Spatial Variation of Malaria Incidence in Young Children from a Geographically Homogeneous Area with High Endemicity

Benno Kreuels,[1] Robin Kobbe,[1] Samuel Adjei,[3] Christina Kreuzberg,[1,2] Claudia von Reden,[1] Kathrin Bäter,[1] Stefan Klug,[1] Wibke Busch,[1] Ohene Adjei,[3] and Jürgen May[1]

[1]Infectious Disease Epidemiology Group, Bernhard Nocht Institute for Tropical Medicine, and [2]Section for Tropical Medicine, Bernhard Nocht Clinic, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [3]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana

Background. In sub-Saharan Africa, malaria is a leading cause of morbidity and mortality among young children. Detailed knowledge of spatial variation of malaria epidemiology and associated risk factors is important for planning and evaluating malaria-control measures.

Methods. The spatial variation of malaria incidences and socioeconomic factors were assessed over 21 months, from January 2003 to September 2005, in 535 children from 9 villages of a small rural area with high *Plasmodium falciparum* transmission in Ghana. Household positions were mapped by use of a global positioning system, and the spatial effects on malaria rates were assessed by means of ecological analyses and bivariate Poisson regression controlling for possible confounding factors.

Results. Malaria incidence was surprisingly heterogeneous between villages, and ecological analyses showed strong correlations with village area ($R^2 = 0.74$; $P = .003$) and population size ($R^2 = 0.68$; $P = .006$). Malaria risk was affected by a number of socioeconomic factors. Poisson regression showed an independent linear rate reduction with increasing distance between children's households and the fringe of the forest.

Conclusions. The exact location of households in villages is an independent and important factor for the variation of malaria incidence in children from high-transmission areas. This fact should be considered in the planning of intervention trials and in spatial targeting of malaria interventions at a local level.

Worldwide, >500 million malaria attacks occur every year, and ~2 million people die of *Plasmodium falciparum* malaria. Sub-Saharan Africa carries most of the burden, and in regions of stable transmission children <5 years of age are at highest risk of malaria morbidity and mortality [1]. Given the magnitude of the problem on the continent, exact targeting of malaria-control measures is needed for cost-effective application of proven and/or new interventions, because it has been shown that only 20% of the population at risk suffers 80% of all infections [2, 3]. Moreover, malaria risk varies markedly across Africa and, most importantly, within countries. For example, a number of studies have shown that malaria vector distribution, transmission rates, and incidence can vary widely over short distances, between neighboring villages, and even within a single settlement as a result of small-area variations in risk factors [4–6]. The identification and understanding of this variation is important, because it allows the detection of high-risk groups and selective targeting of intervention measures [7].

Small-area variation in disease risk can, in theory, be explained by the spatial heterogeneity of exposures to infection—that is, human vector contacts. This has been attributed to differing environments [8] and the varying ecologies of local malaria vectors [9–12] as well as to

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Reprints or correspondence: Dr. Jürgen May, Bernhard Nocht Institute for Tropical Medicine, Infectious Disease Epidemiology, Bernhard-Nocht-Str. 74, D-20359 Hamburg, Germany (may@bni.uni-hamburg.de).

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sociocultural, economic [4, 13], and genetic [14] factors. Many of these parameters have been assessed in areas of unstable seasonal transmission [15–17] and are often considered to be fairly uniform in areas of stable malaria transmission [4, 7]. However, the epidemiology of local malaria is likely to differ even under such conditions. With recently increased support for malaria control in Africa, more research is needed to improve our understanding of the microepidemiology of malaria, especially in settings of high endemicity and to increase the possibility of reducing this preventable and curable disease among high-risk groups.

The aim of the study presented here was to identify factors responsible for variation in malaria incidence at a personal, household, and village level by active monthly follow-ups and passive case detection in an area of high endemicity. Risk factors were assessed through geographic information systems, derived ecological parameters, questionnaires on socioeconomic parameters, and genetic analyses.

SUBJECTS, MATERIALS, AND METHODS

Study population and study area. This study focused on infants from 9 villages of the rural Afigya-Sekyere District in the forest belt of the Ashanti Region in Ghana (figure 1). Economic activity in the district is limited to subsistence farming and small-scale trading. The study area covers ~200 km^2, is geographically homogeneous, and is holoendemic for *P. falciparum* malaria, with perennial transmission and seasonal peaks (high transmission from May to October) [18]. The population ranges from 1492 inhabitants in the smallest village to 12,877 inhabitants in the largest (population census data [year 2000]; available from: Afigya-Sekyere District Assembly, PO Box 1, Agwoya, Ashanti Region, Ghana). The principal malaria vectors are mosquitoes of the *Anopheles gambiae* complex and *Anopheles funestus*. The entomological inoculation rate of *P. falciparum* in the area is >400 infectious bites/year, with a peak of >100 infectious bites/month at the end of the rainy season (authors' unpublished data). The altitude variation is 146 m between the lowest (287 m) and the highest (433 m) village. The temperature variation is 20.4°C–33.5°C, with monthly rainfall between 15 and 214 mm in January and June, respectively [19].

