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PREVALENCE, HETEROGENEITY OF ASYMPTOMATIC MALARIA INFECTIONS AND ASSOCIATED FACTORS IN A HIGH TRANSMISSION REGION
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PREVALENCE, HETEROGENEITY OF ASYMPTOMATIC MALARIA INFECTIONS AND ASSOCIATED FACTORS IN A HIGH TRANSMISSION REGION

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ABSTRACT

Background: **Although current reports have shown a reduction in malaria cases, the disease still remains a major public health problem in Kenya. In most endemic regions, the majority of infections are asymptomatic which means those infected may not even know and yet they remain infectious to the mosquitoes. Asymptomatic infections are a major threat to malaria control programs since they act as silent reservoirs for the malaria parasites.**

Objective: **The study sought to determine the prevalence of asymptomatic malaria infections, whether they show heterogeneity spatially, across age groups and across time as well as their determinants in a high transmission region.**

Study Design: **This was part of a larger prospective cohort study on malaria indices in the HDSS.**

Study Setting: **The study was conducted in the Webuye Health and Demographic Surveillance Site in Bungoma East Sub-County.**

Study Subjects: **Quarterly parasitological surveys were conducted for a cohort of 400 participants from randomly selected households located in known fever "hotspots" and "coldspots". Follow-up of all the participants continued for a period of one year. Generalized estimating equations were used to model risk factors associated with asymptomatic parasitemia.**

Results: **Of the total 321 malaria infections detected during the five cross-sectional surveys conducted over the period of one year, almost half (46.3%) of these were asymptomatic. Overall, most of the asymptomatic cases (67%) were in households within known fever "hotspots". The proportion of infections that were asymptomatic in the coldspots were 73.1%, 31.8%, 13.3%, 55.6% and 48.2% during the first, second, third, fourth and fifth visits respectively. In the known fever "hotspots", the proportion of infections without symptoms was 47.7%, 48.5%, 35%, 41.3% and 47.5% during the first, second, third, fourth and fifth visits respectively. Factors associated with asymptomatic malaria include; the village one lives: people living in village M were twice likely to be asymptomatic (A.O.R:**

2.141, C.I: 0.03 - 1.488), age: children aged between 6 to 15 years were more than twice likely to be asymptomatic (A.O.R: 2.67, C.I. 0.434 - 1.533) and the season: infections during the dry season (January) were less likely to be asymptomatic (A.O.R: 0.26, C.I: -2.289 - 0.400).

Conclusion: **The prevalence of asymptomatic infections in this region is still very high. The highest proportion of asymptomatic infections was registered in a fever coldpspot village which may explain why the village is a fever coldspot in the first place. There is a need for active surveillance to detect the asymptomatic cases as well as treat them in-order to reduce the reservoir. Targeting interventions to the asymptomatic individuals will further reduce the transmission within this region.**

INTRODUCTION

Malaria is a globally important parasitic disease. It is currently responsible for the highest morbidity and mortality among infectious diseases in Sub-Saharan Africa. In endemic regions, children below five years bear the greatest brunt of the disease presenting with a very high risk of morbidity and mortality from the disease [1, 2]. Although there has been a lot of progress in controlling malaria in Kenya, the disease still remains a major public health problem which requires urgent attention [3]. Currently, more than 25 million Kenyans are at risk of malaria every year with about 50% of outpatient attendance being attributed to the disease [3].

Although current reports [4-6] indicate a general reduction in the number of malaria infections, there is a possibility that these current estimates might not be a true reflection of the actual magnitude of malaria infections. This is mainly because facility data is the primary component of these statistics, yet majority of those infected with malaria parasites remain asymptomatic and are therefore not likely to seek health care at a health facility [7]. Asymptomatic parasitemia refers to the detection of asexual or sexual parasites and an absence of any acute clinical symptoms of malaria (usually fever) during a specified time frame. Carriers of the plasmodium parasite though asymptomatic are still able to transmit their infection to a mosquito and therefore act as reservoirs for the malaria infection. They maintain infections during the dry seasons and fuel the infection during the high transmission season [8, 9].

Asymptomatic parasitemia mainly develops because individuals in highly endemic regions are exposed to malaria frequently and as a result develop partial immunity which protects them from developing acute clinical symptoms of malaria [8]. According to Greenwood [10], attitudes to the treatment of a case of malaria may also contribute to local variations in asymptomatic cases of malaria. Communities where individuals seek effective treatment promptly when sick are likely to have fewer cases of asymptomatic cases of malaria compared to a neighboring community where infections are treated with less effective drugs.

