The epidemiology of malaria and challenges to elimination in a low transmission setting in southern Zambia

By

Kelly M. Searle, ScM

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Dissertation Abstract

Background: Recently, malaria has become a major global health priority. As a result there has been renewed interest in malaria control, elimination, and eradication. Zambia is one of the Elimination 8 countries and one of the President's Malaria Initiative focus countries. Southern Province, Zambia has maintained a parasite prevalence of <10% since 2012, and the National Malaria Control Center made a goal of creating 5 malaria free zones in the province. As areas approach elimination, better understanding of the changing epidemiology of malaria transmission should be used to inform and determine how and where to target specific interventions. Additionally, challenges to elimination need to be evaluated to understand the risk for importation and resurgence of transmission.

Methods: The study was conducted in the rural catchment area of Macha Hospital, Choma District, Southern Province, Zambia. First, spatial and temporal trends in passively and actively detected malaria infections were determined. Second, the genetic diversity and complexity of the parasite populations infecting individuals identified through passive and active surveillance was evaluated and compared. Third, a reactive screen-and-treat strategy was evaluated and coverage cascades were developed to inform and improve the intervention. Fourth, the impact of population movement was evaluated using GPS data loggers, in which movement patterns were characterized and quantified, and the amount of time spent in high and low malaria risk was determined.

Results: A fractured spatial pattern was detected for both passively and actively detected infected individuals, and temporally stable, space-time clusters were detected, suggesting the presence of ecologically receptive areas. Phylogenetic analysis showed evidence of two distinct parasite populations from infected individuals identified through passive and active surveillance, with genetic diversity decreasing in actively detected infected individuals but not in passively detected cases. In the initial stages of a reactive

screen-and-treat strategy, challenges such as poor follow-up and coverage, difficulties in maintaining sufficient RDTs, and poor sensitivity of the RDTs impeded the success of the program and a reactive focal drug administration may be more efficient. Most time was spent in the participant's household compound, with time spent in high malaria risk areas was dependent on whether or not the house was located in a high malaria risk area. Seasonal movement patterns were observed, with greater long-distance movements during the dry season.

Conclusions: Temporally stable ecologically receptive areas remain in malaria elimination settings but the chronically infected population may not be contributing to local transmission. Reactive focal drug administration within index case households may be a more efficient at identifying and treating infected individuals than a reactive test and treat strategy. Population movement patterns have the potential to increase the risk of importation at the end of the rainy season when clinical malaria cases peak; however, the risk of malaria importation is likely to be low throughout the remainder of the dry season.

Advisor: William J. Moss, MD MPH

Thesis readers and Final Oral Examination Committee

Committee Members

William J. Moss, MD MPH
Professor, Thesis Advisor
Department of Epidemiology

Frank C. Currierro, PhD
Professor
Department of Epidemiology

Douglas E. Norris, PhD
Professor
Department of Molecular Microbiology and Immunology

Thomas A. Louis, PhD
Professor
Department of Biostatistics

Alternate Committee Members

Priya Duggal, PhD
Associate Professor
Department of Epidemiology

Mufaro Kanyangarara
Assistant Scientist
Department of International Health

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1. Introduction

1.1 Malaria infection and epidemiology

1.1.a. Malaria infection in humans

Malaria is caused by infection with the parasite *Plasmodium,* with five species infecting humans: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*. *Plasmodium falciparum* infection results in the most virulent disease and is the predominant species in sub-Saharan Africa [1]. Malaria is transmitted to humans through the bite of infected female mosquitos of the *Anopheles* genus. *P. falciparum* is transmitted to humans as asexual sporozoites which travel through the blood to the liver where asexual reproduction takes place, with merozoites produced and released into the bloodstream. Merozoites infect red blood cells where they asexually reproduce leading to bursting of the cells. This bursting of red blood cells can lead to anemia and fever. A small proportion of merozoites transform into gametocytes, which are sexually reproducing cells. Gametocytes are transmitted to a female *Anopheles* mosquito when she bites the infected human for a blood meal.

1.1.b. Malaria epidemiology

Malaria transmission is highly dependent upon the distribution and abundance of mosquito vectors. The capacity for transmission of *P. falciparum* from vectors to humans is dependent upon seasonal fluctuations in temperature and availability of breeding sites. An additional factor involved in the capacity for transmission is the joint spatial relationship between competent vectors, the infected human reservoir, and the population densities of vectors and humans.

Nearly half of the global population (3.2 billion people) are at risk of malaria [2]. In 2015, there were an estimated 214 million malaria cases worldwide, with 88% occurring

in Africa [3]. In 2015, 10% of deaths among children under 5 years of age in sub-Saharan Africa were due malaria, where it is the 4th leading cause of death [3]. Typically, pregnant women and children under 5 years of age are at the highest risk of malaria related morbidity and mortality [3]. However, school-age children (5-15 years old), have been shown to be at the highest risk of malaria infection [4-8].

Where malaria is endemic, areas are classified by their level and seasonality of transmission. High transmission areas are classified as holoendemic or hyperendemic, with holoendemic areas those with intense transmission occurring year-long and hyperendemic areas those with intense seasonal transmission. In these areas parasite prevalence is typically >50%. Moderate transmission areas are classified as mesoendemic with regular seasonal transmission and parasite prevalence typically between 10%-50%. Low transmission areas are classified as hypoendemic with intermittent, usually seasonal transmission with parasite prevalence typically <10%.

In endemic areas, partial protective immunity to clinical malaria develops. Immunity is exposure dependent and develops over time. This immunity is complex, as sterilizing immunity against infection has been shown in experimental studies in mice and humans, but it has not been shown in field-based, observational studies [9]. Clinical immunity protects against disease, in which individuals can be infected with *P. falciparum* without suffering from clinical symptoms of malaria [9-12]. The mechanisms that confer immunity to clinical disease are not fully known; however, the process leads to the development of an asymptomatically infected population [13-16]. These asymptomatic individuals have lower parasitemia than symptomatically infected cases, indicating that host immunity may involve mechanisms that control levels of parasitemia [17].

1.1.c. Malaria diagnostic methods

Traditionally, the most commonly used method for the diagnosis of malaria is the clinical presence of fever [18]. However, the World Health Organization (WHO) recommends a parasite-based diagnosis using either microscopy or rapid diagnostic test (RDT) [19]. Microscopy has long been considered the “gold standard” for malaria diagnosis as it is able to quantify the level of parasitemia. The sensitivity and specificity of microscopy, however, are limited by the experience of the microscopist and the level of parasitemia [18-20]. RDTs identify malaria parasite antigens, most commonly histidine rich protein II (HRP-2) and *P. falciparum* lactate dehydrogenase (pLDH) [21]. For clinical malaria cases, RDTs are as sensitive but less specific than microscopy but their sensitivity declines with low levels of parasitemia [18, 19, 22]. RDTs have been shown to give false positive results up to two weeks after treatment, as the target antigens persist in the blood [18, 22, 23]. While not commonly used clinically, PCR detection of parasite DNA is highly specific to detect infection [19, 24]. In areas of low endemicity, PCR is emerging as a new “gold standard” tool to identify cases due to its ability to detect infections with low parasitemia (<100 parsites/μL), where microscopy and RDT may produce false negative results [19, 25].

1.2 Malaria elimination

1.2.a. Early malaria eradication efforts

From the 1950s to the 1970s there were large-scale efforts to eradicate malaria worldwide, known as the Global Malaria Eradication Programme (GMEP) [26]. Substantial investments were made to determine risk factors associated with malaria. These efforts used data on climate, elevation, biting rates, measures of malaria infection (e.g. spleen size, parasite prevalence by microscopy, and febrile episodes), health care records, and vector surveillance [26, 27]. The two main interventions used during the

GMEP were indoor residual spraying (IRS) with DDT and treating passively detected cases with chloroquine. Malaria was eliminated from 15 countries and one territory [2]. Following the conclusion of the GMEP, seven additional countries and one territory eliminated malaria in the 1970s and 1980s. However, these elimination efforts did not fully reach many areas, specifically in sub-Saharan [27]. Additionally, none of the countries that eliminated malaria during the GMEP and shortly afterwards were located in Africa.

1.2.b. Recent malaria elimination efforts

More recently, malaria has become a major global health priority. As a result there has been renewed interest in malaria control, elimination, and eradication [1]. In the past decade, international support and funding for malaria control increased dramatically and targets were set to reduce the burden of malaria by 75% and eliminate malaria in 8-10 countries by 2015 [28]. This renewed commitment to malaria elimination has been made possible with increased coverage of four key interventions: long-lasting insecticide-treated bednets (LLINs and ITNs), indoor residual spraying (IRS), case identification with rapid diagnostic tests (RDT) and treatment with artemisinin-combination therapy (ACT), and intermittent preventive treatment for pregnant women and infants (IPTp and ITPi). Some programs that achieved high coverage with these interventions showed dramatic decreases in the number of malaria cases, hospital admissions and deaths [28-31]. Worldwide, malaria incidence decreased by 37% and malaria mortality decreased by 60% from 2005-2015, and 5 countries received certification of malaria elimination by maintaining 3 consecutive years with no indigenous malaria cases. In 2014, 13 countries reported no indigenous cases of malaria for that year [2]. However, all of these countries are outside of Africa. In Africa, 11 countries demonstrated large (>50%) and sustained decreases in the incidence of clinical malaria

[28]. From 2000-2015, malaria mortality decreased by 65% in all age groups, and by 71% among children under 5 years in Africa [2].

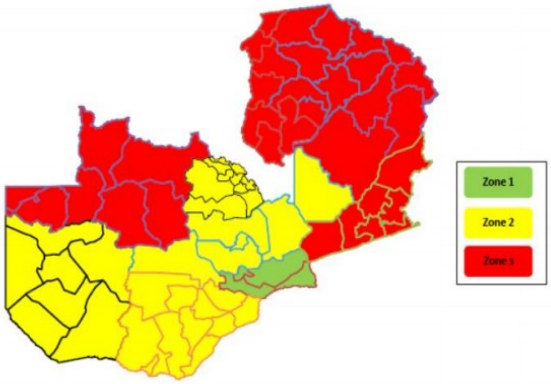
1.2.c. Malaria elimination efforts in Southern Africa

In 2007, as malaria began to decline in Southern Africa, the Southern Africa Development Community (SADC) committed to aid in transitioning eligible countries from control to elimination [32]. This led to the creation of the Malaria Elimination 8 (E8) as a platform for regional malaria elimination in Southern Africa [32]. Botswana, Namibia, South Africa, and Swaziland were identified as the four mainland countries having the greatest potential for malaria elimination and were designated frontline countries. Their four neighbors to the north, Angola, Mozambique, Zambia, and Zimbabwe, with higher malaria transmission were identified as second line countries [32]. Together, these make up the E8, and these countries have committed to coordinated efforts between their National Malaria Control Programmes (NMCPs) to transition from malaria control to elimination [32].

1.2.d. Malaria free zones in Southern Province, Zambia

Zambia is one of the E8 countries and was selected as a President's Malaria Initiative (PMI) focus country in 2007. Zambia has recorded national decreases in malaria deaths among hospital inpatients from 3.9 per 1,000 to 2.4 per 1,000 between 2010 and 2014, and parasite prevalence from 22% in 2006 to 15% in 2012 [33]. The Zambian NMCP classified the country into 3 epidemiologic zones based on reported malaria prevalence. Zone 1 areas are where malaria transmission declined and prevalence in children under 5 years was <1%. Zone 2 areas

Figure: Zambian NMCP epidemiologic zones



are where malaria transmission declined and prevalence in children under 5 years was <14% during peak transmission seasons. Zone 3 areas are where malaria transmission had not been successfully reduced and prevalence in children under 5 years exceeded 14% during peak transmission seasons [33].

Southern Province, Zambia was classified in the zone 2 epidemiologic area of malaria transmission [33]. Parasite prevalence, measured as number of positive tests (RDT or microscopy) per tests performed, decreased from 13% in 2006 to 8.4% in 2012 [33]. Overall, Southern Province has maintained a parasite prevalence of <10% since 2012. However, there remains spatial heterogeneity within the province, with districts on the shore of Lake Kariba having the highest levels of transmission and those further from the lake with lower levels of transmission [34, 35]. Given the overall low parasite prevalence in Southern Province, the NMCC set a goal to create 5 malaria free zones [36, 37]. These malaria free zones will consist of 5 districts where no malaria cases are locally acquired [36]. In creating malaria free zones, traditional interventions such as increasing and sustaining ITN ownership and use will be used in concert with strengthening health systems and surveillance, improving case management in RHCs, and introducing newer, better targeted drug-based interventions.

1.2.d.1. Passive case detection and improving case management

Passive case detection identifies symptomatic individuals who present to a health care facility. Case management in many districts in Southern Province was improved by supplying rural health centers with RDTs and ACT, and training community health workers (CHWs) on testing with RDTs and providing treatment with artemether-lumifantrin (AL) [35]. Thus, passive case detection and quality case management has expanded to be more available to communities in many of the more rural districts in Southern Province. Along with this expansion, an SMS reporting system was

implemented to improve surveillance for passively-detected, symptomatic malaria cases [35]. CHWs and staff at RHCs report monthly numbers of suspected and confirmed malaria cases. Expanding quality case management and improving surveillance for symptomatic malaria cases will aid in reducing malaria morbidity and mortality but will not identify and treat asymptomatic or minimally symptomatic infected individuals.

1.2.d.2. The impact of asymptomatic infections on malaria transmission

In areas of high and moderate malaria transmission, individuals acquire partial protective immunity to clinical malaria. This immunity is sometimes described as age-dependent or exposure dependent as children under five years of age are most likely to suffer from severe malaria disease as they have yet to acquire clinical immunity. With increasing age, severity of malaria disease wanes in the face of persistent exposure, without change to the incidence of infection. As a result, many persons in endemic areas become infected and continue to transmit the parasite but develop minimal or no clinical symptoms [15, 38-40]. Because passive case detection identifies symptomatically infected individuals who present to a health care facility, those who acquired clinical immunity are less likely to present to a health care facility but are able to act as human reservoirs of infection and contribute to ongoing transmission [15, 20, 26, 40].

1.2.d.3. Active case detection and focal drug administration

Active case detection is one method to identify asymptomatic infections, which may be needed to achieve malaria elimination. Active case detection is defined by the WHO as screening for infection among individuals who are at high risk for infection by community health workers at the individual or household level [41]. Active case detection for malaria can be divided into two categories: reactive case detection and proactive case detection [42].

Reactive case detection is defined as the screening of individuals residing in the same household or within a specified distance from the household of a passively detected, infected individual [26, 43-45]. Proactive case detection involves targeted screening and treating those individuals or households considered to be at high risk for malaria infection, usually via mass screen and treat campaigns [42]. Active case detection methods have been recommended for use in low transmission settings attempting to move from malaria control to malaria elimination [41, 42].

A major limitation of test-and-treat strategies is that the currently available RDTs for screening lack the sensitivity to identify many asymptomatic infections due to low levels of parasitemia. Locations transitioning from malaria control to elimination have proposed replacing test-and-treat with focal drug administration programs [46]. Focal drug administration can be reactive or proactive and work just as the test and treat programs; however, instead of screening individuals at risk with an RDT, everyone at risk of infection is presumptively treated with ACTs. In addition to presumptive treatment with ACTs clear parasites from the infected reservoir, single dose treatment with primaquine as a gametocytocide has been suggested to block parasite transmission [47-49].

1.3 Rationale for the current research

In areas approaching malaria elimination, better understanding of the epidemiologic trends and the natural history of malaria elimination will inform how and where to implement interventions. These trends involve the spatial and temporal patterns of symptomatic and asymptomatic malaria infections, and the genetic relationships of parasites infecting the symptomatically and asymptomatically infected individuals. Challenges to achieving and sustaining malaria elimination also need to be evaluated. Operational challenges in the implementation of targeted interventions will

impede the success of elimination programs. Population movement patterns can sustain transmission and lead to parasite importation.

1.3.1 The natural history of malaria elimination

1.3.1.a. Spatial patterns of malaria transmission in areas approaching elimination

The spatial heterogeneity of malaria transmission is well documented [50]. The spatial variation in ecological factors, anopheline bionomics and human movement patterns likely contribute significantly to the level of spatial heterogeneity in malaria transmission. While spatial heterogeneity is well documented, provinces and districts in malarious areas are often treated equally in terms of distribution of interventions [1]. However, changes in spatial patterns in malaria transmission during the transition from control to elimination has not been well documented. These spatial transmission patterns are important when targeting interventions to eliminate malaria [38, 51, 52].

1.3.1.b. Spatial and temporal patterns of passively detected, symptomatic malaria cases

The first line of attack for malaria elimination programs is symptomatic malaria cases that present to health care facilities. Providing quality case management in health care facilities will require the ability to anticipate the annual number of cases to allow timely allocation of resources, including RDTs and antimalarial drugs. As malaria transmission declines and approaches elimination, determining which clinics should be targeted for increased intervention delivery will be essential. Knowing how spatial patterns of symptomatic malaria change in response to declining transmission will help plan and target interventions.

Rural health centers (RHCs) are generally distributed in communities based on population density [53]. RHCs then estimate the size of their catchment area. This estimate is made using a combination of census data with accompanying information on population size and demographics from local authorities [54]. This estimate does not

account for geographic distance from the RHC or actual utilization of a RHC by individuals in the community [55, 56]. Health seeking behaviors depend on many factors, including distance, perceived quality of care, and perceived accessibility of care. [56]. Considering the choice of utilization of an RHC, the catchment population may differ from the estimated population. Information on where individuals report seeking care can assist in more accurately estimating the catchment population served by RHCs. When malaria incidence per population served can be calculated, spatial heterogeneities in symptomatic malaria can be tracked and investigated. This can aid in planning and distributing interventions to health care facilities.

1.3.1.c. Locating hotspots of actively detected, asymptomatic malaria cases

Identifying geographic areas at high risk for asymptomatic malaria will aid in directing targeted interventions such as reactive test-and-treat or focal drug administration, as these individuals will not be detected at health care facilities. Malaria hotspots have been defined as geographical areas where the observed malaria transmission intensity exceeds the expected malaria transmission intensity if transmission were homogeneously dispersed. Hotspots are usually spatially small (<1 km^2), with the micro-epidemiology of malaria transmission in hotspots permissive to sustaining the basic reproductive number (R_0) above 1. The distance from the center of the hotspot to where the observed malaria transmission intensity no longer exceeds the expected malaria transmission intensity determines the borders of a hotspot [38, 52, 57].

Malaria hotpots can be detected through mapping the residence locations of individuals with parasitemia in a specified area [38, 52]. The geographic location of hotspots can then be determined by using spatial scan statistics [58, 59]. This method is based on scanning the map with a window of different sizes to determine if the observed number of cases inside the window is greater than the expected number of cases [58-

60]. The expected number of cases is calculated as the number of cases that would be observed in an area if cases were evenly distributed spatially in the population. The windows with the highest ratio of the number of observed to number of expected cases are identified and the statistical significance of these hotspots is calculated using the likelihood ratio test [58, 59].

To target interventions directed at the asymptomatic reservoir, areas considered to be malaria hotspots should be located [38]. Additionally, as areas transition from malaria control to elimination, the temporal stability of hotspots should be determined. Temporally stable hotspots of actively detected malaria cases as transmission declines provide information on ecologically receptive areas. In these areas, malaria may not be actively transmitted during each season but the populations in these areas are susceptible to infection and the areas are ecologically receptive to maintain malaria transmission.

1.3.1.d. Parasite populations infecting passively and actively detected malaria cases

As areas transition from malaria control to elimination, understanding the epidemiologic patterns of local malaria transmission is crucial to achieving elimination and preventing re-introduction [41, 61, 62]. Of interest is the relative magnitude of malaria infections attributed to locally acquired cases compared to imported cases, particularly elucidating the role the asymptomatically infected reservoir plays in maintaining local malaria transmission [14, 20, 35, 63, 64]. One way to approach this question is to determine the genetic relatedness of parasites between actively detected, predominantly chronically infected asymptomatic malaria cases and passively detected, predominantly acutely infected symptomatic malaria cases [65]. Differences in parasite relatedness between passively and actively detected cases can inform how the parasite population within these different hosts changes as transmission declines [66].

Various methods have been developed to genotype *P. falciparum* parasites. The first method developed, and most commonly used, is based on identification of length-based polymorphisms in 3 loci: merozoite surface protein 1 (*msp-1*), merozoite surface protein 2 (*msp-2*), and glutamine rich protein (*glurp*) [67]. This method relies only on a PCR thermocycler and gel-electrophoresis to determine length of allele polymorphisms [68]. However, this method is limited in tracking infections in populations with high parasite diversity due to the limited number of informative loci [65, 68]. More sophisticated, high resolution methods to determine parasite diversity, such as microsatellite polymorphisms and whole genome sequencing, have been used to overcome this limitation; however these methods are often labor intensive and expensive [65, 68].

A *P. falciparum* molecular barcode was developed to elucidate malaria transmission dynamics by tracking the genomic diversity and complexity of the malaria parasite over time and space [65]. This method was developed specifically for use in local or district-level laboratories, as the highest level of technology required is a polymerase chain reaction (PCR) assay [65]. The molecular barcode consists of 24 unlinked SNPs that characterize unique genomic signatures of circulating *P. falciparum* parasites [65]. The barcode determines whether the major allele, minor allele, or a mixture of major and minor alleles is present in the haploid blood stage of the parasite for each of the 24 SNPs. Variation in the barcode arises as a result of genetic recombination and outcrossing during the sexual stage of infection when gametocytes combine [66]. SNPs with a mixture of the major and minor allele present are referred to as mixed infections, with higher numbers of mixed infections representing higher genetic complexity. The frequency of mixed infections approximates the level of genomic complexity and provides information about the burden of infection due to genetically unique parasites in the population [69].

The molecular barcodes can be used to infer parasite haplotypes infecting individuals [66]. Thus, phylogenetic relationships can be determined for passively and actively detected cases to inform the relative magnitude that each of these populations contribute to the circulating parasite population. The genetic complexity and diversity among passively and actively detected cases can be used to determine how the parasite population changes in each of these populations in response to decreasing transmission.

1.3.2 Challenges to malaria elimination

1.3.2.a. Operational challenges in implementing targeted malaria elimination interventions

Reactive case detection leverages the underlying spatial and temporal clustering of malaria infections [38, 52, 57] and can comprise reactive screen-and-treat or focal drug administration. Methods of implementing reactive case detection have not been fully operationalized and evaluated. Multiple challenges can impact the effectiveness and efficiency of reactive case detection. For example, the optimal screening radius around index cases has not been determined and will vary depending on demographic, ecologic and epidemiologic characteristics [44]. In rural areas, the WHO recommended that programs cover areas as large as the flight range of *Anopheles* mosquitoes, which can be up to 1-2 km [41]. A 1 km screening radius is used in Tanzania, a 300-500 meter screening radius is used in rural Senegal, and a 140-meter screening radius is used in rural Zambia [35, 44, 45]. Screening all index households and their neighbors can be costly and logistically challenging. Even with well-developed protocols, identifying neighboring households within a specified radius from an index household can be difficult in practice. For reactive case detection to be an effective malaria elimination strategy, high coverage levels are needed [64]. For high coverage to be achieved, ample

supplies of RDTs and ACTs must be available to RHCs and rural health posts (RHPs). Additionally, reactive screen-and-treat strategies rely on RDTs to identify infected individuals and the availability and sensitivity of these diagnostics will impact how well a reactive case detection program performs. Given these challenges, it is important to monitor reactive case detection programs as they are implemented to determine how they perform operationally and identify how they can be improved.

1.3.2.b. Human movement and malaria transmission

Population movement is known to contribute to the transmission of vector borne diseases, specifically malaria [70-72]. This is due to differential contact between humans and mosquitos as a result of migration and overlapping activity space [73-75]. During the first global malaria eradication campaign in the 1950s and 1960s, the failure to account for human population movement was one of the factors that contributed to the program's ultimate failure [70, 72].

In areas approaching malaria elimination, human mobility patterns are of particular interest in determining the local dynamics between malaria parasites that are imported and ongoing low-level endemic transmission [76-81]. Population movement patterns can threaten malaria elimination in two ways [70]. The first is through infected individuals traveling into ecologically receptive areas and transmitting parasites to the local population. The second is through uninfected individuals traveling into areas of high malaria transmission, becoming infected and returning to an ecologically receptive area [70]. These movement patterns occur on both large and small spatial scales, each impacting malaria transmission and with the potential to threaten elimination [70, 80-82]

1.4 Summary

With renewed interest in malaria elimination, many areas that have increased interventions have experienced decreases in malaria cases. As areas approach

elimination, transmission becomes more spatially focal and the impact of the asymptomatically infected reservoir on transmission, and how to best target this reservoir, is of interest. The current research describes the epidemiology of malaria in an area of low transmission in Southern Province, Zambia, where elimination strategies are being implemented to create malaria free zones. Spatial and temporal trends in passively detected and actively detected malaria cases were compared, as well as the genetic parasite population infecting each population. Challenges to achieving malaria elimination in this area were described. Operational challenges to reactive case detection strategies, currently being use in the area, were evaluated and the potential impact of these strategies on the infected population was modeled. The impact of population movement patterns on local malaria transmission and risk of importation was explored. To successfully achieve and sustain malaria elimination, the epidemiology of malaria cases, and potential threats to achieving and sustaining elimination should be explored.

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2. Spatial and temporal patterns of malaria in a region of declining transmission in southern Zambia

Authors: Kelly M. Searle, ScM[1], Jailos Lubinda, MSc[2], Harry Hamapumbu[2], Timothy M. Shields, MA[1], Frank C. Curriero, PhD[1], Philip E. Thuma, MD[2], and William J. Moss, MD MPH[1] for the Southern Africa International Centers of Excellence for Malaria Research

[1]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[2]Macha Research Trust, Choma District, Zambia

Abstract

Background: As areas transition from malaria control to elimination, targeted interventions will be needed. Knowledge of changing spatial and temporal patterns of malaria as transmission declines can guide elimination efforts.

Methods: Spatial and temporal changes in malaria incidence and prevalence were measured and analyzed as transmission declined in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia. Passively detected cases of malaria were reported from six rural health centers (RHCs) from August 2008 to September 2015. Seasonal malaria incidence was estimated based on an estimated population size for each catchment area and spatial patterns were evaluated. Parasite prevalence was measured through active case detection using rapid diagnostic tests and PCR in population-based, serial cross-sectional cohort studies conducted from February 2008 to October 2013. Spatial clusters of parasite prevalence were determined and overlap with malaria incidence was assessed.

Results: The number of passively detected malaria cases decreased over the study period. Each transmission season had a dominant RHC catchment area with the highest incidence and adjacent RHC catchment areas had the next highest incidence. However, as the burden of malaria decreased, these spatial relationships changed. More distant, non-adjacent RHC catchment areas frequently had a higher incidence than those adjacent to the dominant RHC. Malaria prevalence as measured by active case detection declined from 9.2% to 0.7% from 2008 to 2013. Statistically significant spatial clusters of prevalent infections were identified during most annual transmission seasons but were not consistent from year to year. Over the entire study period, four statistically significant, temporally stable clusters were detected. These clusters of prevalent infections overlapped with the dominant RHC catchment area with the highest incidence

only during the 2008-2009 season when the burden of malaria was highest. As transmission declined, the spatial distribution of prevalent infections and incident cases no longer overlapped.

Conclusion: As areas approach malaria elimination, information on how spatial and temporal patterns of malaria incidence and prevalence change can be used to plan targeted interventions. The absence of spatial overlap between incident and prevalent cases suggests the emergence of two distinct populations of infected individuals, acutely infected, symptomatic individuals and chronically infected, asymptomatic individuals.

Background

Over the past decade, reductions in the burden of malaria occurred in many countries in sub-Saharan Africa [1] and malaria elimination goals have been proposed [2-4]. Malaria transmission has been shown to be spatially heterogeneous on various spatial scales, from the national level to districts and villages [25-32], but small-scale spatial patterns of malaria transmission as regions move toward elimination have not been fully elucidated. Understanding these epidemiologic patterns of transmission decreases is crucial to achieving and sustaining elimination [2, 3, 5].

Of particular interest is the role of the asymptomatic reservoir in maintaining local malaria transmission [6-10]. The current hypothesis is that asymptomatically infected individuals maintain malaria transmission, whereas individuals lacking clinical immunity develop symptomatic malaria and seek care at health facilities [6, 11, 13, 16, 24]. Thus, areas approaching elimination have increased efforts to detect and treat both symptomatically and asymptomatically infected individuals to achieve elimination [6, 8, 11-23].

This study explored changing spatial and temporal patterns of malaria in a region of southern Zambia where malaria transmission has decreased dramatically and is approaching elimination to better understand the relationship between the asymptomatic reservoir identified through active case detection and symptomatic individuals seeking care at health facilities.

Methods

Study site

The study was conducted in Southern Province, Zambia. The single rainy season lasts from November through April, followed by a cool dry season from April to August and a hot dry season through November. Malaria transmission peaks during the rainy

season [33] and the primary malaria vector is *Anopheles arabiensis*. The study area is populated by villagers living in small, scattered homesteads. Artemisinin combination therapy (ACTs) with artemether-lumefantrine was introduced as first-line anti-malarial therapy in Zambia in 2002 [34] and into the study area in 2004. In Zambia, long lasting insecticide treated nets (LLINs) are distributed through antenatal care clinics and additional mass distribution campaigns [35]. LLINs were widely distributed in the study area in 2007 [36] and more than 11,000 LLINs were distributed from nine health posts in the catchment area of Macha Hospital in 2012, with additional LLINs distributed in 2014 according to the Office of the Macha Hospital Environmental Health Technician.

Passive surveillance through health center reporting

Fourteen rural health centers (RHCs) send weekly reports of malaria cases to study staff in at Macha Research Trust (MRT) [37], including the number of RDTs used, number of RDTs that were positive, and the number of people treated for malaria. Six of these RHCs are located within the active case surveillance study area described below and started reporting in August 2008. One of these RHCs is associated with Macha Hospital.

