

Anopheles gambiae and Anopheles arabiensis population densities and infectivity in Kopere village, Western Kenya

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Abstract

Introduction: This study was conducted in a sugar belt region of western Kenya interfacing epidemic and endemic malaria transmission. We investigated *Anopheles gambiae sensu stricto* (ss) and *Anopheles arabiensis* species compositions and densities, human host choice, and infectivity.

Methodology: Mosquitoes were captured using pyrethrum spray catch technique and first identified based on morphology; species were confirmed by PCR. Blood meal preference and sporozoite rates were determined by ELISA. Parity rates and entomological inoculation rates (EIR) were determined. Seasonal densities were compared against environmental temperatures, relative humidity and rainfall.

Results: In total 2,426 *An. gambiae* were collected. Out of 1,687 female blood-fed mosquitoes, 272 were randomly selected for entomological tests. *An. gambiae* ss and *An. arabiensis* comprised 75% (205/272) and 25% (68/272) of the selection, respectively. *An. gambiae* ss had higher preference for human blood (97%; n=263/272) compared with *An. arabiensis*, which mostly fed on bovines (88%; n=239/272). The sporozoite and parity rates were 6% (16/272) and 66% (179/272) for *An. gambiae* ss and 2% (4/272) and 53% (144/272) for *An. arabiensis* respectively, while EIR was 0.78 infective bites/person/night. Climate (ANOVA; F=14.2; DF=23) and temperature alone (r=0.626; t=3.75; p=0.001) were significantly correlated with vector densities.

Conclusion: *An. gambiae* ss are the most efficient malaria vector mosquito species in Kopere village. Because *An. gambiae* ss largely rests and feeds indoors, use of indoor residual spray and insecticide-treated nets is likely the most suitable approach to malaria vector control in Kopere village and other parts of Kenya where this species is abundant.

Key words: malaria; anopheles; climate; infectivity; transmission

J Infect Dev Ctries 2012; 6(8):637-643.

(Received 23 March 2011 – Accepted 14 October 2011)

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Introduction

Reduction of the malaria burden in endemic and epidemic regions requires knowledge of the malaria-transmitting mosquito species, populations and behavioural characteristics, and malaria exposure risks. The greatest impact of malaria is felt in sub-Saharan Africa where about 20% of deaths among children of younger than five years of age are attributed to malaria alone [1]. Reasons for malaria persistence in sub-Saharan Africa include the presence of highly efficient mosquito vectors as well as various economic and social factors, all of which

play a critical role in malaria transmission. Hay *et al.* [2] described the annual malaria parasite exposure risk across Africa and identified the dearth of information on vector bionomics as a major constraint to malaria control since this information is required to guide malaria control efforts. Similarly, choosing a mosquito control technique requires knowledge of types of vectors and their behavioural characteristics [3]. In addition, parity and sporozoite rates, human blood meal preference, and entomological inoculation rates (EIR) are critical indicators of malaria transmission, but they differ

widely in sub-Saharan Africa, even between villages in the same locality because of variable micro-climates in malaria endemic and epidemic regions.

Mosquito control aimed at reducing malaria transmission requires baseline information upon which monitoring and evaluation of effectiveness of these control efforts can be assessed. Epidemics occur because of accumulation of malaria reservoirs in the zone interfacing malaria endemic lowlands and epidemic-prone high altitudes. High population densities of sporozoite-carrying mosquito vectors emanate from this critical zone to cause malaria epidemics in the highlands. This study investigated malaria transmission in Kopere village, western Kenya, to provide baseline data which can be used to control malaria transmission in an area that lies at the interfacing zone between endemic and epidemic malaria transmission.

Methodology

Study site

This study was conducted in Kopere village, about 20 kilometers southeast of Kisumu Town in western Kenya. The village is located at the interface between endemic and epidemic malaria transmission zones, and lies at the foot of Nandi escarpment, which rises to 1,800 metres along the northern margin of the Kano Plain. The village is drained by several streams that join River Nyando before it flows southwestward into the Kavirondo Gulf of Lake Victoria. These streams are probably utilized by anopheles mosquitoes for breeding to maintain mosquito populations and malaria transmission during the dry seasons.

