RELATIONSHIPS BETWEEN PLASMODIUM FALCIPARUM
TRANSMISSION BY VECTOR POPULATIONS AND THE INCIDENCE
OF SEVERE DISEASE AT NINE SITES ON THE KENYAN COAST

CHARLES N. M. MBODO, ROBERT W. SNOW, CANUTE P. M. KHAMALA,
EPHANTUS W. KABIRU, JOHN H. OUMA, JOHN I. GITHURE, KEVIN MARSH,
AND JOHN C. BEIER

Kenya Medical Research Institute (KEMRI), Kilifi Research Unit, Kilifi, Kenya: Nuffield
Department of Clinical Medicine, John Radcliffe Hospital, Oxford University, Oxford, United
Kingdom: Department of Zoology, University of Nairobi, Nairobi, Kenya: Division of Vector-Borne
Diseases, Ministry of Health, Nairobi, Kenya: Biomedical Sciences Research Centre, KEMRI,
Nairobi, Kenya: Department of Molecular Microbiology and Immunology, School of Hygiene and
Public Health, The Johns Hopkins University, Baltimore, Maryland

Abstract. The transmission of Plasmodium falciparum was studied in relation to the incidence of severe malaria
infections at nine sites in the Kilifi District in Kenya. Intensive mosquito sampling during a one-year period yielded
s.l. was the predominant vector, comprising 98.4% of the total anophelines collected. Overall, 3.5% of 2,868 An.
gambiae s.l. collected indoors and 0.8% of 261 collected outdoors contained P. falciparum sporozoites. Transmission
was detected during 10 months, with peak periods from June to August and December to January. In eight of the
nine sites, entomologic inoculation rates (EIRs) averaged only four infective bites per year (range 0–18); an annual
EIR of 60 was measured for the site with the highest intensity of transmission. The incidence of severe malaria
infections, ranging from 8.6 to 38.1 per 1,000 children (0–4 years), was not associated with EIRs. At these sites on
the coast of Kenya, a high incidence of severe disease occurs under conditions of very low levels of transmission by
vector populations. With respect to conventional approaches for vector control in Africa, decreases in transmission,
even to levels barely detectable by standard approaches, may not yield corresponding long-term reductions in the
incidence of severe disease.

Traditional approaches to malaria epidemiology focus on
relationships between vector-related parameters of transmis-
sion and resulting indices of infection in the community.1 In
malaria-endemic areas, it is clear that most children become
infected but only a small proportion develop severe malari-
a.2,3 Few attempts have been made to evaluate the relations-
ships between transmission patterns and the incidence of se-
vere malaria.

Our baseline epidemiologic studies of malaria at two sites
on the Kenyan coast indicated that low levels of transmission
by vector populations were associated with a high incidence
of severe disease.4 This was unexpected because it has long
been recognized that malaria is a serious health problem in
this area. As an extension of these studies, this paper eval-
uates the transmission of Plasmodium falciparum by vector
populations relative to the incidence of severe malaria
infections in children at nine sites on the Kenyan coast.

MATERIALS AND METHODS

Study area. The study area in Kilifi District, Kenya, 60
km north of Mombasa on the Kenyan Coast, has been the
subject of a series of entomologic and epidemiologic studies
of severe malaria since 1989.4,5 The rural population live
mainly in stick- and mud-built houses with coconut thatch
roofs. Homesteads are scattered and separated from one an-
other by agricultural land. The 1989 Kenya National census
divided clusters of homesteads into enumeration zones (EZs)
according to landmark roads and footpaths. Each EZ com-
prises approximately 100 homesteads and 850 people. Maize
is cultivated for home consumption, and coconut and cashew
nut for cash crops. More than 75% of the population belong
to the Giriama subgroup of the Mijikenda ethnic group.

Survey data for pediatric cases of severe malaria at
the Kilifi District Hospital in Kilifi were used for selecting
at random nine sites. These nine sites were selected from a
total of 74 sites representing census enumeration zones,
where average incidences rates of severe malaria were
available for a three-year period. Three sites each with
high, moderate, and low incidence of severe malaria were
selected based on rankings of incidence rates for the
74 sites divided into the three respective categories (Table
1). Three sites in each of the categories (i.e., high, moderate,
and low) were then selected with the aim of achieving a
representative spatial distribution of sites in each category
throughout the study area (Figure 1). This approach for site
selection was used due to expected differences in vector pop-
ulations and associated intensities of malaria parasite trans-
mision throughout the study area.

Each of the nine sites was subdivided into four equal-sized
sectors based on land areas shown on site maps. At each of
the four sectors per site, a sentinel household was selected
at random from listings of all known houses. The only cri-
teria for selection of the sentinel household was the resi-
dence of at least one child less than five years of age, based
on census data. Additionally, the nearest five households
around the sentinel household were selected. This approach
provided units of six entomologic surveillance households
per sector (i.e., a total of 24 households per site).

