MAJOR ARTICLE

Altitude-Dependent and -Independent Variations in *Plasmodium falciparum* Prevalence in Northeastern Tanzania

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Background. Effective malaria control requires information about intensity of transmission across large areas and populations. Estimates based on entomological factors lack precision and are not cost-effective to obtain. We tested altitude and rainfall measurements as correlates of transmission intensity in different ecological settings.

Methods. We conducted 2 cross-sectional surveys of ~12,000 people (1–45 years old) in 6 altitude transects (150–1800 m) in the Kilimanjaro and Tanga regions of Tanzania. Data were analyzed for associations with altitude and rainfall estimates by use of appropriate regression models.

Results. *Plasmodium falciparum* prevalence showed a negative relationship with altitude (19% and 21% decrease/100-m altitude increase, respectively, in children in Kilimanjaro and Tanga) and rainfall during the 3 months before the survey (46% decrease/100-mm rainfall increase in children in Kilimanjaro). Mean hemoglobin concentrations increased with altitude (0.05 and 0.09 g/dL/100-m altitude increase, respectively, in children in Kilimanjaro and Tanga) and rainfall (0.17 g/dL/100-mm rainfall increase in children and adults in Kilimanjaro).

Discussion. Altitude and rainfall were correlated with parasite prevalence and mean hemoglobin concentration; however, the relationship varied according to ecological setting. Climatological variables alone cannot predict malarial outcomes. Local variations in seasonality of malaria transmission—together with vector species composition, topography, host and parasite genetics, and socioeconomic factors—may influence malaria prevalence.

Despite its considerable public health importance, the relationship between the burden of malarial disease and intensity of transmission of *Plasmodium falciparum* remains unclear [1], in part because of a lack of tools for the rapid and accurate estimation of transmission over large areas and across different transmission intensities. More-precise estimates of transmission intensity would allow improved targeting of novel control tools, such as the introduction of rapid diagnostic tests to optimize the use of expensive combination therapies.

The reference standard for measuring intensity of transmission, the entomological inoculation rate (EIR), is empirically derived from the density of human-biting anopheline mosquitoes, their sporozoite rate, and the human blood index (human biting rate). In Africa, EIRs vary from <1 infectious bite per person per year (ib/p/y) to >800 ib/p/y. However, comparison of EIRs is difficult, because of methodological differences and a paucity of values from areas of moderate and low endemicity, which are typical of much of sub-Saharan Africa [2]. In these areas, obtaining reliable and reproducible estimates of the EIR across a large area is prohibitively time consuming and expensive because of low vector numbers, even lower numbers of infected vectors, and considerable local variations in EIR [3–5]. Transmission estimates based on the prevalence of human infection are more informative, because they represent actual (rather than potential) infections, sufficient numbers of people can relatively easily be tested, and results correlate well with measured EIRs [6]. How-

Received 14 September 2004; accepted 14 December 2004; electronically published 7 April 2005.

Presented in part: Third Multilateral Initiative on Malaria Pan African Malaria Conference, Arusha, Tanzania, 17–22 November 2002 (abstract 318).

Financial support: UK Medical Research Council (grant G9901439); Danish International Development Agency.

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The Journal of Infectious Diseases 2005;191:1589–98

0022-1899/2005/19110-0004$15.00

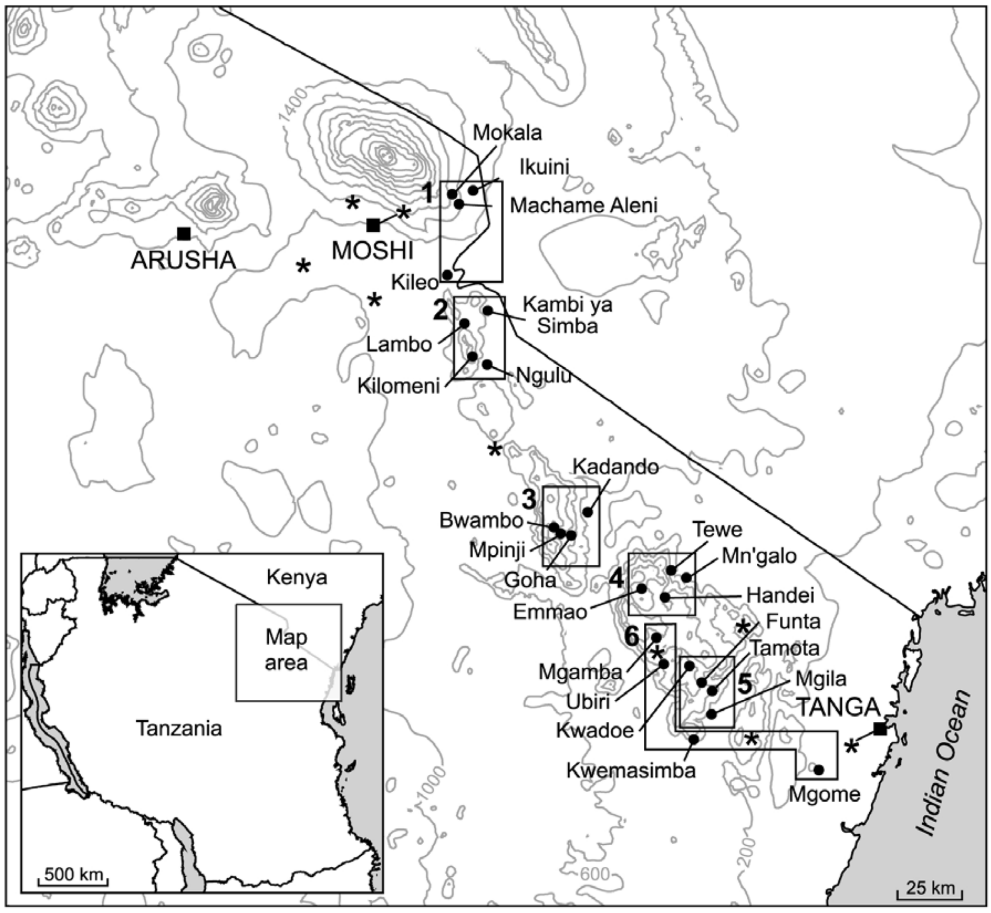

Figure 1. Altitude contour map of the study area. Each box defines a transect of 4 villages *(black circles);* transect numbers match those shown in table 1. Locations of the 8 meteorological stations, from which rainfall and temperature data were used, are also shown *(asterisks).*

ever, parasite prevalence saturates at high transmission and can be affected by seasonal variation in transmission and levels of acquired immunity.