High prevalence of asymptomatic malaria has been shown to occur mainly in endemic regions [7]. This poses a great challenge to elimination efforts because individuals who don't have symptoms are not likely to seek treatment and yet they remain infectious [11]. Previous studies have shown that asymptomatic patients are able to infect

mosquito vectors and also remain infectious longer than the treated symptomatic patients [12].

Most malaria programs have passive malaria surveillance where they mainly detect malaria cases at health facilities and they put them on treatment. The challenge arises with those who do not develop any symptoms and therefore do not present to health facilities for treatment or seek any other form of treatment. These remain as reservoirs for the parasite for a long time and keep re-infecting the mosquitoes which infect the humans. They cannot be detected unless there is active surveillance of cases [13]. This cycle continues and therefore could actually contribute to the persistence of malaria in an area. In addition, individuals with asymptomatic infections may suffer other adverse effects on their health such as chronic anemia, increased tendency to get bacterial infections, slowing mental development among others [14].

Heterogeneity of asymptomatic malaria infections as well as their determinants remains a wide not extensively explored area. This study aimed to determine the prevalence of asymptomatic infections, whether they show heterogeneity spatially, across age groups and across time as well as their determinants in a high transmission region.

METHODS

Study Design

This was a closed prospective cohort study carried out among all household members in selected households within the HDSS in Bungoma East Sub- County for a period of one year.

Study Site

This study was carried out in Bungoma East Sub-County in Bungoma County. Bungoma County is located in the Western part of Kenya 380km west of Nairobi. The County borders Uganda to the West and lies between latitude 0 25.3′ and 0 53.2′ north and longitude 34 21.4′ and 35 04′ East. The total land area is about 3032 km2 [15]. The main inhabitants of Bungoma County are the Luhya, mainly the Bukusu sub-ethnic group. The county has a total population of about 1.37 million [16] with an average density of about 453 persons/km2. More than 60% of the people live below poverty line with no access to social amenities such as water and electricity [15, 17].

Data Collection

This study was conducted as part of a larger prospective cohort study that was set up within the HDSS located in Bungoma East sub-County to investigate malaria transmission indices. Quarterly parasitological surveys were conducted for a cohort of 400 participants from randomly selected households in six sentinel villages representing known fever 'hotspots' and "cold spots". Follow-up was done for all the household members who had been enrolled into the study during the first visit. Trained research assistants visited each of the selected households every three months for a period of one year. During each visit, the head of the household was requested for a written consent to access the household. Each household member had to provide written consent before the testing for malaria was done.

Assessment of Asymptomatic status

Asymptomatic malaria in this study was defined as anybody who had confirmed parasitemia by RDT but had no recent history of symptoms and/or signs of malaria and had not taken antimalarial treatment in the last two weeks. All signs and symptoms were self-reported, or reported by the guardian for minor children. Individuals were tested and those who were found positive after an RDT test were asked if they had any symptoms of malaria on that very day or recently. Those who didn't have symptoms were therefore recorded as asymptomatic. Those who said yes were

further asked to state the symptoms. All household members who were positive for malaria irrespective of whether they had symptoms or not were treated using Artemether Lumefantrine using the National guidelines while those who were sick but did not test positive for malaria were referred to the nearest health centers for treatment.

Data Entry and Analysis

Data were collected using an electronic questionnaire on android mobile devices. Data were entered on the same platform during each quarter. After the data collection process, data were extracted from the phones, each form was checked for completeness and any missing information verified before compiling the quarterly data sets. These quarterly survey data sets were then merged into one data set at the end of the study. Descriptive statistics were used to describe the demographic characteristics of the participants. Generalized estimating equations were used to model risk factors associated with asymptomatic parasitemia and account for correlation between repeated observations on the same individuals, household members and the village.

Results

Of the total 321 malaria infections detected during the five cross-sectional surveys over the course of one year, almost half (46.3%) were asymptomatic infections. Overall, most of these asymptomatic cases (67%) were in the fever hotspots.

Prevalence of Asymptomatic Malaria Infections: The proportion of asymptomatic parasitemia in the fever cold spot was 73.1%, 31.8%, 13.3%, 55.6% and 48.2% during the first, second, third, fourth and fifth visits respectively. In the fever hotspots, the proportion was 47.7%, 48.5%, 35%, 41.3% and 47.5% during the first, second, third, fourth and fifth visits respectively. The highest proportion of asymptomatic cases (both among asymptomatic cases only and also total infections) was between the ages of 6 to 14 years. Interestingly, the older age group (above 50 years) in this cohort was mostly symptomatic (Table 1).

Asymptomatic infections varied across villages as well. The highest number of asymptomatic infections was recorded in one fever hotspot village as well as one fever cold spot village. One of the known fever cold spot village did not have even a single asymptomatic case. All infected cases in this village presented with symptoms.