Infants whose data are reported in this analysis were enrolled in a randomized, double-blind, placebo-controlled study of intermittent preventive treatment, which was conducted from January 2003 until September 2005 [20]. The study was approved by the Committee of Human Research, Publications, and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. To analyze the situation under nearly natural conditions, only data from the placebo arm of the clinical trial were used in this analysis.

Data collection. Children who visited 1 of the health centers in the study area during the recruitment period and met the following criteria were included: age of 3 months (±4 weeks), no chronic diseases, and permanent residence in the study area. Follow-up visits were conducted every month over a period of 21 months until children were 24 months old (10,284 visits in total). Study aims and procedures were explained to parents/guardians; their understanding was confirmed through an interview before written or thumb-printed consent was obtained. At each visit, a standardized medical history was taken and a physical examination was performed, and the acquired information was then stored in the study file. Parasite examination was done according to quality-controlled standardized procedures described elsewhere [21]. A malaria episode was defined as an event of fever (temperature >38.0°C or fever during the preceding 48 h reported by mothers without being asked) accompanied by asexual *P. falciparum* parasitemia of >500 parasites/μL. Parents were encouraged to visit the study team or one of the assigned district health facilities whenever the child was sick.

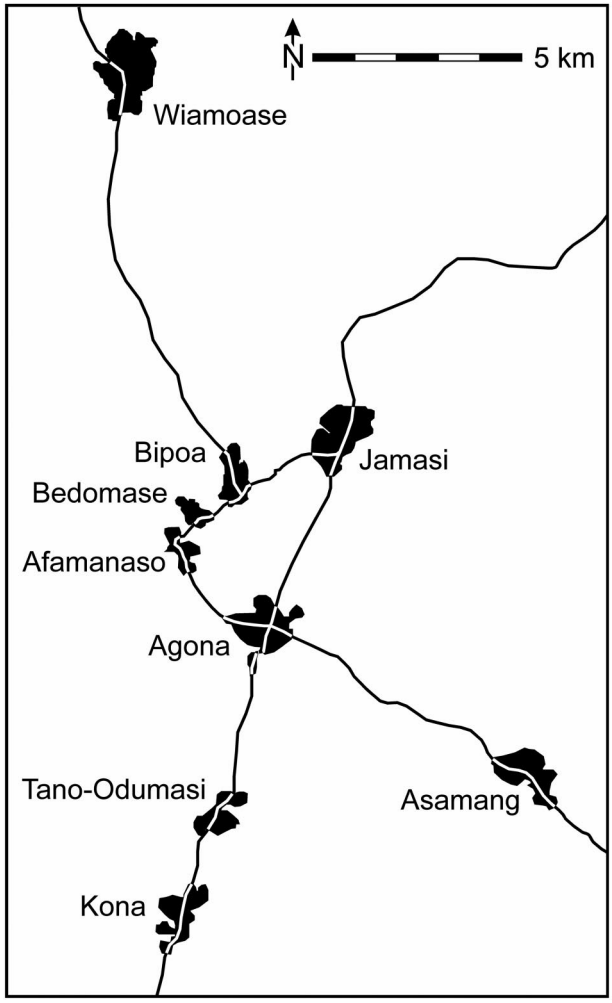

Figure 1. Map of the 9 study villages in the Afigya-Sekyere District, Ashanti Region, Ghana, 2003–2005. Black areas indicate villages, and solid lines indicate main roads.

Medical information was documented in the participants' weighing cards, which were used by the study physician to assess the medical history between active study visits. Information on personal or family characteristics with a possible influence on malaria (sex, ethnic group, birth season, mother's education and occupation, knowledge of transmission, use of protective measures, and family's financial situation) was collected on recruitment during interviews conducted in the local language, Twi, on the basis of a questionnaire. Genotype of the β-globin gene (sickle cell trait) was assessed after the study was completed. Latitude and longitude coordinates and the altitude of households were measured by use of a handheld global positioning system (GPS) receiver (Garmin eTrex GPS; GPS Gesellschaft für professionelle Satellitennavigation). Data from questionnaires and forms were entered within 5 days of each visit, cross-checked, and cleaned before the database was locked. All information on participants and their parents was treated confidentially.

