Distance to *Anopheles sundaicus* larval habitats dominant among risk factors for parasitemia in meso-endemic Southwest Sumba, Indonesia

Christian P. Nixon[1], Christina E. Nixon[2], Dian Sidik Arsyad[2], Krisin Chand[2], Frilasita A. Yudhaputri[2], Wajiyo Sumarto[2], Suradi Wangsamuda[2], Puji B. Asih[2], Sylvia S. Marantina[2], Isra Wahid[3], Gang Han[4], Jennifer F. Friedman[5], Michael J. Bangs[6,7], Din Syafruddin[2,3], J. Kevin Baird[8,9]

[1]Department of Clinical Pathology, University of California San Francisco, USA, [2]Eijkman Institute of Molecular Biology, Jakarta, Indonesia, [3]Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, [4]Yale University School of Public Health, New Haven, CT, USA, [5]Center for International Health Research, Rhode Island Hospital and Alpert Medical School of Brown University, Providence, RI, USA, [6]Public Health & Malaria Control, International SOS, Papua, Indonesia, [7]Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, [8]Eijkman-Oxford Clinical Research Unit, Eijkman Institute of Molecular Biology, Jakarta, Indonesia, [9]The Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, UK

Background: The decline in intensity of malaria transmission in many areas now emphasizes greater importance of understanding the epidemiology of low to moderate transmission settings. Marked heterogeneity in infection risk within these populations creates opportunities to understand transmission and guide resource allocation to greater impact.

Methods: In this study, we examined spatial patterns of malaria transmission in a hypo- to meso-endemic area of eastern Indonesia using malaria prevalence data collected from a cross-sectional socio-demographic and parasitological survey conducted from August to November 2010. An entomological survey performed in parallel, identified, mapped, and monitored local anopheline larval habitats.

Results: A single spatial cluster of higher malaria prevalence was detected during the study period (relative risk = 2.13; log likelihood ratio = 20.7; P < 0.001). In hierarchical multivariate regression models, risk of parasitemia was inversely correlated with distance to five *Anopheles sundaicus* known larval habitats [odds ratio (OR) = 0.21; 95% confidence interval (CI) = 0.14–0.32; P < 0.001], which were located in a geographically restricted band adjacent to the coastline. Increasing distance from these sites predicted increased hemoglobin level across age strata after adjusting for confounders (OR = 1.6; 95% CI = 1.30–1.98; P < 0.001).

Conclusion: Significant clustering of malaria parasitemia in close proximity to very specific and relatively few *An. sundaicus* larval habitats has direct implications for local control strategy, policy, and practice. These findings suggest that larval source management could achieve profound if not complete impact in this region.

Keywords: *Anopheles sundaicus*, Larval habitats, Malaria risk factors, Spatial scan statistic

Introduction

Southeast Asia contributes substantially to the global human population at risk of malaria.[1] Approximately 1.3 billion people living in the nine countries within the spheres of the WHO South East Asia Regional Office (SEARO) and West Pacific Regional Office are at some degree of risk of infection, primarily in hypo- to meso-endemic settings.[1,2] Considering reports of declining incidence of malaria hospitalizations, prevalence, and death across the globe coincident with the scaling up of malaria control efforts,[3–14] understanding low-to-moderate transmission settings such as that typified by Southeast Asia is becoming increasingly important to the adaptation of successful

Correspondence to: C. P. Nixon, Center for International Health Research, Rhode Island Hospital and Alpert Medical School of Brown University, Providence, RI, United States of America. Email: cnixon@lifespan.org

malaria control interventions across a range of transmission intensities. This may be especially true in the context of strategies aiming at the elimination of malaria transmission in amenable localities.

Indonesia, an archipelago of approximately 13 500 islands and a population of over 241 million people in 2012,[15] accounted for approximately 12% of total reported malaria infections to the SEARO in 2012.[1] Using Bayesian model-based geostatistical approaches, Elyazar *et al.* estimated 132.8 and 129.6 million people in Indonesia lived at risk of acquiring *Plasmodium falciparum*[16] and *Plasmodium vivax*[17] respectively in 2010. Although control efforts on the archipelago have historically been hampered by a complex tapestry of political, socioeconomic, and geographic variables,[18] the Indonesian Ministry of Health announced its plan in 2009[19] to achieve elimination phase by 2020 with cessation of malaria transmission in 2030.[20]

Complicating malaria control in Indonesia and elsewhere, is heterogeneity in malaria risk, a frequently encountered yet ill-defined phenomenon that results in widely varied risk between localities or even at the level of individual households.[21–25] It has been postulated that these differences may be due to host genetic factors or specific characteristics of houses or their particular locations that may facilitate contact between humans and vector mosquitoes.[26] Specifically, clustering in households of red blood cell polymorphisms[22,27–31] and host genetic factors that modulate innate and adaptive immunity,[32–34] household proximity to vector breeding sites,[35–39] breeding site longevity and productivity,[40] household design,[21,22,28,41,42] and vicinity of alternative hosts such as cattle[43] all may contribute to or diminish the risk of infection. Since many of these variables are regional and focal, refinement of malaria control efforts through local risk factor assessment has been deemed essential for success, poignantly so in low transmission areas (i.e. malaria incidence below five episodes per 1000 person-years at risk).[44,45]

Spatial prediction models have proven practical and useful in defining at-risk populations at national,[16,17,46–49] sub-continental,[50–52] and global scales,[53,54] yet currently lack the capacity to identify small-scale hotspots of malaria transmission from within larger foci of transmission.[55,56] We determined local spatial patterns in malaria transmission in a hypo- to meso-endemic region of Southwest Sumba District, East Nusa Tenggara Province, Indonesia, using geocoded malaria cross-sectional prevalence, socio-demographic, and vector bionomic data from surveys conducted in 2010, to identify risk factors associated with increased risk of infection. Limited prior knowledge of malaria transmission and vector bionomics in this scarcely studied region were derived from cross-sectional parasitological surveys conducted in 2007 that detected a median prevalence of microscopically patent parasitemia of approximately 6% (range of 0–34%) that was highest along the immediate coastline.[57] Entomological surveys in 2006 documented 11 species of anophelines, with *Anopheles sundaicus* being the overwhelmingly dominant species present in coastal habitats.[58] This study examined the spatial relationships between known larval habitats for this malaria vector mosquito and the prevalence of malaria and anemia among human residents.

Materials and Methods
Study area
The study was carried out in the village of Wainyapu (09.6400°S, 119.02175°E), one of many permanent settlements located along the southwestern coast of Sumba (Fig. 1). Wainyapu averages 15 m above sea-level and is characterized by savannah-like landscapes, isolated pockets of drought-resistant deciduous forest, and low rolling limestone hills. The Indian Ocean comprises the western border of this 22 km² area. A river forms the northern border, with its egress to the sea becoming blocked in the latter portion of the dry season. Air temperatures average 25°C year-round with dry season highs of 32°C and wet season lows of 18°C. Yearly rainfall in western Sumba averages 1200–2450 mm. During the dry season from May to October, southeast trade winds predominate, while in the wet season from November to April, weather patterns become more mercurial, with inconsistent stormy westerly winds and periodic heavy rainfall.

