ABSTRACT

Title of Thesis:	THE ASSOCIATION BETWEEN SUBMICROSCOPIC MALARIA INFECTION AND FEVER: FINDINGS FROM A CROSS-SECTIONAL STUDY IN MALAWI
	Angelica L. Barrall Master of Public Health 2019
Thesis Directed By:	Dr. Typhanye V. Dyer, Assistant Professor Department of Epidemiology & Biostatistics

Individuals with submicroscopic malaria infection are an important reservoir for transmission, but the clinical consequences of these low-density parasitemia infections are poorly understood. Using cross-sectional data from six household-based surveys conducted during the dry and rainy seasons in Malawi from 2012 to 2014, this study examined the association between submicroscopic infection and fever in children and adults. For each survey, 900 households were recruited from three distinct ecological settings in southern Malawi to participate in the study (N=22,145). Overall prevalence of submicroscopic infection in the analytic sample was 8.1%. In a generalized linear mixed model accounting for clustering at the household and neighborhood levels and controlling for age and survey number, submicroscopic infection predicted fever in the dry season only (OR=1.66; 95% CI: 1.04, 2.66). Therefore, fever might not be a consistent marker of submicroscopic infection, but identification and treatment of low parasitemia infections is necessary to eliminate malaria transmission.

THE ASSOCIATION BETWEEN SUBMICROSCOPIC MALARIA INFECTION AND FEVER: FINDINGS FROM A CROSS-SECTIONAL STUDY IN MALAWI

by

Angelica Lynne Barrall

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Advisory Committee:
Professor Typhanye V. Dyer, Chair
Professor Miriam K. Laufer
Professor Tianzhou (Charles) Ma

Table of Contents

List of Tables

List of Figures

List of Abbreviations

EA	Enumeration Area
IPT	Intermittent Preventive Treatment
IRB	Institutional Review Board
ITN	Insecticide-Treated Net
RDT	Rapid Diagnostic Test
rtPCR	Real Time-Polymerase Chain Reaction
SAC	School-Aged Child
qPCR	Quantitative-Polymerase Chain Reaction

Chapter 1: Introduction

According to the World Health Organization, there were an estimated 219 million cases of malaria and 435,000 malaria-related deaths worldwide in 2017 (World Health Organization 2018d). Malaria is the sixth leading cause of death in low-income countries and the ninth leading cause of death in children under five years old worldwide (World Health Organization 2018a; World Health Organization 2018c). Sub-Saharan Africa accounted for 86% of all malaria deaths in 2017 (World Health Organization 2018d). In this region, children bear the brunt of malaria burden with malaria accounting for approximately one in ten deaths in children under the age of five (World Health Organization 2018a). Malawi, the second poorest country in sub-Saharan Africa (The World Bank 2017), suffers a particularly high portion of malaria burden with an estimated 4 million cases occurring in 2017 within a population of about 18 million (World Health Organization 2018d).

In much of sub-Saharan Africa, malaria transmission is endemic. However, acquired immunity protects most of the adult population from infection-related illness and severe clinical complications (Doolan et al. 2009; Jamison et al. 2006). Young children who have not yet acquired immunity against the malaria parasite are therefore, more likely to experience illness and death related to malaria infection.

In communities with high transmission and substantial acquired immunity, individuals can maintain low levels of parasitemia with and without gametocytes that are not detectable by a standard microscopy test (Roper et al. 1996). Individuals with submicroscopic infection are a stable reservoir for the malaria parasite because their

infection goes undetected, but they continue to contribute to onward transmission in the community (Coleman et al. 2004; Nwakanma et al. 2008; Roper et al. 1996). The clinical implications of submicroscopic malaria infection are not well understood.

Specific Aims and Hypotheses

The purpose of this study is to determine if submicroscopic infection is associated with fever, the most common clinical outcome of malaria infection, among a household-based sample in Malawi. This relationship will be examined using two different definitions of fever and within three age groups (i.e., children under five years old, schoolchildren, and adults). Additionally, this study will explore the influence of transmission intensity of malaria in the community, seasonality, and insecticide-treated net (ITN) use on the relationship between submicroscopic malaria infection and fever. See **Figure 1.** for a visual representation of the proposed relationship between study variables.

SPECIFIC AIM 1: To determine if submicroscopic malaria infection is associated with fever in children and adults.

HYPOTHESIS: Fever will be positively associated with submicroscopic infection among children and no association will exist among adults.

SPECIFIC AIM 2: To assess the impact of transmission intensity on the association between submicroscopic infection and fever.

HYPOTHESIS: Children with submicroscopic infection residing in high transmission settings will have a lower rate of fever compared to those in low transmission settings.

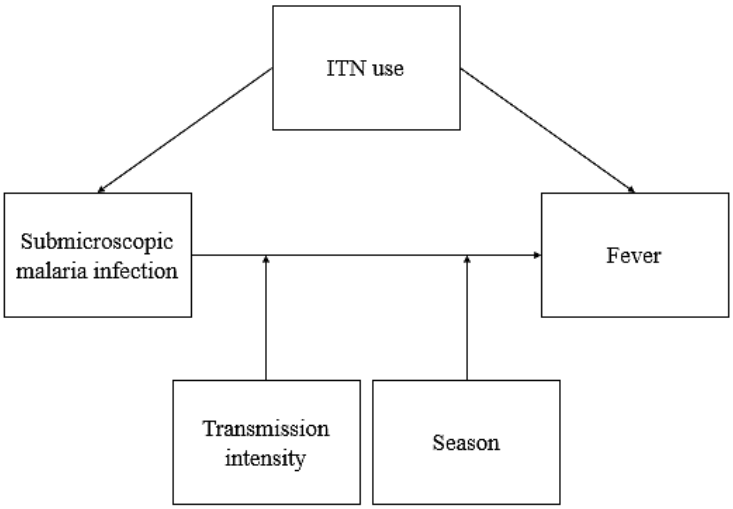
SPECIFIC AIM 3: To examine whether the association between submicroscopic infection and fever is modified by seasonality.

HYPOTHESIS: The magnitude of the association between submicroscopic infection and fever will differ between the rainy season, characterized by high transmission, and the dry season, characterized by low transmission.

SPECIFIC AIM 4: To examine whether the association between submicroscopic infection and fever is confounded or modified by insecticide-treated net (ITN) use.

HYPOTHESIS: ITN use will be associated with submicroscopic infection and decreased likelihood of fever, producing a confounding effect in the model.

Figure 1. Proposed relationship between key study variables



Chapter 2: Literature Review

Malaria in sub-Saharan Africa

Sub-Saharan Africa suffers from disproportionate burden from malaria-related morbidity and mortality compared to the rest of the world. Fifteen countries account for almost 80% of the world's malaria burden and fourteen of those countries are located in sub-Saharan Africa. More than 90% of cases of malaria in 2017 occurred in the World Health Organization (WHO) African Region (World Health Organization 2018d). Several factors contribute to the disproportionate burden of malaria this region carries.

The most dangerous genus of *Plasmodium* parasite to humans is *P. falciparum*. This mosquito is found in tropical and subtropical climates worldwide but is particularly dominant in Africa where it was responsible for nearly all (99.7%) of malaria cases in 2017 (World Health Organization 2018d). *P. falciparum* is responsible for severe symptoms of malaria infection and possibly death. Red blood cells infected with *P. falciparum* can adhere to the walls of blood vessels causing blockages and eventually, severe organ damage. The most common complication from *P. falciparum* infection is cerebral malaria which is characterized by coma occurring after impaired consciousness and seizure (Idro et al. 2010; Mohapatra 2006). Infection with *P. falciparum* can also cause anemia, jaundice, low blood pressure and glucose, and acute respiratory distress (Centers for Disease Control and Prevention 2018; Mohapatra 2006).

Transmission of malaria is more intense in Africa in part because of the specific species of *Anopheles* mosquito found in this region, *Anopheles gambiae*. This species of mosquito is found exclusively in Africa and has a longer lifespan than other *Anopheles* species. The longer lifespan of the *Anopheles gambiae* increases the likelihood that the parasite fully develops within the mosquito and can transmit malaria (World Health Organization 2018b). This species also prefers to bite humans rather than animals, a trait known as anthropophilic (World Health Organization 2018b). Three other highly anthropophilic *Anopheles* mosquitoes are also found in Africa: *An. funestus*, *An. moucheti* and *An. nili* (Sinka et al. 2010).

Social and economic factors specific to the sub-Saharan Africa region further exacerbate the burden of malaria. In terms of socioeconomic growth, the sub-Saharan Africa region has been falling behind the rest of the world. In the past few decades, most countries in this region have experienced gross domestic product (GDP) per capita declines. During this same time, poverty has been increasing in these countries while the rest of the world progresses towards meeting the Millennium Development Goal (MDG) of reducing the number of people living below $1 per day by half. In sub-Saharan Africa, the number of people living at this poverty level increased from 44.6% to 46.4% between 1990 and 2003 (Jamison et al. 2006).

The demographic context of sub-Saharan Africa is conducive to high malaria-related morbidity and mortality. The population of sub-Saharan Africa is remarkably young with about 40% of the population being younger than 15 years old and the median age being about 18 years old (United Nations 2017). Younger populations are less likely to be vulnerable to chronic diseases, therefore infectious diseases,

especially those such as malaria that are particularly severe in children, are a dominant cause of mortality.

The low-income economies, high poverty rates, and high proportions of displaced people in sub-Saharan Africa are considerable barriers to an effective and timely response to malaria in the region. Regions with poor financial prospects are likely to have healthcare systems with poor infrastructure that have less capacity to increase public health interventions or scale-up services.

