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MALARIA "HOTSPOTS" WITHIN A LARGER HOTSPOT; WHAT'S THE ROLE OF BEHAVIOURAL FACTORS IN FINE SCALE HETEROGENEITY IN WESTERN KENYA?
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J.N. Mangeni, D. Ongore, A. Mwangi, J. Vulule, A. Ocala and W.P. O'Meara

ABSTRACT

Background: **Malaria remains a major public health problem in Kenya accounting for the highest morbidity and mortality especially among children. Previous reports indicate that infectious agents display heterogeneity in both space and time and malaria is no exception. Heterogeneity has been shown to reduce the effectiveness of interventions. Previous studies have implicated genetic (both human and parasite) and environmental factors as mainly responsible for variation in malaria risk. Human behaviour and its potential risk for contributing to variation in malaria risk has not been extensively explored.**

Objective: **To determine if there were behavioural differences between the people living in hotspots (high malaria burden) and cold spots (low malaria burden) within a geographically homogeneous and high malaria transmission region.**

Design: **A prospective closed cohort study.**

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

Subjects: **A total of 400 people in randomly selected households in both the fever hotspots and cold spots were tested for malaria at quarterly intervals using malaria rapid diagnostic tests (RDTs).**

Results: **Significant heterogeneity in malaria incidence and prevalence was observed between villages. Incidence of malaria was significantly higher in the hotspots (high malaria burden areas) compared to the cold spots (low malaria burden) (49 episodes per 1000 person months compared to 26/1000, ttest $p < 0.001$). The incidence also varied significantly among the individual villages by season (P: 0.0071). Knowledge on malaria therapy was significantly**

associated with whether one was in the cold spot or hotspot (P: 0.033). Behavioural practices relating to ITN use were significantly associated with region during particular seasons (P: 0.0001 and P: 0.0001 respectively).
Conclusion: **There is marked and significant variation in the incidence of malaria among the villages creating actual hotspots of malaria within the larger hotspot. There is a significant difference in malaria infections between the hotspots and cold spots. Knowledge on malaria therapy and behavioural factors such as ITN use may contribute to the observed differences during some seasons.**

INTRODUCTION

Malaria is a parasitic infection transmitted by the bite of infected anopheline mosquitoes and is endemic in most countries within sub-Saharan Africa. In Kenya, malaria remains a major public health problem accounting for the highest morbidity and mortality especially among children (1- 3). However, recent reports indicate a considerable decline in transmission of malaria in some parts of the country (4-7). Unfortunately this decline is not consistent across all regions; some endemic regions are still experiencing persistently high incidence of malaria while others have had resurgence after years of effective control (8-10).

Studies have shown that there exist variations in transmission of infectious agents even at very small scale (11). Malaria like other infectious diseases demonstrates heterogeneity in its transmission in terms of space and time (12-14). The variation may exist across a wide spectrum of transmission intensity; low, moderate and even high intensity transmission (15). Geographic heterogeneity results in apparent clustering of higher than average transmission forming “hotspots’ of transmission. Hotspots are important because they may maintain malaria infections during the low transmission seasons and fuel further infections during the high seasons (16). This presents a challenge in terms of controlling malaria since such hotspots are often difficult to identify unless adequate surveillance systems are in place.

The causes underlying these small scale variations at the village level are not fully understood and are likely complex and multifaceted. However, some previous studies have implicated environmental factors and genetics (both human and parasite) as playing a major role in determining heterogeneity in malaria transmission (12). Although heterogeneity in human behaviour is also a potential factor that could result in significant spatial variation in malaria risk, it has not been extensively studied. This study was part of a larger study that investigated small scale malaria variations within the Health and Demographic Surveillance Site in Bungoma County. This particular paper reports on the possible role of behaviour in explaining fine scale heterogeneity of malaria.

MATERIALS AND METHODS

Study Design

This was a prospective closed cohort study where all the household members in the selected households were included in the study and followed up for a period of one year.

Study Site

The study was conducted in the Webuye HDSS located in Bungoma East sub-County. The region has been ravaged by malaria for very many years (17). The Webuye HDSS was established in 2007 to provide reliable demographic, health and economic information for planning as well as provide a platform for health research (18).

