VECTOR-RELATED CASE-CONTROL STUDY OF SEVERE MALARIA IN KILIFI DISTRICT, KENYA

CHARLES N. M. MBOGO, EPHANTUS W. KABIRU, GREGORY E. GLASS, DAYO FORSTER, ROBERT W. SNOW, CANUTE P. M. KHAMALA, JOHN H. OUMA, JOHN I. GITHURE, KEVIN MARSH, AND JOHN C. BEIER

Kenya Medical Research Institute (KEMRI), Kilifi Research Unit, Kilifi, Kenya; Biomedical Sciences Research Centre, KEMRI, Nairobi, Kenya; Department of Zoology, University of Nairobi, Nairobi, Kenya; Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya; Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford University, Oxford, United Kingdom; Department of Molecular Microbiology and Immunology, The Johns Hopkins University, Baltimore, Maryland; Department of Tropical Medicine, Tulane University, New Orleans, Louisiana

Abstract. A case-control study examined vector-related and environmental parameters associated with severe malaria in Kilifi District along the coast of Kenya. Over an 11-month period, 119 children identified with severe malaria infections at the Kilifi District Hospital were matched by age with control children who reported to the outpatient clinic with nonsevere infections. Intensive mosquito sampling was done in each of the case-control households over a four-day period, beginning within a week of index case admission. A total of 109 environmental, demographic, behavioral, and animal husbandry variables were characterized for each household. Vector species (Anopheles gambiae s.l. and An. funestus) were detected in 40.1% and 36.1% of case and control houses, respectively. The relative abundance of vectors in individual houses was stable over the two-week resampling periods ($r = 0.9$). Both the overall abundance of anopheline mosquitoes (odds ratio $[OR] = 1.5$) and $P. falciparum$ sporozoite rates (OR $= 1.5$) were not significantly different between case and control houses. In a matched analysis, 11 of 109 house variables associated significantly with severe malaria were also associated with vector abundance, as determined by chi-square linear trend analysis. Under conditions of year-round, low-level transmission on the coast of Kenya, the risk of severe disease in children is multifactorial and not governed strictly by transmission intensity or environmental heterogeneity affecting vector abundance and distributions. This suggests that current interventions that appear to be achievable only in areas where transmission is already low to moderate should be appropriate. However, such interventions should be monitored so that inappropriate and possibly disastrous control activities can be avoided in Africa.

In endemic areas of malaria transmission, most infections are asymptomatic. A major question is why only a small proportion of infected individuals develop severe, life-threatening malaria infections. Relative to disease control, individuals with severe malaria require extensive, supportive health care. In Africa, nearly half of those who develop severe disease in rural areas die, partly because health services are not well equipped to deal with the complications of this disease. Determinants of the clinical outcome of malaria may be related to human host, parasite, vector, or environmental parameters. However, the relative importance of any of these factors is unclear.

In this paper, we used a case-control study design to evaluate the role of anopheline mosquitoes in the occurrence of clinically severe malaria in children residing in Kilifi District, Kenya. Transmission intensity for households of cases and matched controls was examined relative to household and environmental parameters to identify and quantify exposure risk. Determining how children with severe disease differed in their exposure history when compared with infected children who did not develop severe disease provided a basis for evaluating how the epidemiology of severe malaria is influenced by transmission at the household level.

MATERIALS AND METHODS

Study area. The study area in Kilifi District, Kenya, has been described previously. Kilifi District lies on the coastal plain approximately 60 km north of Mombasa. It is an endemic area of Plasmodium falciparum transmission. The population is estimated at 63,834 people, of which 12,000 are children less than five years of age. Approximately 200 cases of severe malaria are admitted to the Kilifi District Hospital annually.

Study population. These studies were approved by the Ethical Committee of the Kenya Medical Research Institute. The study population consisted of all children less than five years of age with a primary diagnosis of severe malaria admitted to Kilifi District Hospital from August 1991 through June 1992. The parents of the children provided written informed consent for their participation in the study. Each child admitted to the pediatric ward at Kilifi District Hospital underwent a full clinical, parasitologic, and hematologic examination. All primary diagnoses of falciparum malaria were further defined as severe malaria if the child was unable to localize pain, or had a hemoglobin level $< 5$ g/dL with a peripheral parasitemia $\geq 10,000/\mu L$ or more, or was unable to sit or stand unaided (prostrated), or had two or more generalized convulsions within 24 hr prior to admission, or had 20% or more $P. falciparum$-infected red blood cells, or the child died without any of these complications. The control group was selected from all children less than five years of age attending the outpatient clinic in the KEMRI Unit at Kilifi District Hospital. These children had parasitemias $\geq 5,000$ parasites/µL of blood, which indicated the presence of significant infection without severe complications.

Cases were matched with controls by age ($\pm 6$ months) and time of admission/treatment (within two weeks). Home addresses were obtained from all individuals enrolled in the study, and interviews and surveys were conducted at the household within one week of identification.

