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An estimation of the entomological inoculation rate for Ifakara: a semi-urban area in a region of intense malaria transmission in Tanzania

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Summary An entomological study on vectors of malaria and their relative contribution to *Plasmodium falciparum* transmission in the semi-urban area of Ifakara, south-eastern Tanzania, was conducted. A total of 32 houses were randomly sampled from the area and light trap catches (LTC) performed in one room in each house every 2 weeks for 1 year. A total of 147 448 mosquitoes were caught from 789 LTC; 26 134 *Anopheles gambiae* s.l., 615 *A. funestus*, 718 other anophelines and 119 981 culicines. More than 60% of the total *A. gambiae* s.l. were found in five (0.6%) LTCs, with a maximum of 5889 caught in a single trap. Of 505 *A. gambiae* s.l. speciated by polymerase chain reaction, 91.5% were found to be *A. arabiensis*. *Plasmodium falciparum* sporozoite enzyme-linked immunosorbent assay tests were performed on 10 108 anopheles mosquitoes and 39 (0.38%) were positive. Entomological inoculation rate (EIR) estimates were generated using a standard method and an alternative method that allows the calculation of confidence intervals based on a negative binomial distribution of sporozoite positive mosquitoes. Overall EIR estimates were similar; 31 *vs.* 29 [95% confidence interval (CI): 19, 44] infectious bites per annum, respectively. The EIR ranged from 4 (95% CI: 1, 17) in the cool season to 108 (95% CI: 69, 170) in the wet season and from 54 (95% CI: 30, 97) in the east of the town to 15 (95% CI: 8, 30) in the town centre. These estimates show large variations over short distances in time and space. They are all markedly lower than those reported from nearby rural areas and for other parts of Tanzania.

keywords *Plasmodium falciparum*, Tanzania, entomological inoculation rate, transmission intensity, *Anopheles arabiensis*, *A. gambiae*

Introduction

The entomological inoculation rate (EIR) estimates the level of exposure to *Plasmodium falciparum*-infected mosquitoes and is the most favoured measure for assessing malaria endemicity and transmission intensity (Burkot & Graves 1995). EIR assessments may be particularly useful when estimating the effect of efforts to reduce human–vector contact. This is an important component of malaria control in many endemic countries is through the use of insecticide treated materials – mosquito nets and curtains – or residual household spraying. Different levels of transmission have been linked to different age and clinical disease patterns, the public health relevance of which has been debated in recent years (Snow *et al.* 1997). The level of transmission has also been correlated with the multiplicity of infection (Arnot 1998) and may be a key factor in the spread of drug-resistant parasites (Hastings & D'Alessandro 2000). Near the area in which this study was conducted, possible associations between EIR and both multiplicity of infection (Fraser-Hurt *et al.* 1999) and morbidity (Smith *et al.* 1998) have been suggested.

The EIR is classically derived from the density of man-biting anopheline mosquitoes, the sporozoite rate within that mosquito population and the human blood index. The human biting catch (HBC) is considered the most accurate method for assessing man-biting rates, although this technique has serious ethical and logistic constraints. Light trap catches (LTC) and pyrethrum spray catches (PSC) represent viable alternatives and have been evaluated against HBC (Lines *et al.* 1991a; Mbogo *et al.* 1993). Apart from practical considerations, an additional problem with EIR calculations

is an inability to estimate their precision. This means that sample size calculations are not possible and makes it difficult to extrapolate the results of malaria intervention studies to other sites on the basis of intensity of transmission.

A recent review of EIR estimates in Africa found marked heterogeneity in malaria risk across the continent (Hay *et al.* 2001). The majority of EIR estimates were from rural areas with an overall mean of 146 infectious bites per person per year (ib/p/y) (range 0–884). In contrast, estimates from more 'urban' areas had a mean of 14 ib/p/year (range 0–43). The review showed Tanzanian EIR estimates to be amongst the highest recorded (mean 367 ib/p/year, range 94–667), although none of these was from an urban area (Hay *et al.* 2001).