Data analysis. Participants were classified into 2 ethnic groups according to their tribal background: the Akan, who are native to the area, and the tribes of northern ethnicity, who have a migratory background but are now permanent residents of the area. Knowledge of malaria transmission was deemed to be adequate if the mother was aware that mosquitoes are involved in the transmission of the disease. Mothers' occupations were grouped into farmers and nonfarmers, because we expected variation in exposure to mosquito bites between the 2 groups. The family's financial situation was defined as good if the kitchen was inside and the house had electricity and piped water. If the family lacked one of these parameters, the situation was defined as poor. A mother was considered to be literate if she had completed at least a primary school education.

Mapping of household positions (according to GPS coordinates) and processing of satellite images was done using Geographic Resources Analysis Support System (GRASS) software (version 6.1), an open-source geographic information system program [22]. Spectral bands recorded by Landsat 5 in March 2002 were acquired from the Earth Science Data Interface at the Global Land Cover Facility [23]. An optimum index factor was calculated to determine the spectral satellite band combination, which showed the maximum information when combined into a composite image. We used the Brovey transformation with 3 multispectral satellite image channels and the panchromatic channel to calculate 3 new color channels of 5 bits/pixel each. These were then combined to form a composite red-green-blue color image with sufficient resolution for further analysis. Village boundaries were defined as the transition between housing and vegetation identified by spectral analysis of these satellite images. After determining whether households were within the area of a village, the shortest distances between the fringe of the forest and the positions of participants' homes were calculated using GRASS by identifying the minimum distance from household positions to the nearest point in the village boundaries. In a second step, we calculated 360 points on a circle of 300-m radius around each household. We then used the number of points outside the village area as a benchmark for the exposure to the forest area around the village.

Statistical analysis of the data was done with Stata/SE software (version 9.2; College Station). Incidence rates (IRs) were calculated for person-times within the 21-month period of follow-up, starting at recruitment at the age of 3 months. Children were not rated as being at risk for malaria 21 days after confirmed malaria episodes or after presumptive antimalarial therapy. The distribution of fixed parameters (sex, ethnic group, birth season, sickle cell trait, mother's education and occupation, knowledge of malaria, use of protective measures, and family's financial situation) among the 9 villages was analyzed using Pearson's χ^2 tests, with differences considered to be significant at $P < .05$. The effects of these predefined parameters on malaria IRs were assessed by bivariate Poisson regression, controlling for possible confounding by the village of residence. Malaria IRs were plotted against village size, population, distance to the forest fringe, and length of the village boundary within 300 m, and the unweighted correlation coefficient *(r)* was calculated. Children with missing values in at least 1 category ($n = 123$) did not differ significantly from others regarding their malaria incidence ($P = .98$). Therefore, these children were included in every bivariate analysis if they were not missing data in the category under consideration. Characteristics with significant effects in the bivariate analysis ($P < .05$) were further analyzed in a multivariate Poisson regression model that also considered possible effect modifications.

RESULTS

Of the 535 children included in the analysis, 357 (67%) experienced at least 1 malaria episode during the observation period,

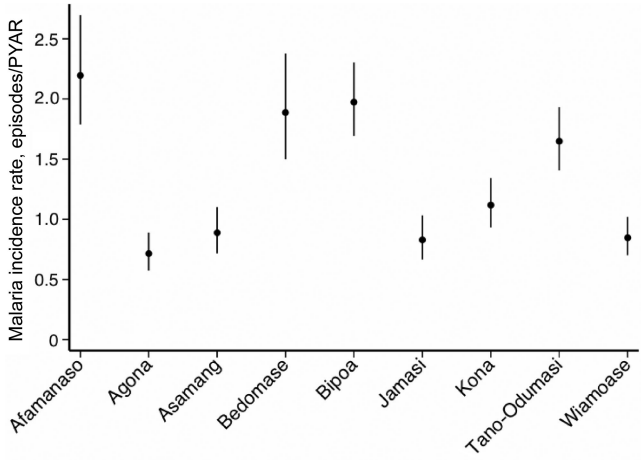

Figure 2. Malaria incidence rates (episodes per person-year at risk [PYAR]) *(dots)* and 95% confidence intervals *(bars),* stratified by the children's village of residence.

Table 1. Distribution of village and individual characteristics among the 9 study villages.