Active surveillance through community surveys

Satellite images were used to develop a sampling frame in a 1,200 km^2 study area. Households were randomly sampled from 2007 to 2013 [28]. The 2007 study area was east of that used from 2008 to 2013, and data collected in 2007 was only used to define the RHC catchment areas. The identification and enumeration of households was done manually to delineate household and non-household structures (kraals, schools, and larger structures) [28]. Households selected from the sampling frame were recruited and enrolled in cross-sectional surveys conducted every other month from February 2008 to December 2013, with a different set of households each month. For each study visit, a questionnaire was administered to collect demographic data, history of recent

malaria symptoms and treatment, healthcare seeking behavior including which RHC they visited the last time they had an illness, knowledge of malaria risk and prevention, LLIN use, and recent travel history. A blood sample was collected by finger prick for a rapid diagnostic test (RDT). Participants who were positive by RDT were offered treatment with artemether-lumefantrine (Coartem®).

Defining RHC catchment areas

The locations of participating households were mapped along with the specified RHC visited during the last illness episode for each resident. Responses from participants from 2007 through 2013 were mapped. The participants from 2008 to 2013 indicated one of the 6 RHCs; the 2007 sample included an additional 2 RHCs. These additional, two RHCs were included to delineate RHC boundaries but were not included in further analyses. Thiessen polygons were created around each household in ArcGIS using the proximity tools. Thiessen polygons define areas of influence around each of a set of points so that any location inside the polygon is closer to that point than any other sample points and the boundaries define the area that is closest to each point relative to all other points. The Thiessen polygons were then dissolved by the reported preferred RHC to create RHC level polygons. The resulting polygons represent the catchment areas most likely served by each RHC [38, 39].

A simulation model was constructed previously which created a simulated population for all of the households in the study area based on the sampled population. The methods used to create this simulated population was previously published [23]. In brief, a prediction model was constructed using data from the population-based, serial cross-sectional surveys to estimate the number of residents per household [23]. These estimates of household size were then spatially joined to the Thiessen polygons representing the RHC catchment areas. Thus, the total number of households and individuals residing in each RHC was estimated.

Calculating incidence rates of passively detected malaria cases

Weekly reports of passively detected malaria cases from the 6 RHCs were aggregated to the month and linked to their RHC catchment area. Monthly incidence rates per catchment area were calculated as the total number of cases per month divided by the total population of the catchment area. As there was a clear seasonal pattern in total malaria cases and incidence rates, the data were aggregated for each annual transmission season from October through September.

Spatiotemporal patterns of incidence of passively detected malaria cases

The RHC with the highest incidence per annual transmission season was classified as the dominant RHC for that season. First-order neighbors of the dominant RHC for each season were neighboring RHCs sharing borders with the dominant RHC. Second-order neighbors of the dominant RHC for each season were neighboring RHCs sharing borders with the first-order neighbors.

Incidence rates per 1,000 residents were calculated for each annual transmission season for the dominant RHC, first-order neighboring RHCs, and second-order neighboring RHCs. Incidence rates ratios comparing dominant and first-order neighboring RHCs, and first-order and second-order neighboring RHCs, were calculated for each annual transmission season.

Cluster detection of actively detected malaria infections

Infection with *Plasmodium falciparum* was confirmed by RDT and PCR during active case detection from the serial cross-sectional surveys from February 2008 to October 2013. Actively detected cases were aggregated by annual transmission season in the same manner as passively detected cases (October through September). SaTScan™ software was used for cluster detection for each annual transmission season using a Bernouli purely spatial model. Clusters were allowed to overlap but were

restricted to having no cluster centers inside other clusters. The maximum cluster size was set to be less than or equal to 25% of the total population at risk.

All annual transmission seasons were combined and a space-time cluster detection analysis was conducted to identify temporally stable clusters. These clusters were also allowed to overlap, were restricted to having no clusters inside other clusters, and the maximum cluster size was set to be less than or equal to 25% of the total population. Statistically significant clusters were mapped in ArcGIS (ESRI 2012. ArcGIS Desktop: Release 10.2. Environmental Systems Research Institute, Redlands, CA, USA).

Results

RCH catchment area populations

A total of 3,235 participants from 735 households were enrolled between 2007 and 2013 in the cross-sectional surveys and had data on their preferred RHC. When linked with the simulated population, 35,148 individuals from 6,589 households were estimated to reside within the catchment areas of the 6 RHCs (Figure 2.1, Figure 2.2). These population estimates were used to calculate incidence rates and create polygons of the RHC catchment areas (Figure 2.2).

Incidence of passively detected malaria at RHCs

The number of passively detected malaria cases declined over the study period, with variation in the incidence of passively detected malaria cases across RHCs (Figure 2.3, Figure 2.4). Annual peaks in incidence were observed in the 2008-2009 and 2009-2010 annual transmission seasons, but biannual peaks in incidence were observed during the remaining time periods (Figure 2.3, Figure 2.4).

Spatiotemporal patterns of incidence of passively detected malaria

Malaria incidence rates for each annual transmission season for the dominant RHC catchment area and first-order and second-order neighboring RHC catchment areas are presented in Table 2.1. The RHC associated with Macha Hospital was the dominant RHC catchment for 4 of the 7 annual transmission seasons (57%), with the Nalube RHC the dominant RHC catchment for two seasons (29%) and the Mangunza RHC for one season (14%) (Table 2.1, Figure 2.5).

Incidence rates in first-order RHCs were consistently higher than or equal to neighboring second order RHCs during the first 4 annual transmission seasons from 2008-2009 through 2011-2012 (Table 2.1, Figure 2.5). After the 2011-2012 season, incidence in second-order neighboring RHCs was greater than first-order RHCs, with the exception of the 2013-2014 season when the incidence in first-order and second-order RHCs were equivalent (Table 2.1, Figure 2.5).

Because the incidence of malaria decreased sharply after the 2010-2011 annual transmission season, analyses were stratified by the higher (2008 through 2011) and lower (2011 through 2015) transmission seasons. A more distinct spatial pattern was observed, with dominant RHC catchments having a higher incidence than their first-order RHCs catchment areas, and first-order RHCs higher than second-order RHCs in the higher transmission period (Table 2.1, Figure 2.6). The incidence rate ratio (IRR) comparing dominant RHCs to their first-order neighbors was 1.95 (1.74-2.19) in the higher transmission period and 1.98 (1.62-2.40) in the lower transmission period (Table 2.2, Figure 2.7). In contrast, the IRR comparing the first- and second-order RHCs was 1.09 (0.97-1.22) in the higher incidence period a 0.79 (0.67-0.94) in the lower transmission period (Table 2.2, Figure 2.7), suggesting less spatial dependence as transmission decreased.

Cluster detection of actively detected infected individuals

Malaria prevalence declined from 9.2% in 2008 to 0.7% in 2013 (Table 2.3, Figure 2.8). The largest decrease in prevalence occurred between the 2008-2009 and 2009-2010 annual transmission seasons when the prevalence declined from 7.1% to 1.7%, and remained low for the remaining 3 transmission seasons (Table 2.3, Figure 2.8). Participant demographic characteristics did not differ by annual transmission season but the proportion of febrile participants was higher in the 2008 and 2012-2013 seasons (Table 2.3).

Spatial clusters were detected using a spatial-only model during each annual transmission seasons except 2010-2011 but these clusters did not overlap (Table 2.4, Figure 2.9). After combining all annual transmission seasons and accounting for temporal trends, four statistically significant, temporally stable clusters were detected using the space-time cluster detection model (Table 2.5, Figure 2.10). Three clusters overlapped due to inclusion of a single household (Table 2.5, Figure 2.10).

Relationship between incidence of passively detected malaria and clusters of actively detected malaria infection

Spatial-only clusters of parasite prevalence overlapped with the dominant RHC catchment area only during the 2008-2009 season. During other annual transmission seasons, spatial-only clusters of parasite prevalence were either in first-order RHCs, second-order RHCs, or overlapping with both. When both malaria incidence and prevalence were low, there was no evidence of spatial overlap between the passively and actively detected infected individuals.

Discussion

These results show changes in spatial and temporal trends of passively and actively detected infections in an area where malaria transmission has declined substantially. To the authors' knowledge, this is the first time spatial and temporal trends

among passively and actively detected cases were compared in an elimination setting. In such areas, targeted interventions to prevent further transmission are critical to achieve elimination but predicting spatial patterns can become difficult as the number of infected individuals decreases.

RHCs estimate their catchment area based on available census data [37]. This estimate does not account for actual utilization of an RHC by residents [40, 41]. Health seeking behavior can depend on many factors including distance, perceived quality of care and accessibility of [41]. Using a novel approach, RHC catchment area population sizes were estimated based on a reported health center use and a simulation model. A hierarchical spatial pattern in malaria incidence was observed based on these estimates, in which incidence was higher in RHC catchment areas adjacent to the RHC with the highest incidence compare to more distant RHCs. However, this spatial dependence was lost as the burden of malaria decreased.

The spatial only model identified clusters of prevalent cases for six of the seven annual transmission seasons and the space-time model detected four temporally stable clusters over the study period. Spatial clusters of actively detected cases and the dominant RHC catchment area overlapped only during the annual transmission season with the highest prevalence. As transmission declined, this spatial relationship between incident and prevalent cases was lost, consistent with a chronically infected reservoir of prevalent cases that are not contributing to incident cases.

A major limitation was the small number of RHC catchment areas used to determine spatial trends in malaria incidence. To estimate the catchment area population sizes, data on health care utilization from community surveys were used, which restricted the number of RHCs to those within the active surveillance study area, and assumed a static population. However, this permitted comparisons of spatial patterns in incidence and prevalent cases. A second limitation was the low number of

prevalent cases available to assess spatial and temporal-spatial clusters, a challenge common to all areas approaching elimination.

Conclusions

As malaria transmission declined in southern Zambia, spatial relationships in malaria incidence changed, with a loss of spatial dependence consistent with a change from endemic malaria transmission to stochastic, parasite importations. Small spatial clusters of prevalent cases were identified but the absence of spatial overlap between incident and prevalent cases suggests the emergence of two distinct populations of infected individuals, acutely infected, incident cases and chronically infected, prevalence cases.

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Table 2.1: Seasonal malaria incidence in dominant catchment areas and first and second order neighbors from 2008 to 2015

	Dominant catchment area		First order neighbors		Second order neighbors	
Season	Incidence per 1,000	95% CI	Incidence per 1,000	95% CI	Incidence per 1,000	95% CI
2008-2009	32.4	28.8, 36.2	40.3	35.3, 45.9	20.8	17.1, 25.0
2009-2010	19.3	15.7, 23.4	13.3	11.2, 15.7	12.9	11.4, 14.6
2010-2011	14.9	11.1, 19.7	6.3	5.4, 7.3	3.8	2.2, 6.0
2011-2012	17.8	14.4, 21.7	10.2	8.3, 12.3	8.4	7.1, 9.8
2012-2013	8.9	6.0, 12.8	5.3	4.5, 6.3	7.1	5.0, 10.0
2013-2014	18.1	14.7, 22.1	7.4	5.9, 9.3	10.0	8.7, 11.5
2014-2015	8.4	6.1, 11.3	4.7	3.4, 6.2	4.3	3.4, 5.3

Table 2.2: Malaria incidence rate ratios between dominant RHC catchment areas and first and second order neighbors for the high (2008-2011) and low (2012-2015) transmission periods

	Dominant catchment vs. first order neighbors			Dominant catchment vs. second order neighbors			First order neighbor vs. second order neighbors		
Season	IRR	95% CI	p-value	IRR	95 % CI	p-value	IRR	95% CI	p-value
2008-2011	2.0	1.7, 2.2	<0.001	2.1	1.9, 2.4	<0.001	1.1	1.0, 1.2	0.075
2012-2015	2.0	1.6, 2.4	<0.001	1.6	1.3, 1.9	<0.001	0.8	0.7, 0.9	0.006

Table 2.3: Demographic characteristics of passively detected cases by annual transmission season

	*2008	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013	p-value
Households	24	98	142	139	151	689	
Participants	153	534	869	746	685	706	
Confirmed malaria cases	14	38	15	13	7	5	
Prevalence (%)	9.2	7.1	1.7	1.7	1.0	0.7	<0.001
Sex							0.9
Male (%)	45.1	45.9	47.2	46.5	44.5	44.9	
Symptomatic	31.4	26.0	21.1	18.4	21.5	48.6	<0.001
Median age (IQR)	14.5 (6.9-35.5)	14.2 (6.6-34.3)	14.7 (6.1-33.3)	14 (5.8-34.5)	13.0 (5.0-13.0)	15 (5.7-34.6)	0.72

*Data collection in 2008 began in February 2008

Table 2.4: Spatial clusters by annual transmission season

Season	Cluster number	Households	Participants	Expected cases	Observed cases	RR	p-value
2008*	1	1	13	1.2	6	8.1	0.008
2008-2009	1	3	30	2.1	11	6.8	0.001
2008-2009	2	2	15	1.6	8	5.9	0.03
2009-2010	1	1	7	0.1	3	30.8	0.04
2010-2011	None detected	NA	NA	NA	NA	NA	NA
2011-2012	1	10	56	0.6	5	28.1	0.02
2012-2013	1	10	39	0.3	4	68.3	0.01

Table 2.5: Space-time clusters of infected individuals identified through active surveillance from 2008 to 2013

Cluster	Households	Participants	Expected cases	Observed cases	RR	p-value
1	63	341	2.6	10	6.4	0.007
2	3	28	0.9	6	6.4	0.01
3	4	25	0.9	6	6.4	0.01
4	67	359	0.7	5	5.7	0.02

Figure 2.1: Map of study households and preferred rural health center

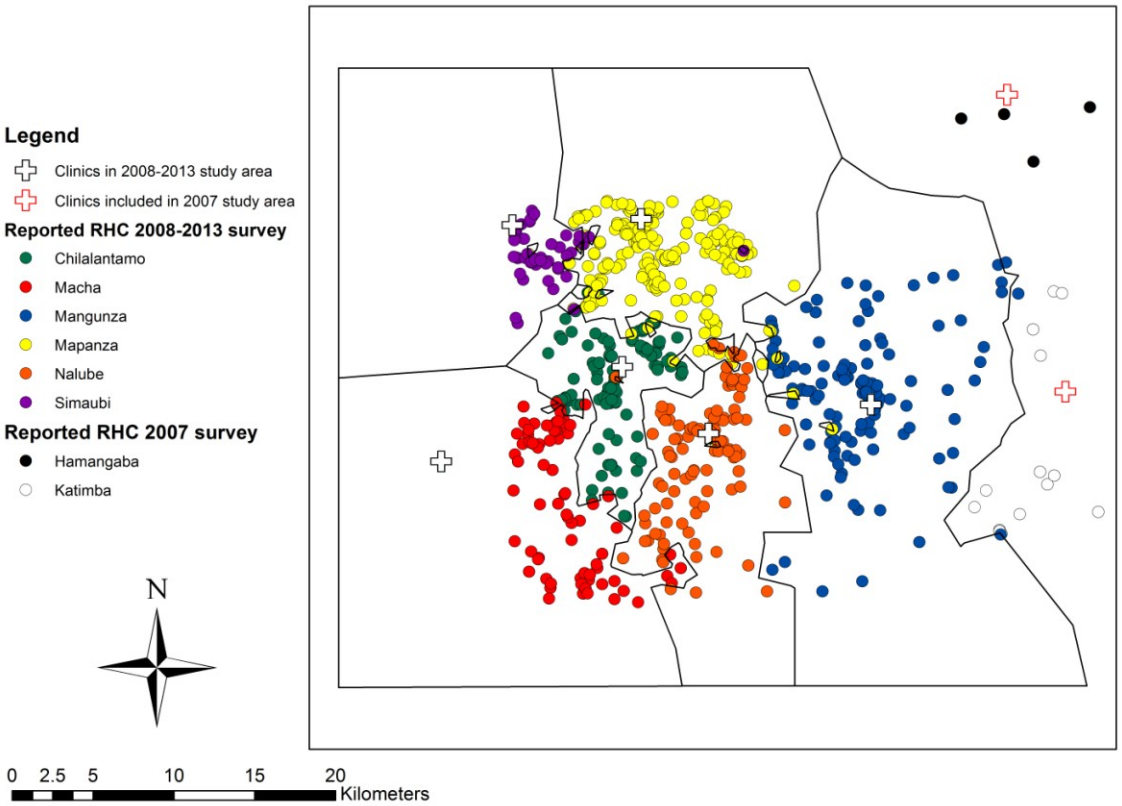


Figure 2.2: Polygons of rural health center catchment areas and population size estimate

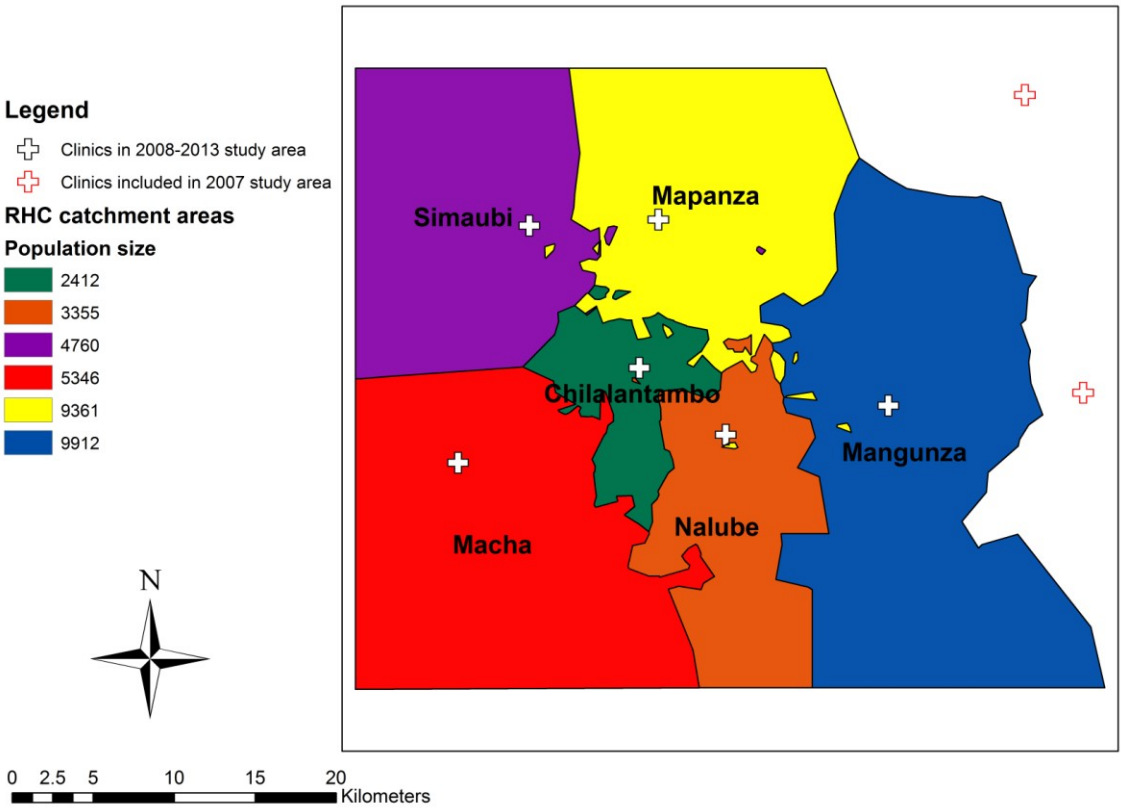


Figure 2.3: Incidence of passively detected malaria per 1,000 population per month by rural health center

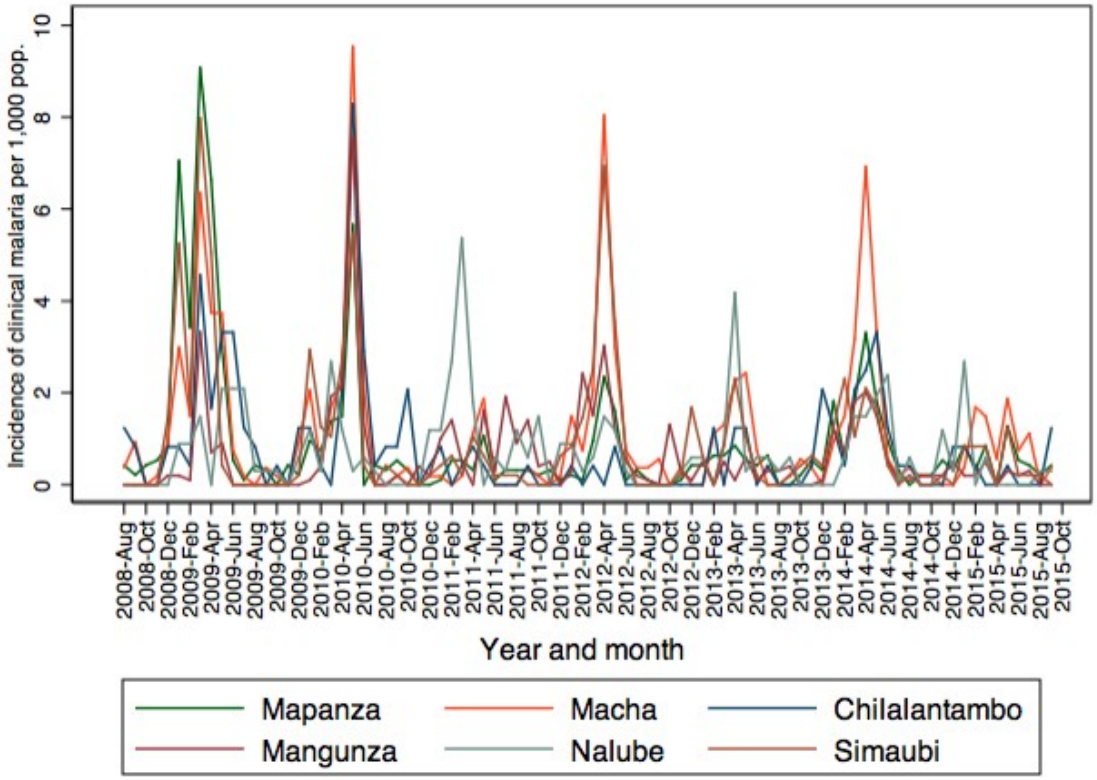


Figure 2.4: Incidence of passively detected malaria per 1,000 population per month stratified by rural health center

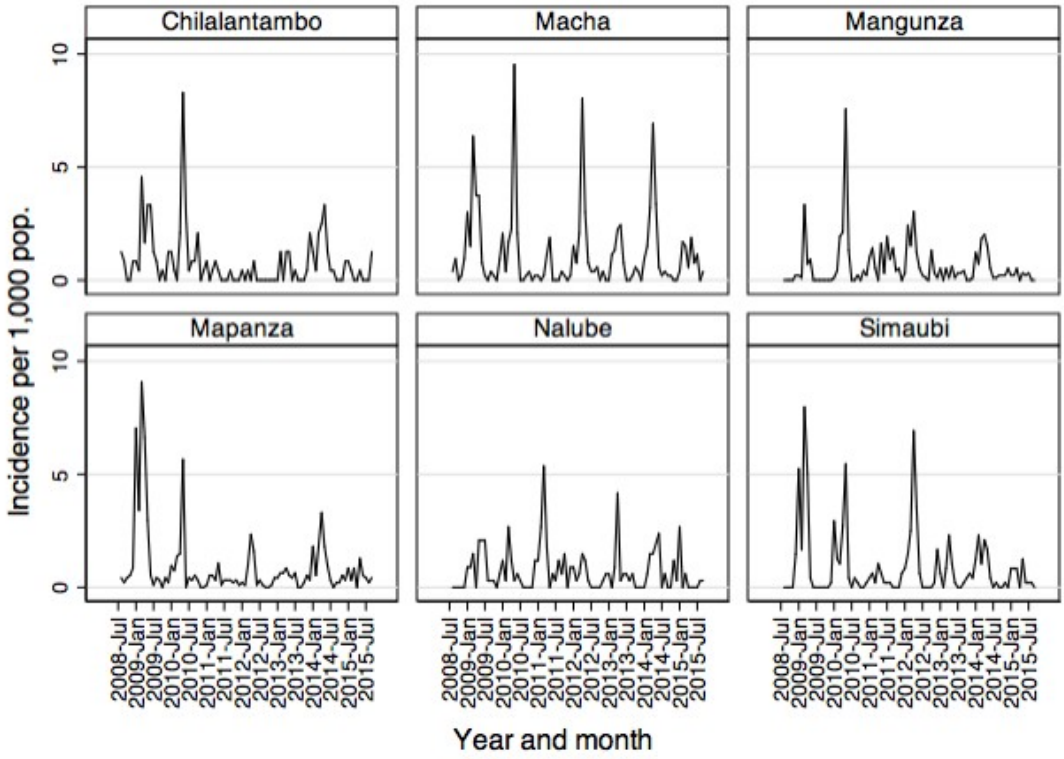


Figure 2.5: Incidence of passively detected malaria for the dominant rural health center and first and second order rural health centers for annual malaria transmission seasons from 2008-2009 through 2014-2015

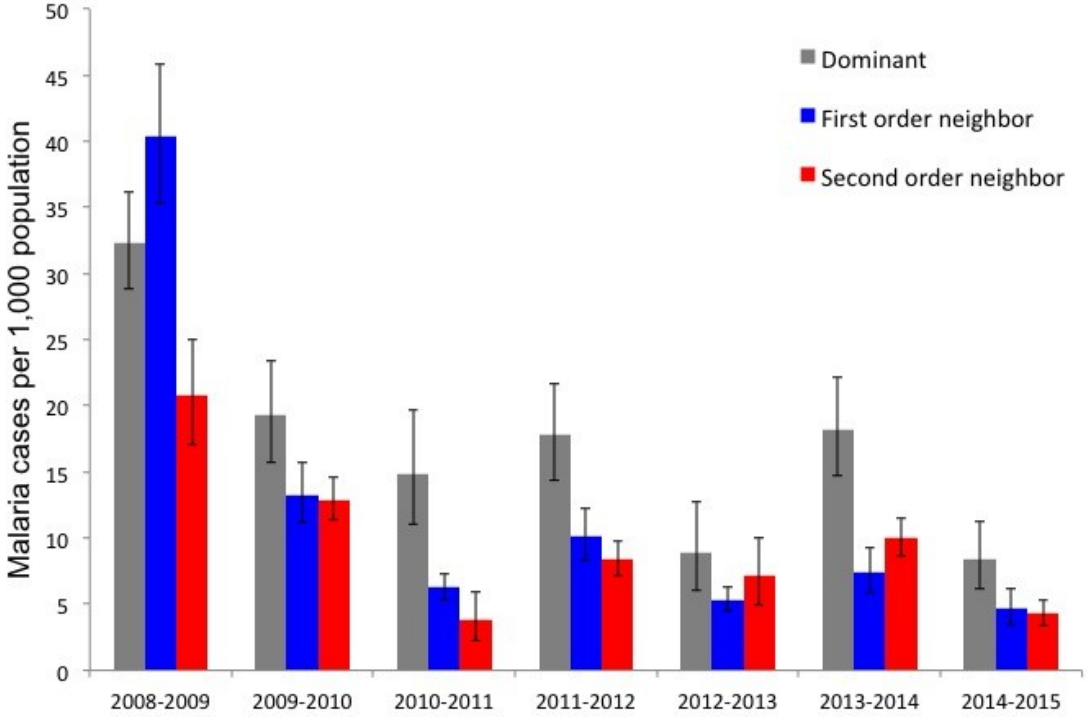


Figure 2.6: Incidence of passively detected malaria for the dominant rural health centers and the first and second order rural health centers for the high (2008-2011) and low (2012-2015) transmission periods

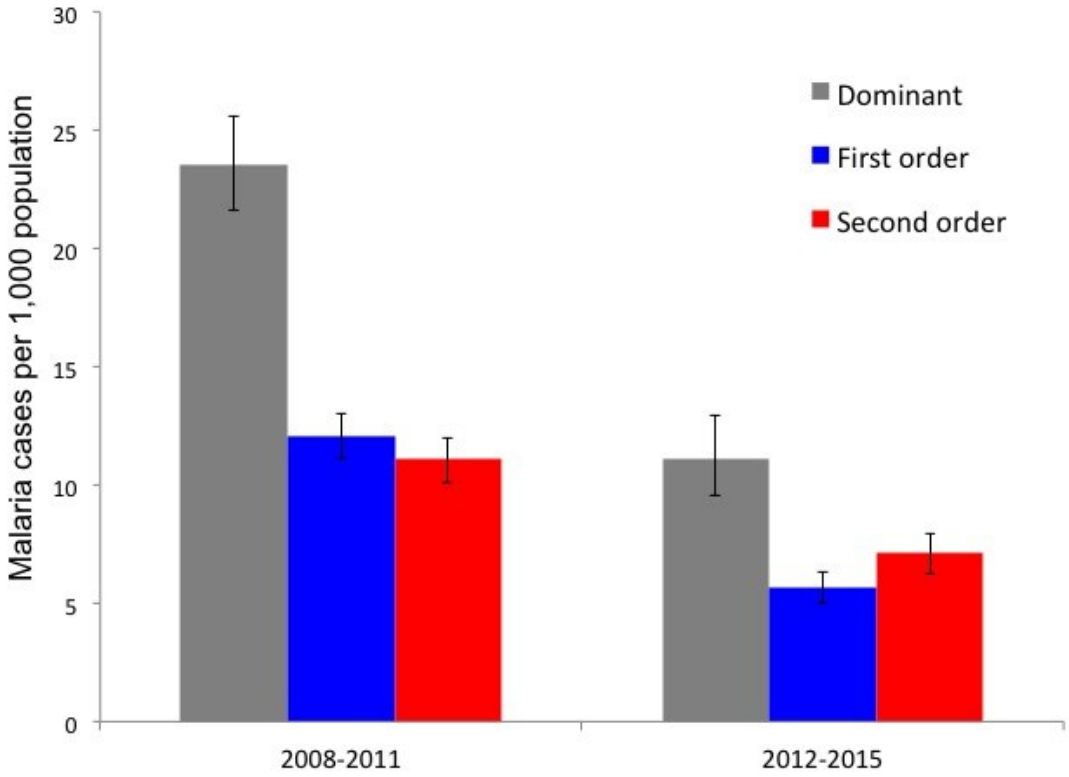


Figure 2.7: Malaria incidence rate ratios between dominant rural health centers and their first and second order rural health centers for the high (2008-2011) and low (2012-2015) transmission periods