Five permanent/semi-permanent ground pool water features are present in a narrow band that extends 220 m from the coastline. The northernmost ground water feature is an oval 1600 m² permanent coastal depression that is situated between the beach and a low coastal limestone hill. Protected by a large sand ridge, the depression is supplied by both fresh and saline water due to periodic exposure to tidal flow with resultant varying depth and water quality throughout the year. The southernmost water feature is a flask-shaped 2105 m² blocked stream mouth, obstructed during the dry season by shifting beach sand. Exposed to tidal flow, this water feature is also supplied by fresh and saline water, although the stream is dry for a good part of the year. The three permanent ground pools sandwiched between these two coastal water features are situated more inland, being located approximately 50 m from the coast and extending to within 220 m from the same. These depressions measure 1201, 3713, and 18535 m², respectively, and are limestone sinks with more or less year-round permanent water of varying depth and quality. The largest limestone sink is the southernmost and is characterized by a large stand of emergent mangrove along the side closest to
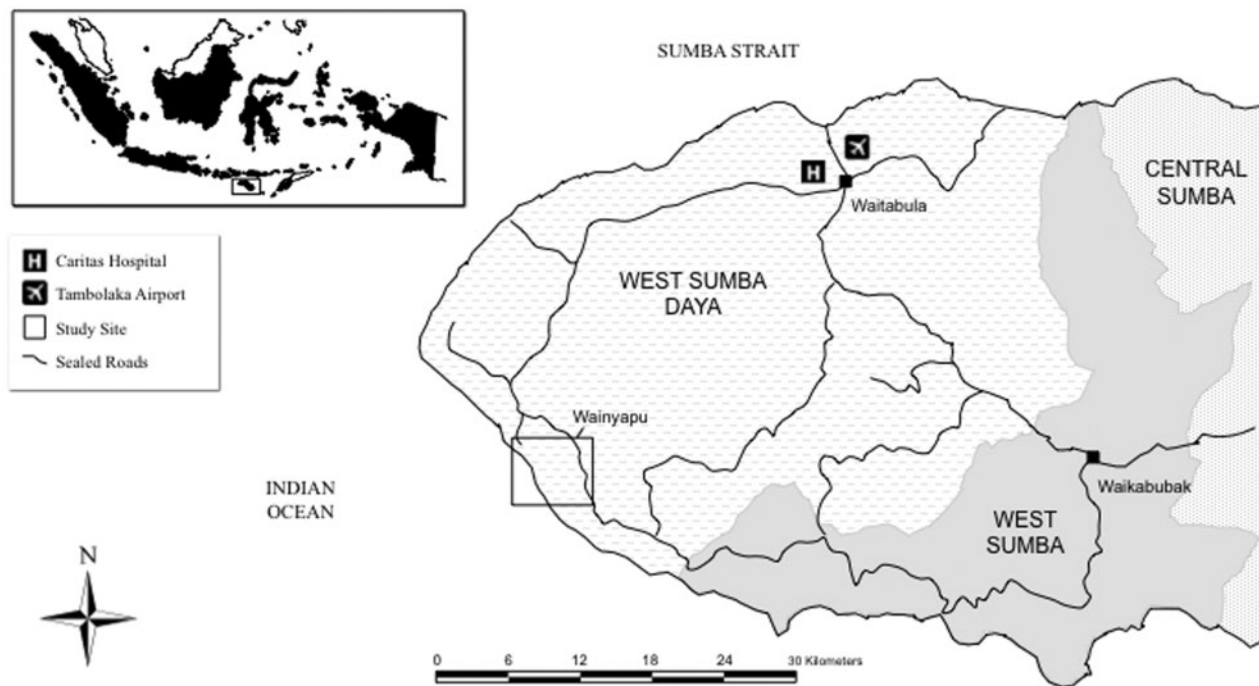
Figure 1 District map of Southwest Sumba, including sealed roads, main towns, study village (Wainyapu), hospital, airport, and the location of Sumba within the Indonesian archipelago (inset).

the coast. All five water features are primarily unshaded with combinations of emergent, floating, and submerged vegetation.

An entomological survey conducted in this region in 2006 identified 11 anopheline mosquito species (Diptera: Culicidae).[58] Species distribution by habit revealed a preponderance of *Anopheles annularis*, *Anopheles subpictus*, and *Anopheles vagus* in upland interior sites, with *An. sundaicus*, *An. subpictus*, and *An. vagus* most commonly found near the coast. *Anopheles sundaicus* was the predominant coastal species identified in evening human-landing collections. Similarly, Barbara *et al.*[59] in 2007 collected Anopheles mosquitoes belonging to 13 species in both interior and coastal localities in western Sumba. Of potential disease vectors, *An. annularis*, *An. subpictus*, and *An. vagus* were the most frequently collected species in the upland interior sites, whereas *An. sundaicus*, *An. subpictus*, and *An. vagus* were most commonly found along the coast. *An. sundaicus* was the predominant species (83 percent of samples) from evening human-landing collections along the coast. More recent use of molecular identification methods by examining the mtDNA of samples of *An. sundaicus* found all specimens assayed from the Wainyapu study site to be *An. sundaicus* (species E),[60] as yet an undescribed (formally named) species in the Sundaicus complex.[61] While species distributions are fairly well known, relatively little is known of the malaria vector bionomics in this area and the rest of Sumba.

Malaria occurs year-round in western Sumba. Prevalence surveys conducted in 2007 included both coastal and inland villages, and reported an overall prevalence of 6.8% in the wet season and 5.0% in the dry.[57] *Plasmodium falciparum* accounted for 70% of infections and was higher in the wet season than the dry (4.9% versus 2.9%), with little difference detected in the inter-seasonal prevalence of *Plasmodium vivax* and *Plasmodium malariae*.

Study population and mapping

A door-to-door census in August 2010 enumerated current residents, most of whom engage in subsistence farming (maize, cassava, cashew, and dry rice cultivation), with village children attending one of two local primary schools. The village, sub-village, household cluster, and household for each individual were recorded, and each individual was given a unique identifier based on these demographic features. Individual household construction type (including wall and roofing materials) along with bednet number, condition, and source, were recorded. The coordinates of every household (taken from the front door) along with schools, churches, health centers, government offices, large cement cisterns, footpaths, two-wheel tracks, main roads, and the borders of standing ground pool water, wells, rivers, wetlands, and the ocean beach were recorded using a hand-held Geographic Positioning System (GPS) (Garmin GPS MAP® 60CSx; Garmin International Inc., Olathe, KS, USA), and processed with MapSource software.[62] Integrated household and vector breeding site distribution maps were produced and analyzed in a Geographic Information Systems (GIS) database[63] using ArcGIS v10.1 software (Environmental Research Systems Institute,

Redlands, CA, USA) to quantify spatial heterogeneity. Generate near table function was used to calculate the Euclidean distance between features, including distance to closest vector larval habitat, distance to closest health facility, and distance to closest permanent cement cistern. Digital data in the GIS were displayed in the WGS 1984 Coordinate system.

Malaria prevalence survey

Fifty percent of households were randomly selected from the census, and every household member (permanent resident) within them was invited to participate in the study. Before enrollment, informed consent was obtained from all adult subjects and from the parents or legal guardians of subjects <18 years of age. The cross-sectional blood survey performed in November 2010, had been reviewed and approved by the Ethical Review Committee of the Eijkman Institute of Molecular Biology, Jakarta, Indonesia. The age, sex, weight, and temperature (tympanic) of all study participants were recorded. Finger-prick blood samples were used for the preparation of standard thick and thin smears on glass microscope slides, blots on filter paper (3M, Watsboro, OR, USA), and hemoglobin measurement using a Hemocue® hemoglobinometer (Hemocue, Angelholm, Sweden). When appropriate, heel-stick blood samples were taken from infants for blood smears only. Blood smears from anyone in the village presenting with signs or symptoms of malaria at time of blood sampling were read within 24 hours. A medical team returned to the village the following day to treat all positive cases with anti-malarial drugs according to Indonesian Ministry of Health guidelines.

Laboratory diagnosis of malaria

Thick and thin smears stained with Giemsa were examined under ×1000 oil immersion light microscopy by certified competent microscopists for the presence of malaria parasites. Parasite densities were measured from positive thick smears by counting the number of asexual parasites per 200 leukocytes and reported as parasites/μl assuming a WBC count of 8000/μl. A minimum of 200 ocular fields were examined before a slide was considered negative. A second certified microscopist reviewed 10% of negative smears and all positive smears. Discordant results were reviewed by a third microscopist, with the majority determination reported and used in the analysis.