Malawi is a small, landlocked country in southern Africa. Malaria is endemic in 95% of the country, except for the highest mountains (President's Malaria Initiative 2018). Transmission of malaria in Malawi occurs throughout the year but peaks after the start of the rainy season in November. Higher transmission areas in the lower lying regions of the country are characterized by high temperatures and humidity, while the lower transmission areas are found in the highlands. The primary vector species in the country is *Anopheles funestus*, though *An. gambiae* and *An. arabiensis* are also present and might dominate in some areas at certain times of the year. *Plasmodium falciparum* accounts for 98% of all malaria infections and all severe malaria-related disease and death in Malawi (President's Malaria Initiative 2018).

Malawi, as one of the poorest countries in sub-Saharan Africa, is particularly vulnerable to the burden of malaria. According to the WHO, an estimated 4.3 million cases of malaria and 7,000 malaria-related deaths occurred in Malawi in 2017 (World Health Organization 2018d). Among the 11 countries in Eastern and Southern Africa, Malawi accounted for 8% of the estimated malaria cases in 2017. Malaria transmission is stable in Malawi and nearly the entire population (18.6 million) is at

risk for malaria infection. Malaria is the primary cause of outpatient visits in the county, accounting for 30% of all visits across all ages (President's Malaria Initiative 2018).

Submicroscopic Malaria Infection

In sub-Saharan Africa, infection with *Plasmodium* parasite is ubiquitous and unlike many other infectious diseases, infection is often not associated with clinical symptoms because of acquired immunity (Jamison et al. 2006). Mortality due to malaria in this region is concentrated in populations with impaired immunity, often observed in individuals with comorbidities or with poorly developed immunity, such as children under 5 years old. Therefore, young children account for the majority of malaria disease burden in Africa. Depending on the strength and maturity of the immune system, this infection might be easily resolved, cause complication, or result in death. The ability to clear mild consequences of infection develops in late childhood, but immunity to a malaria infection that has progressed to red blood cell infection does not develop until adulthood (Jamison et al. 2006).

In addition to the significant risk to children living in regions with stable malaria transmission, chronic infections with *P. falciparum* can also have consequence. Asymptomatic infections can lead to anemia or poor nutrition which increases risk for more severe complication from future infections (Chen et al. 2016). Subclinical infection during pregnancy is associated with premature delivery, lower birthweights, and reduced infant survival rates (Centers for Disease Control and Prevention 2018; Cottrell et al. 2015; Jamison et al. 2006; Mohammed et al. 2013).

The high prevalence of asymptomatic infection in this region can also make accurate diagnosis and surveillance difficult.

Asymptomatic infections represent an important barrier to malaria elimination because they serve as reservoirs that continue transmission, albeit less effectively (Barnes et al. 2008; Bousema et al. 2006; Lin et al. 2014; Nwakanma et al. 2008; Roper et al. 1996), even when parasites are not detected microscopically (Gaye et al. 2015; Ouedraogo et al. 2016). For example, researchers in Malawi have recently discovered that a school-aged child (SAC) are the largest reservoir of infection, responsible for more than 60% of new mosquito infections (Coalson et al. 2018). The majority of SAC infections had a gametocyte density of <1 gametocyte/μL detected. Individuals with asymptomatic malaria are unlikely to visit healthcare facilities or engage in surveillance so their infection goes unaddressed. There is need for innovative strategies to identify and eliminate these low-transmission, asymptomatic parasite reservoirs for the final stages of malaria elimination to be successful (Lamptey et al. 2018; Pava et al. 2016).

The traditional method for laboratory confirmation of malaria infection is microscopy. For this test, a blood sample is spread on as a thick smear, stained, and examined. A trained lab technician examines the smear to detect *Plasmodium* presence, with lower limits of detection being more than 100 parasites/μL in clinical practice (Wongsrichanalai et al. 2007). However, advancements in molecular techniques, such as polymerase chain reaction (PCR), have allowed for more sensitive malaria detection at submicroscopic levels [0.1–10 parasites/μL; (Adams et al. 2015; Bousema et al. 2014; Hofmann et al. 2015; Imwong et al. 2014). Compared

to conventional methods (i.e., microscopy) the application of molecular methods in surveillance surveys has resulted in a 2-10 fold increase in the prevalence of detectable parasitemia (Okell et al. 2012).

Fever and Malaria Infection

Fever is the most common marker of malaria infection. However, in areas of endemicity, it is difficult to define malaria illness and definitively determine the etiology of a fever, especially in cases of submicroscopic infection. The common clinical practice of treating for malaria based on fever and parasitemia results in overdiagnosis of malaria and inappropriate use of antimalaria drugs, increasing the risk of drug-resistance.

Several studies have examined the "pyrogenic threshold," or the parasitic threshold necessary to induce malaria fever. This threshold is influenced by transmission levels, seasonality, immunity of the individual, and stage of infection (Dicko et al. 2005; Gatton et al. 2002; Mmbando et al. 2009). The definition of fever in these studies is consistent with the study described: either self-reported fever in the past 48 hours or a body temperature of $\geq$37.5°C; >38.3°C in Gatton and Cheng (2002).Therefore, the concept of a pyrogenic threshold to create a more sensitive malaria case definition is difficult in practice because it will likely need to be adjusted on a case-by-case basis. It is also unclear if the threshold varies in response to interventions. It is reasonable to assume that transmission intensity, seasonality, and immunity of an individual might also impact the relationship between low-level infection and fever.

This study attempts to determine if submicroscopic malaria infection is a significant predictor of fever, considering transmission levels, seasonality, immunity of the individual (i.e., age), and intervention (i.e., ITN use). The results will have important implications for the evaluation of pyrogenic threshold and clinical practice of treating low-density infections.

Gaps in Knowledge

Despite interest in determining the level of malaria parasitemia that causes fever, few studies have examined the general clinical outcomes of submicroscopic infection. It is possible that low-level parasitemia might be a marker of a parasitic infection that will develop into clinical disease with more severe symptoms. Therefore, determining the physical health consequences of submicroscopic infection could improve estimations of the burden of malaria in healthcare settings.

The need for this research is highlighted by the findings of a study in Gabon of febrile patients. Mawili-Mboumba and colleagues (2017) found that a considerable number of febrile patients (11%) has submicroscopic malaria infection that would not have been identified by standard microscopy. However, this study design did not allow for comparison of submicroscopic infection in children without fever or the prevalence of fever in the general population without any parasitemia.

The strongest study on this topic to date was conducted by Katrak and colleagues (2018) examining the clinical consequences of submicroscopic malaria parasitemia in one district of Uganda with reportedly high, stable transmission. The research team randomly selection 100 households in the district in which all children (aged six months to 10 years old) and one primary adult (aged ≥ 18 years old) were

enrolled in the study. Participants reported to the study clinic every three months and at each visit were interviewed, during which reported fever was collected, and gave a blood sample. Children with submicroscopic infection had 1.42 times the risk of fever compared to children with no infection, while no association was observed in adults. While this study offers highly suggestive evidence that submicroscopic infection and fever are associated among children, the proposed study builds upon this prior research in important ways.

The proposed study utilizes data collected in a variety of transmission areas and in two distinct seasons. As previously suggested by the pyrogenic threshold literature, the association between the level of parasitemia needed to produce fever is likely influenced by transmission levels. This study will be able to determine if malaria transmission in a community and/or season influences the association between submicroscopic infection and fever. Additionally, this study will examine the influence of an intervention, ITN use, on the relationship between submicroscopic infection and fever. Similar to pyrogenic threshold, it is unknown whether intervention would influence this association. Any factors influencing the relationship between submicroscopic infection and a clinical outcome, such as fever, are important and might influence the burden of malaria and the need to treat low-density malaria infections.

Chapter 3: Research Design and Methods

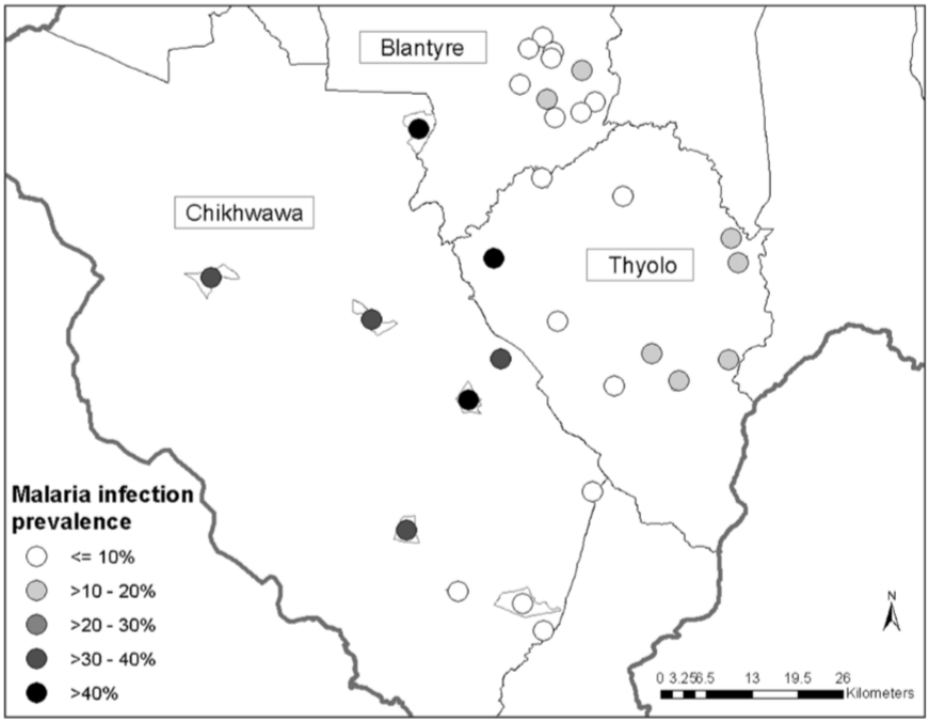
Overall Study Design

This is a cross-sectional analysis of data collected from six household-based, community surveys conducted in three transmission settings in Malawi (i.e., Blantyre City, Chikwawa district, and Thyolo district) between 2012 and 2014. The first survey of each calendar year occurred near the end of the rainy season in April-May and the second was during the dry season in September-October. The survey collected demographics, bed net ownership and use, recent anti-malarial medication use, and recent or current fever. Members of each household that were at least six months of age and present at the time of the survey also provided a temperature and blood sample to allow for microscopy and PCR testing.

