MEASURING GEOSPATIAL AND LONGITUDINAL TRENDS IN MALARIA USING NATIONAL HEALTH SURVEILLANCE DATA IN MALAWI

Hillary M. Topazian

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Approved by:

Audrey Pettifor

Jessie K. Edwards

Michael Emch

Irving Hoffman

Jonathan J. Juliano

Jennifer S. Smith

ABSTRACT

Hillary M. Topazian: Measuring Geospatial and Longitudinal Trends in Malaria Using National Health Surveillance Data in Malawi
(Under the direction of Audrey Pettifor)

Malaria is a significant cause of morbidity and mortality in Malawi, accounting for 30% of outpatient visits. Children report the highest rates of disease, but adults are thought to be an important reservoir to sustained transmission due to persistent asymptomatic infection. However, national malaria measures have been exclusively estimated through Malaria Indicator Surveys which only offer a regional cross-sectional glance at parasite prevalence among children. Malaria is geographically and temporally heterogeneous, making it necessary to understand local and longitudinal transmission patterns before planning targeted interventions. Long-lasting insecticide treated bed nets (LLINs) are integral to Malawi's malaria prevention efforts and are allocated to households every 3-years through mass distribution campaigns. Evidence suggests that the maximum protective lifespan of LLINs is 1-3-years, but a national longitudinal evaluation has never been conducted in Malawi.

The 2015-2016 Malawi Demographic and Health Survey (MDHS) was a nationally representative household survey of asymptomatic individuals ages 15-54. We analyzed 7,393 survey samples, detecting a *P. falciparum* prevalence of 31.1%. Protective factors included urbanicity, greater wealth, higher education, and lower environmental temperatures, but living in a household with a bed net and sleeping under an LLIN were not protective against infection.

To assess clinical malaria over time, we used District Health Information Software 2 (DHIS2) data from January 2018 to June 2020, capturing monthly aggregate reports of confirmed cases from 711 health facilities across Malawi. We found that risk varied at the health facility level and was highest from January to May. Risk decreased one high transmission season following distribution of 10.7 million LLINs and rebounded after two seasons, indicating that LLINs only have a lifespan of 1-2-years. Piperonyl butoxide-treated nets performed better than pyrethroid-treated nets and LLIN effectiveness varied geographically.

Our findings demonstrate a high parasite prevalence among adults, highlighting this population as an important reservoir to sustained transmission. LLINs have a 1-2-year lifespan and insecticide type influences effectiveness. Use of molecular surveillance to supplement ongoing passive data collection creates a natural opportunity to track how large-scale changes affect malaria over time and provide valuable insight to the Malawi Ministry of Health as they plan future interventions.

For my parents, who showed me how to love learning.

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DBS	dried blood spot
DHS	Demographic and Health Survey
HRP-2	histidine-rich protein 2
IRS	indoor residual spraying
ITN	insecticide treated net
LLIN	long-lasting insecticide-treated net
MDHS	Malawi Demographic and Health Survey
MIS	Malaria Indicator Survey
MOH	Ministry of Health
NMCP	National Malaria Control Programme
PBO	piperonyl butoxide
PCR	quantitative polymerase chain reaction
RDT	rapid diagnostic test
SSA	sub-Saharan Africa
UNC	University of North Carolina
USAID	United States Agency for International Development
WHO	World Health Organization

CHAPTER ONE: SPECIFIC AIMS

There were an estimated 7.0 million malaria cases in Malawi in 2018.[1] Malaria remains an important cause of childhood morbidity and mortality, with an estimated annual *Plasmodium. falciparum* parasite rate of 18-19% in children 2-10 years in 2015-2016.[2] While children report the highest proportions of clinical disease,[3] adults are thought to be an important reservoir to sustained transmission due to persistent asymptomatic infections with low parasite densities undetectable by microscopy or rapid diagnostic test.[4] Malaria is also geographically and temporally heterogenous, and risk in Malawi has been found to be modified by several environmental risk factors including altitude, rainfall, temperature, potential evapotranspiration, and proximity to active agriculture.[5–7] Little is known about the prevalence of adult asymptomatic malaria infections, and if risk factors are similar to those among children.

Recent estimates of the malaria burden in Malawi have come from cross-sectional Malaria Indicator Surveys in 2012, 2014, and 2017. While these surveys randomly sampled households at a granular scale, they only offer estimates of malaria prevalence and preventative behaviors among children in broad geographic locations, and at specific points in time. Malawi has established a passive system, the District Health Information Software 2 (DHIS2), which provides monthly malaria statistics from health facilities in a cost-effective and timely manner, but no analysis has utilized DHIS2 to calculate malaria risk at a sub-district level. Long-lasting insecticide-treated nets (LLINs) are Malawi's predominant vector control strategy,[8] with bed nets given to all pregnant women, newborn babies, and to the general population every three years

through mass distribution campaigns.[9] However, there has been no national longitudinal study of the effects of mass distribution campaigns on long-term malaria risk.

The **objective** of the proposed analysis is to identify risk factors for the prevalence of asymptomatic *P. falciparum* among adolescents and adults ages 15-54, and to longitudinally assess the effectiveness of an LLIN mass distribution campaign on the monthly risk of confirmed malaria at the health facility level.

The **specific aims** of this proposal are to:

1. **Identify variations in the prevalence of asymptomatic PCR-positive *P. falciparum*, due to demographic, environmental, and spatial risk factors in adolescent and adults ages 15-54**. Using dried blood spots collected from the Malawi Demographic and Health Survey 2015-2016 (MDHS), we will conduct quantitative PCR to detect parasitemia. We will identify risk factors for infection taken from the MDHS and environmental data sources. Hypothesis: individuals using bed nets treated with non-permethrin insecticides will have a lower infection prevalence than individuals using permethrin-treated nets. Risk factors will be consistent with those observed in asymptomatic children and symptomatic children and adults.
2. **Assess differences in the monthly risk of confirmed malaria across health facilities in Malawi from 2018-2020, before and after a mass distribution campaign at the end of 2018.** Using existing DHIS2 health facility surveillance data, we will compare population risk across Malawi by month. We will stratify estimates between children <5 and persons ≥5 years, and by LLIN insecticide type or application of indoor residual spraying. All malaria cases presenting to clinics are captured in DHIS2 and the population denominator will be estimated from WorldPop geospatial data.[10]

Hypothesis: areas receiving piperonyl butoxide-treated nets will experience a greater reduction in malaria risk estimates as compared to areas receiving pyrethroid-treated nets, after the LLIN mass distribution campaign from September to December 2018.

Understanding the prevalence and spatial distribution of the asymptomatic malaria reservoir in Malawi is essential for implementation and evaluation of control measures. Molecular surveillance of *P. falciparum* in adults can be an important tool for the Malawi Ministry of Health (MOH) to supplement ongoing collection of clinical data through DHIS2 and periodic national surveys of prevalence among children. Assessment of risk measures will provide valuable insight into current and historical malaria case trends and advise the type and frequency of future LLIN mass distribution campaigns.

CHAPTER TWO: BACKGROUND AND INNOVATION

Overview of Malaria

Malaria is caused by infection with a parasite transmitted by female *Anopheles* mosquitoes.[11] There are five species of malaria which infect humans: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi*. The malaria parasite is transmitted from the *Anopheles* mosquito to a human host through a blood meal; the parasite then multiplies in the liver and red blood cells, eventually forming a sexual stage which is taken up by another mosquito to continue the transmission cycle (**Figure 2.1**).

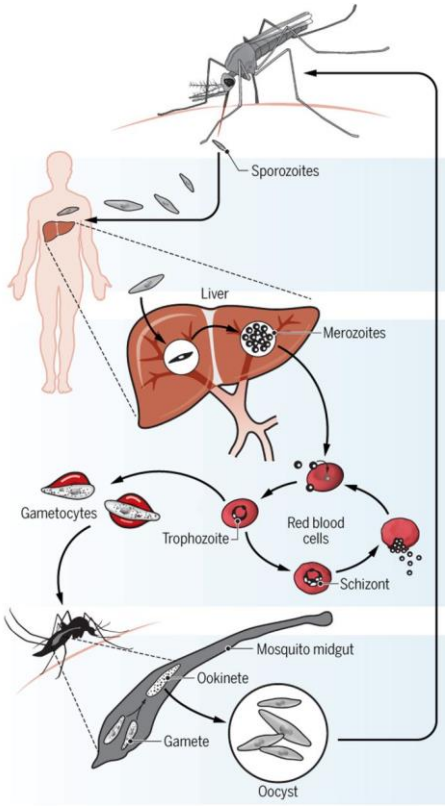


Figure 2.1 Malaria parasite life cycle (adapted).[12]

Once infected, humans experience a 7-30 day incubation period before manifestation of clinical disease, characterized by fever, chills, sweats, headaches, nausea, and vomiting.[11,13] Cases of severe and cerebral malaria can lead to seizures, severe anemia, acute respiratory distress syndrome, and death. Infection with malaria throughout the life-course can generate a certain degree of immunity to clinical disease; as children grow up, parasites can cause sub-clinical infection that remains untreated.[13] Immunity exists against the presence of high parasite densities (anti-parasite) and against development of malarial disease symptoms (anti-disease), and the presence of immunity varies by age and the transmission intensity in the setting of interest (high/moderate/low).[14] As a result, a reservoir exists among people asymptomatically infected with malaria, but whom are still able to transmit to others.[15]

Malaria is diagnosed through point-of-care rapid diagnostic tests (RDTs), microscopic examination looking for parasites in blood, or through nucleic acid amplification-based diagnostics such as polymerase-chain reaction (PCR)-based methods.[13,16] RDTs detect parasite antigens in the blood and are used in outpatient settings to quickly diagnose malaria. RDTs can occasionally result in false-positive results from persistence of histidine-rich protein 2 (HRP-2) antigen after treatment or false-negative results caused by *pfhrp2* gene deletions in some parasite strains.[13,17] Microscopy makes use of a microscope to visually detect parasites in blood smears. PCR requires more advanced technical capacity but can be used to detect an infection with low parasitemia that may not be identified through RDT or microscopy.[13,16] Diagnostic tests are important for providing quick results, but have limited lower limits of detection (microscopy: 50 parasites/μL of blood[18], RDT: 100-200 parasites/μL[19,20]). Microscopy is the standard testing procedure at hospitals, but can suffer from poor quality diagnosis; RDTs are particularly useful in remote areas and for quick management of malaria disease, and PCR is recommended in

research and survey settings due to the high sensitivity of molecular detection techniques.[16] Choice of diagnostic test can lead to drastically differing estimates of infection prevalence due to lower limits of detection; particularly among submicroscopic infections in low transmission areas.[21] In malaria endemic regions, microscopy misses half of *P. falciparum* infections found by PCR,[22] yest theses submicroscopic infections are likely to contribute to sustained transmission in areas where use of microscopy results low infection prevalences.[21]

Global Burden of Malaria

There were 228 million malaria cases globally in 2018, primarily occurring in the World Health Organization's (WHO) Africa (93%) and South-East Asia Regions (3.4%) (**Figure 2.2**).[23] An estimated 405,000 deaths from malaria occurred worldwide in 2018, with children under 5 years representing 67% of all fatalities. The incidence rate of malaria has declined since 2010, although the rate of change has slowed in the last few years. WHO technical strategy goals for 2030 include reducing malaria incidence by 90% and eliminating malaria in 35 countries.[24]

In sub-Saharan Africa, the number of cases decreased from 2010 to 2015, but has risen steadily each year from 2016-2018,[23] indicating the need for increased malaria prevention implementation in the region and the development of new technologies. The number of malaria deaths has declined steadily since 2010 in sub-Saharan Africa. *P. falciparum* is the most common species in Africa, representing 99.7% of all infections.[23]

Funding for malaria control and elimination reached US$ 2.7 billion in 2018, representing key partnerships between endemic countries and the global community, with 70% coming from the international parnters.[23] The majority, 37.8%, of malaria funding from 1997-2013 came from public health research, with 33.8% from clinical trials.[25] Malaria research represents an important part of monitoring malaria trends, determining the effectiveness of

interventions, developing new treatment and prevention strategies, and forming international partnerships.

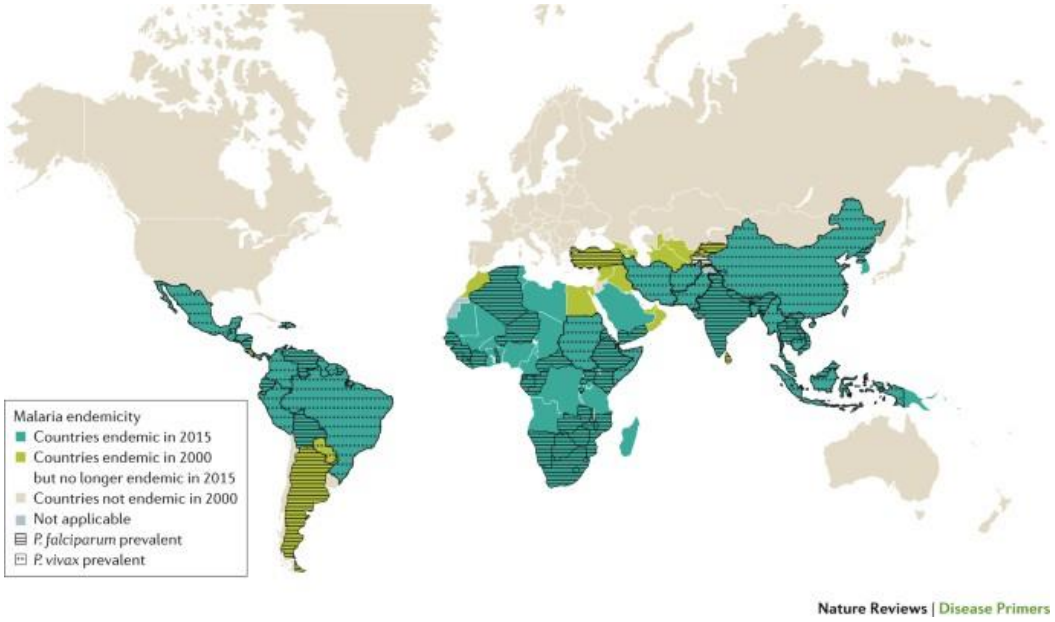


Figure 2.2 Global distribution of malaria.[13]

Malaria Prevention Mechanisms

A plethora of interventions have been developed to reduce mosquito vector prevalence and to interrupt the transmission cycle from human to mosquito, including long-lasting insecticide treated bed nets (LLINs), indoor residual spraying (IRS), and chemoprevention. LLINs act as a physical barrier between humans and mosquito vectors, and as a chemical barrier, killing mosquitoes which land on the insecticide covering the net.[26] Resistance to insecticides such as pyrethroids, carbamates, organochlorines, and organophosphates, are widespread globally,[27] however an intact net treated with an ineffective insecticide can still provide benefit by acting as a physical obstacle. IRS involves spraying insecticide on the walls of a dwelling to kill mosquitoes which land on surfaces. Chemoprevention is also used to reduce seasonal malaria incidence and administered to children and pregnant women through intermittent preventive

treatment. Most recently, the RTS,S/AS01 vaccine has been developed to prevent malaria, but has shown mixed efficacy in sub-Saharan Africa by age group and geographic location in a Phase III trial.[28] However, even with simultaneous mass scale-up of LLINs, IRS, and chemoprevention strategies to 90% coverage, the introduction of novel interventions will still be necessary to even begin to consider global malaria eradication.[29]

Malaria in Malawi

Malaria is endemic in Malawi and the entire population is at risk of infection, particularly during annual high transmission seasons associated with greater rainfall.[30,31] Malaria patterns are geographically and temporally heterogenous in Malawi. Infection rates are higher along the shores of Lake Malawi and in the Shire river valley, and lower in cooler high elevation areas.[9] Cases occur all year round, however, transmission is highest following the annual rainy season, with elevated numbers of cases in January through May.[9] At present, national level clinical malaria data have only been released at the district and national levels, recording 7.0 million confirmed cases in 2018.[32] Malaria cases make up 30% of outpatient visits and 34% of inpatient hospital stays in Malawi,[9] placing a substantial burden of disease on the health care system.

Globally, malaria risk has been found to be modified by several environmental factors, including temperature, precipitation, water pooling, elevation, deforestation, agriculture, and others.[33] In Malawi some of these factors include altitude, rainfall, temperature, potential evapotranspiration, and proximity to active agriculture.[5–7] In Lilongwe, malaria incidence curves among children have been found to closely mimic curves of monthly rainfall, with highest malaria incidence estimates occurring shortly after the months of greatest rainfall (**Figure 2.3**).[34]

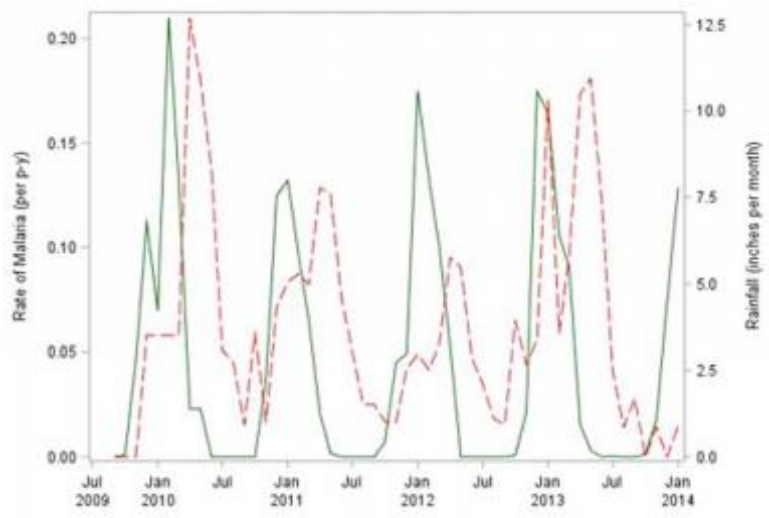


Figure 2.3 Malaria incidence and rainfall patterns, 2009-2014, Lilongwe, Malawi.[34]

Most research on malaria prevalence focuses on *P. falciparum* prevalence among children, an age group particularly at risk for malaria morbidity and death.[3] In 2017, prevalence by microscopy was 26% in the Central and Southern regions and 11% in the Northern region during the high malaria transmission season.[35] Employing models of survey data utilizing microscopy and RDT, the annual *P. falciparum* parasite rate was estimated to be 18-19% in children ages 2-10 years in Malawi in 2015-2016,[2] however there have been no national estimates of malaria prevalence among adults.

Insecticide-treated bed nets (ITNs) are Malawi's predominant vector control strategy,[8] with bed nets given to all pregnant women, newborn babies, and to the general population periodically through mass distribution campaigns. IRS is administered yearly in two districts, with plans for future expansion.[32] In addition, entomological monitoring takes place in targeted locations, and ITN durability monitoring occurs annually.

Prior research studying universal bed net distribution has shown short-term signs of benefit from ITN campaigns, but a lack of sustained effectiveness. One study demonstrated

decreasing estimates of parasite infection and clinical malaria among pregnant women after a mass distribution campaign in 2012, however prevalence began increasing two years following the campaign.[36] Increased malaria prevalence also occurred among children two years after mass distribution of 5.6 million nets, despite reporting increased bed net usage over the same time period.[37] Individual ITN use was associated with a lower individual risk of malaria at baseline, but this association disappeared within two years. Similarly, research among adolescents and adults has shown that sleeping under an LLIN was not associated with reduced risk of malaria prevalence 3-4 years after a mass distribution campaign, even after stratifying by bed net insecticide type.[38] Among school-aged children in Malawi, ITN use increased immediately following a local universal distribution campaign, but usage decreased back to baseline within 3 years, indicating that a universal ITN campaign combined with routine distribution is not sufficient for long term changes in net use.[39] In Malawi, individuals repurpose ITNs for fishing and farming, and sell nets to support their families encountering poverty and food-insecurity,[40] all of which contribute to reduced intervention effectiveness. Older ITNs are also associated with higher infection prevalence, despite the lack of association between net integrity and infection, suggesting that reduced insecticide concentrations over time also hinder effectiveness.[41]

Malawi is also beginning to evaluate programmatic use of RTS,S/AS01, a vaccine developed to reduce incidence of malaria among children in sub-Saharan Africa.[28] Malaria is an incredibly complex parasite, with over 5,000 genes and hundreds of potential targets across the *Plasmodium* life cycle, many of which have been and are currently being explored for vaccine development.[12] RTS,S/AS01 is constructed using the circumsporozoite protein C-terminal as the target antigen to generate a host response to parasite sporozoites in the bloodstream and dormant forms in the liver, prior to the host experiencing symptoms of disease.[42,43] Most recently, a

randomized Phase III trial of RTS,S/AS01 across 11 sites in sub-Saharan Africa from 2009-2013, estimated a study-wide vaccine efficacy of 36.3% (95% CI: 31.8 to 40.5) against clinical malaria in children 5-17 months of age, and 25.9% (95% CI: 19.9 to 31.5) in infants 6-12 weeks.[28] However, efficacy varied widely depending on geographic site. In Lilongwe, Malawi, point estimates of vaccine efficacy were higher than the trial's composite results, at 50.8% (95% CI: 31.4 to 64.7) in children 5-17 months and 38.9% (95% CI: 16.8 to 55.1) in infants 6-12 weeks. The best efficacy estimates were achieved with four doses of the vaccine. Programatic implementation of RTS,S/AS01 in Malawi began in April 2019 and will continue through 2024, with clusters of health facilities randomized to receive or to not receive the vaccine through the Malaria Vaccine Implementation Program evaluation, a partnership between the World Health Organization, the Malawi Ministry of Health, the University of North Carolina, and the Malawi College of Medicine.