Characteristic	Village									P^a
	Afamanaso	Agona	Asamang	Bedomase	Bipoa	Jamasi	Kona	Tano-Odumasi	Wiamoase	
Size, km²	0.35	1.22	0.99	0.27	0.60	1.34	0.80	0.56	1.78	
Population, no.	2508	9321	5277	1492	3875	9096	5853	3453	12,877	
Sex										.61
Male	16 (57.1)	35 (49.3)	28 (46.7)	18 (66.7)	28 (49.1)	38 (52.1)	35 (53.9)	34 (54.8)	40 (43.5)	
Female	12 (42.9)	36 (50.7)	32 (53.3)	9 (33.3)	29 (50.9)	35 (47.9)	30 (46.1)	28 (45.2)	52 (56.5)	
Ethnic group										<.001
Akan	25 (89.3)	55 (78.6)	56 (93.3)	27 (100.0)	53 (93.0)	47 (66.2)	57 (89.1)	50 (82.0)	82 (89.1)	
Northerner	3 (10.7)	15 (21.4)	4 (6.7)	0	4 (7.0)	24 (33.8)	7 (10.9)	11 (18.0)	10 (10.9)	
Birth season										.001
Dry	10 (35.7)	46 (64.8)	30 (50.0)	13 (48.1)	24 (42.1)	47 (64.4)	28 (43.1)	30 (48.4)	63 (68.5)	
Rainy	18 (64.3)	25 (35.2)	30 (50.0)	14 (51.9)	33 (57.9)	26 (35.6)	37 (56.9)	32 (51.6)	29 (31.5)	
Sickle cell trait										.41
HbAA	19 (90.5)	52 (92.9)	49 (89.1)	20 (90.9)	38 (79.2)	49 (87.5)	45 (84.9)	52 (94.5)	61 (85.9)	
HbAS	2 (9.5)	4 (7.1)	6 (10.9)	2 (9.1)	10 (20.8)	7 (12.5)	8 (15.1)	3 (5.5)	10 (14.1)	
Mother's education										.71
Illiterate	2 (7.1)	6 (8.5)	5 (8.3)	3 (11.1)	4 (7.1)	7 (10.0)	4 (6.2)	9 (14.5)	13 (14.1)	
Literate	26 (92.9)	65 (91.5)	55 (91.7)	24 (88.9)	52 (92.9)	63 (90.0)	61 (93.8)	53 (85.5)	79 (85.9)	
Mother's occupation										<.001
Nonfarmer	13 (46.4)	62 (87.3)	47 (78.3)	22 (81.5)	41 (71.9)	68 (93.2)	59 (90.8)	47 (75.8)	61 (66.3)	
Farmer	15 (53.6)	9 (12.7)	13 (21.7)	5 (18.5)	16 (28.1)	5 (6.8)	6 (9.2)	15 (24.2)	31 (33.7)	
Knowledge of malaria										.089
Inadequate	7 (26.9)	16 (25.0)	6 (10.3)	4 (17.4)	10 (18.9)	5 (10.2)	6 (9.7)	6 (10.7)	16 (21.9)	
Adequate	19 (73.1)	48 (75.0)	52 (89.7)	19 (82.6)	43 (81.1)	44 (89.8)	56 (90.3)	50 (89.3)	57 (78.1)	
Protective measure										.045
None	16 (61.5)	25 (39.1)	34 (58.6)	12 (52.2)	25 (47.2)	19 (38.8)	25 (40.3)	30 (53.6)	41 (56.2)	
Bed net	7 (26.9)	26 (40.6)	14 (24.1)	5 (21.7)	19 (35.8)	17 (34.7)	29 (46.8)	21 (37.5)	28 (38.4)	
Window screen	3 (11.5)	13 (20.3)	10 (17.2)	6 (26.1)	9 (17.0)	13 (26.5)	8 (12.9)	5 (8.9)	4 (5.5)	
Family's financial situation										<.001
Poor	21 (84.0)	48 (75.0)	51 (87.9)	17 (77.3)	45 (84.9)	35 (71.4)	50 (84.8)	36 (64.3)	35 (48.0)	
Good	4 (16.0)	16 (25.0)	7 (12.1)	5 (22.7)	8 (15.1)	14 (28.6)	9 (15.2)	20 (35.7)	38 (52.0)	

NOTE. Data are absolute no. (%) of children included in the analysis, unless otherwise indicated. Significant differences indicate that the parameter under consideration is heterogeneously distributed among the villages overall.