Ethical issues

The ethical clearance certificate (No. MU/IPH/IREC/12[B] of 24th August 2001) was granted by the Joint Institutional Research and Ethics Committee (IREC) of the School of Medicine, Moi University, and the Moi Teaching and Referral Hospital. Community entry was organized with the assistance of the local Provincial Administration to explain the purpose of the study, and verbal or written consent to conduct research was received from individual heads of selected households.

Study design and sampling methods

A longitudinal design was used to conduct this study. The sampling frame consisted of all 498 households, and a two-stage sampling technique was used to select 13% (66/498) of the households in the

village from where mosquitoes were collected. This area comprises five (5) blocks, with the smallest block consisting of 27 households. This smaller block was considered as one sampling unit. Consequently, the larger blocks were further divided into units of 27 households each. Three (3) households from each unit were then selected as follows: after a central position was identified, a pen was cast, and the first household pointed to was selected. Systematic sampling with an interval of 8 was used until the required number of 66 households was obtained. The target household was skipped if the household head declined to consent. Such households were replaced by the immediately neighbouring households upon consent.

Pyrethrum spray catch mosquito collection

Indoor-resting mosquitoes were captured by the pyrethrum spray catch technique [4] between December 2001 and November 2003. Twenty percent (20%) emulsified concentrate (EC) of pale pyrethrum extract synergized with piperonyl butoxide was used at a concentration of 0.3% (v/v) in kerosene. The huts were sprayed systematically by two people, one inside and the other one outside the hut. The person inside commenced spraying at the same time as the person outside, with both simultaneously moving in opposite directions, after which the door was closed and only opened after 15 minutes to collect mosquitoes.

Morphological identification of the mosquitoes and determination of other entomological malaria transmission indicators

Preliminary identification of mosquitoes was based on morphological characteristics [5]. After morphological identification, the mosquitoes were counted, sorted into males and females, and preserved in iso-propanol. Abdominal conditions of the female anopheles mosquitoes were determined based on the description of Detinova [6], and they were graded as unfed (UNF), blood fed (BF), half gravid (HG), and gravid (G).

Two hundred and seventy two (272) randomly selected freshly fed female *Anopheles gambiae* mosquitoes were dissected to determine the age composition and parity as described by the World Health Organization (WHO) [4] and Detinova [6]. The remaining portions of the dissected mosquitoes were preserved in iso-propanol for further processing. Parity rate was obtained by multiplying the number

of parous females and dividing the product by the total number of mosquitoes dissected.

Molecular identification of the mosquitoes and determination of blood meal source, sporozoite rates, and entomological inoculation rates were performed using various methods. The iso-propanol-preserved portions of freshly fed (272) female *An. gambiae* mosquitoes were identified to sibling species by the polymerase chain reaction (PCR) technique as previously described [7]. The preserved portions were then analyzed for blood meal sources for human or bovids, and for sporozoite rates following published enzyme-linked immunosorbent assay (ELISA) protocols [8,9]. The EIR were then estimated by multiplying the total number of anophelid mosquitoes/person/night by the sporozoite rate of malaria vectors caught indoors assuming a two-day gonotrophic period in which these vectors skip a blood meal [10].

Data handling and analysis

Mosquito and weather data were stored in an Excel (Microsoft, Redmond, WA, USA) spreadsheet then imported into SPSS version 12 (IBM, Chicago, USA) for analyses. The data for malaria vector infectivity rates were summarized using descriptive statistics. One-sample statistics and linear regression were used to describe mosquito density distributions between months and the effects of weather components on malaria vector densities, respectively. The differences in annual variance were determined using one-way analysis of variance (ANOVA), while the associations of individual climatic components on mosquito population densities were determined by Pearson's correlations.

Results

A total of 2,426 *Anopheles gambiae* were captured indoors between December 2001 and November 2003. Using the vectors' morphological features, 1,687 of the vectors were identified as female mosquitoes. The population densities of these malaria vectors peaked in June 2002 and May 2003 respectively, and the lowest vector density occurred in September 2003 (Figure 1). Overall, the gravid component was high in 2002 and during the same period the following year, while half-gravid and gravid mosquitoes were low in the wet season of 2002 (Figure 2). There was an increase in the number of unfed mosquitoes in 2003, probably as a consequence of reliable and increased rainfall.