Anopheline sampling methods. Mosquitoes were collected from households of children within one week of their enrollment. Mosquito collection methods are described elsewhere. Briefly, collections were made by three techniques;
Centers for Disease Control light traps, human-biting catches, and day-resting indoor collections. On four successive days, each house was sampled for mosquitoes by two all-night light trap collections, one all-night human biting collection and one day resting collection. All anopheline mosquitoes were identified and tested by ELISA for the presence of \textit{P. falciparum} circumsporozoite protein. Additionally, 146 households were resampled two weeks after the initial sampling to evaluate concordance between mosquito abundance at intervals approximating the duration from the time of inoculation exposure to the onset of disease.

**Household and environmental parameters.** A questionnaire to characterize the demography, house construction, animal husbandry practices, and environmental surroundings was completed for each house by a team of trained interviewers. Household information included family size and composition, and activities or behaviors that might affect exposure to vectors such as the use of mosquito repellents and bed nets or the patterns of nocturnal activity. Information on housing construction focused on factors potentially affecting mosquito access to humans, including the type of construction materials of the walls and roof, eaves, sleeping arrangements within the households, and the presence of impediments to mosquito movements such as doors or window screens. Data on numbers and kinds of various domestic animals were obtained to assess the potential roles of various animals as risk factors for vector exposure. Environmental factors included such variables as the location of the house relative to woodland edge, the presence of various crops, temporary pools of water, and materials that might serve as breeding or resting sites for mosquitoes. The questionnaire included 109 parameters.

**Data analysis.** Differences in anopheline abundance between case and control houses were determined by a paired Student’s \( t \)-test. When possible, variables were dichotomized for their presence or absence. Quantitative variables were categorized into quartiles. Odds ratios (ORs) were calculated for matched case-control data to test for associations between the study variables and the occurrence of severe malaria. Conditional logistic regression was used to control for confounding variables. Variables that were statistically significant in the matched analyses were included in the initial regression model and their contributions tested by partial F-tests. The remaining variables then were added and retained if they contributed significantly to the model. After selection of the final model, all possible interactions between variables were assessed and retained if statistically significant (\( P < 0.05 \)). Analyses were performed using the EGRET Program. Patterns of mosquito abundance and infection rates were examined in case and control households for variables found to be significant in the regression model. Trends in the prevalence of mosquito abundance in case and control households were tested by chi-square analysis.

**RESULTS**

During the study period, 136 cases of severe malaria and 126 cases of infection without severe disease (controls) were selected. Temporally matched pairs with completed household surveys were available for 119 pairs (87.5%) of households.

Figure 1 shows the frequency distribution of malaria vectors and \textit{P. falciparum}-infected mosquitoes inside case and control houses. There was no difference in the transmission intensity between case and control houses. Low numbers of malaria vectors per house emphasizes that individual children can develop severe disease by exposure to few if not single infectious bites. Vector species of mosquitoes were detected in 38.2% of 238 houses. Of 1,702 anopheline mosquitoes captured in both case and control houses, 96.2% were \textit{An. gambiae} \textit{s.l.} and 3.8% were \textit{An. funestus}. Overall, the abundance of anopheline mosquitoes was similar in case
DISCUSSION

Vector abundance does not appear to be a major factor affecting the risk of severe malaria on the coast of Kenya. Under the observed conditions of low vector densities,\(^5\) the risk of severe malaria may be multifactorial and not strictly associated with transmission intensity. This highlights the possible role of human host- and parasite-related determinants of severe malaria infections. The low sporozoite inoculation rate, coupled with the high degree of human feeding by vector populations,\(^12\) may be contributing to the efficiency of parasite transmission even at extremely low vector densities. Our earlier studies in the same area indicated that as few as one in 300 or more sporozoite inoculations were associated with severe disease in children.\(^6\)

Several factors potentially modifying vector abundance were associated with the development of severe malaria. For instance, the risk of severe disease increased in houses with more than six people who did not use bed nets and in presence of more than two sheep or goats in a house. Whereas the use of bed nets provides personal protection against mosquitoes, the evidence that the presence of domestic animals in the compound is a risk factor was unexpected, and therefore, this association may have arisen by chance. Such analyses needs to be interpreted with extreme caution. It is possible that household features are associated with socioeconomic status, itself very hard to measure, which in turn affects educational levels of mothers and their malaria treatment seeking behaviors. The fact that it was not possible to demonstrate any effects of the presence of other animals suggests that this finding is either a marker for something else or simply a spurious result given the number of cross-tabulations performed in the final analysis.