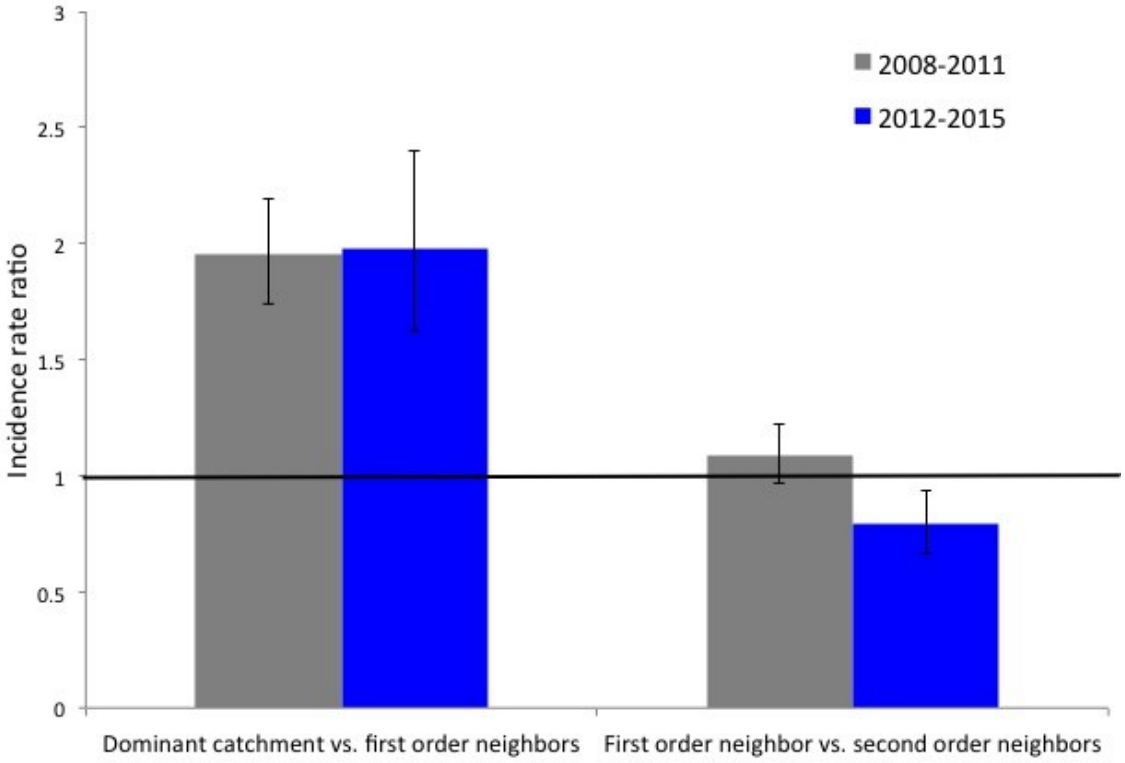
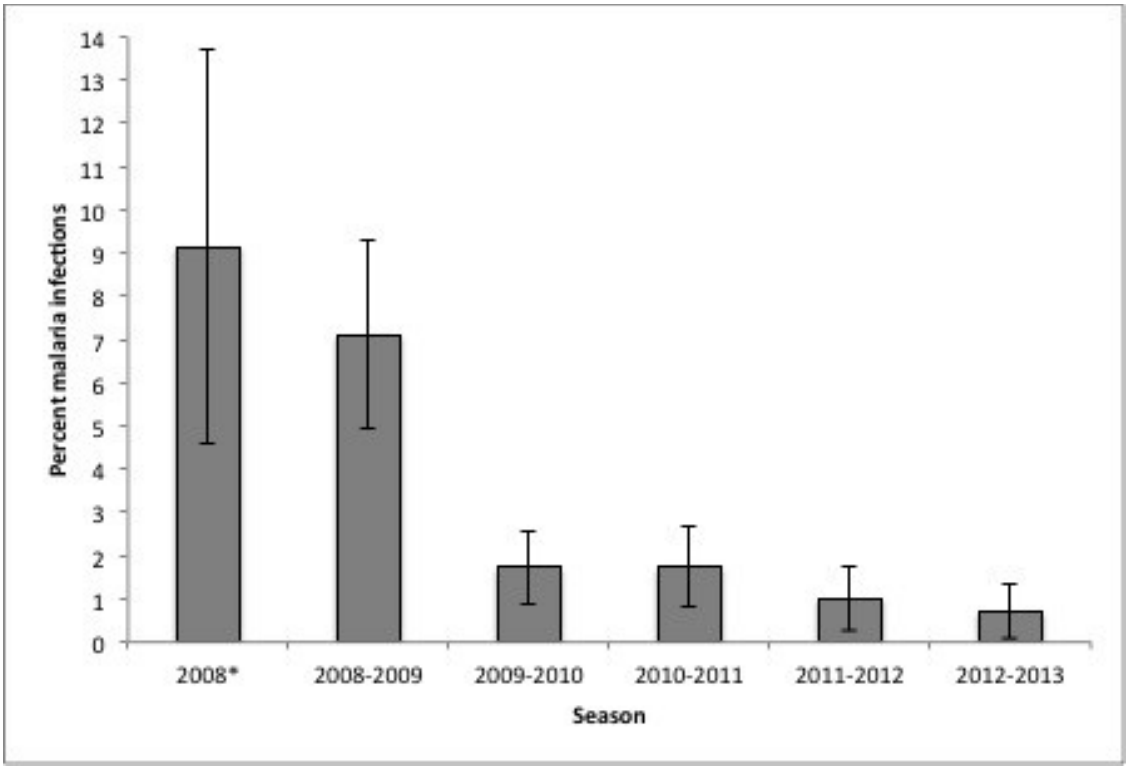


Figure 2.8: Malaria prevalence detected through active surveillance, by annual transmission season



*2008 season began in February 2008

Figure 2.9: Spatial clusters of infected individuals detected through active surveillance in Macha, Zambia (A: *2008, B: 2008-2009, C: 2009-2010, D: 2010-2011, E: 2011-2012, F: 2012-2013)

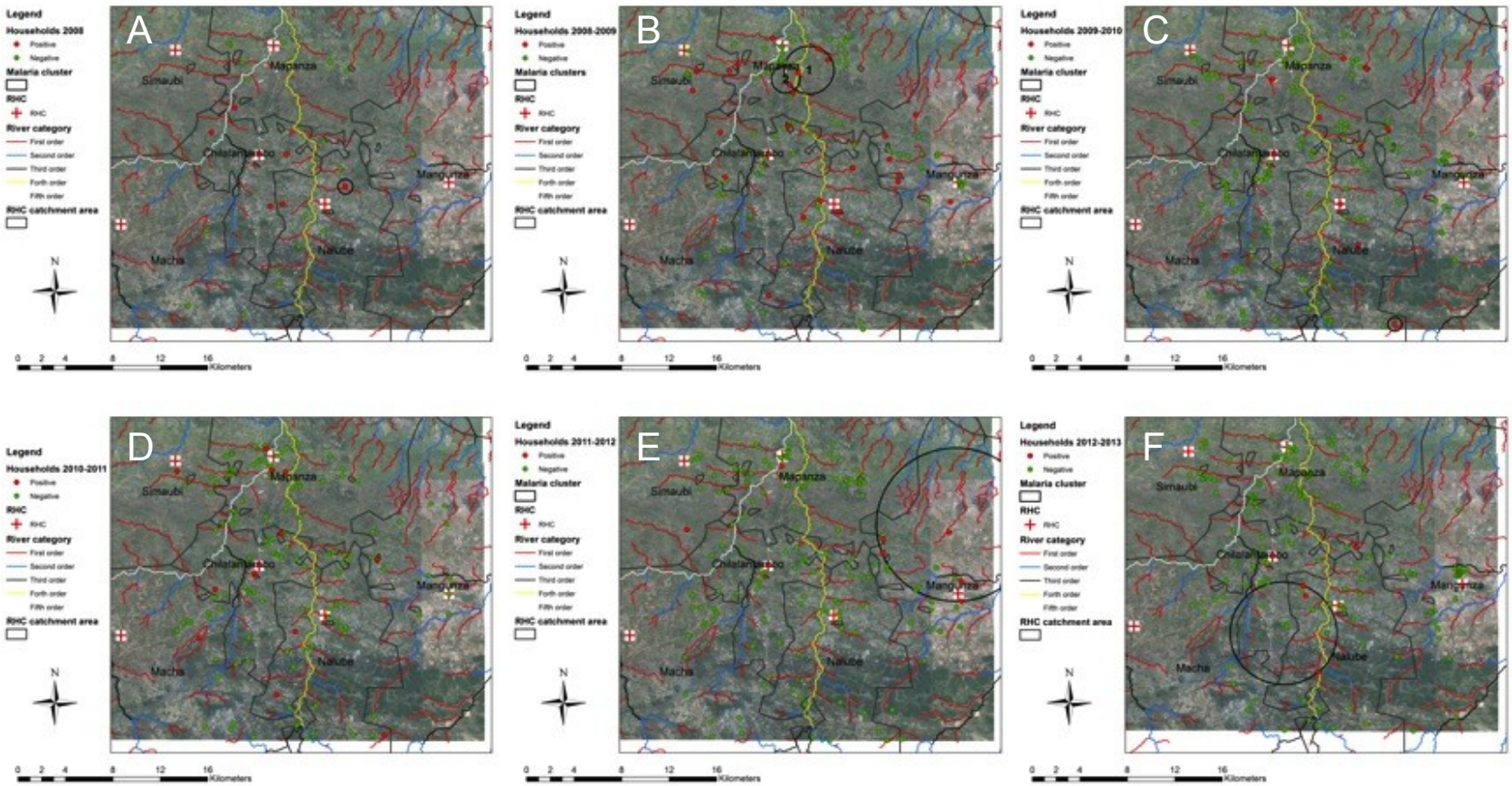
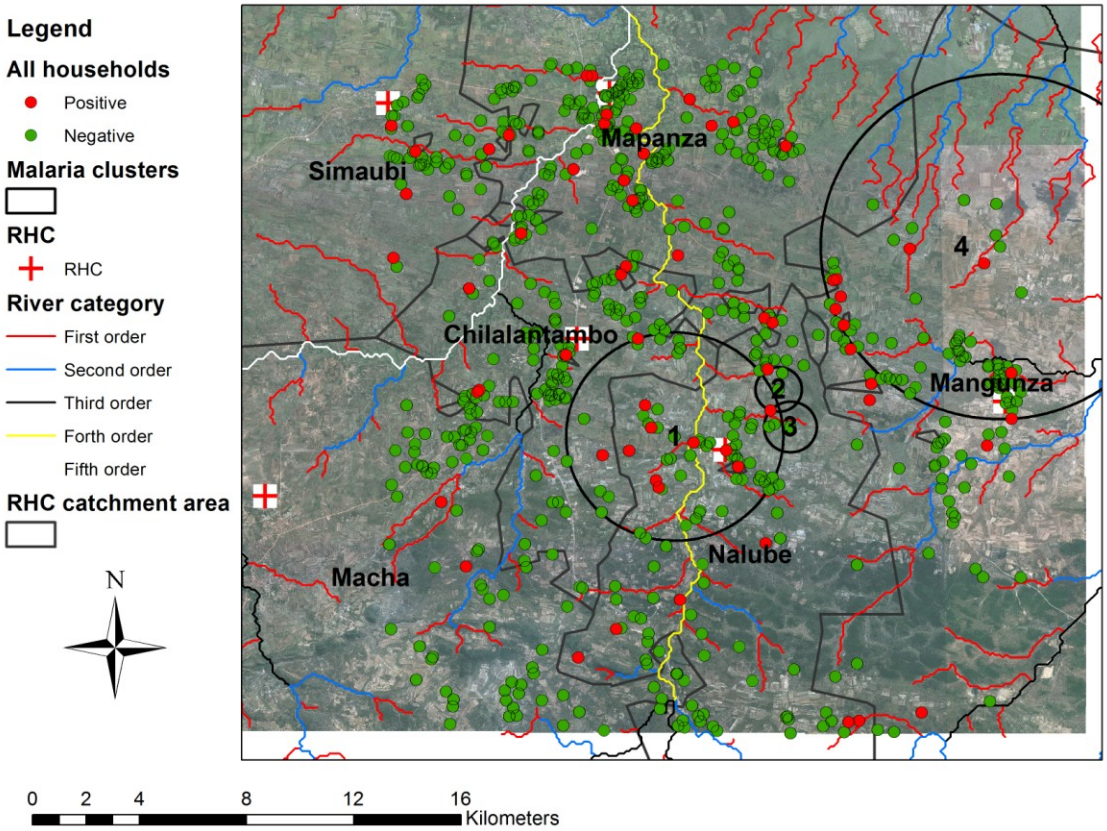


Figure 2.10: Space-time clusters of infected individuals detected through active surveillance from 2008-2013



3. Distinct parasite populations infect individuals identified through passive and active case detection in a region of declining malaria transmission in southern Zambia

Authors: Kelly M. Searle, ScM[1], Ben Katowa, BA[2], Tamaki Kobayashi, PhD MPH[1], Mwiche Siame, MSc[2], Sungano Mharakurwa, PhD[3], Jennifer C. Stevenson, PhD[2,4], Philip E. Thuma, MD[2,4], William J. Moss, MD MPH[1] for the Southern Africa International Centers of Excellence for Malaria Research

[1]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[2]Macha Research Trust, Choma District, Zambia

[3]Biomedical Research Training Institute, Harare, Zimbabwe

[4]Johns Hopkins Malaria Research Institute, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

Abstract

Background: Substantial reductions in the burden of malaria have been documented in parts of sub-Saharan Africa, with malaria elimination goals proposed at regional, national, and subnational levels. As areas transition from high or moderate to low malaria transmission and approach elimination, understanding the epidemiology of low level malaria transmission is crucial to achieving and sustaining elimination. A 24 single nucleotide polymorphism (SNP) *Plasmodium falciparum* molecular barcode was used to characterize parasite populations over time from infected individuals identified through passive and active case detection in an area approaching malaria elimination in southern Zambia.

Methods: The study was conducted in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia, where the parasite prevalence declined over the past decade, from 9.2% in 2008 to less than 1% in 2013. A 24 SNP *P. falciparum* molecular barcode was used to determine the genetic relatedness, diversity and complexity of parasite populations. Parasite haplotypes from actively detected, *P. falciparum*-infected participants enrolled in a population-based, serial cross-sectional cohort study from 2008-2013 and passively detected, *P. falciparum*-infected individuals enrolled at five rural health centers from 2012-2015 were compared. Within each study population, changes in genetic relatedness, diversity and complexity were analyzed as malaria transmission declined.

Results: Infected individuals detected through active and passive surveillance did not differ by basic demographic characteristics. Actively detected cases were most commonly rapid diagnostic test (RDT) negative, asymptomatic and had submicroscopic parasitemia. The phylogenetic tree showed evidence of clustering only during the 2012-2013 transmission season for passively detected cases but no evidence of clustering for

actively detected cases. In a combined phylogenetic tree, there was clear separation of parasite haplotypes from passively and actively detected infections, consistent with two evolutionarily distinct parasite populations. For passively detected infections, the proportion of polyclonal infections was consistently low in all seasons, in contrast with actively detected infections in which the proportion of polyclonal infections was high. The mean genetic divergence for passively detected infections was 34.5% for the 2012-2013 transmission season, 37.8% for the 2013-2014 season, and 30.8% for the 2014-2015 season. The mean genetic divergence for actively detected infections was 22.3% in the 2008 season and 29.0% in the 2008-2009 season but decreased to 9.9% across the 2012-2014 seasons, which were combined because of small sample size.

Conclusions: Distinct parasite populations were identified among infected individuals identified through active and passive surveillance, suggesting the asymptomatic reservoir detected through active surveillance did not contribute substantially to ongoing transmission. As parasite prevalence and diversity within these individuals declined over time, efforts to detect and treat the chronically infected reservoir through reactive case detection or mass drug administration may not be necessary to eliminate malaria in this setting.

Background

Substantial reductions in the burden of malaria have been documented in parts of sub-Saharan Africa [1] and malaria elimination goals have been proposed at regional, national, and subnational levels [2-4]. As areas transition from high or moderate to low malaria transmission and approach elimination, understanding the epidemiologic patterns of transmission is crucial to achieving and sustaining elimination [2, 3, 5]. Of interest is the relative magnitude of infection attributed to locally acquired and imported cases, specifically the role of the chronically infected, asymptomatic reservoir in maintaining local malaria transmission [6-10]. One way to address this question is to determine the genetic relatedness of parasites between actively detected, predominantly chronically infected, asymptomatic infected individuals and passively detected, predominantly acutely infected, symptomatic malaria cases [11]. As areas transition from malaria control to elimination, knowledge of the relative role of chronically infected, actively detected cases and acutely infected, passively detected cases in local malaria transmission dynamics can guide interventions, particularly reactive case detection and focal or mass drug administration strategies.

A *Plasmodium falciparum* molecular barcode assay consisting of 24 unlinked, single nucleotide polymorphisms (SNPs) has been used to characterize unique genetic signatures and track circulating *P. falciparum* parasite populations [11]. The barcode was developed to elucidate malaria transmission dynamics by tracking the genetic diversity and complexity of the parasite over time and space [11]. This method was developed specifically for use in resource-limited settings as the highest level of technology required is a polymerase chain reaction (PCR) assay [11].

This study was conducted in an area of southern Zambia that experienced a dramatic decrease in malaria transmission over the past decade [12]. The molecular

barcode was used to determine the relatedness between parasites over time in infected individuals identified through passive and active surveillance, as well as changes in parasite genetic complexity and diversity.

Methods

Study site and population

The study was conducted in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia, where there is a single rainy season which lasts from November through April, followed by a cool dry season from April until August and a hot dry season from August through November. Malaria transmission peaks during the rainy season [13] and the primary vector is *Anopheles arabiensis* [14, 15]. The hospital catchment area is populated by villagers living in small, scattered homesteads. The prevalence of malaria declined in this area over the past decade, from 9.2% in 2008 to less than 1% in 2013. Artemisinin combination therapy (ACTs) with artemether-lumefantrine was introduced as first-line anti-malarial therapy in Zambia in 2002 [16] and into the study area in 2004. In Zambia, long lasting insecticide treated nets (LLINs) are distributed through antenatal care clinics and additional mass distribution campaigns [17]. LLINs were widely distributed in the study area in 2007 [18] and more than 11,000 LLINs were distributed from nine health posts in the catchment area of Macha Hospital in June 2012, with additional LLINs distributed in 2014 according to the Office of the Macha Hospital Environmental Health Technician.

Active malaria surveillance

Satellite images were used to develop a sampling frame for the random sampling of households to enroll participants in longitudinal and cross-sectional malaria surveys [19]. The identification and enumeration of households was done manually to delineate

household and non-household structures (kraals, schools, and larger structures) [19]. Households selected from the sampling frame were enrolled in either a cross-sectional or longitudinal cohort. Households enrolled in the cross-sectional cohort were surveyed once, whereas households enrolled in the longitudinal cohort were repeatedly surveyed every two months. Surveys were conducted each month, alternating between the cross-sectional cohort and the longitudinal cohort, from February 2008 through October 2013. For each study visit, a questionnaire was administered to collect demographic data, history of recent malaria symptoms and treatment, healthcare seeking behavior, knowledge of malaria risk and prevention and long-lasting insecticidal net use. For these analyses, recent malaria symptoms were defined as having a documented fever greater than 38°C or reporting a fever and chills within the previous 48 hours. A blood sample was collected by finger prick for a rapid diagnostic test (RDT), microscopy and blood was spotted on Whatman 903™ Protein Saver cards. Participants who were positive by RDT were offered treatment with artemether-lumefantrine (Coartem®).

Passive malaria surveillance

Fourteen rural health centers (RHCs) surrounding Macha Hospital sent a weekly text message report of the number of RDTs used, number of positive RDTs, and the number of people treated for malaria to study staff at Macha Research Trust [20]. Five of these RHCs collected demographic data using a short survey and a dried blood spot from individuals with a positive RDT during annual malaria transmission seasons (October-September) from 2012 through 2015.

Laboratory methods

The dried blood spots (DBS) were stored at -20°C in individual plastic bags containing desiccant until DNA extraction. DBS collected from February to September

2008 were initially stored at room temperature, then transferred to -20°C storage afterwards. Parasite DNA was extracted using the Chelex© method from one dried blood spot. The dried blood spots were placed in 1.5 mL microcentrifuge tubes, 1 mL of 0.1% weight by volume saponin in 1x phosphate buffered saline (PBS) was added and the mixture was incubated for 10 minutes at room temperature. The tubes were centrifuged for two minutes at 14,000 rpm, the supernatant discarded and 1 mL of 1X PBS was added. The tubes were centrifuged for 2 minutes at 14,000 rpm, the supernatant discarded and 150 µL of 2% weight by volume Chelex© solution and 50 µL of DNase free water were added and the tubes were boiled for 8 minutes. The tubes were then centrifuged for one minute at 14,000 rpm and approximately 150 µL of DNA was stored at -20°C.

Infection was confirmed using a *Plasmodium* specific nested PCR assay. The nested PCR detected the asexual stage of parasite DNA using two sets of primers targeting a segment of the mitochondrial cytochrome b gene (*cytb*) present in the four major human *Plasmodium* parasites. In the primary PCR step, 6 µL of DNA extract was pipetted into 0.2 mL tubes containing a 19 µL reaction mix made up of DNase free water and final concentrations of dNTPs, 10X PCR buffer, magnesium chloride, forward and reverse primers and DNA Taq polymerase in 25 µL reaction mix. In the nested PCR step, 3 µL of the primary PCR product was added to 0.2 mL PCR tubes containing 22 µL of reaction mix containing DNase free water and final concentrations of dNTPs, 10X buffer, magnesium chloride, forward and reverse primers and Taq DNA polymerase in 25 µL reaction mix. No template controls were included in each experiment and reactions were run in a Techne™ TC-412 thermo cycler (Bibby Scientific Limited, Staffordshire, UK). Amplified product was detected by electrophoresis on 1% agarose gel and viewed under UV light as an 815 base pair DNA band.

The 24 SNP molecular barcode assays were run using a TaqMan protocol at the Macha Research Trust laboratory in Zambia [11]. DNA was extracted from a second DBS for samples positive by nested PCR using the Chelex© method. Due to low parasite DNA concentrations, samples were pre-amplified prior to performing the 24 SNP molecular barcode assay [21]. The pre-amplification step was done by adding 5 µL of DNA for each sample to 10 µL of TaqMan pre-amplification master mix, and 0.2X pooled assay mixture, made up of forward and reverse primers for each of the 24 SNPs [21].

Pre-amplified samples were diluted 1:20 with TE buffer prior to running the 24 SNP molecular barcoding assay [21]. For each of the 24 SNP assays, 2.0 µl of pre-amplified sample DNA was added to 10.0 µl TaqMan master mix, 7.5 µL distilled water, and 0.5 µL TaqMan commercially available primer and probe assay mixture. For each of the 24 SNP assays, 3 known positive controls and 2 negative, no-template controls were run. Positive controls consisted of DNA samples from *P. falciparum* strains obtained from MR4 with known haplotypes for all 24 SNPs. Typically, 12 SNP assays were run for 5 samples at a time with controls on a 96-well plate. The assays were run on the Applied Biosystems StepOnePlus™ (Thermo Scientific, Waltham, MA, USA), and Roche LightCycler 480™ (Roche Diagnostics Corporation, Indianapolis, IN, USA) real time PCR systems.

Parasites sampled from peripheral blood are those in the haploid intra-erythrocytic stage of their lifecycle. Thus, SNP calls were made automatically based on allelic discrimination plots using standardized software programs accompanying the real time PCR systems as one of the two alleles or mixed [22, 23]. In cases where SNP calls could not be made by automatically, the calls were made manually by the study investigators. Otherwise the SNP call was classified as failed. Samples with failed SNP

calls were repeated up to 3 times. If a sample failed on all repeated assays, it was treated as missing data.

Construction of phylogenetic trees

Haplotypes were aligned using the ClustalW method in MEGA6 software, with mixed calls represented as a third possible allele [24]. Maximum likelihood trees were constructed for actively and passively detected malaria infections separately, grouped by malaria transmission season, to explore temporal phylogenetic clustering of haplotypes. Haplotypes for the combined dataset were aligned using the same method and a maximum likelihood tree was constructed.

Parasite genetic complexity and diversity

Parasite genetic complexity was determined by the number of mixed calls at each of the 24 SNPs. Samples with 4 or more mixed calls were categorized as polyclonal infections [25]. Temporal trends were graphed and variation in the proportion of polygenic infections was determined using the Wilcoxon rank-sum test. Samples with more than half missing data were excluded.

Parasite genetic diversity was evaluated by determining divergence from the most common barcode for each transmission season stratified by whether the parasite was identified through passive or active surveillance. The most common barcode for each season was determined by allelic frequency at each SNP to determine the nucleotide diversity. A modified 'SNP π', was developed to account for missing data and mixed allele calls, and was used to measure the seasonal parasite genetic divergence [26]. Details of the methods in calculating the modified 'SNP π' are described in the Appendix.

Results

The 24 SNP molecular barcode was run on 72 samples from actively detected malaria cases and 46 samples from passively detected malaria cases. The median age was 13 years (IQR=7-30) for infected individuals identified through passive case detection and 14 years (IQR=11-21) for those identified through active case detection (Table 3.1). There were no statistically significant differences in sex between the two study populations and no statistically significant differences in median age or sex were observed in passively or actively detected cases across the malaria transmission seasons (Table 3.1). While all actively detected cases were confirmed by PCR, no statistically significant differences were observed in microscopy results across the malaria transmission seasons, with most negative by microscopy (Table 3.1). Statistically significant differences were observed in the RDT results across malaria transmission seasons, with the proportion of RDT positive infections fluctuating, but all seasons had a higher proportion of RDT negative infections (Table 3.1). Statistically significant differences were observed in the proportion of individuals reporting symptoms of malaria for each season, with trends in symptomatic infections decreasing from the 2008 season through the 2010-2011 season, then subsequently increasing (Table 3.1).

Phylogenetic trees

The phylogenetic tree showed evidence of clustering for passively detected cases during the 2012-2013 annual malaria transmission season along with some mixing during the other two seasons (Figure 3.1A). However, there was no evidence of phylogenetic clustering during the 2013-2014 and 2014-2015 annual malaria transmission seasons (Figure 3.1A). For actively detected cases, the phylogenetic tree showed no evidence of phylogenetic clustering during any of the malaria transmission seasons (Figure 3.1B). In a combined phylogenetic tree, there was in general separation

of parasite genotypes from passively and actively detected cases (Figure 3.2), suggesting two evolutionarily distinct parasite populations (Figure 3.2).

Genetic complexity and diversity

Genetic complexity was approximated by calculating the proportion of polyclonal infections in a season. The proportion of polyclonal infections was consistently low for passively detected cases in all seasons, with no polyclonal infections identified during the 2012-2013 and 2014-2015 annual transmission seasons (Table 3.2, Figure 3.3A). In contrast, the proportion of polyclonal infections among actively detected cases was consistently high for all malaria transmission seasons (Figure 3.3B). The last two seasons were combined due to the low numbers of cases but all were infections were polyclonal (Table 3.2, Figure 3.3B). No seasonal trends in genetic complexity were identified (Figure 3.3B).

The mean genetic divergence for passively detected cases was 34.5% for the 2012-2013 season, 37.8% for the 2013-2014 season, and 30.8% for the 2014-2015 season (Table 3.2, Figure 3.4A). The mean genetic divergence for actively detected cases was 22.3% in the 2008 season and 29.0% in the 2008-2009 season (Table 3.2, Figure 3.4B). This decreased to 9.9% in the combined 2012-2014 seasons (Table 3.2, Figure 3.4B). Overall, the genetic divergence remained high among passively detected cases but decreased for actively detected cases (Figure 3.4B).

Discussion

Distinct parasite populations were found in individuals identified through passive and active surveillance in a region of declining malaria transmission in southern Zambia using a SNP-based molecular barcode for *Plasmodium falciparum*. These results

suggest that the population of largely asymptomatic, passively detected infections may not be contributing significantly to ongoing transmission in this setting.

The molecular barcode can be used to document the parasite diversity over time by providing information on the number of unique barcodes present each year, the persistence of unique barcodes between years, and the genetic divergence measured by nucleotide diversity within and between years. The molecular barcode determines whether the major allele, minor allele, or a mixture of major and minor alleles is present in the haploid blood stage of the parasite for each of the 24 SNPs. The resulting barcode represents the parasite's haplotype and can be used to compare the genetic relatedness between individual infections and populations of parasites infecting different groups of people [27]. Variation arises as a result of genetic recombination and outcrossing during the sexual stage of infection when gametocytes combine [24]. SNPs with a mixture of major and minor alleles are referred to as mixed infections, with higher numbers of mixed infections representing higher genetic complexity. The frequency of mixed infections approximates the level of genetic complexity and provides information about the burden of infection due to genetically unique parasites [25].

Typically, as malaria transmission declines, this creates a bottleneck, reducing opportunities for outcrossing in the mosquito midgut, leading to reduced parasite diversity among passively detected, symptomatic malaria cases [24, 27]. This decline in diversity is also accompanied by a decline in the complexity of infection, as there are fewer unique parasites circulating in population [24, 27]. However, parasite diversity among passively detected, symptomatic infections did not decline in southern Zambia, and the complexity of infection was low. As malaria transmission was consistently low between the 2012 and 2015 seasons, the sustained, high parasite diversity may be evidence of imported, locally transmitted parasites among a population susceptible to clinical malaria.

The molecular barcode was used in Senegal to determine the clonal and epidemic expansion of passively detected clinical *P. falciparum* infections [24]. After enhanced deployment of ITNs and use of RDTs and ACTs between 2005 and 2011, reductions in parasite genetic diversity and complexity were observed [24]. Between 2006 and 2013, the molecular barcode was used to determine the decline in malaria transmission among passively detected infections, with re-introduction in 2012 [27]. In Malawi, the molecular barcode was used to determine differences in the complexity of infection between severe clinical malaria cases and cerebral malaria cases among children younger than 5 years of age [25]. Children with cerebral malaria had less complex infections than those with severe malaria [25]. To the authors' knowledge, this is the first time the molecular barcode was used to determine the genetic variation between actively and passively detected infections.

