: Warren S. Warren, $180,000 per year
Main Grant Objective: Develop NMR/MRI applications for Intermolecular Multiple-Quantum Coherences
Principal Responsibilities: Student Investigator to include conducting research on improving contrast in NMR microscopy imaging using multiple quantum coherence techniques in both simulated models and *in vivo*.

ACADEMIC SERVICE

Jan – May, 2014	Teaching Assistant, Spatial Analysis and GIS I & II, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Mar - May, 2013	Teaching Assistant, Clinical and Epidemiological Aspects of Tropical Disease, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Oct- Dec, 2011-2013	Teaching Assistant, Health and Human Rights Seminar, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Jun, 2007 & Jan, 2014	Teaching Assistant & Seminar leader, Problem Solving in Public Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Dec, 2005	Session Chair, Water in Village Life and Livelihoods: Agriculture and Fisheries. Water in Mainland Southeast Asia Conference; November 29 - December 2, 2005; Siem Reap, Cambodia.

ADDITIONAL INFORMATION

- Intermediate Thai and German, basic medical Spanish.
- Proficient in use of: R for statistical computing; ArcGIS and R for GIS and spatial analysis, Atlas.ti for qualitative analysis.