Larval surveillance

An entomological survey was conducted to determine the types and abundance of aquatic habitats where potential vectors might exist in the study site. All possible bodies of water including ponds, earthen and cement-lined wells, wetlands, rivers, earthen depressions, upturned coconut shells, and permanent cement water cisterns were systematically inspected for the presence of mosquito larvae. A standard mosquito dipper was used to collect larvae, which was then poured into 30 × 15 cm white plastic trays for careful observation of all immature life stages (larval instars and pupae). Larvae were collected alive by means of a hand pipette, transferred to labeled Whirl-Pak® sealable plastic bags (Nasco, Fort Atkinson, WI, USA) and transported to the insectary/laboratory. The larvae were carefully reared to the adult stage in plastic trays. Adults were identified to species or species complex using morphological characters.[64]

Statistical analysis

Data were recorded using standardized case report forms and double entered into MS Excel (Microsoft Inc., Redmond, WA, USA). Statistical analysis of data was performed using SPSS version 8.0 (SPSS Inc., Chicago, IL, USA) and JMP version 7.0 (SAS Institute Inc., Cary, NC, USA). The relationship between independent categorical and dichotomous malaria outcomes in bivariate analyses was assessed with Chi-square and Fisher's exact (two-tail) tests. For continuous covariates, we derived Pearson or Spearman correlations for normally and non-normally distributed variables, respectively. Either t-tests or Wilcoxon rank sum tests were used in bivariate analyses addressing between group differences for continuous normal and non-normal data, respectively. We further developed hierarchical logistic regression models in SAS version 9.3 (SAS Institute Inc.) to adjust for non-independence of responses ('clustering') within households. Generalized estimating equations with a log link function were employed. Covariates were selected for evaluation in models if they were associated with the outcome in bivariate analysis (cutoff for evaluation of $P \leq 0.20$). Covariates were retained in the final model if they were significant with $P \leq 0.10$.

Cluster analysis

A Kuldorff spatial scan statistic was obtained using the Bernoulli purely spatial model and SaTScan™ software (Martin Kuldorff; Harvard Medical School, Boston, MA, USA) to identify possible clustering of cases at the household level.[65,66] The software applies an infinite number of variably sized circular windows across the study area, with each circle representing a possible cluster. The observed prevalence of disease within the circle is compared to the expected prevalence across the entire study area by a likelihood ratio test to identify significant clusters of disease. The distribution and statistical significance of the clusters were explored by means of Monte Carlo replication of data sets under the null hypothesis with replications greater than 999 to ensure adequate power for defining clusters. Households that tested

negative during the cross-sectional survey were used as controls in the Bernoulli method.

Bednet distribution

To determine if bednets were clustered or randomly dispersed over a range of distances, multi-distance spatial cluster analysis (Ripley's *K* function) (ArcGIS spatial analytic tool) with boundary correction method (simulate_outer_boundary_values) to correct for underestimates near the edge was applied from 9 to 999 permutations. To calculate the density of features within a defined area, kernel density estimation was used. Based on the quadratic kernel function and a defined radius of 50 m, a kernel estimation surface was used to estimate the density of households with one or more bednets per 50 m radius cell, and the ratio of households with bednets to all households was calculated for each. A smoothed raster image of bednet distribution was generated in ArcMap v10.1 using the ratio of households with bednets to total households for each 100×100 m cell. Changing the kernel radius from 50 to 100 m did not significantly change the estimate of percent of households at each level of coverage.

Results

Study population

The August 2010 census in Wainyapu recorded 2678 individuals residing in 501 homes. Three different methods of home construction were recorded in the village with the majority (75%) classified as 'traditional' (open eaves, grass roofs, bamboo pole walls), and the other 16% and 9% classified as 'semi-traditional' (open eaves, corrugated metal roofs, pleated bamboo walls) and modern 'cement' (open eaves, cement block walls, corrugated metal roofs), respectively. The cross-sectional blood survey in November 2010 enrolled 960 (35.8%) randomly selected individuals from this population. The mean age of study subjects (20 years) did not significantly differ from the total village population ($P = 0.992$). There was no significant difference in gender representation ($P = 0.244$). Parasites were detected in 237 of 960 thick smears analyzed, yielding an unadjusted prevalence of 24.7%. *P. falciparum*, *P. vivax*, and mixed *P. falciparum*/*P. vivax* cases accounted for 57%, 27%, and 11% of infections, respectively. Two mixed *P. vivax*/*P. malariae* infections and eight focally clustered *P. malariae* cases comprised the remainder of the positive smears.

Larval survey

Two months before the cross-sectional parasitological survey, continuous larval surveillance was initiated and maintained on at least once a fortnight schedule, extending through the following dry season. This survey revealed that all five of the ground water features detailed in the methods sections were suitable and productive *An. sundaicus* larval habitats. Peak production at these sites occurred when there was plenty of floating algal mats for females to lay eggs and developing larvae to hide from potential predators. *An. sundaicus* larval were not detected in any other habitat, or in any natural or artificial containers, which is not surprising as these would have been unusual habitats for this species. *An. sundaicus* was typically proportionally less than other species (esp. *An. subpictus*, *An. annularis*) at these sites, except for the northernmost larval habitat where *An. sundaicus* was the predominant species. Although all but one of the five sites were productive year-round to varying degrees, the dry season did have an impact on mosquito production. The southernmost habitat would dry up completely, three others would struggle by the peak/end of the dry season, and the most northern site did not appear to be impacted a great deal. Interestingly, for the largest limestone sink with the emergent mangrove, very small pockets of water created by water buffalo hoof prints in the pond bed during the driest period were found to be perfect haven for anophelines, particularly *An. vagus* and *An. subpictus*. Most importantly, all five larval habitats were productive in the weeks preceeding the cross-sectional prevalence survey.

Cluster analysis

The Bernoulli circular spatial scan statistic identified a single spatial cluster of uncomplicated malaria cases (Fig. 2). In this area, the observed number of cases ($N = 93$) was $\times 1.7$ higher than expected ($N = 53.6$) (relative risk = 2.13; log likelihood ratio = 20.7; $P < 0.001$). The single spatial cluster of malaria was located in close proximity to numerous vector larval habitats. Hot spot analysis of malaria parasitemia scored at the household level using the Getis-Ord Gi∗ statistic (ArcGIS spatial analytic tool), independently predicted similar results as the circular scan statistic (See supplementary material 1 online for this article at www.maneyonline/doi/suppl/10.1179/2047773214Y.0000000167). Furthermore, dividing the village into ten distinct clusters resulted in a near linear decline in prevalence with increasing mean Euclidean distance (calculated from all surveyed individuals in each cluster) from the nearest confirmed larval habitat ($R^2 = 0.862$) (See supplementary material 2 online for this article at www.maneyonline/doi/suppl/10.1179/2047773214Y.0000000167).