Passively detected cases had fewer complex infections, with a low proportion of polyclonal infections, consistent with recent infection with single parasite clones. In contrast, actively detected cases had more complex infections, with a high proportion of polyclonal infections. Between the 2008 and 2009-2010 transmission seasons there was an indication of a non-statistically significant, decreasing trend in the proportion of polyclonal infections; however, this proportion subsequently increased and remained high, indicating that the actively detected population of largely asymptomatic and sub-patent infections was carrying many different parasite clones. While transmission declined, these individuals may be harboring parasites that were acquired over time and maintained at low levels of parasitemia. Chronically infections such as these have been shown to persist for up to a decade [28-31].

Parasite genetic diversity remained relatively high and constant for passively detected cases throughout the observed malaria transmission seasons. This finding, along with the phylogenetic separation from actively detected cases and the paucity of

polyclonal infections, suggests these passively detected cases likely represent recent infections with parasites from outside the study area. In contrast, parasite genetic diversity was lower among actively detected cases, consistent with a chronically infected population. The decreasing parasite diversity among the actively detected cases is consistent with loss of parasites from this chronically infected reservoir as malaria transmission declined over the study period.

There were four major limitations of these analyses. The first was that passive and active case detection did not overlap temporally except for one malaria transmission season. However, given the absence of seasonal phylogenetic clustering for both passively and actively detected cases, the combined phylogenetic tree is likely an accurate representation of the phylogenetic relationship between the two parasite populations. The second was that we identified a high number of mixed infections from which single haplotypes were difficult to resolve. To account for this, a third allele was used to indicate mixed infections in calculating the phylogenetic relatedness and mixed infections were accounted for when calculating genetic divergence. [24, 26]. The third was that the sample size was small, although this is to be expected in a pre-elimination setting. The fourth was the low level of parasitemia. Pre-amplification of parasite DNA was performed to increase the molecular barcode yield, potentially introducing bias in the allele frequency. However, pre-amplification sites were barcode specific to reduce amplification in non-specific parasite DNA, and all samples were pre-amplified regardless of individual parasitemia [21]. After pre-amplification, assays with greater than 3 failures were treated as missing data, however this missing data may have been informative as these samples had very low parasitemia. This limitation is expected in all pre-elimination settings where the parasite prevalence is low.

Conclusions

Distinct parasite populations were found among infected individuals identified through active and passive surveillance, suggesting the asymptomatic reservoir did not contribute substantially to ongoing transmission. As parasite prevalence and diversity within these individuals declined over time, efforts to detect and treat the chronically infected reservoir through reactive case detection or mass drug administration may not be necessary to eliminate malaria in this setting.

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Table 3.1: Demographic characteristics of passively and actively detected individuals infected with *Plasmodium falciparum* by malaria transmission season

	Overall	***2008**	**2008-2009**	**2009-2010**	**2010-2011**	**2011-2012**	**2012-2013**	**2013-2014**	**2014-2015**
Passively detected cases									
Number	46	-	-	-	-	-	12	25	9
Age in years (median [IQR])	13 [7-30]	-	-	-	-	-	11 [7-30]	18 [9-32]	11 [7-11]
Male (%)	64.4	-	-	-	-	-	63.64	60.00	77.78
Actively detected cases									
Number	72	15	19	13	12	7	6		
Age in years (median [IQR])	14 [11-21]	14 [9-18]	13 [11-18]	17 [11-20]	14.5 [10.5-24]	25 [13-31]	12.5 [6-47]		
Male (%)	55.6	66.7	52.6	53.9	50.0	57.1	50.0		
Symptomatic (%)	34.7	60.0	15.8	23.1	16.7	57.1	66.7		
Microscopy positive (%)	11.1	35.0	7.1	11.1	0.0	25.0	0.0		
RDT positive (%)	20.8	20.0	31.6	23.1	8.3	14.3	16.7		

* The 2008 season was truncated as active case detection began in February 2008

Table 3.2: Percent of polyclonal infections and genetic divergence for passively and actively detected individuals infected with *Plasmodium falciparum* by malaria transmission season

	Number	**Percent polyclonal (95% Confidence interval)**	**Percent genetic divergence (95% Confidence interval)**
Actively detected cases			
*2008	15	87 (60-98)	22 (17-28)
2008-2009	19	79 (54-94)	29 (26-32)
2009-2010	13	69 (39-91)	24 (20-28)
2010-2011	12	92 (62-100)	15 (10-19)
2011-2012	7	86 (42-100)	18 (11-26)
2012-2014	6	100 (54-100)	10 (2-17)
Passively detected cases			
2012-2013	12	0 (0-26)	35 (27-42)
2013-2014	25	12 (3-31)	38 (34-42)
2014-2015	9	0 (0-34)	31 (19-43)

* The 2008 season was truncated as active case detection began in February 2008

Figure 3.1: Phylogenetic trees of A: passively detected infections and B: actively detected infections, by malaria transmission season

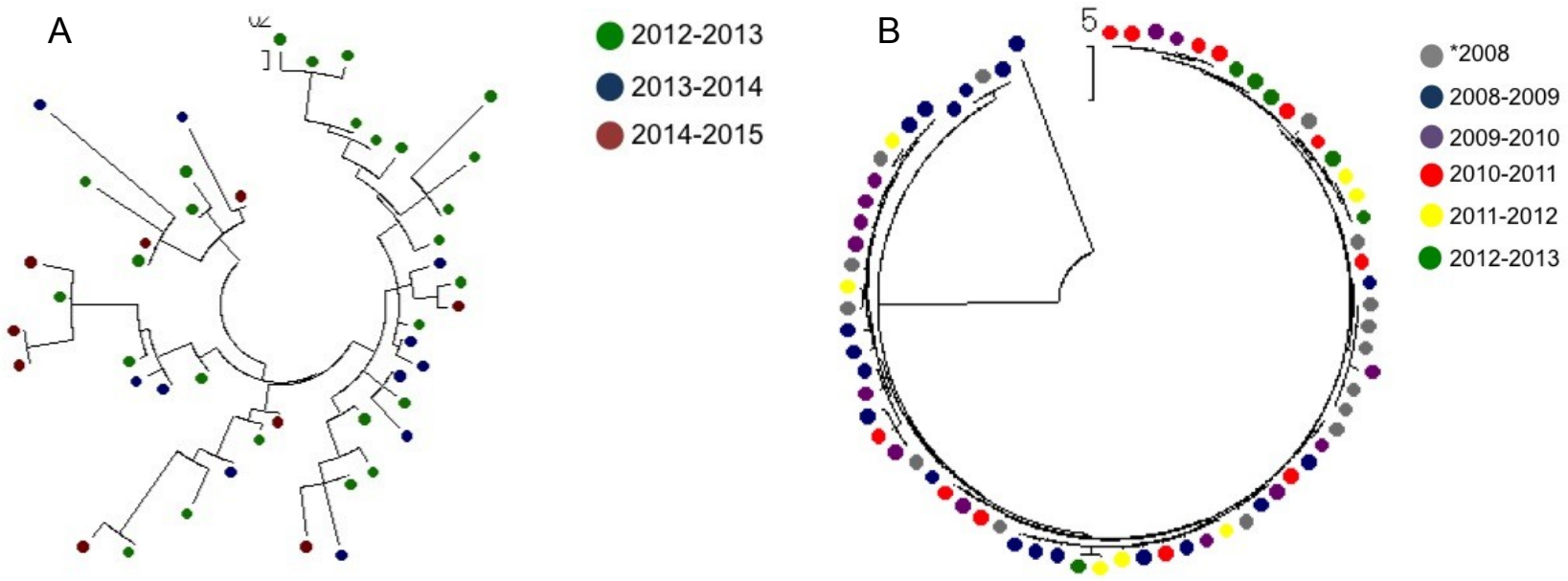


Figure 3.2: Phylogenetic tree of actively and passively detected infections

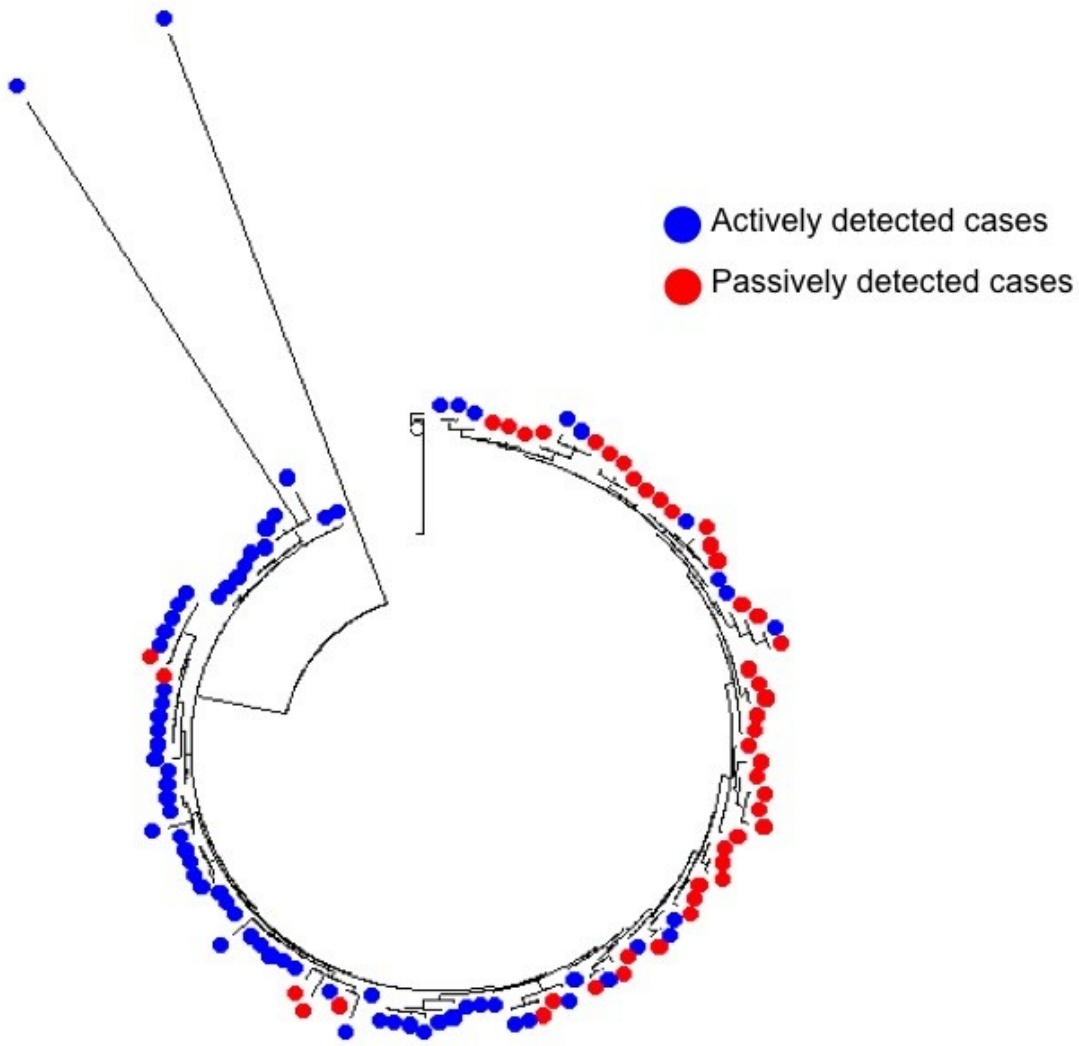


Figure 3.3: Proportion of polyclonal infections by season for A: passively detected infections and B: actively detected infections

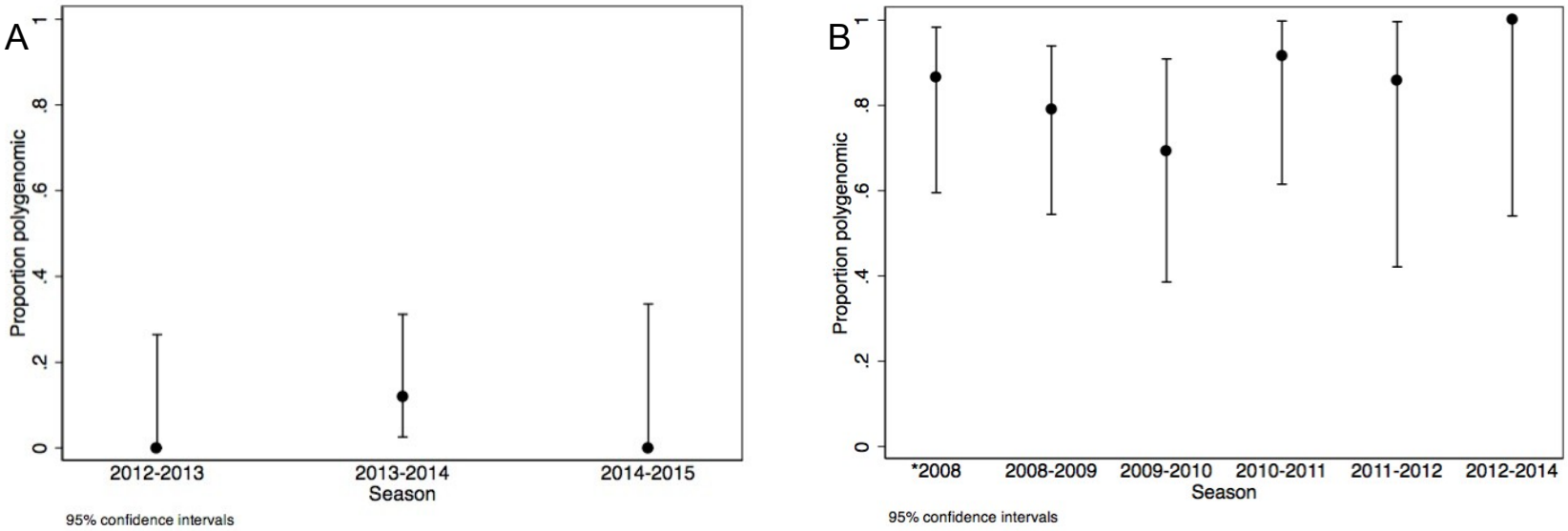
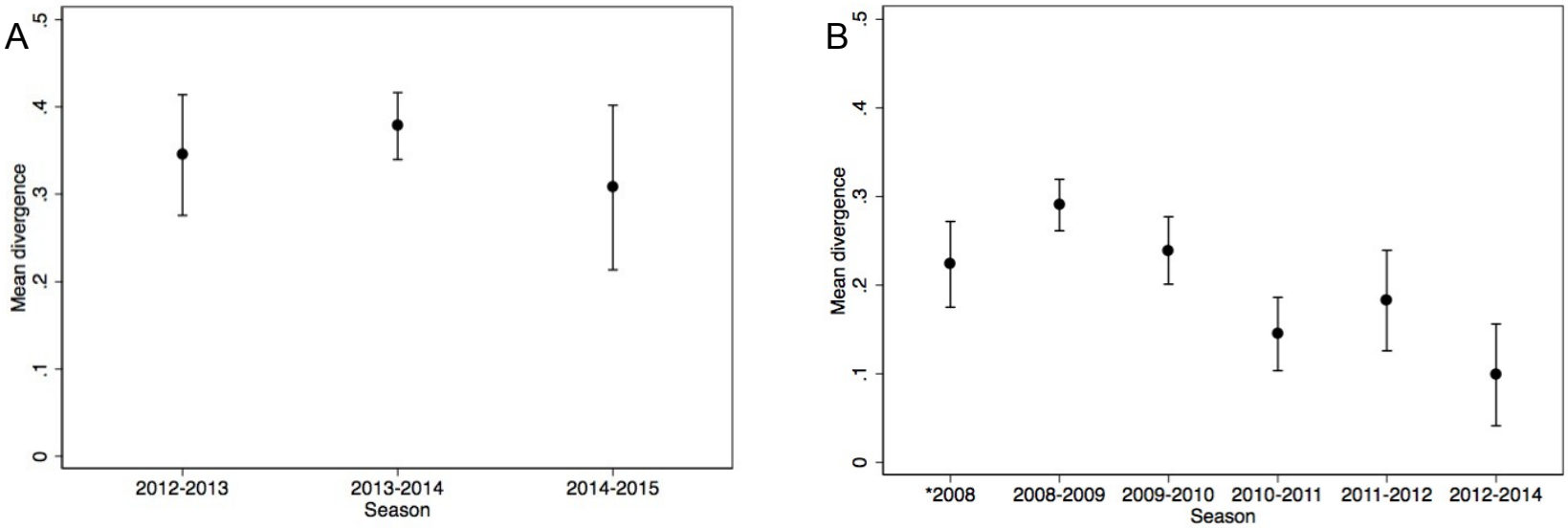


Figure 3.4: Mean genetic divergence by season for A: passively detected infections and B: actively detected infections



Appendix

The modified 'SNP π' used to account for missing data and mixed allele calls was calculated as follows:

1. The most common barcode for each season was determined based on allelic frequency for each of the 24 sites
2. For each sample, the maximum agreement that could be achieved was calculated for all non-missing 24 sites. SNP calls of only allele 1 or allele 2 were given a score of 1 and mixed calls were given a score of 0.5.
3. For each sample, the maximum agreement for all non-missing alleles was summed to give a total potential agreement score. For example, a sample with no missing data and no mixed calls would have a total potential agreement score of 24.
4. The agreement between individual samples and the most common barcode was calculated. For each of the 24 SNPs, each sample received a score of 1 if the allele was the same as the most common barcode and a score of zero otherwise. When the most common allele at a given SNP was a mixed call, the barcode would receive a score of 0.5 for that SNP. If a barcode had a mixed allele assignment at a SNP for which the most common barcode was not mixed, the sample also received a score of 0.5 for that SNP.
5. The agreement across all non-missing sites was summed to create a total observed agreement score.
6. The proportion of agreement for each sample was calculated as the total observed agreement score divided by the total potential agreement score.
7. The proportion of divergence was calculated as 1 minus the percent agreement for each sample.

8. For each season, the mean divergence was calculated to allow for comparisons across seasons.

4. Operational challenges during early implementation of reactive screen-and-treat and implications of simulated reactive case detection strategies for malaria elimination in a region of low transmission in southern Zambia

Authors: Kelly M. Searle, ScM[1,2], Harry Hamapumbu[3], Jailos Lubinda, MSc[3], Timothy M. Shields, MA[1,2], Jessie Pinchoff, PhD MPH[1,2], Tamaki Kobayashi, PhD MPH[1,2], Jennifer C. Stevenson, PhD[2,3], Daniel J. Bridges, PhD[4], David A. Larsen, PhD MPH[4,5], Philip E. Thuma, MD[2,3], and William J. Moss, MD MPH[1,2] for the Southern Africa International Centers of Excellence for Malaria Research

[1]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[2]Johns Hopkins Malaria Research Institute, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[3]Macha Research Trust, Choma District, Zambia

[4]Akros, Cresta Golfview Grounds, Great East Road, Lusaka, Zambia

[5]Department of Public Health, Food Studies and Nutrition, Syracuse University, Syracuse, NY, USA

Abstract

Background: As malaria transmission declines in many regions of sub-Saharan Africa, interventions that target the chronically infected, asymptomatic reservoir are being deployed with the goals of improving surveillance and interrupting transmission. Reactive case detection strategies, in which clinical malaria cases are followed-up at their home where other household residents and neighbors are screened and treated for malaria, are currently being used in Zambia as part of the malaria elimination program. In areas of Southern Province, Zambia, community health workers were trained to conduct home follow-up visits for individuals who tested positive for malaria at a health care facility and to screen all residents and neighbors within 140 meters with a rapid diagnostic test (RDT) and treat those who tested positive.

Methods: The operational challenges during the early stages of implementing a reactive screen-and-treat program in Macha, southern Zambia were assessed using rural health center records, ground truth evaluation of community health worker performance, and data from serial cross-sectional and longitudinal cohorts in the area. The proportion of the total infected population treated was estimated by constructing reactive screen-and-treat and focal drug administration cascades.

Results: In the first six-months of implementing a reactive screen-and-treat strategy, community health workers followed-up 32% of eligible index cases. The main reason reported for low follow-up was lack of RDTs. When index cases were followed-up, 66% of residents were at home in the index households and 58% of neighbors were at home within a 140-meter radius. Forty-one neighboring households of 26 index households were screened, with only 13 (32%) of these households actually falling within the 140-meter screening radius. In the evaluation, the parasite prevalence by RDT was 22% in index households and 5% in neighboring households. In a simulation model with complete follow-up, only 22% of the total infected population would be detected with

reactive screen-and-treat using the currently available RDT, but the proportion treated increased to 57% with reactive focal drug administration, which does not rely on a diagnostic test.

Conclusions: In the initial stages of implementing reactive screen-and-treat, operational challenges impede efficiency, including lack of RDTs at the level of the community health worker, low coverage of the strategy, and difficulty accurately identifying the screening radius. The poor sensitivity of the RDT to detect low-level infections in this low transmission setting was the largest impediment of reactive screen-and-treat. While this barrier is removed with focal drug administration, high levels of follow-up will be needed to treat a high proportion of infected individuals. Reactive screen-and-treat will likely not be sufficient to eliminate malaria and reactive focal drug administration, particularly targeting residents of the index case household, may be more efficient.

Background

Substantial reductions in the burden of malaria have been documented in parts of sub-Saharan Africa and malaria elimination goals have been proposed at regional, national, and subnational levels [1-3]. As areas transition from malaria control to elimination, strategies have been developed to target the population of chronically-infected individuals who are asymptomatic yet can contribute to transmission as an infectious reservoir [4-9]. In malaria endemic areas, individuals develop clinical immunity to disease after repeated exposure to parasites and can remain infectious despite the absence of symptoms or develop low-grade symptoms that would not prompt them to seek care [10, 11]. As malaria transmission declines, the proportion of the total infected population including asymptomatic, chronically-infected individuals with low parasite densities initially increases [12-15], until clinical immunity wanes and more infected individuals develop symptoms. These individuals constitute an asymptomatic reservoir that is less infectious than symptomatically infected individuals due to the low parasite and gametocyte densities, but is capable of transmitting parasites in areas with competent vectors [12, 14, 15].

Several strategies have been developed to identify and treat asymptomatic, chronically infected individuals. Mass drug administration treats populations or high-risk groups based on the fact that current point-of-care diagnostic tests are not sufficiently sensitive to identify individuals with low levels of parasitemia [16, 17]. Active case detection, in contrast, involves screening individuals for malaria infection with rapid diagnostic tests (RDTs) within a defined geographic area (“hot spots”) or high-risk populations (“hot pops”) at regular intervals and treating those who test positive. This method can be used to identify the asymptomatic reservoir by testing and treating individuals who would not be detected by passive case detection [8]. Active case detection, as well as focal and mass drug administration, aims to eliminate parasites

from chronically-infected individuals, thus facilitating the interruption of local transmission [18]. The World Health Organization (WHO) recommends that areas of moderate to low malaria transmission implement active case detection as part of national malaria control and elimination programs [8].

One specific method of active case detection is reactive case detection, which leverages the underlying spatial and temporal clustering of malaria infections [19-21] and can comprise reactive screen-and-treat or focal drug administration. When an infected individual is diagnosed at a health care facility, that person is recorded as an index case triggering reactive case detection. With a reactive screen-and-treat strategy, other residents in the home of the index case are screened using RDTs and treated if positive. Residents of neighboring households may also be screened and treated within a specified distance from the index household [6, 22, 23]. With a focal drug administration strategy, individuals residing in the index case household and possibly neighboring households are treated with antimalarials without testing. The advantage of focal drug administration is that infections, which may have been missed with a low-sensitivity diagnostic, are treated. The assumption underlying these reactive strategies is that temporally stable transmission hotspots exist where chronically-infected individuals reside and ecologic factors support and perpetuate local malaria transmission [19, 24, 25]. Identifying these hotspots and treating chronically-infected individuals has the potential to interrupt transmission and facilitate the transition from malaria control to elimination [20, 23]. Thus, the objective of reactive case detection is to detect areas of recent local transmission using symptomatic index cases as sentinels for malaria transmission hotspots, which are maintained by chronically-infected individuals.

Methods of implementing reactive case detection have not been fully operationalized and evaluated. Multiple challenges can impact the effectiveness and efficiency of reactive case detection. For example, the optimal screening radius around

index cases has not been determined and will vary depending on demographic, ecologic and epidemiologic characteristics [22]. In rural areas, the WHO recommended that programs cover areas as large as the flight range of *Anopheles* mosquitoes, which can be up to 1-2 km [8]. A 1 km screening radius is used in Tanzania, a 300-500 meter screening radius is used in rural Senegal, and a 140-meter screening radius is used in rural Zambia [6, 9, 22]. Screening all index households and their neighbors can be financially costly and logistically challenging. Even with well-developed protocols, identifying neighboring households within a specified radius from an index household can be difficult in practice. However, for reactive case detection to be an effective malaria elimination strategy, high coverage levels are needed [14]. Additionally, reactive screen-and-treat strategies rely on RDTs to identify infected individuals and the availability and sensitivity of these diagnostics will impact how well a reactive case detection program performs. Given these challenges, it is important to monitor reactive case detection programs in their initial stages as they are implemented to determine how they perform operationally and identify how they can be improved.

The initial challenges faced during a recently implemented reactive screen-and-treat program in southern Zambia and its ability to identify infected individuals were evaluated. The impact of operational challenges such as RDT availability, follow-up and coverage, and the sensitivity of RDTs on the potential efficiency of reactive case detection were evaluated using rural health center (RHC) records, assessment of community health worker (CHW) performance, and data from serial cross-sectional and longitudinal cohorts from a population-based study of malaria epidemiology in southern Zambia to construct a simulated reactive case detection cascade.

Methods

Study site and population

The study was conducted in Kalomo, Namwala, and Choma Districts in Southern Province, Zambia, in the catchment area of Macha Hospital in Choma District. Here, a single rainy season lasts from November through April, followed by a cool dry season from April until August and a hot dry season through November. Malaria transmission peaks during the rainy season [26]. The primary malaria vector in the Macha area is *Anopheles arabiensis* [26, 27]. The hospital catchment area consists of villages comprised of small, scattered homesteads. The prevalence of malaria declined in this area over the past decade, from 13.7% in 2006 to less than 1% in 2013 [28]. Artemisinin combination therapy (ACTs) with artemether-lumefantrine was introduced as first-line anti-malarial therapy in Zambia in 2002 [29] and into the study area in 2004. In Zambia, long lasting insecticide treated nets (LLINs) are distributed through antenatal care clinics and additional mass distribution campaigns [30]. LLINs were widely distributed in the study area in 2007 [31], more than 11,000 LLINs were distributed from nine health posts in the catchment area of Macha Hospital in June 2012, with additional LLINs distributed in 2014 according to the Office of the Macha Hospital Environmental Health Technician.

Reactive screen-and-treat in Southern Province, Zambia

The Government of Zambia created a stepped sequence of interventions to achieve malaria elimination [32-34]. Designated as Step A through Step E, these interventions are to be implemented in succession depending on the malaria parasite prevalence and case burden at health facilities [33, 34]. Step D consists of training volunteer CHWs in malaria diagnostics and treatment to expand access to care into the community. CHWs provide this service passively, for symptomatic individuals seeking care, actively and through reactive case detection. Step D is implemented in low transmission settings e.g. when the parasite prevalence is approximately 1% and an average of 10 or fewer malaria cases present to a healthcare facility per week [33]. Step

D activities were implemented through a phased roll-out in selected districts in Southern Province, Zambia with the goals of improving surveillance and creating malaria free zones [9, 32].

When an individual seeks care at a health care facility (hospital, rural health center or rural health post) and tests positive for malaria by RDT, their eligibility for follow-up with reactive screen-and-treat is determined. The CHWs in rural Southern Province, based at rural health posts (RHPs) used travel history at the time of this evaluation, to determine eligibility and excluded from reactive screen-and-treat cases of malaria with a travel history as presumed to be imported. Travel was defined as staying overnight in a place outside their home district within the previous month. RDT positive individuals who had not traveled within the past month were eligible for reactive screen-and-treat. Eligible index cases were to be followed-up in their household within one week of diagnosis. CHWs were trained to visit the households of eligible index cases and neighboring households within 140 meters of an index case, screen all residents with an RDT and treat everyone who tested positive [9].

Record review of reactive screen-and-treat

During the low transmission season from July 2014 to September 2014, a study team from Macha Research Trust visited 10 RHCs in Kalomo, Choma, and Namwala Districts of Southern Province, Zambia. During these visits, the team reviewed and abstracted data on reactive screen-and-treat from January 2014 through June 2014 (within a few months of Step D being implemented) in 20 RHPs serving the catchment areas of the 10 RHCs. RHPs are the lowest level of stationary health care and are staffed by volunteer CHWs. The numbers of RDTs received by the RHP, tests performed, RDT positive malaria cases identified, malaria cases eligible for reactive

screen-and-treat, and the numbers of eligible malaria cases followed-up were recorded for each month.

Ground truth evaluation of reactive screen-and-treat

To ground truth reactive screen-and-treat performance, study staff visited 10 RHPs associated with 7 parent RHCs from July to September 2014, and identified index cases with RDT-confirmed malaria that triggered reactive screen-and-treat. Study staff randomly selected 26 index case households that were screened during Step D activities from January 2014 through June 2014 for ground truth evaluation. The study staff and CHW visited the selected index case households and neighboring households determined to be eligible for screening by the CHW. Household coordinates, the number of residents within each household, the number of residents tested by the CHW, the number of RDT positive residents, the number of residents treated for malaria, and the distance from the index case household to neighboring households collected using a GPS enabled device, were recorded. The time from presentation of the index case to reactive screen-and-treat was calculated when dates were available.

Data on age and sex of individuals, and whether or not eligible residents were screened during the reactive screen-and-treat were collected retrospectively from RHP records of individuals residing in index and neighboring households during the follow-ups for 7 RHPs under 6 parent RHCs.