Transmission intensity varies with climate, of which temperature—which affects the development of the vector and of the parasite within the vector—is a major component; transmission intensity is expected to vary with altitude because of the associated changes in temperature. Mosquito densities and parasite prevalence decrease with increasing altitude [7–10] but are influenced by rainfall, topography [11], land use [12], the socioeconomic status (SES) and genetic composition of the population [13, 14], and the use of antimalarial chemotherapy [15]. As a consequence, previous attempts to estimate malaria transmission by use of climatic models and parasite prevalence on a local scale have failed to accurately predict risk, particularly at low rates of transmission [16].

As part of a program investigating the burden of disease and transmission intensity, the present study aimed to determine the utility of simple indices—altitude and rainfall—to predict the intensity of malaria transmission. We surveyed parasite prevalence and hemoglobin (Hb) concentration in ~12,000 individuals in 24 villages in 6 altitude transects (100–1800 m above sea level) of mountain ranges across northeastern Tanzania.

MATERIALS AND METHODS

Study area. The study area ranged from Mt. Kilimanjaro to the coastal plain of northeastern Tanzania (figure 1). The Usambara Mountains are populated between the altitudes of 300 and 1870 m; the Pare Mountains reach similar altitudes but are much steeper. Mt. Kilimanjaro rises from the plains at 700 m and is inhabited to an altitude of ~2000 m. The area has a short rainy season in November and December and a longer rainy season from March to May; temperatures peak in January and are lowest in July. Intense, perennial *P. falciparum* transmission (EIR, ~700 ib/p/y) occurs on the coastal savannah [17], whereas altitudes >1700 m are believed to be malaria free [18].

Six transects of altitude were defined—3 in the Kilimanjaro region and 3 in the Tanga region (figure 1). Within each transect, 1 village was selected at high (>1200 m), 2 at intermediate (600–1200 m), and, where possible, 1 at low (<600 m) altitude (table 1). Village selection criteria were chosen to minimize

Table 1. Characteristics of study villages.

Transect, village name	Altitude, mean (range), m	Estimated maximum daily temperature, mean, °C[a]	Estimated annual rainfall, mm	Parasite prevalence,[b] %	Hemoglobin concentration, mean, g/dL[b]
Kilimanjaro region					
Rombo					
Mokala	1702 (1788–1623)	16.7	919	0	11.6
Machame Aleni	1421 (1482–1380)	18.9	748	2	11.8
Ikuini	1160 (1215–1118)	20.9	811	3	11.3
Kileo	723 (724–721)	24.2	535	5	11.0
North Pare					
Kilomeni	1556 (1745–1384)	17.4	547	1	11.0
Lambo	1187 (1231–1146)	20.4	562	10	11.2
Ngulu	831 (863–798)	23.0	583	8	11.0
Kambi ya Simba	745 (767–716)	23.8	563	10	11.2
South Pare					
Bwambo	1598 (1643–1564)	16.4	639	3	11.2
Mpinji	1445 (1667–1307)	17.5	639	2	11.1
Goha	1162 (1228–1079)	19.7	665	13	11.1
Kadando	528 (531–525)	24.6	717	25	10.4
Tanga region					
West, Usambara 1					
Emmao	1845 (1872–1810)	14.5	700	0	10.9
Handei	1425 (1517–1372)	17.6	741	17	10.9
Tewe	1049 (1126–965)	20.5	760	22	10.5
Mng'alo	416 (455–406)	25.2	819	55	9.8
West, Usambara 2					
Kwadoe	1523 (1603–1473)	15.8	766	4	10.7
Funta	1279 (1322–1236)	18.3	766	17	10.8
Tamota	1176 (1338–1073)	19.7	766	19	10.2
Mgila	432 (455–406)	25.0	744	34	10.4
West, Usambara 3					
Magamba	1685 (1751–1659)	15.4	730	0	11.4
Ubiri	1216 (1262–1174)	18.9	711	12	10.9
Kwemasimba	662 (691–636)	23.0	744	25	10.5
Mgome	196 (208–165)	25.9	891	61	9.8

[a] Temperature data were based on the altitude and distance from the sea of each village by use of estimates from a model of temperature data from 9 meteorological stations (US National Oceanographic and Atmospheric Association/Climate Prediction Center dekadal rainfall estimates for 1996–2002).

[b] Data from the short rainy season (October–November 2001) for children 0–4 years old.

differences within transects in SES, ethnicity, seasonal migration, and access to health care (as assessed by village-level socioeconomic surveys).

The study area was mapped by use of differential global positioning satellite techniques (Trimble GeoExplorer III and Pro XR receivers; Trimble Navigation), and geographical data were analyzed by use of Arc/Info 7.1 and ArcView 3.2 (ESRI). The long-term (1971–2000) average daily mean temperature was derived from 4 meteorological stations in the Kilimanjaro region and 5 stations in the Tanga region (1 station had data only from 1992 to 2000). Rainfall estimates (RFEs) for the 3-month period preceding each survey were derived from the duration of cold cloud cover for each village, as determined by Meteosat infrared data collected by the Climate Prediction Center at the US National Oceanographic and Atmospheric Administration, by use of dekadal RFE data for 1996–2002. Mean monthly and mean annual duration of cold cloud cover across Africa are strongly correlated with rainfall [19]; dekadal RFE estimates and rain-gauge measurements from neighboring Kenya (available at: http://www.HIMAL.uk.net) are also strongly correlated.

Cross-sectional surveys. Two surveys were conducted in each village, during the short and long rainy seasons, respectively. Written, informed consent was obtained from each participant or guardian of children <15 years old. Ethical clearance was obtained from the institutional review boards of the National Institute of Medical Research of Tanzania and the London School of Hygiene and Tropical Medicine.