Table 1

Proportion of Asymptomatic Malaria infections by Age

Age Category	Percent of infections that were asymptomatic (%)
0-1 year	30
2-5 years	39.6
6-10 years	64.7
10-14 years	60.0
14-21 years	53.5
21-30 years	28.0
30-40 years	21.5
40-50 years	15.4
>50 years	8.4

Asymptomatic Infections by Visit Number/Season

In general, the highest proportion of asymptomatic infections was during the first and last visit although this coincides with an equally high proportion of the total number of malaria infections (Table 2). The two

visits were conducted in July which coincides with the main rainy season. We note, however, the highest proportion of asymptomatic infections (this is in relation to the total malaria infections for that particular quarter and coincides with the dry season, table 2)

Table 2
Distribution of Asymptomatic Infections Over the study Surveys (Seasonal Variations)

	Visit Number				
Asymptomatic infections	**1 (July)**	**2 (Oct)**	**3 (Jan)**	**4 (April)**	**5 (July)**
Yes	22.8%	17.8%	14.4%	13.9%	31.1%
No	32.3%	14.8%	5.8%	14.2%	32.9%

Incidence of Asymptomatic Infections by Village

A fever coldspot village had the highest incidence of asymptomatic cases (29.1 per 1000 person months) followed by fever hotspot villages (22.2 per 1000 person months) (Table 3). ANOVA test was used to check whether there were any differences in the incidence of asymptomatic cases in the villages. The study did not find any statistically significant differences in asymptomatic infections between the villages (p value = 0.68).

Table 3
Incidence of asymptomatic Malaria Infections by Village

Village	Incidence Rate per 1000 person months
Fever hotspot village K	14.3
Fever cold spot village L	0
Fever cold spot village M	29.1
Fever hotspot village N	0
Fever hotspot village S	22.2
Fever hotspot village W	8.4

Factors Associated with Asymptomatic Parasitemia in Fever cold spots and hotspots

A univariate Generalised estimating Equation model (GEE model) controlling for repeated measures and clustering was fitted to determine factors that were associated with asymptomatic parasitemia. Thereafter, a multi-variable adjusted GEE model was fitted to control for confounders and therefore determine factors associated with asymptomatic status in patients who had tested positive for malaria but did not present with any symptom. The Univariate model identified the following factors: Village; People living in village L (cold spot) were 30% less likely to be asymptomatic (O.R: 0.70, C.I; -1.178 - 0.484), region: People living in the hotspots were 5% less likely to be asymptomatic (OR: 0.95, C.I-0.533 - 0.434), age in years: Children above five years were more than twice likely to be asymptomatic when they get a malaria infection (O.R: 2.66, C.I; 0.439 1.518) and the visit number/season: During the third visit which was conducted in January, people were 69% less likely to be asymptomatic (OR: 0.31, C.I; -2.008 - -0.311) (table 13 below).

Table 4
Univariate Logistic Regression GEE model for Factors Associated with Asymptomatic Parasitemia (Unadjusted model)

Variable	Unadjusted Odds Ratio	P value	Unadjusted Odds Ratio (95% CI)	
Village				
Village K	1			
Village L	0.70	0.413	-1.178	0.484
Village M	1.37	0.368	-0.377	1.019
Village N	0.65	0.379	-1.389	0.528
Village S	1.68	0.126	-0.146	1.193
Village W	0.56	0.158	-1.378	0.224
Region				
Coldspot	1			
Hotspot	0.95	0.841	-0.533	0.434
Age in years				
0 – 5	1			
6 – 15	2.66	0.000	0.439	1.518
15– 30	1.02	0.941	-0.677	0.730
30 – 50	0.35	0.062	-2.106	0.050
>50	1.05	0.073	-4.000	0.179
Visit Number				
1	1			
2	0.61	0.153	-1.149	0.180
3	0.31	0.007	-2.008	-0.311
4	0.76	0.456	-0.958	0.430
5	0.78	0.388	-0.792	0.308

In the adjusted multi-variable model (Table 5 below), the village: (A.O.R: 2.14, C.I: 0.03 - 1.488), age: (A.O.R: 2.67, C.I. 0.434 -1.533) and visit number: (A.O.R: 0.26, C.I: -2.289 - 0.400) are the main determinants for asymptomatic parasitemia in the community.