Mosquito sampling. Mosquito collections from June
1992 to May 1993 were made in each of the 24 households
per site. Anopheline mosquitoes were sampled inside and
outside houses by all-night human biting catches and day-
time resting catches.6 Indoor night biting collections (NBC)
were made once a week inside four houses by eight mos-
quito collectors (two per house) at each of the selected sen-
TABLE 1

Incidence of severe malaria per 1,000 children 0–4 years of age (number of cases) in nine enumeration zones in Kilifi District, Kenya between 1989 and 1992

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mukombe</td>
<td>292</td>
<td>27.4</td>
<td>(8)</td>
<td>13.7</td>
<td>54.8</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(16)</td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Fumbini</td>
<td>156</td>
<td>38.5</td>
<td>(6)</td>
<td>19.2</td>
<td>44.9</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(7)</td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Kibarani</td>
<td>216</td>
<td>18.5</td>
<td>(4)</td>
<td>18.5</td>
<td>50.9</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(11)</td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Zowerani</td>
<td>331</td>
<td>9.1</td>
<td>(3)</td>
<td>9.1</td>
<td>21.2</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(7)</td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Kambi ya Wari</td>
<td>202</td>
<td>9.9</td>
<td>(2)</td>
<td>5.0</td>
<td>34.7</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td>(7)</td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Mikingirini</td>
<td>259</td>
<td>3.9</td>
<td>(1)</td>
<td>11.6</td>
<td>30.9</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td>(7)</td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Ufuoni</td>
<td>199</td>
<td>15.8</td>
<td>(3)</td>
<td>5.0</td>
<td>15.1</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td>(3)</td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Kaoyeni</td>
<td>233</td>
<td>12.9</td>
<td>(3)</td>
<td>0.0</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0)</td>
<td>(0)</td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Mtondia</td>
<td>105</td>
<td>0.0</td>
<td>(0)</td>
<td>9.5</td>
<td>9.5</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0)</td>
<td>(1)</td>
<td></td>
<td></td>
<td>Low</td>
</tr>
</tbody>
</table>

* Population enumerated in May 1992, with denominators for incidence rates adjusted by an annual population growth rate of 3.92%.
† Three sites each with high, moderate, and low incidence rates of severe malaria were selected based on rankings of incidence rates for 74 total sites divided into three respective categories. Additionally, sites were selected with the aim of achieving a representative spatial distribution throughout the study area (see Fig. 1).

Day resting indoor (DRI) collections were conducted weekly inside the five additional households surrounding each of the sentinel households (i.e., a total of 20 households per site). In addition to the DRI collections, pyrethrum spray catches (PSC) were done weekly in up to three randomly selected houses in each site during periods when few mosquitoes were being collected by NBC and DRI collections (August 1992 to March 1993). The PSC collections provided a check on the validity of NBC and DRI data, and the additional mosquitoes collected served to increase the total number of mosquitoes processed for the determination of sporozoite rates.

At the laboratory in Kilifi, mosquitoes were identified and prepared for sporozoite testing. Mosquitoes were cut transversely at the thorax between the first and third pair of legs, and the anterior portion was ground in 50 μl of boiled casein blocking buffer with Nonidet P-40; 200 μl of blocking buffer were then added, bringing the final volume to 250 μl. Samples were stored at −20°C and 50-μl aliquots were tested by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies to detect circumsporozoite (CS) proteins of P. falciparum. Samples were assessed visually for positivity.

Demographic data and disease surveillance. The nine sites used for the entomologic surveillance were mapped and the population was enumerated between January and May 1992. All resident individuals were ascribed a unique seven-digit number, coded by site and household number. The unique identification numbers were computerized to provide on-line record linkage with the surveillance system for severe disease at the pediatric ward of Kilifi District Hospital (see below). Incidence rates of severe malaria were calculated for children 0–4 years of age. The denominator for incidence rates was based on census data from May 1992 adjusted by the annual growth rate (3.92%) for the midpoint of the study period. Thus, for each of the nine sites, inci-
TABLE 2
Summary of anopheline mosquito collections and Plasmodium falciparum sporozoite enzyme-linked immunosorbent assay results from Kilifi District, Kenya (June 1992 to May 1993)