Our study was designed to provide an EIR estimate for Ifakara town, the setting for various malaria research studies in the past 5 years (Menendez *et al.* 1997; Hatz *et al.* 1998; Acosta *et al.* 1999; Schellenberg *et al.* 1999, 2001). An EIR estimate of approximately 300 ib/p/y from Namawala, some 30 km away, has been used to describe the endemicity of the trials setting (Smith *et al.* 1993). This figure is similar to other estimates from a number of villages around the town of Ifakara (Tanner *et al.* 1986; Lyimo 1993; Charlwood *et al.* 1998). However, a study of Anopheline malaria vectors in Ifakara town itself in 1965 suggested an overall EIR of only 21–24 ib/p/year (Freyvogel & Kihaule 1968). It may well be that the intensity of transmission in Ifakara town is rather lower than in the surrounding villages, possibly because of fewer and more marginalized breeding sites, reducing the number of mosquitoes. This paper reports an alternative approach to the estimation of the EIR from the semi-urban setting of Ifakara town.

Materials and methods

Study site and mosquito sampling

The town of Ifakara lies in the flood plain of the Kilombero Valley at approximately 36°41′ east and 8°8′ south; the region has an average altitude of 270 m and an annual rainfall range of 1200–1800 mm. Malaria is the foremost health problem reported through the health services and perceived by the local population (Tanner *et al.* 1991). Ifakara town covers an area of approximately 15 km^2. This EIR estimate was confined to an area bounded by the Lumemo River to the west, rice fields to the south and east, and by the ward boundary to the north. A self-weighting sample was chosen as follows: the households within the town are allocated among Balozis (traditionally an individual responsible for a group of 10 houses, although in reality ranging from seven to 23) a list of which was obtained from the local council offices. The town area was arbitrarily divided into three zones: town centre (1), east (2) and south (3). In the town centre, habitation is denser and the majority of houses are of more substantial construction, there is relatively little cultivated land and few trees. The southern zone is less densely populated with large numbers of mature trees and farming of maize, cassava and beans. The eastern zone is the most sparsely populated area and borders on the rice cultivation area.

Balozis were randomly sampled, based on the total number within each area. Each selected Balozi was visited and a list of the heads of households obtained. A single household was selected randomly from each chosen Balozi and visited to assess suitability for inclusion in the study. Households were included if consent was obtained and insecticide was not used in the house either on walls or mosquito nets. One room was selected in each house. For sampling of Balozis and households, three alternative choices were randomly made in case of refusal or unsuitability of the first or subsequent choices.

Mosquito catches were performed in each room once every fortnight over a period of 1 year. Mosquitoes were caught using standard Centre for Disease Control light traps (CDC, Atlanta, GA, USA) hung from the ceiling at the foot end of the bed (Mboera *et al.* 1998). The owner of the house was provided with a new untreated net for the LTC room. Traps were set by trained staff and run for 12 h from 7.00 p.m. Mosquitoes were collected each morning, *Anopheles gambiae* s.l. separated from *A. funestus*, both counted and then stored in separate tubes with silica gel. Other anophelines and culicine mosquitoes were counted and then discarded.

Additional data

Each house in the study was assessed in terms of its structure and proximity to mosquito breeding sites. Study houses were mapped using a hand-held GPS unit (Garmin GPS 12, GARMIN International, Olathe, KS, USA) and distances between each house and the St Francis Designated District Hospital (SFDDH) were calculated using the Haversine formula (Sinnott 1984). A simple weather station was established at Ifakara Health Research and Development Centre to record temperature and rainfall daily.

Laboratory analysis

After catching and counting, mosquitoes were stored on silica gel at room temperature. Mosquitoes sampled for speciation (see below) were split into two tubes with abdomen for speciation and head and thorax for *P. falciparum* circumsporozoite protein (CSP) by enzyme-linked immunosorbent assay (ELISA).

CSP ELISA. It was not possible to assay all mosquitoes by ELISA. As such a large proportion came from five LTCs, ELISAs were run on a sample of mosquitoes from each of these catches. The rationale was that these high mosquito density LTC were the result of waves of emergence of nulliparous adults, as described previously (Charlwood *et al.* 1995a), and these mosquitoes would be uninfected. The CSP ELISA was performed as described previously (Wirtz *et al.* 1987; Collins *et al.* 1988a) with mosquitoes assayed individually. Wells were considered positive when the optical density exceeded the mean + 3 SD of a set of 12 negative controls on the same plate (four *P. berghei* infected *A. stephensi*, four *P. yoelli* infected *A. stephensi* and four uninfected *A. gambiae*). In each plate, four *A. gambiae* infected with *P. falciparum* were used as positive controls. *A. gambiae speciation*. Each LTC was categorized to one of nine space-time points, according to zone and season of collection, and a target sample of 100 mosquitoes randomly selected from each. The sample size was chosen assuming a 1:1 ratio in *A. gambiae* sibling species between *A. gambiae* s.s. and *A. arabiensis* with 10% variation. In some space-time points, <100 mosquitoes were collected, so the total number of mosquitoes speciated was 567. DNA from *A. gambiae* s.l. individual specimens was extracted from the abdomen or wings according to Collins *et al.* (1988b). Samples were identified to species following a protocol described in Pinto *et al.* (1997).