We aimed to recruit 250 individuals (80 who were 0–4 years

old, 80 who were 5–14 years old, and 90 who were 15–45 years old) per village. Sufficient subvillages to fulfil the sample size in the age group of children <5 years old were selected; individuals presented to a central point in the village over a 3-day period and were sampled on a first come, first served basis. Overall, 30%–50% of villagers and >80% of individuals <5 years old were sampled. Demographic, anthropometric, and clinical data were recorded, and a 500-μL finger-prick blood sample was collected. Minor ailments were treated by clinical staff; villagers with more-serious health problems were referred to an appropriate health facility.

Laboratory methods. Giemsa-stained blood films were examined by oil-immersion microscopy. One hundred fields were screened before a slide was deemed to be negative; if parasites were observed, they were counted against 200 white blood cells (WBCs). All slides were read twice; if results were discordant, they were read a third time. The majority result was accepted for slide positivity, and the mean of all 3 readings used to estimate parasite density. Hb concentrations were assessed by hemophotometry (HemoCue). Clinically relevant data were provided to the study physician, and appropriate treatment was provided.

Data management and analysis. Data were double entered and verified in Microsoft Access (Microsoft); range and internal consistency checks and analyses were performed by use of STATA software (version 8; StataCorp). Anthropometric indicators were defined in EpiInfo2002 (NutStat) by use of the US Center for Disease Control and Prevention's 1978 reference population. Stunting was defined as height-for-age values >2 *z*-scores below the mean. The data from both surveys were pooled, and regression models were included to adjust for the effect of season and repeated sampling. Data were adjusted for intravillage correlation by use of random-effects linear regression for mean Hb, population-averaged logistic regression for parasite prevalence, and negative binomial regression with robust SEs for parasite density counts. Separate regression models were fitted for children and adults because of significant age differences in parasite indices and interactions between age and other covariates. A fractional polynomial analysis [20] was used to describe the fit of the age-prevalence data at different altitude bands. To correct for the physiological effect of altitude on oxygen-carrying capacity, Hb values were adjusted for altitude of residence [21].

RESULTS

Participation was high in both surveys, with 97% of the target sample recruited in the first survey pool and 95% recruited in the second pool. The sexes were equally represented in the younger age groups, although 70% of the 15–45-year-olds surveyed were women. After adjustment, there was no significant association between altitude or estimated annual rainfall and stunted growth in children <5 years old. However, the risk of stunting was greater in the Tanga region than in the Kilimanjaro region (odds ratio [OR], 1.97 [95% confidence interval {CI}, 1.42–2.73]; $P < .001$).

Prevalence of malaria infection. A logistic regression model of parasite prevalence found significant effects of age, altitude, and rainfall estimates for the 3 months preceding each survey and significant interactions with region; our analyses include adjustment for these parameters as appropriate. Overall, *P. falciparum* prevalence was 15% during the short rainy season and 21% during the long rainy season; there were very few infections with *Plasmodium malariae* (0.3%) or *Plasmodium ovale* (0.1%). The higher prevalence of infection in the long rainy season was significant in both children (OR, 5.69 [95% CI, 3.49–9.26]; $P < .001$) and adults (OR, 3.00 [95% CI, 1.67–5.40]; $P < .001$).

Parasite prevalence decreased with increasing altitude of residence (figure 2*a*). For every 100-m increase in altitude, the OR for infection was 0.79 (95% CI, 0.72–0.85; $P < .001$) among children (0–14 years old) in Tanga and was 0.81 (95% CI, 0.71–0.92; $P = .002$) among children in Kilimanjaro. In adults, the OR was 0.89 (95% CI, 0.84–0.94; $P < .001$) in Tanga, but the effect of altitude among adults in Kilimanjaro was not statistically significant. For every 100-mm increase in rainfall during the 3 months preceding the survey, the OR of parasitemia was 0.54 (95% CI, 0.42–0.71; $P < .001$) in children in Kilimanjaro; the effect of rainfall among children in Tanga was not statistically significant. In adults, for every 100-mm increase in rainfall during the 3 months preceding survey, the OR was 0.75 (95% CI, 0.58–0.97; $P = .029$) in Tanga and was 0.58 (95% CI, 0.41–0.81; $P = .002$) in Kilimanjaro.

The relationship between parasite prevalence and age (as a continuous variable) was modeled separately for each region and altitude (categorized into altitude bands of <600, 600–1200, and ⩾1200 m), by use of a fractional polynomial logistic regression adjusted for survey timing and within-village correlation (figure 3). Parasite prevalence was higher in Tanga than it was in Kilimanjaro at all ages and at all altitudes. In 4 of 5 region-altitude bands, parasite prevalence peaked in children or adolescents and decreased in adults, which indicates a degree of acquired immunity.

Geometric mean parasite density (GMPD). Parasitemic individuals were classified as symptomatic (measured temperature ⩾37.5°C or reported fever during the preceding 2 days) or asymptomatic. During the short rainy season, 14% of parasite-positive subjects were symptomatic, whereas, during the long rainy season, 35% were symptomatic. In both surveys, the proportion of symptomatic parasite-positive subjects decreased with increasing altitude (28% at <600 m, 26% at 600–1200 m, and 21% at >1200 m; $P = .009$, score test for trend) and with increasing age (31% in 0–4-year-olds, 23% in 5–14-year-olds, and 25% in 15–45-year-olds; $P = .022$, score test for trend).