Study Population

The study sample was selected from three distinct ecological settings in southern Malawi: 1) Blantyre City, an urban setting expected to have low malaria transmission, 2) Chikwawa district, a low-altitude region with intense transmission, and 3) Thyolo district, a high-altitude, rural region with moderate transmission. These three districts border one another, however, and heterogeneity of transmission within each district is expected. Study participants were sampled in two stages: 1) selection of enumeration areas (EAs) within each of the three settings, and 2) selection of households within each EA. Ten EAs were selected within each district using probability proportionate to size, and then 30 households within each EA were selected for the sample using compact segment sampling (Turner et al. 1996). EAs

Figure 2. Selected districts and EAs with malaria infection prevalence (Walldorf et al. 2015)



were excluded if they met any of three criteria: 1) the EA was located on the border between Chikwawa and Thyolo, 2) an EA in Chikwawa was >500m above sea level, or 3) an EA in Thyolo was <500m above sea level. Based on these exclusion criteria, three EAs were replaced with the next randomly selected EA (Walldorf et al. 2015). See **Figure 2.** for a map of the selected communities and their baseline level of malaria infection prevalence based on molecular testing.

All households within an EA were surveyed on the same day. If the household survey was not successfully completed on the same day as the other households in the EA, one additional attempt was made within two weeks to conduct the interview in the selected home. Subsequent rounds of surveying were conducted in the same EAs

and compact segments as the first survey. It is possible that the same households were surveyed repeatedly because it was not possible to collect identifying data to track participants between surveys.

Household members were defined as individuals who had slept in the house for at least two weeks of the previous month. Individuals were excluded from the household-based survey if they were a guest or visitor of the house and if the household did not have an adult to provide consent. If a household was excluded, it was replaced in the study with the nearest household within the compact segment of the EA by convenience selection. Any participant who was ill and required treatment was referred or transported to the health surveillance assistant working with the study team or taken to the nearest healthcare facility.

Data Collection

At each house visit, the study team interviewed the household members present that day in the local language (Chichewa) to collect information about household-level variables and individual-level variables. The household survey questionnaires were adapted from the standardized Malaria Indicator Survey tools (National Malaria Control Programme and ICF International 2010). In the field, responses were collected on tablets using OpenDataKit and managed using Research Electronic Data Capture tools [REDCap; (Harris et al. 2009)].

Nurses working with the field team collected body temperature and blood samples from all household members at least six months old who were present and consented to participate. Drops of blood were collected onto slides as thick smears for microscopy testing and onto filter paper for DNA isolation and PCR testing.

Dependent Variable Definition

The outcome of interest in this study is fever in the past 48 hours. Presence of fever in study participants was measured in three ways: 1) participants were asked if they had had a fever within the last 48 hours and 2) body temperature was collected at the time of the interview and if ≥ 37.5 °C, documented as fever. The final computed outcome variable used in analysis accounts for both self-reported and objective fever measures and is dichotomous (i.e., fever vs. no fever).

Description of Variables

Submicroscopic malaria infection. Blood samples for microscopy and rtPCR testing were collected from participants at least six months of age at the time of the household survey. If there were at least two negative reads from two different miscroscopists, then the participant was considered negative for parasites. If the first two microscopy reads were discrepant, a third read was required. A submicroscopic infection was defined as having a negative microscopy test and a positive rtPCR test. If an individual was positive for the *P. falciparum* lactate dehydrogenase gene they were considered parasite positive by rtPCR (Rantala et al. 2010).

Age. Age was collected from all participants during the household interview. In this study, age is divided into three categories: 1) children under five years old, 2) school-aged children (SACs; 5-15 years old), and 3) adults (over 15 years old).

Wave of data collection. Since this analysis combines data collected from six different cross-sectional waves, the survey number was included as a covariate to control for batch effect.

Transmission intensity. Thirty EAs were sampled for study participants. Site transmission intensity was defined using the average EA prevalence by qPCR across all six surveys. Tertiles of low, moderate, and high transmission communities were created. The lowest tertile was defined as having less than 7% prevalence, the moderate as 7 to 11% prevalence, and the highest as over 11% prevalence, as has been done previously (Buchwald et al. 2017). PCR-based prevalence in the 30 EAS ranges from 0 to ≥ 65%.

Seasonality. In each of the three study years, a wave of data collection occurred near the end of the rainy season in Malawi and again during the dry season. The season of data collection is a dichotomous variable (i.e., rainy season vs. dry season).

Insecticide-treated net (ITN) use. During the household interview, participants were identified as using a bed net by the question, "Which members of the household slept under this bed net last night?" The bed net was further classified as an ITN by the question, "Was this net factory-treated with insecticide prior to acquiring it?" Use of an ITN on the previous night is dichotomous (i.e., yes vs. no).

Blood Testing

Upon delivery to the laboratory at the end of each day of sample collection, blood smears were air dried, methanol fixed, and Giemsa-stained. Thick smears were read by two trained microscopists who each independently recorded the number of parasites observed. Discrepancies between the two reports sent the slide to a third reader. Dried blood spots were tested using rtPCR to detect *P. falciparum* infection by the presence of the lactate dehydrogenase gene.

Data Analysis

Data will be accessed from the Research Electronic Data Capture system and all analyses will be performed using Statistical Analysis System (SAS) version 9.4 (SAA Institute, Cary, NC).

Descriptive statistics of the consented sample were reported. Participants who were microscopically positive for infection, recently received treatment for malaria infection, or were receiving Intermittent Preventive Treatment (IPT) for malaria infection were excluded from analysis. For Aim 1, bivariate analysis using Chi squared tests of association were performed to determine the association between submicroscopic malaria infection and fever within the analytic sample and each of the three age categories. All analytic analyses will be conducted using the combined fever variable as the outcome of interest. Sensitivity analysis were conducted using only the objectively measured fever variable as the outcome to determine if incorporating self-reported fever influences the association. For Aims 2-4, stratification and interaction term analyses tested potential effect modification of community transmission intensity, seasonality, and ITN use on the association between submicroscopic infection and fever. Additionally, bivariate and multivariate analyses were used to confirm if any of these variables of interest confounded the association in this sample. A final mixed effect logistic regression model accounted for clustering at the household and EA levels and controlled for confounding variables and was stratified as appropriate.

Human Subjects

Permission was obtained from village leaders prior to data collection. Informed consent was obtained from all participants or their guardians; assent was also obtained from participants 13-17 years old. The study methods have been approved by the Institutional Review Boards (IRBs) of the University of Malawi College of Medicine, the University of Maryland, Baltimore, and Michigan State University. The University of Maryland, College Park issued a letter of determination that this study does not meet the definition of human subject research (see **Appendix A.**). An additional Data Use Agreement between the University of Maryland, College Park and University of Maryland, Baltimore was also completed (see **Appendix B.**). I have already completed Collaborative Institutional Training Initiative (CITI) training in both Social and Behavioral and Biomedical research as well as in Conflict of Interest, Good Clinical Practice, and Responsible Conduct of Research.

Chapter 4: Results

Sample Characteristics

A total of 22,145 participants were enrolled across the six studies and 19,586 provided signed informed consent. See **Table 1.** for sample characteristics for this sample. The mean age of the sample (*n*=19,586) was 20.0 years old, nearly half (47.9%) were older than 15 years old, and 59% were female. The population distribution between surveys did not vary significantly by district, age, sex, or transmission intensity. The proportion who used ITNs on the previous night, were positive for malarial parasite, had submicroscopic infection, and had fever in the past 48 hours differed significantly between surveys.

A fifth of the sample (22.4%) lived in a community in the lowest tertile for malaria prevalence, a third (33.7%) lived within the moderate tertile, and 43.8% lived in a community in the highest tertile. The Chikhwawa district had the largest proportion of participants living in high transmission communities (59.8%) and Blantyre had the largest proportion living in low transmission communities (41.1%). About three-quarters (74.0%) of households owned an ITN. A larger proportion of children under 5 years old slept under an ITN than adults, and SACs slept under ITNs the least often. Across the six surveys, the average prevalence of malaria infection as determined by rtPCR was 15.8% compared to 10.0% from microscopy. The prevalence of fever in the sample ranged from 14.8% in the first survey to 4.7% in the last. Among those with fever in the past 48 hours (*n*=1,721), 6.8% had submicroscopic infection compared to 6.7% among those without fever (*n*=17,865).

