Area effects of bednet use in a malaria-endemic area in Papua New Guinea


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Abstract

Relationships between area coverage with insecticide-free bednets and prevalence of Plasmodium falciparum were investigated in 7 community-based surveys over a 33-month period in 1990-93 in 6 villages in the Wosera area of Papua New Guinea. Spatial patterns in circumsporozoite rates for P. falciparum, P. vivax, and specific human antigens were determined using ELISAs. P. falciparum prevalence in humans was better explained by bednet coverage in the immediate vicinity than by personal protection alone. Circumsporozoite rates for both P. falciparum and P. vivax were also inversely related to coverage with bednets. There was some increase in zoophagy in areas with high coverage, but relatively little effect on the human blood index or on overall mosquito densities. In this setting, protracted use of untreated bednets apparently reduces sporozoite rates, and the associated effects on prevalence are greater than can be accounted for by personal protection. Even at high bednet coverage most anophelines feed on human hosts, so the decreased sporozoite rates are likely to be largely due to reduction of mosquito survival. This finding highlights the importance of vector ecology for outcomes of bednet programmes and suggests that area effects of untreated bednets should be reassessed in other settings.

Keywords: malaria, Plasmodium falciparum, Plasmodium vivax, prevalence, bednets, Anopheles punctulatus, sporozoite rate, human blood index, Papua New Guinea

Introduction

Village-scale trials have found that insecticide-treated bednets (ITNs) can substantially reduce the survival of malaria vectors, as assessed by parous and sporozoite rates. They have also found measurable decreases in the density of mosquitoes (MAGEE et al., 1991; ROBERT & CARNEVALE, 1991; BEACH et al., 1993; KARCH et al., 1993) and hence of the rate of inoculation of unprotected individuals. It follows that ITNs can protect not only the individuals sleeping under them, but also those living nearby. Indeed, BINKA et al. (1998) found that mortality rates in children without nets were lower if they lived close to concentrations of ITN users.

Mass effects of bednets have been thought to require insecticide treatment of the nets, consistent use over a wide area, and an anthropophilic vector (LINES, 1996). While observational studies e.g., CAMPELL et al. (1987) in The Gambia and GENTON et al. (1994) in Papua New Guinea suggest that untreated nets (UTNs) also protect against Plasmodium falciparum infection, the only published randomized controlled trial of UTNs found no significant protection (SNOW et al., 1988a).

In addition, there were also no significant differences in sporozoite antibody levels between children sleeping under UTNs and those who did not, although those under nets suffered significantly fewer mosquito bites (SNOW et al., 1989).

It has consequently been proposed that the apparent beneficial effects of UTNs in some studies might merely reflect diversion of frustrated host-seeking mosquitoes to unprotected individuals sleeping nearby (SNOW et al., 1988a, 1988b). Differences in infection rates between UTN users and unprotected individuals would then reflect redistribution of bites rather than net decreases in inoculations, and thus widespread use of UTNs would have little overall public health impact, possibly even disadvantageous those who could not obtain them.

In keeping with this hypothesis, in the Papua New Guinea study, GENTON et al. (1994) found that some of the apparent protection might indeed be gained by diverting mosquitoes to non-users of bednets in the same house. While patterns of antimalarial use could account for some variation in prevalence (GENTON et al., 1995) they could not explain the bednet effects (SMITH et al., 2001). The very large apparent efficacy motivated us to look more closely into the effects of these bednets, making use of a geographical information system to determine whether UTNs also affect inhabitants of neighbouring houses.

First, we examined whether the prevalence of P. falciparum parasitaemia was affected by the bednet coverage in nearby houses, and assessed the scale of the effects that we found. Changes in vector behavior in untreated areas must mediate such area effects. We therefore examined whether coverage affects the human blood index (HBI), which measures the degree of anthropophagy. We also assessed rates of zoophagy and examined whether the UTNs affect vector survival, using the sporozoite rate as an indirect measure of this important determinant of malaria-transmission intensity. These analyses enable us to propose a mechanism for the apparent effects of UTNs on parasite prevalence.

Materials and Methods

Study area and malaria prevalence data

The study was carried out in the Wosera area of the East Sepik Province of Papua New Guinea as part of the Malaria Vaccine Epidemiology and Evaluation Project (ALPERS et al., 1992). Hii et al. (1997) described the spatial and temporal patterns in densities of the malaria vectors in this area. The main vectors are Anopheles punctulatus s.s., An. kohensis, An. farauti s.l., and An. karwari. Less frequent anophelines are An. bancroftii and An. longirostris. Malarial surveys in 1990–92 found a high endemicity of P. falciparum, P. vivax and P. malariae (GENTON et al., 1995). The present analyses used parasitological records from these surveys for individuals aged < 20 years whose houses could be geocoded (3459 records).