Construction of simulated reactive case detection cascades

The proportion of infected individuals identified and treated through reactive screen-and-treat in the study area was modeled through a series of steps. First, data collected through repeated cross-sectional surveys were used to estimate the population size of the study area and the number of residents in index households and neighboring

households. Details of the model were previously described [23]. In brief, all households in the study area were enumerated from satellite imagery. Using data from population-based, serial cross-sectional surveys, a prediction model was constructed to estimate the number of residents per household based on the geographic location of the household and ecological features. Using the same data, a prediction model was used to estimate the total number of PCR-positive malaria infections (infections positive for *P. falciparum* by polymerase chain reaction) in the study area. Second, the proportion of symptomatic PCR-positive malaria infections was calculated using data from the population-based, serial cross-sectional surveys. Symptomatic infections were those with a documented fever (tympanic temperature ≥ 38°C) or self-reported fever in the past 48 hours. This proportion was applied to the number of PCR-positive infections in the study area. Third, the proportion of symptomatic PCR-positive infected individuals who sought care from a RHP, RHC, or hospital the last time they had a fever was then estimated using data from the population-based serial cross-sectional surveys. This proportion was applied to the number of symptomatic PCR-positive infected individuals in the study area. Fourth, a sensitivity of 95% was used to determine how many of these symptomatic, PCR-positive infected individuals would be RDT positive when presenting to a health-care facility with symptoms of uncomplicated malaria [35]. These individuals would be identified by passive case detection. Fifth, the record review and ground-truth evaluation data were used to determine the median number of neighboring households per index households that were screened. A median of three neighboring households were screened for each index case and this estimate was used as the number of neighboring households screened through reactive screen-and-treat in the simulation. The record review was also used to determine the RDT positivity in index and neighboring households. Sixth, the sensitivity of the RDT to detect asymptomatic infections in the study area was determined to be 40% in index households and 23% in

neighboring households when compared to PCR, based on a preliminary analyses on comparing RDT results and PCR results on houses screened in this area [Kobayashi, unpublished data]. These sensitivities were used to determine the number of infected individuals in index and neighboring households, based on the RDT positivity rate. The inverse of the RDT sensitivities for index (1/0.40) and neighboring households (1/0.23) was multiplied by the number of RDT positive infections to determine the total number of infections. Last, reactive screen-and-treat and reactive focal drug administration cascades were constructed.

The same model was repeated using observed coverage levels. The observed follow-up of index cases, and the percent of individuals residing in index households and neighboring households at home when visited were used to determine the total infected population that would have been treated under observed coverage.

The sensitivity of the model developed to create the reactive case detection cascades under varying RDT prevalence in index and neighboring households, RDT sensitivity, and level of clinical immunity in the population was evaluated. These three aspects were varied to determine how reactive case detection performed under different conditions. The results of this analysis are presented in the supplemental section (Figures S4.1-S4.3).

Results

Record review of reactive screen-and-treat

The reactive screen-and-treat program started in the study area in May 2013. Records reviewed at the 10 RHCs indicated that 411 malaria cases were passively identified by RDT from January to June 2014 at the 20 RHPs. Of these, 21 cases were excluded by the CHW and 394 were considered eligible for follow-up with reactive screen-and-treat. Of those eligible, 32% (n=126) were followed-up. The primary reason

many households were not followed-up given by the CHW was not having a sufficient number of RDTs.

The expected seasonal pattern of malaria cases was observed, with the number of cases increasing after January and peaking in April (Figure 4.1). As the number of malaria cases increased, the difference between the number of eligible cases and the number of cases followed-up increased (Figure 4.1). When RHPs were stratified by malaria burden (high malaria burden were those with 20 or more eligible cases in a month), high burden RHPs showed a much larger discrepancy between the number of eligible cases and the number of cases followed-up (Figure 4.1), as expected when the burden of reactive case detection exceeded capacity. The largest difference for both high and low burden RHPs occurred in April when the number of malaria cases peaked (Figure 4.1). Over half of the RHPs (n=11) reported at least one month without sufficient RDTs to follow-up eligible index cases with reactive screen-and-treat. Eight of these RHPs reported at least one month without sufficient RDTs to perform passive case detection. Overall, the number of RHPs reporting a lack of RDTs per month increased from January to June. Low burden RHPs reported more months with a lack of RDTs than high burden RHPs. During this time period the parent RHCs were not reporting stock-outs or limited RDTs, suggesting delays or interruptions in distributing sufficient RDTs to the CHWs during the initial stages of implementing the program.

Ground truth evaluation of reactive screen-and-treat

Twenty-six index case households triggering reactive screen-and-treat by a CHW were randomly selected within the catchment areas of 10 RHPs under 7 parent RHCs. The CHW registers identified 63 neighboring households associated with the 26 index case households as eligible for reactive screen-and-treat (89 total households) (Table 4.1). Study staff collected coordinates and household demographics for all 26 index case

households and 89% (n=56) of the 63 neighboring households, as no one was home at 7 households (Figure 4.2). Twenty-two neighboring households were not screened by CHWs (35% of 63), of which 12 (55%) were not screened due to a lack of RDTs, representing 19% of the 63 eligible households. Of the 41 neighboring households that were screened by the CHW, only 13 (32%) were within 140 meters of an index case household (Table 4.1). Study staff identified 21 households within 140 meters of an index household (8 more than the CHWs) and data were collected from 18 (86%) of these households, as no one was home at 3 households (Table 4.1). The percentage of eligible neighboring households within 140 meters of an index household that was screened by the CHWs was 62% (13 of 21) (Table 4.1). The median number of households screened per index case households was three (IQR 1, 3; minimum = 1; maximum = 8).

From the 26 index cases selected for evaluation, 705 individuals residing in 82 households were eligible for reactive screen-and-treat, 261 in index households and 444 in neighboring households (Table 4.1). Overall, 428 individuals (61%) were recorded to have been screened in the CHW registers. In index case households, 66% of the residents were reported as screened compared with 58% of residents in neighboring households ($p = 0.04$) (Table 4.1). During the evaluation 165 eligible individuals were identified in the 18 neighboring households that the study staff collected data from within 140 meters of an index household and 100 (61%) were screened by the CHW (Table 4.1). The parasite prevalence by RDT was 22% among residents of index case households and 5% among residents of neighboring households (Table 4.1).

The median time between when an index case presented to a health care facility and the reactive screen-and-treat was 3 days (IQR=2-5.5; min=1; max=12). The median distance from the index household to the neighboring households screened was 194 meters (IQR=117-303; min=36; max=530 meters). Thirty-two percent of all neighboring

households screened by the CHWs were within 140 meters of the index case household, suggesting the CHWs had difficulty delineating the 140-meter radius. Thirteen RDT positives were detected in index and neighboring households. Only one (7.7%) RDT positive individual detected was within 140 meters of the index case household; this increased to 92% (n=12) of RDT positive individuals with a screening distance of 250 meters (Table 4.2).

Retrospectively, demographic information was collected from 449 individuals eligible for screening by reactive screen-and-treat, 99 from index case households and 350 from neighboring households. No differences in screening by sex were observed overall (49.5% male, 50.7% female, p=0.21), or when stratified by household type (index: 41.6% male, 58.4% female, p=0.28; neighboring: 51.3% male, 48.7% female, p=0.32). Residents of index and neighboring households did not differ by age (index: median age=13.5 years IQR=7-23 years; neighboring: median age=14 years IQR=6-26 years, p=0.75). However, residents who were screened were younger than those who were not (screened: median age=13 years, IQR=6-25 years; not screened: median age=19 years, IQR=12-31 years, p<0.01).

Reactive case detection cascades

A flow diagram of the reactive screen-and-treat and focal drug administration cascade estimates and construction is presented in Figure 4.3. The total population of the study area was estimated to be 32,370 people and the *P. falciparum* parasite prevalence by PCR was estimated to be 2.9% (937 infections) based on the population level simulation model using serial cross-sectional survey data in the study area [23]. Based on data from the serial cross-sectional surveys, 23% (214 individuals) of the 937 PCR positive individuals were estimated to be symptomatic (i.e. febrile), with 36% (n=76) of these individuals estimated to have sought care at a health care facility during their

last febrile episode. Using an RDT sensitivity of 95% for uncomplicated malaria, 73 (95%) of these individuals would have been detected at a health care facility [35]. The proportion of RDT positive residents in the index case households and neighboring households were estimated based on the RDT prevalence observed during the RHC and RHP record reviews (22% in index households and 5% in neighboring households) to capture the local spatial dependence of malaria transmission.

Based on the eligibility criteria used by CHWs, in which persons with recent travel are excluded, 5% of RDT positive symptomatic cases were estimated to be ineligible for reactive screen-and-treat (Figures 4.4 and 4.5). Thus, 95% (n=69) of the RDT positive index cases were estimated to be eligible for reactive screen-and-treat. These cases represented 7% (69 of 937) of the total infected population (Figure 4.4A and 4.5A). Based on household residency figures, it was estimated that 5 residents per response would be screened reactively in each of these 69 index households, yielding 345 residents, with 22% (n=76) estimated as RDT positive per the RDT positivity of index households from the RHC and RHP performance evaluations (Figure 4.4A). Given the 40% sensitivity of the RDT in index households, it was estimated that there were 189 PCR positive infected individuals (20% of total infections) in index households (Figure 4.4A) [36]. With complete follow-up, in which all index case household residents were screened, 16% (73 symptomatic RDT positive index cases and 76 RDT positive secondary infections residing within the index case household) of all infected individuals would have been detected and treated (Figure 4.4A).

When neighboring houses were included in the model, 270 (29% of total infection) infected individuals were estimated to live in 207 neighboring households of 69 index case households (Figure 4.4B). Of these infected individuals, 62 (7% of total infection) were estimated to be RDT positive based on the 23% sensitivity of the RDT. Screening neighboring households of the index case household would increase the

percentage of the total infected population detected and treated from 16% to 22% under complete follow-up of all eligible index cases and neighboring households, with all residents at home and willing to be screened (Figure 4.4B).

Using the same data consisting of 69 index cases, 189 infected individuals in index households, and 270 infected individuals in neighboring households, reactive focal drug administration was modeled. In this analysis the sensitivity of the RDT for asymptomatic individuals did not apply as everyone in index and neighboring households is treated without testing. Under complete follow-up of all eligible index cases, 28% (n=258), of all infected individuals in the population would have been treated (Figure 4.4C). When neighboring households were included, 57% (n=531) of all infected individuals would have been treated (Figure 4.4D).

The same data and model were used to determine the impact on the total infected population treated under the coverage observed in the RHC evaluation and ground truth surveys. Follow-up of eligible index cases was estimated as 32%. In index households 66% of the residents were estimated as being at home during screening and in neighboring households, 58% of the residents were estimated as being home during the screening. Under observed coverage, screening and treating index case households would have detected and treated 9% of the total infected population (Figure 4.5A). When neighboring households were included in the model this increased to 11% of the total infected population that would be detected and treated (Figure 4.5B). The same observed coverage was used to model focal drug administration, where everyone at home is treated without testing during the visit. Under observed coverage of eligible index cases, 11% of all infected individuals in the population would have been treated (Figure 4.5C). This increased to 17% when neighboring households were included in the model (Figure 4.5D).

The cascades develop identified key areas that impact the efficiency of reactive case detection to decrease the pool of infected individuals that could be measured during real time planning and evaluation of programs. These included the sensitivity of the RDT in index and neighboring households and the malaria prevalence in index and neighboring households. Additionally, the impact of the proportion of infected individuals that were symptomatic and care seeking had a large impact on the efficiency of reactive case detection. These areas are displayed on Figure 4.6.

Discussion

The initial implementation of reactive screen-and-treat in this area of southern Zambia faced several operational challenges, as would be expected with a new program using volunteer CHWs to expand clinical services into the community. It was observed that CHWs were applying an exclusion criteria based on recent travel, which was not indicated in the reactive screen-and-treat protocol for rural Southern Province [9]. Approximately one third of eligible index case households resulted in reactive screen-and-treat and coverage decreased to one quarter among RHCs with a higher burden of malaria. This low coverage was likely due to two factors. First, the follow-up screening was logistically difficult for CHWs due to the high number of cases during the peak malaria season. Step D activities were designed to be implemented when the number of malaria cases is approximately 10 per week. Despite the low transmission in this setting, some RHCs reported more than 70 eligible cases per month during the peak transmission season. This overwhelmed the capacity of the CHWs to conduct reactive case detection. During peak transmission times the program would benefit from extra staff. The second challenge was insufficient RDTs as a consequence of the high number of cases and difficulty in anticipating the additional need of RDTs to conduct reactive case detection. Over 50% of CHWs reported having at least one month when reactive

screen-and-treat was not done due to lack of RDTs, and 40% of CHWs reported at least one month when not even passive malaria screening with RDTs could be performed. During this time period, the parent RHCs did not report stock-outs or limited RDTs. This implies that there were delays, interruptions, or failures by RHC staff in distributing sufficient RDTs to the CHWs during the initial stages of implementing the reactive case detection program. Following CHW trainings, a moderate stock of RDTs were provided to CHWs and RHC staff were notified that additional RDTs should be requested and released to CHWs to support Step D. Clearly to implement Step D activities, a reliable and ample supply of RDTs is necessary, however, the rapid seasonal changes (Figure 4.1) makes predicting the number of RDTs required challenging, and surge capacity may not be feasible. Over time, programs should improve their ability to predict need and maintain an adequate stock of RDTs at the level of the RHPs. However, the cost may be that RDTs are stockpiled at these facilities and, if unused, will expire.

When eligible index cases were followed-up, three main challenges were identified that hindered the ability to identify infected individuals through reactive screen-and-treat. First, only one half to two-thirds of residents were at home at the time of screening and residents not at home were older than those at home. Those not at home include school-age children and young adults, the age group comprising the chronically infected reservoir that reactive case detection aims to identify and treat [3]. This challenge could be overcome in response. Notifications could be made to let individuals know when the CHW would be visiting. Households could be re-visited to attempt to access those not able to be at home during the first visit. Second, while theoretically simple, identifying households within 140 meters (the distance of one and a half football fields) of an index case was difficult for the CHWs in practice. Sixty-eight percent of neighboring households screened by CHWs were outside the 140-meter radius. Some CHWs screened neighboring households over half a kilometer from an index case

household. While reactive screen-and-treat programs in other countries screen further from the index household, in this setting nearly all RDT positive individuals were within 250 meters of the index household [6, 22, 37]. This demonstrates not only the difficulty in identifying the appropriate screening radius, but also the burden of unnecessary screening given the lack of RDTs and logistical challenges when the case burden is high. Lastly, as is widely recognized, the low sensitivity of RDTs limits the ability to identify individuals with chronic infection and low-level parasitemia in areas approaching malaria elimination [14, 38, 39]. Even with complete follow-up, only 16% of infected individuals were estimated to be identified by screening all index households and 22% of infected individuals by screening neighboring households. While the infectiousness of very low-parasitemic individuals to mosquitoes is variable, a large portion of the malaria reservoir in this area would be not treated. Given the poor sensitivity of RDTs to detect low parasite levels in this low transmission setting, and the current lack of more sensitive field deployable diagnostics, reactive focal drug administration may be a more efficient use of resources. With complete coverage, nearly 60% of the total infected population in this setting would be treated through reactive focal drug administration. However, complete coverage will be logistically difficult, and many improvements in follow-up strategies and gauging neighboring household distances would need to be made.

A major limitation of this study was the short period of evaluation covering the rainy season, but not the dry season. The study period (January through June of 2014) only represents a 6-month window where the evaluation was implemented. In addition, this period reflects early program implementation. However, other reactive case detection programs can learn from this experience. These results highlight the need for monitoring and evaluation shortly after implementation to identify operational challenges and their potential impact on program performance and impact early on. Additional

analyses are on-going to confirm whether these trends can be reversed or whether reactive case detection should be suspended during the wet season.

A strength of the RHC survey is that demographic data on the number of residents not in the home at the time of screening were collected. The reactive screen-and-treat cascade used population-based survey data from the study area in Choma District. Information from Kalomo and Namwala Districts were not represented in these data; however, the people residing within the three districts are traditional subsistence farmers and are demographically similar. The model did not account for care seeking outside the government health facilities and likely overestimated the number of index cases detected. However, the objective for creating the cascades was to provide estimates of the proportion of infected individual that reactive screen-and-treat and focal drug administration would identify using multiple novel data sources.

Conclusions

A large-scale reactive case detection implementation in Southern Province, Zambia identifies and treats more individuals with malaria than passive case detection alone and can improve surveillance among the chronically infected population. However, several obstacles impede the efficiency of reactive case detection, including the high number of cases during peak months placing burden on current resources and staffing levels, low proportion of residents at home at the time of the screening, the difficulty in identifying households within the specified radius, and the low sensitivity of RDTs in this population. Reactive focal drug administration has the potential to address the latter issue by removing the need for a diagnostic test, although challenges with supply chain will need to be addressed to ensure larger commodities of drug can be accommodated. Irrespective of the inclusion or exclusion of a diagnostic, community sensitization and coordination to enable high target population coverage needs to be addressed. In

summary, with limited resources, coverage, and diagnostic tools, reactive screen-and-treat will likely not be sufficient to achieve malaria elimination in this setting. However, high coverage with reactive focal drug administration could be efficient at decreasing the reservoir of infection and should be considered as an alternative strategy.

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Table 4.1: Ground-truth evaluation of reactive screen-and-treat: household and individual characteristics

Household characteristic	**Index**	**Neighboring**	**Total**
Indicated eligible for RCD by CHW (%)	26 (100)	63 (100)	89 (100)
Identified by study staff (%)	26 (100)	56 (89)	82 (92)
Recorded in CHW register (%)	26 (100)	41(65)	67 75)
Within 140 meters of index household (%)	NA	21(33)	NA
Within 140 meters of index household in CHW register (%)	NA	13 (21)	NA
Resident characteristic			
Indicated eligible for RCD by CHW in 82 households with data (%)	261 (100)	444 (100)	705 (100)
Screened and recorded in CHW register (%)	171 (66)	257 (58)	428 (61)
Within 140 meters of index household (%)	NA	165 (37)	NA
Within 140 meters of index household in CHW register (%)	NA	100 (23)	NA
RDT positive (% of all RDTs)	37 (22)	13 (5)	50 (12)
RDT negative (% of all RDTs)	134 (78)	244 (95)	378 (88)

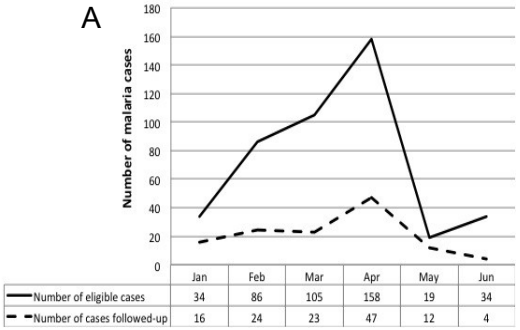
RCD = reactive case detection
CHW = community health worker
RDT = rapid diagnostic test

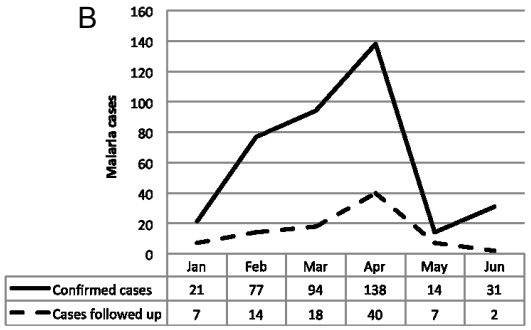
Table 4.2: Cumulative numbers of neighboring households, individuals, and RDT positive cases by distance from index households identified through the ground-truth evaluation of reactive screen-and-treat

	140 meters	**250 meters**	**300 meters**	**350 meters**	**400 meters**	**450 meters**	**500 meters**	**550 meters**
Households indicated as eligible by CHW (% indicated as eligible)	21 (33)	40 (64)	47 (75)	49 (78)	55 (87)	59 (93)	62 (98)	63 (100)
Households screened by CHW (% households screened)	13 (32)	28 (68)	30 (73)	32 (78)	35 (85)	37 (90)	40 (98)	41 (100)
Residents in screened households (% residents screened)	78 (30)	182 (71)	196 (76)	214 (83)	236 (92)	239 (93)	254 (99)	257 (100)
RDT positive cases in screened households (%RDT positive cases)	1 (8)	12 (92)	12 (92)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)

RDT = rapid diagnostic test
CHW = community health worker

Figure 4.1: Malaria cases reported and followed-up with reactive screen-and-treat by month from record review (A: all RHPs, B: high burden RHPs, and C: low burden RHPs)





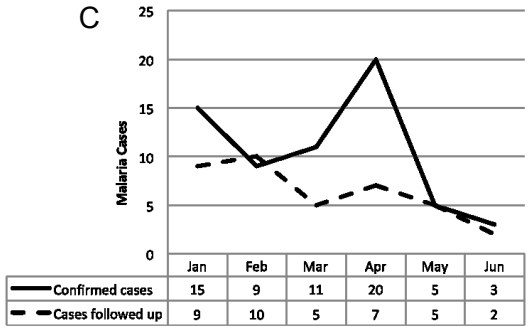


Figure 4.2: Index and neighboring households included in the ground-truth evaluation of reactive screen-and-treat

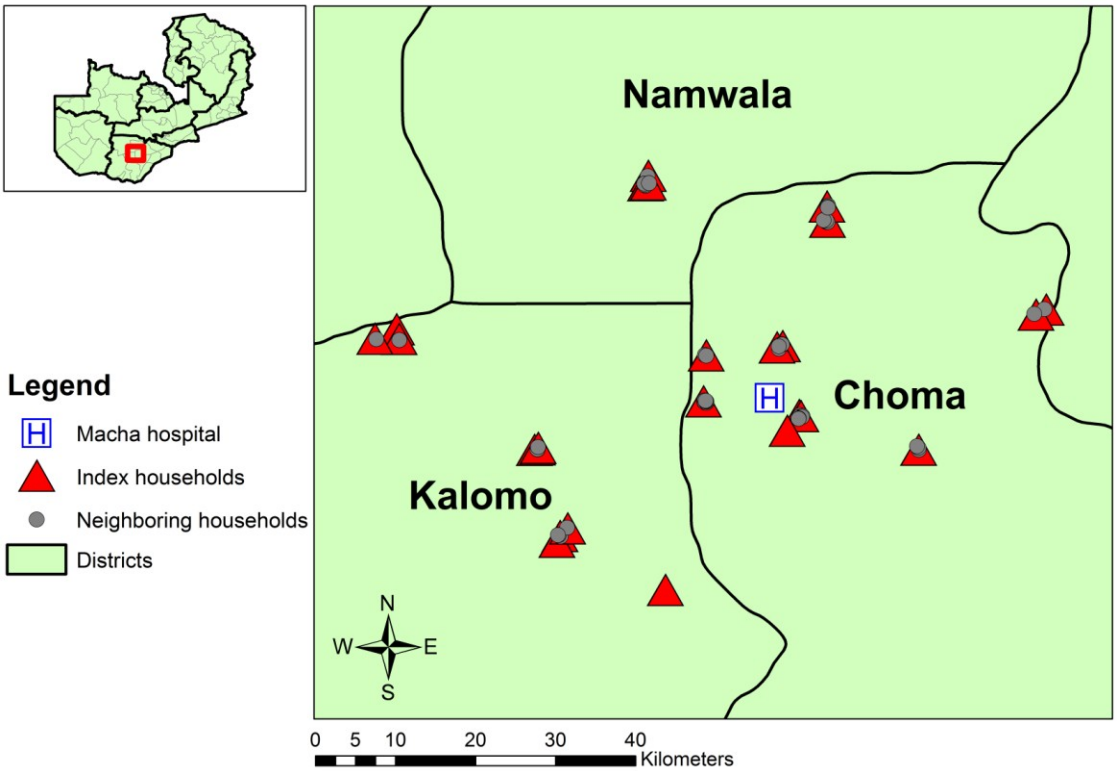
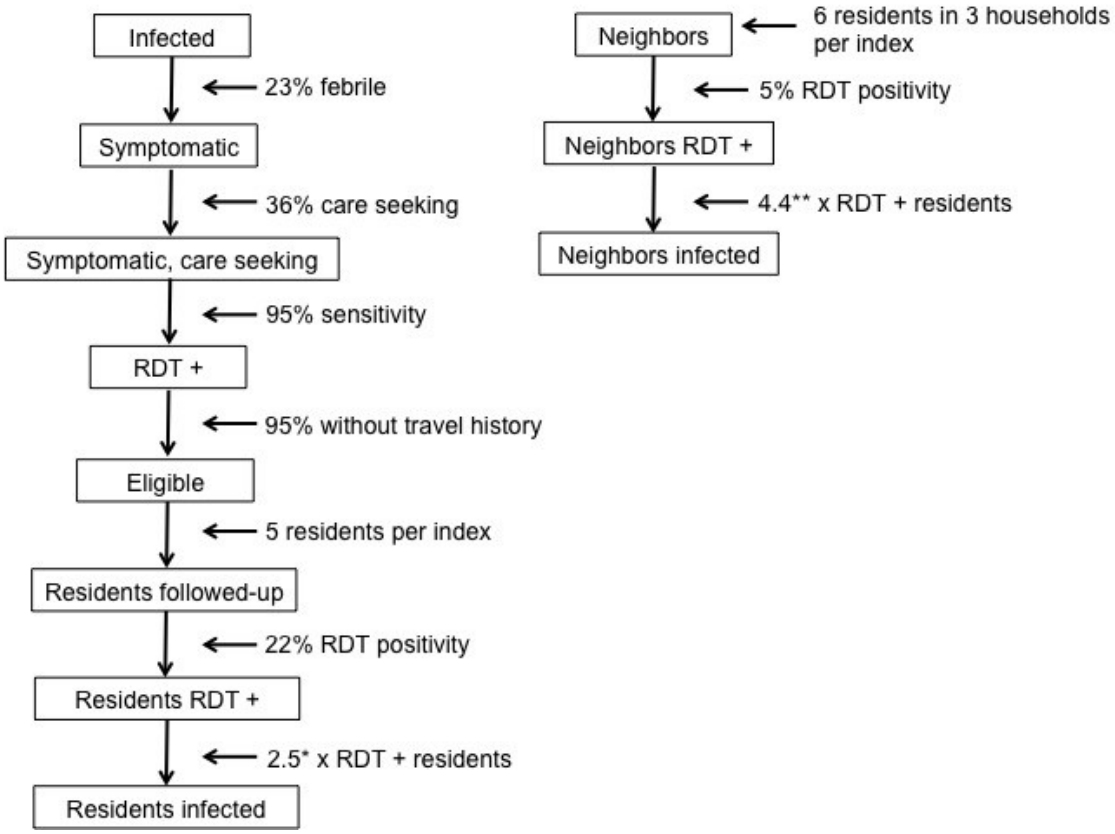


Figure 4.3: Reactive screen-and-treat flow diagram for complete coverage



* 2.5 was derived from the inverse of the sensitivity of the RDT to determine the number of residents infected (1/0.4)

**4.4 was derived from the inverse of the sensitivity of the RDT to determine the number of neighbors infected (1/.0.23)

Figure 4.4: Complete coverage cascade with A: reactive screen-and-treat in index households, B: reactive screen-and-treat in index and neighboring households, C: reactive focal drug administration in index housheolds, D reactive focal drug administration in index and neighboring households. The model assumes complete coverage of index and neighboring households

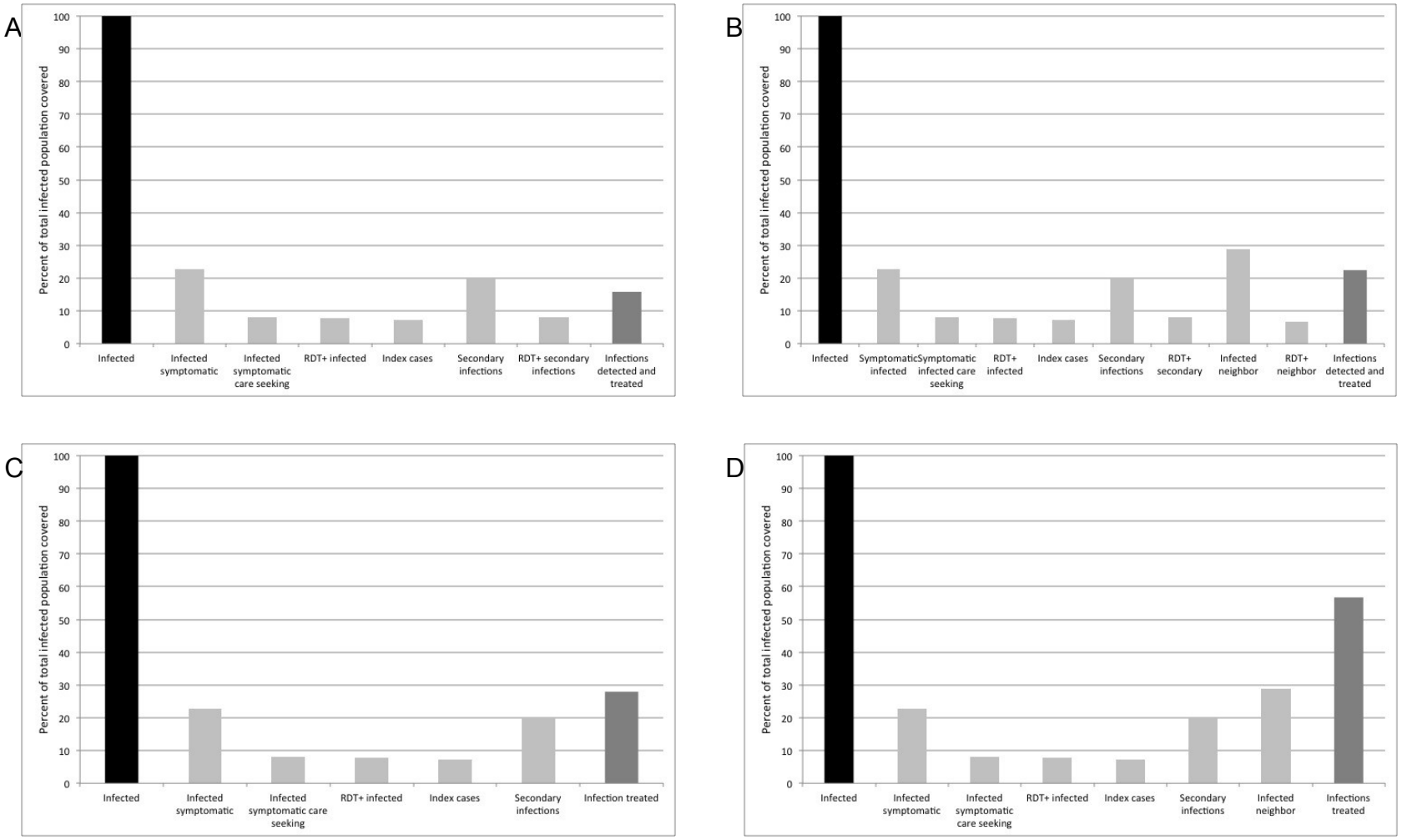


Figure 4.5: Observed coverage cascade with A: reactive screen-and-treat in index households, B: reactive screen-and-treat in index and neighboring households, C: reactive focal drug administration in index housheolds, D reactive focal drug administration in index and neighboring households. The model assumes complete coverage of index and neighboring household. The model assumes coverage of index and neighboring households that was observed during the record review and ground-truth survey