<table>
<thead>
<tr>
<th>Species</th>
<th>NBC</th>
<th>DRI</th>
<th>PSC</th>
<th>No. of mosquitoes per sampling technique*</th>
<th>P. falciparum infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoor collections</td>
<td></td>
<td></td>
<td></td>
<td>No. tested</td>
<td>% positive</td>
</tr>
<tr>
<td>An. gambiae s.l.</td>
<td>2,008</td>
<td>496</td>
<td>364</td>
<td>2,868</td>
<td>3.5</td>
</tr>
<tr>
<td>An. funestus</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>10.5</td>
</tr>
<tr>
<td>An. coustani</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>An. nili</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>An. squamosus</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>An. pharoensis</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Outdoor collections</td>
<td></td>
<td></td>
<td></td>
<td>261</td>
<td>0.8</td>
</tr>
<tr>
<td>An. gambiae s.l.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>An. funestus</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>An. coustani</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>An. squamosus</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>An. pharoensis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* NBC = night-biting collections inside houses, 1,647 human-nights (indoors), and 391 human-nights (outdoors); DRI = day-resting indoor collections, 7,695 collections; PSC = pyrethrum spray collections, 600 collections; – = technique cannot be used outdoors.

RESULTS

Six anopheline species were identified from the nine sites (Table 2). Of the 3,179 Anopheles collected indoors and outdoors during one year at the nine sites, An. gambiae s.l. represented 98.4%, An. funestus 0.6%, An. coustani 0.6%, An. squamosus 0.2%, An. pharoensis 0.1%, and An. nili 0.1%. Plasmodium falciparum sporozoite infections were detected by ELISA in 3.5% of 2,868 An. gambiae s.l. and in 10.5% of 19 An. funestus collected indoors, and in 0.8% of 261 An. gambiae s.l. collected outdoors. No infections were detected in the other four anophelines.

**Indices of malaria parasite transmission.** The EIR was calculated as a product of the human-biting rate and sporozoite rate. Human-biting rates, the number of mosquitoes per human-night, were determined through all-night human-biting catches. Sporozoite rates, the proportion of anophelines testing positive for CS protein per unit time, were based on ELISA testing of mosquitoes obtained by all collection methods. The EIR, a standard measure of transmission intensity, was expressed as the number of infective bites per person per unit time.

**Statistical analysis.** The Kendall coefficient of concordance (W) was calculated to assess the degree of statistical association between the EIR and the incidence of severe malaria infections. This nonparametric test was applied to the data because of the non-normal distribution of EIRs and severe malaria rates, and the limited number of study sites (i.e., n = 9).

**Relationships among human-biting rates, EIRs, and the incidence of severe disease at the nine sites are shown in Figure 2. Low human-biting rates and correspondingly low EIRs were observed in eight of the nine sites. The annual biting rate ranged from 19.4 in Ufuoni to 2,844.4 in Mtondia. Calculation of annual EIRs indicated that residents in eight of the nine sites were exposed to only 0-18 infective bites per person, while in the remaining site (i.e., Mtondia), individuals were exposed to nearly 60 infective bites. The ratio of indoor:outdoor biting by An. gambiae s.l. was 10:1. Overall, about 75% and 80% of all anophelines collected indoors and outdoors, respectively, came from the Mtondia site.

Cases of severe malaria were detected in all sites, including Mikingirini, the only site where transmission was never observed. Spatial patterns of severe disease varied independently of transmission intensity. Yearly rates of se-
were infections averaged 18.6 per 1,000 children 0–4 years of age. Ranking EIRs and the incidence of severe malaria infections for each site indicated that there were no significant associations (W = 0.69, degrees of freedom = 7, V = 0.70) between EIRs determined in each of the nine sites and rates of severe malaria in children less than five years of age.

Table 3 summarizes monthly EIRs and the number of severe *Plasmodium falciparum* infections at the nine sites during the study period. Transmission was detectable during all months except October and March, but monthly patterns varied greatly among sites. The EIRs, when detectable, ranged from 0.0 to 28.7 infective bites per person per month. There was no detectable transmission at Mikingirini throughout the year, yet severe malaria infections were detected during five months of the study period. With the exception of three sites, most transmission was detected between June and August, when most of the severe malaria cases (59%) were admitted to the Kilifi District Hospital.

**DISCUSSION**

This study demonstrates associations between low levels of *P. falciparum* transmission and relatively high incidence rates of severe malaria on the Kenyan coast. Residents in eight of the nine sites were exposed to an average of only 4.1 infective bites per year (range 0–18). In Mtondia, the site with the highest level of transmission, individuals were exposed to nearly 60 infective bites per year. Mtondia differed from other sites in that marshes and coral quarries provided suitable aquatic habitats for anophelines. More than 95% of the indoor transmission was due to *An. gambiae s.l.*, with the remainder by *An. funestus*. The potential for outdoor transmission was at least 10-fold lower than transmission inside houses and involved primarily *An. gambiae s.l.* These findings extend our earlier findings that rates of malaria parasite transmission on the Kenyan coast are exceptionally low, and are atypical of other areas of stable endemicity in Kenya.