Bednet coverage

Individual households in the area have used UTNs for decades for reasons including privacy, reduction of insect nuisance and dust avoidance. During the sample surveys of GENTON et al. (1995) each individual was asked whether he/she had slept under a bednet the previous night. Bednet coverage was then defined for each of a series of radii around each individual's house (25, 50, 100 m). Thus, coverage within 10 m ($C_{10}$) was defined as the proportion of all responses within 10 m that indicated bednet use; $C_{25}$ was the proportion within...
25 m; \( C_0 \) was the coverage within the same house as the index individual.

**Sampling of anophelines**

Mosquito densities were determined by monthly mosquito collections among 5–6 sectors of each village (total of 10 villages) from January 1991 to September 1993. Within each sector, a random inhabitant was selected each month and his or her home defined as the index house. Two men, one inside and the other outside this house carried out landing collections of host-seeking mosquitoes from sunset to midnight. A pair of new collectors resumed collections after midnight until sunrise. To maintain privacy, indoor catches were made some distance from sleeping occupants of the house. Collections were not made under bednets even when these were in use. For further details see Hii et al. (1997).

Indoor resting collections were made at dawn inside the bedrooms of the index house and in 3 neighbouring houses (Hii et al., 1997). Mosquitoes resting on walls and on outside, but not inside surfaces of UTNs were collected. Mosquitoes resting outdoors were collected between 06:30 and 07:30 from the area immediately around the index house and 4 adjacent houses.

**Analysis of blood meals**

Midguts of fully engorged Anopheles mosquitoes from resting collections were tested by ELISA for each of human, pig, dog, cat and goat blood. Details of sampling and ELISA techniques are given by Hii et al. (1997). For each house where resting collections were carried out, both the proportions of blood meals containing human blood (the HBI), and the overall proportion of blood meals containing identified non-human blood were calculated.

**Circumsporozoite rate estimation**

Circumsporozoite antigens for 2 P. vivax phenotypes (PVK210 and PVK247) (Wirtz et al., 1991, 1992), P. falciparum (Wirtz et al., 1987) and P. malariae (Collins et al., 1988) were detected in mosquito pools containing head-thoraces or whole bodies by specific ELISAs. In brief, 96-well microtitre plates (Dynatech Laboratories, Inc., Virginia, VA, USA) were incubated at room temperature overnight with monoclonal antibody (MAb) NSY3 (0.025 \( \mu g/50 \mu L \) of phosphate-buffered saline [PBS]) for PVK210, MAb PV21247-499 (0.1 \( \mu g/50 \mu L \) of PBS) for PVK247, MAb PF2A10 (0.1 \( \mu g/50 \mu L \) of PBS) for P. falciparum, and MAb PB32 (0.25 \( \mu g/50 \mu L \) of PBS) for P. malariae, then incubated with blocking buffer for 1 h at room temperature. One positive and 8 negative controls were run on each plate, and the absorbances were read at 405 nm on a Multiskan ELISA reader. Positive samples were defined as those where the optical density was greater than the mean + 3 standard deviations of a set of 8 negative controls. These samples were re-assayed to confirm them as positive. Control equivalencies were determined to be 100 pg of R32tet32 = 400 sporozoites for P. falciparum, 40 pg of NS1V2 = 400 sporozoites for VK210, 3 ng of VK247 = 500 sporozoites for VK247 and 0.25 pg of PC = 500 sporozoites for P. malariae.

Maps of smoothed spatial patterns in the circumsporozoite rates were produced using conditional auto-regressive statistical models (Bernardinelli & Montomoli, 1992) with distance priors (Vounatsou et al., 2000) and logistic link functions. These models were fitted using the package BUGS (Bayesian Updating using Gibbs Sampling) (Thomas et al., 1992). Additional terms in bednet coverage, measured by \( C_3 \), were added to these models for estimation of coverage effects on circumsporozoite rates, which allowed control of possible spatially structured confounding.

**Results**

**Parasite prevalence and bednet coverage**

The detailed patterns of parasite prevalence by age are described elsewhere (Genton et al., 1995) and the effects of bednet coverage on these patterns are described by Smith et al. (2001). Of the 3459 microscope slides included in the present analyses, 1640 (47%) were positive for asexual stages of P. falciparum.

