Effects of untreated bed nets on the transmission of *Plasmodium falciparum, P. vivax* and *Wuchereria bancrofti* in Papua New Guinea

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Abstract

The impact of untreated bed nets on the transmission of human malaria and filariasis in a village in a hyperendemic area of Papua New Guinea was studied. In anopheline mosquitoes, the *Plasmodium falciparum* sporozoite antigen positivity rate, filarial infection rates and human blood indices dropped significantly after bed nets were introduced. This reduction in human-vector contact did not affect mosquito density as no significant difference in either landing rates or indoor resting catches was found. The number of bed nets in a house and ownership of dogs were factors significantly associated with a reduction in the number of indoor resting mosquitoes. However, the reduction in the *P. falciparum* sporozoite antigen rate in mosquitoes was not accompanied by a reduction in either malaria parasite or antibody prevalences or titres against the *P. falciparum* circumsporozoite protein.

Introduction

The limited success of the initial malaria vaccine trials (BALLOU *et al.*, 1987; HERRINGTON *et al.*, 1987) has focused attention on available methods to reduce malaria transmission. Emphasis has shifted from large centralized control programmes (such as the DDT house spraying programmes) to village-based programmes integrated into existing primary health care systems. This broad approach includes making drugs more readily available for treatment of clinical cases, anti-vector measures such as source reduction against the larval stages, and personal protective measures such as repellents and bed nets against adult mosquitoes.

Impregnating bed nets with permethrin, an insecticide with repellency action, has shown promising results (ROZENDAAL, 1989). In Papua New Guinea, two trials of permethrin impregnated bed nets reduced both the incidence of *Plasmodium falciparum* in young children and the sporozoite antigen positivity rates for *P. falciparum* and *P. vivax* (GRAVES *et al.*, 1987; MILLEN, 1986). Protection appeared to be due to the repellent effect of permethrin as no evidence of decreased survival was seen in the anopheline population (CHARLWOOD & GRAVES, 1987).

However, many villages in Papua New Guinea are not easily accessible and repeated treatment of bed nets with permethrin may prove logistically difficult. Therefore, this study examined the impact of bed nets alone on the transmission of malaria and filariasis. Studies on untreated bet nets in Africa demonstrated that, even though torn bed nets offered some protection from mosquitoes, 19% to 27% of anophelines obtained bloodmeals on occupants sleeping under intact bed nets (data from PORT & BOREHAM, 1982 and LINES *et al.*, 1987, cited by LINDSAY *et al.*, 1989). Subsequently it was shown that the number of anophelines leaving houses in hamlets using bed nets was lower, and fewer had fed on blood, than those leaving houses in hamlets where bed nets were not used (LINDSAY *et al.*, 1989). Some studies in an area of seasonal malaria transmission in The Gambia demonstrated a reduction in splenomegaly (BRADLEY *et al.*, 1986) and parasite prevalence (CAMPBELL *et al.*, 1987) but SNOW *et al.* (1988) could find no such effect. Such mixed results indicate that additional studies in a variety of epidemiological areas are warranted. This study was conducted in a hyperendemic malarious area of Papua New Guinea with perennial transmission, and examined the impact of bed nets on factors affecting transmission of both malaria and filariasis, specifically the degree of man-vector contact and vector density.

Materials and Methods

Study village

The study was conducted in Bukask, a village in the foothills of the Finnesterre range in Madang Province, Papua New Guinea. The area is hyperendemic for malaria, and filariasis caused by *Wuchereria bancrofti* is common (BURKOT *et al.*, 1989c, 1990). *Anopheles punctulatus* is the only known vector of human malaria and filariasis in Bukask (BURKOT *et al.*, 1989c). Bukask is relatively isolated, being one hour's walk from the nearest road or village.

Entomology

Mosquitoes were collected both landing and resting for one week per collection period, as described previously (BURKOT *et al.*, 1987, 1988a, 1988b). Briefly, landing catches were performed by a team of 4 collectors working in pairs. One pair of collectors commenced work at 1800 h with one individual catching landing mosquitoes inside a house while the other individual caught mosquitoes outside. At midnight, the first pair of collectors was relieved by a

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second pair who worked until 0600 h. Mosquitoes were identified on morphological criteria (BELKIN, 1962) and two nights' landing catches were used as a measure of relative abundance and were analysed by enzyme-linked immunosorbent assay (ELISA) for sporozoite antigens of *P. falciparum*, *P. vivax* and *P. malariae* (WIRTZ *et al.*, 1987, 1990; COLLINS *et al.*, 1988).

