Seasonal density, sporozoite rates and entomological inoculation rates of \textit{Anopheles gambiae} and \textit{Anopheles funestus} in a high-altitude sugarcane growing zone in Western Kenya

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Summary

An entomological study was conducted on vectors of malaria and their relative contribution to \textit{Plasmodium falciparum} transmission in Mumias, a high-altitude site and large-scale sugarcane growing zone in Kakamega district, western Kenya. \textit{Anopheles gambiae} s.l., the predominant vector species, represented 84\% ($n = 2667$) of the total Anopheles mosquitoes collected with \textit{An. funestus} comprising only 16\%. Polymerase chain reaction (PCR) identified all 600 specimens of the \textit{An. gambiae} complex tested as \textit{An. gambiae sensu stricto}, an indication that it is the only sibling species represented in the high-altitude sites in western Kenya. \textit{Plasmodium falciparum} sporozoite rates of 6.3\% (133/2118) for \textit{An. gambiae} s.l and 9.5\% (38/402) for \textit{An. funestus} by ELISA were obtained in Mumias. None of 1600 mosquitoes tested for \textit{P. malariae} sporozoites was positive. ELISA tests of mosquito blood meals indicated a high tendency of anthropophagy, a behaviour contributing significantly to malaria transmission by the vector species, with 95.9\%, 4.86\% and 0.2\% having taken at least one blood meal on human, bovine and avian hosts, respectively.

Malaria transmission intensity was low as revealed by the low entomological inoculation rates (EIR) recorded. The EIR values for \textit{An. gambiae} s.l. were 29.2 infective bites per person per year (ib/p/year) and 17.5 ib/p/year for \textit{An. funestus} in Mumias. The highest inoculation rate for both vector species was 7.0 ib/p/month in July. \textit{Plasmodium falciparum} parasite rate among asymptomatic children was 55.4\% and 44\% in the wet (July–September) and dry (December–February) seasons, respectively. These results indicate that malaria transmission intensity in the high-altitude site is low but perennial, with transmission being maintained by \textit{An. gambiae s.s} and \textit{An. funestus}.

keywords \textit{Plasmodium falciparum}, sporozoite rates, entomological inoculation rates, high-altitude

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Introduction

Malaria continues to be a major cause of morbidity and mortality in tropical and subtropical countries of the world despite the enormous investment in control efforts. In Africa, it is responsible for an estimated 1 million deaths, mainly infants and children under the age of 5 years, annually (WHO 1996). In Kenya, malaria is responsible for approximately 30\% of the total out-patient clinic visits and estimates of infant and child mortality on the Kenyan coast show that at least 58 infants per 1000 life births and 12 children per 1000 children aged between 1 and 4 years die each year (Snow \textit{et al.} 1994).

Despite the high prevalences of malaria countrywide, much study on bionomics of malaria vectors and transmission dynamics is based in areas on the shores of Lake Victoria and a few isolated sites on the Kenyan coast. No account exists on the bionomics of vectors of malaria in high-altitude areas. Furthermore, the pattern of malaria transmission appears to be changing rapidly as evidenced by epidemics in areas previously thought to be malaria-free. Epidemics of highland malaria have claimed several lives in Trans Nzoia, Kisii, Uashin Gishu, Kericho and Kakamega districts in recent years (Khan \textit{et al.} 1992). This was the first longitudinal study to be carried out on vector bionomics and malaria transmission in high-altitude ecological zones of...
western Kenya where malaria is endemic. The results provide a better understanding of the intensity and seasonal patterns of malaria transmission in these areas, and represent important baseline data essential for implementing control programmes.

Materials and methods

Study site and mosquito sampling

Mumias, a division in Kakamega District, is a large-scale sugarcane growing zone with an altitude of about 1500 m a.s.l. It has a fairly flat terrain with many streams. The population totals 206 456 inhabitants at a density of approximately 355 persons per km². This district comprises 45 981 households and covers an area of about 581 km² (Republic of Kenya 1989). The homesteads are separated by large sugarcane plantations, which form the main agricultural activity in the area. Cattle, sheep, dogs, goats and chicken are kept by most inhabitants in the villages sampled.