Table 5
Multi-Variate GEE Logistic Regression model for Factors Associated with Asymptomatic Parasitemia (Adjusted model)

Variable	Adjusted Odds Ratio	P value	Adjusted Odds Ratio (95% CI)	
Village				
Village K	1			
Village L	0.74	0.495	-1.165	0.563
Village M	2.14	0.040	0.034	1.488
Village N	0.93	0.896	-1.100	0.962
Village S	1.80	0.090	-0.092	1.269
VillageW	0.67	0.345	-1.205	0.421

Age in years				
0 – 5	1			
6 – 15	2.67	0.000	0.434	1.533
15 - 30	1.10	0.779	-0.611	0.816
30 – 50	0.43	0.126	-1.888	0.233
>50	0.14	0.077	-4.050	0.209
Visit/Season				
July,2013 (1)	1			
Oct,2013 (2)	0.60	0.191	-1.251	0.249
Jan,2014 (3)	0.26	0.005	-2.289	-0.400
April,2014 (4)	0.63	0.248	-1.239	0.320
July,2014 (5)	0.82	0.542	-0.804	0.423

DISCUSSION

Almost half of all those infected with malaria in this region are asymptomatic. This means that if they had not been tested actively during the study, these particular infections would not have been identified since those infected do not feel sick. There is a possibility that the number of asymptomatic infections could be much higher than what our study found because we used RDT for testing. Although the RDT sensitivity has been shown to be very high [18], it's not comparable to PCR [19] especially for low density infections. PCR has a sensitivity of 100% and has been documented as the most effective method for detecting low density parasitemia as well as mixed infections [20]. Therefore, there is likelihood that the very low density parasitemia might not have been detected by the RDT test hence giving a possibility of higher undetected asymptomatic cases in this area. Overall, the highest proportion of asymptomatic infections were found in the fever hotspots compared to the fever coldspots although there was no statistically significant difference between these two regions.

From this study, we can therefore deduce that the prevalence of asymptomatic infections remains very high. However, this is still lower than what has previously been documented in this area up to 93% of malaria infections without symptoms [21]. Given that there is no active surveillance within the HDSS in Bungoma East Sub-County, many people in this area are infected but remain untreated for long periods of time hence maintaining the reservoir within this population. Although hospital records can be used to infer the incidence of clinical malaria in this community, the group of infected asymptomatic individuals cannot be captured by the hospital records since they do not present to the health facilities for treatment. The presence of high asymptomatic cases in endemic regions is a common observation documented by other studies [7, 14]. Previous reports have alluded to the fact that those living in endemic regions get frequent mosquito bites and therefore frequent exposure which leads to development of partial immunity [22]. This partial immunity suppresses the parasite density so it remains below some threshold to cause symptoms [8]. Since asymptomatic individuals do not manifest with any symptoms, they do not seek treatment and therefore remain as actual parasite reservoirs who continue to transmit the infection to the general population [8]. Asymptomatic individuals remain a big threat to malaria control and malaria elimination efforts in this area and Kenya in

particular. Studies have shown that asymptomatic individuals still carry gametocytes and therefore can transmit gametes to the mosquitoes which then infect other people once they have undergone the full cycle [11, 23, 24]. Besides gametocyte carriage, the asymptomatic individual may suffer from other complications related to malaria such as anemia as the parasite continues to destroy red blood cells [25, 26]. Indeed, some studies even advocate for testing of malaria in any individual with a derangement in hematological indicators since they could also be a pointer to chronic infection with malaria [25, 27].

Besides the environmental factors that support a large vector population, asymptomatic infections may explain why malaria is persistent in this region. This evidence is supported by previous reports which attributed persistence of malaria in some of the regions to the existence of the low density parasitemia asymptomatic cases which do not get treated because the infected individuals do not show any symptoms [7, 28]. This is because asymptomatic infections act as the "silent" reservoirs and continue to fuel the infections without being cleared. It still remains unclear how long the asymptomatic parasitemia lasts before clearing from the blood without treatment, although some studies average this to about 70-90 days in children above 10 years and adults but the mean is much higher (179 days) among younger children [29]. This implies that asymptomatic individuals may remain infectious to the mosquitoes for very long periods [27, 29, 30] hence prolonging the infections in the community which is a major threat to the malaria control measures.

This study also explored factors associated with asymptomatic parasitemia in this population. Similar to what was found for malaria infections; generally there were micro-epidemiological differences in asymptomatic parasitemia at village level. The village where one lives is a significant predictor of asymptomatic status when an individual gets infected with malaria. People living in village L (which is one of the villages in the cold spot) are the least likely to be asymptomatic when they get malaria parasitemia. These small scale differences in malaria transmission have been reported before [10, 31]. Most malaria infections in this cold spot village manifested with symptoms. Individuals who manifest with symptoms are more likely to seek treatment for the infection and therefore clear the parasites and cease to be the parasite reservoir. This could partly explain why this village is a coldspot for malaria within the HDSS. On the contrary, in another fever cold spot village M, individuals were more than twice likely to be asymptomatic when they had malaria infection. This may have come about by the fact that the initial classification of villages into cold and hotspots was based on self-reported fevers. As alluded to earlier in the discussion, fevers may also signify other illnesses and not necessarily malaria [32]. Some authors have even argued that the use of fevers in estimation of clinical malaria may result in an over-estimation of clinical cases [33]. Another plausible and supported explanation by previous reports is that symptomatic hotspots are quite unstable and may keep shifting depending on various factors [9, 31, 34, 35]. Arguments against the need to target interventions to hotspots have mostly cited the changing/shifting nature of hotspots especially the febrile/symptomatic hotspots. Last but not least, the other plausible explanation is that since the village has the highest proportion of asymptomatic infections, this means that most sick individuals do not present with any symptoms (especially fever) and therefore fewer cases are detected making it a fever coldspot.