To the question 'Did you sleep in a mosquito net last night?', 2364 individuals from geocoded houses provided 6223 responses of which 2551 (41.0%) were positive. Bednet coverage was highest in the centre and lowest in the western part of the study area (Fig. 1).
In age-adjusted logistic models, the odds for *P. falciparum* infection associated with the individual responses to the question on bednet use the previous night (I) was 0.52 (95% confidence interval [CI] 0.46, 0.60). However, irrespective of the radius (r) used, the bednet coverage, Cc, accounted for more variation in *P. falciparum* prevalence than did these individual responses. In logistic models including terms for coverage within the same house (Cb), within 25 m (Cc), or 50 m (Cc), the additional effect of individual bednet use was small (Fig. 2). However, Cb itself had a somewhat smaller effect on prevalence than did either Cc or Cc (Fig. 2). Correspondingly, the likelihood ratio (LR) statistic testing the effect of Cc (adjuncting for individual responses) was 27.0, compared with 36.4 for Cc, 41.3 for Cc, and 37.8 for Cc (all P < 0.0001). The corresponding LR statistics testing the effect of individual responses to the question on bednet use were 24.6 (adjusting for Cc), 31.7 (adjusting for Cc), 34.9 (adjusting for Cc) and 37.9 (adjusting for Cc). Since the magnitudes of the coverage effects were little affected by the radius used (Fig. 2), further analyses used the smallest radius (25 m) that gave reasonably precise estimates of coverage. This radius corresponded approximately to the radius of a small hamlet.

**Mosquito densities**

Comparison of Figure 1 with maps of biting rates given by HII et al. (1997) indicates that vector densities for *An. punctulatus*, *An. kolensis*, and *An. karwari* are positively correlated with bednet coverage. Those for *An. farauti* s.l., *An. bancrofti* and *An. longirostris* densities are negatively associated with Cc.

**Blood meal analysis**

The HBI in resting *An. punctulatus* and *An. kolensis* was 0.90 and 0.79, respectively (HII et al., 1997). In *An. punctulatus*, HBI tended to decline with increasing Cc (logistic regression: LRc2 = 5.90; P = 0.015) although, even in areas where bednet usage was universal, 78% of resting female *An. punctulatus* had fed on humans. The corresponding decline was not evident in *An. kolensis* (LRc2 = 1.0; P = 0.3). In total, 19.2% *An. punctulatus* and 10.8% *An. kolensis* blood meals tested positive for non-human blood (pig, cat, dog, or goat) with dog the most frequent non-human host, and the proportion of blood meals with non-human blood increased with Cc, in both species (*An. punctulatus*: LRc2 = 11.2; P = 0.0008; *An. kolensis*: LRc2 = 11.3; P = 0.0008). There were insufficient data to test whether rates of either anthropophagy or zoophagy varied with Cc in the minor vectors *An. karwari*, *An. farauti* s.l., *An. bancrofti* and *An. longirostris*.

**Circumsporozoite rates**

From a total of 5484 all-night landing catches, 114973 mosquitoes were tested for circumsporozoite antigens: 754 specimens were positive for circumsporozoite antigen of *P. falciparum*, 361 for *P. vivax* (PVK210 and PVK247 combined), and 99 for *P. malariae* (Table 1). *An. kolensis* and *An. punctulatus* had the highest circumsporozoite rates of the 6 species (Table 1) as well as the highest biting densities (HII et al., 1997). Analyses that did not include spatial autocorrelation indicated marked trends for circumsporozoite rates to decrease as Cc increased. In *An. punctulatus*, this decline was significant and of comparable magnitude for both *P. falciparum* (logistic regression LRc2 = 20.2; P < 0.0001; odds ratio = 0.41; Table 2, Fig. 3a) and *P. vivax* (LRc2 = 5.9; P = 0.023; Fig. 3b). In *An. kolensis* the declines associated with 100% bednet coverage were of similar magnitude to those in *An. punctulatus* (*P. falciparum*: LRc2 = 22.9; P < 0.0001; Fig. 3a; *P. vivax*: LRc2 = 2.8; P = 0.10; Fig. 3b). Data for minor vectors and for *P. malariae* were too sparse for such analyses.

*P. falciparum* circumsporozoite rates were highest in the western and north-eastern parts of the study area, for both *An. punctulatus* (Fig. 4a) and *An. kolensis* (Fig. 4b). This pattern complemented that of bednet coverage. *P. vivax* sporozoites were also more often found in the western part of the study area (Fig. 4c, 4d), but remained low in the north-eastern part (Nale-1 village).