Entomological inoculation rate estimation

The EIR was estimated including a conversion factor for LTC *vs.* man biting catches of 1.605, as described by Lines *et al.* (1991a), without allowance for the number of occupants per room. Two approaches were used to calculate the overall annual EIR, with subsequent estimates calculated by area and season: 1. *standard method*, i.e. 1.605 × (no. of sporozoite positive Elisas/no. of mosquitoes tested) × (no. of mosquitoes collected/no. of catches) × 365; 2. *alternative method*, i.e. 1.605 × (no. of positive Elisas/no. of catches) × 365.

The former approach, being the product of the sporozoite rate and the man-biting rate, is the standard approach and assumed that mosquitoes caught in the five large mosquito catches, but not assayed by ELISA, were sporozoite-negative. This assumption is implicit in the latter approach which also assumes that the vast majority of mosquitoes were assayed. The second approach has the advantage that CIs can be calculated around the resulting EIR estimates.
A comparison of the observed frequencies of sporozoite-positive mosquitoes with those predicted by Poisson and negative binomial distributions (Table 1) showed that the negative binomial distribution produced the better fit and this was used for the subsequent analysis. Analysis was performed in STATA (version 6: TX, USA) and standard commands used to assess the effect of clustering within LTC houses. This produced similar CIs as the cluster-unadjusted analysis and only the former estimates are presented.

Ethics

Ethical permission for the study was obtained from the local ethical committees in Ifakara and the National Institute for Medical Research.

Results

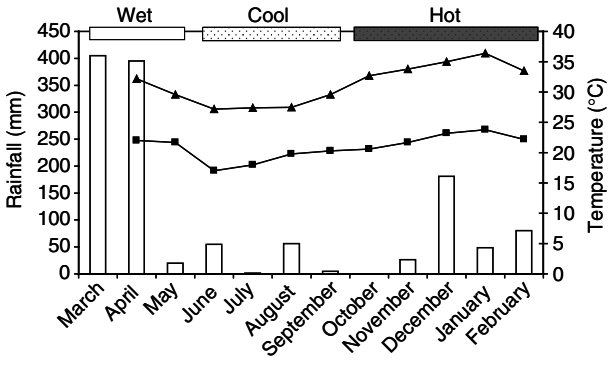
Climatic data and vector abundance

Rainfall and mean monthly minimum and maximum temperature for the year is shown in Figure 1; no data were available for the first month of the study (March 1999) from our source, although other local sources recorded a

Table 1 Table comparing observed numbers of sporozoite-positive mosquitoes in light trap catches (LTC) with those expected, assuming negative binomial and Poisson distributions

No. of sporozoite-positive mosquitoes in LTC	No. of times observed	No. of times expected with negative binomial distribution	No. of times expected with Poisson distribution
0	761	761.1	751.0
1	21	20.6	37.1
2	5	4.9	0.9
3	0	1.5	0.02
4	2	0.5	0.00
≥5	0	0.3	0.00
Total	789	789	789
Chi-squared		5.62 on three degrees of freedom†,‡	21 435.96†

† Some expected values are <5 hence chi-squared test invalid.
‡ Unlikely to be significantly different from observed numbers.



Season	Months	Mean monthly rainfall (mm)	Mean monthly temperature range (min–max °C)
Wet	March–May	273	21.8–30.8
Cool	June–Sept	9	18.7–27.9
Hot	Oct–Feb	22	22.9–34.2

Figure 1 Meteorological information by month during the study period: open bars correspond to rainfall (in millimetres) on lefthand *y*-axis, -■- mean monthly minimum temperature and -▲- mean monthly maximum temperature on the right hand *x*-axis.

figure of 405 mm (C. Golding, Kilombero Valley Teak Company, Tanzania). The year was subsequently divided into seasons (see footnote to Figure 1). May was classified as wet season because of the large amount of surface water despite low rainfall.