Sample Size Calculation

Sample size was calculated using SAS macro based on the poisson regression model used for calculations of sample size for recurrent events (Kuolung Hu, 2008).

The primary outcome in this study is the incidence of malaria R0 = baseline rate, Exp (Bo);

RR = Rate Ratio, denoted as exp (B1)/exp (BO) tt = exposure length

Alpha = alpha level Side =1 or 2

PO =subject allocation factor for the compared (B1), the range of values is (0,1)

Baseline incidence of fevers : 1.2 episodes per person per year

Risk ratio: 1.5

Exposure length: 1 year

Side: 2 sided Power (80%)

Subject allocation factor: 0.67 (2 subjects in the high and 1 subject in the low).

Total sample size = 174 participants

Adjusting sample size for loss to follow up (usually 1.2): (174)1.2 = 209

Adjustment for correlation within clusters (design effect) of 1.7: (209) = 356

There are a total of 73,000 people in the HDSS with a total of 13,000 households.

Since the study was to be conducted at household level, we calculated the approximate number of persons per household by dividing the total population by the number of households = 73,000/13,000 Each household in HDSS has approximately 6 persons. Total number of households for interview = 356/6

Therefore we needed about 60 households for this study. However, we increased these households to 72 households which increased our study population to about 400 persons.

Sampling Procedure

Since this study was part of a larger study that investigated malaria transmission indices in Bungoma East sub-County, we used the sample size and sampling procedure from the main study.

Multi-stage sampling was done. At the first level, six villages were selected purposively based on the spatial clustering of fevers, four in the fever “hotspots” and two in the fever “cold spots”. Longitudinal surveys on self-reported fevers within the HDSS indicated some form of spatial clustering of fever (presumed to be the key malaria symptom) in particular villages while some of the villages had lower than average incidence fevers. In order to explore heterogeneity in malaria burden in the HDSS, we selected six sentinel villages with different levels of fever incidence that were representative of the range of incidence across the study area (19).

At the second stage, one household from each of the villages was selected randomly and a window trap fixed. Two other households within the same village were selected for PSC method. All households selected for entomological surveillance also participated in parasitological surveillance. Three other households closest to each of entomological households were included in the parasitological surveillance (this study only reports the parasitology data in relation to human behaviour - the rest is reported elsewhere).

In total, 400 people from 72 households across the six villages were enrolled and (average per village was 12 households with around 60 persons) were tested for malaria at quarterly intervals for a period of one year (a total of 5 time points). An incident episode of malaria was defined as a new episode diagnosed on testing with RDT or admission by the participant that they had malaria confirmed by the doctor in the last three months (In which case, we had to check the hospital card to confirm that malaria diagnosis was made at a health facility after confirmatory testing for malaria parasites). If a participant tested positive during a quarterly surveillance round but had initiated treatment with anti-malarial in the previous two weeks, this was censored and only the previous episode was recorded.

Data Collection

Data was collected during a face-to-face interview using an electronic questionnaire programmed on the open data platform kit (ODK) on android phones. During each quarterly visit, the head of the household was asked to consent for the household before the interviews were carried out or any testing was initiated. The household heads were then informed that the team would come back after three months. When household members were missing, the field assistants would return to the household at least two to three times until they met the missing members.

Household members who were 18 years and above were asked questions relating to their knowledge on malaria and behaviours that could put them at risk for malaria infections. In addition, all household members were tested for malaria parasites quarterly for a period of one year. We also collected information on ownership and use of ITNs.

Data Management and Analysis

At the end of each round of data collection, the data were reviewed for completeness of the information. Where there was missing data, the field assistants were asked to return to the household and complete the information. The complete electronic questionnaires were compiled and transferred to Stata version 12 for cleaning and preliminary analysis at the end of each quarter.

Data was described using charts and frequency tables. Prevalence of malaria was calculated by round, by region and by village. The incidence of malaria was calculated using survival analysis for repeated events. Chi square test was used to check if there was an association between behavioural factors and region. After examining malaria incidence and prevalence by village, we categorized villages with total incidence above 30/1000 person months as 'hotspots' (sites with higher burden).