Bivariate analyses

Results of bivariate analyses, adjusted for household level clustering of responses are shown in Table 1. Age, but not gender, was significantly associated with risk of parasitemia ($P < 0.001$), with a peak prevalence of 41.6% occurring in children 2 to less than 5 years of age. The mean household size was 5.6
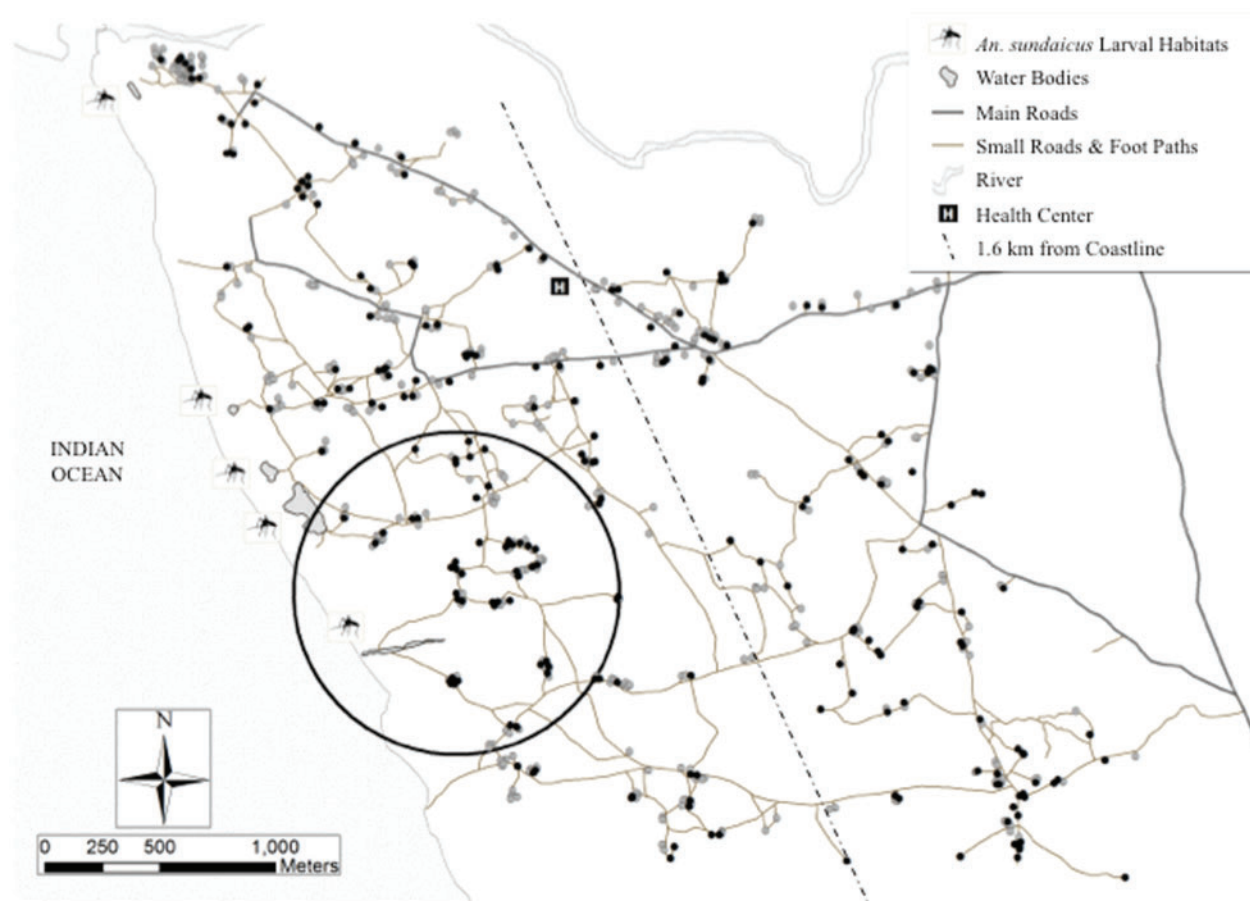
Figure 2   Map of the study village, Wainyapu. Dots denote individual households color-coded by survey participation, with black indicating ≥1 person from household participating in the August 2010 cross-sectional survey (gray households were not surveyed). Circle denotes statistically significant space cluster of malaria parasitemia using Bernoulli purely spatial model (SaTScan^TM software) (relative risk = 2.13; *P* < 0.001).

people (range: 1–16), and the total number of family members (calculated from census, not from study participants) was also significantly associated with risk of infection [odds ratio (OR) = 2.38; 95% confidence interval (CI) = 1.4–4.06; *P* = 0.002] both as a continuous and dichotomous (<6 versus ≥6) variable. Increasing household distance from the nearest confirmed *An. sundaicus* larval habitat (mean = 1366 m, SD = 854, *N* = 229 households) was associated with a declining gradient of prevalence (*P* < 0.001). To capture village wide spatial heterogeneity in prevalence, distance for each household to closest known *An. sundaicus* larval habitat was stratified as < or ≥1600 m (approximately dividing the village in two on a parallel axis from the coastline), with further stratification of this proximal band into <700 m versus 700–1600 m. The likelihood of parasitemia was found to be 4.3-fold higher for those who resided within 1600 m from the closest confirmed *An. sundiacus* larval habitat compared to further distances (95% CI = 3.01–6.23; *P* < 0.001), with no significant difference observed upon further stratification of this proximal 1600 m band into <700 m versus 700–1600 m (OR = 0.75; 95% CI = 0.36–1.58; *P* = 0.58). Increasing distance from a

village health-care facility was associated with a significantly increased likelihood of parasitemia (OR = 1.9, 95% CI = 1.14–3.33, *P* = 0.011) using bivariate analysis. Distance to the closest cement water cistern (See supplementary material 3 online for this article at www.maneyonline/doi/suppl/10.1179/2047773214Y.0000000167) dichotomized as less than or greater than 150 m was not associated with risk of parasitemia (OR = 0.74; 95% CI = 0.44–1.24; *P* = 0.29).

One or more bednets were found installed in 33% of homes. The primary source of bednets was either purchase from market or the local health clinic that would distribute them primarily to pregnant women at no cost. Overall condition of bednets was fair, with 25% of bednets in new condition, 43% with 1–5 holes ('hole' defined as more than 0.5 cm diameter defect), and the remaining 33% with either >5 holes or badly damaged. Computed *K*-function values showed that the distribution of households owning bednets across the 22 km^2 area was significantly more clustered than would be expected from a random distribution of points (See supplementary material 4 online for this article at www.maneyonline/doi/suppl/10.1179/2047773214Y.0000000167). Using a kernel estimation

surface to calculate the density of households with one or more bednets per 50 m radius cell, the highest concentration of bednets was found in the northern half of the study site (Fig. 3). The presence of one or more bednets in a home was not associated with the likelihood of infection after controlling for confounders (OR = 1.18; 95% CI = 0.68–2.07; *P* = 0.573), and there was no association between malaria risk and wall (*P* = 0.372) or roof construction material of the house (*P* = 0.408).

The mean hemoglobin level in parasitemic children less than 10 years of age (10.62 g/dl; 95% CI = 10.33–10.91; *N* = 121) was 0.73 g/dl less than non-parasitemic children of similar age (11.35 g/dl; 95% CI = 11.13–11.56, *N* = 217), a difference that was statistically significant (*P* = 0.001). After controlling for age and sex, hemoglobin levels in all individuals who resided greater than 1600 m from the closest *An. sundaicus* larval habitat was significantly higher than those who resided closer (OR = 1.6; 95% CI = 1.30–1.98; *P* < 0.001).

Multivariate analysis

Hierarchical multivariate logistic regression analysis adjusted for household clustering of responses demonstrated two variables that remained significantly associated with patent parasitemia after adjustment for potential confounders such as age and gender (Table 2). Distance to larval habitats was a strong predictor of risk of infection with a significant reduction of risk when greater than 1.6 km from the closest confirmed *An. sundaicus* larval habitat. Distance from the local health-care facility, along with the number of family members per

Table 1 Bivariate analysis of potential risk factors for patent parasitemia (any species), in 960 individuals residing in 229 study households, Wainyapu, Indonesia, 2010