The 32 households in which traps were set and their position relative to SFDDH are shown in Figure 2. Of 832, 789 (95%) LTCs were included in the final analysis, with operator error and battery malfunction, the main reasons for exclusion. A total of 26 134 *A. gambiae*, 615 *A. funestus*, and 119 981 culicine mosquitoes were caught. Other anophelines, about 718, were caught (*A. pharoensis*, *A. coustani*, *A. squamosus* and *A. zimini*) which were found in all zones in the first 4 months of the study, coinciding with the rains.

Anopheles gambiae were caught in 264 of 789 (33.5%) LTCs and there were only two houses in which no mosquito of this species was caught during the study. Of all *A. gambiae* caught, 60% (15 462) came from five LTCs in households in the eastern zone; four of these catches came from two households in consecutive LTCs. *Anopheles funestus* was found in 125 of 789 traps (15.8%). They were most prevalent in LTC immediately following the rainy season with a maximum of 99 caught in one trap. Culicine mosquitoes were caught in 734 of 789 traps (93%) and were consistently high in the eastern zone throughout the year. Figure 3 shows the breakdown of mosquito density of LTC for each species of mosquito by season.

Laboratory analysis

CSP ELISA. A total of 10 108 mosquitoes were tested for the presence of *P. falciparum* sporozoites in the salivary glands of which 39 (0.39%) were positive. Three of 621 (0.48%) *A. funestus* were sporozoite positive. No sporozoite-positive mosquitoes were collected in 16 of 32 (50%) of the houses, although 10 sporozoite-positive mosquitoes were caught in one house alone. Of 39, 33 (85%) infected mosquitoes were caught during the wet season. The proportion of CSP-positive mosquitoes was lowest during the rains (0.34%) and maximal (3.28%) in the hot season. All 369 samples assayed from the five high mosquito density LTC were negative for CSP.
A. gambiae speciation. Of 567, 505 (89%) mosquitoes selected were successfully speciated by polymerase chain reaction (PCR). Of these 462, 505 (91.5%) were typed as *A. arabiensis*. None of the 45 mosquitoes typed as *A. gambiae* s.s were sporozoite-positive nor were they associated with any particular season or zone.

Entomological inoculation rate estimation

Data from both methods used are presented in Table 2. The column in the table for the number of mosquitoes tested by ELISA includes the number actually assayed by ELISA and the large number of mosquitoes not tested but assumed to be nulliparous and therefore negative (Lines *et al.* 1991b; Charlwood *et al.* 1995b). The overall estimates of 31 for the standard method and 29 ib/p/year (95% CI: 19, 44) for the alternative method was obtained. Seasonal effects were reflected by a maximal EIR of 108 ib/p/year (95% CI: 69, 170) in the wet season and a minimum of four (95% CI: 1, 17) in the cool season. Estimates were also spatially distinct, the lowest estimate found in the town centre 15 ib/p/year (95% CI: 8, 30) and the highest in the east 54 ib/p/year (95% CI: 30, 97).

Household factors

Data on mosquito abundance for both vector and non-vector species and EIR estimates were categorized into tertiles and tested for associations with various internal and external household features using Fisher's exact test. Of the factors investigated, houses within 50 m of a 'shamba' (a cultivated area) ($P = 0.015$), near to a permanent water pool ($P = 0.027$) and owning chickens ($P = 0.013$) were all associated with higher catches of anopheline vectors. When analysed for an association with EIR estimates, these factors were of borderline significance (P range 0.051–0.085), although houses with windows larger than 0.6 m^2 had EIR in the highest tertile ($P = 0.022$) (data not shown).