Table 1. Sample characteristics from six cross-sectional surveys (*n*=19,586)

Sample characteristics *n* (%)	**Rainy 2012 (*n*=2,897)**	**Dry 2012 (*n*=3,544)**	**Rainy 2013 (*n*=3,366)**	**Dry 2013 (*n*=3,248)**	**Rainy 2014 (*n*=3,180)**	**Dry 2014 (*n*=3,351)**
District						
Blantyre	939 (32.4)	1138 (32.1)	1060 (31.5)	1018 (31.3)	1066 (33.5)	1129 (33.7)
Chikwawa	1021 (35.2)	1249 (35.2)	1185 (35.2)	1174 (36.2)	1101 (34.6)	1163 (34.7)
Thyolo	937 (32.3)	1157 (32.7)	1121 (33.3)	1056 (32.5)	1013 (31.9)	1059 (31.6)
Age						
Less than 5 years	514 (19.4)	588 (18.1)	575 (17.1)	538 (16.6)	558 (17.6)	570 (17.0)
5-15 years	991 (37.5)	1202 (36.9)	1217 (36.2)	1209 (37.2)	1196 (37.6)	1312 (39.2)
Over 15 years	1139 (43.1)	1466 (45.0)	1574 (46.8)	1501 (46.2)	1426 (44.8)	1469 (43.8)
Female	1746 (60.3)	1971 (55.6)	1991 (59.2)	1922 (59.2)	1919 (60.4)	2007 (59.9)
Mean household size (SD)*	4.7 (1.8)	5.1 (1.9)	4.8 (1.8)	4.7 (1.7)	4.7 (1.8)	4.8 (1.7)
Living in household with one or more ITNs*	1057 (36.5)	2887 (81.5)	2903 (86.2)	2679 (82.5)	2577 (81.0)	2399 (71.6)

Table 1. Sample characteristics from six cross-sectional surveys (*n*=19,586)

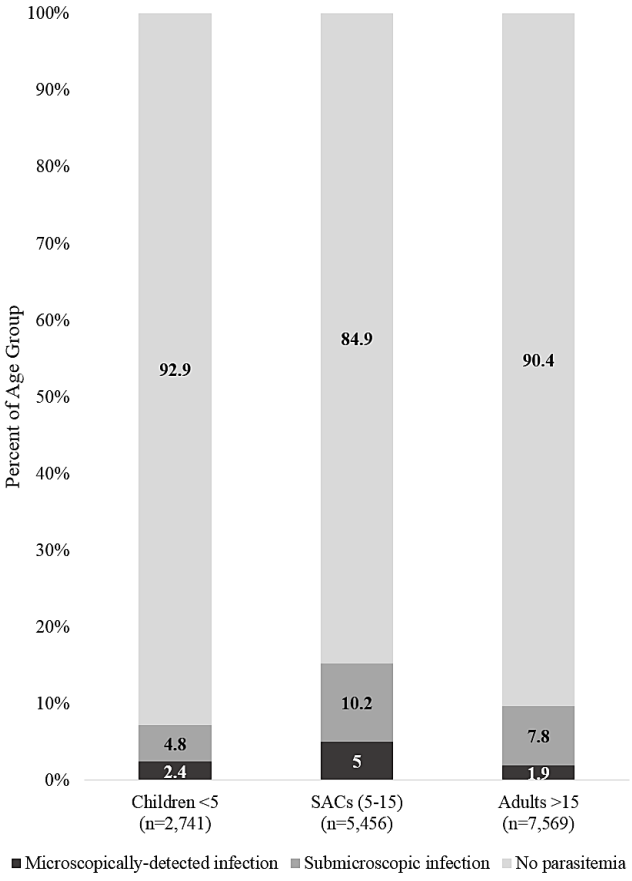
Sample characteristics *n* (%)	**Rainy 2012 (*n*=2,897)**	**Dry 2012 (*n*=3,544)**	**Rainy 2013 (*n*=3,366)**	**Dry 2013 (*n*=3,248)**	**Rainy 2014 (*n*=3,180)**	**Dry 2014 (*n*=3,351)**
Among houses with ITNs (*n*=14,502)						
Children under 5 years who use ITNs*	152 (70.7)	361 (76.3)	433 (86.3)	347 (76.9)	409 (87.0)	277 (66.0)
Children 5-15 years who use ITNs*	171 (44.0)	577 (54.3)	758 (68.7)	592 (56.5)	637 (63.1)	474 (48.2)
Adults over 15 years who use ITNs*	268 (67.2)	760 (67.8)	1052 (81.1)	855 (72.5)	897 (81.7)	651 (65.4)
Transmission intensity						
Low	650 (22.4)	784 (22.1)	730 (21.7)	719 (22.1)	732 (23.0)	779 (23.3)
Moderate	956 (33.0)	1217 (34.3)	1150 (34.2)	1091 (33.6)	1070 (33.7)	1123 (33.5)
High	1291 (44.6)	1543 (43.5)	1486 (44.2)	1438 (44.3)	1378 (43.3)	1449 (43.2)
Parasite prevalence-rtPCR*	401 (17.3)	346 (12.3)	574 (18.8)	316 (10.6)	698 (23.2)	409 (12.8)
Parasite prevalence-microscopy*	323 (13.9)	199 (7.1)	408 (13.2)	194 (6.5)	371 (12.4)	227 (7.0)
Submicroscopic infection*	160 (2.5)	184 (5.2)	214 (6.4)	150 (4.6)	361 (11.4)	238 (7.1)
Fever (past 48 hours)*	430 (14.8)	372 (10.5)	274 (8.1)	220 (6.8)	269 (8.5)	156 (4.7)

*Indicates variable with statistically significant difference ($p < 0.0001$) between surveys by Chi square test or ANOVA.

The prevalence of microscopically-detected malaria infection, submicroscopic infection, and no parasitemia differed significantly ($p<0.0001$) between the three age groups (see **Figure 3.**).

Bivariate and multivariate analyses were restricted to 14,232 participants with complete covariate and demographic data. The majority of excluded participants (70.2%) had incomplete parasite prevalence testing results. Specifically, 3,451 participants were missing microscopy smear results completely, did not have at least two smear results, did not have a third smear result if the first two were discrepant, or did not have a PCR result. An additional 378 participants were excluded due to missing demographic information. Participants were also excluded from analysis if they reported taking an antimalarial medication in the past two weeks or were currently pregnant and were receiving Intermittent Preventive Treatment (IPT) for malaria infection (*n*=1,089). An additional 436 individuals tested positive for malarial infection using microscopic testing and were excluded from analysis. See **Appendix D.** for a comparison of

Figure 3. Prevalence of malaria infection among age groups (*n*=16,232)



sample characteristics among the analytic sample and excluded participants. Excluded and included participants differed significantly on all characteristics tested ($p<0.05$).

Assessment for Effect Modification and Confounding

Stratified regression results are outlined in **Table 2**. To assess for effect modification, the bivariate association between submicroscopic infection and fever was stratified across levels of each variable of interest (i.e., age, transmission intensity, season, ITN use). Although the size of the association appeared to change between the levels of all variables tested, only the odds ratios stratified by season were significantly different as indicated by a Breslow-Day Test ($p=0.01$). The role of season as an effect modifier on the association between submicroscopic infection and fever was further confirmed by modelling a statistically significant interaction term ($p=0.01$). All other interaction terms tested were insignificant ($p>0.05$). In the dry season, those with submicroscopic infection had nearly twice the odds of having a fever (OR=1.80; 95% CI: 1.14, 2.84) compared to those without parasitemia. On the other hand, in the rainy season, those with submicroscopic infection had a 12% reduction in odds (OR=0.88; 95% CI: 0.66, 1.17) of having a fever compared to those without parasitemia although this association was not significant.

The results of bivariate regressions and several multivariate models assessing association between submicroscopic infection and fever are presented in **Table 3**. Being a SAC, living in a high transmission intensity area, season, and ITN use on the previous night were significantly associated with having had a fever in the past 48 hours. To assess for confounding, the crude odds ratio (OR=1.10; 95% CI: 0.86, 1.39)

Table 2. Prevalence of fever and stratified analyses for bivariate association between submicroscopic infection and fever (*n*=14,232)

Characteristic	Fever in past 48 hours (*n*=1,023)		No fever in past 48 hours (*n*=13,209)		Bivariate	
	Submicroscopic infection (*n*=77)	No parasitemia (*n*=946)	Submicroscopic infection (*n*=1,082)	No parasitemia (*n*=12,127)	OR (95% CI)	Breslow-Day Test (p value)
Age						0.54
Children <5	12 (15.6)	207 (21.9)	95 (8.8)	2121 (17.5)	0.77 (0.42, 1.43)	
Children 5-15	25 (32.5)	246 (26.0)	495 (45.8)	4146 (34.2)	1.18 (0.77, 1.79)	
Adults >15	40 (52.0)	493 (52.1)	492 (45.5)	5860 (48.3)	1.03 (0.74, 1.45)	
Transmission intensity*						0.55
Low	3 (3.9)	224 (23.7)	86 (8.0)	3200 (26.4)	2.01 (0.63, 6.40)	
Moderate	17 (22.1)	344 (36.4)	275 (25.4)	4486 (37.0)	1.24 (0.75, 2.05)	
High	57 (74.0)	378 (40.0)	721 (66.6)	4441 (36.6)	1.08 (0.81, 1.44)	
Season*						0.01
Dry	20 (26.0)	477 (50.4)	512 (47.3)	6792 (56.0)	1.80 (1.14, 2.84)	
Rainy	57 (74.0)	469 (49.6)	570 (52.7)	5335 (44.0)	0.88 (0.66, 1.17)	
ITN use						0.57
No	45 (58.4)	494 (52.2)	544 (50.3)	5765 (47.5)	1.04 (0.75, 1.42)	
Yes	32 (41.6)	452 (47.8)	538 (49.7)	6362 (52.5)	1.19 (0.83, 1.73)	