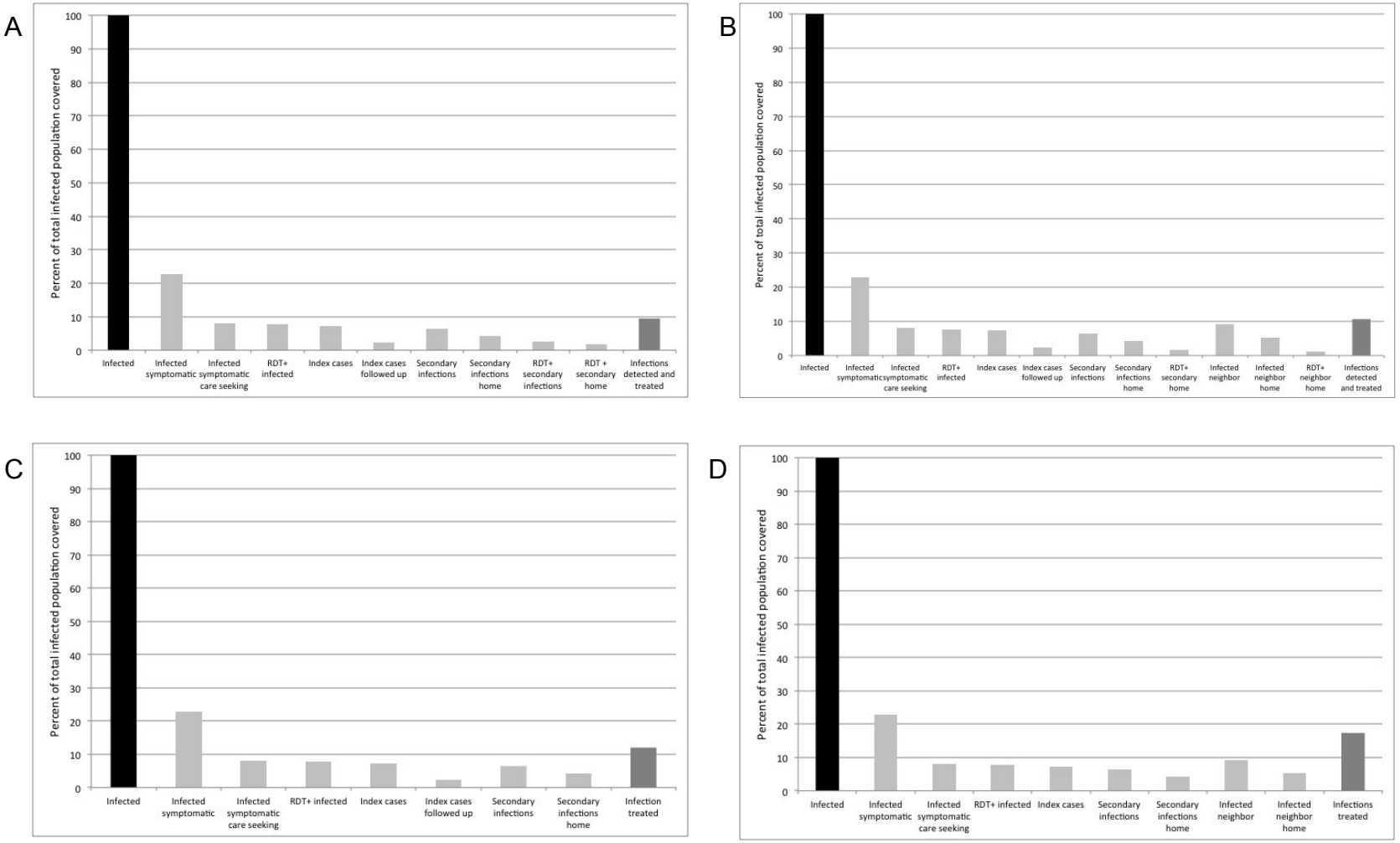


Figure 4.6: Key areas that impact the efficiency of reactive screen-and-treat on index and neighboring households

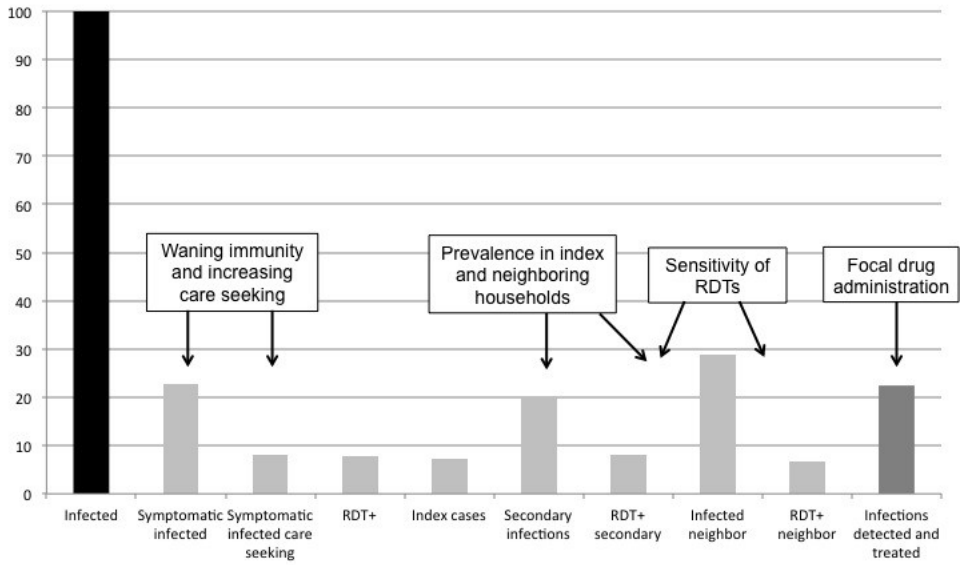


Figure S.4.1: Coverage cascades with malaria prevalence in index and neighboring households observed in preliminary data from Step D activities in Macha for A: reactive test-and-treat in index households; B: reactive focal-drug-administration in index households; C: reactive test-and-treat in index and neighboring households and; D: reactive focal-drug-administration in index and neighboring households

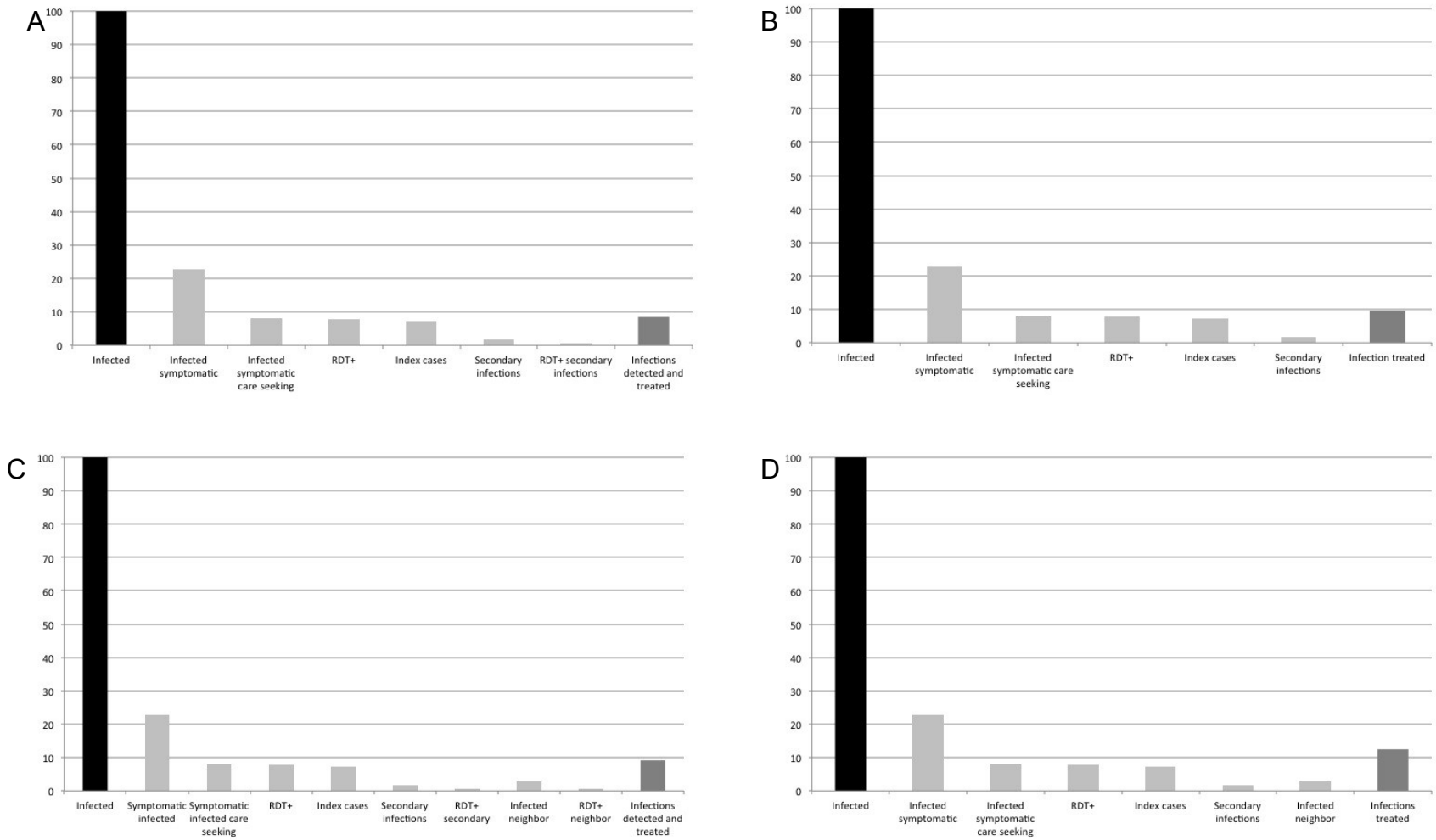


Figure S.4.2: Coverage cascades with malaria prevalence in index and neighboring households observed in the RHP evaluation with the sensitivity of the RDT doubled for index and reactive households for A: reactive test-and-treat in index households; B: reactive focal-drug-administration in index households; C: reactive test-and-treat in index and neighboring households and; D: reactive focal-drug-administration in index and neighboring household

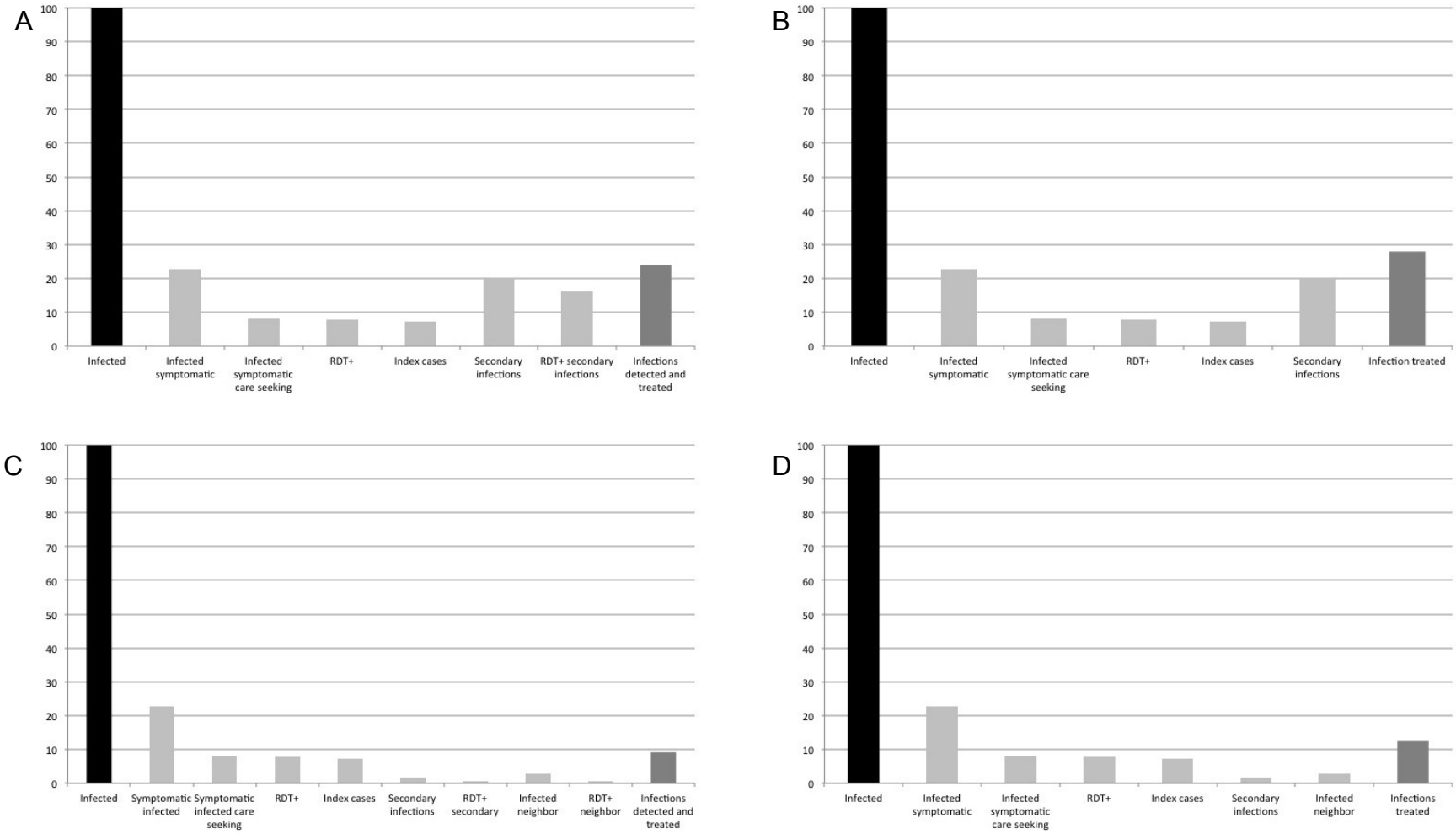
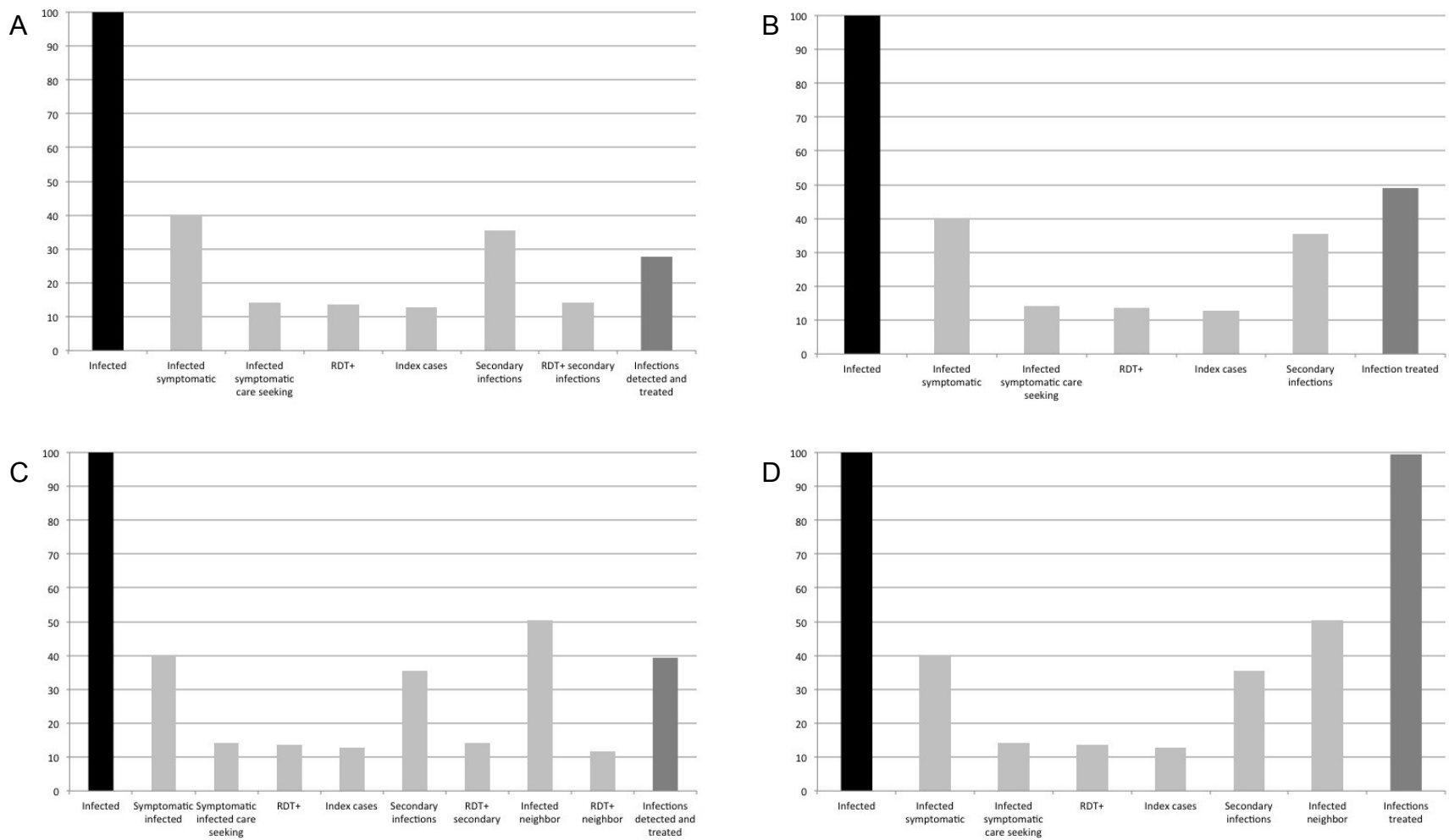


Figure S.4.3: Coverage cascades with malaria prevalence in index and neighboring households observed in the RHP evaluation with the proportion of symptomatic infections doubled for A: reactive test-and-treat in index households; B: reactive focal-drug-administration in index households; C: reactive test-and-treat in index and neighboring households and; D: reactive focal-drug-administration in index and neighboring households



5. Characterizing and quantifying human movement patterns using GPS data loggers in an area approaching malaria elimination in rural southern Zambia

Authors: Kelly M. Searle, ScM[1], Jailos Lubinda, MSc[2], Harry Hamapumbu[2], Timothy M. Shields, MA[1], Frank Curriero, PhD[1], David L. Smith, PhD[3], Philip E. Thuma, MD[2], and William J. Moss, MD MPH[1] for the Southern Africa International Centers of Excellence for Malaria Research

[1]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[2]Macha Research Trust, Choma District, Zambia

[3]Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, USA

Abstract

Background: In areas approaching malaria elimination, human mobility patterns are important in determining the relative proportions of malaria parasites that are imported or the result of low-level, endemic transmission. Commercially available GPS data loggers can be used to investigate individual human mobility patterns and the potential impact on malaria transmission in pre-elimination settings.

Methods: A convenience sample of participants older than 13 years and enrolled in a longitudinal cohort study in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia was selected to participate in a movement study from October 2013 through August 2014. Participants were requested to carry a GPS data logger when active for one month. Geographic position, date and time were logged every 2.5 minutes. Density maps for each month were created to evaluate seasonal trends in movement patterns. The proportion and kernel density of time spent at locations outside the home were plotted by distance from the household. Time spent outside the household compound, including during peak anopheline biting times, and time spent in high and low malaria risk using a previously published malaria risk map were calculated.

Results: A total of 69 of 173 eligible participants in the longitudinal cohort agreed to carry a GPS data logger. The convenience sample of participants was older than the eligible study population but there were no differences by sex, and the age distribution of participants varied slightly by month. Long-distance movement density maps showed evidence of seasonal patterns. There was less long-distance movement during the rainy season and no participants left the study area. Long-distance movement increased at the end of the rainy season and throughout the dry season, with participants traveling outside the study area and staying away longer. A median of 10.6% (IQR: 5.8-23.8) of

time was spent outside the household compound. This decreased by nearly half during peak anopheline biting times to a median of 5.6% (IQR: 1.7-14.9). The percent of time spent in areas of high malaria risk for participants residing in areas of high risk ranged from 83.2% to 100.0% (median: 96.4%, IQR: 91.1-98.1), and the percent of time spent in areas of high malaria risk for participants residing in areas of low risk ranged from 0.0% to 36.7% (median 0.0%, IQR: 0.0-.66). During peak biting times, the median amount of time spent within the household compound in high malaria risk areas was 44.3% (IQR: 33.9-49.2), higher than the amount of time spent outside of the household compound during peak biting times in both high risk areas (5.4% [IQR: 0.98-14.4]) and low risk areas (2.0% [IQR: 0.0-18.2]; p=0.001).

Conclusions: Malaria control interventions targeted at the household level, such as insecticide treated nets and reactive case detection, are likely made more effective by the less frequent, long-distance movement during the rainy season, with limited movement to and from high and low risk areas. The long-distance movement patterns during the dry season were consistent with Lévy random walks. This long-distance movement may increase the risk of importation at the end of the rainy season when the number of clinical malaria cases peaked; however, the risk of malaria importation is likely to be low throughout the remainder of the dry season.

Background

Population movement is known to contribute to the transmission of infectious diseases [1-3]. The spatial dependency and heterogeneity of infectious diseases makes them susceptible to spread via population movement. Transmission of vector-borne diseases is particularly heterogeneous [3-5]. For mosquito-borne diseases, specifically malaria, this heterogeneous transmission typically is due to differential contact between humans and mosquitos as a result of migration, overlapping activity space and unequal biting rates [4, 6, 7]. During the first global malaria eradication campaign in the 1950s and 1960s, failure to account for human population movement was identified as one of the factors that contributed to the program's failure [1, 3].

In areas approaching malaria elimination, human mobility patterns are important in determining the relative proportions of malaria parasites that are imported or the result of low-level, endemic transmission. [8-13]. Population movement patterns can threaten malaria elimination in three primary ways [1, 8]. The first is through uninfected residents traveling to higher malaria risk areas and transmitting parasites to local vectors upon returning home. The second is through infected visitors transmitting to local vectors [1, 8], and the third is through infected migrants re-locating and transmitting to local vectors [8]. As human movement patterns and malaria transmission are dynamic processes, individuals can be passive acquirers before becoming active transmitters upon return to their residence [2, 3]. These movement patterns occur on both large and small spatial scales, impacting malaria transmission and potentially threatening elimination [1, 12-14]

Many methods to measure human mobility have been explored to describe the impact of human movement on malaria transmission [15]. Long-distance migratory patterns have been characterized using census data on birth-place and prior residence [15, 16]. Many countries include questions on recent travel in malaria indicator surveys

[15]. Recent travel history also is used to classify passively detected case as imported. In research settings, movement diaries and travel histories have been used to measure human movement. However, these methods are subject to recall and social desirability bias, and small-scale movement is difficult to determine from survey data [15].

Mobile phone data to track individual movement is a convenient and accurate method for measuring human movement [8, 15]. However, there are limitations with mobile phone data to measure human movement patterns, particularly in rural settings. Use of mobile phone data to measure movement assumes that individuals who own a mobile phone are representative of the population and that a single person uses the phone [17]. In many areas, multiple mobile service providers are available and individuals have different subscriber identify module (SIM) cards under different provider accounts. Mobile phone coverage may be limited rural areas [18], limiting the ability to detect movement in these settings, and cannot readily measure movement across international borders. Finally, mobile phone data are reported at a population level without individual demographic information. Mobile phone data are primarily useful for capturing long-distance movement but do not capture short-distance human mobility patterns that impact the micro-epidemiology of malaria transmission [8, 15, 17, 19].

On both large and small spatial scales, human movement can be measured using commercially available GPS data loggers to describe individual movement patterns [20-23]. Their low cost and ease of use makes GPS data loggers ideal for tracking short-distance human movement to infer risk over specific time periods, including peak transmission seasons and vector biting times [24]. Commercially available GPS data loggers have been used to investigate individual human mobility and its impact on the transmission of several infectious diseases, including schistosomiasis, hookworm, and dengue virus [20-22, 24]. Use of commercially available GPS data loggers to track individual movement has been validated under different geographic and

environmental conditions [25]. Commercially available GPS data loggers were used to examine movement patterns in a study of influenza at a university in New Jersey, USA [26], and of dengue in Iquitos, Peru [22, 24]. However, most studies reporting data using commercially available GPS data loggers were conducted in North and South America, and in urban or peri-urban settings [20, 22, 24, 26-28]. One exception was a study conducted in northern Tanzania among the Hadza hunter-gatherer population to explore foraging patterns consistent with Lévy random walks [29]. Otherwise, little is known of small-scale movement patterns in rural sub-Saharan Africa and how these patterns may impact malaria transmission, control and elimination.

Commercially available GPS data loggers were used to determine movement patterns among a population of rural, agrarian participants in a longitudinal cohort study of malaria epidemiology in Southern Province, Zambia. These analyses aid in explaining the micro-epidemiology of malaria transmission and the risk of imported malaria as elimination is achieved and sustained [15]. Knowledge of mobility patterns and their potential impact on malaria transmission can inform the planning of malaria elimination strategies, particularly the targeting of interventions that account for spatial and seasonal variations in mobility.

Methods

Study site and population

The study was conducted in the rural catchment area of Macha Hospital in Choma District, Southern Province, Zambia, 70 km from the nearest town of Choma and approximately 1,200 km^2 in area. The single rainy season lasts from November through April, followed by a cool dry season from April until August and a hot dry season through November. Malaria transmission peaks during the rainy season [30] and the primary vector is *Anopheles arabiensis* [31, 32]. The hospital catchment area is populated by

villagers living in small, scattered homesteads. The parasite prevalence declined in this area over the past decade, from 9.2% in 2008 to less than 1% in 2013 [33]. Artemisinin combination therapy (ACTs) with artemether-lumefantrine was introduced as first-line anti-malarial therapy in Zambia in 2002 [34] and into the study area in 2004. In Zambia, long lasting insecticide treated nets (LLINs) are distributed through antenatal care clinics and additional mass distribution campaigns [35]. LLINs were widely distributed in the study area in 2007 [36] and more than 11,000 LLINs were distributed from nine health posts in the catchment area of Macha Hospital in 2012, with additional LLINs distributed in 2014 according to the Office of the Macha Hospital Environmental Health Technician.

Satellite images were used to develop a sampling frame for the random sampling of households to recruit and enroll individuals into longitudinal and cross-sectional surveys of malaria parasitemia starting in 2008 [36]. The identification and enumeration of households was done manually to delineate household and non-household structures (kraals, schools and larger structures) [36]. Households randomly selected from the sampling frame were recruited and enrolled in either one of two cohorts: cross-sectional or longitudinal. Households enrolled in the longitudinal cohort were repeatedly surveyed every two months whereas households enrolled in the cross-sectional cohort were visited once. Two hundred twenty individuals from 34 households were included in the longitudinal cohort. For each study visit, a questionnaire was administered to gather information on demographic characteristics, recent malaria symptoms and treatment history, knowledge of malaria risk and prevention, insecticide treated net (ITN) use, and recent travel history. A blood sample was collected by finger prick for a malaria rapid diagnostic test (RDT).[36].

GPS data loggers

Criteria for selection of the GPS devices were developed to accommodate the study population, ensure participants would not be responsible for charging the devices, and to protect privacy. These criteria included size, weight, water resistance, battery life, memory size, programing capabilities, motion detection, and validity. IgotU ® GT-600 (Mobile Action Technology) GPS loggers were selected as they were shown to be accurate (point accuracy of 4.4 meters and line accuracy of 10.3 meters) and acceptable in Iquitos, Peru [20]. These devices were light weight (37 g), had a large battery (750 mAh), were programmable, could collect up to 262,000 waypoints with 64 Mb of memory, and were water resistant [20-22]. The loggers could be password protected and accessed only with the accompanying software when connected to a computer with a custom USB connection. The data loggers could be worn using a Velcro strap or lanyard, or carried in a pocket or a bag, with the only requirement that they be carried with the participant continuously during their normal daily movement. As the devices were motion activated, they could be removed when participants were sleeping or sedentary to preserve battery life.

A convenience sample was selected from the longitudinal cohort during study visits from October 2013 through August 2014. Participants in the longitudinal cohort who were 13 years and older were invited to participate during alternate months. The study staff aimed to enroll 12 participants per month and have at least 10 complete the full month. Up to three participants per household were permitted to participate with no more than two individuals participating concurrently in a single month. Enrolled participants were requested to carry the GPS data logger at all times they were active for a one-month period. This allowed for a full year of data collection to assess seasonal patterns in population movement. The observed rainfall collected at the study site using a HOBO weather station (Onset Computer Corporation, Bourne, MA, USA) was graphed to document seasonal rainfall patterns. To prevent data loss due to limited battery life,

participants carried one logger for two weeks at which time the device was exchanged for a fully charged logger during a household visit by the study team.

Serial numbers of the GPS data loggers were matched to participant unique identification numbers. The power button was locked and the GPS data loggers were password protected so that only study staff could access the data. Geographic position was logged every 2.5 minutes. The loggers were programmed to be motion activated and hibernate when not in motion to conserve battery life. Data collected from the loggers contained date, time, longitude and latitude. Study staff maintained a monthly record of the date and time the devices were distributed and collected.