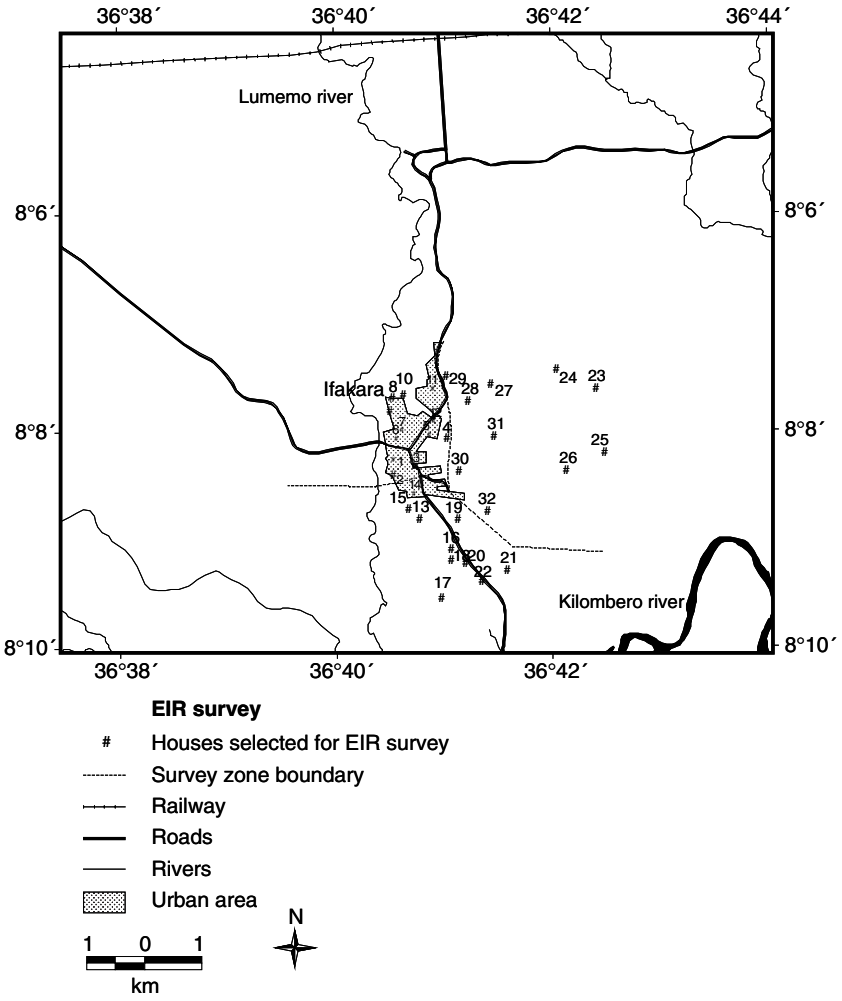


Figure 2 Map of Ifakara town and the study area. The solid lines represent the arbitrary boundary to define study zones. Numbered points represent individual houses in the study: 1–12 in town centre, 13–22 south and 23–32 in the east.

Discussion

This paper describes a year-long entomological study of malaria vectors in the semi-urban area of Ifakara, southern Tanzania. Overall EIR estimates of 31 and 29 infectious bites per person per annum were generated using two different methods. The advantage of the second method is that it allows the calculation of CIs. This is complicated in the standard method, as the man-biting rate and the sporozoite rate are not independent of each other: an inverse association over time is expected between sporozoite rate and population density, as observed in this study and others (Charlwood *et al.* 1995a). Hence an estimate of the covariance of these terms is necessary to generate a CI around the EIR figure. The second method assumes that ELISA results are available for all mosquitoes caught in a light trap. These represent the numerator and denominator of the man-biting rate and sporozoite rate,

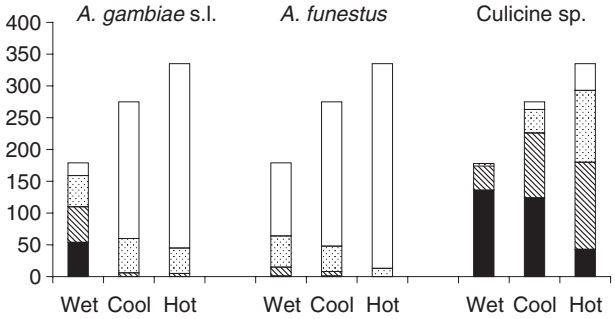


Figure 3 Graph showing the number of light trap catches with 51+ ■, 6–50 ▧, 1–5 ⬚ and 0 □ mosquitoes for vector and non-vector species by season in the study area.

respectively, and as such cancel each other out in calculation. In our data set we have 25 256 ELISA results (including those from the five large LTCs which we

Table 2 Entomological inoculation rate (EIR) estimates for Ifakara town by zone and by season

Category	No. of mosquitoes caught	No. tested by ELISA†	No. sporozoite-positive	Proportion sporozoite positive	EIR standard method‡	EIR alternative method (95% CI)§	
Season							
Wet	26 228	24 758 (94%)	33	0.0013	114.4	108.0	68.7–169.9
Cool	387	375 (96.9%)	2	0.0053	4.4	4.3	1.1–17.0
Hot	134	123 (91.8%)	4	0.0325	7.6	6.9	1.9–25.3
Zone							
Town	3310	3175 (95.9%)	8	0.0025	15.8	15.1	7.6–30.2
South	2574	1939 (75.3%)	8	0.0041	27.2	20.5	7.1–59.4
East	20 865	20 142 (96.5%)	23	0.0011	55.8	53.9	29.8–97.4
Overall	26 749	25 256 (94.4%)	39	0.0015	30.7	28.9	19.0–44.2