In symptomatic children, there was no effect of either altitude

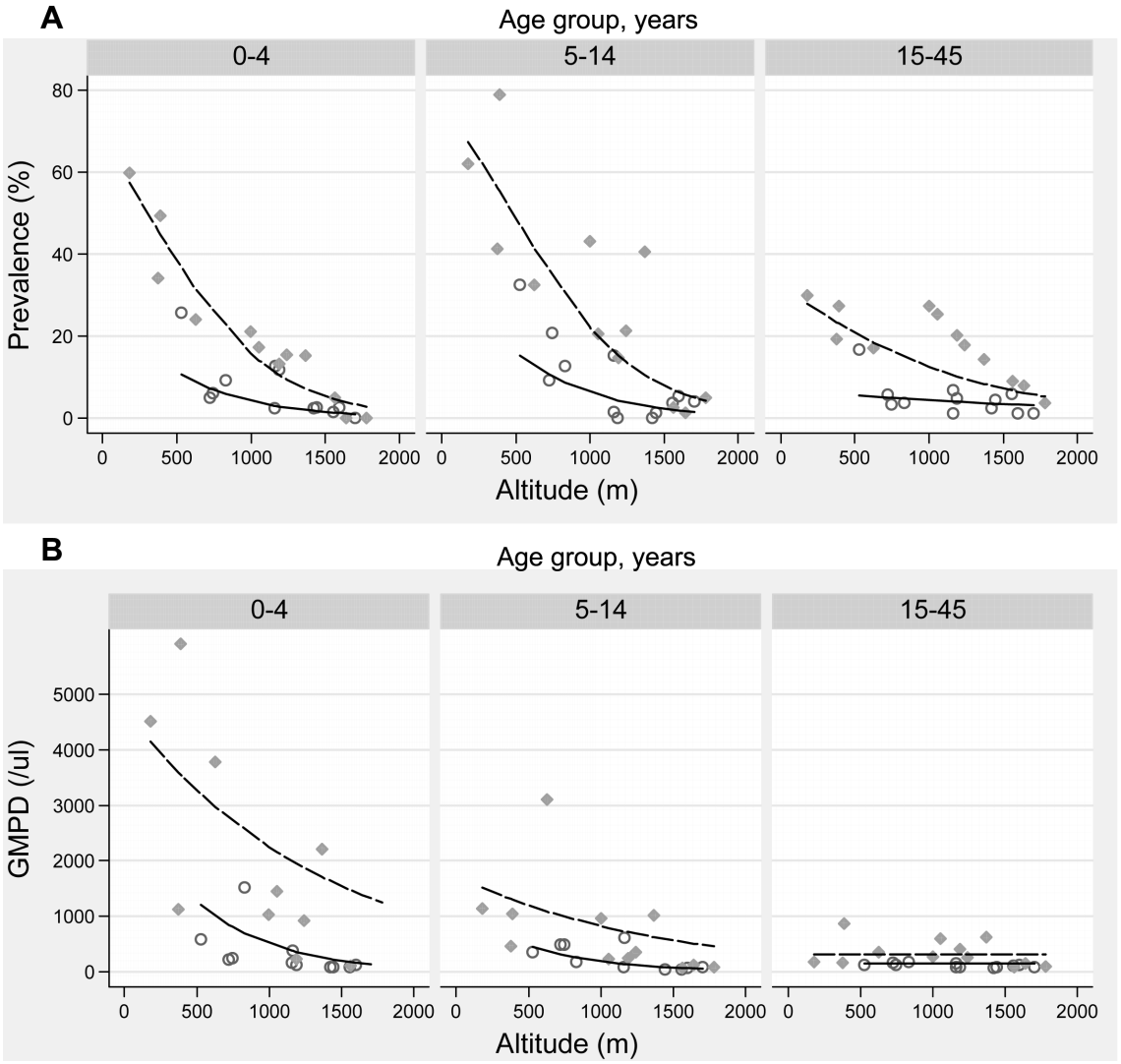

Figure 2. Association between altitude and malariometric parameters. Associations are shown between altitude and actual and predicted mean parasite prevalence *(A)* or asymptomatic geometric mean parasite density (GMPD) *(B),* by age group, for the short rainy season (October–November 2001). Predicted values are from a logistic regression model adjusting for intracluster correlation within villages *(A)* or a negative binomial model of GMPD in asymptomatically infected individuals with no prior treatment *(B),* adjusting for intracluster correlation within villages. *Circles,* villages in Kilimanjaro; *diamonds,* villages in Tanga.

or rainfall on GMPD; however, GMPD decreased by 65% in older children, compared with younger children (density ratio, 0.35 [95% CI, 0.24–0.50]; $P < .001$); it was twice as high in those who reported having received antimalarial treatment during the preceding week than in those who did not (density ratio, 2.03 [95% CI, 1.41–2.92]; $P < .001$). This apparent paradox probably reflects the low efficacy or improper use of the available antimalarial drugs in this area of extremely high drug resistance [22, 23].

In asymptomatic individuals, after adjusting for survey timing and antimalarial treatment during the preceding week, GMPD decreased with increasing altitude in children but not in adults (figure 2*B*). In adults, there was an interaction between GMPD and recent rainfall in Tanga, with a reduction in GMPD of 14% per 100-mm increase in rainfall (density ratio, 0.86; [95% CI, 0.75–0.98]; $P = .024$), but no significant effect was seen in Kilimanjaro. Asymptomatic GMPD in adults was also significantly higher in Tanga than in Kilimanjaro (density ratio, 2.43 [95% CI, 1.18–5.01]; $P = .016$). In children, for every 100-m increase in altitude, asymptomatic GMPD decreased by 7% (density ratio, 0.93; [95% CI, 0.88–0.98]; $P = .009$) in Tanga and by 17% (density ratio, 0.83 [95% CI, 0.78–0.88]; $P < .001$) in Kilimanjaro. Older children (5–14 years old) had significantly lower GMPDs than younger children (density ratio, 0.36 [95% CI, 0.26–0.51]; $P < .001$). GMPD was 2.83 times higher in asymptomatic children (95% CI, 1.45–5.50; $P = .002$) and 2.27