Note. *Identifies significant difference (p value <0.05) between prevalence of fever between infection groups as determined by a Chi square test.

from the bivariate association between submicroscopic infection and fever was compared to the adjusted odds ratios for each covariate of interest. None of the adjusted models produced odds ratios that differed appreciably from the crude estimate. Given that the measure of association between submicroscopic infection and fever did not change when adjusting for transmission intensity, season, or ITN use, none of these variables were included in the final model as confounders. Additionally, transmission intensity was not associated with fever independently of submicroscopic infection as determined by a Chi square test ($p=0.06$) and ITN use was not associated with submicroscopic infection ($p=0.07$) strengthening the justification for not controlling for these covariates in the final model. Although adjustment for age did not change the size of the association between submicroscopic infection and fever, age met criteria for a confounding variable in the model. Among those without submicroscopic infection, SACs had 1.65 times the odds of children under 5 years old of having fever (95% CI: 1.36, 1.99) and adults had 1.16 times the odds of fever compared to children under 5 (95% CI: 0.98, 1.38) although that association was not significant ($p>0.05$). Both SACs and adults were less likely than children under the age of 5 to have submicroscopic infections (SACs vs. children <5: OR=0.39, 95% CI: 0.31, 0.48; Adults vs. children <5: OR=0.55, 95% CI: 0.44, 0.68). Given the biological influence of age on the relationship between malaria infection and clinical outcomes, the estimation of effect would likely be bias without controlling for age in the final model. Including age as a confounder in models predicting outcomes of malaria infection is also a standard in the field.

Association Between Submicroscopic Infection and Fever

To account for the effect modification of season on the association between submicroscopic infection and fever, the final model was stratified by the dry and rainy seasons. In the dry season, individuals with submicroscopic infection had 1.66 times the odds (95% CI: 1.04, 2.66) of having a fever compared to those without parasitemia. In the rainy season, individuals with submicroscopic infection were less likely to have a fever compared to those without parasitemia, although this association did not remain significant (aOR=0.77; 95% CI: 0.56, 1.05). Being a SAC was also a significant predictor of fever compared to children under 5 years old in both seasons (in dry, aOR=1.80, 95% CI: 1.37, 2.36; in rainy, aOR= 1.47, 95% CI: 1.11, 1.94). Being an adult was not a significant predictor of fever in either season.

Sensitivity Analysis

The results of the sensitivity analysis in which an alternative definition of the fever outcome was utilized in analysis is outlined in **Appendix E**. Using the objective temperature definition, only 1.1% of the sample met criteria for fever compared to 7.2% using the combined temperature and self-report variables for fever. In stratified analysis, none of the odds ratios between levels of any of the covariates of interest (i.e., age, transmission intensity, season, ITN use) were significantly different ($p>0.05$) as determined by a Breslow-Day Test.

When using the objective definition of fever only, the bivariate association between submicroscopic infection and fever remained insignificant (OR=1.34; 95% CI: 0.71, 2.55). The bivariate associations between fever and age, season, and ITN

Table 3. Crude and adjusted analyses predicting fever in the past 48 hours (*n*=14,232)

	Bivariate	**Multivariate**			**Final Models**	
		Model 1	**Model 2**	**Model 3**	**Dry (*n*=7,801)**	**Rainy (*n*=6,431)**
Submicroscopic infection	1.10 (0.86, 1.39)	1.16 (0.91, 1.47)	1.13 (0.89, 1.43)	1.10 (0.87, 1.40)	**1.66 (1.04, 2.66)**	0.77 (0.56, 1.05)
Age						
Children <5	Ref	-	-	-	Ref	Ref
Children 5-15	**1.69 (1.41, 2.04)**	-	-	-	**1.80 (1.37, 2.36)**	**1.47 (1.11, 1.94)**
Adults >15	1.18 (1.00, 1.39)	-	-	-	1.24 (0.98, 1.58)	1.09 (0.85, 1.37)
Transmission intensity						
Low	Ref	Ref	-	-	-	-
Moderate	0.91 (0.77, 1.08)	0.91 (0.76, 1.08)	-	-	-	-
High	**0.82 (0.69, 0.97)**	**0.81 (0.68, 0.96)**	-	-	-	-
Season						
Dry	Ref	-	Ref	-	-	-
Rainy	**0.76 (0.67, 0.87)**	-	**0.76 (0.67, 0.87)**	-	-	-
ITN use						
No	Ref	-	-	Ref	-	-
Yes	**1.22 (1.07, 1.38)**	-	-	**1.22 (1.07, 1.39)**	-	-

Note. Odds ratios and 95% confidence intervals from bivariate, multivariate, and mixed effect logistic regression. Bold text indicated significant association (p<0.05). Model 1: submicroscopic infection+transmission intensity. Model 2: submicroscopic infection+season. Model 3: submicroscopic infection+ITN use. Final models: submicroscopic infection+survey number (1-6)+age.

use also became insignificant using the objective definition. The association between living in a high transmission community and fever remained significant, however the size of the effect was strengthened. Using the objective fever definition, individuals in high transmission areas had 49% reduced odds of fever (OR=0.51, 95% CI: 0.33, 0.81) compared to individuals in low transmission settings. Similar to the original analysis, using the objective fever definition did not produce adjusted estimates that differed from the crude estimate of association between submicroscopic infection and fever.

In the final models, submicroscopic infection was not a significant predictor of objective fever in either season (in dry, OR=1.96, CI: 0.61, 6.26; in rainy, OR=1.03, CI: 0.47, 2.27). Given there was no evidence of effect modification using the objective fever definition, the association between submicroscopic infection and fever was also tested without stratification between seasons. Submicroscopic infection remained an insignificant predictor of fever when controlling for age and survey number (aOR=1.31; 95% CI: 0.69, 2.51).

Chapter 5: Discussion

Submicroscopic infections, or low parasitemia malaria infections, are known to be common in both low and high transmission settings in sub-Saharan Africa. However, little is known about the clinical implications of these low-level infections. This study examined the association between submicroscopic infection and fever, the most common indicator of malaria infection, among a population residing in communities with varying levels of transmission and during both the dry and rainy seasons. This study also accounted for the influence of a widespread intervention, use of ITNs, on the association. In a sample of 14,232 individuals from three districts in Malawi, submicroscopic infection was a significant predictor of fever in the dry season only. Specifically, individuals with submicroscopic infection in the dry season had 1.66 times the odds of having fever compared to individuals with no parasitemia. These results indicate that fever might not be a consistent indicator of low parasitemia infections.

Unexpectedly and in contrast with previous literature (Katrak et al. 2018), age was not a powerful modifying factor for the association between submicroscopic infection and fever. The prevalence of fever in this sample did not significantly differ among the age groups by submicroscopic infection (see **Table 2.**). It was expected that children, but not adults, with submicroscopic infection would have increased risk of fever. It is possible that inclusion of children less than 2 years old in the younger age group might have influenced the strength of this association. Infants might have persistent fetal hemoglobin, or other age-related differences in their immune systems,

that protects against the clinical effects of low-parasitemia infections (Dechavanne et al. 2015; Dobbs et al. 2016). However, the youngest participants in this study were 6 months old and not expected to have fetal hemoglobin. Further, it is possible that age was an inaccurate proxy for an individual's level of immunity to infection. Individuals with more exposure to malaria infection, typically adults and older children, would be less likely than children who have little immunity to experience clinical outcomes, such as fever, from a low-parasitemia infection. Currently, there is no standardized way to measure an individual's level of immunity, but such a method would have important clinical implications.

Similarly, likelihood of fever among individuals with submicroscopic infection did not differ by ITN use. ITN use was hypothesized to be associated with submicroscopic infection and decreased likelihood of fever, but ITN use did not have a significant relationship with submicroscopic infection in this study. The weak association between ITN use and low-density infection is confirmed in a previous study of this sample (Buchwald et al. 2017). ITNs were shown to protect against microscopically-detected infections, but those associations were weakened when infections identified by PCR were added to the model. Further, ITNs only protect from indoor biting, therefore other factors, such as an individual's occupation, might impact exposure to mosquito bites more than ITN use (Cotter et al. 2013; Zaw et al. 2017).

Further, transmission intensity did not have a significant impact on the association between submicroscopic infection and fever. It was hypothesized that individuals with low parasitemia in higher transmission settings would have a lower

rate of fever compared to those in low transmission settings. However, among those with submicroscopic infection, the prevalence of fever was much greater in the high transmission settings compared to the low transmission settings (see **Table 2.**). It is possible that transmission setting was not associated with fever because transmission setting might not accurately capture an individual's prior exposure to malaria infection. As previously mentioned, a more precise measure of an individual's exposure history is needed to determine whether transmission intensity has a significant impact on clinical symptoms experienced by those with low parasitemia. Another possibility is that the fever cases identified in this study has another disease causing their illness. This would confound the association between their fever and their low parasitemia infection. A longitudinal study monitoring the health status of each participant in a more comprehensive way would be needed to control for this possible confounding.