A higher proportion of asymptomatic cases were from the fever hotspots as compared to the fever coldspots although this is not statistically significant. Studies have shown that people living in the hotspots for malaria transmission have a much higher tendency to be asymptomatic reservoirs of the parasite [7, 29] . This can be explained by the fact that those in areas of high transmission tend to develop some partial immunity due to repeated exposures to the malaria parasite and this shields them from developing clinical symptoms when they get infected. This has been demonstrated by previous studies [22]. Nevertheless, there is evidence that the natural immunity acquired from the repeated exposures does not necessarily shorten the length of these asymptomatic infections [29] and therefore the infections may become chronic [27]. These persistent infections are detrimental to the affected individual posing serious health challenges through chronic low-grade hemolysis which leads to anemia as well as cognitive impairment in school going children [14, 26]. The implications on malaria control programs are enormous. The presence of high prevalence of asymptomatic infections threatens to reverse the gains already made and documented. Effective control programmes therefore need to reach everyone at risk in order to reduce the prevalence. Notwithstanding, people with no symptoms will definitely not seek treatment even with the best treatment guidelines and subsidized effective drugs yet they remain infectious. The greater challenge is tracking down all infected people and offering treatment.

The study also found that the age of an individual is a significant predictor of asymptomatic parasitemia. The younger children (below 5 years) have a much higher tendency to develop symptoms while those between 6 to 10 years are more likely to remain asymptomatic when they get malaria infection. Similar evidence has been reported elsewhere [36]. Infants (0-6months) are mostly asymptomatic when they get infected by malaria parasites because of the acquired maternal antibodies [37]. However, this passive immunity from the mother begins to wane after six months and the infant exposed to the parasite may begin to get very severe malaria infections [38]. Older infants and young children in holoendemic regions become more prone to manifestation of clinical disease after a parasite infection because they don't have immunity against the parasite [38, 39]. However, these repeated exposures to the malaria parasite enables them to acquire immunity specific to the parasite [22, 40-42] making the children semi-immune to the parasite and therefore less prone to the severe attacks. The semi-immune status makes them less prone to development of clinical symptoms whenever they get infected with the malaria parasite. Nevertheless, the acquired immunity against malaria is partial and not lifelong [22]. When semi immune individuals move out of an endemic area, the exposure reduces and therefore they begin to lose their immunity [22] and if taken back to an endemic region, they are likely to develop severe malaria. Contrary to previous studies that have indicated that older people living in malaria endemic regions are less likely to develop malaria [41] and when they do, they are not likely to present with symptoms, our results present an opposite picture. Similar to the younger children below five years, older adults infected with malaria in this cohort were more likely to present with symptoms. This is an interesting finding that may form a basis for further research on what changes take place in the partial immunity for malaria as one grows older. We interpret this with caution given the proportion of older people in our cohort was quite small. Some reports have shown greater vulnerability to malaria by older adults, however these studies have mainly been

conducted among the non-immune populations especially the tourists [43]. Age is therefore a major predictor for asymptomatic disease. Our findings are corroborated by those of other studies that have shown age as one main predictor of whether one develops symptoms or not [41, 42, 44].

These findings have implications on malaria control in a high transmission region. The younger children present with symptoms when infected and therefore are taken for treatment while older children and adults likely remain asymptomatic carriers of the parasite and therefore keep re-infecting the children in their households. Since the older children don't present with symptoms when infected, they may suffer debilitating effects of chronic infection such as chronic anemia, increased co-bacterial infections and impaired cognition that may lead to low performance in school among others [14].