Variable	*n*	Cases (%)*	Odds ratio[†]	95% CI[‡]	*P* value[§]
Individual					
Age group (years)					
<2	64	14 (21.9)	1.0	…	…
2–<5	125	52 (41.6)	2.54	1.27–5.08	0.01
5–<10	167	61 (36.5)	2.06	1.05–4.02	0.04
10–<20	226	71 (31.4)	1.64	0.85–3.15	0.16
20+	378	39 (10.3)	0.41	0.21–0.81	0.01
Age (continuous)	960	237 (24.7)	…	…	<0.001
Gender					
Male	466	116 (24.9)	1.0	…	…
Female	494	121 (24.5)	0.98	0.73–1.31	0.29
Household					
Mosquito net					
None	152	81 (53.3)	1.0	…	…
≥1 mosquito net	77	44 (57.1)	1.17	0.67–2.03	0.58
Distance to *An. sundaicus*					
Larval habitats (m)					
<700	67	50 (74.6)	1.0	…	…
700–1600	74	51 (68.9)	0.75	0.36–1.58	0.58
>1600	88	24 (27.3)	0.13	0.06–0.26	<0.001
Distance to cement water					
Cisterns (m)					
<150	113	66 (58.4)	1.0	…	…
≥150	116	59 (50.9)	0.74	0.44–1.24	0.29
Distance to health					
Facility (m)					
<1600	137	84 (61.3)	1.0	…	…
≥1600	92	41 (44.6)	1.9	1.14–3.33	0.014
Home construction					
Materials					
Roof					
Grass	177	94 (53.1)	1.0	…	…
Metal sheet	52	31 (59.6)	1.3	0.7–2.44	0.43
Wall					
Cement	22	14 (63.6)	1.0	…	…
Bamboo	207	111 (53.6)	0.66	0.27–1.64	0.5
Household members					
<6 residents	112	49 (43.8)	1.0	…	…
≥6 residents	117	76 (65.0)	2.38	1.4–4.06	0.002

Note: *Numeric values represent number of individuals (proportion of each group) with parasitemia due to any of the three *Plasmodium* parasites of humans detected by conventional light microscopy. For household level data, numeric values represent number of households (proportion of each group) with at least one person with parasitemia.
[†]The likelihood of parasitemia versus non-parasitemia compared to the referent.
[‡]95% CI = 95% confidence interval adjusted for within household clustering of cases.
[§]The *p*-value is Fisher's exact (two-tailed) Chi-square for categorical variables and *t*-test for age as continuous variable. The mean age of parasitemic individuals was 12.05 years (95% CI = 9.92–14.19 years) versus 22.4 years (95% CI = 21.19–23.64 years) for non-parasitemic individuals.
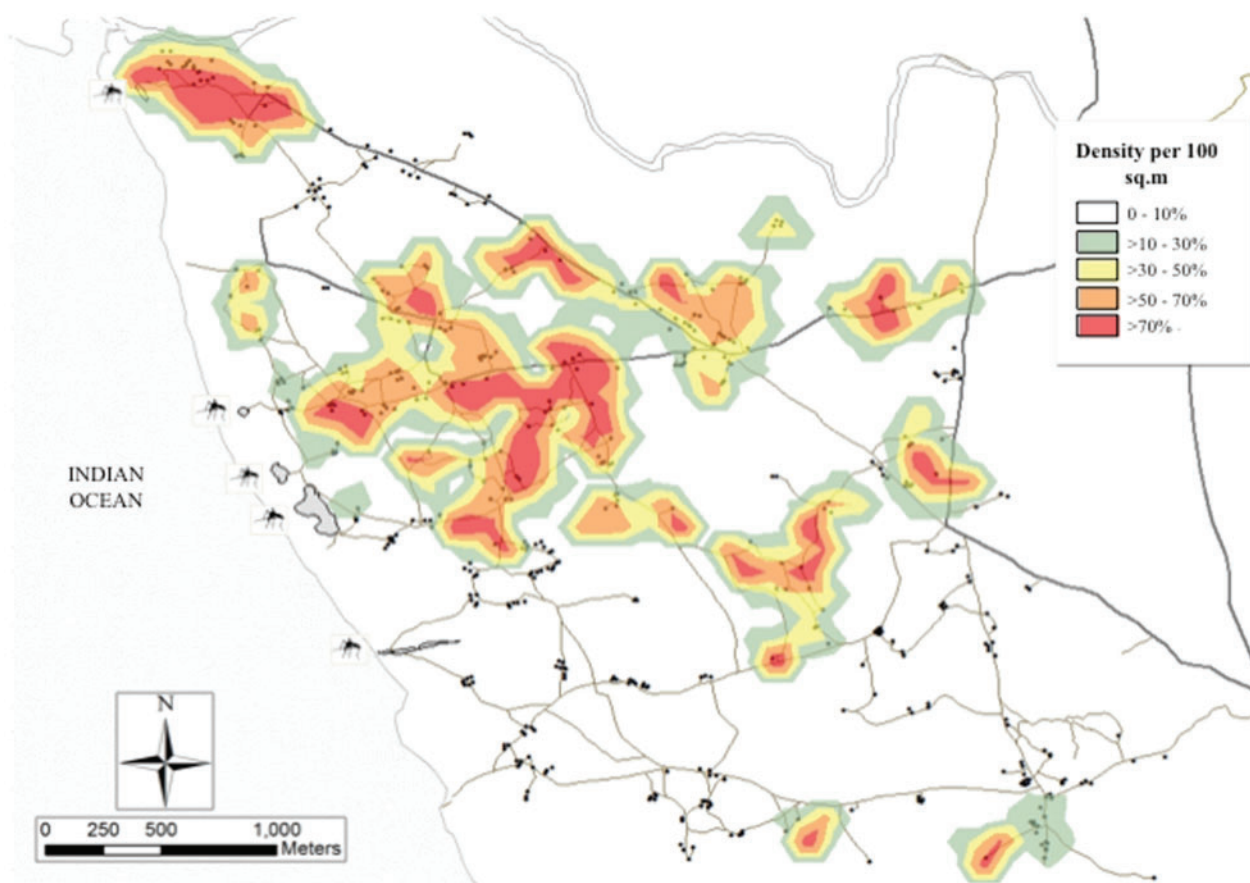
Figure 3 Spatial distribution of household-level bednet coverage in Wainyapu. Coverage raster represents the corresponding percentage of households with ≥1 bednet per 100 m². Areas with 0–10%, >10–30%, >30–50%, >50–70%, and >70+ community coverage are shown in white, green, yellow, orange, and red, respectively. *Anopheles* vector larval habitats (squares with mosquitoes), footpaths (fine lines), main roads (darker lines), and village households (black dots) are depicted.

household, failed to retain significance in multivariate models, suggesting that this relationship was likely confounded by distance to larval habitats (Fig. 2). Risk of infection was significantly associated with age, with the highest infection prevalence in the 2–<5 year old age group.

Table 2 Hierarchical multivariate logistic regression model of significant variables associated with risk of parasitemia in Wainyapu, Indonesia

Variable*	Odds ratio†	95% CI‡	P value
Age	…	…	<0.001
Distance to *An. sundaicus* Larval habitats (m)			
<1600 m	1.0	…	…
≥1600 m	0.21	0.14–0.32	<0.001
Distance to health Facility (m)			
<1600 m	1.0	…	…
≥1600 m	1.0	0.99–1.01	0.95
Number of household Members			
<6 residents	1.0	…	…
≥6 residents	1.0	0.93–1.09	0.85

Note: *Age modeled as a continuous variable.
†Odds ratio adjusted for household clustering of responses.
‡95% CI = 95% confidence interval.

Discussion

This study analyzed spatial heterogeneity in malaria transmission in a hypo- to meso-endemic region of Southwest Sumba District, East Nusa Tenggara Province, Indonesia, to facilitate individual and household level risk factor identification. Household proximity to the five identified and confirmed *Anopheles sundaicus* larval habits was a dominant factor predicting risk of acquiring malaria infection, with a strong, near linear decline in prevalence with increasing Euclidean distance from the coastline ($R^2 = 0.862$). Cluster analysis was performed to identify spatial hot spots of transmission, with a single significant cluster identified in close proximity to several productive vector larval habitat sites. Hemoglobin level analysis after adjusting for confounders indicated significantly higher hemoglobin levels across age strata further away from the larval habitats, consistent with the observed spatial heterogeneity in uncomplicated malaria infections.