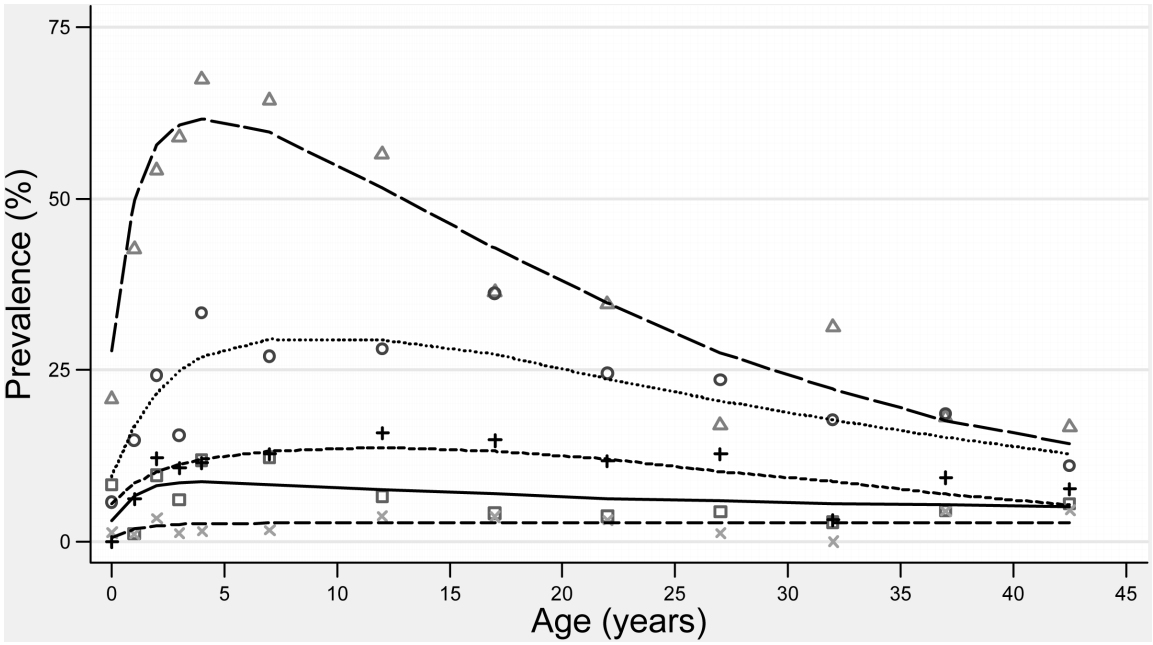

Figure 3. Association among age, altitude, and parasite prevalence. Age prevalence curves by altitude band (<600, 600–1200, and >1200 m) and region (Kilimanjaro and Tanga) were modeled by use of fractional polynomial logistic regression adjusted for survey timing and within-village correlation. The 1 village in Kilimanjaro that lay below 600 m was excluded because it provided insufficient data in that band. *Triangles,* Tanga, <600 m (predicted, long-dashed line); *circles,* Tanga, 600–1200 m (predicted, dotted line); *squares,* Kilimanjaro, 600–1200 m (predicted, short-dashed line); *plus signs,* Tanga, > 1200 m (predicted, solid line); *crosses,* Kilimanjaro, >1200 m (predicted, dashed line).

times higher in asymptomatic adults (95% CI, 1.17–4.41; $P = .016$) who reported having received treatment for malaria during the preceding week, compared with those who did not report having received treatment.

Prevalence and severity of anemia. The prevalence of moderate anemia (Hb concentration, 8 g/dL) decreased significantly with increasing altitude (7.2% at <600 m, 2.8% at 600–1200 m, and 2.1% at >1200 m; $P < .001$, score test for trend) and was significantly higher in Tanga than in Kilimanjaro (4.97% vs. 1.51%; $P < .001$). Severe anemia (Hb concentration, 5 g/dL) was also more prevalent in Tanga than in Kilimanjaro (0.43% vs. 0.09%, respectively; $P < .001$).

The mean Hb concentration was higher in older (5–15 years old) than in younger (0–4 years old) children (difference, 1.58 g/dL [95% CI, 1.51–1.64]; $P < .001$) and was higher in girls than in boys (difference, 0.14 g/dL [95% CI, 0.07–0.21]; $P < .001$). Children (0–4 years old) with stunting had lower Hb concentrations than their nonstunted counterparts in both Kilimanjaro (difference, −0.20 g/dL [95% CI, −0.36 to −0.04]; $P = 0.017$) and Tanga (difference, −0.32 g/dL [95% CI, −0.47 to −0.17]; $P < .01$). As expected, women had a lower mean Hb concentration than men (difference, −1.65 g/dL [95% CI, −1.77 to −1.52]; $P < .001$).

Mean Hb concentrations were lower in Tanga than in Kilimanjaro in both children (difference, −1.04 [95% CI, −1.59 to −0.49]; $P < .001$) and adults (difference, −1.48 [95% CI, −1.61 to −0.66]; $P < .001$). The mean Hb concentration was positively associated with altitude in both regions (figure 4). In children, the Hb concentration increased by 0.09 g/dL (95% CI, 0.06–0.12; $P < .001$) for every 100-m increase in altitude in Tanga and by 0.05 g/dL (95% CI, 0.01–0.08; $P = .016$) for every 100-m increase in altitude in Kilimanjaro. In adults, the Hb concentration increased by 0.08 g/dL (95% CI, 0.04–0.12, $P < .001$) for every 100-m increase in altitude in Tanga. The mean Hb concentration was positively associated with rainfall in Kilimanjaro, increasing by 0.17 g/dL (95% CI, 0.10–0.23; $P < .001$) for every 100-mm increase in estimated 3-month rainfall in children and by 0.17 g/dL (95% CI, 0.06–0.28; $P = .002$) in adults. There was no significant effect of recent rainfall on mean Hb concentration in either children or adults in Tanga.

DISCUSSION

The present study describes the effect that altitude and estimated rainfall have on indices of malaria infection over a large area encompassing a wide range of intensities of transmission. The inclusion of children and adults in the study allowed us to look for interactions between intensity of transmission and the acquisition of immunity. Overall, altitude was closely correlated with multiple measures of malaria exposure (parasite prevalence, asymptomatic parasite density in children, and mean Hb concentration). Similarly, in a study of hospital admissions