The other two nights' landing catches per collection period were dissected immediately to determine malaria infection rates (oocysts and sporozoites), with the remaining material stored in 70% ethanol until stained and dissected for filarial worms (NELSON, 1959). Malaria vector infection rates were used to determine mosquito survivorship during the extrinsic incubation period (P_e) and during the feeding cycle (P_f), the individual mosquito vectorial capacity (IC) and the mosquito infection probability (K) as described previously (GRAVES *et al.*, 1990; SAUL *et al.*, 1990; BURKOT *et al.*, 1991).

Resting mosquitoes were collected in each house in Buksak for 4 mornings per collection period between 0600 and 0700 h. Outdoor resting mosquitoes were collected between 0700 and 0800 h. Mosquitoes were transported to the laboratory in an insulated container, identified to species on morphological criteria (BELKIN, 1962) and graded as fully engorged, partially fed or unfed. Engorged mosquitoes from all 4 mornings' outdoor resting collections and 2 mornings' indoor resting collections were squashed on filter paper for later ELISA analysis for host blood meal source identification using polyclonal rabbit antisera against the 8 most common hosts in the area (human, dog, cat, pig, rat, chicken, marsupial and bird; BURKOT *et al.*, 1988a). The other 2 mornings' indoor resting collections were kept in an insectary for 3 d; then their stomachs were removed for oocyst examination and their salivary glands removed for sporozoite antigen analyses by ELISA as described above.

Demography and parasitology

At the start of the study, basic demographic information (name, names of mother and father, date of birth, sex, and house location) were collected. This information was updated before each malariometric survey. Malariometric surveys were conducted 3 times during the study in May 1986, October 1986 and March 1987. At each survey, thick and thin blood films were made for later malaria parasite detection and identification by light microscopy, as described by BURKOT *et al.* (1987). Thick films were examined at 1000× magnification and densities recorded as the number of parasites per 200 white blood cells counted or per 100 fields for light infections. Splenomegaly was assessed using Hackett's grading at each survey.

At each survey, residents were examined by a physician and treatment given for common complaints. Referrals to the provincial hospital were made and free transportation arranged for those needing more specialized medical attention. In addition, during each entomological survey, institute technicians had aspirin, chloroquine and amodiaquine available for treatment of suspected symptomatic malaria cases.

During the first survey in May 1986 (survey 186), 10 ml blood samples were collected between 2200 and 0100 h. 2 ml were stored in ethylediaminetetraacetic acid and filtered for microfilariae detection, and the remaining 8 ml allowed to clot for serum collection. During the third survey in March 1987 (survey 187), fingerprick blood samples were collected in Microtainers® for serum separation. Following analysis of venous blood samples, residents were informed of their malaria and filariasis parasite status and the options for treatment explained.

Serology

Sera were assayed by ELISA for the presence of immunoglobulins G and M (IgG and IgM) recognizing the circumsporozoite (CS) proteins of *P. falciparum* and *P. vivax* and antibody units (AU) of activity calculated as described previously (BURKOT *et al.*, 1989b; WIRTZ *et al.*, 1989). Briefly, a recombinant molecule comprising 32 repeats (NANP) of the *P. falciparum* CS protein coupled to the tetracycline resistance gene ($R_{32}tet_{32}$) (YOUNG *et al.*, 1985) or two copies of the 9 amino acid repeat portion of the *P. vivax* CS protein (DRAA/DGQPAG) (ARNOT *et al.*, 1985) linked to bovine serum albumen were used as antigens. Sera were tested in duplicate with individual controls at a 1:100 dilution and their activity compared to that of a standard curve derived from 2-fold dilutions of a strongly positive serum.

Malaria intervention measures

Chloroquine usage was monitored in Buksak by Dill-Glazko analysis of urine samples collected during the 186 and 187 malariometric surveys (LELIJVELD & KORTMANN, 1970). Houses were inspected for bed nets during the second malariometric survey in October 1986 (survey 286), before distribution of bed nets. During the first week of 1987, numbered bed nets were assigned to the residents of Buksak. Enough bed nets were distributed to enable all residents requesting a bed net to have one. At the time of bed net distribution, the proper way to hang a bed net was demonstrated and their correct use was explained at a village meeting. Domestic animal numbers were also determined by interviewing heads of households at the 286 and 187 surveys. Houses were also re-examined for bed nets at the time of the 187 survey.