The Malawi Ministry of Health has drafted specific goals to reduce malaria incidence and malaria-related deaths in the coming years. Malawi's Health Sector Strategic Plan aims to cut the malaria incidence rate of presumed and confirmed cases from 2015 levels of 380 per 1,000 to 200 per 1,000 by 2021.[44] The same plan also hopes to cut inpatient malaria deaths per year from 23 per 100,000 to 14 per 100,000 within the same time period.

Malaria Surveillance

In Malawi, historical estimates of the malaria burden and malaria prevention behaviors have only been estimated at the regional level at single points in time, such as from cross-sectional Demographic and Health Surveys (DHS) in 2010 and 2015/2016,[45,46] and Malaria Indicator Surveys (MIS) in 2012, 2014, and 2017.[35,47] Funded through USAID, DHS surveys are nationally-representative, randomized household surveys conducted in over 90 countries every

five years, collecting data on a multitude of heath indicators.[48] DHS surveys obtain blood samples from adults for HIV testing purposes; though they do not test individuals for malaria infection, surveys capture information on malaria prevention and treatment behaviors. In contrast, MIS surveys are specific to malaria and conducted more frequently. MIS surveys test children <5 years for malaria infection using RDTs and microscopy, and collect data on malaria indicators through household surveys in nearly 30 countries.[49] While both DHS and MIS national surveys randomly sampled households at a granular scale, they can only offer a high-level cross-sectional glance at malaria prevalence among children and preventative behaviors (such as bed net ownership and use) in broad geographic locations. For example, MIS 2017 found a national malaria prevalence of 24% in children by microscopy and could only produce three broad prevalence measures for the Northern, Central, and Southern regions, as these surveys only generate reliable estimates down to the regional level.[47] However, malaria in Malawi was proven to be geographically heterogenous at the sub-district level in the Northern region of the country from 2004-2006,[50] and has even been shown to be heterogenous down to the household level within a rural area of a single district.[51] Surveys also sample at specific points in time; DHS can take place at any time over the course of the year, and MIS is administered only during the high malaria transmission months; this temporal variation prohibits estimation of malaria patterns over both wet and dry seasons. DHS and MIS results have been compared in Cameroon and show varying malaria estimates when conducted in different transmission seasons.[52] DHS was also unable to capture high malaria prevalence clusters in certain geographical spaces, revealing the limitations of single cross-sectional surveys in areas with seasonal patterns and varying ecosystems. Additionally, these surveys require extensive time and resources dedicated to active surveillance data collection efforts; the average cost of a DHS survey is $1.6 million, involving

active visits to 27,516 individual households.[46,53] Other research involving *P. falciparum* age-standardised annual parasite rates in children[54] and predicted malaria risk across smaller land areas[55] have traditionally only been able to achieve a more granular focus by relying on models based on survey data.

With the introduction of District Health Information Software 2 (DHIS2), used as a health management information system in Malawi since 2015, each health facility reports malaria case statistics (and other health indicators) to the Ministry of Health monthly. This enables ascertainment of population malaria counts to calculate cumulative incidence continuously over time through passive surveillance. DHIS2 is a system used in 72 low and middle-income countries, covering 2.3 billion people.[56] The application is country-specific and web-based, but can be accessed offline.[57] Users can control the interface to analyze data, generate reports, and add modules tailored to a country's specific health needs. DHIS2 malaria data have already been analyzed in Uganda, Zimbabwe, and Kenya to determine the effect of interventions, assess DHIS2 monthly reporting completeness, and conduct spatial mapping studies.[58–61] In Malawi, DHIS2 has been in use by the Ministry of Health since 2015. Health Surveillance Assistants collect community-level data on paper forms which are then sent to health facilities to be entered into registers.[8] Patient data from registers are consolidated into de-identified summary reports and submitted to the District Health Program Coordinator on paper forms, where data are verified and then entered electronically into DHIS2. Reports include outpatient aggregated counts on the number of positive microscopy and rapid diagnostic test results, outpatient data on the number of suspected and confirmed cases, inpatient data on the number of suspected and confirmed cases, the number of inpatient malaria deaths, number of bed nets distributed to pregnant women and babies, and the number of Lumefantrine-Artemether

treatments administered.[8] Data are aggregated by health facility and month, and variables are stratified into children <5 years and persons ≥5 years.

In addition to collecting malaria case information, Malawi also administers insecticide resistance studies to determine the potential effectiveness of LLINs and IRS for future campaigns. Entomological monitoring takes place across various districts and is assessed by sampling mosquitoes of different species to determine sporozoite infection rates and to test susceptibility to various insecticides.[62] In 2017, high levels of pyrethroid and carbamate resistance were found, indicating the need for future testing of additional insecticides.

Innovation

Malaria is geographically heterogeneous, and malaria risk measures in Malawi vary at a fine geographical scale.[51] The proposed study would be the first to nationally evaluate malaria in Malawi at a sub-district level, using routinely collected health facility surveillance data to estimate variations in malaria cumulative incidence both geographically and temporally. Geographically granular estimates of malaria can be used to target interventions to specific villages, with ongoing surveillance data creating a natural opportunity to study how interventions affect malaria incidence over time. This will also be one of the first studies in Malawi to utilize DHIS2 data to study the effects of a health intervention. The design of this study will serve as a model for other countries that also use DHIS2 and whose governments could replicate this design for implementation of other health interventions through public and private health facilities. We are uniquely positioned to take advantage of this national data source to evaluate Malawi's largest malaria prevention effort, making efficient use of time and resources already being allocated to ongoing passive surveillance.

Asymptomatic and submicroscopic malaria are thought to be important reservoirs for malaria transmission as asymptomatic infections persist for months, allowing more time for the parasite to transmit.[22] This is the first molecular study in Malawi to create a national assessment of malaria infection among adolescents and adults. Assessment of asymptomatic malaria across Malawi and an analysis of risk factors will provide an understanding of the prevalence and spatial distribution of underlying infections to inform malaria prevention efforts and identify key target groups. Results can also serve as a baseline assessment for future evaluations of asymptomatic malaria prevalence following the 2021-2022 MDHS. In partnering with the Malawi Ministry of Health, our goal is to use malaria epidemiologic data to inform current interventions and future health policy and programs. Questions about granular risk measures at the health facility level, effects of LLIN mass distribution campaigns, and the prevalence of the asymptomatic malaria reservoir must be answered before population-level malaria policy decisions can be made, in order to fully grasp the benefits and long-term sustainability of interventions against infection.

CHAPTER THREE: ASYMPTOMATIC *PLASMODIUM FALCIPARUM* MALARIA PREVALENCE AMONG ADOLESCENTS AND ADULTS IN MALAWI, 2015-2016 [1]

Introduction

Malaria remains a significant cause of morbidity and mortality in Malawi, with an estimated 7.0 million confirmed cases reported in 2018.[32] *Plasmodium falciparum* is the most virulent of the malaria species, giving rise to 98% of infections in-country.[32] While children report the highest proportion of clinical disease,[3] adults are thought to be an important reservoir to sustained transmission due to persistent asymptomatic infection. Underlying infection is unlikely to be diagnosed and treated as individuals often do not exhibit signs and symptoms of disease and consequentially do not seek care. Even if an individual is tested, parasite densities in asymptomatic infections are usually low and may be undetectable by microscopy or rapid diagnostic test (RDT),[4,63] further reducing the likelihood of diagnosis and treatment. Asymptomatic infections can persist for months and older age is associated with an increased duration of persistent infection, allowing the parasite the opportunity to transmit for a prolonged period of time.[64] The annual *P. falciparum* parasite rate was estimated to be 18-19% in children ages 2-10 years in Malawi in 2015-2016,[2] however, little is known about the prevalence of adult asymptomatic *P. falciparum* infection.

[1] This chapter previously appeared as an article in *Scientific Reports*. The original citation is as follows: Topazian HM, Gumbo A, Puerto-Meredith S, Njiko R, Mwanza A, Kayange M, Mwalilino D, Mvula B, Tegha G, Mvalo T, Edwards JK, Emch M, Pettifor A, Smith JS, Hoffman I, Meshnick SR, Juliano JJ. Aymptomatic *Plasmodium falciparum* malaria prevalence among adolescents and adults in Malawi, 2015-2016. *Scientific Reports*; 2020. **10**:18740.

Malaria is geographically and temporally heterogenous. In Malawi, malaria risk has been found to be modified by several environmental factors, including elevation, rainfall, temperature, and proximity to active agriculture.[5–7] Insecticide-treated bed nets (ITN) are Malawi's primary vector control strategy,[8] with bed nets given to all households with pregnant women, newborn babies, and to the general population every three years through mass distribution campaigns.[9] ITNs act as both a physical and chemical barrier to repel and kill mosquitoes which land on the net.[26] Bed net use has been shown to have protective individual and community-level associations with *P. falciparum* infection in children under five living in Lilongwe, Malawi,[65] and more broadly across Africa,[66] however these relationships have not been established among Malawian adults. In addition, malaria vectors have varying levels of resistance to different types of insecticide used on ITNs, such as alpha-cypermethrin, permethrin, and deltamethrin,[62] however, the effect of ITN insecticide type on malaria prevalence has not been assessed at the population level.

The objective of the current analysis is to characterize and identify changes in the prevalence of asymptomatic *P. falciparum*, due to demographic, environmental, and spatial risk factors in Malawian adolescents and adults ages 15-54 years. Using dried blood spots collected from the 2015-2016 Malawi Demographic and Health Survey (2015-16 MDHS), we conducted quantitative polymerase chain reaction (PCR) to detect parasitemia. We then identified risk factors for infection using data taken from the 2015-16 MDHS and other spatial and environmental information sources.[67–69] A particular focus was placed on ownership and self-reported use of bed nets. Understanding the prevalence and spatial distribution of underlying infection is essential for implementation and evaluation of future interventions. Molecular surveillance of *P. falciparum* in adults can be an important tool for Ministries of Health to

supplement ongoing collection of clinical data through national health management information systems and periodic national household surveys of malaria prevalence among children.

Methods

Study design and population

The 2015-16 MDHS was a cross-sectional, nationally-representative survey enrolling 26,361 households, 24,562 individual female participants, and 7,478 individual male participants, from a total of 850 clusters between October 2015 and February 2016, during the annual transition between dry and rainy seasons.[46] In addition to household and individual interviews, women ages 15-49 and men ages 15-54 were also asked to contribute dried blood spots (DBS), collected on filter paper, to measure HIV prevalence. Of the 15,125 eligible individuals who contributed DBS for HIV testing, 7,393 (48.9%) unique DBS cards were subsequently located and found to have enough sample remaining for use in the current study. Informed consent was obtained from all individuals and/or their parent or legal guardian for participation in the 2015-16 MDHS, collection and storage of DBS, and additional testing of their samples. Ethical approval for our analysis was obtained from Institutional Review Boards through the National Health Sciences Research Committee at the Malawi Ministry of Health (#19/08/2381) and the University of North Carolina at Chapel Hill (#19-2882), and all research was performed in accordance with relevant guidelines and regulations.

DNA amplification and genotyping

DBS were punched into 96 well plates at UNC Project-Malawi and shipped to the University of North Carolina at Chapel Hill for testing. DNA was extracted from filter paper using Chelex and stored at -80°C. Further details on the DNA extraction protocol and PCR assay validation are included in the Technical Methods found in the Supplemental Material. We tested

each individual sample with a real time PCR assay targeting the *P.f. lactate de-hydrogenase* gene (*pfldh*) to identify individuals with *P. falciparum* malaria infection, our primary outcome.[70] Samples amplified with a PCR cycle threshold (C_T) value above 39 were considered negative. Sensitivity analyses considered a range of C_T value cut-offs to determine the impact of altering the sensitivity of the assay (**Supplementary Figure A1**).

Spatial and ecological variables

De-identified 2015-16 MDHS survey and geospatial data were linked to each sample's PCR results through random sample barcode. As part of the DHS methodology, GPS coordinates are collected in the field, marking the center of each cluster of households. The DHS program maintains participant confidentiality by displacing the GPS coordinates for all survey clusters: urban clusters are displaced up to a maximum of 2km and rural clusters up to 5km, with an additional 1% subset of rural clusters displaced up to 10km.[71] PCR *P. falciparum* prevalence was mapped onto a smoothed surface using a constructed semivariogram and simple kriging to predict regional variation in malaria prevalence. Simple kriging assumes that observed malaria prevalence is spatially autocorrelated and that there is a known mean trend which is stationary across our study area.

Individual and cluster level risk factors were selected based on directed acyclic graphs and known associations from relevant literature.[5–7,33,72] Individual level factors included sex, age group, wealth quintile, education level, owning livestock, source of drinking water, living in a household with a bed net, sleeping under a long-lasting insecticide-treated net (LLIN), LLIN insecticide type, living in a household with at least 1 net per 1.8 household members, and anemia (women only). Cluster level covariates included region, urban/rural place of residence, elevation, month of data collection, landcover, the proportion of a cluster with bed nets, and the proportion

of a cluster that slept under an LLIN. As transmission intensity is seasonal in Malawi, with peak transmission between January and May due to greater rainfall,[9,34] we also examined environmental variables at the cluster level including current month's average daily maximum temperature, and the prior month's precipitation. Modeled monthly precipitation and monthly average daily maximum temperature raster files, created from both in-situ weather station data and satellite imagery, were acquired from the Climate Hazards Center at the University of California, Santa Barbara.[67,68] Clusters were assigned precipitation and temperature values by averaging raster cells which fell within the 2km and 10km buffers surrounding urban and rural clusters, respectively, similar to DHS methodology for ecological variables.[73] Land cover estimates were obtained from satellite imagery classified by the Regional Center for Mapping of Resources for Development and SERVIR-Eastern and Southern Africa into settlement areas, forest, grassland, cropland, wetland, and other land cover types.[69] Clusters were assigned the majority land cover value within the 2km and 10km buffers surrounding urban and rural clusters.

Statistical analysis

We estimated prevalence differences to quantify the associations between demographic and environmental risk factors and the probability of being infected with *P. falciparum*. To power findings within subgroups of interest, the DHS two-stage sampling design selects population clusters with unequal probability, followed by households within clusters, violating the independence of observations assumption in standard regression. Weighting individual observations is required to appropriately specify individual and group-level variation. We accounted for the 2015-16 MDHS complex sample survey design when estimating bivariate and multivariate associations using 2015-16 MDHS HIV sample weights incorporated into linear risk regression models fit using generalized estimating equations. As the subset of DBS used in this

analysis was not a simple random sample (**Supplementary Table A4**), we also incorporated standardized inverse probability of selection weights[74] into regression models by calculating stabilized propensity scores for the probability of each individual being selected into the analysis based on their respective covariate pattern. Bivariate models were run between all risk factors and the malaria prevalence outcome, to determine high-risk populations and areas for targeted interventions. Multivariate adjusted analyses were performed to assess the association between bed net use, insecticide type, and malaria prevalence, after controlling for confounding variables, as bed nets have the potential for use in future interventions. Multivariate models were run among the total study population and in a sub-analysis of pregnant women. All weighted tabulations, models, and maps were run using R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria), using the *survey* (*v3.35-1*; Lumley, 2019) and *sf* (*v0.9-2*; Pebesma, 2020) packages.

Results

Of all 7,393 samples analyzed, 2,125 were PCR-positive for *P. falciparum*, and 5,268 were negative. After incorporating survey weights, the weighted *P. falciparum* prevalence was 31.1% (standard error = 1.1) among adolescents and adults ages 15-54 (**Table 3.1**). Samples represented 497 out of 850 (58.5%) clusters. The median number of individuals per cluster was 16 (IQR: 12 to 30; range 1 to 34). Over half of positive samples (55.6%) had parasitemias ≤10 parasites/μL (**Figure 3.1**), and parasitemia was not correlated with age. The intra-class coefficient at the cluster level was 22.3%, indicating that individual risk is associated with cluster-level risk.

Table 3.1 Characteristics of the study population, stratified by *P. falciparum* PCR status, 2015-2016 Malawi Demographic and Health Survey.

Variable		PCR-negative		PCR-positive		Total	
		n	%	n	%	n	%
Unweighted total number*		5268		2125			
Weighted count proportion**		4799	68.9	2170	31.1	6969	
Individual level covariates**							
Sex	Male	2191	45.7	1124	51.8	3315	47.6
	Female	2608	54.3	1045	48.2	3654	52.4
Age group (years)	15-24	1936	40.3	1096	50.5	3031	43.5
	25-34	1421	29.6	583	26.9	2004	28.8
	35-44	1004	20.9	349	16.1	1353	19.4
	45-54	439	9.1	142	6.5	581	8.3
Wealth quintiles	Poorest	712	14.8	530	24.4	1242	17.8
	Poorer	849	17.7	524	24.1	1372	19.7
	Middle	943	19.6	490	22.6	1433	20.6
	Richer	1004	20.9	366	16.9	1370	19.7
	Richest	1291	26.9	260	12.0	1551	22.3
Education	None/preschool	373	7.8	186	8.6	559	8.0
	Primary	2816	58.7	1525	70.3	4341	62.3
	Secondary	1381	28.8	442	20.4	1823	26.2
	Higher	209	4.4	12	0.5	221	3.2
Owns livestock, herds, or farm animals	No	2207	46.0	946	43.6	3153	45.2
	Yes	2593	54.0	1224	56.4	3816	54.8
Source of drinking water	Piped	1222	25.5	250	11.5	1472	21.1
	Unpiped	3577	74.5	1920	88.5	5497	78.9
Household has a bed net	No	1606	33.5	749	34.5	2355	33.8
	Yes	3193	66.5	1421	65.5	4614	66.2
Slept under an LLIN last night	No	3014	62.8	1408	64.9	4422	63.4
	Yes	1786	37.2	762	35.1	2547	36.6
Insecticide of LLIN individual slept under (out of individuals sleeping under nets)	Permethrin	1141	63.9	507	66.6	1649	64.7
	Non-permethrin	642	36.0	254	33.4	897	35.2
At least 1 net per 1.8 household members	No	731	15.2	243	11.2	975	14.0
	Yes	2462	51.3	1178	54.3	3640	52.2

Anemia (women only)	Not anemic	1821	69.8	628	60.1	2449	67.0
	Mild	601	23.1	327	31.3	929	25.4
	Moderate	173	6.6	81	7.8	254	7.0
	Severe	10	0.4	9	0.9	19	0.5
Cluster level covariates**							
Region	Northern	822	17.1	254	11.7	1076	15.4
	Central	1653	34.4	914	42.1	2567	36.8
	Southern	2325	48.4	1001	46.1	3326	47.7
Place of residence	Urban	941	19.6	152	7.0	1093	15.7
	Rural	3858	80.4	2018	93.0	5876	84.3
Elevation (m)	<500	719	15.0	287	13.2	1005	14.4
	≥ 500 & <1000	1673	34.9	1006	46.4	2678	38.4
	≥ 1000 & <1500	2217	46.2	849	39.2	3067	44.0
	≥ 1500	191	4.0	28	1.3	219	3.1
Month of data collection	October '15	867	18.1	635	29.3	1503	21.6
	November '15	1580	32.9	639	29.5	2219	31.8
	December '15	730	15.2	234	10.8	963	13.8
	January '16	1462	30.5	568	26.2	2029	29.1
	February '16	161	3.4	94	4.3	255	3.7
Landcover	Settlement	819	17.1	116	5.4	935	13.4
	Forest	605	12.6	313	14.4	917	13.2
	Grassland	333	6.9	185	8.5	518	7.4
	Cropland	2743	57.1	1410	65.0	4153	59.6
	Wetland	220	4.6	131	6.0	350	5.0
	Other	80	1.7	15	0.7	96	1.4
		mean	**se**	**mean**	**se**	**mean**	**se**
Proportion of cluster with bed nets		64.4	0.9	63.6	1.1	64.2	0.8
Proportion of cluster that slept under an LLIN last night		31.5	0.7	30.6	0.8	31.2	0.6
Current month's average daily maximum temperature (°C)		31.1	0.1	31.5	0.1	31.2	0.1
Prior month's precipitation (mm)		67.3	4.0	62.3	4.7	65.7	3.9