[a] For comparisons across villages (Pearson's χ^2 test).

and the maximum number of episodes during this period was 10 in a child from 1 village. The overall malaria IR was 1.20 (95% confidence interval [95% CI], 1.12–1.28) episodes/person-year at risk (PYAR). However, IRs differed greatly between the villages, with the lowest being 0.71 (95% CI, 0.57–0.89) episodes/PYAR in Agona and the highest being 2.20 (95% CI, 1.79–2.70) episodes/PYAR in Afamanaso for the entire observation time (figure 2).

Personal and family characteristics. First, we analyzed the distribution of sex, ethnic background, infant's birth season, sickle cell trait (HbAS compared with HbAA), mother's education and occupation, mother's knowledge of malaria transmission, use of protective measures (bed nets or window screens), and the family's financial situation among the 9 villages. Overall heterogeneity of distribution was found for ethnic groups, birth season, family's financial situation, occupation, and the use of protective measures (table 1). All other parameters were evenly distributed.

Then a bivariate Poisson regression analysis was performed to assess the effect of these parameters on malaria IRs. A significantly lower malaria incidence was seen for children born during the rainy season (IR ratio [IRR] adjusted for village of residence, 0.81; $P = .002$), children with literate mothers (IRR, 0.77; $P = .011$), children from households that used protective measures (IRR for bed net usage, 0.70; $P < .001$) (IRR for window screen usage, 0.50; $P < .001$), and children from families with a good financial situation (IRR, 0.66; $P < .001$). In contrast, a higher risk for malaria was found in children of northern ethnicity (IRR, 1.33; $P = .002$) and those whose mothers worked as farmers (IRR, 1.36; $P < .001$). The β-globin genotype (HbAS compared with HbAA), sex, and mother's knowledge of malaria transmission did not show significant influence (table 2). Ad-

Table 2. Personal characteristics with a possible influence on malaria incidence.

Characteristic	Rate, episodes/PYAR	Crude IRR[a] (95% CI)	P[b]	Adjusted IRR[c] (95% CI)	P[d]
Sex					
Male (n = 272)	1.27	1		1	
Female (n = 263)	1.12	0.88 (0.78–1.00)	.054	0.92 (0.81–1.05)	.23
Ethnic group					
Akan (n = 452)	1.19	1		1	
Northerner (n = 78)	1.32	1.11 (0.93–1.33)	.25	1.35 (1.12–1.62)	.002
Birth season					
Dry (n = 291)	1.22	1		1	
Rainy (n = 244)	1.17	0.96 (0.84–1.09)	.51	0.81 (0.71–0.92)	.002
Sickle cell trait					
HbAA (n = 385)	1.21	1		1	
HbAS (n = 52)	1.04	0.86 (0.69–1.08)	.20	0.82 (0.66–1.03)	.09
Mother's education					
Illiterate (n = 53)	1.46	1		1	
Literate (n = 478)	1.18	0.80 (0.66–0.98)	.032	0.77 (0.63–0.94)	.011
Mother's occupation					
Nonfarmer (n = 420)	1.08	1		1	
Farmer (n = 115)	1.64	1.52 (1.32–1.75)	<.001	1.36 (1.18–1.58)	<.001
Knowledge of malaria					
Inadequate (n = 388)	1.33	1		1	
Adequate (n = 76)	1.17	0.88 (0.74–1.05)	.15	0.89 (0.75–1.07)	.22
Protective measures					
None (n = 227)	1.50	1		1	
Bed net (n = 166)	1.01	0.68 (0.58–0.78)	<.001	0.70 (0.60–0.81)	<.001
Window screen (n = 71)	0.73	0.48 (0.39–0.61)	<.001	0.50 (0.39–0.63)	<.001
Family's financial situation					
Poor (n = 338)	1.33	1		1	
Good (n = 121)	0.84	0.63 (0.53–0.75)	<.001	0.66 (0.55–0.79)	<.001

NOTE. CI, confidence interval; IRR, incidence rate ratio; PYAR, person-year at risk.

[a] Without adjustment.
[b] Bivariate Cox regression analysis (P values for 2-sided test).
[c] Adjusted for children's village of residence.
[d] Multiple Cox regression analysis (P values for 2-sided test).

justment for the influence of village of residence did not change the crude IRRs considerably. Only the IRRs of the Northerner ethnic group differed >10% after adjustment for village residence (IRR, 1.11 vs. 1.35).

Village characteristics. In an ecological analysis, we correlated the geographic village characteristics of altitude, area, and population size with malaria IRs. Although altitude was not significantly correlated with malaria rates ($R^2 = 0.14$; $P = .32$), village area ($R^2 = 0.74$; $P = .003$) and population size ($R^2 = 0.68$; $P = .006$) showed a strong positive correlation with lower malaria rates (figure 3*A* and 3*B*).