A number of prior studies have utilized cluster analysis to identify spatial and temporal foci of malaria transmission.[67–70] The circular scan statistic used in this study has been previously validated for plotting and understanding local malaria space–time

clusters,[71–74] and has proven useful in targeting control strategies in areas such as the Kenyan highlands.[75] Particularly well suited to local risk factor assessment and identification, the spatial scan statistic is useful to detect clusters, but the fixed scan window can limit detection of irregularly shaped clusters.[76,77]

A survey of *Anopheles* mosquitoes in western Sumba in 2007 in both upland interior and coastal sites captured and identified numerous potential disease vectors.[59] The predominant species from evening human landing collections (mosquitoes per human) were *An. subpictus* and *An. vagus* in the upland interior and *An. sundaicus* along the coast. These findings were recapitulated in the recently completed companion spatial repellency study.[60] Village transect studies indicated that flight distances of coastal vectors were short (approximately 300 m), predicting a relatively higher concentration of mosquitoes closer to the coast. Although larval sampling revealed the presence of *An. sundaicus* in the coastal habitats, lesser potential malaria vectors such as *An. subpictus* and *An. vagus* were generally much more prevalent overall. Conversely, subsequent studies involving the capture of adult mosquitoes found the overwhelming majority of human-biting species to be *An. sundaicus*.[60]

Computed *K*-function values showed that the distribution of households owning bednets was significantly more clustered than would be expected from a random distribution of points. Although the presence of one or more bednets was not significantly associated with risk of parasitemia, it is likely that overall poor bednet condition, frequency of use, and unequal distribution mitigated the efficacy of this proven cost-effective prophylactic measure.[78,79] The strength of this study is that bednet ownership was assessed for every single home by a fieldworker, avoiding self-reporting of bednets which can be biased if the absence of bednets is perceived as a means for receiving a new or additional bednets. However, the ability of this study to extrapolate the results to predict protection was limited by the fact that we did not distinguish between treated and untreated bednets, or estimate bednet usage on an individual level. Bednet ownership coverage also appeared to be influenced by household location in one of the four village sub-districts (See supplementary material 2 online for this article at www.maney online/doi/suppl/10.1179/2047773214Y.0000000167) potentially exposing socioeconomic variability that was not captured in this study.

Overlay of the bednet distribution map with the cluster analysis, suggests that poor bednet coverage in conjunction with proximity to vector larval sites may have synergized to produce the single significant spatial cluster of malaria. However, functional inequality in breeding site productivity and longevity may have played contributory roles as well. Two of the breeding sites were either markedly reduced in size or became dry during the height and end of the dry season or flooded during the peak of the rainy season, highlighting potential breeding site variability not captured in the cross-sectional nature of the study design. Nevertheless, 2 weeks before the cross-sectional parasitological survey, all five *An. sundaicus* larval habitats were productive, likely contributing to the observed spatial heterogeneity in risk of parasitemia.

In general, children less than 5 years old had the highest frequencies of parasitemia, pointing to age-dependent determinants of protection that may or may not be the product of cumulative exposure to infection during life.[80,81] Increasing household distance to a health facility, a known risk factor for malaria,[82,83] was also identified as a significant risk factor for patent parasitemia in bivariate analysis. However, this relationship failed to retain significance in the multivariate model, likely confounded by the distance to larval habitats. Alternatively, the relatively small square area of the Wainyapu study site, made long travel times or lack of transportation to the health facility unlikely explanations for the observed spatial gradient of infection (Fig. 2). Additional risk factors not recorded such as presence/absence or proximity to domestic and penned livestock may have also contributed to malaria risk. Preliminary analysis of two red blood cell polymorphisms prevalent in the study population, glucose 6-phosphate dehydrogenase deficiency (G6PD) and Southeast Asian ovalocytosis (SAO), suggest that the latter may modulate risk of uncomplicated infection due to *P. vivax* in this population (See supplementary material 4 online for this article at www.maneyonline/doi/suppl/10.1179/2047773214Y.0000000167), a finding that is in keeping with a recent report of reduced risk of *P. vivax* malaria in Papua New Guinean children with SAO.[84]

In summary, a relatively high risk of malaria was linked to proximity to very specific and few (e.g. five) *Anopheles sundaicus* larval habitats in our study. Before the advent of indoor residual spraying with dichloro-diethyl-trichloro-ethane in the mid-twentieth century, larval source management through environmental interventions was a highly effective malaria control method employed insuch areas as the Panama Canal Zone[85,86] and peninsular Malaysia.[87] These early successes bolstered the concept of species sanitation[87,88] that was widely applied to great effect in other malaria endemic regions including Indonesia (then called Netherlands Indies) between 1920 and 1935.[88,89] The principle of species sanitation is

straightforward: identify the dominant vector, identify its preferred larval sites, and eliminate those sites by whatever means possible. Although simple in principle, larval source management can be complex, requiring an in-depth understanding of local malaria epidemiology, entomology, and vector ecology to be effective.[90,91] In our study, we have refined the approach of the pioneers of species sanitation through the application of modern GPS and GIS to facilitate vector larval habitat identification by proximity/risk analysis. Our findings illustrate and inform the key question of any species sanitation operation: where are the sites causing the most malaria? The answer to that question directly guides the location and nature of measures to be taken to reduce malaria transmission.

Disclaimer Statements

Acknowledgements

References

1 World Health Organization. World malaria report 2012. Geneva: WHO; 2012. Available from: http://www.who.int/malaria/publications/world_malaria_report_2012/report/en/index.html

2 Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present, and future. Lancet Infect Dis. 2004;4:327–36.

3 Nyarango PM, Gebremeskel T, Mebrahtu G, Mufunda J, Abdulmumini U, Ogbamariam A, et al. A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004: the effect of combination of control methods. Malar J. 2006;5:33.

4 Ceesay Sj, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJ, et al. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. Lancet. 2008;372:1545–54.

5 WHO. Impact of long-lasting insecticidal-treated nets (LLINs) and artemisinin-based combination therapies (ACTs) measured using surveillance data, in four African countries. Geneva: World Health Organization Global Malaria Programme; 2008.

6 O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, et al. Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet. 2008;372:1555–62.

7 O'Meara WP, Mwangi TW, Williams TN, McKenzie FE, Snow RW, Marsh K. Relationship between exposure, clinical malaria, and age in an area of changing transmission intensity. Am J Trop Med Hyg. 2008;79:185–91.

8 Behrens RH, Carroll B, Beran J, Bouchaud O, Hellgren U, Hatz C, et al. The low and declining risk of malaria in travelers to Latin America: is there still an indication for chemoprophylaxis. Malar J. 2007;6:114.

9 Geiger C, Agustar HK, Compaore G, Coulibay B, Sie A, Becher H, et al. Declining malaria parasite prevalence and trends of asymptomatic parasitaemia in a seasonal transmission setting in north-western Burkina Faso between 2000 and 2009–2012. Malar J. 2013;12:27.

10 Sutcliffe CG, Kobayashi T, Hamapumbu H, Shields T, Kamanga A, Mharakurwa S, et al. Changing individual-level risk factors for malaria with declining transmission in southern Zambia: a cross-sectional study. Malar J. 2011;10:324.

11 Meyrowitsch DW, Pedersen EM, Alifrangis M, Scheike TH, Malecela MN, Magesa SM, et al. Is the current decline in malaria burden in sub-Saharan Africa due to a decrease in vector population? Malar J. 2011;10:188.

12 Sievers AC, Lewey J, Musafiri P, Franke MF, Bucyibaruta BJ, Stulac SN, et al. Reduced paediatric hospitalizations for malaria and febrile illness patterns following implementation of community-based malaria control programme in rural Rwanda. Malar J. 2008;7:167.

13 Delacollette C, D'Souza C, Christophel E, Thimasarn K, Abdur R, Bell D, et al. Malaria trends and challenges in the Greater Mekong Subregion. Southeast Asian J Trop Med Public Health. 2009;40(4):674–91.

