The effect of permethrin-impregnated bednets on house entry by mosquitoes (Diptera: Culicidae) in The Gambia

S.W. Lindsay and J.H. Adiamah

Medical Research Council Laboratories, Fajara, The Gambia

J.R.M. Armstrong

Tropical Health Epidemiology Unit, London School of Hygiene and Tropical Medicine, Keppel St, London, WC1E 7HT, UK

Abstract

A trial was carried out using experimental huts to determine whether mosquitoes deterred from entering a building where a person slept under a permethrin-impregnated bednet would enter neighbouring dwellings where people used untreated nets. In each of the six experimental huts a man slept under a bednet for six weeks during the dry season in The Gambia. One bednet was impregnated with permethrin, the other five nets were untreated. The design of the trial was based on a series of Latin squares, so that both men and nets were rotated between huts on different nights. At the end of the trial each man had slept under each net in each hut. Mosquitoes collected from the huts each morning were used to estimate the total number of mosquitoes which had entered each hut during the night. Fewer *Anopheles gambiae* Giles (*sensu lato*), *A. pharoensis* Theobald and *Mansonia* spp. entered a hut with an impregnated bednet than those with untreated nets. However, there was no evidence that mosquitoes deterred from entering a hut with a treated net entered neighbouring huts with untreated nets. Thus it appears unlikely that people using untreated bednets in a community where most use impregnated nets would receive more bites than if everyone had an untreated net. However, the responses of mosquitoes to permethrin-impregnated bednets were found to depend both upon their species and nutritional condition since *A. rufipes* Gough, banded *Culex* spp. and non-human bloodfed *A. gambiae* (*sensu lato*) freely entered huts with a treated net.

Introduction

Bednets (mosquito nets) impregnated with pyrethroid insecticides are being used increasingly to control malaria in many parts of the tropics (Curtis *et al.*, 1989; Rozendaal, 1989; WHO, 1989). This control strategy has been most effective at combating malaria in areas of low seasonal transmissio4949 Bch as The Gambia (Alonso *et al.*, 1991; Lindsay *et al.*, 1989; S.W. Lindsay *et al.*, unpublished data; Snow *et al.*, 1988). In this country, a recent large malaria control trial carried out using permethrin-impregnated bednets reduced mortality in children aged between one to four years old by 63% compared to those sleeping under untreated nets (Alonso *et al.*, 1991).

Correspondence: Dr S.W. Lindsay, Department of Zoology, The University, Glasgow G12 8QQ, UK.

Permethrin-treated nets protect people from mosquito bites in a number of ways. Firstly, mosquitoes may be killed when landing on impregnated fabric (Darriet *et al.*, 1984; Lindsay *et al.*, in press a; Lines *et al.*, 1987; Snow *et al.*, 1987). Secondly, some mosquitoes which come into contact with treated netting may become physically irritated by the insecticide and fly away thereby reducing the time spent searching for a bloodmeal (Hossain & Curtis, 1989; Miller, 1990). Lastly, a proportion of mosquitoes will be deterred from entering a building with a permethrin-treated bednet (Darriet *et al.*, 1984; Lindsay *et al.*, in press b; Miller *et al.*, in press; Rozendaal *et al.*, 1989). This effect is unlikely to be a consequence of the permethrin itself, which is relatively non-volatile (Wells *et al.*, 1986), but of the volatile chemicals present in the emulsifiable concentrate formulation (Lindsay *et al.*, in press b).

Here we describe a study to determine whether mosquitoes deterred from entering a building with a permethrin-treated bednet attempt to feed in an adjacent house where the occupant sleeps under an untreated net. The study was designed to detect whether or not mosquitoes diverted from a hut with a treated net would be more likely to enter neighbouring huts than those further away. Although there is evidence to show that marking mosquitoes does not affect their behaviour (Service, 1976), we chose to use a technique which did not involve handling the insects. Six experimental huts based on a traditional African house design were built along a straight line, equal distances apart. In each hut a man slept under a bednet; one net was treated with permethrin and the other five were left untreated. The number of mosquitoes entering each hut was then estimated from collections made each morning.

