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

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**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
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 falciparum16 and Plasmodium vivax17 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 200919 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 polymorphisms27–31 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 cattle43 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 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 soadicus 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...
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). Species distribution by habitat 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. 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), as yet an undescribed (formally named) species in the Sundicus complex. 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. 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. Integrated household and vector breeding site distribution maps were produced and analyzed in a Geographic Information Systems (GIS) database 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, Watboro, 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 6 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/ml assuming a WBC count of 8000/ml. 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 306 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 Kulldorff spatial scan statistic was obtained using the Bernoulli purely spatial model and SaTScan™ software (Martin Kulldorff; 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_outside_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 1006 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 preceding 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 6 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
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 \( \geq 6 \) ) variable. Increasing household distance from the nearest confirmed \textit{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 \textit{An. sundaicus} larval habitat was stratified as < or \( \leq 1600 \) 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 \textit{An. sundaicus} 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/supp/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/supp/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 \textit{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 \textit{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, Walnyapu, Indonesia, 2010

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group (years)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>64</td>
<td>14 (21.9)</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>2–&lt;5</td>
<td>125</td>
<td>52 (41.6)</td>
<td>2.54</td>
<td>1.27–5.08</td>
</tr>
<tr>
<td>5–&lt;10</td>
<td>167</td>
<td>61 (36.5)</td>
<td>2.06</td>
<td>1.65–4.02</td>
</tr>
<tr>
<td>10–&lt;20</td>
<td>226</td>
<td>71 (31.4)</td>
<td>1.64</td>
<td>0.85–3.15</td>
</tr>
<tr>
<td>&gt;20</td>
<td>378</td>
<td>39 (10.3)</td>
<td>0.41</td>
<td>0.21–0.81</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>960</td>
<td>257 (26.4)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>466</td>
<td>116 (24.9)</td>
<td>0.98</td>
<td>0.73–1.31</td>
</tr>
<tr>
<td>Female</td>
<td>494</td>
<td>121 (24.5)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Household</td>
<td></td>
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</tr>
<tr>
<td>Mosquito net</td>
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<td></td>
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<tr>
<td>None</td>
<td>152</td>
<td>81 (53.3)</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>$\leq$ 1 mosquito net</td>
<td>77</td>
<td>44 (57.1)</td>
<td>1.17</td>
<td>0.67–2.03</td>
</tr>
<tr>
<td>Distance to \textit{An. sundaicus} Larval habitats (m)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;700</td>
<td>67</td>
<td>50 (74.6)</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>700–1600</td>
<td>74</td>
<td>51 (68.9)</td>
<td>0.75</td>
<td>0.36–1.58</td>
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<tr>
<td>&gt;1600</td>
<td>88</td>
<td>24 (27.3)</td>
<td>0.13</td>
<td>0.06–0.26</td>
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<tr>
<td>Distance to cement water</td>
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<td>Cisterns (m)</td>
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<tr>
<td>&lt;150</td>
<td>113</td>
<td>66 (58.4)</td>
<td>1.0</td>
<td>...</td>
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<tr>
<td>$\leq$ 150</td>
<td>116</td>
<td>59 (50.9)</td>
<td>0.74</td>
<td>0.44–1.24</td>
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<tr>
<td>Distance to health Facility (m)</td>
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<tr>
<td>&lt;1600</td>
<td>137</td>
<td>84 (61.3)</td>
<td>1.0</td>
<td>...</td>
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<tr>
<td>$\leq$ 1600</td>
<td>142</td>
<td>41 (44.6)</td>
<td>1.9</td>
<td>1.14–3.33</td>
</tr>
<tr>
<td>Home construction</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Materials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roof</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass</td>
<td>177</td>
<td>94 (53.1)</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>Metal sheet</td>
<td>52</td>
<td>31 (59.6)</td>
<td>1.3</td>
<td>0.7–2.44</td>
</tr>
<tr>
<td>Wall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cement</td>
<td>22</td>
<td>14 (63.6)</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>Bamboo</td>
<td>207</td>
<td>111 (53.6)</td>
<td>0.66</td>
<td>0.27–1.64</td>
</tr>
<tr>
<td>Household members</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$\leq$ residents</td>
<td>112</td>
<td>49 (43.8)</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>$\leq$ 5 residents</td>
<td>117</td>
<td>76 (65.0)</td>
<td>2.38</td>
<td>1.4–4.06</td>
</tr>
</tbody>
</table>

Note: *Numeric values represent number of individuals (proportion of each group) with parasitemia due to any of the three \textit{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.

$^{1}$The likelihood of parasitemia versus non-parasitemia compared to the referent.

$^{2}$95% CI = 95% confidence interval adjusted for within household clustering of cases.

$^{3}$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.
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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>...</td>
<td>...</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance to An. sundaicus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larval habitats (m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1600 m</td>
<td>1.0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>≤1600 m</td>
<td>0.21</td>
<td>0.14–0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance to health facility (m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1600 m</td>
<td>1.0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>≥1600 m</td>
<td>1.0</td>
<td>0.99–1.01</td>
<td>0.95</td>
</tr>
<tr>
<td>Number of household members</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6 residents</td>
<td>1.0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>≥6 residents</td>
<td>1.0</td>
<td>0.93–1.09</td>
<td>0.85</td>
</tr>
</tbody>
</table>

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 habitats 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. The circular scan statistic used in this study has been previously validated for plotting and understanding local malaria space–time
clusters, and has proven useful in targeting control strategies in areas such as the Kenyan highlands. 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.

A survey of *Anopheles* mosquitoes in western Sumba in 2007 in both upland interior and coastal sites captured and identified numerous potential disease vectors. The predominant species from evening human landing collections (mosquitoes per human) were *A. subpictus* and *A. vagus* in the upland interior and *A. sundaicus* along the coast. These findings were recapitulated in the recently completed companion spatial repellency study. 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 *A. sundaicus* in the coastal habitats, lesser potential malaria vectors such as *A. subpictus* and *A. 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 *A. sundaicus*.

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. 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.maneyonline/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 *A. 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. Increasing household distance to a health facility, a known risk factor for malaria, 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 ovovaculosis (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.

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-dimethyl-trichloro-ethane in the mid-twentieth century, larval source management through environmental interventions was a highly effective malaria control method employed in such areas as the Panama Canal Zone and peninsular Malaysia. These early successes bolstered the concept of species sanitation that was widely applied to great effect in other malaria endemic regions including Indonesia (then called Netherlands Indies) between 1920 and 1935. 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.\textsuperscript{90,91} In our study, we have refined the approach of the pioneers of species identification 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 identification 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
Contributors Conceived and designed field research: MJB DS JKB. Performed the field research: CPN CEN DSA KC FAY WS SW PBA SSM IW MJB DS JKB. Analyzed the data: CPN CEN DSA GH JFF JKB. Wrote the first draft of the manuscript: CPN. Contributed to the writing of the manuscript: CPN CEN JFF MJB DS JKB. Agree with manuscript results and conclusions: CPN CEN DSA KC FAY WS SW PBA SSM IW GH JFF MJB DS JKB.

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Conflicts of interest The authors of this publication have no conflicts of interest to disclose.

Ethics approval Ethical approval for this study was reviewed and approved by the Ethical Review Committee of the Eijkman Institute of Molecular Biology, Jakarta, Indonesia.

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Distance to Anopheles sundaicus larval habitats