As expected, the magnitude of association between low parasitemia infection and fever differed between seasons. Bivariate results revealed that individuals with submicroscopic infection had 1.80 times the odds of individuals with no parasitemia for having a fever in the dry season (see **Table 2.**). While the dry season put individuals more at risk, the rainy season appeared to be a protective factor although this association was not significant. These results indicate that fever might not be the best indicator of submicroscopic infection, especially in months of high mosquito density. Previous longitudinal research has found that submicroscopic infection was associated with increased risk of fever among children 2 to 5 years old (Katrak et al.

2018). However, this study did not account for seasonal differences in its model and did not recruit from areas with varying transmission intensity.

In adjusted regression analysis, being a SAC was a significant predictor of fever in both seasons compared to younger children under the age of 5. In the dry season, SACs had 1.80 times the odds of younger children for having a fever and in the rainy season, their odds were 1.47 times greater (see **Table 3.**). SACs also had higher prevalence of submicroscopic infection reinforcing research showing that SACs are important reservoirs of malaria infection in this sample (Coalson et al. 2018) and similar populations in sub-Saharan Africa (Lamptey et al. 2018). These results support the need to target SACs, who often have low-density infections and are asymptomatic, to facilitate successful interventions aimed at eliminating malaria transmission.

Study Strengths and Limitations

A strength of this study is its ability to characterize the burden of malarial disease at the individual-level within communities with varying transmission intensity. This study offers a cost-effective way to examine the relationships between a potential clinical consequence of low-level infection and both behavioral and environmental factors related to malaria. This study also specifically enrolled school-aged children and young adults, populations that account for the majority of new infection in this population, but have been ignored in similar research examining clinical outcomes of submicroscopic infection (Katrak et al. 2018; Mawili-Mboumba et al. 2017). Importantly, this study utilizes a large, household-based sample rather than a sample obtained from a healthcare setting. Therefore, this study is more

representative of infection in the community and does not rely on the participants to self-report their illness. This study is unbiased by confounding factors, such as severity of physical illness or access to a healthcare facility, that might lead an individual to seek treatment.

One limitation of this study is the possibility that the identification of fever was mischaracterized. Given the nature of cross-sectional data, it is more difficult to distinguish between fever caused by a low-level malaria infection and some other febrile illness. Future research requires access to accurate health records or longitudinal follow-up to confirm the relationship between submicroscopic infection and clinical outcomes.

The sensitivity analysis revealed that the inclusion of self-reported fever in the past 48 hours did impact the results of the study. When using only the objective definition of fever, a temperature of ≥ 37.5 °C measured on the day of the interview, the association between submicroscopic infection and fever became insignificant in both the dry and rainy seasons. Given the reduction in the number of fever cases when using the objective measure (n=161 compared to n=1,023), the final models might not have been properly powered to detect significant associations. Another limitation to including a self-reported variable is the possibility that those who self-reported fever in the past 48 hours might have been mistaken. If non-differential misclassification bias occurred, it would have biased the final results towards the null.

Additionally, the comparison between excluded and included participants further revealed that the excluded participants differed from the analytic sample on all characteristics and notably had a significantly higher prevalence of fever and lower

prevalence of submicroscopic infection. Therefore, if the participants included in the analytic sample were not representative of the population and underrepresented prevalence of fever and overrepresented submicroscopic infection, the association might have been weakened.

The field team works during the day, so it is possible that SAC

and adults are less likely to be present to participate in the study compared to younger children and mothers. Therefore, selection bias might have influenced the association between submicroscopic infection and fever if certain age groups were more likely to have provided samples and report on physical health. If younger children were more likely to be in the study, the analytic sample is not representative of the population of interest and the results are not generalizable. It is also likely that the SACs and adults that were home during the interviews were more likely to be sick rather than at school or work. This would bias the association between submicroscopic infection and fever towards the null because the prevalence of fever would be overrepresented. However, the research team did make efforts to alert the community of the survey date and by waiting at the site until the end of the day. It is possible that recall bias might also result in underestimation of fever in older compared to younger children if the mother is reporting on recent symptoms.

Significance

Although fever might not be a consistent marker of low parasitemia, individuals with submicroscopic malaria infection are important reservoirs of transmission that might need to be identified and eliminated to fully interrupt malaria transmission. This study suggests that treating low parasitemia infections might not

be worthwhile for the general population in high transmission countries. However, research shows that submicroscopic infection is associated with considerable risk in certain sub-populations. For example, a recent, longitudinal study following 637 Ugandan women during pregnancy found that even women with submicroscopic infection have an increased risk of placental malaria (Briggs et al. in press). All of the women with low parasitemia infection were febrile. Another sub-population of concern might be travelers from endemic to malaria naïve areas. A recent study of immigrants and travelers from sub-Saharan Africa showed a relatively high prevalence of submicroscopic malaria infection [8.9%; (Pousibet-Puerto et al. 2019)]. Given the risk of spreading a malaria reservoir to countries without endemicity, systematic screening programs for migrants from sub-Saharan Africa and other endemic areas might be justified.

Additional research is needed to determine what factors best predict who will have clinical consequences of low parasitemia infections. This study found that season, or mosquito density, in a community influences the risk of fever in individuals with submicroscopic infection. Several other factors such as occupation and immunity were not measured directly in this study but could be important predictors of clinical consequences of submicroscopic infection. In this study, age and transmission setting did not appear to be adequate proxies for prior exposure to infection and so other measures that are clinically meaningful need to be determined. Longitudinal studies that comprehensively monitor health status should determine if other febrile infections confound the association between low parasitemia and fever and distinguish between an active submicroscopic infection and a recently treated

malaria infection. Future studies should also follow-up with participants who experienced fever as a result of submicroscopic infection to determine if their health outcomes differ from those who did not experience fever.

Ultimately, public health strategies attempting to eliminate malaria transmission in sub-Saharan Africa, where low-density infections are ubiquitous, will require diagnostic tools sensitive enough to identify all of those who make up the infectious reservoir. These sensitive diagnostic tools are in development but require continued testing and evaluation of implementation in the field (Tao et al. 2019). More sensitive diagnostic tools in clinical practice might also reduce misdiagnosis of malaria in febrile patients, thereby reducing the opportunity for drug-resistance to develop.

Conclusion

In this study, submicroscopic infection predicted fever among all ages in the dry season but not in the rainy season. This study adds important findings to a small body of literature on the clinical implications of low-density malaria infections. The transmission intensity of the community did not impact the relationship between submicroscopic infection and fever nor did utilization of a common malaria prevention strategy, ITNs. The use of fever as a marker for low-density infections is not reliable during months of high mosquito density and the low effect size in months of low mosquito density suggests that fever is not a clinically relevant marker for submicroscopic infection. Therefore, it might not be reasonable to test for and treat all low-density infections at the individual-level. However, this study did find a considerable proportion of submicroscopic infection in the population which would

not have been detected without more sensitive testing, such as PCR. If malaria transmission is to be eliminated, more sensitive diagnostic tests must be used routinely to detect low parasitemia. Future studies should determine if other factors predict fever among individuals with submicroscopic infection and longitudinal research is needed to characterize the clinical implications of fever among this group.

Appendix A. Institutional Review Board: Determination of Not Human Subject Research

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

1204 Marie Mount Hall
College Park, MD 20742-5125
TEL 301.405.4212
FAX 301.314.1475
irb@umd.edu
www.umresearch.umd.edu/IRB

DATE:	February 27, 2019
TO:	Angelica Barrall
FROM:	University of Maryland College Park (UMCP) IRB
PROJECT TITLE:	[1388824-1] The association between submicroscopic malaria infection and fever: Findings from a cross-sectional study in Malawi
REFERENCE #:	
SUBMISSION TYPE:	New Project
ACTION:	DETERMINATION OF NOT RESEARCH
DECISION DATE:	February 27, 2019

Thank you for your submission of New Project materials for this project. The University of Maryland College Park (UMCP) IRB has determined this project does not meet the definition of human subject research under the purview of the IRB according to federal regulations.

We will retain a copy of this correspondence within our records.

If you have any questions, please contact the IRB Office at 301-405-4212 or irb@umd.edu. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within University of Maryland College Park (UMCP) IRB's records.

Appendix B. Data Use Agreement between University of Maryland-Baltimore and University of Maryland-College Park

UNIVERSITY OF MARYLAND
Research and Development

DATA TRANSFER AND USE AGREEMENT

This Data Transfer and Use Agreement ("Agreement") is entered into upon the last dated signature ("Effective Date") by and between The University of Maryland, Baltimore a public institution of the University System of Maryland and an agency of the State of Maryland, with a place of business at 620 W. Lexington Street, Baltimore, MD 21201 ("Provider"), and University of Maryland, College Park ("Recipient"), and concerns the transfer of data by Provider to Recipient for the purposes and under the terms and conditions set forth herein.