Materials and methods

Study area

The study was carried out at Wali Kunda in The Gambia in April and May, during the dry season in 1990. Six experimental huts were built in a straight line some 12 m apart in an area of open Sudan Savannah woodland. The line of huts ran parallel to the side of a large ricefield which was the principal mosquito breeding site at this time of year. Each hut was built to an original design by Rapley (1961) and Smith (1965), based on a traditional African house. The huts were of a similar size, made with mud walls and a thatched roof, with a verandah extending from the base of each of the four outside walls (Lindsay *et al.*, in press b; Miller *et al.*, in press). Each building was ant-proofed by being raised from the ground on concrete legs surrounded with water-filled channels. On two opposite sides of each hut, perpendicular to the ricefield, the verandahs were enclosed to collect roughly half of the mosquitoes which left the room through the eaves during the night. Mosquitoes leaving the hut were also collected in window traps on the same sides of the building as the enclosed verandahs. Cotton wool buds saturated with 10% sucrose were suspended from the inside of the closed verandahs to act as a food source for hungry mosquitoes.

Bednets

Six intact bednets were made from nylon netting, each with a total area of some 14 m^2. One net was treated with a target dose of 500 mg/m^2 permethrin (25% Emulsifiable concentrate, 'Imperator', ICI Public Health) five days before the start of the trial. As far as we were able to ascertain from the manufacturers of the permethrin, the volatile components of the emulsifiable concentrate were the same as used in all experimental hut trials carried out in The Gambia (Lindsay *et al.*, in press b, Miller, 1990; R.J. Pleass *et al.*, unpublished data). However, the precise composition of the permethrin formulations used in the different trials was not known. Five bednets were left untreated.

Human baits

Six men slept in the huts for six weeks, with a one night break at the end of each week. The men went to bed at 22.00 hrs and left the huts at 07.00 hrs the following morning. These men were paid for their work and took regular malaria chemoprophylaxis.

Mosquito collections

Each hut was searched for 60 man-minutes, during which time mosquitoes were collected from the room, both enclosed verandahs and the two window traps. In each of the three habitats, female mosquitoes were identified according to their species. *Anopheles gambiae* (*sensu lato*) were scored as bloodfed or unfed and bloodmeals identified as human or non-human using a modification of the ELISA technique described by Burkot *et al.* (1981).

Experimental design

The design was based on a series of Latin squares (Cochran & Cox, 1957) to allocate the six sleepers to a different hut each week, the nets to different huts on each of the six nights of the week, and to allow for systematic variation between nights of the week.

Statistical analysis

Mosquito collections were related to the distance of each hut from the one with a treated net. The permethrin-impregnated bednet was used on each of the 36 nights of the study. There were 60 collections made from nets immediately adjacent to the treated net, and 48, 36, 24 and 12 made from huts at a distance of 2, 3, 4 and 5 huts from the treated net, respectively. Counts of mosquitoes were log transformed ($\log_e [n+1]$) and proportions angularly transformed ($\sin^{-1} \sqrt{p}$) to stabilize the variance. Analysis of variance was carried out using the statistical package GLIM (Payne, 1986). Estimated numbers or proportions of mosquitoes found in each hut each morning were adjusted relative to those found in hut one (sleeper one and net one) at the start of the study. Adjusted means are presented in graphical form and an estimate of the overall standard deviation (SD) was calculated. The standard error of the difference between any two means (SE) may be derived using the formula shown below:

$$SE = s\sqrt{1/n_2 + 1/n_2} \tag{1}$$

where the sample size for the two means are n_1 and n_2 respectively.

To detect whether the deterrent effect of the permethrin-treated bednet decreased over time, an estimate of deterrency was calculated for each night of the study. Nightly deterrency was determined using the following calculation:

Deterrency on night i =

$$\left(\frac{\text{no. of mosqs. in hut with treated net, night i, +1}}{\text{no. of mosqs. in 5 huts with untreated nets, night i, +1}}\right) \quad (2)$$

Linear regression analysis was carried out using GLIM to detect whether there was a significant deterioration in deterrency during the study.