An overall EIR of 0.78 infective bites/person/night was obtained. The species composition within the *An. gambiae* consisted of 75% (n = 205) *An. gambiae ss* and 25% (n = 68) *An. arabiensis*. *An. gambiae ss* had a parity rate of 66% (n = 179), human blood meal of 97% (n = 263), and a sporozoite rate of 6% (n = 16) compared with a parity rate of 53% (n = 144), bovine host preference of 88% (n = 239), and a sporozoite rate of 2% (n = 4) for and *An. arabiensis*.

Vector densities were not uniformly distributed across months (ANOVA; F = 4.7, DF = 23, p = 0.008). Linear regression showed that combined climatic variables significantly affected the mosquito vector densities (ANOVA; F = 14.2, DF = 23, p = 0.001). However, this statistical method could explain only 49.9% of the variance in the data (Adjusted R² = 0.34, and R² = 0.499). Independently, partial correlation showed that temperature was the only climatic component which had a significant correlation with the vector densities (r = 0.626; with t = 3.75; p = 0.001). The lack of positive correlation between the mosquito densities and other climatic components, namely rainfall, relative humidity (RH) and maximum temperatures, probably accounted for 51% of the variance in the data.

Discussion

The entomological parameters investigated demonstrated efficiency in malaria transmission of the local vectors. The majority of vectors captured indoors in Kopere village, located at an elevation ranging between 1,260 metres and 1,440 metres above sea level, were *An. gambiae ss*; only a small proportion consisted of *An. arabiensis*. However, *An. arabiensis* populations diminish as the elevation rises above 1,400 meters and is not known to thrive in high relative humidity [11]. For instance, Githeko *et al.* [12] documented an increased presence of *An. arabiensis* below the Nandi foothills, where it is the dominant member of *An. gambiae* found at low altitude in the expansive Kano Plain, especially in the Ahero rice fields where it was shown to be a poor malaria vector.

However, this study deviates from others that have investigated the bionomics of malaria vectors around Ahero where data was extrapolated to represent the expansive Kano Plain. Based on these reports malaria is considered endemic in this region [13-16]. The species composition, EIR, and vectorial capacity of *An. gambiae* found in the current study site differ from those of Ahero, which lie within the

Figure 1. Rainfall patterns and malaria vector density December 2001 - November 2003