1. Definitions. For the purposes of this Agreement, the following terms have the same meaning and effect as those set forth in 45 CFR Parts 160 and 164 ("HIPAA Privacy Rule").

a. "Covered Entity" (45 CFR § 160.103) means an organization, individual, institution, or other entity that is subject to the standards, requirements, and implementation specifications of the HIPAA Privacy Rule with respect to Protected Health Information (45 CFR § 160.103).

b. "De-identified information" (45 CFR § 164.514) means information that formerly contained individually identifiable health information but which has had all unique identifying information, numbers, characteristics, and codes removed such that the information a record contains cannot be used alone or in combination with other information to identify the individual who is the subject of the information. Identifying information includes but is not limited to the eighteen (18) categories of identifiers described in 45 CFR 164.514(b)(2).

c. "Protected Health Information ("PHI")" (45 CFR § 164.103) means any information, whether oral or recorded in any form or medium: (i) that relates to the past, present, or future physical or mental condition or an individual; the provision of healthcare to an individual; or the past, present or future payment for the provision of health care to an individual, and (ii) that identifies the individual or with respect to which there is a reasonable basis to believe the information can be used to identify the individual.

d. "Limited Data Set ("LDS")" (45 CFR 164.514(e)(2)) means Protected Health Information that excludes the sixteen (16) direct identifiers listed in that section. Any such information that identifies the individual who is the subject of the PHI, his or her relatives, employers, or household members must be removed for the PHI to constitute an LDS. Unlike de-identified PHI, an LDS may contain postal address information, including a

town, city, State, or zip code; age; specific dates, for example, dates of birth, death, admission, treatment, or release; and any other information not specifically listed in that section, that could be used alone or in combination with other information to identify a specific individual.

2. Description of Data. The data to be transferred to Recipient is data collected under the project entitled, "Epidemiology of Malaria in Malawi: Human hosts and parasites in three districts Part 2: Cross-sectional surveillance" ("Data")

   If Data constitutes or includes any Protected Health Information, such PHI shall be transferred to Recipient in the form of an LDS. Provider shall remove all direct identifiers from the LDS prior to transfer to Recipient. For the purpose of this Agreement, "Data" shall include any LDS.

3. Collection of Data. Provider represents that the collection and transfer of Data has been and will be conducted in compliance with all applicable laws and regulations and that all necessary approvals, authorizations and assurances have been obtained.

4. Transfer and Use of Data. Recipient shall use Data solely to: analyze the data ("Purpose"). To the extent that any LDS is transferred to Recipient hereunder, Provider and Recipient expressly intend that this Agreement will constitute a "data use agreement" in accordance with 45 CFR § 164.514(e)(4), and that Recipient's use of the LDS shall be solely for the purpose as authorized hereunder.

5. Responsibilities and Authorizations of Recipient.

   a. Recipient agrees to use and disclose Data in accordance with all applicable laws, regulations, and policies. Recipient represents that Recipient has obtained all approvals, authorizations, and assurances necessary for receipt and use of the Data, including but not limited to approval of Recipient's Institutional Review Board.

   b. Recipient agrees that it will only use the Data for the Purpose, and shall not use or disclose any LDS in any manner that would constitute a violation of any law or regulation (including the HIPAA Privacy Rule) if such use or disclosure was made by Provider.

   c. Recipient is not authorized and shall not further disclose Data other than as permitted by this Agreement or as required by law. Recipient shall not distribute Data to any third party without prior written consent from Provider.

   d. Recipient shall use appropriate administrative, technical, and physical safeguards to prevent use or disclosure of an LDS other than as authorized by this Agreement.

   e. Recipient shall notify Provider in writing within five (5) working days of its discovery of any use or disclosure of any LDS that is not authorized by this Agreement, of which Recipient, its officers, employees, or agents become aware. Recipient shall take (i)

prompt corrective action to cure any deficiencies or (ii) any action pertaining to such unauthorized disclosure required by applicable law.

f. Recipient shall not identify or contact any donor or living relative who is associated with any Data received from Provider pursuant to this Agreement. Recipient shall not attempt to obtain or otherwise acquire any PHI associated with the Data beyond that provided in an LDS by the Provider.

g. Recipient shall retain and shall comply with the terms of this Agreement for as long as Recipient retains Data, in perpetuity.

6. Breach or Violation; Hold Harmless. Provider shall not be responsible for any breach or violation of this Agreement or of any law or regulation applicable to the Data. Provider shall have the right to report any such breach or violation of this Agreement with respect to PHI or an LDS by Recipient to the Secretary of the Department of Health and Human Services.

The state of Maryland to the extent provided by the Maryland Tort Claims Act ("the Act'), Title 12, Subtitle 1, State Government Article, Annotated Code of Maryland, which permits, under certain circumstances and subject to limitations provided by law, claims in tort against the State of Maryland related to negligence of University of Maryland employees, will be responsible for all damages to persons or property incurred due to either party's activity performed under this agreement. In order to file a claim under the Act, a claimant must submit a written claim to the Treasurer of the State of Maryland or a designee of that office within one year after the injury to the person or property that is the basis for the claim.

7. Intellectual Property. Provider shall retain ownership of all Data and shall have the unrestricted right to use, disclose, and transfer Data to third parties. Recipient acknowledges and agrees that nothing herein shall be deemed to grant to Recipient any intellectual property rights in any Data.

8. Results of Data Use. Recipient shall notify Provider, in confidence, of results arising from the use of the Data by providing Provider with a draft manuscript describing such results.

9. Disclaimer. Subject to the limited representations in Section 3 of this Agreement, Data is provided WITHOUT REPRESENTATION OR WARRANTY OF ANY KIND, EITHER EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY OTHER WARRANTY, EXPRESS OR IMPLIED. PROVIDER MAKES NO REPRESENTATION OR WARRANTY THAT THE USE OF THE DATA WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHT.

10. Return or Destruction of Data. Upon written request of the Provider, Recipient shall return all Data to Provider, or shall destroy all Data as directed by Provider.

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Provider:

University of Maryland, Baltimore

Stephen Peterson

Digitally signed by Stephen Peterson
DN: cn=Stephen Peterson, o=University of Maryland, Baltimore, ou=Sponsored Programs, email=speterson@umaryland.edu, c=US
Date: 2019.02.01 10:49:43 -05'00'

Name: Stephen Peterson
Title: Contracts Manager
Date: 2/1/2019

Recipient:

University of Maryland, College Park

Katie McKeon

Name: Katie McKeon
Title: Assistant Director, ORA

Date: 01/22/2019

Read and Understood:

Provider Scientist:

Name: Miriam K. Laufer, MD, MPH
Title: Professor of Pediatrics
Date: 01/24/2019

Recipient Scientist:

Name: Angelica Barrall

Date: 1/21/19

Appendix C. MPH Competencies Addressed in Thesis

The MPH competencies that will be addressed in this thesis are:

- Identify vital statistics and other key sources of data for epidemiological purposes.
- Describe a public health problem in terms of magnitude, person, time and place.
- Comprehend basic ethical and legal principles pertaining to the collection, maintenance, use and dissemination of epidemiologic data.
- Explain the importance of epidemiology for informing scientific, ethical, economic and political discussion of health issues.
- Apply the basic terminology and definitions of epidemiology.
- Calculate basic epidemiology measures.
- Communicate epidemiologic information to lay and professional audiences.
- Differentiate among the criteria for causality.
- Draw appropriate inferences from epidemiologic data.
- Describe epidemiologic study designs and assess their strengths and limitations.
- Evaluation the strengths and limitations of epidemiologic reports.
- Calculate advanced epidemiology measures.
- Design, analyze, and evaluate an epidemiologic study.

Appendix D. Comparison of included and excluded participants (n=19,586)

Sample characteristics *n* (%)	**Excluded (*n*=5,354)**	**Included (*n*=14,232)**	**p value**
District			
Blantyre	1498 (28.0)	4852 (34.1)	<0.0001
Chikwawa	2391 (44.7)	4502 (31.6)	
Thyolo	1465 (27.4)	4878 (34.3)	
Age			
Less than 5 years	908 (18.9)	2435 (17.1)	<0.0001
5-15 years	2215 (46.0)	4912 (34.5)	
Over 15 years	1690 (35.1)	6885 (48.4)	
Female	2916 (54.5)	8640 (60.7)	<0.0001
Mean household size (SD)	4.9 (1.79)	4.8 (1.80)	<0.0001
Living in household with one or more ITNs	3760 (70.2)	10742 (75.5)	<0.0001
Among houses with ITNs			
Children under 5 years who use ITNs	550 (81.9)	1429 (76.9)	0.007
Children 5-15 years who use ITNs	882 (55.7)	2209 (58.9)	0.03
Adults over 15 years who use ITNs	855 (70.1)	3746 (73.0)	0.04
Transmission intensity			
Low	881 (16.5)	3513 (24.7)	<0.0001
Moderate	1485 (27.7)	5122 (36.0)	
High	2988 (55.8)	5597 (39.3)	
Submicroscopic infection	148 (2.8)	1159 (8.1)	<0.0001
Fever (past 48 hours)	698 (13.0)	1023 (7.2)	<0.0001

Note. P value of <0.05 identifies significant difference as determined by a Chi square test.

Appendix E. Crude and adjusted analyses predicting fever measured by temperature (n=14,232)

	Bivariate	**Multivariate**			**Final Models**	
		Model 1	**Model 2**	**Model 3**	**Dry (*n*=7,801)**	**Rainy (*n*=6,431)**
Submicroscopic infection	1.34 (0.71, 2.55)	1.54 (0.80, 2.95)	1.34 (0.71, 2.56)	1.34 (0.70, 2.55)	1.96 (0.61, 6.26)	1.03 (0.47, 2.27)
Age						
Children <5	Ref	-	-	-	Ref	Ref
Children 5-15	1.24 (0.80, 1.91)	-	-	-	1.52 (0.86, 2.70)	0.87 (0.43, 1.73)
Adults >15	1.27 (0.84, 1.91)	-	-	-	1.50 (0.88, 2.57)	0.97 (0.50, 1.87)
Transmission intensity						
Low	Ref	Ref	-	-	-	-
Moderate	**0.62 (0.39, 0.98)**	**0.61 (0.38, 0.97)**	-	-	-	-
High	**0.51 (0.33, 0.81)**	**0.49 (0.31, 0.78)**	-	-	-	-
Season						
Dry	Ref	-	Ref	-	-	-
Rainy	0.99 (0.73, 1.36)	-	0.99 (0.72, 1.35)	-	-	-
ITN use						
Yes	Ref	-	-	Ref	-	-
No	1.06 (0.78, 1.45)	-	-	1.06 (0.78, 1.45)	-	-

Note. Odds ratios and 95% confidence intervals from bivariate, multivariate, and mixed effect logistic regression. Bold text indicated significant association (p<0.05). Model 1: submicroscopic infection+transmission intensity. Model 2: submicroscopic infection+season. Model 3: submicroscopic infection+ITN use. Final models: submicroscopic infection+survey number (1-6)+age.