* Counts do not incorporate sample weights, and are not representative of the weighted populations used in the table
** Sampling weights applied
Note: LLIN = long-lasting insecticide-treated net

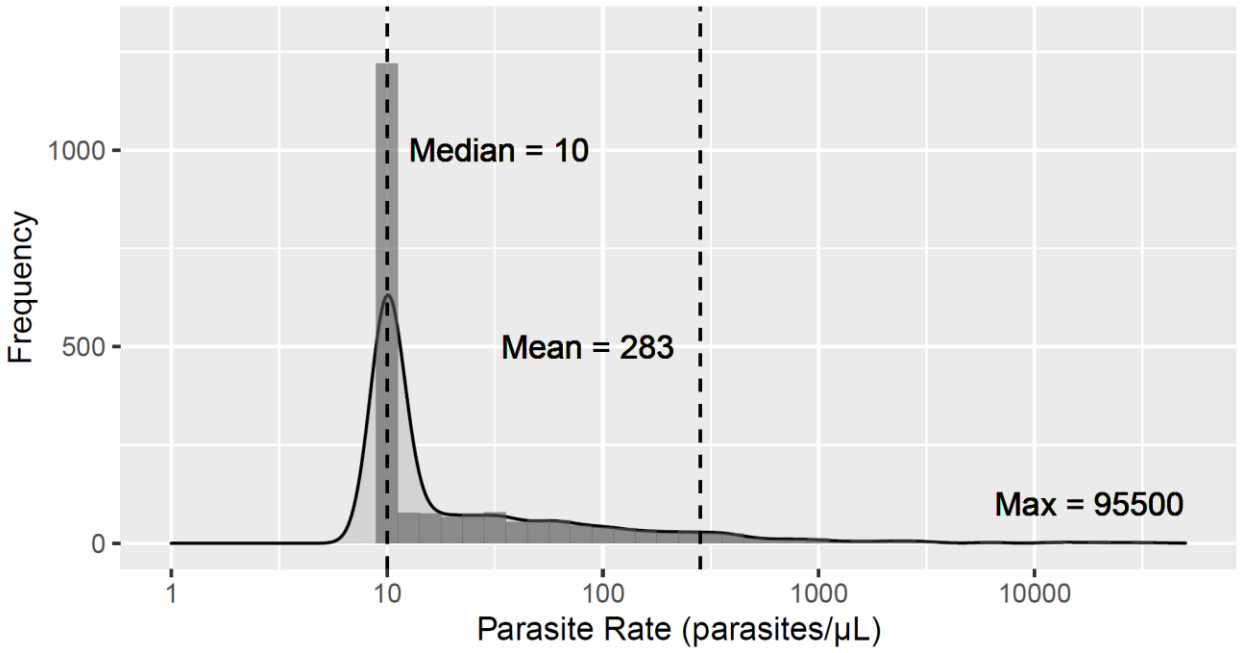


Figure 3.1 Distribution of PCR *P. falciparum* positive parasitemia values (n=2,215). Values <10 parasites/µL are rounded up to 10 parasites/µL. The density plot's solid line represents a normal distribution using the observed counts.

Demographics

After incorporating weights to account for survey design and selection bias, 52.4% of participants were female, 43.5% were between the ages of 15 to 24, and primary school was the highest level of education attended by 62.3% (**Table 3.1**). Most participants came from the Central (36.8%) and Southern regions (47.7%), and 84.3% were from rural areas. Most individuals (59.6%) were from clusters located on cropland. Data collection was spread unevenly across months, ranging from 3.7% of samples collected in February 2016 to 31.8% in November 2015. Two-thirds (66.2%) of participants lived in a household with a bed net, but only 36.6% reported sleeping under a long-lasting insecticide-treated bed net (LLIN) the night prior; 64.7% under permethrin treated LLINs and 35.2% under non-permethrin (alpha-cypermethrin or deltamethrin) treated LLINs. Over half (52.2%) of individuals lived in households meeting the World Health Organization's universal coverage criteria of a minimum of 1 net per 1.8 people.

Simple kriging *P. falciparum* prevalence estimates ranged from 2.5% to 83.5% across Malawi, demonstrating high spatial heterogeneity (**Figure 3.2**). *P. falciparum* prevalence was higher in the Northeastern part of the country along the lakeshore, and in the central southern region near Ntcheu. Prevalence was lowest in the southern tip of Malawi and in the Northwest highland areas. Estimates should be interpreted regionally, as standard errors are high in areas where data do not exist.

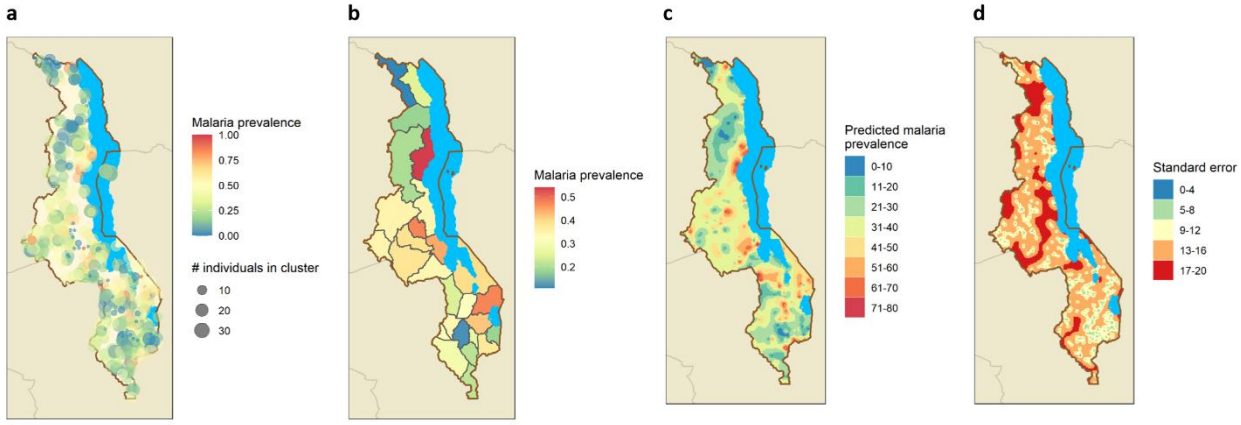


Figure 3.2 Spatial distribution of 2015-16 MDHS clusters. a) *P. falciparum* prevalence by cluster and cluster size, b) weighted *P. falciparum* prevalence by district, c) smoothed PCR *P. falciparum* prevalence estimates using simple kriging, d) smoothed *P. falciparum* standard error estimates using simple kriging. Clusters with fewer than five observations were removed prior to kriging to reduce the influence of extreme values due to small sample sizes. Smoothed surfaces are meant to demonstrate regional differences and should not be used for interpretation into areas where data do not exist. All maps were run using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) and the *sf* (*v0.9-2*; Pebesma, 2020) package.

Risk factor analysis

In weighted bivariate analysis, female sex was associated with a lower prevalence of *P. falciparum* infection (prevalence difference: -0.05, 95% confidence intervals: -0.08 to -0.03) (**Table 3.2**). Prevalence decreased as age increased and was lowest among those 45-54 years as compared to those 15-25 years (PD: -0.12, 95% CI: -0.17 to -0.06). Parasite prevalence was lower in urban versus rural areas (PD: -0.20, 95% CI: -0.24 to -0.17) and among populations with piped drinking water versus unpiped (PD: -0.18, 95% CI: -0.22 to -0.14). Other protective factors included greater wealth (PD: -0.26, 95% CI: -0.31 to -0.21, richest vs. poorest), higher education (PD: -0.28, 95% CI: -0.34 to -0.22, higher vs. none). Living in wetlands (PD: 0.25, 95% CI: 0.15 to 0.34) and grasslands (PD: 0.23, 95% CI: 0.13 to 0.33) were associated with the highest prevalence of infection, as compared to settlement areas. Increased geographic average daily maximum temperature was associated with greater malaria prevalence (PD: 0.02, 95% CI: 0.01 to 0.03 per 1°C increase). Individuals living at elevations between 500 and 1,000 meters above sea level, along the lake shore, had the highest prevalence of infection (37.6%), with lowest prevalence among those living at or above elevations of 1,500 meters above sea level (12.7%), although other geographic factors likely contributed to variations in estimates.

Table 3.2 Bivariate associations between demographic and environmental risk factors and *P. falciparum* prevalence using weighted survey data.

Covariates	Variable	*P. falciparum* Prevalence	Prevalence Difference	95% Confidence Interval		p-value
Sex	Male	0.339	-	-	-	-
	Female	0.286	-0.05	-0.08	-0.03	<0.001
Age group (years)	15-24	0.361	-	-	-	-
	25-34	0.291	-0.07	-0.11	-0.04	<0.001
	35-44	0.258	-0.10	-0.15	-0.06	<0.001
	45-54	0.245	-0.12	-0.17	-0.06	<0.001
Wealth quintiles	Poorest	0.427	-	-	-	-
	Poorer	0.382	-0.05	-0.09	0.00	0.05
	Middle	0.342	-0.08	-0.14	-0.03	0.001
	Richer	0.267	-0.16	-0.20	-0.11	<0.001
	Richest	0.167	-0.26	-0.31	-0.21	<0.001
Education	None	0.332	-	-	-	-
	Primary	0.351	0.02	-0.03	0.07	0.4
	Secondary	0.243	-0.09	-0.14	-0.04	0.001
	Higher	0.053	-0.28	-0.34	-0.22	<0.001
Owns livestock, herds or farm animals	No	0.300	-	-	-	-
	Yes	0.321	0.02	-0.01	0.05	0.2
Source of drinking water	Piped	0.170	-	-	-	-
	Unpiped	0.349	0.18	0.14	0.22	<0.001
Household has a bed net	No	0.318	-	-	-	-
	Yes	0.308	-0.01	-0.04	0.02	0.6
Slept under an LLIN last night	No	0.318	-	-	-	-
	Yes	0.299	-0.02	-0.05	0.01	0.2
Insecticide of LLIN individual slept under (out of individuals sleeping under nets)	Permethrin	0.308	-	-	-	-
	Non-permethrin	0.284	-0.02	-0.08	0.03	0.4
At least 1 net per 1.8 household members	No	0.249	-	-	-	-
	Yes	0.324	0.07	0.03	0.12	0.001
Number of months ago that sleeping net was obtained (treated or untreated)	<12	0.302	-	-	-	-
	12-23	0.294	-0.01	-0.07	0.05	0.8
	24-35	0.281	-0.02	-0.10	0.06	0.6
	≥36	0.265	-0.04	-0.09	0.02	0.2

Anemia (women only)	Not anemic	0.256	-	-	-	-
	Mild	0.352	0.10	0.05	0.14	<0.001
	Moderate	0.319	0.06	-0.01	0.14	0.1
	Severe	0.478	0.22	-0.05	0.49	0.1
Region	Northern	0.236	-	-	-	-
	Central	0.356	0.12	0.07	0.17	<0.001
	Southern	0.301	0.06	0.01	0.12	0.02
Place of residence	Urban	0.139	-	-	-	-
	Rural	0.343	0.20	0.17	0.24	<0.001
Elevation (m)	<500	0.285	-	-	-	-
	≥ 500 & <1000	0.376	0.09	0.03	0.16	0.007
	≥ 1000 & <1500	0.277	-0.01	-0.07	0.05	0.8
	≥ 1500	0.127	-0.16	-0.23	-0.09	<0.001
Month of data collection	October '15	0.423	-	-	-	-
	November '15	0.288	-0.13	-0.21	-0.06	<0.001
	December '15	0.243	-0.18	-0.26	-0.10	<0.001
	January '16	0.28	-0.14	-0.21	-0.08	<0.001
	February '16	0.369	-0.05	-0.16	0.06	0.3
Landcover	Settlement	0.124	-	-	-	-
	Forest	0.341	0.22	0.14	0.29	<0.001
	Grassland	0.356	0.23	0.13	0.33	<0.001
	Cropland	0.34	0.22	0.18	0.25	<0.001
	Wetland	0.373	0.25	0.15	0.34	<0.001
	Other	0.16	0.04	0.00	0.07	0.03
Proportion of cluster with bed nets (scaled)	Mean	0.311	-	-	-	-
	10% increase	-	-0.01	-0.02	0.01	0.4
Proportion of cluster that slept under an LLIN last night (scaled)	Mean	0.311	-	-	-	-
	10% increase	-	-0.01	-0.03	0.00	0.2
Current month's average daily maximum temperature (°C) (scaled)	Mean	0.311	-	-	-	-
	1°C increase	-	0.02	0.01	0.03	<0.001
Prior month's precipitation (mm) (scaled)	Mean	0.311	-	-	-	-
	100 mm increase	-	-0.02	-0.05	0.01	0.2

Note: LLIN = long-lasting insecticide treated net

After adjusting for age, sex, wealth, and household size, there was no difference in the prevalence of infection between households with and without bed nets (PD: 0.02, 95% CI: -0.02 to 0.05) or between individuals who reported sleeping under an LLIN the previous night and

those who did not (PD: 0.01, 95% CI: -0.02 to 0.04) (**Table 3.3**). After adjusting for age, sex, and wealth, sleeping under a non-permethrin versus permethrin treated LLIN (PD: -0.02, 95% CI: -0.07 to 0.02) was not protective. However, a sub-analysis among the study population's 319 pregnant women showed a protective prevalence difference between those who slept under non-permethrin treated LLINs, including alpha-cypermethrin and deltamethrin, (PD: -0.16, 95% CI: -0.34 to 0.02) as compared to permethrin treated LLINs (**Table 3.4**), although this did not reach statistical significance. Meeting the WHO universal coverage criteria of 1 net per 1.8 household members was not protective in either population. Community-level household bed net coverage and the community-level proportion who slept under an LLIN were not protective against P. falciparum infection in the general population.

Sensitivity analyses defining malaria positivity as PCR amplification which crossed the threshold line below CT values of 37 and 38 found similar relationships between covariates and P. falciparum prevalence in bivariate and multivariate analyses (**Supplementary Tables A5-A8**).

Table 3.3 Multivariate associations between bed net associated risk factors and *P. falciparum* prevalence using weighted survey data.

Exposure	Model	Prevalence Difference	95% Confidence Interval		p-value
Household has a bed net	Unadjusted	-0.01	-0.04	0.02	0.6
	Adjusted*	0.02	-0.02	0.05	0.3
Slept under an LLIN last night	Unadjusted	-0.02	-0.05	0.01	0.2
	Adjusted*	0.01	-0.02	0.04	0.6
Individual slept under LLIN treated with non-permethrin (vs. permethrin)	Unadjusted	-0.02	-0.08	0.03	0.4
	Adjusted†	-0.02	-0.07	0.02	0.4
At least 1 net per 1.8 household members	Unadjusted	0.07	0.03	0.12	<0.001
	Adjusted§	0.02	-0.01	0.06	0.2

* models adjusted for age, sex, wealth, and household size

† model adjusted for age, sex, and wealth

§ model adjusted for wealth and district

Note: LLIN = long-lasting insecticide treated net

Table 3.4 Multivariate associations between bed net associated risk factors and *P. falciparum* prevalence using weighted survey data among pregnant women in the 2015-16 MDHS who contributed samples to the analysis (n=319).

Exposure	Model	Prevalence Difference	95% Confidence Interval		p-value
Household has a bed net	Unadjusted	-0.09	-0.21	0.03	0.2
	Adjusted*	-0.09	-0.21	0.03	0.1
Slept under an LLIN last night	Unadjusted	-0.06	-0.20	0.07	0.4
	Adjusted*	-0.06	-0.19	0.08	0.4
Individual slept under LLIN treated with non-permethrin (vs. permethrin)	Unadjusted	-0.19	-0.37	-0.02	0.04
	Adjusted†	-0.16	-0.34	0.02	0.08
At least 1 net per 1.8 household members	Unadjusted	0.02	-0.15	0.18	0.8
	Adjusted§	0.03	-0.09	0.15	0.6

* models adjusted for age, wealth, and household size

† model adjusted for age and wealth

§ model adjusted for wealth and region

Note: LLIN = long-lasting insecticide treated net

Discussion

This study represents the first national survey in Malawi to determine *P. falciparum* infection in adolescents and adults. Nearly a third of individuals ages 15-54 are infected with *P. falciparum*, primarily with low-density infections undetectable through microscopy or RDT. Protective factors against asymptomatic *P. falciparum* infection include older age, urban residence, greater wealth, higher education, and lower geographic average daily maximum temperature. Living in a household which owned a bed net and reporting sleeping under an LLIN were not protective against infection among our study population, even after stratifying by insecticide type. However, among pregnant women, sleeping under alpha-cypermethrin or deltamethrin-treated nets appeared protective, as compared to permethrin treated nets. Underappreciating the significance of the extensive reservoir of asymptomatic infection among adolescents and adults neglects an important source of sustained *P. falciparum* transmission in Malawi and hinders understanding of key target groups for intervention.

Demographic and environmental risk factors associated with *P. falciparum* infection among adolescent and adults in our study resemble those previously found among asymptomatic children and individuals of all ages presenting with clinical symptoms; these risk factors in Malawi include low elevation, higher temperatures, younger age, rurality, and region.[5–7] Similar to the cross-sectional nationally representative Malawi Malaria Indicator Surveys (MIS) among children in 2014 and 2017,[35,75] the highest malaria prevalence was found in the Central region, followed by the Southern and Northern regions, although results are likely influenced by regional data collection during different months of the year (**Supplementary Table A9**). The national prevalence estimate of 31% in our study is comparable to results using similar methodology among adults from the Democratic Republic of the Congo in 2007 and 2014, which also found

that younger age, male sex, and lower wealth indicators were risk factors for increased infection.[76,77] Our prevalence estimate is much higher than modeling predictions of *P. falciparum* annual parasite rates of 18-19% in children 2-10 years in 2015-2016, however these predictions were generated from multiple community-based survey measurements in the literature and from other unpublished sources which used RDT and microscopy as opposed to PCR.[2] While prevalence estimates were high, parasitemia values were low among our study population; however this finding is consistent with research showing that malaria infection is more likely to be submicroscopic among older children and adults versus younger children, hypothesized to stem from acquired immunity.[21] Risk factor analysis is important for identifying key populations to target for malaria prevention and control, and our results suggest that malaria prevention measures might be best focused towards men, younger age groups, poor communities, and rural areas.