ETHICAL CONSIDERATIONS

This was a sub-study within the MESA funded malaria study in Western Kenya. The study was approved by the Moi University/MTRH Institutional review ethics Committee (IREC) via an amendment No. 000778 and also UON/ KNH Institutional Review Board refNo. KNH-ERC/A/298. Additionally, after explaining the importance of the study, an informed written consent was obtained from the head of the household. All household members were also requested for informed written consent before RDT was done. Further, children were asked to assent before their parents/guardians could consent for them. Everyone who was found to have malaria was treated using the current Ministry of Health treatment guidelines.

RESULTS

Demographic Characteristics of the Participants

i) Age Distribution of the Participants by Region

A total of 400 people (all age-groups) were observed quarterly for a period of twelve months. There was no significant difference in the age distribution of participants between the cold spots and hotspots. Majority of the participants were children between the ages of 6 - 10 years (See Table 1 below).

Table 1
Age Distribution of the participants By Region (Frequencies and Percentages)

Age Category	Cold spot	Hotspot	Total
0 – 1	5 (3.62)	6 (2.43)	11 (2.86)
2 - 5	26 (18.84)	38 (15.44)	64 (16.67)
6 – 10	27 (19.56)	52 (21.13)	79 (20.57)
11 -14	14 (10.14)	26 (10.56)	40 (10.41)
15 -21	19 (13.76)	37 (15.04)	56 (14.58)
22 -30	19 (13.76)	22 (8.94)	41 (10.67)
31-40	11 (7.97)	29 (11.78)	40 (10.41)
41-50	8 (5.79)	15 (6.09)	23 (5.98)
>50	9 (6.52)	21 (8.53)	30 (7.81)
Total	138 (100)	246 (100)	384 (100.0)

Percentage in parenthesis

ii) Education of the Participants by Region (Only Adults and Older Children)
Most of the adult participants had primary school education level. There were no major differences in education between the cold spots and hotspots (Table 2)

Table 2
Education Level of the Participants by Region (Frequencies and Percentages)

Education status	Cold spot		Hotspot		Total	
None	1	(1.03)	6	(3.44)	7	(2.58)
Not Applicable	1	(1.03)	0	(0.00)	1	(0.36)
Pre-primary	1	(1.03)	0	(0.00)	1	(0.36)
Primary-some	61 (62.88)		113	(66.08)	174	(64.2)
Primary-finished	10	(10.3)	26	(14.94)	36	(13.28)
Secondary-some	9	(9.27)	14	(8.04)	23	(8.48)
Secondary-finished	8	(8.24)	10	(5.74)	18	(6.64)
post secondary_some	1	(1.03)	1	(0.57)	2	(0.73)
Post-secondary_ finished	5	(5.15)	4	(2.29)	9	(3.32)
Total	97	(100)	174	(100)	271	

**Percentage in Parenthesis*

iii) Employment Status (Adults Only)
Most of the participants were not formally employed. Most people engage in farming for their household consumption.

Table 3
Employment status of Respondents by Region (Adults only, children were excluded)

Employment status	Colds pot		Hotspots		Total	
Below Employment age	14	(17.94)	21	(17.64)	35	(17.76)
Employed	6	(7.69)	11	(9.24)	17	(8.62)
Self-employed	18	(23.07)	19	(15.96)	37	(18.78)
Manual labourer	9	(11.53)	14	(10.92)	23	(11.16)
Unemployed	28	(35.89)	50	(42.01)	78	(39.59)
Retired	3	(3.84)	4	(3.36)	7	(3.55)
Total	78	(100)	119	(100)	197	(100)

Household Characteristics

i) ITN ownership

Every household in both the fever hotspot and fever cold spots had at least one Insecticide Treated Net (ITN). This however does not imply that each individual has access to an Insecticide Treated Net (ITN).There were a total of 167 sleeping spaces, of which 125 (74.8%) had an ITN while 42 (25.2%) did not. There were no statistically significant differences in the proportion of sleeping spaces that had an ITN between the hotspots and cold spots P= 0.742).