Geographic position of the household within the village. Not surprisingly, the size of the village area strongly correlated with the mean of the shortest distances from each household to the forest fringe ($R^2 = 0.97$; $P < .001$). This led to the question of whether the mean distance between households and the forest, as an area with a potentially higher density of mosquito breeding sites, could explain the correlation between malaria incidence and village size.

Indeed, malaria IRs steadily decreased by 0.20 episodes/PYAR every 50 m with increasing distance from the village-forest border toward the center of the village (95% CI, −0.26 to −0.14; $P < .001$) (figure 4*A*). In agreement with that observation, children living outside the villages ($n = 73$) had IRs very similar to children living within the outermost 50 m of the villages (IRR, 0.85 [95% CI, 0.68 to 1.06]; $P = .15$).

We then used a circle with a radius of 300 m around each household and the segment of that circle crossing the area outside the village, expressed in degrees, as a benchmark for the exposure to the forest. In agreement with the observation of lower malaria IRs toward the center of the villages, we found an increase of 0.25 episodes/PYAR with every 60° increase in segment size outside the village area (95% CI, 0.19–0.31; $P < .001$) (figure 4*B*). The effect of the distance to the forest fringe and that

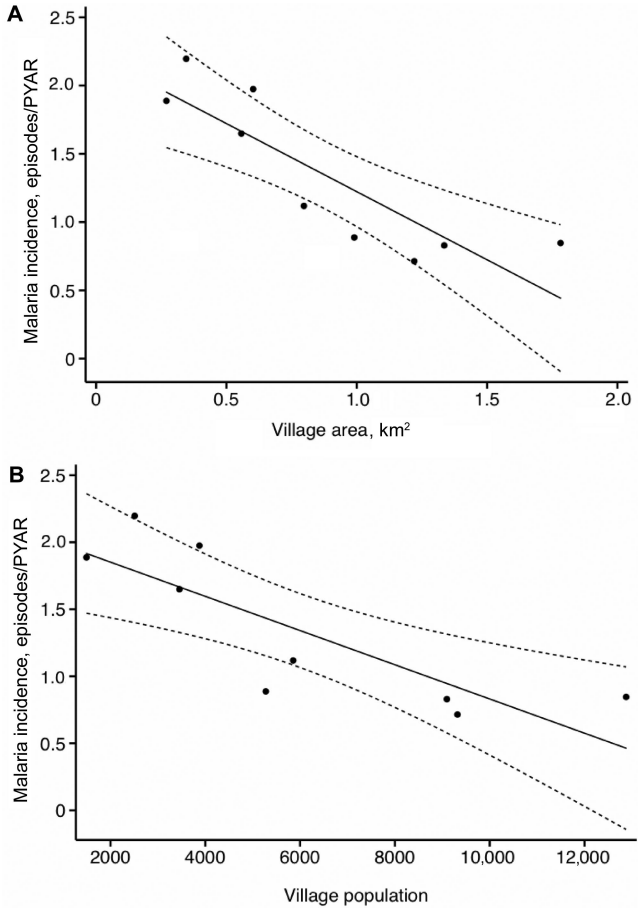

Figure 3. Correlation between malaria incidence rates (episodes/person-year at risk [PYAR]) and village size, shown as a regression line *(solid line)* with 95% confidence interval *(dotted lines). A,* Village size expressed as the area in square kilometers ($R^2 = 0.74$; $P = .003$). *B,* Village size expressed as population size ($R^2 = 0.68$; $P = .006$) (population census data [year 2000]; obtained from the Afigya-Sekyere District Assembly).

of the circle segment outside the village on malaria incidence could not be quantitatively compared and adjusted for in a single multiple regression model because of the high collinearity of the 2 exposures.

The relative positions of the households as well as the socioeconomic factors with an effect on malaria incidence and an unequal distribution among the villages were then included in a multivariate Poisson regression analysis. Adjustment of IRRs for all individual factors with an influence on malaria incidence shifted IRRs toward 1 (table 3). The low *P* value for the log likelihood ratio together with the lower absolute value of the log likelihood indicates that the adjusted model offers a better explanation of the malaria incidence and is superior to the crude model. However, an effect of the village of residence on malaria IRs, not explained by the model, still remained. Adjusted IRRs in villages with >5000 inhabitants were significantly lower than those in villages with <5000 inhabitants (IRR, 0.60 [95% CI, 0.50–0.72]; $P < .001$), indicating that the parameters adjusted for could not completely explain the inconsistency in rates between the villages.