Surprisingly, household ownership of bed nets, individual use of LLINs for sleeping, owning 1 net per 1.8 household members, and community bed net coverage were not associated with asymptomatic *P. falciparum* prevalence. While two-thirds of individuals resided in households which owned bed nets, only 36.6% reported sleeping under an LLIN the previous night, indicating low LLIN usage. Prevalence was higher among men as compared to women; females have been shown to clear asymptomatic *P. falciparum* infections faster than males, leading to the appearance of lower infectivity, and highlighting the importance of biological differences.[78] Other reasons for prevalence differences between males and females could relate to gender norms and behavioral factors around sleeping arrangements, gender-differentiated access to education about malaria or treatment and screening services, and gendered division of outdoor labor.[79]

Bed net ownership and use indicators were not associated with *P. falciparum* prevalence among our overall study population but insecticide type was found to have a near protective association among pregnant women. Pregnant women had similar patterns of sleeping under an LLIN the previous night as compared to the overall study population (38.7% vs. 36.6% respectively), however of those who slept under LLINs, 43.6% of pregnant women used nets treated with alpha-cypermethrin or deltamethrin, compared with 35.2% in the general population. Additionally, 49.0% of the nets used by pregnant women for sleeping were less than one year old, compared with 37.2% in the general population. While the general population receives bed nets every three years as part of regular mass distribution campaigns, Malawi's National Malaria Control Programme has given out free LLINs to pregnant women through antenatal care clinics since 2006[9] with 79-87% of pregnant women who attended antenatal clinics receiving LLINs in 2015-16.[9] Although WHO recommendations allow for up to three years between mass distribution campaigns,[80] field research in Benin, Malawi, Rwanda, Senegal, and Tanzania suggests that ITNs have a limited lifespan of two years before protection is compromised by holes, insecticide-resistance, and reduced concentrations of insecticide.[37,81–84] In Burkina Faso, a third of people had stopped using LLINs within a year of distribution.[85] Pregnant women in our study appear to have been the recipients of relatively newer nets which might have greater effectiveness, while households without pregnant women would have primarily received nets through Malawi's first national mass distribution campaign in 2012, or through limited follow-up mop-up campaigns in six districts in 2014.[30] In areas where nets are used at night, there is also evidence to suggest that long term use of insecticides inside the home can shift *Anopheles spp.* mosquitos from indoors to outdoors host seeking behavior, increasing the likelihood of an individual becoming infected, and perhaps contributing to reduced effectiveness of LLINs

against malaria.[86,87] Bed net durability and mosquito biting behavior were not measured as part of the 2015-16 MDHS, but could have contributed to the lack of association found between net ownership and use, and malaria prevalence among our study population.

The major strength of this study is the efficient use of a large number of nationally collected samples from adolescents and adults, a population which is understudied in malaria transmission research. Using molecular and epidemiologic methods, we better characterized the reservoir of asymptomatic *P. falciparum*, a pool of infection which is likely contributing to sustained transmission in Malawi. The results presented can serve as baseline assessment; repeated use of DHS samples over time can create a picture of shifting trends across the entire country while using resources cost-effectively to supplement intermittent MIS iterations estimating malaria prevalence among children. Characterizing the prevalence of *P. falciparum* among adults and identifying key target groups will be informative as the Malawi Ministry of Health designs future mass distribution campaigns and other interventions against malaria.

The primary limitation of this analysis is the cross-sectional nature of available samples. The 2015-16 MDHS captured DBS from participants at a single time point, allowing for estimation of marginal associations between risk factors and infection to identify groups at high risk, but limiting evaluation of causal relationships. Additionally, the samples used in the study were collected during the 2015-16 MDHS survey period from October to February, limiting inference to the remainder of the year and hindering comparison with published prevalence estimates among children from different time periods; however, as the study period occurred during the transition from dry to rainy season, we anticipate that results are somewhat representative of a yearly average. We used inverse probability of selection weights to make our results generalizable to the broader DHS cohort, but additional bias could still result due to

unmeasured confounding. Our analysis was also constrained by the aggregated geographical classification of individuals. As part of DHS methodology, individuals are geolocated at the center of their study cluster, which is then displaced up to 5km for 99% of rural clusters and 2km for urban clusters. There is an inherent lack of precision in land cover, temperature, and precipitation data, nonetheless, our methods attempt to account for geographic displacement by using average values falling within a cluster's potential buffer area. Although our study measures presence of *P. falciparum*, we were not able to ascertain the presence of gametocytes within each infection, limiting the extent to which we can predict how these infections continue to sustain transmission. Results from elsewhere in Africa show that infection with *P. falciparum* gametocytes is associated with low asexual parasite densities and asymptomatic disease.[4] Comparisons between microscopy and PCR have found that microscopy can miss over 90% of gametocyte carriers due to limited sensitivity for low-density infections,[22] further highlighting the importance of molecular surveillance tools in understanding infection transmission dynamics in this population.

Despite existing limitations, this analysis provides valuable input into an understudied yet critical group to consider in efforts to interrupt ongoing malaria transmission. Malawi spent an estimated $82 million on malaria control in 2016[88] and malaria accounts for 30% of all outpatient visits and 34% of inpatient hospital admissions.[9] Households often amass high direct and indirect costs due to clinical malarial disease, despite free diagnosis and treatment.[89] One of the primary goals of the Malawi Malaria Strategic Plan 2017-2022 is to achieve universal LLIN coverage for all households.[9] Research from Madagascar shows that while LLIN mass distribution campaigns may only provide community protection for one year, protection can be sustained when campaigns are followed by continuous LLIN distribution to eligible households,

including recently married couples, immigrants, children of vaccination age, and homes with uncovered sleeping areas.[90] Malawi could benefit from education on consistent and correct use of bed nets, and through expansion of continuous LLIN distribution services to additional populations beyond pregnant women, targeting younger individuals living in rural areas with high prevalence of infection for more frequent net replacement. Treating malaria at the population level through mass drug administration can clear parasite presence and prevent transmission of gametocytes from asymptomatic infections. However, mass drug administration is only recommended in settings considering malaria elimination, and requires low malaria prevalence, effective vector control, access to treatment, and extensive community participation as prerequisites for implementation.[91,92]

This study presents unique insight into the national prevalence of asymptomatic *P. falciparum* infection among adolescent and adults in Malawi. Use of molecular and epidemiological surveillance methods in tandem demonstrates that demographic and environmental risk factors for infection parallel those found in children and among individuals with symptomatic disease. Within the current framework of mass distribution frequency and community education, presence of bed nets in the household and use of LLINs by the individual did not appear to provide protective benefits, regardless of insecticide type, most likely due to bed net age and low frequency of use. Results from this study provide valuable guidance to decision makers in Malawi as the National Malaria Control Programme designs bed net distribution programs following mid-term review of the 2017-2022 National Malaria Control Strategy. Future work to replicate this analysis following the 2021-22 MDHS will enable assessment of changes in asymptomatic *P. falciparum* prevalence and other genetic markers in adolescents and adults across time.

CHAPTER FOUR: EFFECTIVENESS OF A NATIONAL MASS DISTRIBUTION CAMPAIGN OF LONG-LASTING INSECTICIDE-TREATED NETS ON CLINICAL MALARIA IN MALAWI, 2018-2020

Introduction

Malaria is endemic in Malawi, accounting for 30% of outpatient visits and 34% of inpatient admissions in-country.[9] Historically, national malaria measures have been exclusively estimated cross-sectionally, such as through USAID-funded Malaria Indicator Surveys (MIS) in 2012, 2014, and 2017. MIS are designed to offer a high-level glance at malaria prevalence among children 6-59 months in Malawi's three regions, however, malaria in Malawi is known to be geographically heterogenous at the sub-district level,[50] varies at the household level,[51] and differs across age groups and seasons. MIS surveys only sample during high malaria transmission months, prohibiting estimation of how patterns fluctuate over the course of the year, and require extensive time and resources dedicated to active data collection. Other research involving annual parasite rates in children[54] and predicted risk across smaller land areas[55] have only achieved a detailed focus by modeling survey data.

Through District Health Information Software 2 (DHIS2), a health management information system used in Malawi since 2015, each health facility reports paper-based monthly composite statistics to the Ministry of Health. Malaria data includes total confirmed case counts for persons <5 years of age and ≥5 years of age each month, which can be used to track the burden of disease continuously over time through passive surveillance. Similarly constructed DHIS2 malaria data have already been analyzed in Uganda, Zimbabwe, and Kenya to determine the effects of vector control and artemisinin-based combination therapy, assess DHIS2 reporting

completeness, and map the spatial distribution of malaria by district.[59–61] At present, Malawi's DHIS2 malaria data have only been assessed at the district and national levels, recording 7.0 million confirmed cases in 2018.[32] No analyses have been conducted at the health facility level, providing the detail necessary to target local areas for intervention.

Long-lasting insecticide-treated bed nets (LLINs) are integral to Malawi's malaria prevention efforts, alongside broad use of rapid diagnostic tests (RDTs) and artemisinin-based combination therapies, and fundamental to achieving the reduction goals outlined in Malawi's Malaria Strategic Plan 2017-2022. LLINs provide a physical and chemical barrier to mosquito bites, and nets treated with various insecticides are selected for implementation based on vector-resistance and cost. Implementation occurs through mass distribution campaigns and continuous distribution to pregnant women at antenatal care visits and to newborns following birth.[9] Insecticide-treated nets (ITNs) provide individual and community level protective benefits within a year of distribution,[65] but there is no consensus on the maximum LLIN lifespan. World Health Organization recommendations suggest conducting mass distribution campaigns every three years,[80] however, field research in Eastern, Southern, and Western Africa suggests that nets may have a much shorter lifespan, and rapidly become ineffective due to holes, reduced concentrations of insecticide, and repurposing.[81–83,93] Prior research on mass distribution campaign effectiveness in Malawi has been limited by the use of cross-sectional survey data[37] and narrow geographic range.[94]

We present the first national longitudinal analysis of confirmed malaria risk at the health facility level, using DHIS2 to estimate geographical and temporal variations from January 2018 to June 2020 by month and age strata. We assess risk before and after a mass bed net distribution campaign in late 2018 and stratify by intervention type including pyrethroid-treated nets,

piperonyl butoxide (PBO)-treated nets, and indoor residual spraying (IRS). Given that DHIS2 is now routinely used in 72 low-and middle-income countries, capturing metrics from 2.3 billion people,[56] this study design and analysis can be replicated across the globe to inform national and regional health policies for malaria and other diseases.

Methods

Study design and population

Malawi's population was over 17.5 million in 2018, with 2.6 million (15%) under the age of 5 years.[95] The entire population lives in moderate to high malaria transmission zones and are at risk of acquiring infection. The outcome of interest was monthly confirmed malaria cumulative incidence (cases/population). Cases included individuals presenting at health facilities with a confirmed malaria diagnosis captured through DHIS2 from January 2018 through June 2020. Confirmed cases are recorded as aggregated monthly counts at the health facility level within strata of age, test result, and other variables; DHIS2 contains no individual-level patient data. Population estimates were extracted from WorldPop population distribution data.[10] Although it is possible for an individual to experience more than one malaria episode per month, we expected these instances to be rare, and refer to cumulative incidence as "risk" for simplicity. The exposure of interest was the 2018 LLIN mass distribution campaign.

Ethical approval was obtained from Institutional Review Boards through the National Health Sciences Research Committee at the Malawi Ministry of Health (#19/08/2381) and the University of North Carolina at Chapel Hill (#19-2882).

DHIS2

Malawi DHIS2 data are available at the health facility level from 2018 onward. Indicators were summed within age groups to create aggregate counts of malaria cases confirmed through

microscopy or RDT in the following settings: outpatient, inpatient, and community village health clinic (applicable to children <5 years). DHIS2 captures data from both public and private health facilities but does not capture asymptomatic cases, symptomatic cases that do not present for care, individuals who die at home without a diagnosis, and those that seek diagnostic and treatment services through traditional medicine.

A total of 756 facilities recorded data in 2018, 925 in 2019, and 926 as of June 2020, resulting in 25,099 total observations (**Figure 4.1**). Health facilities were linked to geographic coordinates through existing data sources[96] and cross-referenced using Google Maps and OpenStreetMap. Health centers with duplicate coordinates and names were combined into single entries. This analysis included 711 facilities with DHIS2 data in 2018, 2019, and 2020, encompassing 20,962 (of 25,099; 83.5%) total health facility reports, but capturing 97.1% (16.6 M/17.1 M) of total confirmed malaria cases reported into DHIS2 (**Supplementary Table B1**).

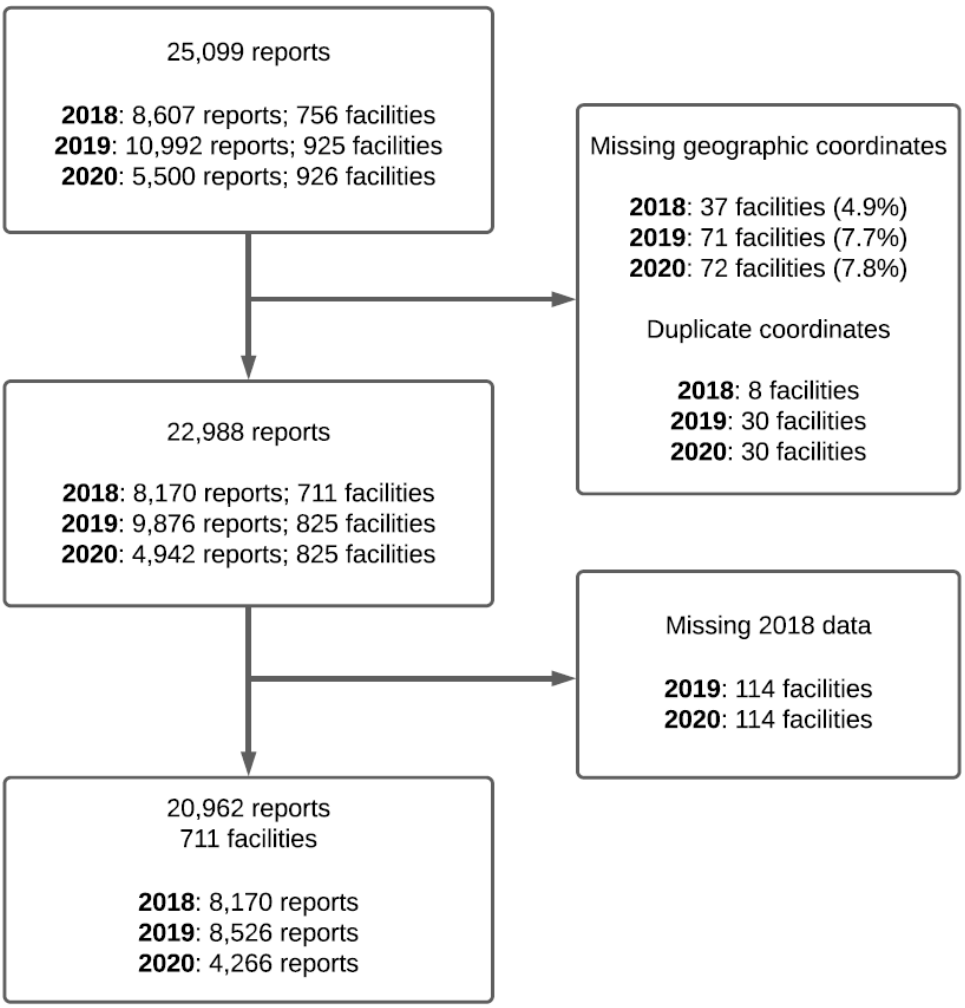


Figure 4.1 Health facilities and facility reports from Malawi's District Health Information Software 2 which were included in the analysis, January 2018 to June 2020.

Spatial analysis and population measurements

Population estimates were derived from the WorldPop project which releases population raster maps modelled from census counts, nighttime lights, settlement buildup, landcover, roads, and other variables, downscaled to 100x100m grid squares, and stratified by <5 and ≥5 year age groups.[10] Since existing WorldPop rasters were created using UN population projections based on the 2008 Malawi census, we used an open-source algorithm[97] to recalculate up-to-date estimates using 2018 census counts within each Traditional Authority boundary.[98]

To determine population denominators for each health facility, we delineated catchment areas using Thiessen polygons (**Figure 4.2**). A Thiessen polygon is defined as a region that contains all areas that are closer to a particular point (health facility) than to any other neighboring points. These catchment areas were overlaid on the recalculated WorldPop rasters and <5 and ≥5 population counts were summed within each area.

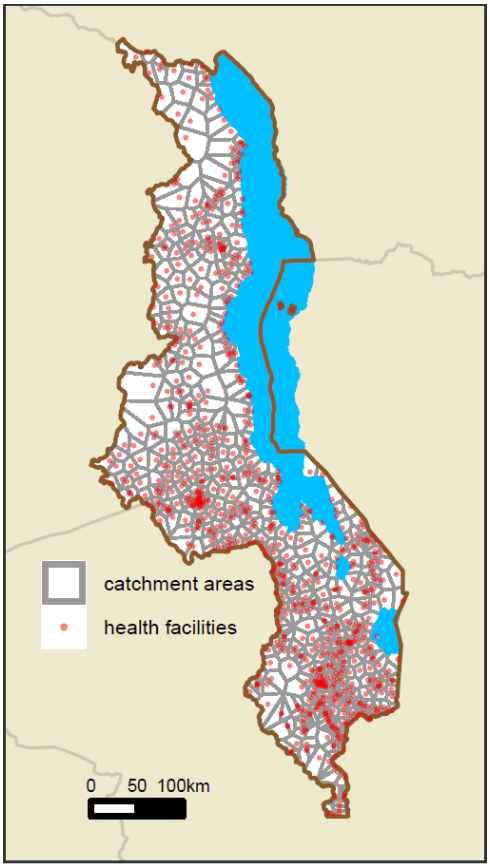


Figure 4.2 Health facility catchment areas for health facilities with data in Malawi's District Health Information Software 2 in 2018-2020 (n=711), calculated by Euclidean distance using Thiessen polygons.

Longitudinal environmental data were used to assess bias in malaria risk estimates between years. We used Climate Research Unit gridded Time Series (CRU TS) v.4 which

interpolates values from in-situ weather station data to create national monthly estimates of average temperature and total precipitation.[99]

Bed net mass distribution campaign

Malawi's most recent mass distribution campaign took place from September to December 2018, allocating LLINs across 27 of Malawi's 28 districts. Each district administered either permethrin- or deltamethrin- (Olyset® or Permanent®; "pyrethroid") treated nets, or permethrin + PBO-treated nets (Olyset® Plus; "PBO"), with one district, Mchinji, distributing both (**Figure 4.3**). Mchinji was assigned pyrethroid-treated nets in analyses of risk, as 69.4% of LLINs were pyrethroid-treated. Net type assignment was determined by assessment of malaria burden and population size within each district, but there was no association between intervention assignment and baseline 2018 malaria risk either at the health facility ($p=0.3$) or district levels ($p=0.3$). Health facilities coordinated household distribution via centralized locations within their catchment areas such as primary schools, health posts, and churches. LLINs were distributed by 648 health facilities and 602 (of 648, 92.9%) were linked to DHIS2 and used in the health facility level risk analysis. All distribution facilities were included in district level calculations. Due to a national shortage of nets, one district, Nkhotakota, was assigned to receive IRS instead of LLINs. Mangochi received pyrethroid-treated nets in 2018 and IRS in 2019 but was not included in adjusted modeling. IRS with pirimiphos-methyl CS was administered from October to November 2018, and pirimiphos-methyl CS & clothianidin from October to November 2019 in Nkhotakota and November to December 2019 in Mangochi.

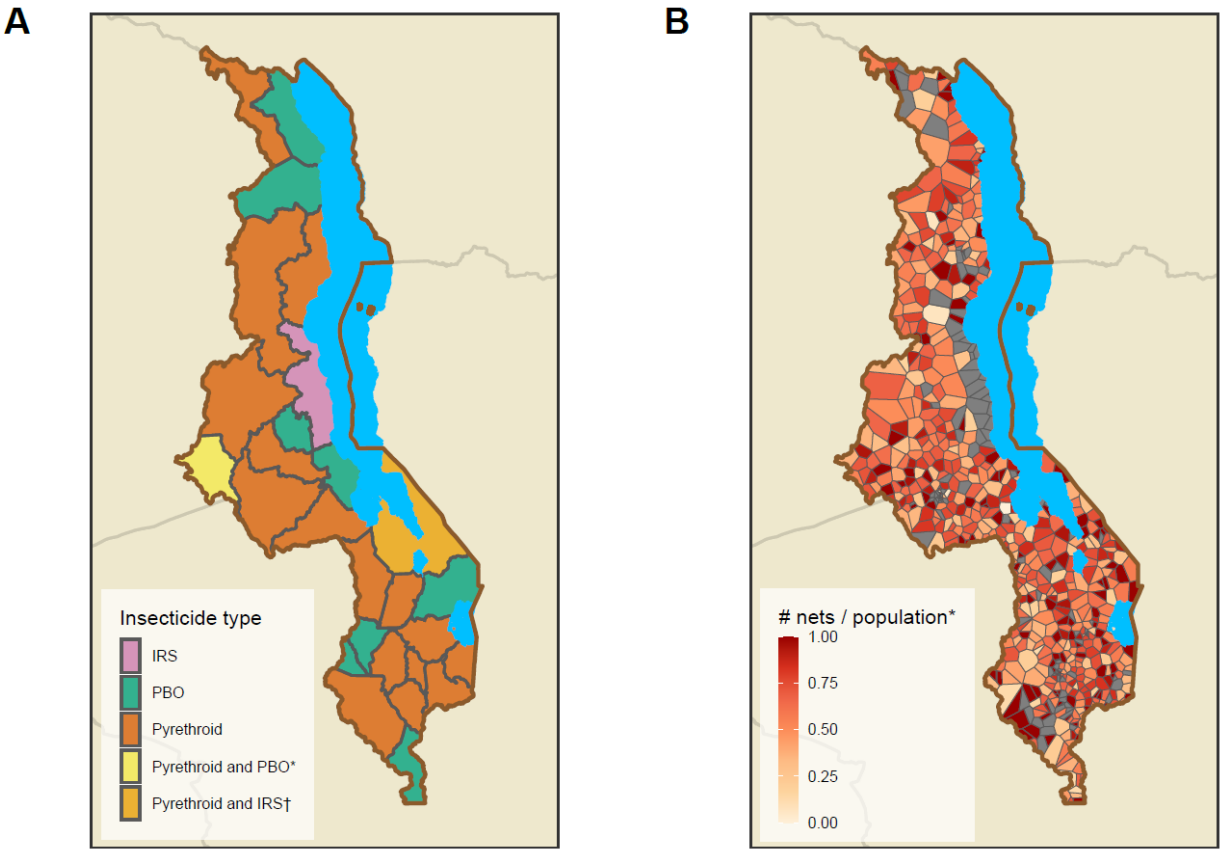


Figure 4.3 Distribution of insecticide-treated nets from September to December 2018 during Malawi's mass distribution campaign, and implementation of IRS in October and November 2018 and in November and December 2019. A) Assigned intervention and insecticide type by district. * Mchinji distributed 69.4% pyrethroid-treated nets and 30.6% PBO-treated nets, † IRS in 2019 only. B) Density of nets (number of nets per person) by health facility catchment area. * values >1 set to 1.
Note: IRS = indoor residual spraying, PBO = piperonyl butoxide.