Most of the ITNs (68%) were acquired free of charge during the mass ITN campaign in the area that was conducted in 2011. In addition, some families were also able to acquire more ITNs freely during antenatal visits and when they took their babies to child welfare clinics during the postnatal period. About 9% of the participants reported having bought additional ITNs at an approximate cost of between Ksh.50 and Ksh.150. In some of the cases, some people sold the same ITNs that they had been given freely at a small cost of only ksh.50. All the ITNs acquired during the campaigns were Long Lasting Insecticide Nets (LLINs) and were therefore already treated at the time they were acquired. Despite the fact that the nets were already treated, 7% of the respondents tried to retreat their nets even though this is not recommended for LLINS.

Incidence and prevalence of Malaria

The incidence of malaria was much higher (49/1000 person months) in the designated hotspots (actual high malaria burden area) compared to the cold spots (26/1000 person months). This difference was statistically significant (ttest p < 0.001).

Prevalence of Malaria by Region

Prevalence of malaria was consistently higher in hotspots (actual higher malaria burden areas) compared to cold spots (actual lower malaria burden areas).

Figure 1
Incidence of Malaria by Village

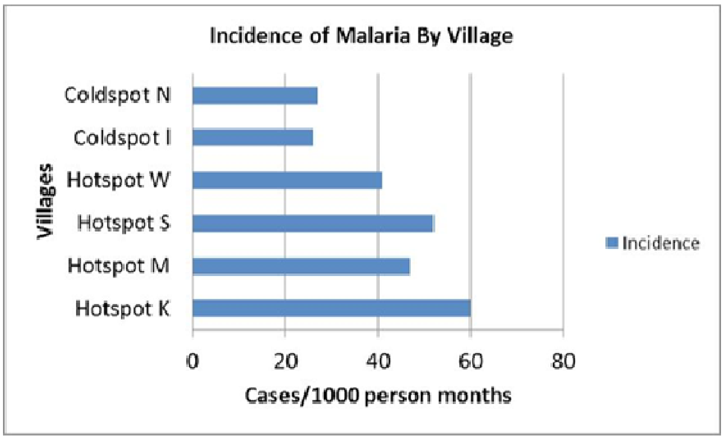
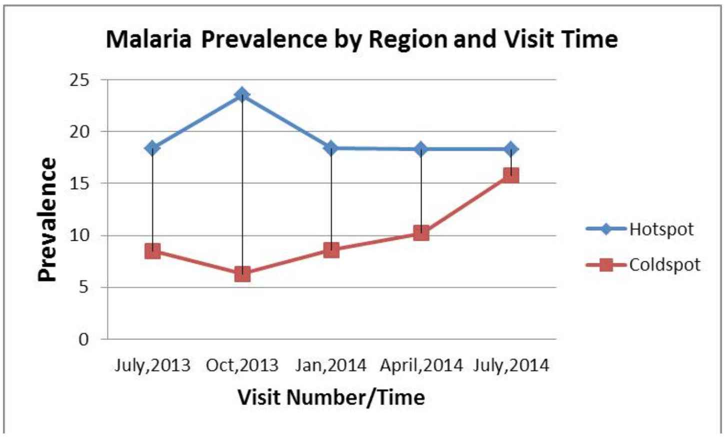


Figure 2
Malaria Prevalence by Region and Visit Number



Prevalence of malaria was highest in the hotspot villages (high burden areas) throughout the five visits compared to the cold spots. Seasonal variation in prevalence was apparent in both hotspots and cold spots.

Overall, the highest prevalence was recorded in a hotspot village and the lowest was in a cold spot village. There are statistically significant differences in the incidence of malaria among the villages (Anovaf =3.24, p=0.0071).

Association between Malaria Knowledge and Region (Fever Hotspot or Cold spot)

Although most participants were able to correctly state that the mosquito bite was the main mode of spreading malaria, some cited dirty water, unhygienic conditions and a small group stated that they didn't know the mode of spread (table 4).