Comparing ranks of the EIRs and severe malaria rates for the nine sites showed no significant association between the most common measure of sporozoite challenge and disease. The only comparable data are those from The Gambia and Congo where transmission is also low but markedly seasonal, with site-specific EIRs ranging from 4 to 24 infective bites in The Gambia, and 1 to < 100 in Congo. The considerable variation from 100 infective bites in the semi-rural, peripheral areas of Brazzaville and the central area, where people could expect to receive one infective bite every three years, was not associated with any differences in hospital admissions for pernicious malaria or hospital-based mortality from malaria.

Malaria parasite transmission within the majority of the sampled houses appeared to occur at extremely low levels of vector abundance. Despite this, hospital-based rates of severe malaria in this area were still high, with children having an annual 1.8% minimum risk of developing severe malaria infections. The intensity of mosquito sampling was greater than that used by other investigators in areas with higher vector abundance. Despite this intensive surveillance, the estimated annual number of *An. gambiae s.l.* bites per person ranged from only 19 to 159 in eight of the nine sites. Such extremely low levels of host contact by the predominant vector, and correspondingly low EIRs, highlight the inherent difficulties in determining low levels of transmission intensity. As shown in Table 3, there is no doubt that transmission was occurring at rates below our entomologic threshold for detection because many of the severe malaria cases were detected during prolonged periods when our entomologic surveillance indicated average monthly EIRs of zero.

Spatial and temporal patterns of severe disease also varied independently of transmission intensity. In particular, the Mtondia site, with an annual EIR of 59.6 infective bites per person, had similar incidence rates of severe malaria as Kambi ya Wari with 3.6 infective bites per person per year (Figure 2). Aside from technical difficulties associated with measuring low EIRs, it was clear that children were developing severe malaria infections following exposure to very few sporozoite inoculations. It was normal to see severe malaria cases during periods when little or no transmission was detectable. This highlights the probability that many severe cases were due to single bites from infected mosquitoes.

Within sites, there was pronounced heterogeneity in transmission intensity and disease. An unequivocal clustering of
mosquitoes within the study sites demonstrates the apparent focal nature of transmission in some zones. For instance, in the Kibarani site, more than 70% of all the anopheline mosquitoes were caught in one house and all infected mosquitoes came from this house. Such overdispersion of vectors has implications for randomized-controlled trials of vector control strategies where communities are the units of randomization. Such marked heterogeneity of mosquito distributions, particularly in an area like Kibarani, confounds attempts to investigate correlations between transmission and disease on a small geographic scale.

The current basis for malaria control in Africa is that malaria-specific mortality can be reduced by vector control measures such as the use of insecticide-impregnated bednets. Although studies in Africa have shown decreases in childhood mortality by the use of insecticide-impregnated bednets, levels of transmission that are acceptable from the public health perspective have not yet been established. More data are required on the relationships between disease and EIRs at higher levels of transmission. Our findings that high rates of severe disease can occur even at very low levels of transmission raise questions regarding the long-term impact of vector control efforts in Africa and elsewhere. It is clear that significantly reducing high rates of transmission, even to levels where annual EIRs are < 10, will not prevent unacceptably high levels of severe disease.

Acknowledgments: We are grateful for the assistance of all scientific and technical staff at the Kilifi Research Unit, particularly Dr. Dayo Forster, Samuel K. Muiruri, Arnold M. Mwakala, Nathaniel Baya, and Dr. N. M. Peshu. We thank Dr. Robert Wirtz for providing monoclonal antibodies through a grant from the World Health Organization. This paper was published with the permission of the Director of the Kenya Medical Research Institute.

Financial support: This study was supported by funds from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Robert W. Snow is a Wellcome Trust Senior Fellow in Basic Biomedical Sciences, Kevin Marsh is a Wellcome Trust Senior Fellow in Clinical Sciences, John C. Beier was supported by grant AI-29000 from the National Institute of Health.

Authors’ addresses: Charles N. M. Mbogo, Kenya Medical Research Institute (KEMRI), Kilifi Research Unit, PO Box 428, Kilifi, Kenya. Robert W. Snow and Kevin Marsh, Kilifi Research Unit, Oxford University, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford OX3 3DU, United Kingdom. Canute P. M. Khamala, Department of Zoology, University of Nairobi, Nairobi, Kenya. Ephantes W. Kaburu and John H. Ouma, Division of Vector Borne Diseases, Ministry of Health, PO Box 20750, Nairobi, Kenya. John I. Githure, Kenya Medical Research Institute, Biomedical Sciences Research Centre, PO Box 54840, Nairobi, Kenya. John C. Beier, Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University Medical Center, 1501 Canal Street, 5th Floor, New Orleans, LA 70112-2824.

Reprint requests: Charles N. M. Mbogo, Kenya Medical Research Institute, Kilifi Research Unit, PO Box 428, Kilifi, Kenya.

REFERENCES

21. Smith T, Charliwood JD, Kihonda J, Mwankuse S, Billingsley