Results

Mosquito abundance

During the trial 9996 *A. gambiae* (*sensu lato*), 3741 *A. rufipes* Gough, 824 *A. pharoensis* Theobald, 459 banded *Culex* spp., 384 *Mansonia africanus* Theobald, 133 *M. uniformis* Theobald and 72 *Aedes aegypti* Linnaeus were collected from the experimental huts. There were too few *A. aegypti* collected to warrant further analysis.

House entry

For all species investigated there were no significant differences between the estimated numbers of mosquitoes entering huts with untreated bednets (table 1). However in all cases, except for *A. rufipes* and banded *Culex* spp., there were significant differences between nets when the permethrin-impregnated net was included in the analysis (table 1). Thus permethrin-treated bednets significantly reduced the numbers of *A. gambiae* (*sensu lato*), *Mansonia* spp. and *A. pharoensis* entering huts compared with untreated nets (fig. 1). The standard error of the difference (SE) between any two means can be calculated from the overall standard deviation (SD) values in table 1 using formula (1). There was no evidence that the deterrency of the permethrin-treated net against *A. gambiae* (*sensu lato*) ($F = 2.7$, 1 and 24 d.f., ns) and *A. pharoensis* ($F = 0.4$, 1 and 24 d.f., NS) declined during the study. However, the reverse was true for *Mansonia* spp., where the deterrent effect of the treated net waned significantly during the trial ($F = 10.7$, 1 and 24 d.f., $P < 0.01$).

Of the 2058 *A. gambiae* (*sensu lato*) bloodmeals analysed 741 (36.0%) were of human origin and these were assumed to have come from the bait in the hut in which the specimens were collected. There was no evidence that the treated net deterred the entry of non-human bloodfed *A. gambiae* (*sensu lato*) into huts as similar numbers of mosquitoes were found in all six buildings (fig. 2, $F = 0.8$, d.f.= 5 and 165, ns). However fewer human bloodfed *A. gambiae* (*sensu lato*) were found in the hut with the treated bednet compared to those with untreated nets (fig. 2, $F = 2.2$, d.f.= 5 and 165, $P < 0.05$). There was no significant increase in human bloodfed mosquitoes in huts with untreated bednets nextdoor to a hut with a treated net relative to the more distant huts with untreated nets (fig. 2, $F = 0.5$, d.f.= 4,130, ns). Dead human-bloodfed *A. gambiae* (*sensu lato*) mosquitoes were found on more mornings in huts with the permethrin-treated net than in those with untreated nets (fig. 3, $\chi^2 = 22.9$, 5 d.f., $P < 0.001$). However the results did not show an increased mortality of human-bloodfed mosquitoes in adjacent huts with untreated nets (fig. 3, $\chi^2 = 5.0$, 4 d.f., ns).

Feeding success

Proportionately fewer *A. gambiae* (*sensu lato*) fed on a man sleeping under an untreated bednet on nights when there were large numbers of mosquitoes searching for a bloodmeal compared with when there were small numbers (fig. 4). The data appear to fall into two distinct groups; one where there was evidence of density dependent-feeding success and one where no human-bloodfed mosquitoes were collected from the huts. Hut searches when no man-fed mosquitoes were found were not evenly distributed between individual sleepers ($\chi^2 = 14.8$, d.f. = 5, $P < 0.025$), with 18 of these 30 searches occurring with the same two men. Excluding the 30 searches where no man-fed *A. gambiae* (*sensu lato*) were collected, there was a significant inverse relationship between the proportion of man-fed mosquitoes and the total number entering each hut with an untreated net ($r = -0.381$, $n = 150$, $P < 0.001$).

Discussion

Using an experimental design based on a series of Latin squares we were able to determine the relative deterrency or attractiveness of individual bednets, whilst allowing for the large nightly variation in mosquito numbers. However, a disadvantage of this design was that the trial was not balanced, with huts closer to the one with a treated net being observed more often than more distant huts.