Similar to other studies, we found asymptomatic status is associated with the season/timing. We recorded a high proportion of asymptomatic cases during the peak seasons (July). However, the highest proportion of asymptomatic infections in comparison to total infections was recorded in January. Some of the previous reports conducted in similar endemic settings have reported similar findings [45]. Asymptomatic infections are usually highest during the dry season mainly because of the reduced incidence of new infections and a larger proportion of infections remaining untreated from the previous season [46]. In many endemic regions, malaria transmission is seasonal peaking during the rainy season. This is because mosquito breeding is at its highest immediately after the long rains due to availability of many breeding sites [47-51]. The asymptomatic cases carried through the dry season then ignite transmission during this high transmission season. Mosquitoes bite the infected asymptomatic individuals and flare up the infection by passing it on to others [28]. Given that malaria infections during the rainy season are more likely to have high density parasitemia as compared to the dry season, this can possibly explain why majority of those infected during the rainy season manifest with symptoms.

During the rainy season, there was a higher number of symptomatic disease in the fever hotspots than the fever coldspots while during the dry seasons, there were more symptoms in the fever coldspots than the fever hotspots. This is in agreement with a study conducted in Mali that showed an increase in fevers during the rainy seasons [52]. Asymptomatic individuals in the hotspots act as reservoirs of the parasite and fuel new infections during the rainy seasons. This finding is supported by evidence from a study conducted by Bousema , Drakeley [35]. It is therefore important to identify hotspots of asymptomatic infections because they form foci of transmission where individuals with asymptomatic disease act as the main reservoirs of the parasite during the dry season and fuel new infections during the rainy seasons which spread to the neighboring areas [13, 28, 53].

CONCLUSION

The study has shown a very high prevalence of asymptomatic parasitemia in this region. Given that asymptomatic individuals do not present with clinical symptoms, they continue to act as silent reservoirs for the parasites without being eliminated. Such individuals can only be detected if there is active surveillance. Therefore, there is need to incorporate actual testing of malaria as part of the active surveillance for the county for a period of time and then design appropriate interventions for the hotspots within this larger hotspot in Western Kenya. This will reduce the reservoir and together

with the other targeted interventions, malaria transmission can be reduced with the hope of elimination.

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REFERENCES

1. Snow, RW, Guerra, CA, Noor, AM, Myint, HY and Hay, SI, The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature. 2005; 434: p. 214-7.
2. World Health Organization, W, The World Malaria Report 2005. 2005: Geneva, Switzerland.
3. Division of Malaria Control, D, Kenya Malaria Fact sheet. 2015, KEMRI: Nairobi.
4. Okiro, EA,Hay, SI,Gikandi, PW,Sharif, SK,Noor, AM,Peshu, N, et al., The decline in paediatric malaria admissions on the coast of Kenya. Malaria Journal. 2007; 6: p. 151.
5. O'Meara, WP,Bejon, P,Mwangi, TW,Okiro, EA,Peshu, N,Snow, RW, et al., Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet. 2008; 372(9649): p. 1555-62.
6. O'Meara, WP, Mangeni, JN, Steketee, R and Greenwood, B, Changes in the burden of malaria in sub-Saharan Africa. Lancet Infect Dis. 2010; 10(8): p. 545-55.
7. Bottius, E, Guanzirolli, A, Trape, J, Rogier, C, Konate, L and Druilhe, P, Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. Transactions of The Royal Society of Tropical Medicine and Hygiene. 1996; 90(1): p. 15-19.
8. Lindblade, KA, Steinhardt, L, Samuels, A, Kachur, SP and Slutsker, L, The silent threat: asymptomatic parasitemia and malaria transmission. Expert Rev Anti Infect Ther. 2013; 11(6): p. 623-39.
9. Bejon, P,Williams, TN,Liljander, A,Noor, AM,Wambua, J,Ogada, E, et al., Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. PLoS Med. 2010; 7(7): p. e1000304.
10. Greenwood , BM, The microepidemiology of malaria and its importance to malaria control. Trans R Soc Trop Med Hyg. 1989; 83 Suppl: p. 25-9.
11. Alves, FP, Gil, LHS, Marrelli, MT, Ribolla, PEM, Camargo, EP and da Silva, LHP, Asymptomatic carriers of Plasmodium spp. as infection source for malaria vector mosquitoes in the Brazilian Amazon. Journal of Medical Entomology. 2005; 42(5): p. 777–779.
12. Alves, FP, Gil, LH, Marrelli, MT, Ribolla, PE, Camargo, EP and Da Silva, LH, Asymptomatic carriers of Plasmodium spp. as infection source for malaria vector mosquitoes in the Brazilian Amazon. J Med Entomol. 2005; 42(5): p. 777-9.
13. Bousema , T, Okell, L, Felger, I and Drakeley, C, Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol. 2014; 12(12): p. 833-40.
14. Chen, I,Clarke, SE,Gosling, R,Hamainza, B,Killeen, G,Magill, A, et al., "Asymptomatic" Malaria: A Chronic and Debilitating Infection That Should Be Treated. PLoS Med. 2016; 13(1): p. e1001942.
15. District Statistics Office, B, Bungoma District Profile. 2006.
16. Kenya National Bureau of Staistics, K, Population distribution by administrative units. 2010: Nairobi,Kenya.
17. Staistics., KNBo, Population distribution by administrative units. 2010: Nairobi,Kenya.
18. Katharine, A,Jonathan, JD,Piero, LO,Cho-Min, N,Sally, MJ,Yemisi, T, et al., Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries. Cochrane Infectious Diseases Group. 2011.