Data management

After each two-week collection period, the data were downloaded from each device using the @trip software (Mobile Action Technology, Inc., New Taipei City, Taiwan). The unique participant and household identification numbers were added manually. The data were checked for inconsistent logging and device errors, such as battery failure or unrealistically few locations logged. Raw data for each two-week period were uploaded to a secure REDCap (Research Electronic Data Capture) server [37]. The monthly record was used to remove data points where the logger was in transit with study staff to and from study households.

Mapping movement patterns

Movement data were projected into UTM Zone 35S, WGS 1984, and imported into ArcGIS (ESRI 2012. ArcGIS Desktop: Release 10.2. Environmental Systems Research Institute, Redlands, CA, USA) for pre-processing and analysis. Pre-processing was done by removing erroneous data points based on the shape, speed, or abrupt change in direction in the movement tract using a software extension developed for GPS

based trajectory analysis in ArcScene by Qi et al [26]. The cleaned and processed movement tracts were used to determine the cumulative amount of time spent at each location. High-resolution movement density maps were created by calculating the kernel density of the tract paths per 100 m^2 using the ArcScene software extension [26].

A movement density map was created to display the movement trajectory density for each participant and overlaid on a map of the study area with the enumerated households to represent the cumulative amount of time each participant spent in different areas. Density maps for participants were aggregated up to the month of collection to evaluate seasonal trends in movement patterns. These movement densities were normalized to be on the same scale for each month to make direct comparisons. Short-distance, local movement patterns were evaluated by overlaying the density map on a high-resolution satellite image of the study area. Long-distance movement patterns were evaluated by overlaying the density map on a satellite image obtained from ESRI. A 3-dimensional density map was created and overlaid on a previously published malaria risk map of the study area [36] to visualize movement patterns in and out of areas of higher and lower malaria risk.

Calculating activity space

Time was converted from date, hour, minute and second format to a numeric format. The total time participants carried a GPS data logger for each two-week time period was calculated independently to permit inclusion of individuals who only carried the logger for the first two-week period or experienced battery failure during the two-week period. The time elapsed between two consecutively logged geographic locations was then calculated. To account for differences in the total amount of time recorded by the GPS data logger for each participant, the proportion of time spent in each location was calculated. The proportion of time and kernel density of time spent in locations were

plotted against distance from the household to determine the distribution of the movement patterns.

The proportion of time participants spent within their household compound was calculated. A typical household compound in the study area has one or more domestic structures with several smaller structures, such as cooking houses or animal kraals. A household compound was defined as a grouping of these structures that function as a family unit. During each study visit, geographic coordinates of the household were collected from the front entrance of the main domestic structure using a tablet computer. To account for the household compound layout, and error due to the limits of spatial resolution of the GPS logger and the tablet used to collect the GPS coordinates, the household was defined as a 100-meter circular buffer around the measured household coordinates. Trajectories for each of the two-week periods for each participant were joined to the household buffer. This allowed for the movement to be defined as being within or outside the household compound.

As the primary vector *An. arabiensis* is known to have exophilic feeding behavior, the amount of time spent outside the household compound during peak biting time was estimated [31, 32]. We were not able to estimate the time outdoors due to the complex household structure and spatial resolution of the GPS loggers. Peak biting times for *An. arabiensis* were estimated to be between 19:00 and 6:00 hours in the study area [31]. First, the proportion of time each participant was within and outside the household compound was plotted to determine seasonal patterns in time spent outside of the household compound. The trajectories for each two-week period for each participant were then stratified by within and outside peak vector biting times. The subset of trajectories during the peak biting time was used to calculate the proportion of time spent outside of the household compound during peak biting times and graphed by month to determine seasonal patterns.

The proportion of time spent in areas of high malaria risk was calculated using a previously constructed malaria risk map [36]. The risk map was created using community-based surveys and environmental features obtained from satellite imagery and remotely-sensed data to predict the probability of malaria infection in the study area. Areas were categorized as being high and low malaria risk based on this map. Polygons of areas of high and low malaria risk were created from the raster formatted malaria risk map. The trajectories for each two-week period for each participant were spatially joined to the high malaria risk polygons. The proportion of time spent in areas of high and low risk was calculated for each participant and aggregated by month to assess seasonal patterns. Participants were stratified by household compound location as being within an area of high or low malaria risk. The proportion of time spent within and outside the household compound was calculated for participants residing in areas of high malaria risk. However, only the proportion of time spent outside the household compound was calculated for participants residing in areas of low malaria risk, as they did not have the opportunity to spend time in an area of high malaria risk within their household compound.

Results

During the study period, 173 eligible participants from 49 households in the longitudinal cohort were visited, of whom 69 agreed to carry a GPS data logger. All completed the first two weeks of data collection and 62 completed the second two weeks. Data from one participant was excluded from all analyses as they reported they were ill and gave the GPS data logger to other family members. The other six participants who did not complete the second two weeks declined further participation. The GPS data loggers were well accepted among participants and even became popular within the community. The age distribution of participants varied slightly by month but

there were no differences by sex (Table 5.1). The convenience sample of participants was older than the eligible study population but not different by sex (Table 5.1).

The long-distance movement density maps, which display movement patterns outside the study area, showed evidence of seasonal patterns in population movement (Figure 5.1). There was less long-distance movement during the rainy season (December and February), with no participants leaving the study area (Figure 5.1). From April through August, long-distance movement increased as participants traveled outside the study area and stayed further from home for longer periods (Figure 5.1).

The short-distance maps, which display high-resolution movement patterns within the 1,200 km^2 study area, showed no evidence of seasonal mobility patterns (Figure 5.2). Evidence of seasonal patterns in long-distance but not short-distance movement was supported by kernel density plots of the proportion of movement trajectory by distance from the household compound (Figure 5.3) and showed longer trips, farther from home beginning in April as the rainy season ended (Figure 5.3). These kernel density plots also showed that participants spent most of their time close to their household compound with seasonal, longer trips that included shorter movements around these distant locations (Figure 5.3). This seasonal pattern of increased long-distance movement following the end of the rainy season coincides with an increase in clinical malaria cases. The measured monthly rainfall was consistent with the expected seasonal rainfall patterns (Figure 5.4).

Movement density in areas of high and low malaria risk was mapped (Figure 5.5). There was no evidence of a seasonal trend in the percentage of time spent away from the household compound (Figure 5.6). Overall, a median of 10.6% (IQR: 5.8-23.8) of time was spent away from the household compound. This decreased by nearly half during peak anopheline biting times to a median of 5.6% (IQR: 1.7-14.9) of time spent outside the household compound (Figure 5.7). The amount of time spent in areas of high

malaria risk was dependent on whether the household compound was located in an area of high malaria risk (Figure 5.8). The percent of time spent in areas of high malaria risk for participants residing in areas of high malaria risk ranged from 83.2% to 100% (median: 96.4%, IQR: 91.1-98.1), and the percent of time spent in areas of high malaria risk for participants residing in areas of low malaria risk ranged from 0% to 36.7% (median 0%, IQR: 0.0-.66).

The amount of time spent in high-risk areas outside the household compound during peak vector biting times was not different between participants residing in household compounds in high malaria risk (median: 5.5%, IQR: 0.98-14.4) and low malaria risk (median: 2%, IQR: 0-18.2) (p=0.4) areas (Figure 5.8). During peak biting times, the median time spent within the household compound in high malaria risk areas was 44.3% (IQR: 33.9-49.2), higher than the amount of time spent away from the household compound during peak biting times in both high risk areas (5.4% [IQR: 0.98-14.4]) and low risk areas (2.0% [IQR: 0.0-18.2]) (p=0.001) (Figure 5.8).

Discussion

Residents of rural, southern Zambia primarily spent time close to their household compound, with frequent short movements around their household compound and infrequent longer trips that included shorter movements around these distant locations. Long-distance movement patterns showed clear seasonal patterns. During the rainy season, participants did not travel far from their household compound, presumably to stay closer to their farms but perhaps also because roads became impassable. As the rainy season ended, participants began to travel further from their household compound and stayed there for longer durations.

The long-distance movement patterns at the end of the rainy season and during the dry season are consistent with Lévy random walk patterns and other random walk

searches [29]. These random walk patterns have been described primarily for animal foraging patterns but also more recently among nomadic hunter-gatherers in northern Tanzania [38] [29]. Lévy walks are random walk search strategies used when searching for heterogeneously distributed food [29]. This pattern consists mainly of shorter movements (e.g. frequent short-term trips close to home) combined with fewer farther movements (e.g. infrequent longer-term trips far from home) [29, 39]. While movement patterns are important for understanding malaria transmission, challenges remain as to how best to incorporate these patterns into malaria transmission models [40]. This study showed that human movement patterns follow seasonal patterns, with Lévy random walk patterns during the dry season but not during the rainy season.

Malaria prevalence declined dramatically in parts of southern Zambia but the region remains receptive to malaria transmission and clinical cases typically occur each year throughout the rainy season, increasing at the end of the rainy season. This seasonal increase in clinical malaria cases coincides with when the population becomes more mobile and begins to display Lévy random walk patterns. While malaria prevalence is low in the study area, some surrounding areas have higher malaria prevalence. Movement to these areas for extended periods and traveling back home may result in imported infections. However, this may only be important at the end of the rainy season in the month of April, as this marks the beginning of the dry season and vectors subsequently are not available to maintain transmission. Thus, these long-distance, seasonal movement patterns may result in imported infections but are unlikely to facilitate transmission during the dry season.

Participants spent approximately 5% of time away from their household compound during peak biting times. However, the spatial resolution of the GPS data loggers and the satellite imagery limited the ability to determine if participants were inside a domestic structure. For short-distance movement patterns, the proportion of

time spent in areas of high malaria risk was mainly dependent on whether or not the participant's household compound was in an area of high malaria risk. In this study area where the ecologic risk of malaria was estimated, individuals who resided in areas of higher and lower malaria risk did not spend much time in areas of the opposite risk. However, even a small amount of time spent in a high malaria risk area could result in infection and the introduction into low malaria risk areas, propagating local transmission. Therefore, malaria elimination interventions implemented at the household level, such as ITNs, IRS and reactive case detection, may benefit from the less frequent, long-distance movement during the rainy season.

The human mobility patterns observed in this study would be described as circulatory rural-rural movement in Prothero's typology [1]. These types of movement patterns and their seasonality should be considered when planning malaria elimination strategies. Because of restricted mobility during the rainy season, interventions directed at households may be more effective. In areas at higher ecological risk, interventions could be targeted at households during the rainy season, as mobility outside of high-risk areas during this time is minimal.

Conclusions

Malaria control interventions targeted at the household level, such as insecticide treated nets and reactive case detection, are likely made more effective by the less frequent, long-distance movement during the rainy season, with limited movement to and from high and low risk areas. The long-distance movement patterns during the dry season were consistent with Lévy random walks. This long-distance movement may increase the risk of importation at the end of the rainy season when clinical malaria cases peak; however, the risk of malaria importation is likely to be low throughout the remainder of the dry season.

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Table 5.1: Demographic characteristics of the study participants by month and comparison with the remaining eligible population

	Number	**Age in years (median [IQR])**	**Percent male (% [95% CI])**
Study participants	68	39.1 [19.8-54.7]	50.0 [38.0-62.0]
October	12	54.5 [42.6-61.7]	41.7 [12.0-71.3]
December	12	45.8 [20.3-57.7]	50.0 [20.0-80.0]
February	11	19.3 [16.6-51.4]	36.4 [6.0-66.7]
April	11	39.0 [23.2-41.6]	54.6 [23.1-86.0]
June	12	40.8 [26.9-48.2]	50.0 [20.0-80.0]
August	10	21.5 [14.5-30.2]	70.0 [39.5-100.0]
Remaining eligible individuals	105	23.8 [16.0-42.8]	48.6 [38.9-58.2]

Figure 5.1: Long-distance movement density, October 2013 to August 2014

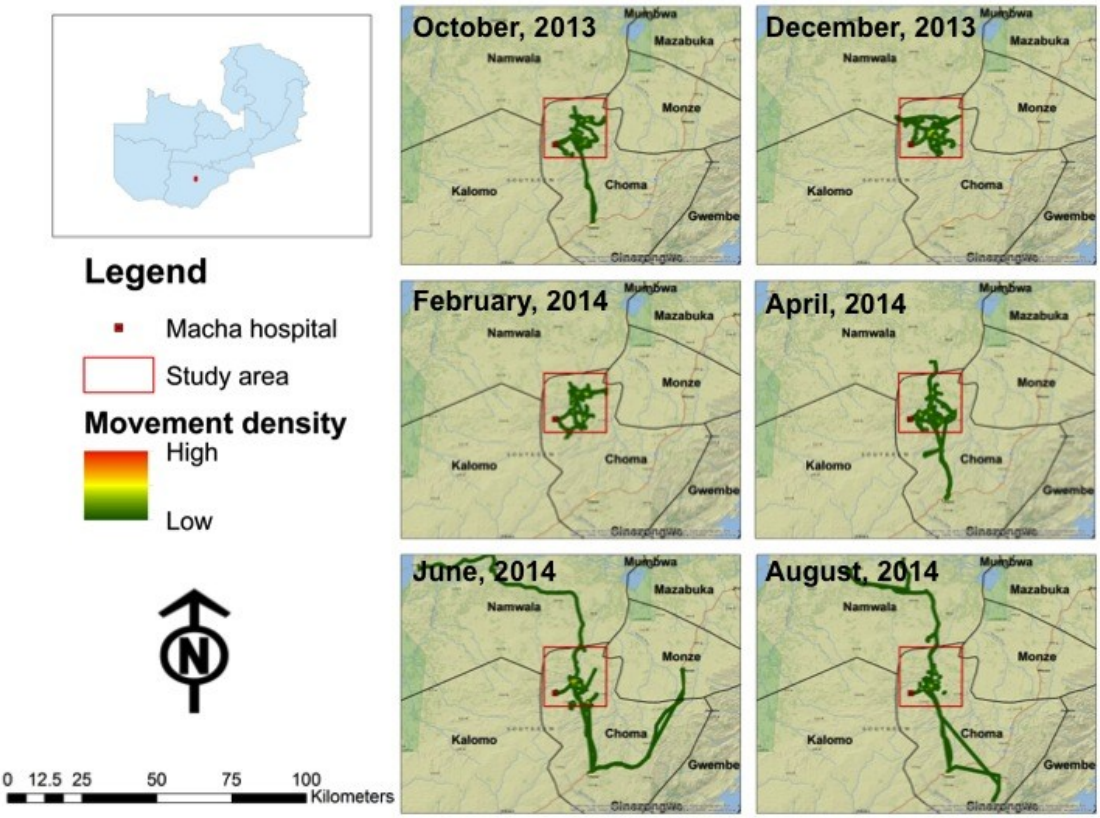


Figure 5.2: Short-distance movement density, October 2013 to August 2014

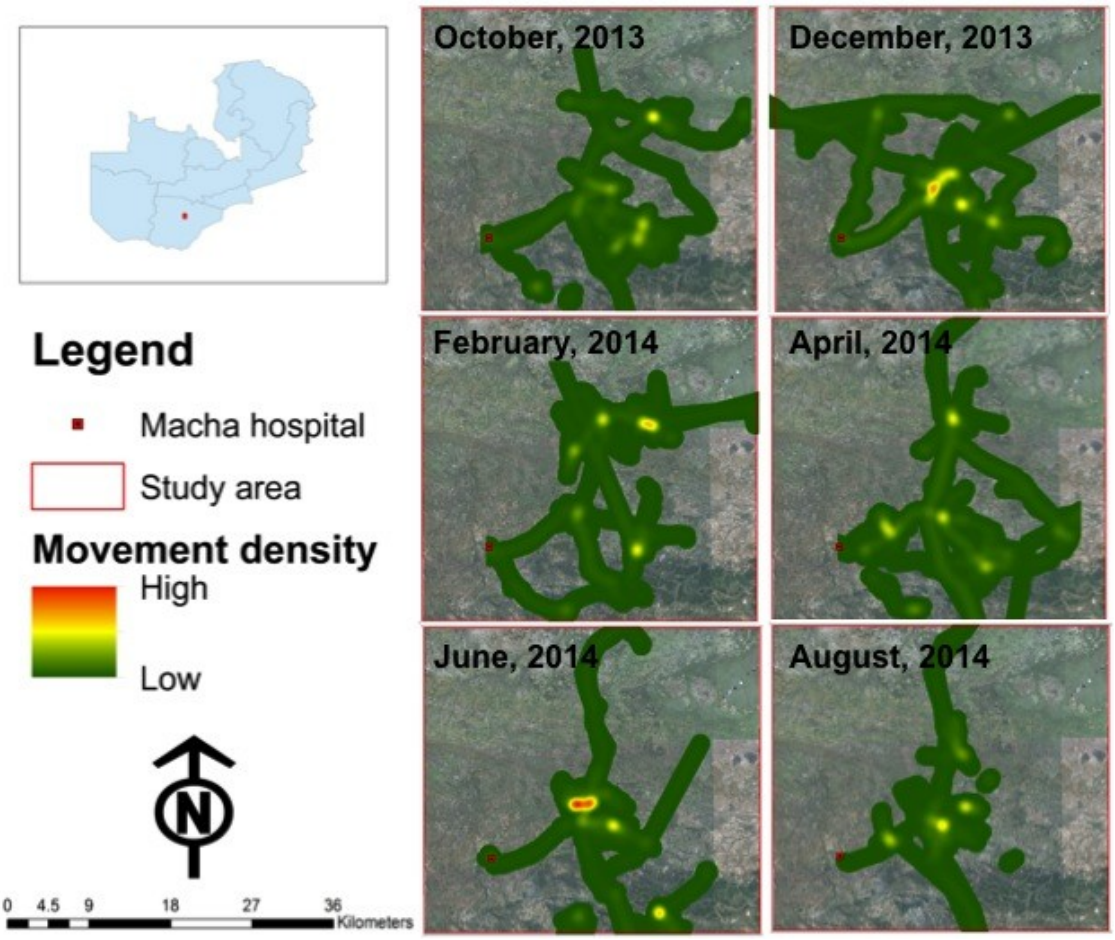


Figure 5.3: Proportion of movement trajectory by distance from participant's home for each month from October 2013 to August 2014

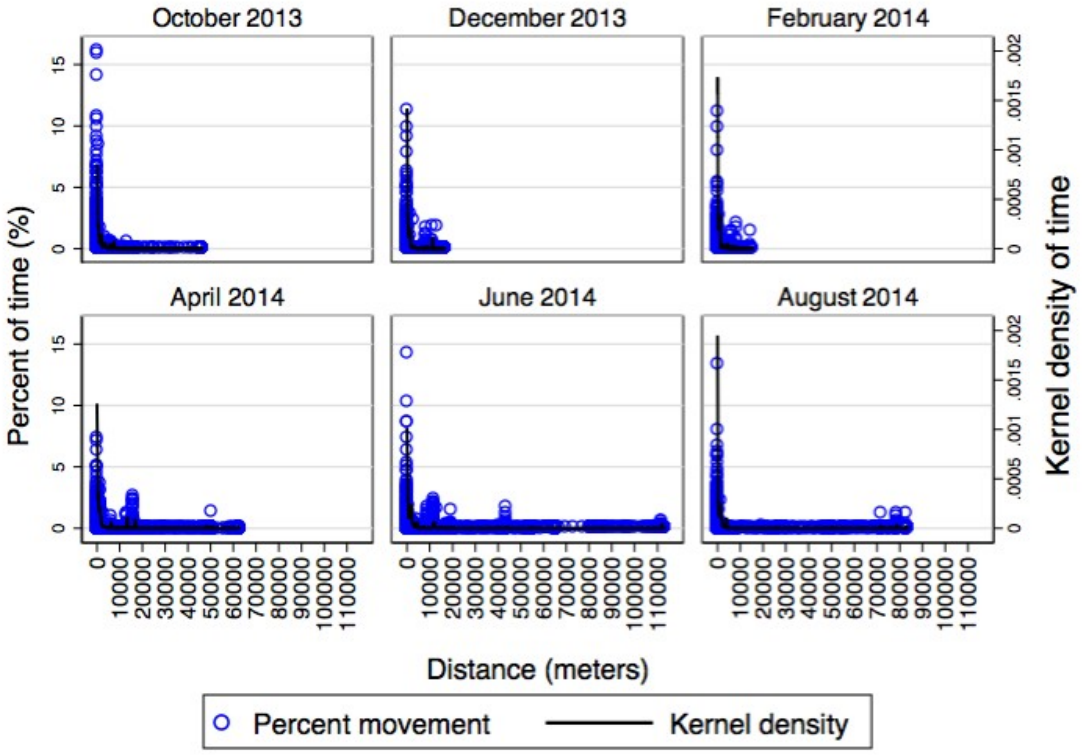


Figure 5.4: Total rainfall per month collected by HOBO weather station in Macha, Zambia

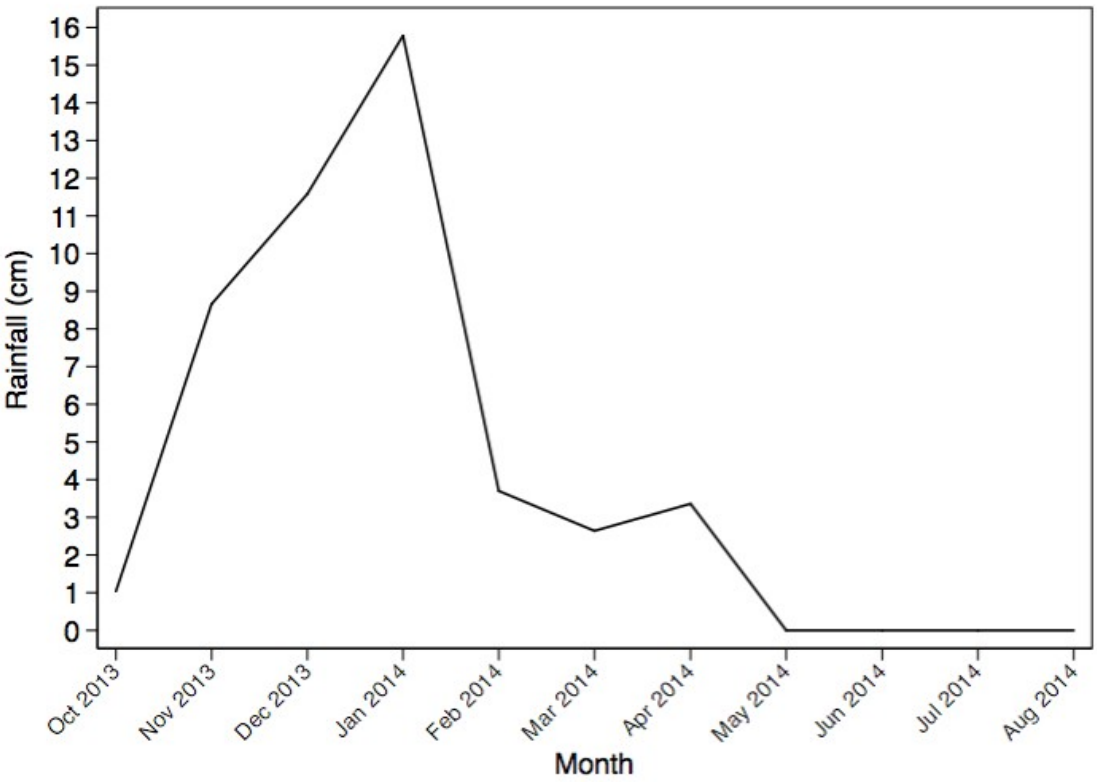


Figure 5.5: Short-distance movement density in areas of different malaria risk, October 2013 to August 2014

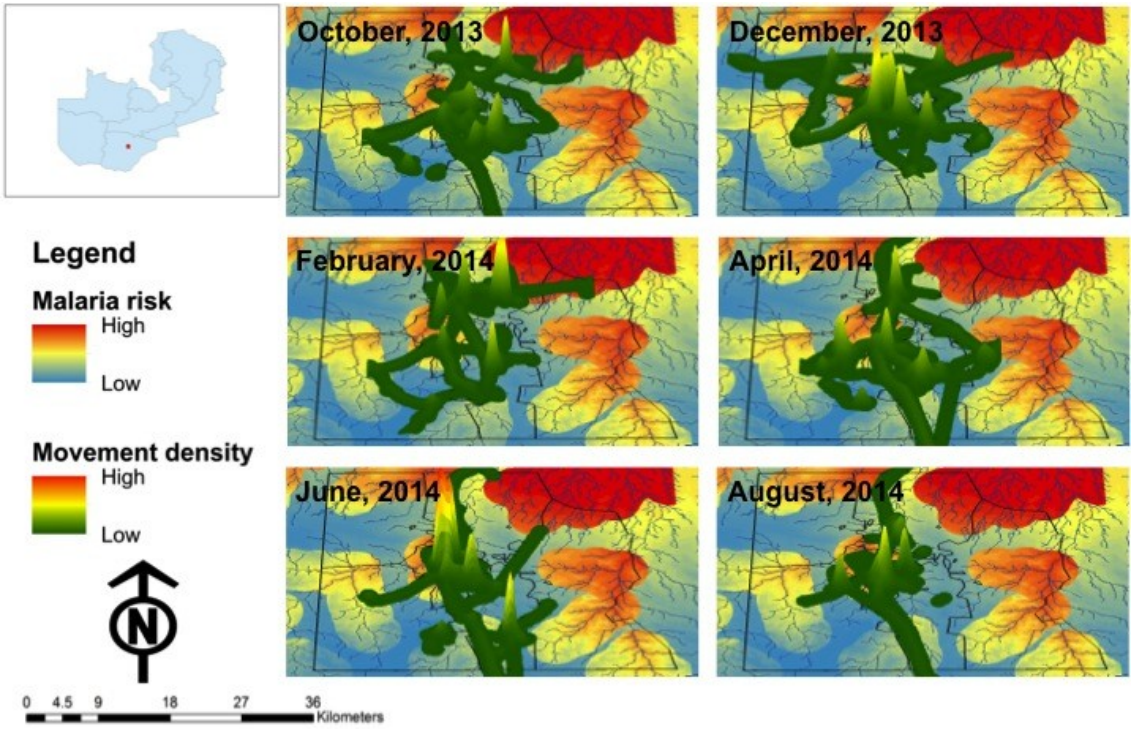


Figure 5.6: Percent of time spent away from the household compound

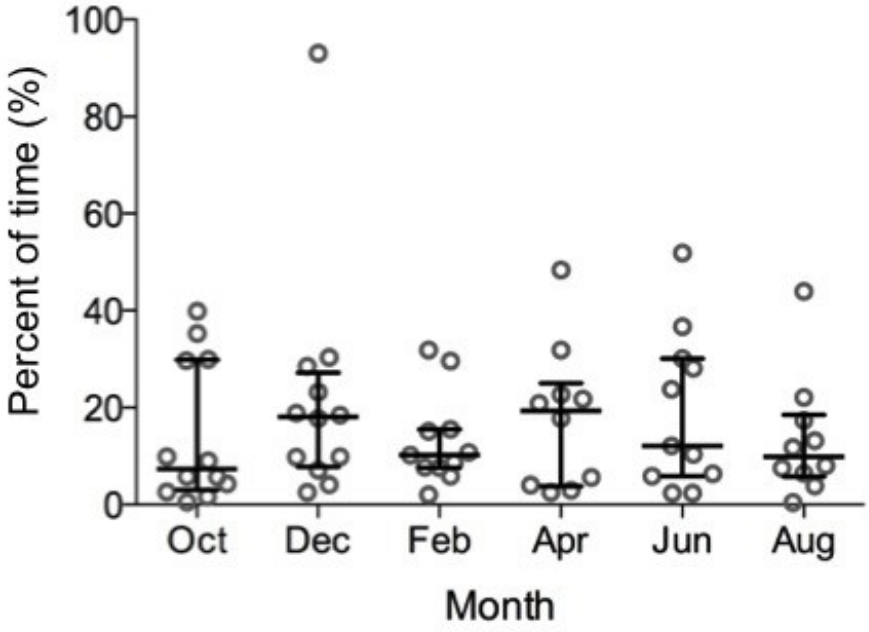


Figure 5.7: Percent of time spent away from the household compound during peak anopheline biting times

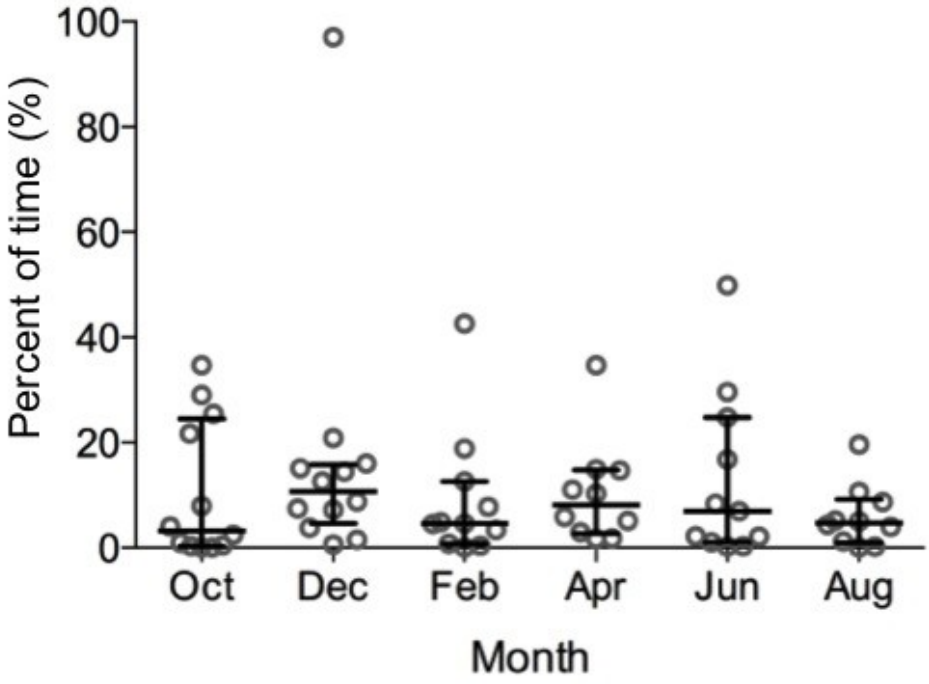
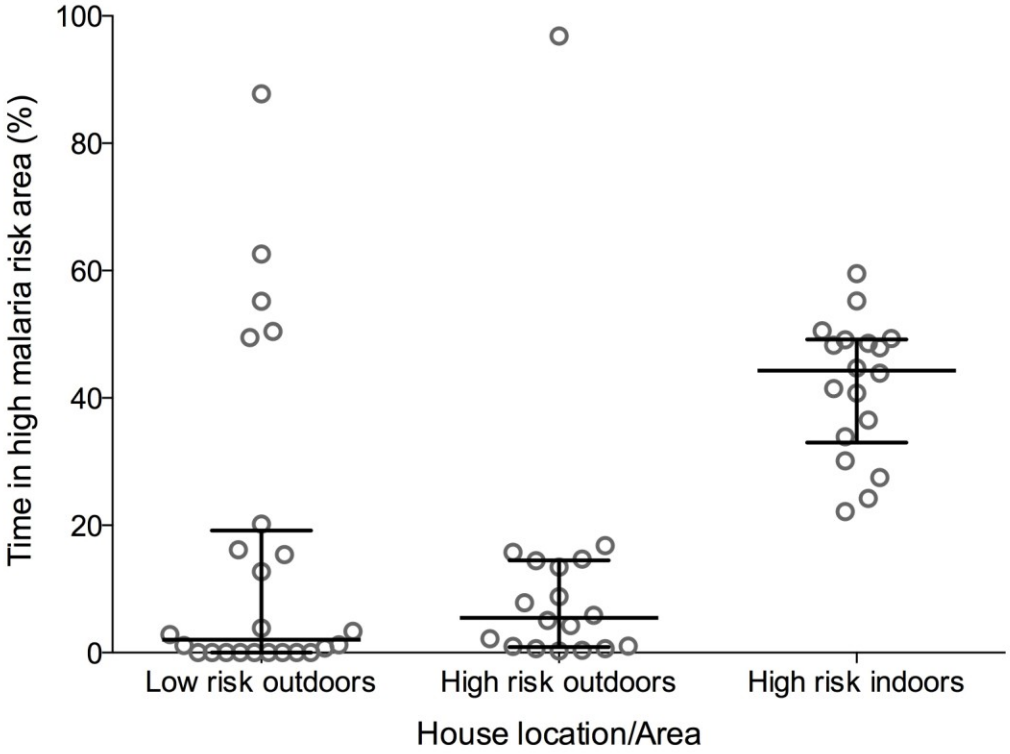


Figure 5.8: Percent of time spent away from the household compound during peak anopheline biting times in areas of high malaria risk by household location



6. Conclusions

6.1 Summary of findings

In the Macha study area of Southern Province, Zambia, changes in the spatial and temporal patterns of passively detected cases were observed as transmission declined. As areas approach malaria elimination, information on how spatial and temporal patterns of passively and actively detected infected individuals change in response to declining transmission can be used to target interventions. Transitions from annual seasonal peaks in passively detected malaria cases to biannual seasonal peaks allow for applications of interventions, such as focal drug administration, prior to the expected peak in cases, although a shift to fractured spatial patterns complicates the spatial targeting of interventions. However, enhanced surveillance systems will allow seasonal spatial patterns to be detected and targeted. Real time monitoring of monthly malaria cases presenting at health care facilities could be used to detect areas with increases in cases prior to and during the transmission season. These areas can then be targeted for increased delivery of interventions to detect and treat symptomatic cases, and reactively treat asymptomatic cases to decrease onward transmission.