Statistical analysis

Risk values at each health facility and district were visualized using maps and graphs stratified by time and age group. Risk differences were calculated by comparing risk in high (January to May) and low (June to December)[9] transmission seasons before and after the 2018 mass distribution campaign. Quasi-poisson regression models were used to estimate and compare risks under scenarios where all health facilities received pyrethroid-treated nets, PBO-treated nets, or IRS. Models adjusted for baseline 2018 malaria risk and LLIN coverage, and the delta

method was used to estimate 95% confidence intervals. DHIS2 2018 risk values were compared to 2017 estimates of *Plasmodium falciparum* parasite rates in children ages 2 to 10 years, modeled using MIS results and local survey data,[8] in order to compare risk estimates to prevalence estimates from similar time periods by district. All tabulations, models, and maps were run using R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) and the *sf* (*v0.9-2*; Pebesma, 2020) and *ggalt* (*v0.4.0*; Rudis, 2017) packages.

Role of the funding source

The funders had no role in the study design, data collection, analysis, interpretation, or writing. All authors had full access to the data in the study and had final responsibility for the decision to submit for publication.

Results

The analysis included 711 health facilities; the majority (580/711, 81.6%) reported data for the entire 30-month study period and nearly all facilities (709/711, 99.7%) reported data for at least 24 months. Over 10.7 million bed nets were distributed in 2018; 1 net was distributed for every 1.6 (range: 1.3-2.2) people nationally (**Supplementary Table B2**). Nkhotakota sprayed 112,264 structures with insecticide in 2018, resulting in a coverage rate of 95%.[32] IRS coverage in 2019 was 89% in Nkhotakota (population = 393,077) and 78% in Mangochi (population = 1,148,611).

Risk varied geographically and seasonally among all age groups, with elevated estimates during high transmission seasons nationally and year-round along the shores of Lake Malawi (**Figure 4.4**). Risk was higher among children <5 years than among individuals ≥5 years and seemed to be heterogenous within district boundaries, indicating that aggregate measures across large geographic areas can obscure local disease variation.

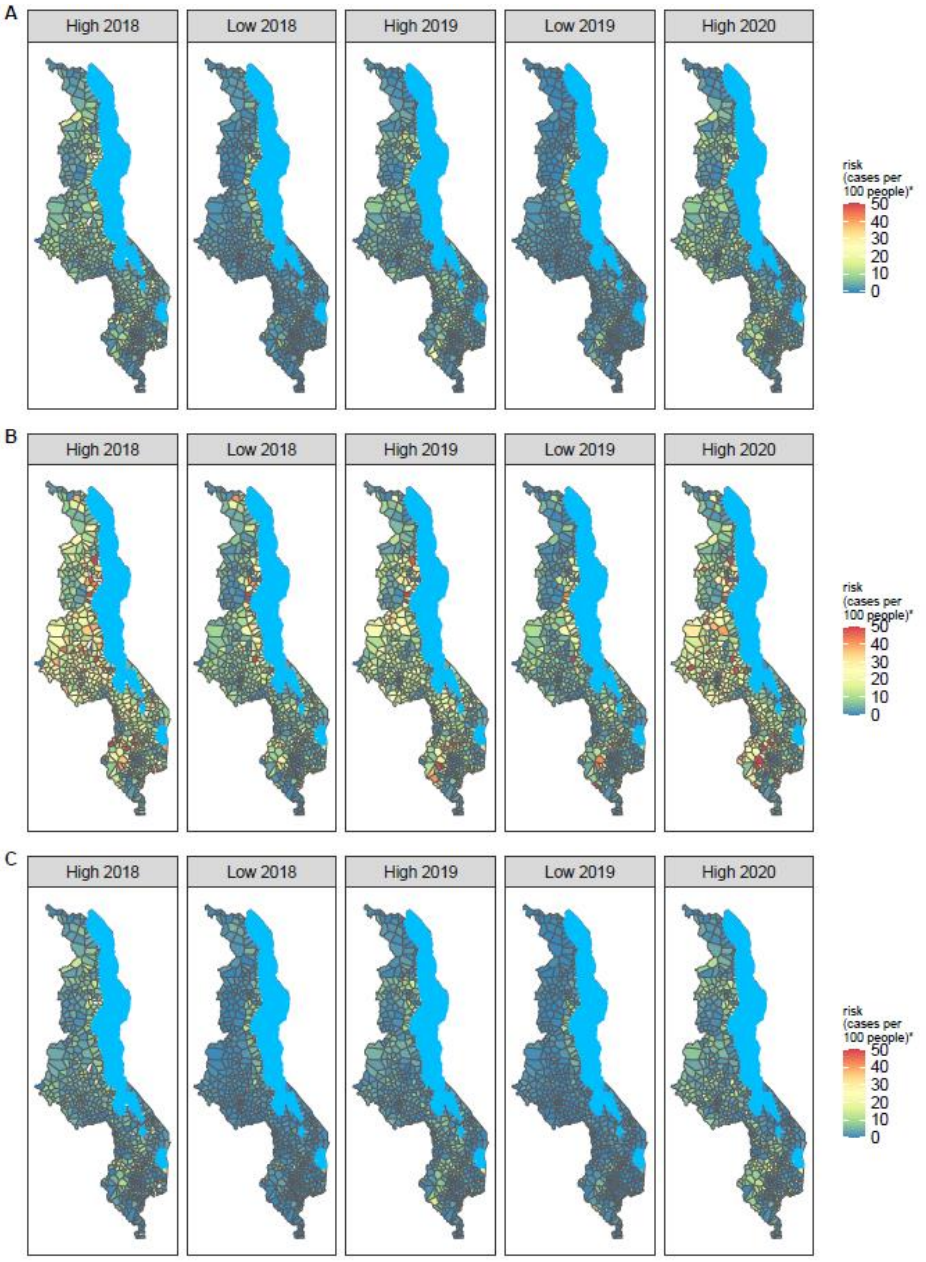


Figure 4.4 Monthly median risk of malaria (cases per 100 people) by high (January to May) and low (June to December) malaria transmission season and health facility catchment area, 2018-2020, among A) the total population, B) children <5 years, and C) individuals ≥5 years of age. Confirmed case data are taken from Malawi's District Health Information Software 2 and population denominators from adapted WorldPop 2018 estimates. * risk values >50 are set at 50 cases per 100 people.

During high transmission seasons, there were 4.3 million recorded malaria cases nationally in 2018, 3.0 million in 2019, and 4.1 million in 2020. Spanning all seasons, there were

6.8 million cases in 2018 and 5.1 million in 2019, an overall annual reduction of 1.7 million cases following the LLIN campaign. Malaria risk was lower during high transmission months in 2019 as compared to 2018, an association which held for all age groups and intervention types (**Figure 4.5, Supplementary Table B3**). Risk rebounded across all groups during the 2020 high transmission season, except for areas receiving IRS. Malaria risk in high transmission seasons decreased from 25.6 cases per 100 people in 2018 to 16.7 cases per 100 people in 2019 (**Table 4.1**), a crude risk difference of -8.9. Confidence intervals were precise to one tenth of a percent due to the thousands of individuals included in each age group. Risk increased up to 23.2 cases per 100 people in 2020, with a seasonal risk difference of -2.4 comparing 2018 and 2020. Variations in risk were greater among children <5 years with seasonal risk differences of -30.2 and -16.8 cases per 100 people, comparing risk in 2019 and 2020 to 2018, respectively; estimates among individuals ≥5 years showed similar trends (-5.3 and 0.1). Malaria risk was less varied during low transmission seasons, with seasonal risk differences of -8.0, -1.4, and -2.4 cases per 100 people among <5 years, ≥5 years, and all ages comparing 2019 to 2018.

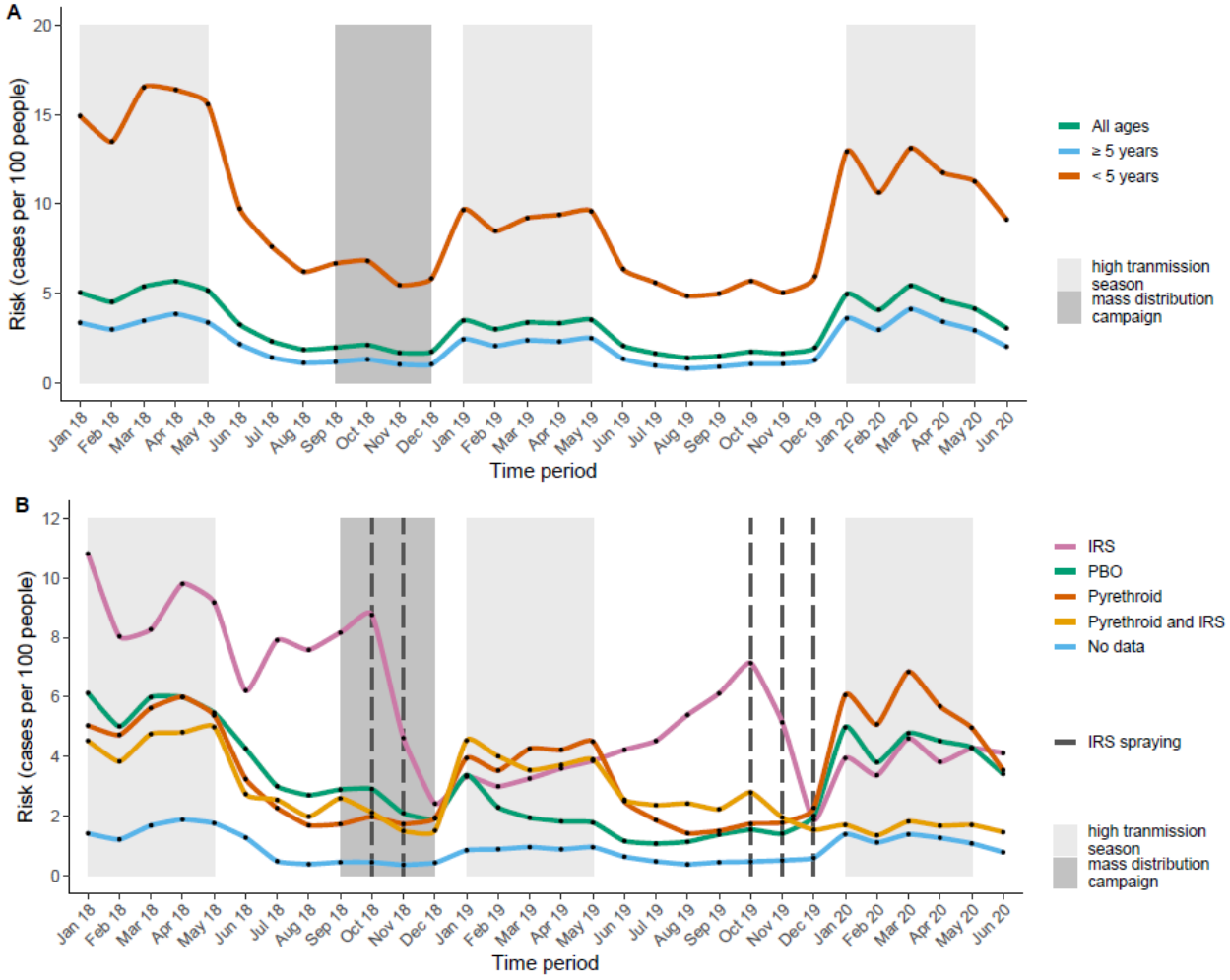


Figure 4.5 Risk of malaria (cases per 100 people) from January 2018 to June 2020, stratified A) by age group, and B) by insecticide type. 'No data' refers to health facilities which did not have any bed net distribution or IRS information. Dark gray color blocks represent months where the mass distribution campaign occurred (September to December 2018) and light gray blocks represent months which fall during the yearly high malaria transmission season (January to May). Risk was measured monthly and curves are smoothed using X-splines. Note: IRS = indoor residual spraying, PBO = piperonyl butoxide.

IRS: 1 district; 22 health facilities; 387,523 people; PBO: 9 districts; 127 health facilities; 3,150,863 people; Pyrethroid: 17 districts; 423 health facilities; 10,854,018 people; Pyrethroid and PBS: 1 district; 48 health facilities; 1,141,817 people; No data: 91 health facilities; 2,221,396 people

Table 4.1 Risk of malaria (cases per 100 people) by high (January to May) and low (June to December) malaria transmission seasons, stratified by age group and insecticide type, January 2018 to June 2020. High transmission estimates represent 5-month risks, low transmission estimates represent 7-month risks.

		High transmission* (cases per 100 people)						**Low transmission*** (cases per 100 people)		
		2018 risk	**2019 risk**	**2020 risk**	**RD 2019 vs. 2018**	**RD 2020 vs. 2018**		**2018 risk**	**2019 risk**	**RD 2019 vs. 2018**
Age group	<5 years	76.5	46.4	59.7	-30.2	-16.8		46.5	38.4	-8.0
	≥5 years	16.9	11.6	17.0	-5.3	0.1		8.8	7.4	-1.4
	All ages	25.6	16.7	23.2	-8.9	-2.4		14.3	11.9	-2.4
Insecticide type	IRS	46.1	17.0	20.0	-29.1	-26.1		44.0	34.4	-9.6
	Pyrethroid	26.8	20.5	28.6	-6.3	1.9		14.3	13.0	-1.3
	PBO	28.6	11.2	22.4	-17.4	-6.2		18.8	9.7	-9.1
	Pyrethroid / IRS	22.8	19.7	8.2	-3.2	-14.6		14.1	15.8	1.8
	No data	7.8	4.5	6.2	-3.3	-1.6		3.4	3.5	0.0

* High transmission season: January to May; Low transmission season: June to December
IRS = indoor residual spraying, PBO = piperonyl butoxide, RD = risk difference

Risk followed a similar pattern of decline and rebound when stratified by LLIN type. Administration of PBO-treated nets resulted in risk differences of -17.4 and -6.2 comparing 2019 and 2020 to 2018 high transmission seasons, followed by pyrethroid-treated nets (-6.3 and 1.9). Use of IRS sustained protection, with a risk difference of -29.1 cases per 100 people comparing 2019 and 2018 high transmission seasons and a risk difference of -26.1 comparing 2020 and 2018. In Mangochi, pyrethroid-treated nets somewhat reduced risk in 2019 (RD: -3.2), but IRS spraying in 2019 dramatically reduced risk in 2020 (RD: -14.6). IRS and PBO-treated nets moderately reduced risks during the 2019 low transmission season (RD: -9.6, -9.1) relative to 2018, but there was minimal change in areas receiving pyrethroid-treated nets (RD: -1.3).

Implementation of IRS, PBO-, and pyrethroid-treated nets resulted in varying effects by district (**Figure 4.6, Supplementary Figure B1**). Ntchisi, receiving PBO-treated nets,

underwent the greatest reduction in risk of -38.4 cases per 100 people comparing high transmission seasons in 2019 and 2018 (**Supplementary Table B4**). Overall, 12 of the 28 districts (42.9%) experienced increases in malaria risk between 2018 and 2020 high transmission seasons. Nkhotakota, receiving IRS, experienced the greatest reduction in risk (-26.1) between 2018 and 2020 high transmission seasons.

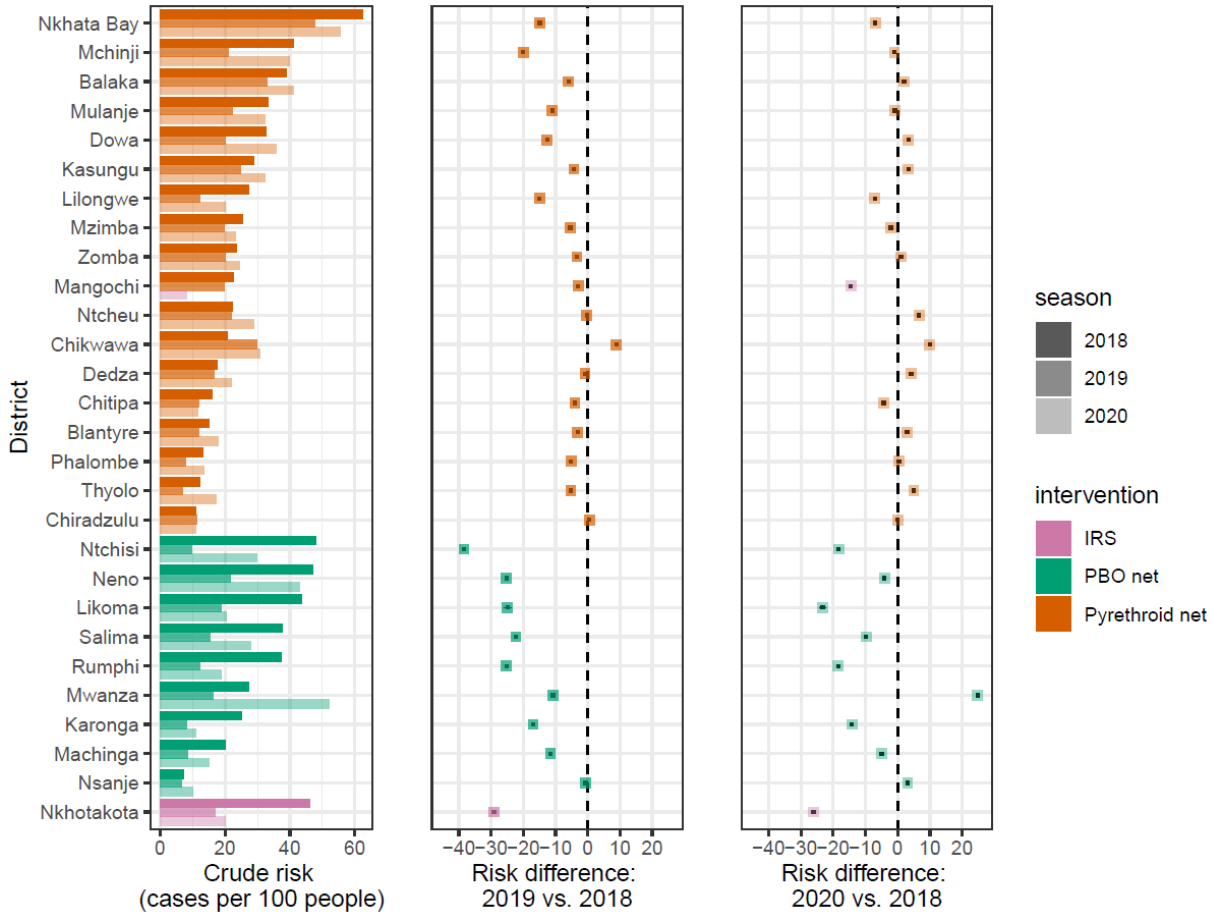


Figure 4.6 Risk of malaria (cases per 100 people) and risk differences from 2018 to 2019 and 2020, stratified by district and type of intervention. Data are shown for months falling within the high malaria transmission season (January to May), and estimates represent 5-month risks. Risk difference point values and 95% confidence intervals are shown by black dots and lines underlying each colored point, although confidence intervals are not readily visible because of the narrow range. Mchinji's net distribution was comprised of 69.4% pyrethroid-treated nets and 30.6% PBO-treated nets. Note: IRS = indoor residual spraying, PBO = piperonyl butoxide.