Results

Demography and parasitology

During the study, the population in Buksak village fluctuated between 93 and 129. The village was hyperendemic for malaria, with *P. falciparum*, *P. vivax* and *P. malariae* commonly identified in blood films (Table 1). Overall malaria parasite prevalences ranged from 52% to 70%. Spleen rates ranged from 64% to 71%, with average enlarged spleens ranging from 2·3 to 2·6 over the time of the study. 31% of the population had *W. bancrofti* microfilariae, with an average of 2083 worms per ml of blood (Table 2).

Malaria intervention measures

At the start of the study in 1986, only 7 of 93 residents slept under 4 bed nets. At the end of the study, 78% of residents were sleeping under bed nets. Reasons for less than 100% usage included a fluctuating population in which residents came to and left

Table 1. Results of parasite surveys in Buksak for prevalence of *Plasmodium falciparum* asexual stages, *P. falciparum* gametocytes *P. vivax*, *P. malariae* and splenomegaly[a]

Age (years)	Malaria parasite rates (%) No. tested	Pf	Pfg	Pv	Pm	Total	Splenomegaly No. tested	Rate (%)	AES
Survey 186									
<5	12	67	8	25	50	75	12	100	3·2
5–<10	17	59	0	6	35	82	17	88	2·8
10–<15	20	35	5	5	25	50	20	100	2·6
15–<20	12	33	8	33	25	75	12	58	2·6
≥20	39	18	0	3	10	26	39	62	2·3
Total	100	36	3	10	24	52	100	71	2·6
Survey 286									
<5	10	100	10	50	60	100	10	90	3·0
5–<10	19	74	0	32	37	79	19	95	2·3
10–<15	18	44	11	28	22	78	18	89	2·2
15–<20	11	64	9	36	36	91	11	45	1·8
≥20	35	17	0	14	17	46	35	37	2·0
Total	93	48	4	27	29	70	93	66	2·3
Survey 187									
<5	7	5	14	29	29	100	5	80	1·2
5–<10	16	81	12	6	19	94	10	100	2·7
10–<15	18	28	0	28	22	67	14	93	2·8
15–<20	17	35	0	24	12	65	11	64	2·0
≥20	37	16	5	8	8	30	27	41	2·4
Total	95	35	5	16	15	59	66	68	2·4

[a]Abbreviations: Pf, *P. falciparum* asexual stages; Pfg, *P. falciparum* gametocytes; Pv,. *P. vivax*; Pm, *P. malariae;* AES, average enlarged spleen (Hackett's scale).

Table 2. Results of parasite survey in Buksak for prevalence of *Wuchereria bancrofti* microfilariae (survey 186)

Age (years)	Microfilariae No. tested	Positive (%)	Mean density (per ml)
<5	10	10	392
5–<10	16	19	98
10–<15	20	25	1374
15–<20	12	17	200
≥20	38	50	2870
Total	96	31	2083

Buksak throughout the study, small houses which would not accommodate sufficient numbers of bed nets for all residents, and complaints that bed nets were too hot to sleep under comfortably.

Anti-malarial drugs were not readily available in the village; only 3 of 82 urine samples at the first survey were positive by the Dill-Glazko test. The study did not alter the availability of chloroquine and utilization was not changed at the conclusion of the study as only 2·4% of 83 urine samples were positive by the Dill-Glazko test.

Serology

The prevalence of persons with IgG recognizing either *P. falciparum* or *P. vivax* CS repeats dropped between the 186 and 187 surveys from 65·1% (54/83) to 55·4% (51/92) and from 25·3% (21/83) to 13·0% (12/92), respectively. This drop in prevalence was not significant ($P>0·75$ and $P>0·25$; logistic regression analysis allowing for interactions of age and survey). Neither were there significant differences in the prevalence of IgM antibodies between the 186 and 187 surveys against the CS repeats of *P. falciparum* (3·6% and 3·3%) or *P. vivax* (4·8% and 8·7%) (logistic regression analysis; $P>0·10$ and $P>0·25$, respectively). Significant differences were not seen in the average AUs of IgG or IgM recognizing *P. falciparum* CS repeats or the average AUs of IgG or IgM recognizing *P. vivax* CS repeats for any of the age groups (Mann-Whitney test, $P>0·10$) between the 186 and 187 surveys.