There were no statistically significant differences in knowledge about malaria, including mode of spread of malaria, breeding sites for vectors, mosquito biting time and malaria prevention between the hotspots and the cold spots. However, there was a statistically significant difference in knowledge regarding medications used for malaria between the cold spots and hotspots (X2 =P:0.033) (Table 4a). A higher proportion of individuals in a hotspot reported the government recommended first-line therapy as the best treatment for malaria.

Figure 3
Prevalence of Malaria by Village and Visit Number

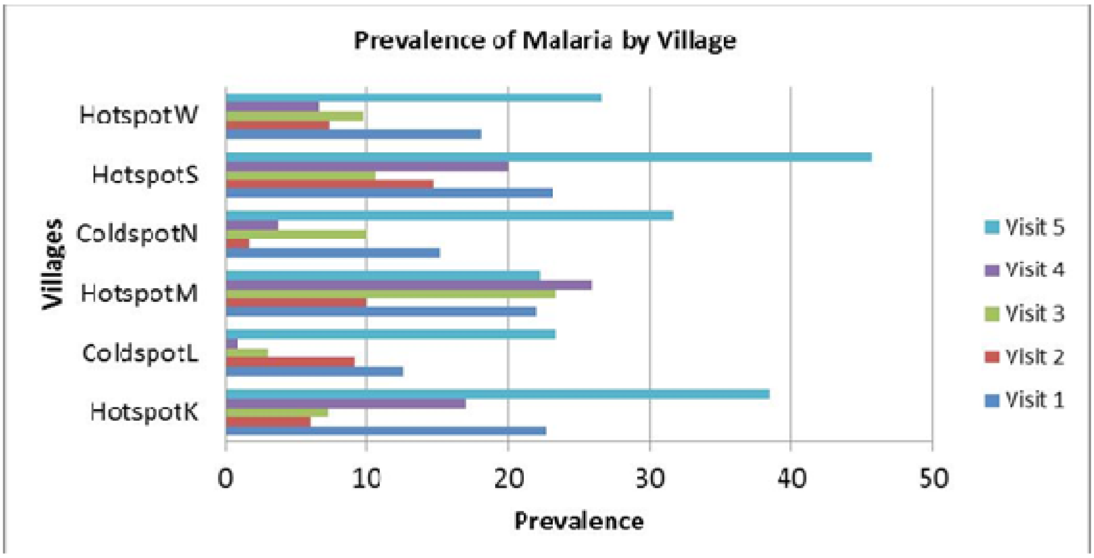


Table 4(a)

Relationship between Knowledge Related variables and Region

Knowledge on mode of spread of malaria (row percentages)

	Mosquito Bite	Dirty water	Unhygienic conditions	Don't know
Cold spot	83.33% (40)	8.33% (4)	4.16%(2)	4.16% (2)
Hotspot	89.77 % (79)	1.13 % (1)	2.27%(2)	6.81% (6)
Fishers Exact		P = 0.133		

Knowledge on Mosquito breeding Sites (Row percentages)

Region	Correct Answer	Incorrect Answer	Don't know
Cold spot	79.59% (39)	14.29% (7)	6.12% (3)
Hotspot	70.45% (62)	21.59% (19)	7.95% (7)
Pearson chi2 = 1.3862		P = 0.500	

Knowledge on biting time (row percentages)

Region	Night	Dawn	Dusk	Sunrise & sunset
Cold spot	38.77% (19)	8.16% (4)	48.97%(24)	4.08% (2)
Hotspot	42.52% (37)	5.74% (5)	47.12%(41)	4.59% (4)
Pearson chi2 = 1.1106		Pr = 0.775		

Knowledge on Malaria Prevention Measures

Region	Use of ITNs	Drain stagnant water	Use of mosquito coil	Others
Cold spot	69.39% (34)	12.24% (6)	8.16% (4)	10.20% (5)
Hotspot	73.86% (65)	10.23% (9)	5.68% (5)	10.23% (9)
Pearson Chi2 =6.6018		Pr=0.086		

Knowledge on medications used for malaria treatment (row percentages)