19. Johnston, SP, Pieniazek, NJ, Xayavong, MV, Slemenda, SB, Wilkins, PP and da Silva, AJ, PCR as a confirmatory technique for laboratory diagnosis of malaria. J Clin Microbiol. 2006; 44(3): p. 1087-9.
20. Laban, NM,Kobayashi, T,Hamapumbu, H,Sullivan, D,Mharakurwa, S,Thuma, PE, et al., Comparison of a PfHRP2-based rapid diagnostic test and PCR for malaria in a low prevalence setting in rural southern Zambia: implications for elimination. Malar J. 2015; 14: p. 25.
21. Hamel, MJ, Odhacha, A, Roberts, JM and Deming, MS, Malaria control in Bungoma District, Kenya: a survey of home treatment of children with fever, bednet use and attendance at antenatal clinics. Bull World Health Organ. 2001; 79(11): p. 1014-23.
22. Doolan, DL, Dobaño, C and Baird, JK, Acquired Immunity to Malaria. Clinical Microbiology Reviews. 2009; 22(1): p. 13-36.
23. Bousema, JT,Gouagna, LC,Drakeley, CJ,Meutstege, AM,Okech, BA,Akim, IN, et al., Plasmodium falciparum gametocyte carriage in asymptomatic children in western Kenya. Malar J. 2004; 3: p. 18.
24. Karl, S, Gurarie, D, Zimmerman, PA, King, CH, St. Pierre, TG and Davis, TME, A Sub-Microscopic Gametocyte Reservoir Can Sustain Malaria Transmission. PLoS ONE. 2011; 6(6): p. e20805.
25. Maina, RN,Walsh, D,Gaddy, C,Hongo, G,Waitumbi, J,Otieno, L, et al., Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. Malaria Journal. 2010; 9(3): p. 1-11.
26. Newton, CR,Warn, PA,Winstanley, PA,Peshu, N,Snow, RW,Pasvol, G, et al., Severe anaemia in children living in a malaria endemic area of Kenya. Trop Med Int Health. 1997; 2(2): p. 165-78.
27. Bottius, E, Guanzirolli, A, Trape, JF, Rogier, C, Konate, L and Druilhe, P, Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. Trans R Soc Trop Med Hyg. 1996; 90(1): p. 15-9.
28. Das, NG,Dhiman, S,Talukdar, PK,Goswami, D,Rabha, B,Baruah, I, et al., Role of asymptomatic carriers and weather variables in persistent transmission of malaria in an endemic district of Assam, India. Infect Ecol Epidemiol. 2015; 5: p. 25442.
29. Bretscher, MT, Maire, N, Felger, I, Owusu-Agyei, S and Smith, T, Asymptomatic Plasmodium falciparum infections may not be shortened by acquired immunity. Malar J. 2015; 14: p. 294.
30. Agusto, FB,Del Valle, SY,Blayneh, KW,Ngonghala, CN,Goncalves, MJ,Li, N, et al., The impact of bed-net use on malaria prevalence. J Theor Biol. 2013; 320: p. 58-65.
31. Bejon, P,Williams, TN,Nyundo, C,Hay, SI,Benz, D,Gething, PW, et al., A micro-epidemiological analysis of febrile malaria in Coastal Kenya showing hotspots within hotspots. Elife. 2014; 3: p. e02130.
32. O'Meara, WP,Mott, JA,Laktabai, J,Wamburu, K,Fields, B,Armstrong, J, et al., Etiology of pediatric fever in western Kenya: a case-control study of falciparum malaria, respiratory viruses, and streptococcal pharyngitis. Am J Trop Med Hyg. 2015; 92(5): p. 1030-7.
33. Rooth, I and Björkman, A, Fever episodes in a holoendemic malaria area of Tanzania: parasitological and clinical findings and diagnostic aspects related to malaria. Transactions of The Royal Society of Tropical Medicine and Hygiene. 1992; 86(5): p. 479-482.
34. Ernst, KC, Adoka, SO, Kowuor, DO, Wilson, ML and John, CC, Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. Malar J. 2006; 5: p. 78.
35. Bousema , T,Drakeley, C,Gesase, S,Hashim, R,Magesa, S,Mosha, F, et al., Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis. 2010; 201(11): p. 1764-74.
36. Riley, EM, Wagner, GE, Akanmori, BD and Koram, KA, Do maternally acquired antibodies protect infants from malaria infection? Parasite Immunol. 2001; 23(2): p. 51-9.
37. Snow, RW, Nahlen, B, Palmer, A, Donnelly, CA, Gupta, S and Marsh, K, Risk of severe malaria among African infants: direct