The principal malaria vectors in the study area were mosquitoes of the *A. gambiae* complex. During the dry season *A. arabiensis* Patton predominated over *A. gambiae* (*sensu stricto*), whilst in the rainy season only *A. gambiae* (*sensu stricto*) were found in mosquitoes collected between 1987 and 1990 (Lindsay *et al.*, in press c). In this

Table 1. Results of ANOVA of numbers of mosquitoes entering experimental huts; five huts with untreated bednets and one with a permethrin-impregnated net. SD is the overall standard deviation. ns denotes P>0.10

Mosquito species	Variation between huts with						
	Untreated nets			All nets			
	F	d.f.	*P*	F	d.f.	*P*	SD
A. gambiae (*s.l.*)	0.4	4,130	ns	10.8	5,165	<0.001	0.31
A. pharoensis	0.8	4,130	ns	2.3	5,165	<0.05	0.59
Mansonia spp.	0.7	4,130	ns	3.0	5,165	<0.05	0.29
A. rufipes	1.3	4,130	ns	1.2	5,165	ns	0.26
Banded *Culex* spp.	0.4	4,130	ns	1.3	5,165	ns	0.14

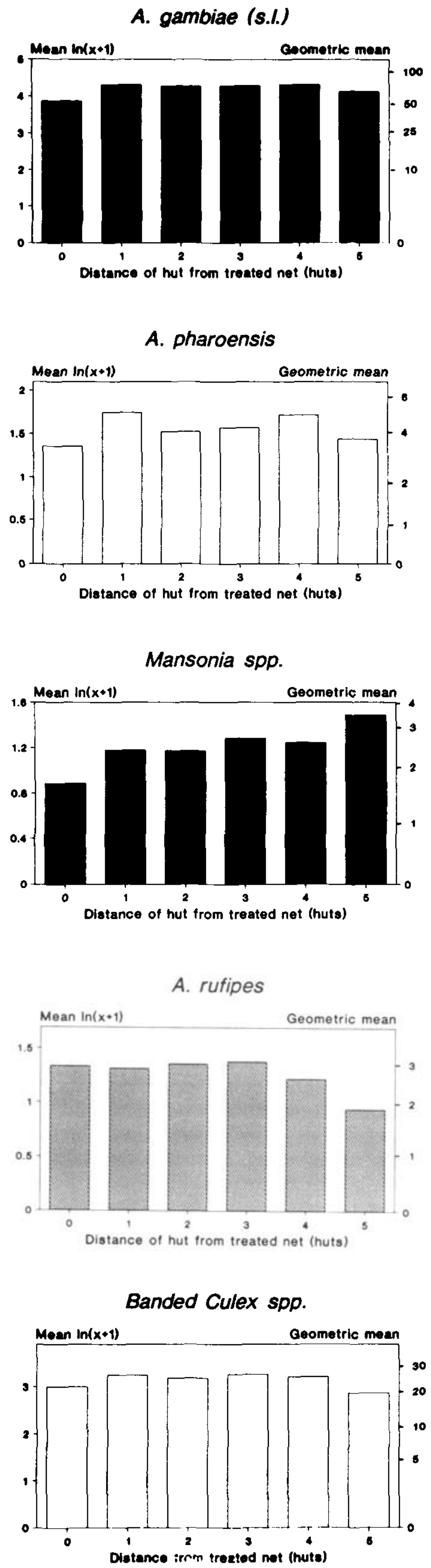

Fig. 1. Estimated and adjusted mean numbers of mosquitoes entering six man-baited experimental huts at various distances from a permethrin-treated net. Huts were some 12 m apart.