Mosquitoes were collected once weekly for 11 months from 10 houses (1995–96) in 4 villages by pyrethrum spray collection (PSC) from 0600 h to 0930 h. All female anopheline mosquitoes were preserved on damp absorbent paper in a cool box and later identified to species level by morphological criteria (Gillett 1972). Legs and wings of all An. gambiae s.l. were preserved individually in 70% ethanol for sibling species identification by PCR. The rest of the mosquito was placed in individual vials and dried on calcium sulphate (driarite) for at least 4 days for sporozoite and blood meal ELISA tests. On average a total of 40 houses were sampled per month.

PCR identification of Anopheles gambiae sibling species

DNA was extracted from single mosquito legs using the method described by Collins et al. (1987). Eight µl of the sample DNA were then used as template for PCR amplification. PCR reaction conditions were performed as described by Kampen et al. (1995). Denaturing of double-stranded DNA, primer annealing and polymerization took place at 94 °C, 50 °C, and 72 °C for 30 s, respectively. A total of 30 cycles were run. To analyse the polymerase chain reaction products, 10 µl of each amplified sample were run in 1.8% horizontal agarose-TBE gels and visualized by a UV transilluminator.

Entomological inoculation rate (EIR)

The EIR, expressed as the number of infective bites per person per unit time, was derived as the product of the sporozoite rate and the mosquito biting density/rate on humans (Molineaux et al. 1988). The man biting rates were calculated by dividing the number of blood-fed anopheline mosquitoes caught by PSC by the number of occupants in the house where they were collected multiplied by the human blood index (Githeko et al. 1993).

Plasmodium falciparum sporozoite rates

The head and thorax of each mosquito was carefully separated from the abdomen and tested for the presence of P. falciparum circumsporozoite protein (CS) as described by Beier et al. (1987). Briefly, mosquitoes were ground individually in 50 µl bovine casein containing Nonidet 40 and final volume brought to 250 µl with blocking buffer. 50 µl of the triturate was used in ELISA tests. Absorbance was measured using an ELISA reader (Titertek) at 414 nm. Samples were considered positive (infected) when absorbance values exceeded the mean plus 3 standard deviations of the mean absorbance of eight negative controls. The sources of mosquito blood meals were determined to derive the human blood index. The ELISA procedure for blood meal determination has been described previously (Beier et al. 1988).

Results

Identification and abundance of vector species

A total of 2524 anopheline mosquitoes belonging to two species, An. gambiae s.l. and An. funestus, were caught by PSC in indoor collections. The monthly densities for An. gambiae in Mumias ranged from 2.9 to 11.4 females per household and 0.3 to 2.6 for An. funestus.

Despite the observation that the mosquito population appeared to show seasonal variation with highest densities occurring in the wet season or soon after the rains, no significant correlation was established between monthly rainfall totals and relative densities of the two species at this site (An. gambiae s.l. r = −0.167, d.f. = 9, P = 0.624, An. funestus r = 0.129, d.f. = 9, P = 0.705).

Some 28% (n = 600) of the total An. gambiae s.l. analysed using PCR to further characterize them into respective sibling species were all An. gambiae s.s. No An. arabiensis was identified. This indicates that An. gambiae s.s. is the only sibling species of the An. gambiae Giles complex represented at high-altitude sites in western Kenya. This species, together with An. funestus, is therefore responsible for malaria transmission in high-altitude areas of western Kenya.

Plasmodium falciparum sporozoite rates

The mean monthly sporozoite rates for both An. gambiae s.l. and An. funestus are shown in Table 1. The difference
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Table 1  Sporozoite rates and inoculation rates for An. gambiae s.l. and An. funestus in Mumias