† Includes data from five light trap catches in which large numbers of mosquitoes were caught (15 462) but not assayed on the assumption they were nulliparous.
‡ Standard method: 1.605 × (no. of sporozoite-positive Elisas/no. of mosquitoes tested) × (no. of mosquitoes collected/no. of catches) × 365.
§ Alternative method: 1.605 × (no. of positive Elisas/no. of catches) × 365.

assumed to be negative) and 26 759 mosquitoes from LTC, i.e. results for 94% of mosquitoes. In any study, it is unlikely that the number of catches used to calculate an EIR will fully represent the total number of person nights within a village and therefore the calculation of CIs allows the estimate to be placed in context. Sample size calculations can also be made on the basis of the expected CI, which may be particularly important in areas of low endemicity where vector densities (and sporozoite positivity) are low.

The speciation data strongly implicate *A. arabiensis* as the principal malaria vector in Ifakara town. This species has been described previously in detail in a nearby rural area (Charlwood *et al.* 1995a,b). Pertinent to this study, the investigators described a high proportion of *A. arabiensis* in a large number of nulliparous females trapped in LTC immediately after rain, high human blood index in blood-fed *A. arabiensis* and a range of sporozoite rates. A recent study from Northern Tanzania has shows that *A. arabiensis* has a memorized home range and that mosquitoes tend to return to the same houses to feed (McCall *et al.* 2001). This might also have contributed to the clustering of malaria transmission even within a relatively small town. In this study more than one quarter (10 of 39) of sporozoite-positive mosquitoes were collected in one house and in half the houses, no infected mosquitoes were found.

In 1965, Freyvogel and Kihaule (1968) estimated an EIR of 21–24 ib/p/year for Ifakara, which is very similar to the estimate from this study. However, the earlier result was based on relatively few mosquitoes caught in PSC and visual examination of the salivary glands, and is thus likely to be an underestimate. Ifakara is a district town in rapid transition characterized by increases in population, quality of housing and availability of goods. These goods are very likely to include mosquito control and protection methods, in demand because of nuisance mosquitoes which our data show are present in large numbers all year round. There has also been an active social marketing programme distributing insecticide treated mosquito nets (Armstrong Schellenberg *et al.* 2000). It may therefore be that increasing 'urbanization' has caused a decrease in transmission intensity, but we were unable to confirm this.

EIR estimates for the rural sites close to Ifakara have suggested the region is highly endemic for malaria (Tanner *et al.* 1986; Lyimo 1993; Smith *et al.* 1993; Charlwood *et al.* 1998) although our data suggest that this may not be the case for the town itself. Whilst not statistically significant, the estimate for the town centre is less than that for the eastern side of the town. This presumably reflects distance from breeding sites with houses in the town centre further from these sites. Analysis of household data, whilst limited, supports this by showing that an increased density of malaria vectors and EIR were associated with proximity to farmland or 'shambas' and permanent water collections. These findings are in accordance with observations from other studies in the same area showing an increasing incidence of clinical malaria as distance from the hospital increased (Acosta *et al.* 1999; Schellenberg *et al.* 2001). The phenomenon has also been described in an urban area in Mozambique, where the EIR decreased by 50% every 90 m moved further away from breeding sites (Mendis *et al.* 2000) and, in Kenya, where protection from severe malaria was correlated with higher surrounding population density, a surrogate for urbanization (Snow *et al.* 1999). The contrast in our estimates and those 10-fold higher from the rural areas 10–30 km away will be due to a variety of factors. These are expected to be primarily decreased because of proximity to breeding sites, increased human density and

dilution of the infectious reservoir, higher quality housing and greater coverage of mosquito nets in the urban setting.

A consistent finding in the two Ifakara town-based studies was the marked seasonal change in estimates, both reporting the highest number of sporozoite-positive mosquitoes and EIR in the wet season. The overall EIR from our study was within the range of estimates from urban sites in Africa (0–43), although the wet season figure (108) shows that, at times, malaria transmission is as intense as in more rural settings (Hay *et al.* 2001). However, taken together, the Ifakara studies suggest a likely difference in EIR estimates from areas in close proximity to each other and with differing patterns of land use.