Region	First line Therapy	Old Therapy	Other	Don't know
cold spot	42.86% (21)	20.41 % (10)	22.45%(11)	14.29 % (7)
Hotspot	53.41 % (47)	4.55 % (4)	23.86%(21)	18.18 % (16)
Pearson chi2 = 8.7677		P= 0.033		

Table 4b
Relationship between Behavioural Factors and Region

	Dinner Time (Row percentages)			
Region	**before_7pm**	**between_7_and_8_30pm**	**after_8_30pm**	**Others**
Cold spot	16.32 % (8)	81.63% (40)	2.04% (1)	0.00%(0)
Hotspot	10.22 % (9)	85.22% (75)	3.40% (3)	1.14%(1)
Fishers exact		Pr = 0.635		
	Resting Location after Dinner (Row percentages)			
Region	**Indoor**	**Outdoor**	**Both**	**Others**
Cold spot	85.71 % (42)	12.24% (6)	2.04 % (1)	0.00%(0)
Hotspot	76.14 % (67)	12.50 % (11)	10.23 % (9)	1.14%(1)
Fishers Exact		Pr = 0.250		
	Bed time (Row Percentages)			
Region	**At 8pm**	**At 10pm**	**At 12midnight**	**Between 9&midnight**
Cold spot	18.36 % (9)	46.93% (23)	0.00% (0)	34.69% (17)
Hotspot	17.04 % (15)	29.54% (26)	1.13% (1)	52.27% (46)
Pearson chi$_2$(6) = 10.2104		Pr = 0.116		
	Regular ITN use (Self-reported)			
Region	**Daily**	**Sometimes/Occasionally**	**Never**	**Other**
Cold spot	81.63 % (40)	6.12% (3)	10.20 % (5)	2.04%(1)
Hotspot	73.86 % (65)	13.63% (12)	12.50% (11)	0.00%(0)
Pearson chi$_2$(4) = 4.2106		Pr = 0.378		
	Keeps Open Containers			
Region	**Yes**	**No**		
Cold spot	71.11 % (96)	28.89% (39)		
Hotspot	78.90 % (187)	21.10% (50)		
Pearson chi$_2$ = 2.8690		Pr = 0.090		

Relationship between Behavioural Factors and Region

Individuals were asked about the time and place they take their dinner, their resting location after dinner, the time they go to bed, whether they had travelled in the last three months, whether they kept open containers with water, if they slept under the net the previous night, how often they use the ITN in a week and what prompted them to use the ITN (table 4b). Questions on dinner time, resting location after dinner, time to bed were asked only once in the first round of data collection. However, questions regarding their travel history in the last three months and net use were asked during every round.

There were no significant differences in most of the variables that denoted behaviour related to malaria risk in both the hotspots and the cold spots. However, having slept under the net the previous night was significantly associated with the region in the second and fourth quarters of data collection (X2 = 16.827 P< 0.0001) (Table 4c). Initially, a higher proportion of individuals in hotspots reported sleeping under an ITN consistently, but the difference narrowed until the proportion consistently sleeping under an ITN in the hotspot was smaller in the final round compared to the cold spots, although this difference in the final round did not reach statistical significance at the 0.05-level. Despite higher levels of consistent use in hotspots, a significantly larger proportion of individuals reported never sleeping under an ITN in the hotspot compared to the cold spots in rounds 2 and 4.