evidence of clinical protection during early infancy. J Infect Dis. 1998; 177(3): p. 819-22.
38. Sehgal, VM, Siddjiqui, WA and Alpers, MP, A seroepidemiological study to evaluate the role of passive maternal immunity to malaria in infants. Trans R Soc Trop Med Hyg. 1989; 83 Suppl: p. 105-6.
39. Baird, JK, Host age as a determinant of naturally acquired immunity to Plasmodium falciparum. Parasitol Today. 1995; 11(3): p. 105-11.
40. Bruce, MC,Donnelly, CA,Packer, M,Lagog, M,Gibson, N,Narara, A, et al., Age- and species-specific duration of infection in asymptomatic malaria infections in Papua New Guinea. Parasitology. 2000; 121 (Pt 3): p. 247-56.
41. Ladeia-Andrade, S, Ferreira, MU, de Carvalho, ME, Curado, I and Coura, JR, Age-dependent acquisition of protective immunity to malaria in riverine populations of the Amazon Basin of Brazil. Am J Trop Med Hyg. 2009; 80(3): p. 452-9.
42. Eli, S, Siegal, S, Havi, M and David, R, Age as a Risk Factor for Severe Plasmodium falciparum Malaria in Nonimmune Patients. Clin Infect Dis. 2001; 33(10): p. 1774-1777.
43. Stich, A, Zwicker, M, Steffen, T, Kohler, B and Fleischer, K, [Old age as risk factor for complications of malaria in non-immune travellers]. Dtsch Med Wochenschr. 2003; 128(7): p. 309-14.
44. Frederick , NB,Yaw, AA,Dolphine, AA,Mariangela, B,David, MM,Goufa, Z, et al., High Prevalence of Asymptomatic Plasmodium falciparum Infections in a Highland Area of Western Kenya: A Cohort Study. The Journal of Infectious Diseases. 2009(200): p. 66–74.
45. Dhiman, S, Goswami, D, Rabha, B, Yadav, K, Chattopadhyay, P and Veer, V, Absence of asymptomatic malaria in a cohort of 133 individuals in a malaria endemic area of Assam, India. BMC Public Health. 2015; 15: p. 919.
46. Geiger, C,Agustar, HK,Compaore, G,Coulibaly, B,Sie, A,Becher, H, et al., Declining malaria parasite prevalence and trends of asymptomatic parasitaemia in a seasonal transmission setting in North-Western Burkina Faso between 2000 and 2009-2012. Malar J. 2013; 12: p. 27.
47. Singh, N and Sharma, VP, Patterns of rainfall and malaria in Madhya Pradesh, central India. Ann Trop Med Parasitol. 2002; 96(4): p. 349-59.
48. Briet, OJ, Vounatsou, P, Gunawardena, DM, Galappaththy, GN and Amerasinghe, PH, Temporal correlation between malaria and rainfall in Sri Lanka. Malar J. 2008; 7: p. 77.
49. Galardo, AK,Zimmerman, RH,Lounibos, LP,Young, LJ,Galardo, CD,Arruda, M, et al., Seasonal abundance of anopheline mosquitoes and their association with rainfall and malaria along the Matapi River, Amapa, [corrected] Brazil. Med Vet Entomol. 2009; 23(4): p. 335-49.
50. Hamad, AA,Nugud Ael, H,Arnot, DE,Giha, HA,Abdel-Muhsin, AM,Satti, GM, et al., A marked seasonality of malaria transmission in two rural sites in eastern Sudan. Acta Trop. 2002; 83(1): p. 71-82.
51. Binka, FN, Morris, SS, Ross, DA, Arthur, P and Aryeetey, ME, Patterns of malaria morbidity and mortality in children in northern Ghana. Trans R Soc Trop Med Hyg. 1994; 88(4): p. 381-5.
52. Dicko, A,Mantel, C,Kouriba, B,Sagara, I,Thera, MA,Doumbia, S, et al., Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. Trop Med Int Health. 2005; 10(6): p. 550-6.
53. Bousema , T,Griffin, JT,Sauerwein, RW,Smith, DL,Churcher, TS,Takken, W, et al., Hitting hotspots: spatial targeting of malaria for control and elimination. PLoS Med. 2012; 9(1): p. e1001165