

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

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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 houses 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.^{1,2} 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.³⁻⁵ Determinants of the clinical outcome of malaria may be related to human host, parasite, vector, or environmental parameters.^{1,2} 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.^{5,6} 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.⁷ 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 $\geq 10,000/\mu\text{l}$ 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.⁵ 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 (± 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.⁸ Briefly, collections were made by three techniques;

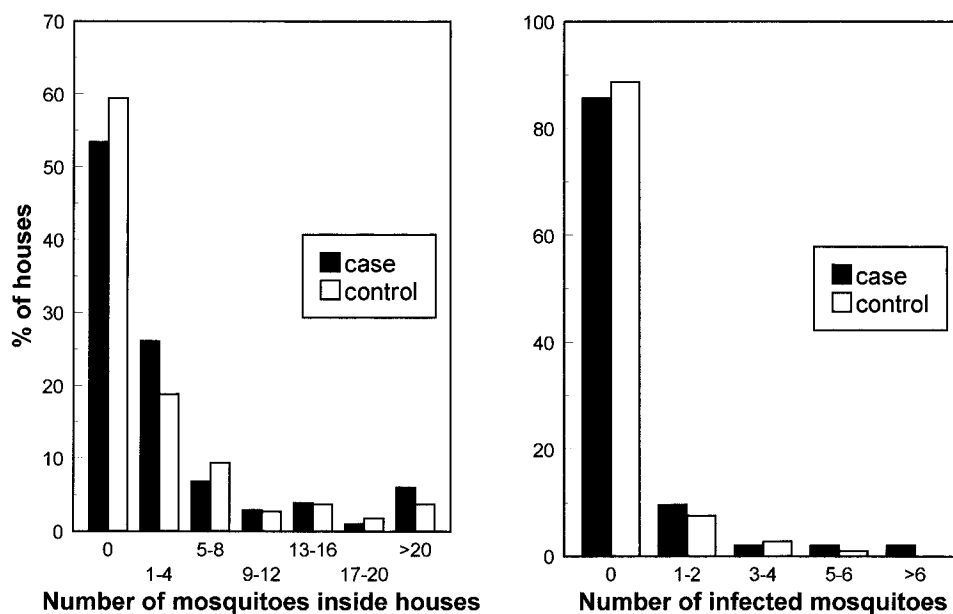


FIGURE 1. Frequency distribution of malaria vectors and the number infected with *Plasmodium falciparum* inside case and control houses.

Centers for Disease Control light traps, human-biting catches, and day-resting indoor collections.⁹ 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 *P. falciparum* circumsporozoite protein.¹⁰ 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.¹¹ 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 *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 *An. gambiae s.l.* and 3.8% were *An. funestus*. Overall, the abundance of anopheline mosquitoes was similar in case

TABLE 1

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)

Trapping technique*	Mean number of anopheline mosquitoes		<i>t</i>	<i>P</i>
	Case	Control		
HBI	4.9	3.9	0.55	0.59
LT	3.4	5.3	0.74	0.46
DRI	0.4	0.4	0.15	0.89
Total	9.1	7.2	0.85	0.40

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

and control houses ($t = 0.85$; degrees of freedom = 103, $P = 0.399$, by paired Student's *t*-test) (Table 1). The abundance of either species of mosquito (none, 1–7, or > 7 mosquitoes) was not associated with severe disease ($P > 0.05$ for all comparisons, by chi-square test for trend). The relative abundance of anopheline vectors in the houses was stable over a two-week period based on comparisons for 146 houses re-sampled ($r = 0.9$).

The proportions of anopheline mosquitoes infected with *P. falciparum* were also similar in case and control houses (*An. gambiae s.l.* OR = 1.5, 95% confidence interval [CI] = 0.9, 2.5; *An. funestus* OR = 3.4, 95% CI = 0.3, 171.5) (Table 2). Of the 49 case houses with at least one mosquito collected, 16 (33.3%) yielded *P. falciparum* sporozoite-positive mosquitoes compared with 13 (30.2%) of 43 in control houses. The mean sporozoite rate per house was similar in case and control houses (OR = 0.96; 95% CI = 0.39, 2.33).

In matched analyses, 11 of 109 environmental and demographic variables were associated ($P < 0.05$) with severe malaria (Table 3). The risk of severe disease decreased with more than three adults in the household, more than two children less than seven years of age in the household, houses with two bedrooms, households with more than one dog, and houses with small temporary pools of water (Table 3). The risk of severe disease increased in households with more than six individuals who did not use bed nets, households with less than two children less than seven years of age, houses that had at least two or more windows or doors that could not be sealed, and households with more than two sheep (Table 3). Ninety-eight household and environmental parameters were not significant.

Seven variables met the criterion for inclusion in the final conditional logistic regression model (Table 3). The risk of severe disease increased in houses with more than six individuals who did not use nets, and in houses with more than two sheep. However, the risk of severe disease decreased in houses with more than three adults greater than 20 years of age, more than two children less than seven years of age, houses with more than one dog, and houses with two bedrooms. There were no significant interactions among the variables. Despite the absence of an overall difference in mosquito abundance between case and control houses, there were significant differences in the patterns of mosquito presence between case and control houses for variables in the final regression model (Figure 2).