14 Teklehaimanot HD, Teklehaimanot A, Kiszewski A, Rampao HS, Sachs JD. Malaria in São Tomé and principe: on the brink of elimination after three years of effective antimalarial measures. Am J Trop Med Hyg. 2009;80:133–40.

15 Available from: http://www.prb.org/pdf12/2012-population-data-sheet_eng.pdf

16 Elyazar IR, Gething PW, Patil AP, Rogayah H, Kusriastuti R, Wismarini DM, et al. Plasmodium falciparum malaria endemicity in Indonesia in 2010. PLoS One. 2011;6:e21315.

17 Elyazar IR, Gething PW, Patil AP, Rogayah H, Sariwati E, Palupi NW, et al. Plasmodium vivax malaria endemicity in Indonesia in 2010. PLoS One. 2012;7:e37325.

18 Elyazar IR, Hay SI, Baird JK. Malaria distribution, prevalence, drug resistance and control in Indonesia. Adv Parasitol. 2011;74:41–175.

19 Departemen Kesehatan, Keputusan Menteri Kesehatan Republik Indonesia Nomor 293/MENKES/SK/IV/2009 28 April 2009. Tentang Eliminasi Malaria di Indonesia. Jakarta: Direktorat Pemberantasan Penyakit Bersumber Binatang, Departemen Kesehatan Indonesia; 2009. p. 31.

20 Global Malaria Programme. Malaria elimination: a field manual for low and moderate endemic countries. Geneva: World Health Organization; 2007. p85.

21 Oesterholt MJ, Bousema JT, Mwerinder OK, Harris C, Lushino P, Masokoto A, et al. Spatial and temporal variation

in malaria transmission in a low endemicity area in northern Tanzania. Malar J. 2006;5:98.

22 Greenwood BM. The microepidemiology of malaria and its importance to malaria control. Trans R Soc Trop Med Hyg. 1989;83(Suppl):25–9.

23 Creasey A, Giha H, Hamad AA, El Hassan IM, Theander TG, Arnot DE. Eleven years of malaria surveillance in a Sudanese village highlights unexpected variation in individual disease susceptibility and outbreak severity. Parasitology. 2004;129: 263–71.

24 Sintasath DM, Ghebremeskel T, Lynch M, Kleinau E, Bretas G, Shililu J, *et al.* Malaria prevalence and associated risk factors in Eritrea. Am J Trop Med Hyg. 2005;72:682–7.

25 Branch OL, Casapia WM, Gamboa DV, Hernandez JN, Alava FF, Roncal N, *et al.* Clustered and local transmission and asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* malaria infections in a recently emerged, hypoendemic Peruvian Amazon community. Malar J. 2005;4:27.

26 Lindsay SW, Snow RW. The trouble with eaves; house entry by vectors of malaria. Trans R Soc Med Hyg. 1988;82:645–6.

27 Rogier C, Trape JF. Malaria attack in children exposed to high transmission: who is protected? Trans R Soc Trop Med Hyg. 1993;87:245–6.

28 Clark TD, Greenhouse B, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Staedke SG, *et al.* Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda. J Infect Dis. 2008;198:393–400.

29 Mockenhaupt FP, Ehrhardt S, Gellert S, Otchwemah RN, Dietz E, Anemana SD, *et al.* Alpha$^+$-thalassemia protects African children from severe malaria. Blood. 2004;104:2003–6.

30 Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, *et al.* Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature. 1995;376(6537):246–9.

31 Allen SJ, O'Donnell A, Alexander ND, Mgone CS, Peto TE, Clegg JB, *et al.* Prevention of cerebral malaria in children in Papua New Guinea by Southeast Asian ovalocytosis band 3. Am J Trop Med Hyg. 1999;60:1056–60.

32 Driss A, Hibbert JM, Wilson NO, Iqbal SA, Adamkiewicz TV, Stiles JK. Genetic polymorphisms linked to susceptibility to malaria. Malar J. 2011;10:271.

33 Griffiths MJ, Shafi MJ, Popper SJ, Hemingway CA, Kortok MM, Wathen A, *et al.* Genomewide analysis of the host response to malaria in Kenyan children. J Infect Dis. 2005;191:1599–611.

34 Ockenhouse CF, Hu WC, Kester KE, Cummings JF, Stewart A, Heppner DG, *et al.* Common and divergent immune response signaling pathways discovered in peripheral blood mononuclear cell gene expression pattens in presymptomatic and clinically apparent malaria. Infect Immun. 2006;74:5561–73.

35 Carter R, Mendis KN, Roberts D. Spatial targeting of interventions against malaria. Bull World Health Organ. 2000;78:1401–11.

36 Ghebreyesus TA, Hailie M, Witten KH, Getachew A, Yohannes M, Lindsay SW, *et al.* Household risk factors for malaria among children in the Ethiopian highlands. Trans R Soc Trop Med Hyg. 2000;94:17–21.

37 Thomas CJ, Lindsay SW. Local-scale variation in malaria infection amongst rural Gambian children estimated by satellite remote sensing. Trans R Soc Trop Med Hyg. 2000;94:159–63.

38 Ernst KC, Adoka SO, Kowuor DO, Wilson ML, John CC. Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. Malar J. 2006;5:78.

39 Staedke SG, Nottingham EW, Cox J, Kamya MR, Rosenthal PJ, Dorsey G. Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children. Am J Trop Med Hyg. 2003;69:244–6.

40 Fillinger U, Sombroek H, Majambere S, van Loon E, Takken W, Lindsay SW. Identifying the most productive breeding sites for malaria mosquitoes in The Gambia. Malar J. 2009;8:62.

41 Kreuels B, Kobbe R, Adjei S, Kreuzberg C, von Reden C, Bäter K, *et al.* Spatial variation of malaria incidence in young children from a geographically homogeneous area with high endemicity. J Infect Dis. 2008;197:85–93.

42 Gamage-Mendis AC, Carter R, Mendis C, de Zoysa AP, Herath PR, Mendis KN. Clustering of malaria infections within an endemic population: risk of malaria associated with the type of housing construction. Am J Trop Med Hyg. 1991;45:77–85.

43 Molineaux L, Gramiccia G. The Garki Project. Research on the epidemiology and control of malaria in the Sudan savannah of West Africa. Document 78/4320/1-6000. Geneva: World Health Organization; 1980.

44 Bousema T, Griffin JT, Sauerwein RW, Smith, DL, Churcher TS, Takken W, *et al.* Hitting hotspots: spatial targeting of malaria for control and elimination. PLoS Med. 2012; 9:e1001165.

45 Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, *et al.* Operational strategies to achieve and maintain malaria elimination. Lancet. 2010;376(9752):1592–603.

46 Reid H, Vallely A, Taleo G, Tatem AJ, Kelly G, Riley I, *et al.* Baseline spatial distribution of malaria prior to an elimination programme in Vanuatu. Malar J. 2010;9:150.

47 Kazembe L, Kleinschmidt I, Holtz T, Sharp B. Spatial analysis and mapping of malaria risk in Malawi using point-referenced prevalence of infection data. Int J Health Geogr. 2006;5:41.

48 Brooker S, Leslie T, Kolaczinski K, Mohsen E, Mehboob N, Saleheen S, *et al.* Spatial epidemiology of *Plasmodium vivax*, Afghanistan. Emerg Infect Dis. 2006;12:1600–2.

49 Noor Am, Clements A, Gething PW, Moloney G, Borle M, Shewchuk T, *et al.* Spatial prediction of *Plasmodium falciparum* prevalence in Somalia. Malar J. 2008;7:159.

50 Gosoniu L, Vounatsou P, Sogoba N, Maire N, Smith T. Mapping malaria risk in West Africa using a Bayesian nonparametric non-stationary model. Comput Stat Data Anal. 2009;53:3358–71.

51 Kleinschmidt I, Omumbo J, Briet O, van de Giesen N, Mensah NK, Windmeijer P, *et al.* An empirical malaria distribution map for West Africa. Trop Med Int Health. 2001;6(10):779–86.