Table 4©
Association between ITN Use and Region by Visit Number

Visit One (July)	**Sleeps under the Net (Row percentages)**		
Region	**Daily**	**Sometimes**	**None**
Cold spot	40.9% (54)	50.8% (67)	8.3% (11)
Hotspot	50.4% (118)	44.9% (105)	4.7% (11)
Pearson chi_2 = 4.1016		Pr = 0.129	
Visit Two (Oct)	**Sleeps under the Net (Row percentages)**		
Region	**Daily**	**Sometimes**	**None**
Cold spot	30.3% (40)	66.7% (88)	3.03% (4)
Hotspot	51.03% (124)	35.4% (86)	13.6% (33)
Pearson chi_2 = 36.0825		Pr = 0.0001	
Visit Three (Jan)	**Sleeps under the Net (Row percentages)**		
Region	**Daily**	**Sometimes**	**None**
Cold spot	43.5% (60)	44.9% (62)	11.6% (16)
Hotspot	42.28% (104)	46.75% (115)	10.98% (27)
Pearson chi_2 = 0.1237		Pr = 0.940	
Visit Four (April)	**Sleeps under the Net (Row percentages)**		
Region	**Daily**	**Sometimes**	**None**
Cold spot	36.1% (39)	71.9% (100)	0.00% (0)
Hotspot	40.9% (97)	49.8% (118)	9.3% (22)
Pearson chi_2 = 16.827		Pr = 0.0001	
Visit Five	**Sleeps under the Net (Row percentages)**		
Region (July)	**Daily**	**Sometimes**	**None**
Cold spot	43.07% (59)	49.64% (68)	7.30% (10)
Hotspot	34.31% (82)	59.83% (143)	5.86% (14)
Pearson chi_2 = 3.6776		Pr = 0.159	

DISCUSSION

This study investigated whether differences exist in malaria related knowledge and behaviour between villages with high malaria burden (hotspots) and those with relatively lower burden (cold spots).We found significant heterogeneity in both prevalence and incidence between villages in the HDSS.

Knowledge and behaviour related to malaria risk and whether these were associated with malaria burden at the village-level was investigated. Variables measuring knowledge included the following; causative agent for malaria, mode of spread, medications used for malaria, breeding sites, common biting time and preventive measures for malaria. Human risky behavioural practices included: staying out late in the night, sleeping very late, travelling outside Bungoma County and not using ITN while sleeping at night.

Heterogeneity in human behaviour is likely to influence exposure to malaria vectors and hence the subsequent transmission of the parasites to man. Knowledge and human behaviour has also been shown to influence the in/ appropriate use of anti-malaria treatment in some studies. Previous studies have shown that the level of knowledge on preventive measures or treatment may impact directly on the use of these preventive measures as well as uptake of treatment (20, 21). On the contrary, some reports indicate that despite high levels of knowledge on malaria and preventive measures, this is not always accompanied by correct practice (22). However, most studies are in agreement that there is a clear interdependence between human behaviour and development of malaria (23, 24).

The common malaria vector in the Western region is a member of the Anopheles gambie complex which mainly bites indoors and mostly late night when people have gone to sleep. Behaviour such as staying up very late in the night would likely increase chances of being bitten by mosquitoes, hence exposure to parasites consequently leading to infection. In addition, as the ITN coverage increases, there is increased possibility of mosquitoes adapting new behaviour where they bite during non-peak hours or bite outside the house (25). Current reports have documented the change in mosquito behaviour whereby the mosquito vectors are now biting outside the house and during earlier hours/time than previously documented (26-29). This could imply that those who like to stay outside in the night till very late or those who work mainly in the night might be predisposed to an increased number of bites which can lead to a disproportionately larger number of infections among those who practice these habits.

We however did not find significant differences in malaria related knowledge on the mode of transmission, biting times and protection against malaria between those living in the hotspots and cold spots. Most studies on knowledge relating to malaria have documented a high level of knowledge regarding the causative organism and mode of spread though the respondents may not necessarily know the details of how transmission occurs (21, 30, 31). Although the study does not show any major differences in the knowledge variables regarding malaria, there was a significant association in the knowledge relating to medications used for malaria between the hotspots and cold spots. This finding is supported by a study from Columbia by Forero, Chaparro (32) that found significant differences in knowledge regarding malaria medications impacted on practice. Mwenesi, Harpham (33) also found that mothers of children at Kilifi withdrew antimalarial treatment from children who developed complications such as convulsions because of lack of appropriate knowledge. The higher proportion of individuals who know about first line treatment in the hotspots could indicate that they mainly contact the health system for malaria treatment more often and are therefore more familiar with first-line treatment. It is important for the community to have the right information because knowledge on malaria prevention measures has been previously documented to influence the practice or uptake of malaria control measures (20, 31, 34, 35).