We estimate that, if all districts had received pyrethroid-treated nets, malaria risk would have been 5.1 cases per 100 people (95% CI: 4.8 to 5.3) in 2019 and 7.1 (95% CI: 6.8 to 7.4) in 2020 high transmission seasons. If all districts had received PBO-treated nets, malaria risk would have been lower in both years at 2.8 (95% CI: 2.5 to 3.1) and 5.6 (95% CI: 5.2 to 6.0). If all districts had received IRS, malaria risk in 2019 would have been 3.6 (95% CI: 3.1 to 4.1) and 4.3 (95% CI: 3.7 to 4.8) in 2020. Had all districts received PBO-treated nets, risk would have been -2.3% lower (95% CI: -2.7 to -1.9) in 2019 compared to 2018 high transmission seasons, and -1.5% lower (95% CI: -2.0 to -1.0) in 2020 compared to 2018, relative to pyrethroid-treated nets. Had all districts had received IRS, risk would have been 1.4% lower (95% CI: -2.0 to -0.9) in 2019 compared to 2018 high transmission seasons, and 2.8% lower (95% CI: -3.5 to -2.2) in 2020 compared to 2018, relative to pyrethroid-treated nets.

The 2018 Malawi census recorded 17,563,749 million people;[95] using our adjusted risk values we estimate that relative to national roll-out of pyrethroid-treated nets, distribution of PBO-treated nets in all districts in 2018 would have averted 665,522 (95% CI: 505,858 to 825,185) confirmed cases and application of IRS would have averted 752,771 (95% CI: 532,559 to 972,983) cases over the 2019 and 2020 high transmission seasons.

National average monthly temperature was similar in the months preceding 2018 and 2019 high transmission seasons (**Supplementary Figure B2**). National average precipitation was slightly higher in the months preceding the 2019 high transmission season as compared to previous years, potentially influencing underlying malaria transmission.

Discussion

Over 30 months, risk of malaria varied locally and was highest from January to May and among children <5 years of age. Malawi experienced declining national risk in 2019 and

subsequent rebound in 2020, however, patterns varied by district and intervention type. In 2020, 12 of 28 districts (42.9%) experienced increases in malaria risk, suggesting that protective effects disappear 1 to 2 years after LLIN distribution. PBO-treated nets performed better than pyrethroid-treated nets in adjusted analyses. Annual IRS application sustained low malaria risk over two transmission seasons, and a single IRS application in 2019 reduced malaria risk in a district where pyrethroid-treated nets had no effect. Our findings suggest that national roll-out of PBO-treated LLINs or IRS would have the potential to avert hundreds of thousands of cases relative to pyrethroid-treated nets. Policy and programmatic prevention decisions have the potential to substantially alter Malawi's malaria burden.

Longitudinal analysis demonstrates a reduction in malaria risk following mass LLIN distribution among our study population, but a subsequent re-bounding of disease suggests that LLINs do not universally retain effectiveness through two high transmission seasons. Prior research in Malawi has depicted increased malaria prevalence among children two years after mass distribution of 5.6 million nets, despite reporting increased bed net usage over the same time period.[37] Individual ITN use was associated with a lower individual risk of malaria at baseline, but this association disappeared within two years. Similarly, research among adolescents and adults has shown that sleeping under an LLIN was not associated with reduced risk of malaria prevalence 3-4 years after a mass distribution campaign, even after stratifying by bed net insecticide type.[38]

Risk of malaria was heterogeneous at the health facility level, with wide variations depending on geographic location and month, aligning with estimated differences at the sub-district level.[50,51] Environmental risk factors contribute to fluctuations in malaria transmission, and elevation and rainfall[55] are potential contributors to the spatial heterogeneity of LLIN effects

by district. Fine-scale longitudinal mapping of malaria risk allows for local monitoring and evaluation, including targeting of resources to small areas during periods of high transmission.

Potential sources of a short LLIN lifespan include repurposing, damage, and insecticide-resistance, and attenuation of insecticide concentration. Mosquito collection studies in Malawi show increasing insecticide-resistance over time to pyrethroid-treated nets[100], but retention of efficacy with added PBO, similar to our study findings. An estimated 50% of ITNs are lost from households in sub-Saharan Africa after 23 months, another likely contributor to reduced intervention effects over time.[101] In Malawi, individuals repurpose ITNS for fishing and farming, and sell nets to support their families encountering poverty and food-insecurity,[40] all of which contribute to reduced intervention effectiveness.

The frequency of LLIN distribution also influences effectiveness. The World Health Organization recommends mass distribution campaigns every 3 years, allocating one LLIN for every 1.8 people.[80] However, evidence from field research in Eastern and Southern Africa questions this recommendation, consistently finding that malaria incidence rebounds 1-3 years following ITN or LLIN distribution.[81–83,93,94] Qualitative studies are necessary to discover local reasons for LLIN effectiveness variation. Sites in Madagascar followed similar disease trends as our study, experiencing rebounding malaria 2-3 years following a mass distribution campaign; however, malaria incrementally declined in areas receiving additional continuous LLIN distribution to households including young children, immigrants, pregnancy women, and recently married couples.[90] Malawi distributes nets continuously to pregnant women and newborns, but the protection provided by mass LLIN campaigns could be potentially sustained through introduction of additional distribution channels or expanded inclusion criteria.

Our study represents one of the largest published analyses of LLIN effectiveness over time, capturing records from 711 health facilities over a 30-month period and representing the most granular national estimates published to-date. Data were reported at a near capacity rate each month, with 98% completeness in 2018 and 96% in 2019. Approximately 98% of all suspected cases were confirmed nationally in 2018, indicating high testing coverage of individuals presenting for care.[32] Use of routinely collected DHIS2 data, provided by the National Malaria Control Programme, represents an efficient use of resources to study the effectiveness of Malawi's most comprehensive malaria prevention intervention.

The main limitations of our analysis stem from missing data; we excluded facilities lacking spatial coordinates and missing 2018 data. However, many of the excluded health facilities were small, and our analysis still captured 97.1% (16.6 M/17.1 M) of confirmed DHIS2 malaria cases and 99.7% (709/711) of facilities reported data for at least 24 months during the study period. We are also missing malaria cases who do not report to a clinic for diagnosis; a potentially substantial population as only 54% of children <5 years with fever sought advice or treatment in 2017.[35] Our Thiessen polygons were built using Euclidean distance, assuming that individuals attend the nearest health facility and that everyone in the catchment area accesses the facility with equal probability. Facility size and quality of care also likely influences health seeking behavior, and referral hospitals may double count cases already recorded at smaller clinics. We updated projected WorldPop rasters using 2018 census data; while this provides more accurate population estimates, modeled population values are imperfect. We were not able to examine synergistic effects of IRS and LLINs, but future analysis will compare various intervention combinations as Malawi expands IRS coverage. Additional influences on malaria

counts include potential underreporting in April to June 2020 due to the COVID-19 pandemic, and introduction of limited RTS,S malaria vaccine roll-out to children in April 2019.

Our study design can serve as a model for other countries collecting health metrics through DHIS2. Use of a routine surveillance system provides a natural opportunity to study disease trends over time and create quasi-experimental designs to measure interventions on a large scale without the cost and limited scope of primary data collection. Assessment of malaria risk provides valuable insight into current and historical case trends as the Ministry of Health plans future mass distribution campaigns. Decisions regarding type of insecticide and frequency of net distribution have the potential to substantially alter the malaria landscape in Malawi, averting morbidity, mortality, and a strain on health system resources for diagnosis and treatment.

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

Review of Aims

One of the goals of this manuscript was to understand the landscape of both asymptomatic and clinically confirmed malaria at fine geospatial scales. Aim 1 sought to identify which adolescents and adults are infected with asymptomatic *P. falciparum* infection, populations which are often omitted in malaria prevalence studies, but key to transmission in an endemic country. Through use of 2015-2016 MDHS dried blood spots collected from adolescents and adults ages 15-54 years, we conducted quantitative PCR to detect parasitemia. By linking PCR data with survey responses and other spatial and environmental data sources, we identified risk factors for infection including male sex, rurality, poverty, lower education, living in wetlands and grasslands, and higher environmental temperatures. Living in a household with a bed net (prevalence difference: 0.02, 95% CI: -0.02 to 0.05), living in a household with 1 net per 1.8 household members (PD: 0.02; 95% CI: -0.01 to 0.06), and sleeping under an LLIN (PD: 0.01; 95% CI: -0.02 to 0.04) were not protective against infection. When *P. falciparum* prevalence was mapped, cluster estimates ranged from 2.5% to 83.5% indicating high spatial heterogeneity. Prevalence estimates were much higher than modeled predictions and household survey measurements among children. Identifying risk factors for asymptomatic adult infection is essential for targeted interventions to high-risk sub-populations.

The goal of Aim 2 was to assess differences in the risk of clinically confirmed malaria at the health facility level before and after a mass LLIN distribution campaign. Using DHIS2 health facility surveillance data and WorldPop geospatial data, we compared risk in each month from

January 2018 to June 2020 among children <5 and persons ≥5 years. 711 health facilities contributed 20,962 facility reports. We created geospatial maps by high and low malaria transmission seasons to create the first visualizations of risk of malaria at the health facility level, enabling identification of high-risk areas and time periods. We stratified results by intervention type including PBO-treated nets, pyrethroid-treated nets, and/or application of IRS. Malaria risk decreased from 25.6 to 16.7 cases per 100 people from 2018 to 2019, and rebounded to 23.2 in 2020, resulting in risk differences of -8·9 in 2019 and -2·4 in 2020 as compared to 2018. Piperonyl butoxide-treated (PBO) LLINs were more effective than pyrethroid-treated LLINs, with adjusted risk differences of -2.3 (95% CI: -2.7 to -1.9) cases per 100 people comparing 2018 to 2019 and -1.5 (95% CI: -2·0 to -1·0) comparing 2018 to 2020. Relative to pyrethroid-treated nets, national roll-out of PBO-treated nets in 2018 would have averted 665,522 (95% CI: 505,858 to 825,185) confirmed cases of malaria over the 2019 and 2020 high transmission seasons following the mass campaign and national use of IRS would have averted 752,771 (95% CI: 532,559 to 972,983) cases. Findings suggest that while the World Health Organization recommends mass LLIN distribution every 3 years, the protective effects of LLINs disappear 1 to 2 years after LLIN distribution in Malawi, leaving millions unprotected. Policy decisions regarding the type of LLIN used and application of IRS in national rollouts have the potential to substantially alter Malawi's malaria burden, preventing morbidity, mortality, and a strain on health center resources for diagnosis and treatment.

Conclusions

Answers to the research questions posed here contribute to filling gaps in knowledge about patterns of malaria risk in Malawi among adolescents and adults, at fine geospatial scales, and across months of the year. Use of molecular methods allowed a national molecular

assessment of malaria prevalence among adolescents and adults for the first time in Malawi, benefiting the MOH as the government prioritizes resources and interventions to high-risk areas and population groups. Malawi also benefits from analysis of DHIS2 data, which can become an ongoing surveillance tool to continuously monitor malaria risk and the benefits or lack of benefit contributed by roll-out of national or regional interventions against malaria. Use of MDHS samples and DHIS2 provides opportunities to use existing resources to answer additional research questions about malaria, without extra funds and manpower dedicated to primary data collection. Household surveys require extensive time and resources dedicated to active surveillance data collection efforts; the average cost of a DHS survey is $1.6 million and involves active visits to 27,516 individual households.[46,53] Malawi spent an estimated $82 million on malaria control in 2016[88] and malaria accounts for 30% of all outpatient visits and 34% of inpatient hospital admissions.[9] Households also often amass high direct and indirect costs due to clinical malarial disease, despite free diagnosis and treatment.[89] Use of national existing surveillance data to inform policy makers can efficiently make use of both financial and human capital while reducing the malaria burden.

Malawi could benefit from more frequent mass distribution campaigns or additional expansion of annual IRS to a greater number of areas.[9] Research from Madagascar shows that while LLIN mass distribution campaigns may only provide community protection for one year, protection can be sustained when campaigns are followed by continuous LLIN distribution to eligible households, including recently married couples, immigrants, children of vaccination age, and homes with uncovered sleeping areas.[90] Malawi may also benefit from increased education on consistent and correct use of bed nets, and through expansion of continuous LLIN distribution

services to additional populations beyond pregnant women, targeting younger individuals living in rural areas with high prevalence of infection for more frequent net replacement.

Future Directions

The enclosed findings represent a starting point for continued molecular and epidemiological malaria surveillance in Malawi. 2015-2016 MDHS dried blood spots can be further assessed for the presence of *P. vivax*, *P. ovale,* and *P. malariae*, species which are thought to be rare in sub-Saharan Africa, but which have never been measured nationally in Malawi using PCR-based methods. Instances of *P. ovale* and *P. malariae* have been identified among children using microscopy,[35] but prevalence has not been assessed in adults, a large potential reservoir for transmission. Additionally, 2015-2016 MDHS dried blood spots can be subsequently tested for drug-resistant alleles, similar to what has been done in the Democratic Republic of the Congo.[102] Similar molecular testing following the 2021 MDHS and future iterations of DHS among adults can be used to track changes in *P. falciparum*, other *Plasmodium spp*., and drug resistance alleles over time to determine the potential effectiveness of interventions. Collection of dried blood spots can also be added to MIS among children to better assess parasite prevalence and other genetic markers among all ages of the population.

As the RTS,S/AS01 malaria vaccine is programmatically tested in sub-Saharan Africa, molecular surveillance can also help monitor genetic changes in the population over time. RTS,S/AS01 has been shown to be more effective against malaria strains which match the vaccine construct as opposed to strains which mis-match,[103] but researchers do not know the geographic prevalence of strain type across Malawi. 2015-2016 MDHS dried blood spots can be assessed for malaria strain prevalence, with analysis repeated after the 2021 MDHS to determine if introduction of the vaccine among children in 2019 causes specific strain-selection among

adults. Large-scale data collection across multiple years is necessary to track vaccine-induced variant selection on a population level. Antimalarial drug use creates known selective pressure towards drug resistance and other loci in Malawi,[104] and within Africa and Asia,[105] causing ebbs and flows of resistant strains with use and disuse of medications,[106–108] and prompting hypotheses that similar selection could occur with the introduction of RTS,S/AS01. Genomics can be a useful tool to characterize parasite populations where malaria vaccines are more or less effective, monitor parasite populations where RTS,S/AS01 implementation has occurred, predict the effect of vaccine roll-outs in certain areas, and contribute to future vaccine development.[109]

DHIS2 can undergo frequent analysis, similar to the results provided here, to monitor clinically confirmed malaria risk among subpopulations and the effect of new interventions on disease risk over time. DHIS2 creates a sustainable feedback loop between health officials at the health facility, district, and national levels in Malawi, and the addition of regular analysis forms a partnership with research institutions to supplement MIS and DHS cross-sectional prevalence study results. Additional DHIS2 variables could be added to analysis to record aggregate case counts among school-aged children, and existing variables such as suspected malaria case counts and drug administration could be analyzed to determine geospatial and temporal differences in testing rates and drug stockouts.

To supplement results showing that LLINs in Malawi have a 1-2-year lifespan, future qualitative research can also be performed to identify local reasons for LLIN disuse. Along the shores of Lake Malawi, individuals have been found to repurpose ITNS for fishing and farming, and to sell nets to counter poverty and food-insecurity,[40] but research is needed in other areas of the country to determine the best ways to extend LLIN lifespan or to better encourage LLIN use in households. Additionally, research questions can investigate different combinations of

interventions including variation in LLIN distribution, introduction of new LLIN types, expansion of continuous distribution to additional subpopulations, and co-application of LLINs and IRS within the same households. Cost-effectiveness studies can supplement program evaluation findings to create a more nuanced national strategy for malaria prevention.

Future replication of the analyses presented here will form a more complete longitudinal picture of asymptomatic infection and confirmed clinical disease in Malawi. Malawi's Health Sector Strategic Plan aims to cut the malaria incidence rate of presumed and confirmed cases from 2015 levels of 380 per 1,000 to 200 per 1,000 by 2021.[44] The same plan also hopes to cut inpatient malaria deaths per year from 23 per 100,000 to 14 per 100,000 within the same time period. Ongoing surveillance of disease and evaluation of prevention programs create opportunities to understand where to target interventions and which interventions to utilize to interrupt the transmission cycle most effectively. These and future data provide the MOH with valuable insight to avert malaria cases and save lives affected by a preventable disease.

APPENDIX A: CHAPTER THREE SUPPLEMENTARY MATERIALS

Technical Methods

DNA amplification and genotyping

DBS were punched in triplicate into 96 well plates using a 6 mm hole-punch at UNC Project-Malawi and shipped to the University of North Carolina at Chapel Hill for testing. DBS samples were stored at -20°C prior to parasite DNA extraction using a Tween-Chelex protocol modified from Teyssier et al. 2019. One milliliter of 0.5% Tween 20 in 1X PBS was added to each well and plates were incubated at room temperature overnight on shakers. After centrifuging, Tween-PBS was aspirated, and samples were washed with 1 mL of 1X PBS and underwent a 15-30-minute incubation at 4°C. After centrifuging again, PBS was aspirated and 150 µL of a 1:2 solution of 20% Chelex 100 resin in water was added to each well and plates were incubated for 10 minutes at 95°C, occasionally shaking. Plates were centrifuged at 1,500 rmp for 10 minutes and supernatant was transferred to non-skirted 96 well plates and centrifuged again at 1,500 rpm for 5 minutes, removing the supernatant to an Eppendorf LoBind DNA 96-well plate for storage. DNA was extracted and stored at -80°C until qPCR.

Samples were run in singular in 384 well plates alongside 2 negative controls and 2 of each concentration of positive controls across a wide range of parasitemias: 100,000; 1,000; 100; 10; and 1 parasite/µL. PCR primers and probe are noted in **Supplementary Table A1**. Real time PCR was conducted in 12 µL volumes, using Roche Universal Probe Master Mix and 2 µL of input DNA. The PCR cycles were 50C for 2 minutes and 95C for 10 minutes, followed by 40 cycles of 95C denaturation for 15 seconds and 60C annealing/extension for 1 minute. Parasitemias were calculated using a trendline based on the Ct values of the positive control standards within each plate.

PCR assay validation

In total we conducted 908 control reactions, including 480 positive controls and 428 negative controls (**Supplementary Table A2**). Negative controls were either water (n=48) or human DNA (0.1ng/µL) from a non-exposed population (n=380). Positive controls were generated across a range of parasitemias, from 10,000 parasites/µL to 1 parasite/µL. Cultured parasites (strain 3D7, MRA-102, BEI Resources, Manassas VA) were quantified using repeated counts on a hemocytometer and mixed with human whole blood in order to mock desired parasitemias. The spiked blood was dried onto filter paper at 70 µL per spot, and DNA was extracted from these filter papers using the same methodology as the clinical samples. Extracted spiked blood DNA was used for all control reactions and would approximate any losses of DNA through the extraction process. We conducted a higher number of replicates at the lowest parasitemia value (n=194) to mimic the expected distribution of parasitemias in the clinical samples. All assay results and amplification curves were manually evaluated on the machine prior to exportation to the database. Based on these data, we estimated the sensitivity, specificity, negative predictive value (NPV) and positive predictive values (PPV) across a range of prevalence estimates (**Supplementary Table A3**). Samples amplified with a PCR cycle threshold (CT) value above 39 were considered negative in the final analysis to remain conservative in our classification of *P. falciparum* infected individuals. A 39-cycle cutoff is approximately one standard deviation above the mean Ct value for our 194 replicates of 1 parasite/µl, our lowest positive control standard. The resultant data suggests that the assay has an extremely low false positivity rate.

Reference:

Teyssier, N.B. *et al.* Optimization of whole-genome sequencing of *Plasmodium falciparum* from low-density dried blood spot samples. *bioRxiv*. (2019)

Supplementary Table A1 Primers and reaction conditions; each reaction consisted of 12 µL.