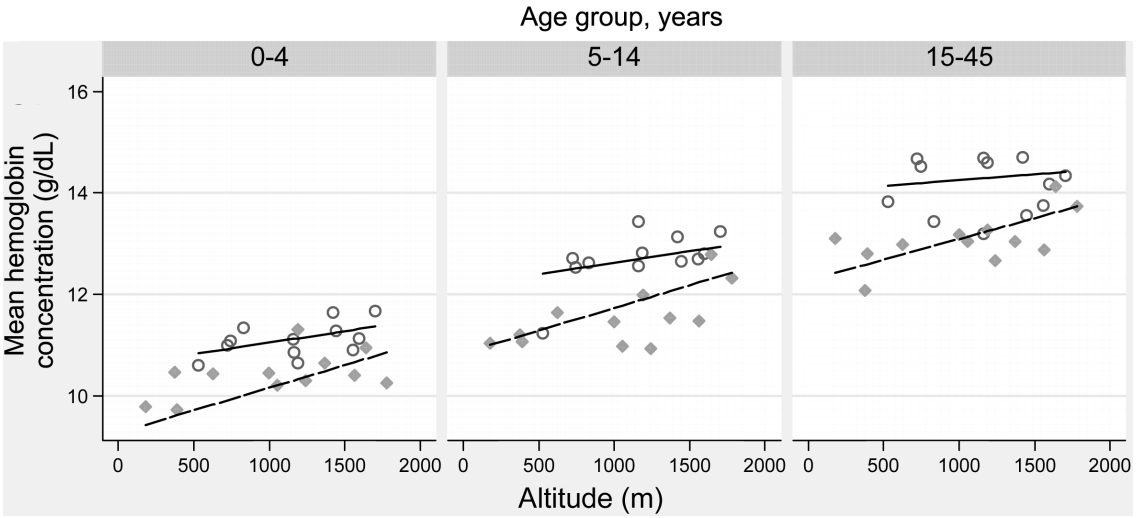

Figure 4. Association between altitude and mean hemoglobin (Hb) concentration (in g/dL), by age group. Associations are shown between altitude and actual and predicted mean Hb for the short rainy season (October–November 2001) for males only and for children without stunting. The fitted lines are predicted from a linear regression model that adjusted for intracluster correlation within villages and independent effects of altitude on Hb (0.1 g/dL for steps of 100–300 m, according to the exponential model of Dirren et al. [21]). *Circles,* villages in Kilimanjaro; *diamonds,* villages in Tanga. Predicted values: *solid lines,* villages in Kilimanjaro; *broken lines,* villages in Tanga.

in the same study area, we found clear evidence of altitude-related differences in the age distribution and manifestations of severe malaria [24].

Previous studies have shown a logarithmic relationship between parasite prevalence in children and EIR [6] and between EIR and altitude [8]. Our data thus confirm that intensity of transmission is directly related to altitude and that parasite prevalence (adjusted for covariates) is a good marker of this. (The lack of association between altitude and parasite prevalence among adults in Kilimanjaro may reflect their greater mobility vs. that of children, particularly in high-altitude villages, where adults migrate to the lowlands to farm during the rainy seasons [data not shown].) Applying the criteria developed by Beier et al. [6], we estimate that only 1 village in our study, Mgome, had an EIR >100 ib/p/y. EIRs in the other lowland villages (<600 m) were 11–100, those in midrange villages (600–1200 m) were <10 ib/p/y, and those in high-altitude villages (>1200 m), with the exception of the outliers described below, were <1 ib/p/y.

The mean Hb concentration is another indicator of malaria infection and (after adjusting for the direct physiological effects of altitude) revealed a pattern similar to that of parasite prevalence. In children <5 years old, both parasite prevalence and mean Hb concentration correlated equally well with altitude, which suggests that mean Hb concentration reflects recent malaria exposure in this age group. However, with increasing age, the mean Hb concentration tended to correlate more closely with altitude than did parasite prevalence; this effect was particularly apparent for adults in the Tanga region, which suggests that, among individuals who have a significant degree of acquired immunity, the examination of a single peripheral blood film may underestimate the burden of disease due to chronic, low-density parasitemia. Indeed, our data suggest that, in the absence of other common causes of anemia, such as heavy hookworm infection [17, 25], Hb concentration may be a more reliable measure of *P. falciparum* exposure than parasite prevalence.

After adjusting for altitude, parasite prevalence decreased (and mean Hb concentrations increased) with increasing rainfall (estimated during the 3 months preceding the survey), which indicates that malaria transmission decreases as rainfall increases. At first glance, this seems counterintuitive, because, in this region of Tanzania, malaria transmission in lowland sites has consistently been shown to increase during the rainy season [8, 10, 26]. However, there is no direct, predictable relationship between rainfall and the intensity of malaria transmission [27]. In other settings, very heavy rainfall can lead to a paradoxical decrease in malaria transmission [28] because of flushing of breeding sites, which reduces larval numbers and decreases nutrient availability [11, 29–31]. Heavy rainfall at high altitude in Kilimanjaro is also associated with reduced ambient temperature, which would further limit vector development.

The acquisition of immunity with age and increasing transmission intensity is apparent from the shift in peak parasite prevalence to older age groups with increasing altitude [32] and from the less marked effect of altitude on parasite prevalence among adults, compared with that among children. The decrease in asymptomatic GMPD with increasing altitude indicates that the fever threshold is lower in children with lower

levels of exposure, which is consistent with observations published elsewhere [33], in which pyrogenic thresholds differed with intensity of transmission [34, 35]. The lack of association between GMPD and altitude in adults is consistent with evidence that the ability to control parasite replication is acquired earlier than the ability to prevent infection per se [36]. The observation that GMPD was significantly lower in adults than in children, even at the highest altitudes, implies that adults can develop a considerable degree of antiparasite immunity even under conditions of very low transmission. Furthermore, our observation of an increase in symptomatic parasitemic individuals during the long transmission season is consistent with the notion that clinical episodes of malaria are associated with new infections by genetically distinct parasites [37].

Despite the clear association between malariometric parameters and altitude within transects, marked altitude-independent differences were observed; after adjustment for altitude and potential confounding factors, the risk of malaria infection and that of anemia were both 5-fold higher in children in Tanga than in children in Kilimanjaro. This might be explained by differences in vector composition. In coastal Tanga, the principal vectors are *Anopheles gambiae* (sensu stricto) and *Anopheles funestus* [10], whereas, around Mt. Kilimanjaro, vectors are almost exclusively *Anopheles arabiensis* [38, 39], which is a significantly less efficient vector than *A. gambiae* (sensu stricto) [40]. All 3 vectors are present at various times throughout the year in the Pare Mountains [41]. These vector distributions correspond very well with the parasite prevalence data that we recorded and with those from more extensive comparisons of malaria stability and vector composition [42]. Differences between transects might also reflect differences in SES and health-seeking behavior (including the use of bed nets and antimalarial drugs) [43]; preliminary data have suggested higher SES in villages in Kilimanjaro (data under analysis), but the prevalence of alleles associated with drug resistance is similar in both regions [22, 23]. Finally, the prevalence of hemoglobinopathies might decrease with increasing altitude [44] and thus confound the relationship between Hb concentration and transmission intensity, but there is little evidence that they affect parasite prevalence per se.