Estimates, allowing for spatial structure, of the effects of Cc on circumsporozoite rates had broader confidence intervals than unadjusted estimates (Table 2). However, although some of these intervals overlapped with unity, the estimated coverage effects were of similar magnitude for both *Plasmodium* species, and also for both major

**Table 1. Overall circumsporozoite rates (%) by *Plasmodium* and mosquito species (Wosera, Papua New Guinea, 1991–93)**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Total mosquitoes evaluated</th>
<th><em>P. falciparum</em></th>
<th><em>P. vivax</em></th>
<th><em>P. malariae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. punctulatus</em></td>
<td>42.584</td>
<td>0.76 (0.68, 0.84)</td>
<td>0.34 (0.29, 0.40)</td>
<td>0.09 (0.07, 0.12)</td>
</tr>
<tr>
<td><em>An. kolensis</em></td>
<td>49.000</td>
<td>0.77 (0.69, 0.85)</td>
<td>0.19 (0.17, 0.22)</td>
<td>0.09 (0.07, 0.13)</td>
</tr>
<tr>
<td><em>An. karwari</em></td>
<td>15.007</td>
<td>0.32 (0.24, 0.42)</td>
<td>0.07 (0.05, 0.11)</td>
<td>0.04 (0.02, 0.09)</td>
</tr>
<tr>
<td><em>An. farauti</em> s.l.</td>
<td>44.38</td>
<td>0.14 (0.06, 0.30)</td>
<td>0.03 (0.01, 0.10)</td>
<td>0.14 (0.06, 0.31)</td>
</tr>
<tr>
<td><em>An. longirostris</em></td>
<td>0.509</td>
<td>0.21 (0.13, 0.30)</td>
<td>0.05 (0.01, 0.13)</td>
<td>0.01 (0.01, 0.02)</td>
</tr>
<tr>
<td><em>An. bancrofti</em></td>
<td>855</td>
<td>0.23 (0.06, 0.93)</td>
<td>0.12 (0.03, 0.47)</td>
<td>0.12 (0.02, 0.83)</td>
</tr>
</tbody>
</table>

Circumsporozoite rate was calculated by ELISA; positive mosquitoes captured on human bait. Figures in parentheses are exact binomial 95% confidence intervals.

*Sporozoite-positives found in whole bodies only.*
Table 2. Effects of untreated bednet coverage on sporozoite rate, adjusted for spatial pattern (Wosera, Papua New Guinea)

<table>
<thead>
<tr>
<th>Vector</th>
<th>Parasite</th>
<th>No allowance for spatial pattern</th>
<th>Analysis allowing for spatial pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Odds of CSP positivity(^a)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odds of CSP positivity(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI)</td>
</tr>
<tr>
<td>An. punctatus</td>
<td>P. falciparum</td>
<td>0.41 (0.28, 0.59)</td>
<td>0.30 (0.17, 0.49)</td>
</tr>
<tr>
<td>An. kolensis</td>
<td>P. falciparum</td>
<td>0.41 (0.28, 0.58)</td>
<td>0.62 (0.37, 1.00)</td>
</tr>
<tr>
<td>An. punctatus</td>
<td>P. vivax</td>
<td>0.46 (0.24, 0.88)</td>
<td>0.35 (0.16, 0.81)</td>
</tr>
<tr>
<td>An. kolensis</td>
<td>P. vivax</td>
<td>0.55 (0.28, 1.12)</td>
<td>0.58 (0.25, 1.31)</td>
</tr>
</tbody>
</table>

\(^a\)Estimated odds for a sampled house with \(C_{25} = 100\%\) compared with a house with \(C_{25} = 0\%\).

CSP, circumsporozoite protein; \(C_{25}\), coverage of households with bednets within a 25-m radius.

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Discussion

The demonstration that ITNs are an effective intervention against malaria mortality has encouraged their widespread introduction in malaria-endemic areas. However, there are major operational difficulties in ensuring that ITNs receive their periodic re-treatment (ZIMICKI, 1996). Many remote communities will undoubtedly be left with high coverage of effectively untreated nets.
The only published randomized controlled trial of UTNs (SNOW et al., 1988a) found no significant protective effect. Yet the efficacy of UTNs in community randomized trials with UTNs as control was lower than that where control groups had had no nets, suggesting that UTNs have a positive, although small, efficacy (LENGELE, 1998). The UTN effects found in Wosera (GENTON et al., 1994) were surprisingly large, and demand an explanation. Confounding can never be ruled out in observational studies, but neither the type of house construction, nor repellent effects of wood fires (GENTON et al., 1994), nor variations in antimalarial treatment of the hosts (GENTON et al., 1995; SMITH et al., 2001) account for the bednet effects. The present analyses attempt to explain the bednet effects by analysing entomological processes that might be involved, rather than by seeking further possible confounders.