52 Gemperli A, Sogoba N, Fondjo E, Mabaso M, Bagayoko M, Briet OJT, *et al.* Mapping malaria transmission in West and Central Africa. Trop Med Int Health. 2006;11:1032–46.

53 Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, *et al.* A world malaria map: *Plasmodium falciparum* endemicity in 2007. PLoS Med. 2009;6:e1000048.

54 Guerra C, Gikandi P, Tatem A, Noor A, Smith D, Hay S, *et al.* The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. PLoS Med. 2008;5:e38.

55 Bousema T, Drakeley C, Gesase S, Hashim R, Magesa S, Mosha F, *et al.* Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis. 2010;201:1764–74.

56 Bejon P, Williams TN, Liljander A, Noor AM, Wambua J, Liljander A, *et al.* Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. PloS Med. 2010;7: e1000304.

57 Syafruddin D, Krisin, Asih P, Sekartuti, Dewi RM, Coutrier F, *et al.* Seasonal prevalence of malaria in West Sumba District, Indonesia. Malar J. 2009;8:8.

58 Asih PB, Dewi RM, Sekartuti, Sadikin M, Sumarto W, Sinaga B, *et al.* Efficacy of artemisinin-based combination therapy for uncomplicated falciparum malaria in West Sumba district, East Nusa Tenggara Province, Indonesia and the genotypic profiles of the parasite. Am J Trop Med Hyg. 2009;80:914–8.

59 Barbara KA, Sukowati S, Rusmiarto S, Susapto D, Bangs MJ, Kinzer MH. Survey of *Anopheles* mosquitoes (Diptera: Culicidae) in West Sumba District, Indonesia. Southeast Asian J Trop Med Public Health. 2011;42:71–82.

60 Syafruddin D, Bangs MJ, Sidik D, Elyazar I, Asih PBS, Nurlela S, *et al.* Impact of the spatial repellent on malaria incidence in two villages in Sumba, Indonesia. Am J Trop Med Hyg. 2014;91:1079–87.

61 Dusfour I, Blondeau J, Harbach RE, Vythilingam I, Baimai V, Trung HD, *et al.* Polymerase chain reaction identification of three members of the *Anopheles sundaicus* (Diptera: Culicidae) complex, malaria vectors in Southeast Asia. J Med Entomol. 2007;44:723–31.

62 Garmin Ltd. GPSMAP® 60CSx with sensors & maps. Olathe, KS: Garmin Ltd.

63 ESRI. ArcGIS 10.0 of Arc View, ArcMap, ArcCatalog, ArcToolbox and spatial analyst user's guide. Redlands, CA: ESRI.

64 Atmosoedjono S, Bangs MJ. Illustrated key to the *Anopheles* of Indonesia (O'Connor CT, Soepanto A, 1979): an English translation and revision. Jakarta: Ministry of Health; 1989. p. 1–40.

65 Kulldorff M. A spatial scan statistic. Commun Stati Theor Method. 1997;26:1481–96.

66 Kulldorff M, Nagarwalla N. Spatial disease clusters — detection and inference. Stat Med. 1995;14:799–810.

67 Gaudart J, Poudiougou B, Dicko A, Ranque S, Toure O, Sagara I, et al. Space–time clustering of childhood malaria at the household level: a dynamic cohort in a Mali village. BMC Public Health. 2006;6:286.

68 Winskill P, Rowland M, Mtove G, Malima R, Kirby M. Malaria risk factors in north-east Tanzania. Malar J. 2011;10:98.

69 Coleman M, Coleman M, Mabuza A, Kok G, Coetzee, Durrheim D. Using the SaTScan method to detect local malaria clusters for guiding malaria control programmes. Malar J. 2009;8:68.

70 Brooker S, Clarke S, Njagi JK, Polack S, Mugo B, Estambale B, et al. Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. Trop Med Int Health. 2004;9:757–66.

71 Boscoe F, McLaughlin C, Schymura M, Kielb C. Visualization of the spatial scan statistic using nested circles. Health Place. 2003;9:273–7.

72 Kulldorf M, Feuer EJ, Miller BA, Freeman LS. Breast cancer in northeastern United States: a geographical analysis. Am J Epidemiol. 1997;146:161–70.

73 Nkhoma ET, Hsu CE, Hunt VI, Harris AM. Detecting spatiotemporal clusters of accidental poisoning mortality among Texas counties, United States, 1980–2001. Int J Health Geogr. 2004;3:25.

74 Odoi A, Martin SW, Michel P, Middleton D, Holt J, Wilson J. Investigaton of clusters of giardiasis using GIS and a spatial scan statistic. Int J Health Geogr. 2004;3:11.

75 Bostoen K, Chalabi Z. Optimization of household survey sampling without sample frames. Int J Epidemiol. 2006;35:751–5.

76 Aamodt G, Samuelson SO, Skrondal A. A simulation study of three methods for detecting disease clusters. Int J Health Geogr. 2006;5:15.

77 Patil GP, Taille C. Geographic and network surveillance via scan statistics for critical area detection. Stat Sci. 2003;18:457–65.

78 Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database of Systematic Reviews 2004. Chichester: John Wiley & Sons, Ltd; 2004.

79 Noor A, Mutheu J, Tatem A, Hay S, Snow R. Insecticide-treated net coverage in Africa: mapping progress in 2000–2007. Lancet. 2009;373(9657):58–67.

80 Doolan D, Dobano C, Baird JK. Acquired immunity to malaria. Clin Microbiol Rev. 2009;22:13–36.

81 Kurtis JD, Mtalib R, Onyango FK, Duffy PE. Human resistance to *Plasmodium falciparum* increases during puberty and is predicted by dehydroepiandrosterone sulfate levels. Infect Immun. 2001;69:123–8.

82 Lawpoolsri S, Chavez I, Yimsamran S, Puangsa-art S, Thanyavanich N, Maneeboonyang W, et al. The impact of human reservoir of malaria at a community-level on individual malaria occurrence in a low malaria transmission setting along the Thai–Myanmar border. Malar J. 2010;9:143.

83 Lowassa A, Magizo HD, Mahande AM, Mwang'onde BJ, Msangi S, Mahande MJ, et al. Social economic factors and malaria transmission in Lower Moshi, northern Tanzania. Parasite Vectors. 2012;5:129.

84 Rosanas-Urgell A, Lin E, Manning L, Rarau P, Laman M, Senn N, et al. Reduced risk of *Plasmodium vivax* malaria in Papua New Guinean children with Southeast Asian ovalocytosis in two cohorts and a case–control study. PLoS Med. 2012;9:e1001305.

85 Ross R. An address on the prevention of malaria in British possessions, Egypt, and parts of America. Lancet. 1907;2:879.

86 Chamberlain WP. Twenty-five years of American medical activity on the Isthmus of Panama 1904–1929; a triumph of preventive medicine. Mount Hope: Panama Canal Press; 1929.

87 Watson M. The prevention of malaria in the Federated Malay States: a record of 20 years' progress. London: John Murray; 1921.

88 Takken W, Snellen WB, Verhave JP, Knols BCJ, Atmosoedjono S. Environmental measures for malaria control in Indonesia: an historical review on species sanitation. Wageningen: Wageningen Agricultural University; 1990. Wageningen Agricultural Papers 90.7.

89 Bradley DJ. Watson, Swellengrebel and species sanitation: environmental and ecological aspects. Parassitologia. 1994;36:137.

90 Fillinger U, Kannady K, William G, Vanek MJ, Dongus S, Nyika D, et al. A tool box for operational mosquito larval control: preliminary results and early lessons from the urban malaria control program in Dar es Salaam, Tanzania. Malar J. 2008;7:e20.

91 Vanek MJ, Shoo B, Mtasiwa D, Kiama M, Lindsay SW, Fillinger U, et al. Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania. BMC Public Health. 2006;6:154.