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (mm)</th>
<th>Mean temp. °C</th>
<th>An. gambiae s.l.</th>
<th>An. funestus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MBR SR (%) EIR</td>
<td>MBR SR (%) EIR</td>
</tr>
<tr>
<td>May</td>
<td>279.2</td>
<td>22.6</td>
<td>1.122 9.0 0.101</td>
<td>0.173 7.7 0.013</td>
</tr>
<tr>
<td>Jun</td>
<td>180.0</td>
<td>22.2</td>
<td>1.550 7.6 0.118</td>
<td>0.942 12.2 0.112</td>
</tr>
<tr>
<td>Jul</td>
<td>151.0</td>
<td>21.4</td>
<td>0.902 9.1 0.082</td>
<td>1.011 6.9 0.070</td>
</tr>
<tr>
<td>Aug</td>
<td>162.8</td>
<td>21.9</td>
<td>0.699 7.6 0.053</td>
<td>0.466 11.8 0.055</td>
</tr>
<tr>
<td>Sep</td>
<td>156.9</td>
<td>21.1</td>
<td>0.811 2.5 0.021</td>
<td>0.677 13.1 0.089</td>
</tr>
<tr>
<td>Oct</td>
<td>134.3</td>
<td>22.4</td>
<td>0.689 6.9 0.048</td>
<td>0.411 5.3 0.022</td>
</tr>
<tr>
<td>Nov</td>
<td>133.3</td>
<td>22.3</td>
<td>1.796 0.0 0.000</td>
<td>0.349 0.0 0.000</td>
</tr>
<tr>
<td>Dec</td>
<td>65.4</td>
<td>22.3</td>
<td>1.747 5.7 0.100</td>
<td>0.360 9.5 0.034</td>
</tr>
<tr>
<td>Jan</td>
<td>131.8</td>
<td>22.3</td>
<td>0.776 9.8 0.076</td>
<td>0.380 7.1 0.027</td>
</tr>
<tr>
<td>Feb</td>
<td>129.4</td>
<td>22.5</td>
<td>1.515 6.1 0.092</td>
<td>0.308 0.0 0.000</td>
</tr>
<tr>
<td>Mar</td>
<td>153.9</td>
<td>23.0</td>
<td>2.377 6.9 0.164</td>
<td>0.436 10.0 0.044</td>
</tr>
</tbody>
</table>

MBR, Man biting rate; SR, Sporozoite rate; EIR, Entomological inoculation rate.

between the mean P. falciparum sporozoite rates for An. gambiae s.l. (6.3%, 133/2115) and An. funestus (9.5%, 38/402) over the 11 month study period was significant ($\chi^2 = 5.33$, d.f. = 1, $P < 0.05$). Except for November when no An. gambiae s.l. positives for P. falciparum sporozoite antigens were recorded and September when only 2.5% positives were obtained, sporozoite rates were generally high during the study period (range 5.7%–9.8%). High positivity rates were recorded for An. funestus with the peak mean monthly rate occurring in September (13.1%). A high human blood index (HBI), calculated from proportions of mosquitoes that had human blood and mixed blood feeds of human and bovine and avian origin was recorded in Mumias (0.959).

Entomological inoculation rate

The inoculation rates, calculated as a product of the man biting rates, the human blood index and the mean monthly sporozoite rates for An. gambiae s.l. and An. funestus are shown in Table 1. The mean sporozoite inoculation rate in Mumias over the 11-month study period was 0.08 ib/p/night for An. gambiae s.l. and 0.048 ib/p/night for An. funestus, giving a ratio of 1:7:1 for the two species. The combined sporozoite inoculation rate for both species was 0.13 ib/p/night giving an average of 47.5 ib/p/year at this site. The mean monthly EIR for An. gambiae s.l. increased progressively from May to July (wet season), then dropped gradually between August to November with decreasing rainfall amounts, and showed an increase in the dry season (December to March) giving a double peaked transmission pattern (Figure 1). Despite the apparent seasonal variation in inoculation rates observed, correlation analysis revealed no significant linear relation between EIR and weather variables (temperature, $r = 0.395$, d.f. = 9, $P = 0.230$, rainfall, $r = 0.140$, d.f. = 9, $P = 0.683$). An individual in Mumias would expect to receive 29.2 infective bites per year by An. gambiae s.l with an average time to a single infective inoculation of 12.5 days by this species with a range of 6.1 days in March to 47.6 days in September.

An. funestus seemed to account for a relatively minor but important role in malaria transmission owing to the low level of sporozoite inoculation rates for this species. The highest

Figure 1 Monthly changes in inoculation rates for An. gambiae s.l. (■) and An. funestus (□) in Mumias.
EIRs for this species were experienced from June to September (range 0.06–0.12 ib/p/yr) corresponding to the wet season and on average it would take 20.8 days for an individual to receive an infective bite from An. funestus.