Entomology

An. punctulatus comprised more than 99·9% of anophelines collected in landing catches in Buksak, with the remainder being *An. koliensis*. The increased availabilty of bed nets in Buksak had a marked effect on many entomological measures. Significant reductions in the human blood index (HBI) for anophelines collected in both indoor and outdoor resting catches were observed between the 1986 and the 1987 collections (Table 3). In the outdoor resting catches, the proportion of anophelines feeding on dogs rose 5-fold from 8% to 40%, while the proportion feeding on pigs more than doubled, from 6% to 14%. Considering indoor resting mosquitoes, the introduction of bed nets resulted in twice as many anophelines feeding on dogs (9%) inside houses as before bed nets were introduced (4%). The significant reduction in man-vector contact demonstrated by the lower HBIs was corroborated by significant drops in the *P. falciparum* sporozoite antigen positivity rate and the *W. bancrofti* infection rates for all mosquito stages of the parasite (Table 3). Of equal interest was the failure of the sporozoite antigen positivity rate for *P. vivax* to diminish following widespread use of bed nets. One mosquito positive for *P. malariae* sporozoite antigen was detected in 1987, but none was found in 1986. The total sporozoite antigen rate for all species dropped by 27% from 1·35% in 1986 to 0·99% in 1987 ($\chi^2=2·78$, $P>0·05$).

There was no evidence that bed nets affected the anopheline density in Buksak. Landing catches were performed during 14 weeks in both 1986 and 1987. Although the landing rates on humans (expressed as the average number of anophelines collected in the indoor and outdoor landing catches from 1800 h to 0600 h) rose from an average of 135 anophelines per man per night in 1986 to 250 anophelines per man per night in 1987 (Table 4), this increase was not significant. An inverse relationship can be seen between an average nightly landing catch of anophelines and rainfall (Figure). During the prolonged drought in 1987, average landing rates increased to 657 per man per 12 h collection period.

A total of 10918 and 13430 anophelines were captured resting inside Buksak houses in 1986 and 1987, respectively. The Wilcoxon matched pairs signed rank test was used to compare catches of resting mosquitoes in 1986 and 1987 in the same 29 houses (Table 4). No significant change was seen in the average number of resting mosquitoes per house per night (either blood-fed or the total of blood-fed and unfed) between 1986 and 1987 when bed nets were made widely available.

Significant differences were not found in mosquito

Table 3. Entomological measures of vector-host contact

Entomological measure	1986 Positive % (no./total)	1987 Positive % (no./total)	χ^2	P
Blood indices				
Outdoor resting				
Human	84 (188/225)	46 (79/171)	60·04	<0·0001
Dog	8 (18·5/225)	40 (68·5/171)	55·60	<0·0001
Pig	6 (14·5/225)	14 (23·5/171)	5·15	<0·025
Indoor resting				
Human	96 (1565·5/1637)	90 (1990·5/2207)	39·38	<0·0001
Dog	4 (57·5/1637)	9 (195·5/2207)	39·38	<0·0001
Pig	1 (21·5/1637)	<1 (17/2207)	2·28	>0·10
Sporozoite antigen rates				
P. falciparum	0·82 (39/4760)	0·40 (31/7694)	8·39	<0·005
P. vivax	0·44 (21/4760)	0·52 (40/7694)	0·23	>0·50
P. malariae	0 (0/4760)	0·01 (1/7694)	–	>0·50[c]
Total	1·35 (64/4768[a])	0·99 (76/7699[b])	2·78	>0·05
Filarial infection rates				
L1 & L2	12·96 (253/1952)	6·79 (113/1664)	36·92	<0·0001
L3	5·38 (105/1952)	1·62 (27/1664)	34·90	<0·0001
Total	15·78 (308/1952)	7·87 (131/1664)	51·90	<0·0001

[a]In 1986, 8 mosquitoes were lost following dissection but before analysis could be performed for sporozoite antigen identification by ELISA; of these, 4 were identified as sporozoite positive.
[b]In 1987, 5 mosquitoes were lost following dissection but before analysis could be performed for sporozoite antigen identification by ELISA; of these, 4 were identified as sporozoite positive.
[c]Fisher's exact test.