In addition to this regional variation, we also observed microheterogeneity in intensity of transmission at the village level. There were 3 villages (Ikuini, Handeni, and Funta) in which the parasite prevalence was higher than expected, given their altitude, and 1 village (Kileo) in which the parasite prevalence was lower than expected. Handeni lies in a sheltered basin, which may provide long-term vector breeding sites [11], whereas Funta has previously been identified to be epidemic prone. Kileo reported extremely low rainfall at the time of both surveys [45, 46]. None of these unexpected parasite prevalences were matched by outlying mean Hb concentrations, which suggests that they reflect short-term fluctuations in malaria transmission intensity rather than any long-term aberration in transmission; serological analysis of samples from these villages confirmed this interpretation. Moreover, serological parameters were extremely highly correlated with altitude, irrespective of transect or region [47], which indicates that intertransect differences may be largely explained by short-term seasonal variation.

In conclusion, the present results demonstrate that altitude is a useful proxy for malaria transmission across a very wide range of transmission intensities, but only when there is sufficient information to adjust for the confounding effects of seasonal variation in rainfall and vector composition. However, given that both altitude and rainfall can be rapidly and accurately measured by use of geographical information systems approaches and that surveys of vector composition are much cheaper and quicker than measuring EIR, we believe that this approach has potential for the rapid assessment of malaria transmission intensity over large areas. Although microheterogeneity of transmission may obscure these relationships in small studies, we have demonstrated the utility of altitude measures for characterizing *P. falciparum* transmission with a precision that is appropriate for epidemiological or intervention studies. Furthermore, this relatively small, well-populated geographical area harbors a wide range of intensities of malaria transmission and is an optimal site for evaluation of such interventions.

Acknowledgments

This study was conducted as part of the Joint Malaria Programme (JMP), a collaboration between the National Institute for Medical Research (NIMR), Kilimanjaro Christian Medical College (KCMC), the London School of Hygiene and Tropical Medicine (LSHTM), and the Centre for Medical Parasitology, University of Copenhagen (CMP). We thank Dr. Andrew Kitua (NIMR), Brian Greenwood (LSHTM), John Shao (KCMC), and Ib Bygbjerg (CMP), for their ongoing support and advice; Faustin Lazier, Mary Mosha, Bruno Paul Mmbando, all members of the JMP community studies team, Frank Magogo, Esther Lyatuu, Juma Akida, and laboratory staff at KCMC and NIMR Amani; regional medical officers for Tanga (Dr. William Mwengee) and for Kilimanjaro (Dr. Olomi), for assistance and support; Jane Bruce and Dr. Neal Alexander, for statistical advice; and Drs. Lasse Vestergaard, Daniel Chandramohan, and Chris Whitty, for comments on the manuscript.

References

1. Snow RW, Marsh K. The consequences of reducing transmission of *Plasmodium falciparum* in Africa. Adv Parasitol **2002**; 52:235–64.
2. Hay SI, Rogers DJ, Toomer JF, Snow RW. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, Internet access and review. Trans R Soc Trop Med Hyg **2000**; 94:113–27.
3. Mbogo CM, Mwangangi JM, Nzovu J, et al. Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. Am J Trop Med Hyg **2003**; 68: 734–42.
4. Drakeley C, Schellenberg D, Kihonda J, et al. An estimation of the entomological inoculation rate for Ifakara: a semi-urban area in a region