DISCUSSION

It has been suggested that small-area variation of malaria incidence is more pronounced in areas of low and seasonal endemicity and is generally negligible in areas of high endemicity [7]. In areas of low endemicity, the risk of malaria is expected to be crucially dependent on the density of mosquitoes transmitting malarial parasites and can therefore be described by the entomological inoculation rate (infectious mosquito bites per person per year). Under these conditions, transmission to human hosts

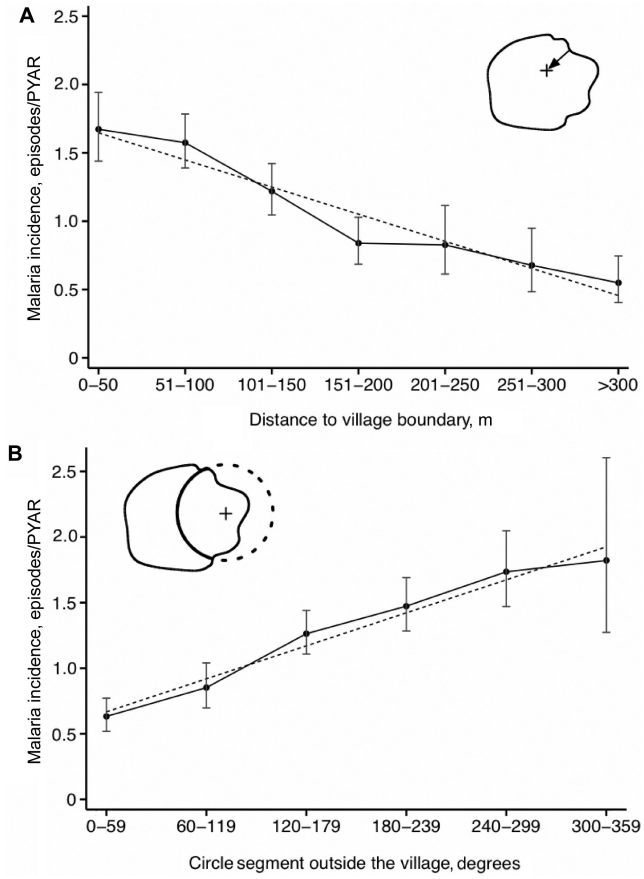

Figure 4. Association between malaria incidence rates (episodes/person-year at risk [PYAR] *[dots]* with 95% confidence intervals [CIs] *[bars]*) and relative position of households. Predicted malaria incidence is based on a linear regression *(dotted lines). A,* Distance to the forest area surrounding the villages (slope of dotted line, −0.20 [95% CI, −0.26 to −0.14]; $P < .001$). The sketch shows a household *(cross),* the village border *(irregular line),* and the shortest distance between the household and the fringe of the forest *(arrow). B,* Exposure to the forest area surrounding the villages (slope of dotted line, 0.25 [95% CI, 0.19 to 0.31]; $P < .001$). The sketch shows a household *(cross),* the village border *(irregular line),* and the segment of a circle of 300-m radius around the household that is outside the village border *(dotted segment).*

Table 3. Crude and adjusted malaria incidence rate ratios (IRRs), stratified by village of residence.

Village	Population, no.	Crude IRR[a]	*P*	Adjusted IRR[b]	*P*
Bedomase	1492	1		1	
Afamanaso	2508	1.20	.31	1.03	.87
Tano-Odumasi	3453	0.88	.43	1.05	.77
Bipoa	3875	0.93	.65	1.05	.77
Asamang	5277	0.51	<.001	0.64	.023
Kona	5853	0.56	.001	0.67	.031
Jamasi	9096	0.48	<.001	0.65	.058
Agona	9321	0.43	<.001	0.54	.002
Wiamoase	12,877	0.47	<.001	0.62	.030
Log likelihood[c]		−2355.4		−2304.0	

NOTE. IRRs are sorted by population size, with Bedomase (the smallest village) as the comparator.

[a] Without adjustments.

[b] Adjusted for all individual factors with an influence on malaria: distance to the forest, circle segment outside the village, ethnic group, birth season, mother's education and occupation, use of protective measures, and family's financial situation.

[c] Log-likelihood ratio test, $\chi^2 = 102.7$ ($df = 16$); $P < .0001$ (P value for 1-sided test). Only individuals with information in every category were included ($n = 349$).

should be dependent on the distance of households to *Anopheles* breeding sites. Such a dependency is not necessarily to be expected in areas of high and perennial transmission, largely because of homogeneous vector densities, saturated exposure, and a high level of acquired immunity starting early during childhood.