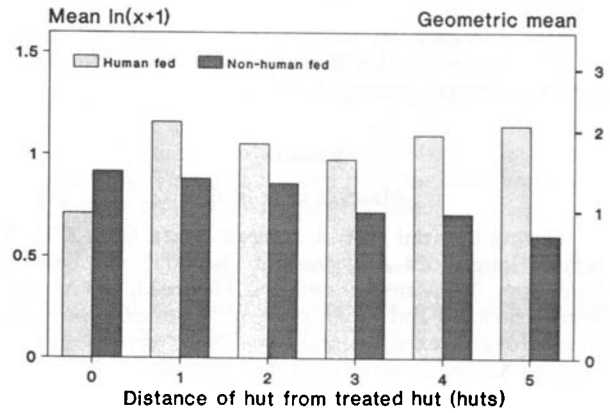

Fig. 2. Hut entry by human and non-human bloodfed *A. gambiae* (*sensu lato*) mosquitoes into experimental huts at various distances from a permethrin-treated net. Huts were approximately 12 m apart. The standard deviation for human fed mosquitoes = 0.45 and for non-human fed mosquitoes = 0.36.

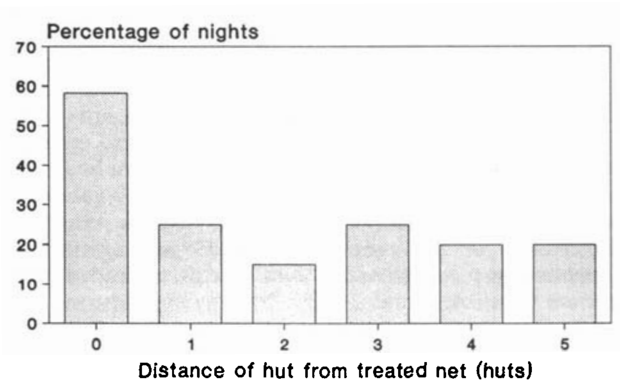

Fig. 3. Of the 174 occasions when human-fed *A. gambiae* (*sensu lato*) were found in each hut, the figure shows the proportion when mortality occurred among the man-fed mosquitoes. Huts were about 12 m apart.

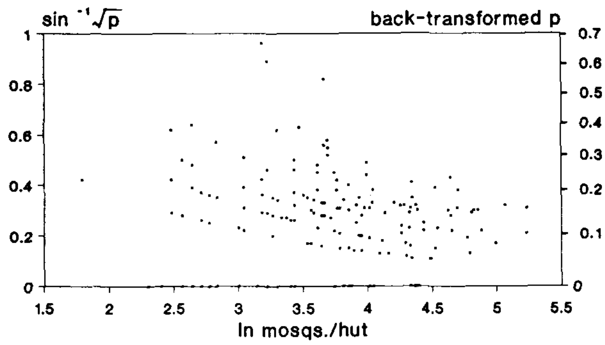

Fig. 4. Relationship between the number of mosquitoes entering a hut, excluding non-human bloodfed mosquitoes, and the proportion of mosquitoes (p) bloodfeeding on man.

Table 2. Entry rates of mosquitoes in experimental huts with permethrin-treated bednets compared to those with untreated nets (- sign indicates a lower rate with treated bednets than untreated nets, ± indicates no consistent effect).

Country	Permethrin formulation & target dosage	Source of Permethrin	Vectors	House entry	Authors
Burkina Faso	20% EC 80mg/m²	Wellcome	*A. gambiae* (*s.1.*) & *A. funestus*	-	Darriet *et al.*, (1984)
Suriname	25% EC 500mg/m²	ICI	*A. darlingi*	-	Rozendaal *et al.*, 1989
Tanzania	10% EC 200mg/m²	Wellcome	*A. arabiensis*	±	Lines *et al.*, 1987
The Gambia	25% EC 500mg/m²	ICI	*A. gambliae s.s.*	-	Miller *et al.*, in press
The Gambia	25% EC 5-500mg/m²	ICI	*A. gambiae s.s.* & *A. arabiensis*	-	Lindsay *et al.*, 1991
The Gambia	25% EC 500& 1000mg/m²	ICI	*A. gambiae s.s.*	±	Pleass *et al.*, unpublished data

dry season study, fewer *A. gambiae* (*sensu lato*) entered huts with a permethrin-impregnated bednet than those with untreated nets. This deterrent effect is probably due to volatile components of the emulsifiable concentrate, not the permethrin alone (Lindsay *et al.*, in press b). Detterrency has also been observed in other trials in the tropics where Wellcome, as well as ICI, formulations have been tested (table 2). However results from Tanzania and one trial in The Gambia were equivocal (Lines *et al.*, 1987; R.J. Pleass *et al.*, unpublished data).