The highest EIR estimate was 108 ib/p/year in the wet season, corresponding to more than one infectious bite per night for this season. In contrast, the transmission intensity in the cool and hot seasons were significantly lower and equates to approximately one infectious bite per month. Interestingly, hospital admissions for malaria peak after the wet season (Freyvogel & Kihaule 1968; Guinovart 2001). A possible explanation is that clinical attacks are caused by infections with new clones generated from increased sexual recombination of the parasite (Ofosu-Okyere *et al.* 2001), although the data are also consistent with seasonal changes in access to preventive and curative health services.

In summary, we have used a novel approach to the calculation of the EIR which enables an assessment of the CI surrounding the estimate. This approach may facilitate comparison of different EIR estimates and can be used as a basis for rational sample size calculations for entomological studies. This study has also shown that *A. arabiensis* was the principal vector of *P. falciparum* transmission in the semi-urban setting of Ifakara and that the intensity of transmission was rather lower than in the more rural surrounding areas. This helps to set in context a number of recent malaria intervention studies.

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References

Acosta CJ, Galindo CM, Schellenberg D *et al.* (1999) Evaluation of the SPf66 vaccine for malaria control when delivered through the EPI scheme in Tanzania. *Tropical Medicine and International Health* **4**, 368–376.

Arnot D (1998) Unstable malaria in Sudan: the influence of the dry season. Clone multiplicity of *Plasmodium falciparum* infections in individuals exposed to variable levels of disease transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 580–585.

Armstrong Schellenberg JRM, Abdulla S, Minja H *et al.* (1999) KINET: a social marketing programme of treated bednets and net re-treatment for malaria control in Tanzania, with evaluation of child health and long-term survival. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 225–231.

Burkot TR & Graves PM (1995) The value of vector-based estimates of malaria transmission. *Annals of Tropical Medicine & Parasitology* **89**, 125–134.

Charlwood JD, Smith T, Kihonda J, Heid B, Billingsley PF & Takken W (1995a) Density independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania. *Bulletin of Entomological Research* **85**, 29–35.

Charlwood JD, Kihonda J, Sama S *et al.* (1995b) The rise and fall of Anopheles arabiensis (Diptera: Culicidae) in a Tanzanian village. *Bulletin of Entomological Research* **85**, 37–44.

Charlwood JD, Smith T, Lyimo E *et al.* (1998) Incidence of *Plasmodium falciparum* infection in infants in relation to exposure to sporozoite-infected anophelines. *American Journal of Tropical Medicine and Hygiene* **59**, 243–251.

Collins FH, Procell PM, Campbell GH & Collins WE (1988a) Monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for detection of *Plasmodium malariae* sporozoite in mosquitoes. *American Journal of Tropical Medicine and Hygiene* **38**, 283–288.

Collins FH, Mehaffey PC, Rasmussen MO, Brandling-Bennet AD, Odera JS & Finnerty V (1988b) Comparison of DNA-probe and isoenzyme methods for differentiating *Anopheles gambiae* and *Anopheles arabiensis* (Diptera: Culicidae). *Journal of Medical Entomology* **25**, 116–120.

Fraser-Hurt N, Felger I, Edoh D *et al.* (1999) Effect of insecticide-treated bed nets on haemoglobin values, prevalence and multiplicity of infection with *Plasmodium falciparum* in a randomized controlled trial in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93** (Suppl. 1), 47–51.

Freyvogel TA & Kihaule PM (1968) Report on a limited Anopheline survey at Ifakara, South Eastern Tanzania. *Acta Tropica* **25**, 18–26.

Guinovart C (2001) Patterns of morbidity and mortality among paediatric inpatients at a district hospital in rural Tanzania. MSc Thesis, LSHTM, London.

Hastings IM & D'Alessandro U (2000) Modelling a predictable disaster: the rise and spread of drug-resistant malaria. *Parasitology Today* **16**, 340–347.

Hatz C, Abdullah S, Mull R *et al.* (1998) Efficacy and safety of CGP56697 (artemether and benflumetol) compared with chloroquine to treat acute falciparum malaria in Tanzanian children aged 1–5 years. *Tropical Medicine and International Health* **3**, 498–504.