of intense malaria transmission in Tanzania. Trop Med Int Health **2003**;8: 767–74.
5. Shililu J, Ghebremeskel T, Mengistu S, et al. High seasonal variation in entomologic inoculation rates in Eritrea, a semi-arid region of unstable malaria in Africa. Am J Trop Med Hyg **2003**; 69:607–13.
6. Beier JC, Killeen GF, Githure JI. Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. Am J Trop Med Hyg **1999**; 61:109–13.
7. Attenborough RD, Burkot TR, Gardner DS. Altitude and the risk of bites from mosquitoes infected with malaria and filariasis among the Mianmin people of Papua New Guinea. Trans R Soc Trop Med Hyg **1997**; 91:8–10.
8. Bodker R, Akida J, Shayo D, et al. Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. J Med Entomol **2003**; 40:706–17.
9. Akhwale WS, Lum JK, Kaneko A, et al. Anemia and malaria at different altitudes in the western highlands of Kenya. Acta Trop **2004**; 91:167–75.
10. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, Curtis CF. Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. Malar J **2003**; 2:28.
11. Balls MJ, Bodker R, Thomas CJ, Kisinza W, Msangeni HA, Lindsay SW. Effect of topography on the risk of malaria infection in the Usambara Mountains, Tanzania. Trans R Soc Trop Med Hyg **2004**; 98:400–8.
12. Lindblade KA, Walker ED, Onapa AW, Katungu J, Wilson ML. Land use change alters malaria transmission parameters by modifying temperature in a highland area of Uganda. Trop Med Int Health **2000**; 5: 263–74.
13. Attenborough R, Porteous R, Gardner D. Longitudinal weight growth patterns in the highland fringes of West Sepik Province, Papua New Guinea: a comparison of three groups. Ann Hum Biol **1995**; 22:131–50.
14. Schanfield MS, Ohkura K, Lin M, Shyu R, Gershowitz H. Immunoglobulin allotypes among Taiwan aborigines: evidence of malarial selection could affect studies of population affinity. Hum Biol **2002**; 74: 363–79.
15. Vercruysse J, Jancloes M, Van de Velden L. Epidemiology of seasonal falciparum malaria in an urban area of Senegal. Bull World Health Organ **1983**; 61:821–31.
16. Omumbo JA, Hay SI, Guerra CA, Snow RW. The relationship between the *Plasmodium falciparum* parasite ratio in childhood and climate estimates of malaria transmission in Kenya. Malar J **2004**; 3:17.
17. Ellman R, Maxwell C, Finch R, Shayo D. Malaria and anaemia at different altitudes in the Muheza district of Tanzania: childhood morbidity in relation to level of exposure to infection. Ann Trop Med Parasitol **1998**; 92:741–53.
18. Lengeler C, Armstrong-Schellenberg J, D'Alessandro U, Binka F, Cattani J. Relative versus absolute risk of dying reduction after using insecticide-treated nets for malaria control in Africa. Trop Med Int Health **1998**; 3: 286–290.
19. Hay SI, Tucker CJ, Rogers DJ, Packer MJ. Remotely sensed surrogates of meteorological data for the study of the distribution and abundance of arthropod vectors of disease. Ann Trop Med Parasitol **1996**; 90:1–19.
20. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol **1999**; 28:964–74.
21. Dirren H, Logman MH, Barclay DV, Freire WB. Altitude correction for hemoglobin. Eur J Clin Nutr **1994**; 48:625–32.
22. Alifrangis M, Enosse S, Khalil IF, et al. Prediction of *Plasmodium falciparum* resistance to sulfadoxine/pyrimethamine in vivo by mutations in the dihydrofolate reductase and dihydropteroate synthetase genes: a comparative study between sites of differing endemicity. Am J Trop Med Hyg **2003**; 69:601–6.
23. Pearce RJ, Drakeley C, Chandramohan D, Mosha F, Roper C. Molecular determination of point mutation haplotypes in the dihydrofolate reductase and dihydropteroate synthase of *Plasmodium falciparum* in three districts of northern Tanzania. Antimicrob Agents Chemother **2003**; 47:1347–54.
24. Reyburn H, Mbatia R, Drakeley C, et al. Association of transmission intensity and age with manifestations and case fatality of severe *Plasmodium falciparum* malaria. JAMA **2005**; 293:1461–70.
25. Lusingu JP, Vestergaard LS, Mmbando BP, et al. Malaria morbidity and immunity among residents of villages with different *Plasmodium falciparum* transmission intensity in north-eastern Tanzania. Malar J **2004**; 3:26.
26. Ijumba JN, Mosha FW, Lindsay SW. Malaria transmission risk variations derived from different agricultural practices in an irrigated area of northern Tanzania. Med Vet Entomol **2002**; 16:28–38.
27. Craig MH, Snow RW, le Sueur D. A climate-based distribution model of malaria transmission in sub-Saharan Africa. Parasitol Today **1999**; 15:105–11.
28. Lemnge MM, Kamugisha ML, Njunwa KJ, Salum FM, Msangeni HA, Kitua AY. Exploratory study of malaria situation in Hanang and Babati Districts after a reported malaria epidemic: I. Health facility based information on malaria morbidity and mortality. Tanzania Health Res Bull **2001**; 3:18–34.
29. Depinay JM, Mbogo CM, Killeen G, et al. A simulation model of African *Anopheles* ecology and population dynamics for the analysis of malaria transmission. Malar J **2004**; 3:29.
30. Patz JA, Strzepek K, Lele S, et al. Predicting key malaria transmission factors, biting and entomological inoculation rates, using modelled soil moisture in Kenya. Trop Med Int Health **1998**; 3:818–27.
31. Zhou G, Minakawa N, Githeko AK, Yan G. Association between climate variability and malaria epidemics in the East African highlands. Proc Natl Acad Sci USA **2004**; 101:2375–80.
32. Woolhouse ME, Hargrove JW. On the interpretation of age-prevalence curves for trypanosome infections of tsetse flies. Parasitology **1998**; 116:149–56.
33. Maitland K, Williams TN, Bennett S, et al. The interaction between *Plasmodium falciparum* and *P. vivax* in children on Espiritu Santo island, Vanuatu. Trans R Soc Trop Med Hyg **1996**; 90:614–20.
34. Smith T, Schellenberg JA, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. Stat Med **1994**; 13:2345–58.
35. Schellenberg JR, Smith T, Alonso PL, Hayes RJ. What is clinical malaria? Finding case definitions for field research in highly endemic areas. Parasitol Today **1994**; 10:439–42.
36. Struik S, Riley EM. Does malaria suffer from a lack of memory. Immunol Rev **2004**; 201:268–90.
37. Contamin H, Fandeur T, Rogier C, et al. Different genetic characteristics of *Plasmodium falciparum* isolates collected during successive clinical malaria episodes in Senegalese children. Am J Trop Med Hyg **1996**; 54: 632–43.
38. Ijumba J, Mosha F, Lindsay S. Malaria transmission risk variations derived from different agricultural practices in an irrigated area of northern Tanzania. Med Vet Entomol **2002**; 16:28–38.
39. Kulkarni M, Kweka E, Nyale E, et al. Entomological evaluation of malaria vectors at different altitudes in Hai District, North East Tanzania. Med Vet Entomol (in press).
40. White GB. *Anopheles gambiae* complex and disease transmission in Africa. Trans R Soc Trop Med Hyg **1974**; 68:278–301.
41. Mnzava AEP, Kilama WL. Observations on the distribution of the *Anopheles gambiae* complex in Tanzania. Acta Trop **1986**; 43:277–82.
42. Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J. A global index representing the stability of malaria transmission. Am J Trop Med Hyg **2004**; 70:486–98.
43. National Bureau of Statistics. Household budget survey 2000/01: appendix C2002. Dar es Salaam, Tanzania: National Bureau of Statistics, **202**:24–32 .
44. Moormann AM, Embury PE, Opondo J, et al. Frequencies of sickle

cell trait and glucose–6-phosphate dehydrogenase deficiency differ in highland and nearby lowland malaria-endemic areas of Kenya. Trans R Soc Trop Med Hyg **2003**; 97:513–4.
45. Lindsay SW, Bodker R, Malima R, Msangeni HA, Kisinza W. Effect of 1997–98 El Nino on highland malaria in Tanzania. Lancet **2000**; 355: 989–90.
46. Bodker R, Kisinza W, Malima R, Msangeni H, Lindsay SW. Resurgence of malaria: Usambara Mountains, Tanzania, an epidemic of drug resistant parasites. Global Change Hum Health **2000**; 1:134–53.
47. Drakeley C, Corran PH, Coleman PG, et al. Estimating medium and long term trends in malaria transmission using serological markers of malaria exposure. Proc Natl Acad Sci USA (in press).