Earlier studies conducted in The Gambia have shown that at night a small proportion of bloodfed *A. gambiae* (*sensu lato*) may move between houses where people use untreated bednets (Boreham & Port, 1982). However in the present study, there was no evidence that mosquitoes which fed on men sleeping under treated nets rested in adjacent buildings where the occupant had an untreated net. Both the numbers and mortality of man-fed *A. gambiae* (*sensu lato*) mosquitoes were similar in huts with untreated nets, irrespective of whether they were next door to the hut with a treated net or not.

One interesting finding was that proportionately fewer *A. gambiae* (*sensu lato*) fed on men under untreated bednets when there were high numbers of mosquitoes attempting to feed, compared with when there were few vectors. One explanation for this density-dependent feeding success was that men behaved differently when attacked by a large number of mosquitoes from when there were few biting. For example, men experiencing high levels of attack may avoid sleeping next to the side of the net where they would be bitten by mosquitoes feeding through the netting. Instances when no human-bloodfed mosquitoes were collected from a hut probably reflect the behaviour of some of the sleepers who may be particularly careful to avoid mosquito bites by tucking their nets in well and sleeping away from the walls of the net.

Whilst unfed *A. gambiae* (*sensu lato*) mosquitoes were deterred from huts with an insecticide-impregnated bednet, the entry of non-human bloodfed *A. gambiae* (*sensu lato*) (probably cattle-fed) into huts with a treated net was similar to that found in control huts. One explanation for this apparent discrepancy may be related to the relative risks a mosquito is exposed to during its life. Perhaps the most vulnerable period for a female mosquito is when she attempts to take a bloodmeal and risks being killed by a host. At this time, the sensory perception of a host-seeking mosquito may become heightened, as indicated by a general increase in activity (Jones & Gubbins, 1978; Davis *et al.*, 1987), resulting in the insect being deterred from entering a hut with a permethrin-treated net. Conversely bloodfed mosquitoes become relatively quiescent and unresponsive shortly after feeding (Jones & Gubbins, 1978) during the process of finding a resting site to assimilate their meal. Bloodfed mosquitoes may become less sensitive to various stimuli including odours emanating from a treated net, and thus enter huts regardless of whether a treated net is present or not.

The present study demonstrated that different species of mosquitoes vary in their behaviour toward permethrin-treated bednets. Although most species are to some extent deterred from entering a hut with an impregnated bednet, the presence of this insecticide failed to restrict the entry of *A. rufipes* or banded *Culex* spp. into huts. This is important since in The Gambia the deterrent properties of permethrin formulations contribute greatly to the overall reduction in human biting rates when people use treated nets (Lindsay *et al.*, in press b; Miller, 1990; Miller *et al.*, in press). However, if treated nets are introduced as a malaria control strategy in areas of the tropics where vectors are undeterred by the insecticide formulation then the effectiveness of this intervention may not be so great. We therefore recommend that further studies using experimental huts should be carried out to assess the impact of treated nets against major vectors in other parts of the world, before they are used on a large scale in the community.

Mosquitoes deterred from entering a hut with a permethrin-treated net did not enter adjacent buildings in exceptional numbers as shown by the numbers of mosquitoes found in all five huts with untreated nets being similar. Perhaps mosquitoes deterred from entering a hut with a permethrin-treated net cease their normal house-entry activities for a period of time and disperse widely. It would be of interest to know whether these displaced mosquitoes experience an increased rate of mortality as a consequence of this dispersal compared with unfed mosquitoes entering huts with treated or untreated nets.