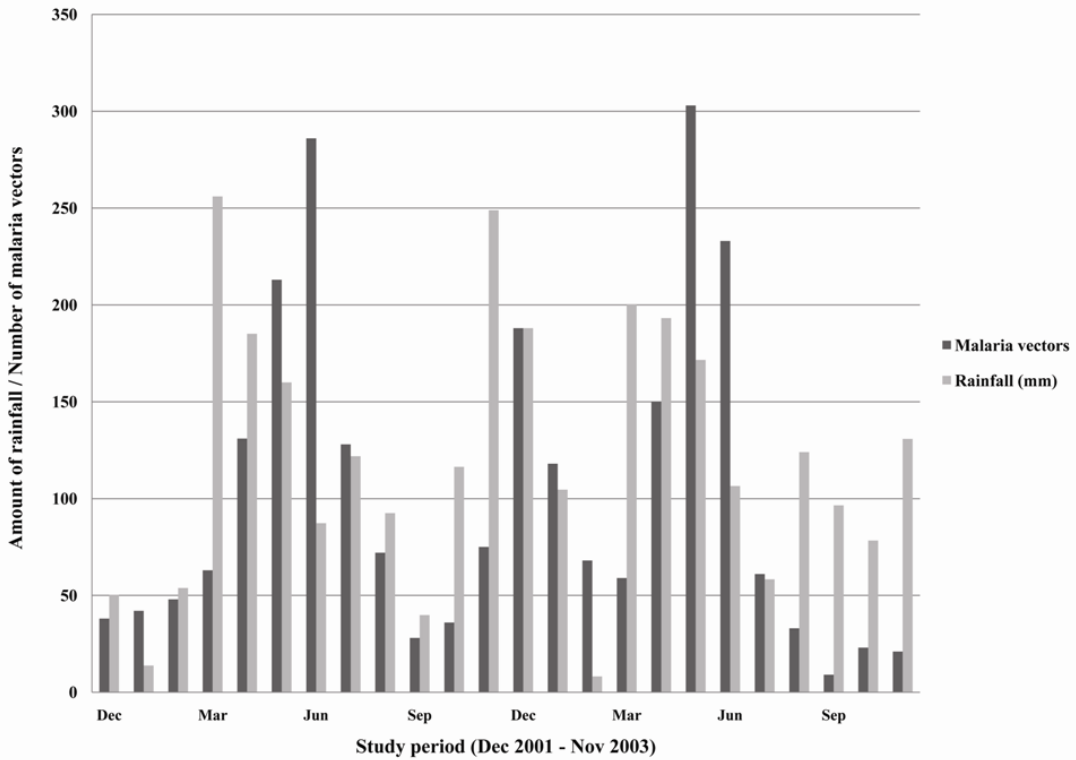
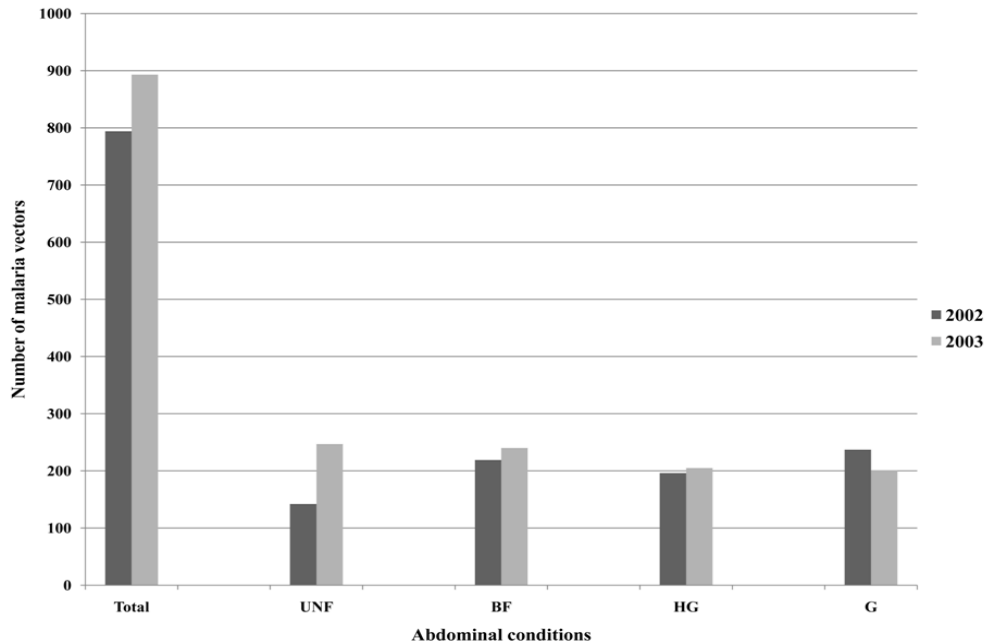


Figure 2. The abdominal conditions of malaria vectors between 2002 and 2003



flood basin of River Nyando, as well as other malaria transmission ranges located at high elevations but geographically belong to the expansive Kano Plain. Within the Kano Plain alone, the data reported in Ahero, Miwani and the Kopere village demonstrate non-uniform malaria exposure risks in areas that are separated by very short distances but tend to exhibit unique ecological niches because of variable weather.

These findings are consistent with the observations of Hay *et al.* [2] who identified Kenya and Senegal as countries in sub-Saharan Africa with high EIR variation due to the sub-regional ecological niches and the variable climate which affect malaria transmission at the local level. Because we did not sample mosquitoes from other areas, we cannot extrapolate our data across the expansive Kano Plain since malaria transmission differs significantly even between villages located a mile apart [2].