In addition to changing spatial and temporal patterns, differences in parasite genetics were observed between passively and actively detected cases. Parasites infecting the passively and actively detected populations were phylogenetically separated. Analysis of the complexity of infection indicated that passively detected cases were primarily monoclonal whereas actively detected cases were primarily polyclonal. This implies that passively detected cases are being infected with single parasites, while actively detected cases are harboring multiple parasites, potentially between transmission seasons. The genetic divergence between seasons was constant for passively detected cases but decreased over seasons for actively detected cases.

These differences are consistent with the hypothesis that the actively detected cases represented chronic, sub-patent infections in contrast to the acute infections of the passively detected cases. The parasites infecting the passively detected cases can be cleared through successful case management and treatment at health care facilities. As these are likely seasonally acquired acute infections, increased passive surveillance at health care facilities can effectively treat this population. The chronic infections may not need to be actively sought out and treated to decrease or halt transmission. Additionally, as these infections are harbored through multiple transmission seasons, they could be targeted through reactive case detection programs, specifically reactive focal drug administration. .

Targeted interventions, such as reactive case detection, can accelerate malaria elimination. However, several obstacles impede the efficiency of reactive case detection, including the high number of cases during peak months burdening resources and staffing levels, the low proportion of residents at home at the time of the screening, the difficulty in identifying households within the specified radius, and the low sensitivity of RDTs in this transmission setting. Reactive focal drug administration has the potential to address the latter obstacle by removing the need for a diagnostic test, although challenges with supply chain will need to be addressed to ensure a larger volume of antimalarials can be accommodated. Irrespective of the inclusion or exclusion of a diagnostic test, community sensitization and coordination to enable high target population coverage needs to be addressed. With limited resources, coverage, and diagnostic tools, reactive screen-and-treat will likely not be sufficient to achieve malaria elimination in this setting. However, high coverage with reactive focal drug administration could be more efficient in decreasing the reservoir of infection and should be considered as an alternative strategy.

In this area most individuals spent the majority of their time around their household compound, especially during peak vector biting times. The proportion of time spent in areas of higher malaria risk was determined by the risk status of the participant's household. Therefore, interventions targeted at the household level, such as LLINs/ITNs and IRS, take advantage of the restricted, small-scale movement patterns. The long-distance movement patterns displayed seasonal patterns consistent with those of Lévy random walks, where individuals make more frequent long-distance, long-term trips from their home, highlighting the risk of malaria importation. As these movement patterns were observed during the dry season, interventions such as reactive case detection or reactive focal drug administration could take advantage of this seasonality and begin implementation at the end of the rainy season, in advance of long-distant movements.

Malaria elimination is a dynamic process that can create fractured spatial patterns of transmission and a segregation of malaria infections into a chronically infected asymptomatic population and an acutely infected symptomatic population. The symptomatically infected population can be identified and treated simply with passive case detection and improving the quality of case management. The chronically infected, and asymptomatically infected populations are much more difficult to identify and treat. While more targeted interventions, such as reactive focal drug administration, may overcome this challenge, high coverage levels will need to be achieved. Additionally, populations are not static, and mobility can be highly seasonal, which impacts the ability to reach all of the population with targeted interventions. To achieve malaria elimination, one intervention or even some combination of interventions may not be efficient in all settings. As malaria transmission decreases and elimination becomes the goal of interventions, the underlying epidemiology of malaria needs to be evaluated in to provide the most appropriate combination of interventions that will be implemented. Advanced

planning should also be made to deliver the interventions to the most appropriate areas, maintain high coverage over a long time frame until elimination is achieved, and prevent reintroduction and resurgence.

6.2 Strengths and limitations

A major strength of these findings is that most of the work was conducted over an eight year period when malaria transmission was decreasing. This study had the ability to document the spatial and temporal trends in passively and actively detected infections, store biological samples from cases, and determine the genetic relationships between parasites. This allowed for a large repository of data and samples that led to the ability to describe the natural history of malaria elimination on a local scale. Additionally, these data highlight the importance of having enhanced surveillance systems in areas moving from malaria control to elimination, as passive case detection only reveals part of the story of malaria elimination.

The strong relationship between the study staff at MRT and the community of Macha was integral in the success of the population movement study using GPS data loggers. The community was invested in the study and was willing to participate. The data collected showed evidence of seasonal movement patterns. These movement patterns allow for interventions distributed at the household level to be timed during the transmission season to ensure high coverage. Also, to our knowledge, this is the first study describing and quantifying movement patterns of a rural sub-Saharan African population and its potential impact of malaria elimination.

During the course of achieving malaria elimination, new interventions will be implemented in settings such as Macha. In this setting, we evaluated the newly introduced reactive test and treat strategy in its initial stages. The study staff were able to leverage an established relationship with the RHCs and CHWs to conduct the

evaluation. The findings can help guide the process of improving the current intervention and develop next steps from the programmatic and policy levels of malaria elimination.

While the data provide a great level of detail and long time frame, there were few malaria cases in later years (2010 onwards) to draw definitive conclusions. However, in an elimination setting, the paucity of cases is expected and inferences from the small numbers can be made. Assumptions were made when mapping the incidence of symptomatic malaria cases, particularly that there was no substantial change in the underlying population over time. Additionally, the population was derived using a simulation model based on a random sample of the population, and assumes this was representative of the entire population.

The molecular barcode was used to determine the population complexity and diversity from those with confirmed malaria infection. However, due the low levels of parasitemia in the actively detected cases, some assays failed leading to missing data. As many of the actively detected cases had high numbers of mixed calls, there was a high proportion of polyclonal infections. Typically, haplotypes with missing data and polyclonal infections are not included in analyses of population genetics as it is not possible to determine the proportion of each allele present in a mixed call and exact haplotypes cannot be determined. This was overcome by including a third possible allele representing mixed calls and using the percent agreement, accounting for missing data and mixed calls.

The human movement study was conducted among a small number of participants from the already established longitudinal cohort study. However, this was designed in part to determine the feasibility and acceptability of the using GPS data loggers to measure population movement patterns in rural sub-Saharan Africa. The GPS data loggers were well received among the population and the study was feasible and manageable. Although we used a convenience sample rather than a random sample,

the study population included a large proportion of all eligible participants in the longitudinal cohort and did not differ from those not included in the study population.

The reactive case detection evaluation was conducted over a short period that only covered the rainy and not the dry season. The study period (January through June of 2014) only represented a 6-month window where the evaluation was implemented and was early in the program's implementation. However, other reactive case detection programs can learn from this experience. The results highlight the need for monitoring and evaluation shortly after implementation to identify operational challenges and their potential impact on program performance and impact.

6.3 Recommendations for future research and policy

The Macha research site in Choma District, Southern Province, Zambia, provides a setting where the transition from malaria control to elimination can be assessed and obstacles to elimination evaluated. Surveillance of passively detected malaria cases is ongoing. The temporal and spatial trends can be measured in real time to determine when and where increases in passively detected, symptomatic cases occur. Additionally, surveillance of passively detected cases in this area can be used to identify when and where outbreaks of symptomatic malaria are occurring during the process of malaria elimination.

Active case detection, via reactive surveillance, may be able to identify foci of malaria transmission. Genetic relatedness between infections in index cases and infections in individuals residing in the same household and their neighbors can provide insight into the transmission patterns between symptomatic and asymptomatic malaria cases. Investigation into this genetic relatedness of parasites infecting index cases, household members of index cases, and their neighbors is currently being explored.

Research into how to most effectively target the asymptomatic population is ongoing. Reactive test and treat programs may increase case detection of asymptomatically infected cases in transmission foci and treat them to prevent ongoing transmission. However, the sensitivity of RDTs has shown that it is not sufficient to detect low parasitemic, asymptomatic malaria infections, as it was developed to detect symptomatic malaria infections. Therefore, test and treat programs using the currently available RDTs for case detection of asymptomatic infections may not be the best use of resources. In areas approaching malaria elimination, reactive focal drug administration should be considered.

The role of population movement patterns and malaria transmission in this area will need to be explored further. Simulated, agent based models of vector and human movement patterns will be applied to this setting. These complex interactions will be modeled to determine the impact of human population movement patterns on malaria transmission. While that is ongoing, the timing of interventions targeted at households can be shifted to before and after the rainy season to increase their impact. Targeting interventions at the household before the onset of the rainy season will aid in reducing the parasite population prior to the peak transmission season, thus reducing local transmission when people are less likely to travel long distances. Interventions targeted after the rainy season could prevent or decrease the onward transmission of parasites acquired during long-distance travel that occurs towards the end of the rainy season.

CURRICULUM VITAE

Kelly M. Searle

Email: ksearle1@jhu.edu
Phone: (814)-603-3140
Address: 2712 Woodsdale Avenue, Baltimore, MD 20214

EDUCATION

Doctor of Philosophy in Epidemiology (PhD)
Johns Hopkins Bloomberg School of Public Health (2012-2016)
Concentration: Infectious Disease
Dissertation Topic: The epidemiology of malaria transmission and threats to achieving elimination in an area of effective malaria control in southern Zambia

Master of Science in Epidemiology (ScM)
Johns Hopkins Bloomberg School of Public Health (2010-2012)
Concentration: Infectious Disease
Thesis Topic: Evaluation of reactive case finding to target focal malaria transmission in two different settings using representative household samples and simulation of population level data

Bachelor of Science in Pathobiology and Veterinary Sciences (BS)
University of Connecticut (2001-2005)

PROFESSIONAL EXPERIENCE

Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology (August 2013-present)
ICEMR Doctoral Student

- Developed and implemented a population movement study using GPS data loggers in Macha, Zambia and Nchelenge, Zambia.
- Performed analyses on population movement patterns from GPS data loggers in Macha, Zambia.
- Trained laboratory scientists on molecular barcoding methods to evaluate *P. falciparum* parasite diversity and complexity.
- Developed and implemented study protocols for the use of molecular barcoding methods for samples obtained from passive and active malaria case detection in Macha, Zambia.
- Conducted various spatial analyses to determine the distribution of malaria cases over space and time.
- Assisted in the development of study protocols for and enhanced reactive case detection sampling system.
- Evaluated reactive case detection as a potential tool for malaria elimination.
- Assisted in data management and quality control for data from all three sites of the ICEMR study.

Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology (April 2013-October 2015)
Research Assistant

- Assist in data management and quality control for the Pediatric Antiretroviral Therapy (PART) study and Early Infant Diagnosis (EID) study, two longitudinal cohorts of HIV infected infants, children, and adolescents.
- Conduct weekly quality control and quality assurance on enrollment and follow up study visit data collected at the field site in Macha, Zambia.
- Conduct monthly quality control on enrollment and follow up data collected at the field site in Macha, Zambia.
- Maintain codebooks to assist in data analysis using different survey versions.
- Develop SAS programs to translate data collected on electronic tablets to usable datasets for analysis.
- Conduct preliminary analyses and create summary reports.
- Aided in the design, implementation, and analysis of a measles and rubella immunology case control study.

The United States National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Immunoregulation (June 2011-August 2011)
Research Fellow

- Assisted in the design of a sub-study investigating neurologic effects of human immunodeficiency virus (HIV) within an established cohort of individuals beginning antiretroviral treatment with advanced HIV.
- Developed the study plan and presentation for the scientific review process
- Developed the Institutional Review Board (IRB) protocol and consent forms
- Identified eligible study participants
- Developed laboratory protocol for immunological biomarkers of neuroinflammation
- Adhered to strict sample management under Biological Safety Level 2 and 3 regulations
- Adhered to IRB protocol and maintenance of patient confidentiality

Johns Hopkins Biological Repository and Infectious Disease Laboratory (December 2010-April 2013)
Research Assistant

- Assist in the intake, processing and management of a wide variety of human biological samples.
- Assist in the data management of various large observational cohort studies.
- Adhere to all protocols of a research and clinical laboratory.
- Adhere to strict biological sample management under Biological Safety Level 2 and 3 regulations.

Duke University Medical Center, Department of Ophthalmology (February 2008 – July 2010)
Research Assistant / Laboratory Manager

- Conducted research projects collaboratively with two primary investigators at Duke University Medical Center within the field of Ophthalmology.
- Managed a laboratory consisting of the primary investigators, post-doctoral fellows and undergraduate students.

- Was responsible for the maintenance, breeding, identification, genotyping, and pedigrees of a large mouse colony.
- Assisted in the development and amending of two separate Duke Institutional Animal Care and Use Committee (IACUC) protocols.
- Worked in cooperation with all laboratory personnel and the Duke IACUC to maintain compliance in all animal procedures described in each animal protocol.
- Developed, oversaw and adhered to all laboratory safety procedures in cooperation with, and in compliance to the Duke Occupational and Environmental Safety Office.
- Performed all necessary scientific experiments as the laboratory technician; analyzed the data and recorded results for independent and collaborative projects.
- Managed all financial records of the laboratory, including purchasing, receiving and reconciliation for funding account codes.

University of Connecticut Pathobiology Department (May 2004 – June 2005)
Research Assistant

- Assisted in the research of the treatment of Lyme's Disease as well as the testing of new West Nile vaccines for equines
- Organized and monitored field research by taking scheduled blood samples from selected horses as well as administering oral, intravenous, and intramuscular treatments and vaccines.
- Participated in the reporting of observations of the physical animal as well as organizing the data and statistical results of the studies.

TEACHING EXPERIENCE

Johns Hopkins Bloomberg School of Public Health (September 2014-December 2014)
Teaching Assistant, Fundamentals of Epidemiology

- Independently lead weekly lab discussions focused on the application of methods introduced in lecture.
- Hold office hours to provide additional assistance to students in need.
- Attended and participated in weekly instructors' meetings to discuss material to be presented in lecture and lab the following week
- Evaluated student assignments and examinations
- Assisted in the development in the course curriculum; planned, prepared, and presented a new lecture for the course.

Johns Hopkins Bloomberg School of Public Health (August 2014-December 2014)
Comprehensive Exam Teaching Assistant

- Develop a bank of comprehensive exam questions based on past exams
- Organize past questions by content area and course
- Conduct analysis to evaluate the performance of each question
- Determine which questions are of use and which should be eliminated from the question bank, based on analysis and knowledge of the courses and subject matter

Johns Hopkins Bloomberg School of Public Health (March 2014-May 2015)
Teaching Assistant, Practical Epidemiology for Basic Scientists

- Assisted instructors in the development of lecture material
- Developed and gave lectures
- Attended lectures and assisted in instruction and clarification of material
- Held office hours to provide additions assistance to students in need of clarification or more in depth explanation of methods and/or applications
- Developed and modified quizzes, labs, and exams
- Evaluated quizzes and exams
- Provided feedback to instructors on lectures and students' progress and understanding of the material

Johns Hopkins Bloomberg School of Public Health (January 2014-present)
Teaching Assistant, Spatial Analysis and Geographic Information Systems I-IV

- Lead lab discussions, focused on application of geographic information systems and introductory spatial statistics
- Edited course material prior to presentation and use in the class
- Held office hours to provide additional assistance to students in need of clarification or more in depth explanation of methods and/or applications
- Evaluated student assignments and provided constructive feedback

Johns Hopkins Bloomberg School of Public Health (January 2014-March 2014)
Lead Teaching Assistant, Epidemiologic Methods III

- Assisted instructors in the development of laboratory materials for instructors, teaching assistants, and students
- Attended and participate in weekly instructors' meetings to discuss material to be presented in lecture and lab the following week
- Finalized code used in analyses conducted in laboratories
- Developed and edited student assignments
- Designed grading rubrics for student assignments and oversaw the grading by teaching assistants, to ensure consistency and fairness
- Assisted instructors in the development of examinations
- Evaluated student assignments and examinations

Johns Hopkins Bloomberg School of Public Health (August 2013-May 2014)
Teaching Assistant, Epidemiologic Methods I, II, & IV

- Assisted in instruction of the first, second, and fourth course in a four course series, focused on epidemiologic methods for research in public health
- Lead group lab discussions focusing on the application of methods presented in lectures
- Attended and participated in weekly instructors' meetings to discuss material to be presented in lecture and lab the following week
- Held office hours to provide additional assistance to students in need of clarification or more in depth explanation of methods and/or applications
- Evaluated student assignments and examinations

Johns Hopkins Bloomberg School of Public Health (January 2013-March 2013)
Teaching Assistant, Epidemiologic Methods III

- Assisted in instruction of the third course in a four course series, focused on epidemiologic methods for research in public health
- Lead group lab discussions focusing on the application of methods presented in lectures
- Attended and participated in weekly instructors' meetings to discuss material to be presented in lecture and lab the following week
- Held office hours to provide additional assistance to students in need of clarification or more in depth explanation of methods and/or applications
- Evaluated student assignments and examinations

Lead Teaching Assistant, Epidemiology and Public Health Impact of HIV/AIDS, Advanced Topics in Control and Prevention of HIV/AIDS

- Assisted in curriculum planning and course design of introductory and advanced level HIV/AIDS courses focusing on the biological and social aspects of HIV
- Maintained course website
- Provided assistance and further explanation to students in need
- Conducted meetings with student groups for planning of student lead discussions aimed at providing additional, supplemental and/or personal information and experience with instructors and peers
- Provided assistance to instructors in regards to content of lecture material and teaching methods
- Wrote, reviewed, proctored, and graded examinations focused on evaluating the extent of knowledge and experience gained throughout the course
- Assisted students in the development of research papers, followed by the editing and evaluating of papers
- Accepted anonymous feedback from students and alter course content and presentation accordingly

Guest Lectures

- Cluster detection – Spatial analysis III: Spatial statistics
- Epidemiology and public health programs in the field – Fundamentals of Epidemiology
- Biomarkers – Practical Epidemiology for Basic Scientists
- Reliability – Practical Epidemiology for Basic Scientists
- Measures of Association I – Practical Epidemiology for Basic Scientists
- Measures of Association II – Practical Epidemiology for Basic Scientists
- Temporal Exploration – Spatial analysis I: ArcGIS
- Spatial data issues – Spatial analysis II: Spatial data technologies
- Computing resources for spatial data – Spatial analysis II: Spatial data technologies
- GIS and public health – Medical Geography

INTERNATIONAL EXPERIENCE

Global Health Established Field Placement in Macha, Zambia; Johns Hopkins Center for Global Health (June 2013-August 2013)

- Developed an IRB amendment to implement a human movement study, using GPS data loggers, within an existing malaria study in Macha, Zambia
- Developed study materials for a human movement study, using GPS data loggers; including information pamphlets, consent documents, and databases.
- Trained study staff and field teams on the human movement study and use of GPS data loggers.
- Worked with the study staff and field teams to ensure cultural sensitivity, as well as the purpose of the movement study amongst study participants.
- Optimized protocols for molecular assays used in determining malaria parasite diversity
- Trained laboratory staff on how to perform molecular assays used in determining malaria parasite diversity.
- Developed databases for the recording of results from molecular assays.
- Collaborated with laboratory scientists from the Zambian National Malaria Control Center (NMCC) to standardize methods and protocols for molecular assays between the Macha study site and the NMCC to aid in future results comparisons.

United States Peace Corps Mozambique, Southern Africa (September 2005 – November 2007)

Secondary School Biological Sciences Education Volunteer

- Worked in cooperation with the Mozambican Ministry of Education and Culture, as well as the faculty of Armando Emilio Guebuza Secondary School in the community of Catandica.
- Taught Biology at the pre-university level and acted as the head of the Biology Department, overseeing colleagues as well as advising student teachers.
- Developed Chemistry and Biology Laboratories, trained all colleagues in the methods of proper care and use of equipment, in doing so facilitated the integration of the practical into the theory.
- Organized and executed local and regional science fairs to promote science and technology in the school system by working on the Science Fair Organizational Commission of Peace Corps Mozambique, in collaboration with the Mozambican Organization for Science and Technology.
- Promoted development relating to gender ideologies on the local and national level through starting girls' and boys' clubs and orchestrating conferences which discussed the impact of gender roles on society, focusing on the spread of HIV as well as other sexually transmitted diseases prevalent in the country.
- Trained and worked with local community health activists to promote awareness to the impact of HIV on their community.
- Participated in the Peer Support Network, trained in crisis counseling, providing support and assistance to other volunteers in country.

HONORS AND AWARDS

Johns Hopkins Malaria Research Institute Pre-doctoral Fellowship 2015, Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health

Louis I. and Thomas D. Dublin Award 2014, Departments of Biostatistics and Epidemiology, Johns Hopkins Bloomberg School of Public Health

Abraham Lilienfeld Teaching Assistantship 2013, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

Miriam E. Brailey Scholarship 2013, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

Global Health Established Field Placement 2013, Johns Hopkins Center for Global Health

New England Scholar 2005, University of Connecticut

PUBLICATIONS

Searle KM, Hamapumbu H, Lubinda J, Shields TM, Pinchoff, J, Kobayashi T, Stevenson JC, Bridges DJ, Larsen DA, Thuma PE, and Moss WJ, for the Southern Africa International Centers of Excellence for Malaria Research. Operational challenges during early implementation of reactive screen-and-treat and implications of simulated reactive case detection strategies for malaria elimination in a region of low transmission in southern Zambia. *Malaria Journal*. *Submitted*.

Sutcliffe CG, **Searle KM**, Matakala HK, Biomed D, Greenman M, Rainwater-Lovett K, Thuma PE, Moss WJ. Measles and rubella seroprevalence among HIV- infected and uninfected Zambian youth. *PIDJ. Submitted.*

Shields TM, Pinchof J, Lubinda J, Hamapumbu H, **Searle K**, Kobayashi T, Thuma PE, Moss WJ, Curriero FC. Spatial and temporal changes in household structure locations using high-resolution satellite imagery for population assessment: an analysis of household locations in southern Zambia between 2006 and 2011. *Geospatial Health, In Press.*

Searle KM, Shields T, Hamapumbu H, Kobayashi T, Mharakurwa S, Thuma PE, David L. Smith DL, Glass G, Moss WJ. Efficiency of household reactive case detection for malaria in rural southern Zambia: simulations based on serial cross-sectional surveys. *PLoS ONE* 8(8): e70972. doi:10.1371/journal.pone.0070972

Cho KI, **Searle K**, Webb M, Yi H, Ferreira PA
Ranbp2 haploinsufficiency mediates distinct cellular and biochemical phenotypes in brain and retinal dopaminergic and glia cells elicited by the Parkinsonian neurotoxin, 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Cellular Molecular Life Sciences* (2012) 69:3511-3527

Cho KI, Yi H, Tserentsoodol N, **Searle K**, Ferreira PA. Neuroprotection resulting from insufficiency of RANBP2 is associated with the modulation of protein and lipid homeostasis of functionally diverse but linked pathways in response to oxidative stress. *Disease Models and Mechanisms* 2010 Sep-Oct; 3(9-10):595-604.

Walsh MM, Yi H, Friedman J, Cho KI, Tserentsoodol N, McKinnon S, **Searle K**, Yeh A, Ferreira PA. Gene and protein expression pilot profiling and biomarkers in an experimental mouse model of hypertensive glaucoma. *Experimental Biology and Medicine.* 2009 Aug; 234(8):918-30.

Cho KI, Yi H, Yeh A, Tserentsoodol N, Cuadrado L, **Searle K**, Hao Y, Ferreira PA Haploinsufficiency of RanBP2 is neuroprotective against light-elicited and age-dependent degeneration of photoreceptor neurons. *Cell Death and Differentiation*. 2009 Feb; 16(2):287-97.

POSTER PRESENTATIONS

Searle KM, Shields TM, Lubinda J, Hamapumbu H, Kobayashi T, Thuma PE, Curriero F, Moss WJ for the Southern Africa International Centers of Excellence for Malaria Research. Temporal instability of spatial clusters of *Plasmodium falciparum* infection in an area of low malaria transmission in southern Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Searle KM, Katowa B, Kobayashi T, Siame M, Mharakurwa S, Moss WJ for the Southern Africa International Centers of Excellence for Malaria Research. Patterns of *Plasmodium falciparum* genotypes in an area of low malaria transmission in southern Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Pringle J, Das S, Kobayashi T, **Searle KM**, Siame M, Katowa B, Chaponda M, Muleba M, Mulenga M, Norris D, Moss WJ. *Plasmodium falciparum* Multiplicity of Infection Pre- and Post-Vector Control Campaigns in Nchelenge District, Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Laban NM, Kobayashi T, Hamapumbu H, **Searle KM**, Thuma P, Moss WJ. The asymptomatic malaria reservoir in a region of declining malaria transmission in Southern Province, Zambia: demographic characteristics and associated risk factors. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Pinchoff J, **Searle KM**, Shields TM, Hamapumbu H, Kobayashi T, Laban NM, Thuma PE, Moss WJ, Curriero FC. Efficiency of reactive case detection with focal drug administration for malaria elimination: simulation study based on cross-sectional surveys in rural southern Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Hast MA, Chaponda M, **Searle KM**, Lupiya J, Lubinda J, Kobayashi T, Shields TM, Mulenga M, Curriero FC, Moss WJ. The use of GPS data loggers to describe spatio-temporal movement patterns and correlations with malaria risk in an area of hyperendemic malaria in northern Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Hast MA, Chaponda M, Bridges DJ, Larsen D, **Searle KM**, Lupiya J, Kobayashi T, Shields TM, Mulenga M, Curriero FC, Moss WJ. The effectiveness of a targeted indoor residual spray campaign with pirimiphos-methyl in Nchelenge District, northern Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2015.

Searle KM, Lubinda J, Shields T, Hamapumbu H, Curriero F, Thuma P, and Moss WJ. Use of GPS data loggers to describe spatio-temporal human movement patterns in an area of effective malaria control in southern Zambia. Poster presented at the American Society for Tropical Medicine and Hygiene conference 2014.

Sutcliffe C, **Searle K**, Matakala H, Greenman M, Rainwater-Lovett K, Thuma P, Moss WJ. Measles and rubella seroprevalence among HIV-infected and uninfected Zambian children and adolescents. Poster presented at the Johns Hopkins Vaccine Day 2014.

Searle KM, Lubinda J, Shields T, Hamapumbu H, Moss WJ. Use of GPS data loggers to track human movement patterns in an area of effective malaria control in southern Zambia. Poster presented at the Johns Hopkins Malaria Research Institute World Malaria Day conference 2014.

ACADEMIC SERVICE

Epidemiology Student Organization Curriculum Committee Chair. Academic year 2014-2015
Infectious Disease Epidemiology Journal Club Student Coordinator. Academic year 2012-2013
Epidemiology Student Organization Sports Chair. Academic year 2012-2013
Johns Hopkins University Peace Corps Group. Active member, academic year 2012-2013

FOREIGN LANGUAGES

Portuguese – Advanced high level of speaking, reading and writing