Environmental heterogeneity affects vector distribution and abundance, and consequently transmission potential. The existence of severe disease in houses with undetectable vector mosquitoes, coupled with the lack of association among > 90 house and environmental parameters suggests that severe disease can occur in all environments despite interesting time-space clustering patterns.\(^5\) The sporozoite rate among vector mosquitoes caught in households of children who had severe or mild malaria were similar. However, the number of \(P.\) falciparum-positive mosquitoes obtained in each group was low so that no firm conclusions can be drawn from this finding. Overall, these results indicate that children who develop severe malaria were under similar levels of exposure to malaria-infected mosquitoes as children who developed mild malaria at the time of presentation at the hospital, and that this was also probably the case at the time of their infection. It is difficult to know whether chil-

---

**Table 1**

<table>
<thead>
<tr>
<th>Trapping technique*</th>
<th>Mean number of anopheline mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
</tr>
<tr>
<td>HBI</td>
<td>4.9</td>
</tr>
<tr>
<td>LT</td>
<td>3.4</td>
</tr>
<tr>
<td>DRI</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>9.1</td>
</tr>
</tbody>
</table>

*HBI = human-biting indoor catches; LT = Centers for Disease Control light traps; DRI = day-resting indoor collections.

**Table 2**

<table>
<thead>
<tr>
<th>Species</th>
<th>% (n) of mosquitoes positive by ELISA</th>
<th>(\chi^2)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(An.) gambiae s.l.</td>
<td>4.9 (906)</td>
<td>0.56</td>
<td>0.45</td>
</tr>
<tr>
<td>(An.) funestus</td>
<td>11.4 (35)</td>
<td>0.41</td>
<td>0.52</td>
</tr>
<tr>
<td>Total</td>
<td>5.1 (941)</td>
<td>1.01</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Arithmetic means for combined totals of \(Anopheles\) gambiae s.l. and \(An.\) funestus caught in case and control houses by three trapping techniques in Kilifi District, Kenya, (August 1991 to June 1992).
Table 3
Odds ratio, 95% confidence interval (CI), and conditional logistic regression for some variables associated with malaria disease in Kilifi, Kenya

<table>
<thead>
<tr>
<th>Variable</th>
<th>Matched analysis</th>
<th>Conditional logistic regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>People in the house</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3 people &gt;20 years old</td>
<td>0.38</td>
<td>(0.15, 0.96)</td>
</tr>
<tr>
<td>&gt;6 people exposed</td>
<td>4.55</td>
<td>(1.16, 3.94)</td>
</tr>
<tr>
<td>&gt;2 children &lt;7 years old</td>
<td>0.63</td>
<td>(0.36, 1.12)</td>
</tr>
<tr>
<td>&lt;2 children &lt;7 years old</td>
<td>2.08</td>
<td>(1.05, 4.15)</td>
</tr>
<tr>
<td>Presence of animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;2 sheep</td>
<td>11.00</td>
<td>(1.42, 85.20)</td>
</tr>
<tr>
<td>&gt;1 dog</td>
<td>0.43</td>
<td>(0.20, 0.93)</td>
</tr>
<tr>
<td>Openings (doors plus windows)</td>
<td>1.22</td>
<td>(1.01, 1.47)</td>
</tr>
<tr>
<td>Two bedrooms</td>
<td>0.53</td>
<td>(0.31, 0.89)</td>
</tr>
<tr>
<td>Temporary pools</td>
<td>0.12</td>
<td>(0.02, 0.99)</td>
</tr>
</tbody>
</table>

In conclusion, evidence is presented that the risk of severe malaria infections in children living on the coast of Kenya is not associated with vector abundance and associated environmental heterogeneity. This suggests that current interventions that appear to be achievable only in areas where transmission is already low to moderate should be appropriate. However, such interventions should be monitored so that...
Acknowledgments: We are grateful for the assistance of all scientific and technical staff at the Kilifi Research Unit, particularly Dr. N. M. Peshu, Joseph M. Nzovu, Samuel K. Muiruri, Arnold M. Mwakala, and Nathaniel Baya. We thank Dr. Robert Wirtz for providing monoclonal antibodies through a grant from the World Health Organization. This paper is published with the permission of the Director of the Kenya Medical Research Institute.

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

Authors’ addresses: Charles N. M. Mbogo, Kenya Medical Research Institute, Kilifi Research Unit, PO Box 428, Kilifi, Kenya. Ephantus W. Kabiru and John H. Ouma, Division of Vector Borne Diseases, Ministry of Health, PO Box 20750, Nairobi, Kenya. Gregory E. Glass, Department of Molecular Microbiology and Immunology, The Johns Hopkins University, School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205. Dayo Forster, Robert W. Snow, and Kevin Marsh, Kenya Medical Research Institute, Kilifi Research Unit, PO Box 428, Kilifi, Kenya and Nufﬁeld Department of Clinical Medicine, John Radcliffe Hospital, Oxford University, Oxford OX3 3DU, United Kingdom. Canute P. M. Khamala, Department of Zoology, University of Nairobi, PO Box 30197, Nairobi, Kenya. John C. Beier, Department of Tropical Medicine, Tulane University, 1501 Canal Street, New Orleans, LA 70112.

REFERENCES