Factor	Final Concentration	Sequence
Forward primer	300 nM	ACGATTTGGCTGGAGCAGAT
Reverse primer	300 nM	TCTCTATTCCATTCTTTGTCACTCTTTC
Probe	250 nM	FAM-AGTAATAGTAACAGCTGGATTTACCAAGGCCCCA-TAMRA

Supplementary Table A2 Control reactions. Negative controls represent water (n=48) or human DNA (n=380).

Parasites/µL	N	# positive at Ct 39	% positive at Ct 39	Mean Ct	Median Ct	Standard deviation Ct
0	428	0	0	-	-	-
1	194	148	76.3	37.5	37.1	1.61
5	48	48	100	34.3	34.3	0.34
10	84	83	98.8	33.7	33.4	1.12
100	50	50	100	30.8	30.6	1.25
1,000	52	52	100	26.5	26.3	0.89
10,000	52	52	100	22.6	22.6	0.63

Supplementary Table A3 Sensitivity, specificity, negative predictive value, and positive predictive values across a range of *P. falciparum* prevalence estimates.

Prevalence	Sensitivity	Specificity	Positive predictive value	Negative predictive value
-	90.2	100	-	-
0.1	-	-	100	98.9
0.2	-	-	100	97.6
0.3	-	-	100	96.0
0.4	-	-	100	93.9

Supplementary Table A4 Characteristics of 2015-16 MDHS samples which were available for the current analysis and those which were unavailable.

Variable		Unavailable samples n (%)	Available samples n (%)	p-value*
Total		7732	7393	
# clusters		633 (74.5)	497 (58.5)	
Sex	Male	3569 (46.2)	3472 (47.0)	0.3
	Female	4163 (53.8)	3921 (53.0)	
Age group (years)	15-24	3257 (42.1)	3285 (44.4)	0.03
	25-34	2312 (29.9)	2093 (28.3)	
	35-44	1554 (20.1)	1429 (19.3)	
	45-54	609 (7.9)	586 (7.9)	
Wealth quintiles	Poorest	1273 (16.5)	1100 (14.9)	<0.001
	Poorer	1454 (18.8)	1338 (18.1)	
	Middle	1536 (19.9)	1330 (18.0)	
	Richer	1639 (21.2)	1471 (19.9)	
	Richest	1830 (23.7)	2154 (29.1)	
Education	None	610 (7.9)	548 (7.4)	0.001
	Primary	4701 (60.8)	4336 (58.7)	
	Secondary	2129 (27.5)	2244 (30.4)	
	Higher education	277 (3.6)	238 (3.2)	
	Missing	15 (0.2)	27 (0.4)	
Owns livestock, herds or farm animals	No	3366 (43.5)	3354 (45.4)	0.02
	Yes	4366 (56.5)	4039 (54.6)	
Source of drinking water	Piped	1772 (22.9)	2387 (32.3)	<0.001
	Unpiped	5960 (77.1)	5006 (67.7)	
Household has a bed net	No	2253 (29.1)	2299 (31.1)	0.009
	Yes	5479 (70.9)	5094 (68.9)	
Slept under an LLIN last night	No	4957 (64.1)	4677 (63.3)	0.3
	Yes	2775 (35.9)	2716 (36.7)	
Insecticide of LLIN individual slept under last night	Permethrin	1691 (60.9)	1641 (60.4)	0.6
	Non-permethrin	1084 (39.1)	1074 (39.5)	
	Missing	0 (0.0)	1 (0.0)	
1 net per 1.8 household members	No	7705 (99.7)	7357 (99.5)	0.4
	Yes	21 (0.3)	29 (0.4)	

Anemia (women only)	Not anemic	2770 (66.5)	2585 (65.9)	0.6
	Mild	1064 (25.6)	1027 (26.2)	
	Moderate	296 (7.1)	283 (7.2)	
	Severe	32 (0.8)	23 (0.6)	
	Missing / NA	1 (0.0)	3 (0.1)	
Region	Northern	1025 (13.3)	1896 (25.6)	<0.001
	Central	3046 (39.4)	2357 (31.9)	
	Southern	3661 (47.3)	3140 (42.5)	
Place of residence	Urban	1541 (19.9)	1671 (22.6)	<0.001
	Rural	6191 (80.1)	5722 (77.4)	
Elevation (m)	<500	1480 (19.1)	1474 (19.9)	<0.001
	≥ 500 & <1000	3190 (41.3)	2828 (38.3)	
	≥ 1000 & <1500	2891 (37.4)	2881 (39.0)	
	≥ 1500	171 (2.2)	210 (2.8)	
Month of data collection	October '15	1528 (19.8)	1038 (14.0)	<0.001
	November '15	3070 (39.7)	2005 (27.1)	
	December '15	1627 (21.0)	904 (12.2)	
	January '16	1335 (17.3)	3078 (41.6)	
	February '16	172 (2.2)	368 (5.0)	
Proportion of cluster with bed nets	mean (SD)	0.68 (0.18)	0.68 (0.19)	0.5
Proportion of cluster that slept under an LLIN last night	mean (SD)	0.32 (0.16)	0.33 (0.15)	<0.001

* significance testing uses t-tests with an alpha=0.05 for continuous variables and chi-squared tests for categorical variables, comparing between strata of available and unavailable samples.

Supplementary Table A5 Bivariate associations between demographic and environmental risk factors and *P. falciparum* prevalence using weighted survey data. Samples amplified with a qPCR cycle threshold value above 38 were considered negative.

Covariates	Variable	*P. falciparum* Prevalence	Prevalence Difference	95% Confidence Interval		p-value
Sex	Male	0.303	-	-	-	-
	Female	0.263	-0.04	-0.06	-0.01	0.002
Age group (years)	15-24	0.335	-	-	-	-
	25-34	0.256	-0.08	-0.11	-0.05	<0.001
	35-44	0.227	-0.11	-0.15	-0.07	<0.001
	45-54	0.224	-0.11	-0.16	-0.06	<0.001
Wealth quintiles	Poorest	0.396	-	-	-	-
	Poorer	0.351	-0.04	-0.09	0.00	0.04
	Middle	0.308	-0.09	-0.14	-0.04	<0.001
	Richer	0.239	-0.16	-0.20	-0.11	<0.001
	Richest	0.144	-0.25	-0.30	-0.20	<0.001
Education	None	0.305	-	-	-	-
	Primary	0.322	0.02	-0.03	0.07	0.5
	Secondary	0.211	-0.09	-0.15	-0.04	0.001
	Higher	0.039	-0.27	-0.32	-0.21	<0.001
Owns livestock, herds or farm animals	No	0.270	-	-	-	-
	Yes	0.292	0.02	-0.01	0.05	0.1
Source of drinking water	Piped	0.148	-	-	-	-
	Unpiped	0.318	0.17	0.13	0.21	<0.001
Household has a bed net	No	0.283	-	-	-	-
	Yes	0.281	0.00	-0.03	0.03	0.9
Slept under an LLIN last night	No	0.289	-	-	-	-
	Yes	0.271	-0.02	-0.05	0.01	0.2
Insecticide of LLIN individual slept under (out of individuals sleeping under nets)	Permethrin	0.279	-	-	-	-
	Non-permethrin	0.256	-0.02	-0.07	0.02	0.3
At least 1 net per 1.8 household members	No	0.230	-	-	-	-
	Yes	0.295	0.07	0.02	0.11	0.002
Anemia (women only)	Not anemic	0.233	-	-	-	-
	Mild	0.324	0.09	0.04	0.14	<0.001
	Moderate	0.317	0.08	0.01	0.16	0.03
	Severe	0.478	0.24	-0.03	0.52	0.08

Region	Northern	0.208	-	-	-	-
	Central	0.318	0.11	0.06	0.16	<0.001
	Southern	0.279	0.07	0.02	0.12	0.006
Place of residence	Urban	0.112	-	-	-	-
	Rural	0.314	0.2	0.16	0.24	<0.001
Elevation (m)	<500	0.266	-	-	-	-
	≥ 500 & <1000	0.347	0.08	0.02	0.14	0.01
	≥ 1000 & <1500	0.244	-0.02	-0.08	0.04	0.5
	≥ 1500	0.096	-0.17	-0.23	-0.11	<0.001
Month of data collection	October '15	0.394	-	-	-	-
	November '15	0.255	-0.14	-0.21	-0.07	<0.001
	December '15	0.220	-0.17	-0.26	-0.09	<0.001
	January '16	0.250	-0.14	-0.21	-0.08	<0.001
	February '16	0.349	-0.04	-0.16	0.07	0.4
Landcover	Settlement	0.096	-	-	-	-
	Forest	0.314	0.22	0.15	0.29	<0.001
	Grassland	0.322	0.23	0.13	0.32	<0.001
	Cropland	0.310	0.21	0.18	0.25	<0.001
	Wetland	0.354	0.26	0.16	0.36	<0.001
	Other	0.107	0.01	-0.02	0.04	0.5
Proportion of cluster with bed nets (scaled)	Mean	0.282	-	-	-	-
	10% increase	-	-0.05	-0.17	0.08	0.5
Proportion of cluster that slept under an LLIN last night (scaled)	Mean	0.282	-	-	-	-
	10% increase	-	-0.11	-0.25	0.03	0.1
Current month's average daily maximum temperature (°C) (scaled)	Mean	0.282	-	-	-	-
	1°C increase	-	0.02	0.01	0.03	<0.001
Prior month's precipitation (mm) (scaled)	Mean	0.282	-	-	-	-
	100 mm increase	-	-0.02	-0.04	0.01	0.2

Note: LLIN = long-lasting insecticide treated net

Supplementary Table A6 Bivariate associations between demographic and environmental risk factors and *P. falciparum* prevalence using weighted survey data. Samples amplified with a qPCR cycle threshold value above 37 were considered negative.

Covariates	Variable	*P. falciparum* Prevalence	Prevalence Difference	95% Confidence Interval		p-value
Sex	Male	0.266	-	-	-	-
	Female	0.229	-0.04	-0.06	-0.01	0.003
Age group (years)	15-24	0.296	-	-	-	-
	25-34	0.226	-0.07	-0.1	-0.04	<0.001
	35-44	0.198	-0.10	-0.14	-0.06	<0.001
	45-54	0.171	-0.13	-0.17	-0.08	<0.001
Wealth quintiles	Poorest	0.352	-	-	-	-
	Poorer	0.314	-0.04	-0.08	0.00	0.08
	Middle	0.272	-0.08	-0.13	-0.03	0.001
	Richer	0.204	-0.15	-0.19	-0.11	<0.001
	Richest	0.117	-0.24	-0.28	-0.19	<0.001
Education	None	0.258	-	-	-	-
	Primary	0.286	0.03	-0.02	0.07	0.2
	Secondary	0.175	-0.08	-0.14	-0.03	0.002
	Higher	0.039	-0.22	-0.27	-0.16	<0.001
Owns livestock, herds or farm animals	No	0.234	-	-	-	-
	Yes	0.257	0.02	0.00	0.05	0.1
Source of drinking water	Piped	0.120	-	-	-	-
	Unpiped	0.280	0.16	0.13	0.19	<0.001
Household has a bed net	No	0.247	-	-	-	-
	Yes	0.246	0.00	-0.03	0.03	0.9
Slept under an LLIN last night	No	0.256	-	-	-	-
	Yes	0.231	-0.02	-0.05	0.00	0.1
Insecticide of LLIN individual slept under (out of individuals sleeping under nets)	Permethrin	0.237	-	-	-	-
	Non-permethrin	0.220	-0.02	-0.06	0.03	0.5
At least 1 net per 1.8 household members	No	0.193	-	-	-	-
	Yes	0.260	0.07	0.03	0.11	0.001
Anemia (women only)	Not anemic	0.201	-	-	-	-
	Mild	0.285	0.08	0.04	0.13	<0.001
	Moderate	0.279	0.08	0.01	0.15	0.04
	Severe	0.415	0.21	-0.05	0.48	0.1

Region	Northern	0.188	-	-	-	-
	Central	0.278	0.09	0.04	0.14	<0.001
	Southern	0.241	0.05	0.01	0.10	0.02
Place of residence	Urban	0.082	-	-	-	-
	Rural	0.277	0.19	0.16	0.22	<0.001
Elevation (m)	<500	0.238	-	-	-	-
	≥ 500 & <1000	0.306	0.07	0.01	0.13	0.02
	≥ 1000 & <1500	0.210	-0.03	-0.08	0.02	0.3
	≥ 1500	0.073	-0.17	-0.22	-0.11	<0.001
Month of data collection	October '15	0.343	-	-	-	-
	November '15	0.226	-0.12	-0.19	-0.05	0.001
	December '15	0.194	-0.15	-0.23	-0.07	<0.001
	January '16	0.216	-0.13	-0.19	-0.06	<0.001
	February '16	0.306	-0.04	-0.14	0.07	0.5
Landcover	Settlement	0.074	-	-	-	-
	Forest	0.273	0.20	0.14	0.26	<0.001
	Grassland	0.274	0.20	0.11	0.28	<0.001
	Cropland	0.276	0.20	0.17	0.23	<0.001
	Wetland	0.301	0.23	0.14	0.32	<0.001
	Other	0.062	-0.01	-0.05	0.02	0.5
Proportion of cluster with bed nets (scaled)	Mean	0.247	-	-	-	-
	10% increase	-	-0.05	-0.17	0.06	0.4
Proportion of cluster that slept under an LLIN last night (scaled)	Mean	0.247	-	-	-	-
	10% increase	-	-0.13	-0.25	0.00	0.06
Current month's average daily maximum temperature (°C) (scaled)	Mean	0.246	-	-	-	-
	1°C increase	-	0.02	0.01	0.03	<0.001
Prior month's precipitation (mm) (scaled)	Mean	0.247	-	-	-	-
	100 mm increase	-	-0.01	-0.04	0.01	0.3

Note: LLIN = long-lasting insecticide treated net

Supplementary Table A7 Multivariate associations between bed net associated risk factors and *P. falciparum* prevalence using weighted survey data. Samples amplified with a qPCR cycle threshold value above 38 were considered negative.

Exposure	Model	Prevalence Difference	95% Confidence Interval		p-value
Household has a bed net	Unadjusted	0.00	-0.03	0.03	0.9
	Adjusted*	0.02	-0.01	0.06	0.2
Slept under an LLIN last night	Unadjusted	-0.02	-0.05	0.01	0.2
	Adjusted*	0.01	-0.02	0.04	0.6
Individual slept under LLIN treated with non-permethrin (vs. permethrin)	Unadjusted	-0.02	-0.07	0.02	0.3
	Adjusted†	-0.02	-0.06	0.02	0.4
At least 1 net per 1.8 household members	Unadjusted	0.07	0.02	0.11	0.002
	Adjusted§	0.01	-0.03	0.04	0.8

* models adjusted for age, sex, wealth, and household size

† model adjusted for age, sex, and wealth

§ model adjusted for wealth and district

Note: LLIN = long-lasting insecticide treated net

Supplementary Table A8 Multivariate associations between bed net associated risk factors and *P. falciparum* prevalence using weighted survey data. Samples amplified with a qPCR cycle threshold value above 37 were considered negative.

Exposure	Model	Prevalence Difference	95% Confidence Interval		p-value
Household has a bed net	Unadjusted	0.00	-0.03	0.03	0.9
	Adjusted*	0.00	-0.03	0.03	1.0
Slept under an LLIN last night	Unadjusted	-0.02	-0.05	0.00	0.1
	Adjusted†	0.00	-0.02	0.03	0.9
Individual slept under LLIN treated with non-permethrin (vs. permethrin)	Unadjusted	-0.02	-0.06	0.03	0.5
	Adjusted†	-0.01	-0.05	0.03	0.7
At least 1 net per 1.8 household members	Unadjusted	0.07	0.03	0.11	0.001
	Adjusted§	0.01	-0.01	0.04	0.3

* models adjusted for age and sex

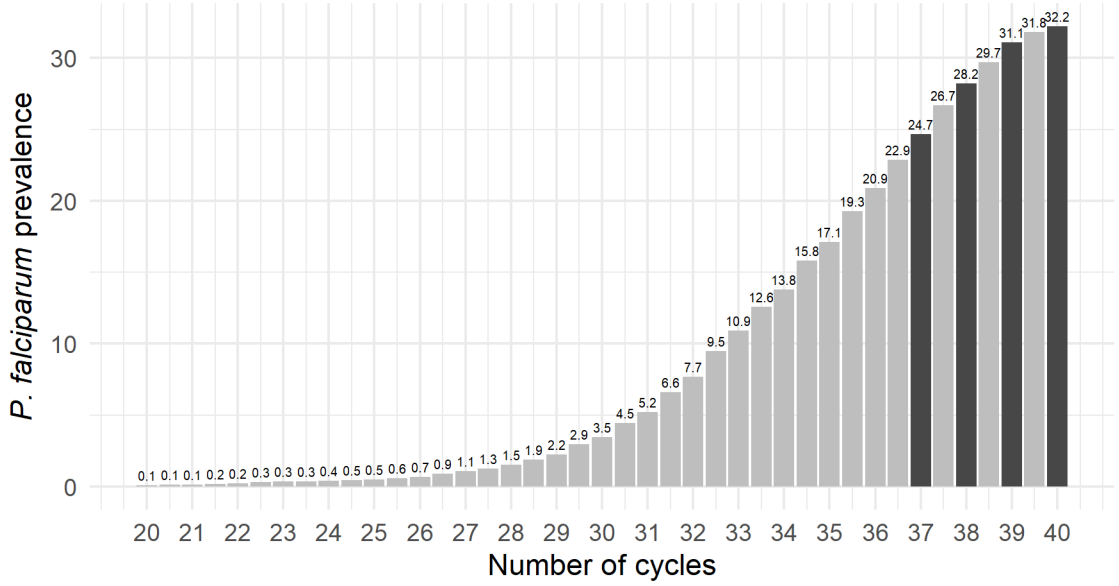
† model adjusted for age, sex, and wealth

§ model adjusted for wealth and district

Note: LLIN = long-lasting insecticide treated net

Supplementary Table A9 *P. falciparum* prevalence stratified by month and region.

Month	Region	N	Percent of samples collected during the month	*P. falciparum* prevalence
January	Northern	278	9.0	0.291
	Central	1094	35.5	0.282
	Southern	1706	55.4	0.264
February	Northern	213	57.9	0.268
	Central	137	37.2	0.467
	Southern	18	4.9	0.444
October	Northern	65	6.3	0.246
	Central	454	43.7	0.377
	Southern	519	50.0	0.428
November	Northern	824	41.1	0.223
	Central	486	24.2	0.358
	Southern	695	34.7	0.273
December	Northern	516	57.1	0.188
	Central	186	20.6	0.285
	Southern	202	22.3	0.243



Supplementary Figure A1 Weighted *P. falciparum* prevalence at various C_T values (n=7,393). The q-PCR cycle number shows how many cycles occurred before the sample's reaction curve crossed the threshold for a positive result.

APPENDIX B: CHAPTER FOUR SPPLEMENTARY MATERIALS

Supplementary Table B1 Proportion of health facilities, health facility reports, and confirmed malaria cases included in the study analysis and in DHIS2, by year.

	Health facilities				Health facility reports				Confirmed malaria cases		
Year	**included in study**	**DHIS2**	**%**		**included in study**	**DHIS2**	**%**		**included in study**	**DHIS2**	**%**
2018: Jan-Dec	711	756	94.0		8,170	8,607	94.9		6,833,895	6,997,921	97.7
2019: Jan-Dec	711	925	76.9		8,526	10,992	77.6		5,074,442	5,229,573	97.0
2020: Jan-Jun	711	926	76.8		4,266	5,500	77.6		4,664,899	4,839,038	96.4
TOTAL	711	926	76.8		20,962	25,099	83.5		16,573,236	17,066,532	97.1

DHIS2 = District Health Information Software 2

Supplementary Table B2 Bed net coverage by district, calculated using 2018 Malawi census population counts and bed net distribution counts for all health facilities included in the analysis (n=711). Nkhotakota did not distribute bed nets, but sprayed 112,264 structures in 2018, resulting in a coverage rate of 94.9% (PMI Malawi Malaria Operational Plan FY 2020).