The study also found that there were no major differences in behaviour such as sleeping time, where dinner is eaten and where people rest after dinner. Most of the people in both the hotspots and cold spots sleep between 9pm and 12pm just before the peak biting time for mosquitoes. Majority of the people also had their dinner early and mainly inside the house. The people in the study share common cultural practices and behavioural characteristics. Although these behaviours may be a risk factor for malaria exposure, these risk factors did not vary between these villages in close proximity.

We did not find significant differences in travel history between the hotspots and cold spots throughout the five rounds of data collection. This is again related to the fact that this is a predominantly rural setting and therefore there is less travel and general movement compared to an urban setting. Travelling was therefore not associated with whether someone was in the hotspots or cold spots in any of the data collection rounds. On the contrary, travel has previously been documented as a key risk factor for malaria especially in low transmission settings (36). Travel especially to endemic regions (for those from non-endemic regions) may predispose individuals to malaria infections and hence those infected may travel back home with the disease incubating but later manifest with symptoms of malaria while in their own home area. This is basically imported malaria and may sometimes be mistaken for an original transmission episode in the area if history is not well taken. However, travel does not necessarily play any major role in increasing the number of malaria cases in endemic regions given that transmission is intense and always present in this population throughout the year unless in cases where travel is associated with sleeping outside the bed net like in the cases where they attend funerals and may have to spend the nights outside.

ITN use is also a behavioural practice which is predominantly informed by appropriate knowledge and attitudes. Using a chi-square test to check whether there was an association between ITN use and region (hotspots and cold spots), the study found a significant association between the ITN use and region during the second (October) and fourth (April) data collection rounds. This might be associated with the use of ITNs related to mosquito abundance that become a nuisance especially during the rainy seasons (37). After the rainy season, the mosquito numbers reduce and the nuisance also reduces, this may lead to some people not sleeping under the bed nets(38). The second visit was conducted in October, at this point there are likely to be fewer mosquitoes and therefore some people who normally get prompted to use ITNs by the buzzing sound of mosquitoes may stop using the ITNs when they don't get this nuisance. The same scenario is reflected in the fourth visit which was carried out in April. The heavy rains begin around this time and therefore mosquito larvae are washed off by the running water and hence there will be fewer mosquitoes which also mean less buzzing and less nuisance until later in the month or the following months. Those who mainly protect themselves because of the nuisance produced by the noise from mosquitoes may therefore not use the ITNs on some of the days when they don't hear this noise. This is an important finding to note because proper and consistent use of ITNs has been extensively studied and documented by previous studies to protect individuals and families against mosquito bites and therefore malaria infections (39-42). This finding is also supported by evidence from previous studies that have also shown seasonal variations in the use of ITNs recording a higher use of ITNs during the rainy season which also coincides with high mosquito abundance (38, 43, 44). The finding may partly explain the differences in the incidence of malaria between the fever hotspots and cold spots although the possible difference in use of ITNs is only noted and recorded at two survey points. Malaria prevention behaviour such as appropriate use of ITNs has been shown to influence prevalence of malaria in an area (25, 45-47). Although ITN distribution campaigns were done in the whole region and there is 100% ownership of ITNs, this does not translate to access to ITNs, the choice to sleep under a net or not remains largely an individual's decision which is affected by many factors. Differences in ITN use may therefore result in differences in malaria prevalence even on a small scale. When there is a group of households using ITNs well and another group of households not using them correctly, there is bound to be differences in malaria prevalence in the two groups. This may provide some answers to some of the differences noted between hotspots and cold spots within the HDSS. However, this interpretation is done with a lot of caution as we do not have sufficient information beyond just an association and this is only present in the second and fourth rounds of data collection.

CONCLUSION

There is significant heterogeneity in the incidence of malaria across the villages within the HDSS in Bungoma East County creating actual hotspots within the larger hotspot. The incidence of malaria is higher in the malaria hotspots compared to the cold spots. Knowledge and behaviour related to malaria is strongly associated with region (hotspot or coldspot) during particular seasons and may therefore likely influence the variation in the incidence of malaria. Malaria control programmes may need to take into consideration human behaviour before they roll out any of the interventions to the population.

COMPETING INTERESTS

The authors declare no competing interests.

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