However, our results demonstrate a pronounced spatial heterogeneity of malaria incidence at a local level, even in a region of vegetational and altitudinal homogeneity with a high and stable perennial transmission. Varying malaria attack rates were observed in the villages of our study area, which were at most 18 km distant from each other. To find potential causes for these differences, we identified a number of sociodemographic, personal, and household characteristics with differing distributions between the villages and significant effects on malaria risk. The important influence of such sociodemographic factors as ethnic group, birth season, mother's education and occupation, use of protective measures, and the family's financial situation has been reported in a number of studies [13, 24–26], although others did not find significant associations [27]. The lower malaria incidence for children born during the rainy season may not seem plausible at first consideration. During the first months of their lives, however, infants are protected from malaria by maternal antibodies [28]. Thus, children who are born in the rainy season gain vulnerability to malaria during the low-transmission season, whereas those born in the dry season lose protection during the high transmission season. Furthermore, the number of malaria episodes for children born in the rainy season may have been underestimated, because assessment of malaria episodes did not start until the age of 3 months, and, accordingly, malaria episodes during the first 3 months were not counted.

Sickle cell trait had only a slight and less significant effect in our study, which seems surprising but was most probably due to lack of statistical power. Analysis of a larger group of children from the same area and time span showed that this condition conferred protection against malaria at a similar level (20% protection [authors' unpublished data]) with, however, statistical significance.

Malaria incidence showed a strong correlation with population size and the area of the villages. Our analyses indicated that, in addition to a strong variation even in rural areas of high transmission, the risk of malaria decreases linearly with increasing distance to the peripheral area of the villages or, alternatively, with the area of exposure to putative mosquito breeding grounds in the forest. Although the association of the entomological inoculation rate and vector density with closeness of breeding sites is obvious [5, 15–17], it is more difficult to find evidence of a direct association between distance from breeding sites and health impact. In particular, this association has not yet been demonstrated convincingly in children <2 years of age in areas of stable transmission. In areas with unstable transmission, some studies have shown such a relationship [6, 10].

Despite these distinctive relationships, differences in malaria risk among the 9 study villages were not sufficiently explained by heterogeneity of distances from the forest fringe or exposure to forest area alone. In addition, after adjustment for sociodemographic and geographic factors, 2 major groups of villages with different malaria risks could be distinguished: 4 villages with <5000 inhabitants and 5 villages with >5000 inhabitants. Other village characteristics associated with village size may have an additional effect on malaria incidence. The presence of a clinic or hospital in the 5 larger villages may improve the availability of

prompt malaria treatment, and a higher health education standard could have an additional effect on malaria rates. Factors that were not tested might also account for the remaining differences. For example, because we did not map locations of breeding sites, we cannot make a statement as to the exact distances between these sites and houses in the individual villages. We also did not assess the density of breeding sites around the individual villages, and their density may vary from one village to the next. The remaining differences, however, also imply that there are still factors of influence that have not yet been identified.

In conclusion, the results of the study have several implications. First, the obvious variability in malaria risk in small areas might be important for the planning and conduct of interventional trials. Appropriate analyses should be applied to overcome the unbalanced distribution of risk factors and confounders in apparently homogeneous areas.

Second, the data suggest a strong relationship between the risk of malaria and living close to the forest fringe. This observation allows indirect conclusions on vector densities in villages and the distribution of most relevant mosquito-breeding grounds. In areas of perennial transmission, it can be difficult to identify these entomological parameters and their health impact, because both permanent and temporary breeding sites contribute to vector density [29]. This is especially true for *A. gambiae,* the main vector in our study area, which is able to breed in the smallest puddles, tire ruts, and buckets [30]. Nonetheless, our findings suggest that the highest vector pressure comes from the forest area surrounding the villages, and this hypothesis is supported by the observation that vector densities are increased for houses on the outskirts of villages [31].

Third, under difficult conditions and with low budgets, malaria control measures have to focus on high-risk areas to increase efficiency and cost-effectiveness. The gradient of malaria risk from the surrounding area to the center of villages should be considered in the planning of control strategies. In view of recent World Health Organization recommendations on the resumption and expansion of indoor residual spraying with dichlorodiphenyltrichloroethane (DDT) and other insecticides [32], our results support considerations about focal spraying of houses in the outer areas of villages to minimize the environmental pressure of insecticides [31]. With such a strategy, a contextual effect on the central parts of villages is conceivable and should be investigated in further studies.

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