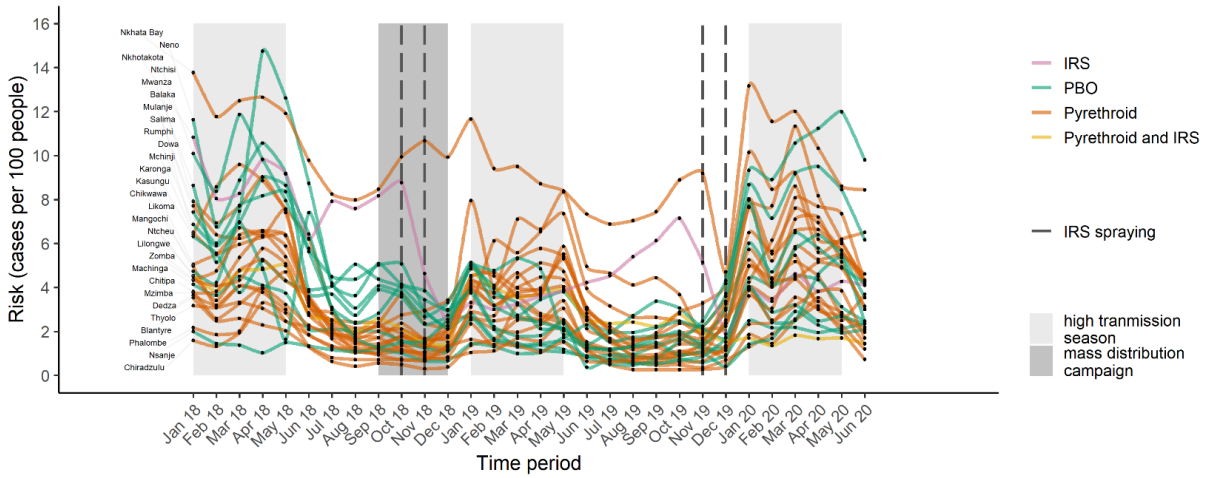
District	Intervention	Population size (2018)	Number of bed nets distributed	Coverage (nets per 100 people)	Number of people per net
Balaka	Pyrethroid	448,706	301,033	67.1	1.5
Blantyre	Pyrethroid	1,251,484	692,646	55.3	1.8
Chikwawa	Pyrethroid	534,779	396,272	74.1	1.3
Chiradzulu	Pyrethroid	356,875	238,011	66.7	1.5
Chitipa	Pyrethroid	234,927	129,579	55.2	1.8
Dedza	Pyrethroid	830,512	518,538	62.4	1.6
Dowa	Pyrethroid	772,569	461,366	59.7	1.7
Karonga	PBO	365,028	253,486	69.4	1.4
Kasungu	Pyrethroid	842,953	513,735	60.9	1.6
Likoma	PBO	14,527	8,535	58.8	1.7
Lilongwe	Pyrethroid	2,626,901	1,441,780	54.9	1.8
Machinga	PBO	735,438	518,742	70.5	1.4
Mangochi	Pyrethroid / IRS	1,148,611	784,210	68.3	1.5
Mchinji	Pyrethroid / PBO	602,305	413,605	68.7	1.5
Mulanje	Pyrethroid	684,107	466,162	68.1	1.5
Mwanza	PBO	130,949	84,698	64.7	1.5
Mzimba	Pyrethroid	1,161,456	672,652	57.9	1.7
Neno	PBO	167,409	81,198	48.5	2.1
Nkhata Bay	Pyrethroid	284,681	179,657	63.1	1.6
Nkhotakota	IRS	393,077	..	..	..
Nsanje	PBO	503,412	230,374	45.8	2.2
Ntcheu	Pyrethroid	659,608	392,781	59.5	1.7
Ntchisi	PBO	317,069	190,242	60.0	1.7
Phalombe	Pyrethroid	398,666	285,977	71.7	1.4
Rumphi	PBO	229,161	140,902	61.5	1.6
Salima	PBO	478,346	327,771	68.5	1.5
Thyolo	Pyrethroid	721,456	481,419	66.7	1.5
Zomba	Pyrethroid	860,604	583,576	67.8	1.5
TOTAL	..	17,755,616	10,788,947	68.8	1.6

IRS = indoor residual spraying, PBO = piperonyl butoxide

Supplementary Table B3 Risk of malaria (cases per 100 people) by month from January 2018 to June 2020, stratified by age group and intervention type. 'No data' refers to health facilities which did not have any bed net distribution or IRS information.

	Age group risk (cases per 100 people)				Insecticide type risk (cases per 100 people)				
	<5 years	**≥5 years**	**All ages**		**IRS**	**Pyrethroid**	**PBO**	**Pyrethroid / IRS**	**No data**
18-Jan	14.9	3.4	5.0		10.8	5.0	6.1	4.5	1.4
18-Feb	13.5	3.0	4.5		8.0	4.7	5.0	3.8	1.2
18-Mar	16.5	3.5	5.4		8.3	5.6	6.0	4.8	1.7
18-Apr	16.4	3.8	5.7		9.8	6.0	6.0	4.8	1.9
18-May	15.6	3.4	5.2		9.2	5.4	5.5	5.0	1.8
18-Jun	9.7	2.2	3.3		6.2	3.2	4.3	2.7	1.3
18-Jul	7.6	1.4	2.3		7.9	2.3	3.0	2.5	0.5
18-Aug	6.2	1.1	1.8		7.6	1.7	2.7	2.0	0.4
18-Sep	6.7	1.2	2.0		8.2	1.7	2.9	2.6	0.4
18-Oct	6.8	1.3	2.1		8.8	2.0	2.9	2.1	0.4
18-Nov	5.5	1.0	1.7		4.6	1.7	2.1	1.5	0.4
18-Dec	5.8	1.0	1.7		2.4	1.9	1.9	1.5	0.4
19-Jan	9.7	2.4	3.5		3.3	3.9	3.4	4.5	0.8
19-Feb	8.5	2.1	3.0		3.0	3.5	2.3	4.0	0.9
19-Mar	9.2	2.4	3.4		3.3	4.3	1.9	3.5	0.9
19-Apr	9.4	2.3	3.3		3.6	4.2	1.8	3.7	0.9
19-May	9.6	2.5	3.5		3.9	4.5	1.8	3.9	0.9
19-Jun	6.4	1.3	2.1		4.2	2.5	1.2	2.5	0.6
19-Jul	5.6	1.0	1.6		4.5	1.9	1.1	2.4	0.5
19-Aug	4.8	0.8	1.4		5.4	1.4	1.1	2.4	0.4
19-Sep	5.0	0.9	1.5		6.1	1.5	1.4	2.2	0.4
19-Oct	5.7	1.1	1.7		7.1	1.7	1.5	2.8	0.5
19-Nov	5.0	1.1	1.6		5.1	1.8	1.4	1.9	0.5
19-Dec	5.9	1.3	2.0		1.9	2.3	2.0	1.5	0.6
20-Jan	12.9	3.6	5.0		3.9	6.1	5.0	1.7	1.4
20-Feb	10.6	3.0	4.1		3.4	5.1	3.8	1.3	1.1
20-Mar	13.1	4.1	5.4		4.6	6.8	4.8	1.8	1.4
20-Apr	11.8	3.4	4.6		3.8	5.7	4.5	1.7	1.3
20-May	11.3	2.9	4.1		4.3	5.0	4.3	1.7	1.1
20-Jun	9.1	2.0	3.0		4.1	3.5	3.4	1.5	0.8

IRS = indoor residual spraying, PBO = piperonyl butoxide



Supplementary Figure B1 Risk of malaria from January 2018 to June 2020, stratified by district, all ages. Line colors represent the type of invention which households in each district received Dark gray color blocks represent months where the mass distribution campaign occurred (September to December 2018) and light gray blocks represent months which fall during the yearly high malaria transmission season (January to May). Risk was measured monthly and curves are smoothed using X-splines. Note: IRS = indoor residual spraying, PBO = piperonyl butoxide.

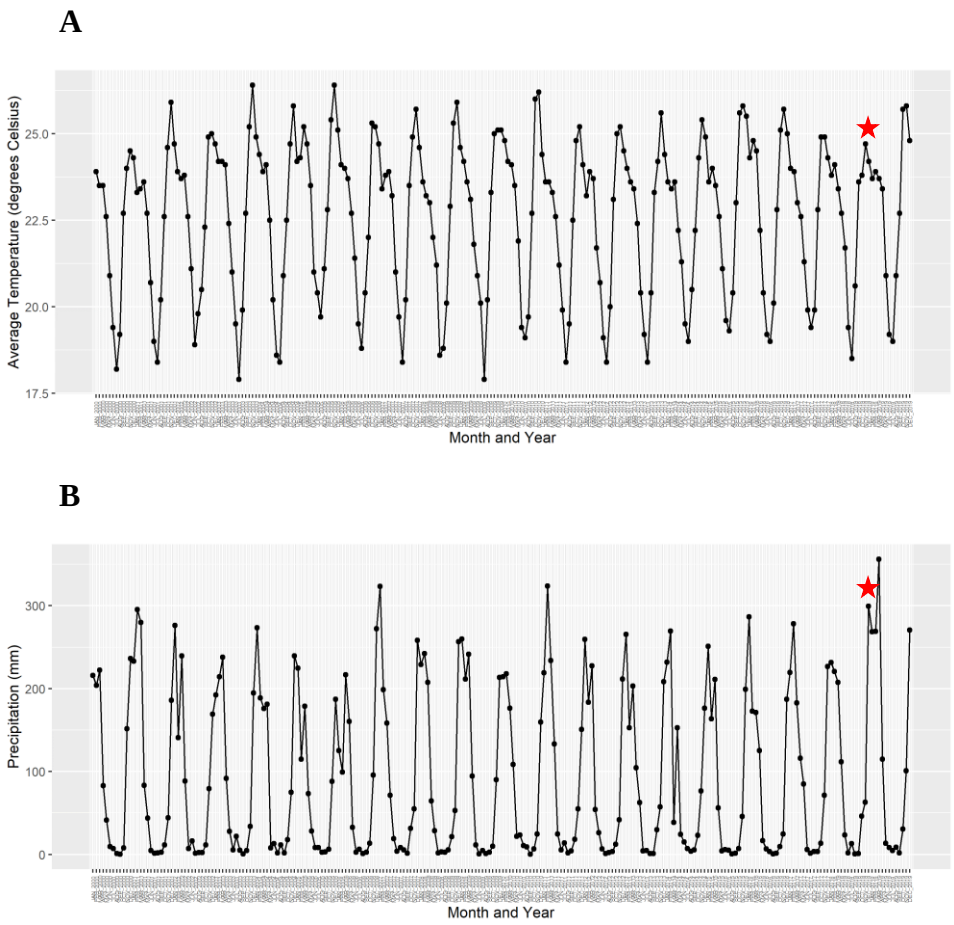
Supplementary Table B4 Changes in malaria risk (cases per 100 people) by district from January 2018 to June 2020, stratified by age group and high and low malaria transmission seasons.

		High transmission* risk (cases per 100 people)														
District	**Insecticide**	**<5 years**					**≥5 years**					**All ages**				
		2018	**2019**	**2020**	**RD 19 vs. 18**	**RD 20 vs. 18**	**2018**	**2019**	**2020**	**RD 19 vs. 18**	**RD 20 vs. 18**	**2018**	**2019**	**2020**	**RD 19 vs. 18**	**RD 20 vs. 18**
Balaka	Pyrethroid	79.6	58.5	70.4	-21.1	-9.1	31.7	28.5	35.8	-3.3	4.0	39.0	33.0	41.0	-6.0	2.0
Blantyre	Pyrethroid	48.6	33.6	45.0	-15.0	-3.6	10.1	8.7	14.1	-1.4	3.9	15.1	11.9	18.0	-3.2	3.0
Chikwawa	Pyrethroid	60.5	70.6	70.3	10.1	9.8	13.7	22.3	23.6	8.6	10.0	20.8	29.7	30.8	8.9	10.0
Chiradzulu	Pyrethroid	31.8	23.1	21.2	-8.8	-10.6	7.8	9.6	9.4	1.8	1.6	11.0	11.4	11.0	0.5	0.0
Chitipa	Pyrethroid	31.4	23.4	21.0	-8.0	-10.4	13.7	10.3	10.3	-3.4	-3.4	16.1	12.0	11.8	-4.0	-4.3
Dedza	Pyrethroid	61.2	49.6	62.1	-11.6	0.8	10.2	11.2	14.9	1.0	4.8	17.6	16.8	21.8	-0.9	4.2
Dowa	Pyrethroid	103.9	60.3	99.6	-43.6	-4.3	21.3	13.6	25.9	-7.7	4.6	32.6	20.0	35.9	-12.6	3.3
Karonga	PBO	56.9	21.5	23.3	-35.5	-33.7	20.0	6.0	8.9	-14.0	-11.1	25.1	8.1	10.9	-17.0	-14.2
Kasungu	Pyrethroid	101.1	78.9	103.8	-22.2	2.8	16.9	15.6	20.3	-1.3	3.4	29.0	24.7	32.4	-4.3	3.3
Likoma	PBO	64.9	38.5	34.5	-26.4	-30.4	41.0	16.3	18.7	-24.7	-22.3	43.8	18.8	20.5	-24.9	-23.3
Lilongwe	Pyrethroid	99.9	41.7	62.8	-58.2	-37.0	14.9	7.4	13.1	-7.6	-1.8	27.2	12.2	20.1	-15.0	-7.1
Machinga	PBO	51.6	22.2	35.7	-29.4	-15.8	13.7	5.8	11.0	-8.0	-2.8	20.1	8.5	15.1	-11.6	-5.0
Mangochi	Pyrethroid / IRS	68.9	57.8	25.4	-11.1	-43.5	12.9	11.6	4.6	-1.3	-8.3	22.7	19.7	8.2	-3.0	-14.5
Mchinji	Pyrethroid / PBO	114.8	56.6	99.0	-58.2	-15.8	28.2	14.8	29.8	-13.4	1.6	41.0	21.0	40.0	-20.0	-1.0
Mulanje	Pyrethroid	97.5	60.6	86.4	-36.9	-11.1	23.2	16.3	24.0	-6.9	0.8	33.3	22.3	32.5	-11.0	-0.8
Mwanza	PBO	114.4	60.0	149.7	-54.4	35.3	12.2	8.9	35.2	-3.3	23.0	27.2	16.4	52.0	-10.8	24.8
Mzimba	Pyrethroid	65.2	50.6	50.6	-14.6	-14.6	18.7	14.8	18.8	-3.8	0.1	25.3	19.9	23.2	-5.4	-2.1
Neno	PBO	107.8	42.0	77.4	-65.7	-30.4	36.1	18.1	36.7	-18.0	0.5	47.0	21.8	42.9	-25.2	-4.1
Nkhata Bay	Pyrethroid	186.1	135.9	144.5	-50.2	-41.6	42.7	33.4	41.2	-9.3	-1.4	62.6	47.7	55.6	-14.9	-6.9
Nkhotakota	IRS	128.2	43.5	53.5	-84.7	-74.7	30.8	12.1	13.7	-18.7	-17.0	46.1	17.0	20.0	-29.1	-26.1
Nsanje	PBO	15.4	13.3	18.2	-2.2	2.7	5.6	5.3	8.8	-0.4	3.2	7.1	6.5	10.2	-0.7	3.1
Ntcheu	Pyrethroid	75.6	62.2	74.2	-13.3	-1.4	13.3	15.2	21.3	1.9	8.0	22.3	22.0	28.9	-0.3	6.6
Ntchisi	PBO	144.3	33.8	79.0	-110.5	-65.2	31.3	5.5	21.2	-25.9	-10.2	48.1	9.7	29.8	-38.4	-18.3
Phalombe	Pyrethroid	29.3	16.1	23.9	-13.2	-5.4	10.3	6.5	11.7	-3.8	1.4	13.2	7.9	13.6	-5.2	0.4
Rumphi	PBO	72.2	26.9	31.7	-45.3	-40.5	31.7	9.7	16.8	-22.0	-14.9	37.4	12.1	18.9	-25.2	-18.5
Salima	PBO	111.1	46.3	74.5	-64.7	-36.6	23.5	9.4	18.9	-14.1	-4.6	37.7	15.4	27.9	-22.3	-9.7
Thyolo	Pyrethroid	40.5	20.6	47.0	-20.0	6.5	7.9	4.9	12.8	-3.0	4.8	12.2	7.0	17.2	-5.3	5.0
Zomba	Pyrethroid	60.6	49.7	62.2	-10.9	1.6	17.4	15.2	18.4	-2.2	1.0	23.4	20.0	24.5	-3.4	1.1

* High transmission season: January to May; Low transmission season: June to December; IRS = indoor residual spraying, PBO = piperonyl butoxide, RD = risk difference

District	Insecticide	Low transmission* risk (cases per 100 people)								
		<5 years			≥5 years			All ages		
		2018	2019	RD 19 vs. 18	2018	2019	RD 19 vs. 18	2018	2019	RD 19 vs. 18
Balaka	Pyrethroid	41.5	47.8	6.3	10.5	14.7	4.2	15.2	19.7	4.5
Blantyre	Pyrethroid	23.5	27.3	3.8	5.1	5.7	0.6	7.5	8.5	1.0
Chikwawa	Pyrethroid	44.0	67.2	23.2	8.1	17.2	9.1	13.6	24.8	11.3
Chiradzulu	Pyrethroid	12.1	9.4	-2.7	2.9	2.2	-0.6	4.1	3.2	-0.9
Chitipa	Pyrethroid	27.4	17.0	-10.4	10.1	3.2	-6.8	12.4	5.1	-7.3
Dedza	Pyrethroid	39.4	30.5	-8.9	5.9	5.1	-0.7	10.8	8.9	-1.9
Dowa	Pyrethroid	56.8	42.1	-14.8	8.8	7.1	-1.8	15.4	11.9	-3.5
Karonga	PBO	72.9	16.8	-56.1	19.0	3.7	-15.3	26.4	5.5	-20.9
Kasungu	Pyrethroid	52.6	56.1	3.6	6.3	7.1	0.9	13.0	14.2	1.3
Likoma	PBO	71.0	46.5	-24.5	27.6	13.7	-13.8	32.6	17.5	-15.1
Lilongwe	Pyrethroid	35.4	25.8	-9.6	4.9	3.4	-1.4	9.2	6.6	-2.6
Machinga	PBO	32.7	26.4	-6.2	6.5	4.6	-1.9	10.9	8.3	-2.6
Mangochi	Pyrethroid / IRS	45.6	52.3	6.8	7.3	8.1	0.8	14.0	15.8	1.9
Mchinji	Pyrethroid / PBO	68.7	53.7	-15.0	13.6	11.9	-1.7	21.7	18.0	-3.7
Mulanje	Pyrethroid	58.0	52.1	-5.9	12.8	11.1	-1.7	18.9	16.6	-2.2
Mwanza	PBO	98.4	54.8	-43.5	16.4	9.1	-7.2	28.4	15.9	-12.6
Mzimba	Pyrethroid	34.4	26.0	-8.4	6.8	5.6	-1.2	10.7	8.5	-2.2
Neno	PBO	55.5	31.8	-23.7	16.4	10.8	-5.6	22.3	14.0	-8.3
Nkhata Bay	Pyrethroid	198.0	159.9	-38.1	42.2	33.9	-8.3	63.9	51.5	-12.5
Nkhotakota	IRS	121.7	96.2	-25.5	29.7	22.9	-6.8	44.2	34.4	-9.8
Nsanje	PBO	14.5	18.5	4.0	5.3	5.6	0.3	6.7	7.6	0.9
Ntcheu	Pyrethroid	46.7	42.5	-4.3	7.8	8.4	0.6	13.4	13.3	-0.1
Ntchisi	PBO	85.2	36.5	-48.7	13.1	6.4	-6.6	23.8	10.9	-12.9
Phalombe	Pyrethroid	22.7	14.3	-8.4	6.5	3.9	-2.6	8.9	5.5	-3.5
Rumphi	PBO	35.0	14.5	-20.5	11.1	3.9	-7.2	14.5	5.4	-9.0
Salima	PBO	91.2	40.5	-50.7	15.6	5.6	-10.0	27.8	11.3	-16.5
Thyolo	Pyrethroid	24.0	27.1	3.1	3.4	4.4	1.0	6.1	7.4	1.2
Zomba	Pyrethroid	40.0	42.2	2.2	9.5	9.8	0.3	13.7	14.3	0.6

* High transmission season: January to May; Low transmission season: June to December; IRS = indoor residual spraying, PBO = piperonyl butoxide, RD = risk difference



Supplementary Figure B2 A) average monthly temperature and B) total monthly precipitation, Malawi, 2000 to 2019. Stars indicate peaks in November and December 2018, respectively, immediately before the start of the 2019 high malaria transmission season.

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