We first considered whether the associations between malaria prevalence and UTN use arose because of protection of individual hosts, or via effects of the nets on the mosquito population. Individual responses will give an exaggerated idea of variation in use within households if UTN usage varies from night to night. In such circumstances $C_{2}$ could be a better measure of individual level protection over time than the questionnaire response assigned to an individual. Indeed in the nearby Madang province, BURKOT et al. (1989) found that the number of bednets in a house provides a better predictor of long-term bednet use than interview data. In principle, apparent coverage effects might therefore simply reflect individual protection together with imperfect measurement of bednet usage.

In Wosera, spot checks of actual bednet usage confirmed that data for self-reported use the previous night were reasonably accurate (GENTON et al., 1994) and should thus give a reasonably unbiased estimate of coverage. At the same time, the finding of GENTON et al. (1994), that non-net-users in the same houses tended to be more often infected than people in houses where no one sleeps under a net, suggests that patterns of UTN use within households do not change continuously. If different people used the UTNs on different nights this would reduce within-house variation in prevalence. These observations suggest that our modelling procedures correctly distinguish area and individual effects, and hence that the better fit of logistic models with terms in $C_{1}$ and $C_{2}$ than corresponding models in $C_{3}$ is evidence for genuine area effects. The relatively small residual effect of $C_{2}$ reported in Table 3.1 suggests that the area effects of UTNs are larger than the individual effects. The area term indicates that the major impact of UTNs in Wosera is a result of area effects rather than individual protection.

Following LINES et al. (1987) and SNOW et al. (1988a, 1988b), GENTON et al. (1994) contended that diversion of mosquitoes on to unprotected individuals explained why non-users in the same house as users can be at high risk. There is no contradiction between this finding and that of overall protective effects of net coverage: most of the victims of diversion are in areas of low net-coverage, while the protective effects of UTN coverage appear to be more important at high $C_{3}$.

The effects of $C_{3}$ on the HBI and on rates of animal feeding confirm that the UTNs divert mosquitoes on to alternative hosts. Zooprophylactic effects have also been observed in the Madang area of Papua New Guinea (CHARWOOD et al., 1986). However, the less anthropophilic vector An. farauti is more important in the Madang area, and the people there keep many more pigs, so their toll is more potential for effective zooprophylaxis in Madang than in Wosera.

We do not believe that diversion can be the main reason for the area effects in Wosera. First, the effect of UTN use on HBI was rather small, and even when net coverage was high, reaching 82% in one village [see GENTON et al. (1994)], most of the fed mosquitoes belonging to the 2 major vector species had still managed to find a human host. Secondly, the protection against parasitaemia associated with high coverage, even in models that adjusted for individual-level UTN use, argues that diversion is a secondary phenomenon. If diversion were important, we would expect a large individual protection effect but a negative effect of coverage. It appears that the effects of diversion on to unprotected human hosts are more than compensated for by the effects on the circumsokozoite rate.

In such circumstances it could be a better measure of individual level protection over time than the questionnaire response assigned to an individual. Indeed in the nearby Madang province, BURKOT et al. (1989) found that the number of bednets in a house provides a better predictor of long-term bednet use than interview data. In principle, apparent coverage effects might therefore simply reflect individual protection together with imperfect measurement of bednet usage.

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In summary, the Wosera data indicate that, if coverage is high, long-term use of UTNs may have substantial impact on prevalence, more because of effects on vector biomics than of individual protection. This interpretation follows from parallels between the patterns of circumsporozoite rates in *P. vivax* and *P. falciparum*, which both appear to be strongly affected by UTN coverage. It challenges the view that ‘mass-killing effects’, with reductions in survivorship, sporozoite rate and density of the mosquito vector population at a village level, occur specifically when ITNs are used consistently over a wide area against an anthropophilic vector (LINES, 1996). We propose that such effects can occur even when coverage is patchy and the nets are not impregnated with insecticide. It is important that ITNs have similar effects elsewhere, especially in areas of sub-Saharan Africa where *An. funestus* and the *An. gambiae* complex are the main vectors.

Acknowledgements

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**Announcements**

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This meeting will bring together the Bangladesh Association for Advancement of Tropical Medicine and the Royal Society of Tropical Medicine and Hygiene.

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