Parasite rates among asymptomatic children in the study site were high. 55.4% and 44% of the children examined in a transverse study in the wet (July–September) and dry season (December–February) had malaria parasites in their blood streams, respectively (Table 2).

**Table 2** Distribution of malaria infection and *Plasmodium falciparum* parasite densities among asymptomatic children in Mumias

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. examined</th>
<th>% parasitaemic</th>
<th>Geometric mean parasite density</th>
<th>No. examined</th>
<th>% parasitaemic</th>
<th>Geometric mean parasite density</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>14</td>
<td>42.9</td>
<td>2345.6</td>
<td>11</td>
<td>45.5</td>
<td>2465.9</td>
</tr>
<tr>
<td>1–4</td>
<td>36</td>
<td>61.1</td>
<td>1748.6</td>
<td>29</td>
<td>48.3</td>
<td>848.1</td>
</tr>
<tr>
<td>5–9</td>
<td>36</td>
<td>69.4</td>
<td>1092.6</td>
<td>40</td>
<td>45.0</td>
<td>763.7</td>
</tr>
<tr>
<td>10–15</td>
<td>29</td>
<td>48.3</td>
<td>926.7</td>
<td>27</td>
<td>37.0</td>
<td>761.0</td>
</tr>
</tbody>
</table>

Discussion

PCR analysis identified all the mosquitoes tested to be *An. gambiae* s.s. showing that this could be the only member of the *An. gambiae* Giles complex represented in the highland areas to the north of Kisumu. The absence of *An. arabiensis* could be attributed to high rainfall and cooler temperatures in these areas, both of which do not seem to favour this species (Table 1). In some areas of Africa, increases in the relative frequencies of *An. gambiae* s.s. have been observed to coincide with rainy seasons, whereas *An. arabiensis* is better able to exploit drier areas and seasons (Rishikesh et al. 1985). White (1972), in a study in the highland areas around Kisumu on the shores of Lake Victoria, found *An. gambiae* s.s to be the only species of the *An. gambiae* complex represented.

The mean sporozoite rates for *An. gambiae* s.l. over the 11 months of the study (6.3%) were comparable to those obtained by Githeko et al. (1993) at Miwani (6.0%) and by Beier et al. (1990) at Kisian (5.5%) in Kisumu district. However, these values were lower than those recorded for the same species (13.1%) in Saradidi (Beier et al. 1990). The mean sporozoite rate for *An. funestus* (9.5%) was much higher than that obtained at Ahero and Miwani (4.3%), Kisian (6.0%) and Saradidi (4.9%). However, this may not necessarily mean that *An. funestus* has greater vectorial importance in Mumias than in Kisumu district, as very low densities of this species observed in the high-altitude areas may generally compromise its vectorial significance.

The study demonstrates that malaria transmission in Kakamega district is perennial with high wet season inoculation rates. However, the inoculation rates obtained in this study were generally lower than those recorded in most studies in areas close to Lake Victoria. In the Ahero and Miwani study areas near Kisumu, the annual *P. falciparum* inoculation rates have been estimated at 91 and 416 ib/p/year, respectively (Githeko et al. 1993). Beier et al. (1990) reported comparably high inoculation rates in Kisian (299 ib/p/year) and Saradidi (237 ib/p/year), confirming a very high intensity in the sites. The comparatively low inoculation rates recorded for *An.gambiae s.l* (29.2 ib/p/year) and *An. funestus* (17.5 ib/p/year) is a reflection of the low densities of the species in the study site. The magnitude of the entomological inoculation rate is influenced by the rate at which vectors feed on humans, which is largely dependent on the overall mosquito density and to some extent the feeding habits of the vector species. Nonetheless, evidence indicates that even low levels of malaria transmission may contribute to significant levels of malaria in the population (Mbogo et al. 1995). This confirms the occurrence of fairly high parasite rates recorded in the wet (55.4%) and dry season (44%) among asymptomatic children in the high-altitude site of Mumias in Kakamega district.

The considerable variation in intensity of malaria transmission within a given locality shows the extent of the limitation of most studies which do not take into account the diversity of epidemiological situations existing in a small area. Since each locality presents a different malaria situation as depicted by the present study, an understanding of the local vectors, their bionomics and the factors that affect transmission intensity are vital for successful control of the disease.

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