Bibliography

Adams M, Joshi SN, Mbambo G et al. An ultrasensitive reverse transcription polymerase chain reaction assay to detect asymptomatic low-density Plasmodium falciparum and Plasmodium vivax infections in small volume blood samples. Malar J 2015; 14 (1):520.

Barnes KI, Little F, Mabuza A et al. Increased gametocytemia after treatment: An early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. J Infect Dis 2008; 197 (11):1605-13.

Bousema JT, Schneider P, Gouagna LC et al. Moderate effect of artemisinin-based combination therapy on transmission of Plasmodium falciparum. J Infect Dis 2006; 193 (8):1151-9.

Bousema T, Okell L, Felger I et al. Asymptomatic malaria infections: Detectability, transmissibility and public health relevance. Nat Rev Microbiol 2014; 12 (12):833-40.

Briggs J, Ategeka J, Kajubi R et al. Impact of microscopic and submicroscopic parasitemia during pregnancy on placental malaria in a high-transmission setting in Uganda. J Infect Dis in press.

Buchwald AG, Coalson JE, Cohee LM et al. Insecticide-treated net effectiveness at preventing *Plasmodium falciparum* infection varies by age and season. Malar J 2017; 16:32.

Centers for Disease Control and Prevention. Malaria. 2018 [cited 2018 November 3].

Chen I, Clarke SE, Gosling R et al. "Asymptomatic" malaria: A chronic and debilitating infection that should be treated. PLoS Med 2016; 13 (1):e1001942.

Coalson JE, Cohee LM, Buchwald AG et al. Simulation models predict that school-age children are responsible for most human-to-mosquito Plasmodium falciparum transmission in southern Malawi. Malar J 2018; 17 (1):147.

Coleman RE, Kumpitak C, Ponlawat A et al. Infectivity of asymptomatic Plasmodium-infected human populations to Anopheles dirus mosquitoes in western Thailand. J Med Entomol 2004; 41 (2):201-8.

Cotter C, Sturrock HJ, Hsiang MS et al. The changing epidemiology of malaria elimination: New strategies for new challenges. Lancet 2013; 382 (9895):900-11.

Cottrell G, Moussiliou A, Luty AJ et al. Submicroscopic Plasmodium falciparum infections are associated with maternal anemia, premature births, and low birth weight. Clin Infect Dis 2015; 60 (10):1481-8.

Dechavanne C, Cottrell G, Garcia A et al. Placental malaria: Decreased transfer of maternal antibodies directed to Plasmodium falciparum and impact on the incidence of febrile infections in infants. PLoS One 2015; 10 (12):e0145464.

Dicko A, Mantel C, Kouriba B et al. Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. Trop Med Int Health 2005; 10 (6):550-6.

Dobbs KR, Dent AE. Plasmodium malaria and antimalarial antibodies in the first year of life. Parasitology 2016; 143 (2):129-38.

Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clin Microbiol Rev 2009; 22 (1):13-36.

Gatton ML, Cheng Q. Evaluation of the pyrogenic threshold for Plasmodium falciparum malaria in naive individuals. Am J Trop Med Hyg 2002; 66 (5):467-73.

Gaye A, Bousema T, Libasse G et al. Infectiousness of the human population to Anopheles arabiensis by direct skin feeding in an area hypoendemic for malaria in Senegal. Am J Trop Med Hyg 2015; 92 (3):648-52.

Harris PA, Taylor R, Thielke R et al. Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009; 42 (2):377-81.

Hofmann N, Mwingira F, Shekalaghe S et al. Ultra-sensitive detection of Plasmodium falciparum by amplification of multi-copy subtelomeric targets. PLoS Med 2015; 12 (3):e1001788.

Idro R, Marsh K, John CC et al. Cerebral malaria: Mechanisms of brain injury and strategies for improved neurocognitive outcome. Pediatr Res 2010; 68 (4):267-74.

Imwong M, Hanchana S, Malleret B et al. High-throughput ultrasensitive molecular techniques for quantifying low-density malaria parasitemias. J Clin Microbiol 2014; 52 (9):3303-9.

Jamison D, Feachem R, Makgoba M et al. Disease and mortality in Sub-Saharan Africa, 2nd edition. Washington, DC: The International Bank for Reconstruction and Development / The World Bank; 2006.

Katrak S, Nayebare P, Rek J et al. Clinical consequences of submicroscopic malaria parasitaemia in Uganda. Malar J 2018; 17 (1):67.

Lamptey H, Ofori MF, Kusi KA et al. The prevalence of submicroscopic Plasmodium falciparum gametocyte carriage and multiplicity of infection in children, pregnant women and adults in a low malaria transmission area in Southern Ghana. Malar J 2018; 17 (1):331.

Lin JT, Saunders DL, Meshnick SR. The role of submicroscopic parasitemia in malaria transmission: What is the evidence? Trends Parasitol 2014; 30 (4):183-90.

Mawili-Mboumba DP, Ndong RN, Rosa NB et al. Submicroscopic falciparum malaria in febrile individuals in urban and rural areas of Gabon. Am J Trop Med Hyg 2017; 96 (4):815-8.

Mmbando BP, Lusingu JP, Vestergaard LS et al. Parasite threshold associated with clinical malaria in areas of different transmission intensities in north eastern Tanzania. BMC Med Res Methodol 2009; 9 (1):75.

Mohammed AH, Salih MM, Elhassan EM et al. Submicroscopic Plasmodium falciparum malaria and low birth weight in an area of unstable malaria transmission in Central Sudan. Malar J 2013; 12:172.

Mohapatra M. The natural history of complicated Falciparum malaria - A prospective study. J Assoc Phys India 2006; 54:848-53.

National Malaria Control Programme and ICF International. Malawi malaria indicator survey (MIS). Lilonge, Malawi and Calverton, Maryland.

Nwakanma D, Kheir A, Sowa M et al. High gametocyte complexity and mosquito infectivity of Plasmodium falciparum in the Gambia. Int J Parasitol 2008; 38 (2):219-27.

Okell LC, Bousema T, Griffin JT et al. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun 2012; 3:1237.

Ouedraogo AL, Goncalves BP, Gneme A et al. Dynamics of the human infectious reservoir for malaria determined by mosquito feeding assays and ultrasensitive malaria diagnosis in Burkina Faso. J Infect Dis 2016; 213 (1):90-9.

Pava Z, Burdam FH, Handayuni I et al. Submicroscopic and asymptomatic Plasmodium parasitaemia associated with significant risk of anaemia in Papua, Indonesia. PLoS One 2016; 11 (10):e0165340.

Pousibet-Puerto J, Cabezas-Fernández MT, Lozano-Serrano AB et al. Submicroscopic malaria in migrants from Sub-Saharan Africa, Spain. Emerg Infect Dis 2019; 25 (2):349-52.

President's Malaria Initiative. Malawi: Malaria Operational Plan FY 2018. Washington, DC.

Rantala AM, Taylor SM, Trottman PA et al. Comparison of real-time PCR and microscopy for malaria parasite detection in Malawian pregnant women. Malar J 2010; 9:269.

Roper C, Elhassan IM, Hviid L et al. Detection of very low level Plasmodium falciparum infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. Am J Trop Med Hyg 1996; 54 (4):325-31.

Sinka ME, Bangs MJ, Manguin S et al. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis. Parasit Vectors 2010; 3 (1):117.

Tao D, McGill B, Hamerly T et al. A saliva-based rapid test to quantify the infectious subclinical malaria parasite reservoir. Science translational medicine 2019; 11 (473):1-13.

The World Bank. GDP per capita, all countries and economies. 2017 [cited 2018 November 6].

Turner AG, Magnani RJ, Shuaib M. A not quite as quick but much cleaner alternative to the Expanded Programme on Immunization (EPI) cluster survey design. Int J Epidemiol 1996; 25 (1):198-203.

United Nations. World population prospects: The 2017 revision, Volume II: Demographic profiles. New York, NY.

Walldorf JA, Cohee LM, Coalson JE et al. School-age children are a reservoir of malaria infection in Malawi. PLoS One 2015; 10 (7):e0134061.

Wongsrichanalai C, Barcus MJ, Muth S et al. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg 2007; 77 (6 Suppl):119-27.

World Health Organization. Global health estimates 2016: Deaths by cause, age, sex, by country and by region, 2000-2016. Geneva, Switzerland.

World Health Organization. Malaria - Fact Sheet. 2018b.

World Health Organization. World health statistics 2018: Monitoring health for the Sustainable Development Goals. Geneva, Switzerland.

World Health Organization. World malaria report, 2018. . Geneva, Switzerland.

Zaw MT, Thant M, Hlaing TM et al. Asymptomatic and sub-microscopic malaria infection in Kayah State, eastern Myanmar. Malar J 2017; 16 (1):138.