Table 4. Entomological measures of anopheline density

Measure of mosquito density	1986 (mean)	1987 (mean)	P
Nightly human landing rates	130	250	>0·05[a]
Indoor resting per house			
Human blood-fed/house/morning	8·11	6·34	>0·05[b]
Total no. blood-fed and unfed	8·74	7·52	>0·05[b]

[a]Wilcoxon rank sum test.
[b]Wilcoxon matched pairs signed rank test.

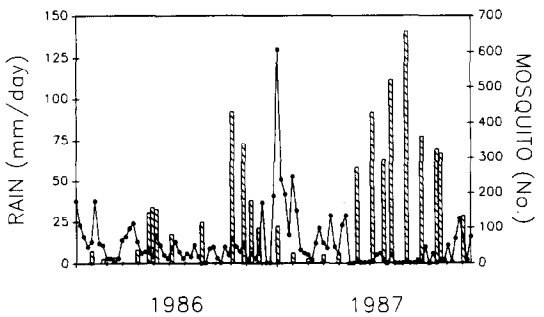

Figure. Anopheline landing rates (number per person per night; hatched bars) in Buksak and rainfall patterns in 1986–1987. Rainfall measurements given were from the nearest rainfall recording station at the Papua New Guinea Institute of Medical Research, approximately 50 km north of Buksak.

survival either per feeding cycle or per extrinsic incubation period between 1986 and 1987. Survival per feeding cycle diminished from 0·695 (standard error [SE]=0·047) in 1986 to 0·582 (SE=0·075) in 1987 (z=1·274, P>0·05), while mosquito survival per extrinsic incubation period changed from 0·191 (SE=0·034) in 1986 to 0·200 (SE=0·057) in the first 13 weeks of 1987 (z=0·133, P>0·05). The individual mosquito vectorial capacity dropped insignificantly from 0·538 (SE=0·148) in 1986 to 0·339 (SE=0·116) (z=1·058, P>0·05) in the first 13 weeks after widespread use of bed nets, while the mosquito infection probability increased non-significantly from 0·062 (SE=0·011) in 1986 to 0·108) in 1987 (z=0·426, P>0·05).

Table 5. Regression analyses of some possible variables affecting number of mosquitoes caught resting indoors (dependent variable)[a]

Independent variable	Year	Slope	df	t	P
Single regression analyses					
Dogs	1986	−0·538±0·694	27	0·775	>0·05
	1987	−0·867±0·306	27	2·838	<0·005
Pigs	1986	−0·572±0·360	27	1·589	>0·05
	1987	0·003±0·187	27	0·016	>0·05
Nets	1987	−0·739±0·322	28	2·290	<0·05
In-net	1987	−0·356±0·229	28	1·554	>0·05
Out-net	1987	0·203±0·354	28	0·573	>0·05
Multiple regression analyses					
Dogs	1987	−0·761±0·292	25	2·61	<0·02
Nets	1987	−0·657±0·278	25	2·36	<0·05

[a]Abbreviations: df, degrees of freedom; t, Student's t test; P, probability of difference occurring by chance.

No correlation was found in 1986 between the numbers of indoor resting mosquitoes and either house construction (built on posts or on the ground), ownership of dogs, pigs, cats or chickens, the number

of adults or humans sharing a house, or various ratios of these quantities when analyzed by the statistical package GLIM (McCULLAGH & NELDER, 1983). After introduction of bed nets in 1987, the analysis described above was repeated with the addition of the following variables: numbers of bed nets in a house (nets), the number of inhabitants in a house using bed nets (in-net) and the number of inhabitants sleeping outside bed nets (out-net) (Table 5). Significant negative associations were found between numbers of resting mosquitoes and both dog ownership and the number of bed nets in the house. Multiple regression analyses showed the best fitting model for resting mosquito numbers (the dependent variable) included dogs and bed nets as independent variables (Table 5). This model explained approximately one-third of the variation in the data (model sum of squares=47·23, df [degrees of freedom]=2; residual sum of squares=80·42, df=25).

Discussion

Significant effects on the malaria and filaria parasite rates in the human population were not expected in this study because of the small village population and the very high inoculation rates recorded. For these reasons, the study concentrated on detailed analyses of the effects of bed nets on the entomological components of malaria and filariasis transmission.