TABLE 2

Plasmodium falciparum infection rates for *Anopheles gambiae s.l.* and *An. funestus* caught in case and control houses in Kilifi, Kenya

Species	% (n) of mosquitoes positive by ELISA		χ^2	<i>P</i>
	Case	Control		
<i>An. gambiae s.l.</i>	4.9 (906)	4.0 (731)	0.56	0.45
<i>An. funestus</i>	11.4 (35)	3.7 (27)	0.41	0.52
Total	5.1 (941)	4.0 (758)	1.01	0.32

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,⁶ 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 suggest 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 3

Odds ratio, 95% confidence interval (CI), and conditional logistic regression for some variables associated with malaria disease in Kilifi, Kenya

Variable	Matched analysis		Conditional logistic regression analysis	
	Odds ratio	95% CI	Odds ratio	95% CI
People in the house				
>3 people >20 years old	0.38	(0.15, 0.96)	0.08	(0.02, 0.35)
>6 people exposed	4.55	(1.16, 3.94)	6.27	(2.28, 17.27)
>2 children <7 years old	0.63	(0.36, 1.12)	0.45	(0.22, 0.94)
<2 children <7 years old	2.08	(1.05, 4.15)		
Presence of animals				
>2 sheep	11.00	(1.42, 85.20)	11.35	(1.33, 96.96)
>1 dog	0.43	(0.20, 0.93)	0.28	(0.09, 0.80)
Openings (doors plus windows)				
Two bedrooms	1.22	(1.01, 1.47)		
Temporary pools	0.53	(0.31, 0.89)	0.34	(0.16, 0.69)
	0.12	(0.02, 0.99)	0.08	(0.01, 0.88)

dren who developed severe malaria were exposed to a greater sporozoite inoculum than those who developed mild malaria. However, since both groups were exposed to similar levels of bites and abundance, it is reasonable to postulate that the risk of contracting severe malaria is not governed by level of exposure but the dose of sporozoite inoculum. It has been shown in low transmission conditions where infection is accompanied by disease that each episode is likely to have arisen from a single infective bite.^{6,13} This suggests that

children in these environments can develop severe disease by exposure to few if not single infectious bites.

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

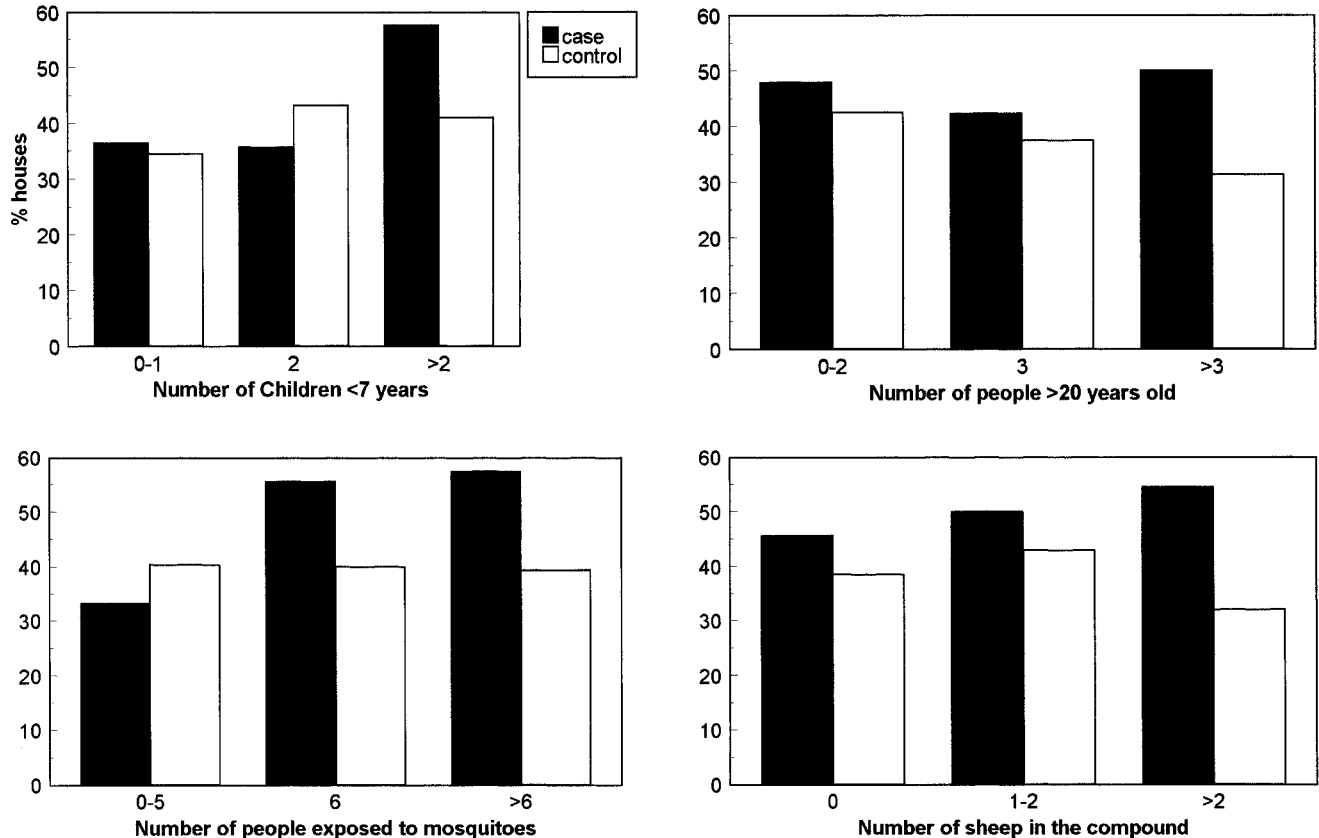


FIGURE 2. Trends in mosquito occurrence in case and control houses relative to the variables that were significant in the conditional regression analysis.

inappropriate and possibly disastrous control activities can be avoided in Africa.

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