Hay SI, Rogers DJ, Toomer JF & Snow RW (2001) Annual *Plasmodium falciparum* entomological inoculation rates (EIR)

across Africa: literature survey, Internet access and review. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 113–127.

Lines JD, Curtis CF, Wilkes TJ & Njunwa TJ (1991a) Monitoring human biting mosquitoes in Tanzania with light traps hung beside mosquito nets. *Bulletin of Entomological Research* **81**, 77–84.

Lines JD, Wilkes TJ & Lyimo EO (1991b) Human malaria infectiousness measured by age specific sporozoite rates in *Anopheles gambiae* in Tanzania. *Parasitology* **102**, 167–177.

Lyimo E (1993) Some aspects of the adult bionomics of *Anopheles gambiae* s.l. in relation to malaria transmission in southeastern Tanzania. PhD Thesis, Wageningen Agricultural University, The Netherlands.

McCall PJ, Mosha FW, Njunwa KJ and Sherlock K (2001) Evidence for memorized site-fidelity in *Anopheles arabiensis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 587–590.

Mboera LEG, Kihonda J, Braks M and Knols BGJ (1998) Short report: Influence of centres for disease control, light trap position, relative to a human-baited bed net, on catches of *Anopheles gambiae* and *Culex quinquefasciatus* in Tanzania. *American Journal of Tropical Medicine and Hygiene* **59**, 595–596.

Mbogo CN, Glass GE, Forster D *et al.* (1993) Evaluation of light traps for sampling anopheline mosquitoes in Kilifi, Kenya. *Journal of American Mosquito Control Association* **9**, 260–263.

Mendis C, Jacobsen JL, Gamage-Mendis A *et al.* (2000) *Anopheles arabiensis* and *An. funestus* are equally important vectors of malaria in Matola coastal suburb of Maputo, southern Mozambique. *Medical and Veterinary Entomology* **14**, 171–180.

Menendez C, Kahigwa E, Hirt R *et al.* (1997) A randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for the prevention of severe anaemia and malaria in Tanzania infants. *Lancet* **350**, 844–850

Ofosu-Okyere A, Mackinnon MJ, Sowa MP *et al.* (2001) Novel *Plasmodium falciparum* clones and rising clone multiplicities are associated with the increase in malaria morbidity in Ghanaian children during the transition into the high transmission season. *Parasitology* **123**, 113–123.

Pinto J, Arez AP, Franco AS, do Rosário VE & Snounou G (1997) Simplified methodology for PCR investigation of midguts from mosquitoes of the *Anopheles gambiae* complex, in which the vector and *Plasmodium* species can both be identified. *Annals of Tropical Medicine and Parasitology* **91**, 217–219.

Schellenberg D, Menendez C, Kahigwa E *et al.* (1999) African children with malaria in an area of intense *Plasmodium falciparum* transmission: features on admission to the hospital and risk factors for death. *American Journal of Tropical Medicine and Hygiene* **61**, 431–438.

Schellenberg D, Menendez C, Kahigwa E *et al.* (2001) Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. *Lancet* **357**, 1471–1477.

Sinnott RW (1984) Virtues of the Haversine. *Sky and Telescope* **68**, 159.

Smith T, Charlwood JD, Kihonda J *et al.* (1993) Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Tropica* **54**, 55–72.

Smith T, Charlwood JD, Kitua AY *et al.* (1998) Relationships of malaria morbidity with exposure to *Plasmodium falciparum* in young children in a highly endemic area. *American Journal of Tropical Medicine and Hygiene* **59**, 252–257.

Snow RW, Omumbo JA, Lowe B *et al.* (1997) Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* **349**, 1650–1654.

Snow RW, Peshu N, Forster D *et al.* (1999) Environmental and entomological risk factors for the development of clinical malaria among children on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 381–385.

Tanner M, Del Guidice G, Betschart B *et al.* (1986) Malaria transmission and development of anti-sporozoite antibodies in a rural African community. *Memoires Instituto de Oswaldo Cruz* **86** (Suppl. II), 199–205.

Tanner M, De Savigny D, Mayombana C *et al.* (1991) Morbidity and mortality at Kilombero, Tanzania 1982–88. In: *Disease and Mortality in Sub-Saharan Africa* (eds RG Feachem & DT Jamison), Oxford University Press, Oxford, pp. 286–305.

Wirtz RA, Burkot TR, Graves PM & Andre RG (1987) Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *Journal of Medical Entomology* **24**, 433–437.

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