The introduction of bed nets into a Papua New Guinea village resulted in a significant reduction in man-vector contact but no effect on mosquito density. The reduction in man-vector contact was reflected in a significant decrease in the HBI with a concomitant 5-fold rise in the rate at which the vectors in Buksak fed on dogs. A drop in the HBI would predict decreases in the sporozoite antigen positivity rates (BURKOT *et al.*, 1989a) and indeed there was a significant drop in the infection rates for *P. falciparum* sporozoite antigen and *W. bancrofti* worms. Interestingly there was no significant drop in the *P. vivax* sporozoite antigen positivity rate after the introduction of bed nets. Introduction of bed nets into this village, where *An. punctulatus* was the malaria vector, resulted in a 27% reduction (not statistically significant) in the sporozoite antigen rate for all species. However, in an earlier study in Madang villages, permethrin impregnated bed nets resulted in a significant 67% drop in the total sporozoite antigen rate for *An. punctulatus* with a significant reduction in *P. falciparum* incidence in young children (GRAVES *et al.*, 1987).

In the present study, the significant drop in *P. falciparum* sporozoite antigen rate was insufficient to affect parasite rates in the human population or the proportion of individuals positive for antibodies against the *P. falciparum* CS protein. This finding may reflect the very high sporozoite inoculation rates occurring even after the introduction of bed nets as well as the small village population. It has been suggested that antibody prevalence and titres against CS proteins reflect transmission levels, and measuring these antibodies has been proposed as an alternative way of measuring sporozoite transmission (DRUILHE *et al.*, 1986; ESPOSITO *et al.*, 1988). However, in Papua New Guinea villages, correlations between inoculation rates and antibody prevalences have not been found (BURKOT *et al.*, 1989b). SNOW *et al.* (1989) also failed to find a difference in seropositivity rates or mean antibody titre between children sleeping under bed nets and those who did not, even though the children sleeping under bed nets suffered significantly fewer mosquito bites than children sleeping without nets. As the immunodominant epitope of the *P. falciparum* CS protein has been reported in blood stages of the parasite (COCHRANE *et al.*, 1989; COPPEL *et al.*, 1985), it may be that these antibodies reflect parasitaemia and not transmission.

The reduction in man-vector contact did not affect the anopheline density in Buksak, as there were no significant differences in either the landing rate or the numbers of anophelines resting indoors captured before and after introduction of bed nets. It appears that the dominant factor determining the anopheline density is the availability of larval habitats and not host accessibility. The major larval habitat of *An. punctulatus* in this area was a large stream. During the wet season, when the stream floods, the population of adult mosquitoes plummets. In the dry season, as the stream level drops, greater numbers of larval habitats are created in the quiet pools and edges of the streams and consequently the adult population increases. This effect was particularly evident during the prolonged drought in 1987 when the landing rates per man increased to 657 compared to an average of 13 in week 9 of 1987 during the rainy season.

The introduction of bed nets was associated with a significant reduction in the number of anophelines captured resting inside a house if that household owned dogs, but there was no effect of owning other domestic animals. This confirms an earlier study showing that dogs were preferred hosts to humans for *An. punctulatus* and that humans were preferentially selected over pigs (BURKOT *et al.*, 1988a). No association was found between the number of mosquitoes caught resting and the number of either adults or all humans sleeping in a house. This result is not surprising as the earlier study showed no relationship between body size and feeding success for the members of the *An. punctulatus* complex (BURKOT *et al.*, 1988a).

The number of mosquito nets in a house was associated with a significant reduction in number of resting mosquitoes, but no association was found with the number of individuals inside bed nets or the number sleeping outside bed nets (or their ratio). While the number of bed nets inside houses was confirmed by visual inspection, information on the numbers of individuals sleeping under, and the numbers sleeping outside, bed nets was obtained by interview on a limited number of occasions. It appears that the number of bed nets in a house is a better predictor of bed net use over a long period than information on their use obtained by interviewing individuals.

Although owning dogs and the presence of bed nets in the house were associated with a reduction in the numbers of resting mosquitoes, a demonstrable effect on measures of malaria in the host were not shown. The reduction in man-vector contact resulted in a lowering of the *P. falciparum* sporozoite antigen rate from 0·008 in 1986 to 0·004 in 1987 and the stage 3 *W. bancrofti* rate from 0·0538 in 1986 to 0·0162 in 1987. When multiplied by the average landing rate in 1987 (250/d), the estimates of relative daily inoculation

rates for *P. falciparum* and *W. bancrofti* were 1 and 4, respectively. These inoculation rates were still much too high to have had an effect on parasite rates. However, bed net usage as part of an integrated control scheme may have a measureable impact on malaria indicators and host morbidity.

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