The population densities of the malaria vectors peaked in June and May 2002 and 2003 respectively, and the lowest vector densities occurred in September 2003 in tandem with low precipitation experienced during the same period. The fluctuations in precipitation patterns were also significantly associated with non-uniform distribution of malaria vectors ($p < 0.001$). These observations are consistent with those of other reports [17-19] which showed that mosquitoes are sensitive to environmental changes that affect the breeding habitats and the longevity of adult mosquitoes.

We found high-density populations of small and unfed anopheles mosquitoes resting indoors most likely as a consequence of crowded breeding habitats which may have produced small and weak adults that could not take part in malaria transmission probably due to short longevity. This is consistent with a report from West Africa where a majority of smaller females survived to mate but did not survive beyond the nulliparous stage as shown by the absence of small females among older age classes captured indoors [20]. Furthermore, Okanda *et al.* [21] observed that large females easily attracted mates and had greater fecundity than smaller females. Lyimo and Takken [22] have also recorded two to three blood meal repeats among smaller females before completion of the first gonotrophic cycle, a behaviour that may increase malaria transmission risks if these blood meals were obtained from different hosts even though the pre-gravid state is associated with incomplete feeds. It is unlikely that *Plasmodium falciparum* sporozoite infectivity is due to short periods between such feeds.

Because all of the mosquitoes were captured indoors, the parity rate and the human blood meal preference reported here suggest that a majority of these mosquitoes were endophilic and anthropophilic. The human blood meal preference found in our study is higher than those reported for *An. gambiae ss* within the Kano Plain [23,24]. This is probably because the sibling species composition favoured in the studies by Anon [23] and Service [24] (*An. Arabiensis*) has low human blood meal preference [12]. However, the human blood meal preference we found is consistent with that of Mwangangi *et al.* [25], who reported slightly higher values for human blood and sporozoite rates at the Kenyan coast than those found in our study area, suggesting higher vectorial capacity among malaria vectors at the coast than in Kopere village. Alternatively, higher blood meal preference at the coast may have been due to the higher proportion of *An. gambiae ss* at the coast compared to that in our study site (79% vs 76%). *An. gambiae ss* is more endophilic and anthropophilic than *An. arabiensis*. Unlike *An. gambiae ss*, *An. arabiensis* expresses heterogeneous behavioural characteristics and is readily diverted to other domesticated animals for blood meal, which compromises its ability to transmit malaria. In a recent study, Bayoh *et al.* [26] found a lower human host choice for *An. gambiae ss* (70%), while *An. arabiensis* most frequently fed on bovine (65%) in Seme and Asembo in Nyanza Province of Kenya where the wide usage of insecticide-treated nets had a profound effect on the densities of *An. gambiae ss*. Interestingly, in central Kenya the only sibling species of the *An. gambiae* group of species was *An. Arabiensis*, which has low human blood meal preference [27].

The data presented here needs cautious interpretation considering the possible impact of more recent vigorous interventions such as the use of insecticide treated nets and indoor residual spray [26]. However, it is important to evaluate malaria transmission patterns from different sites of Kano Plain which might help in assessing the effectiveness of control efforts in this endemic area and in western Kenya.

The *Anopheles gambiae ss* species were the most abundant and most infective indoor resting mosquito vectors found in Kopere village. These findings suggest that the indoor residual spray and other house modifications recently verified in Ahero [16] for vector control could appropriately be used in Kopere

village since the most abundant vector species we found appeared to rest and feed exclusively indoors.

Acknowledgements

We express our sincere gratitude to the residents of Kopere village for their cooperation during the fieldwork component of this study. We are grateful to laboratory staff at the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, as well as KEMRI, Kilifi who analyzed the mosquitoes for sibling species identification of *Anopheles gambiae*, human blood meal preference and sporozoite infectivity rates.

This study was jointly supported through a training grant from the Research Foundation of the State University of New York at Buffalo, USA (Grant No. D43-TW: 001505), and the Moi University (K) – VLIR UOS (Belgium) Collaborative programme.

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Conflict of interests: No conflict of interests is declared.