Our present findings are supported from entomological studies carried out in Gambian villages where most people slept under permethrin-impregnated bednets (Lindsay *et al.*, unpublished data). In these communities men who slept on their own with an untreated net were used as baits for estimating the abundance of mosquitoes in each settlement. It was found that the numbers of mosquitoes entering these houses were not increased compared to similar homes in villages where the majority of people used an untreated net. This finding suggests that people sleeping under an untreated bednet in a village with insecticide-treated nets did not get bitten more frequently indoors by malaria mosquitoes than would be the case if only untreated nets were used in the community. Indeed, in a number of field trials it has been shown that the large-scale use of insecticide-treated bednets reduces malaria transmission within a village due to a fall in mosquito numbers and a decrease in the proportion of vectors with sporozoites (data of Carnevale *et al.* and Wilkes & Njunwa in Curtis *et al.*, 1989).

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References

Alonso, P.L., Lindsay, S.W., Armstrong, J.R.M., Conteh, M., Hill, A.G., David, P.H., Fegan, G., de Francisco, A., Hall, A.J., Shenton, F.C., Cham, K. & Greenwood, B.M. (1991) The effect of insecticide-treated bednets on mortality of Gambian children. *Lancet* **337**, 1499–1502.

Boreham, P.F.L. & Port, G.R. (1982) The distribution and movement of engorged females of *Anopheles gambiae* Giles (Diptera: Culicidae) in a Gambian village. *Bulletin of Entomological Research* **72**, 489–495.

Burkot, T.R., Goodman, W.G. & DeFoliart, G.R. (1981) Identification of mosquito blood meals by enzyme linked immunoabsorbent assay. *American Journal of Tropical Medicine and Hygiene* **30**, 1336–1341.

Cochran, W.G. & Cox, G.M. (1957) *Experimental designs*. 611 pp. New York, Wiley.

Curtis, C.F., Lines, J.D., Carnevale, P., Robert, V., Boudin, C., Halna, J.-M., Pazart, L., Gazin, P., Richard, A., Mouchet, J., Charlwood, J.D., Graves, P.M., Hossain, M.I., Kurihara, T., Ichimori, K., Li Zuzi, Lu Baolin, Majori, G., Sabatinelli, G., Coluzzi, M., Njunwa, K.J., Wilkes, T.J., Snow, R.W. & Lindsay, S.W. (1989) Impregnated bednets and curtains against malaria mosquitoes. pp. 5-46 *in* Curtis, C.F. (*Ed.*) *Appropriate technology for vector control*. Boca Raton, C.R.C.

Darriet, F., Robert, V., Tho Vien N. & Carnevale, P. (1984) *Evaluation of the efficacy of permethrin impregnated intact and perforated mosquito nets against vectors of malaria*. WHO mimeographed document (WHO/VBC/84.899). 12 pp. Geneva, World Health Organization.

Davis, E.E., Haggart, D.A. & Bowen, M.F. (1987) Receptors mediating host-seeking behaviour in mosquitoes and their regulation by endogenous hormones. *Insect Science and its Applications* **8**, 637–641.

Hossain, M.I. & Curtis, C.F. (1989) Permethrin-impregnated bednets: behavioural and killing effects on mosquitoes *Medical and Veterinary Entomology* **3**, 367–376.

Jones, M.D.R. & Gubbins, S.J. (1978) Changes in the circadian flight activity of the mosquito *Anopheles gambiae* in relation to insemination, feeding and oviposition. *Physiological Entomology* **3**, 213–220.

Lindsay, S.W., Snow, R.W., Broomfield, G.L., Semega Janneh, M., Wirtz, R.A. & Greenwood, B.M. (1989) Impact of permethrin-treated bednets on malaria transmission by the *Anopheles gambiae* complex in The Gambia. *Medical and Veterinary Entomology* **3**, 263–271.

Lindsay, S.W., Hossain, M.I., Bennet, S. & Curtis, C.F. (in press a) Preliminary studies of the insecticidal activity and wash fastness of 12 synthetic pyrethroid treatments impregnated into bednetting assayed against mosquitoes. *Pesticide Science*.

Lindsay, S.W., Adiamah, J.H., Miller, J.E. & Armstrong, J.R.M. (in press b) Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. *Medical and Veterinary Entomology* **5**.

Lindsay, S.W., Wilkins, H.A., Zieler, H.A., Daly, R.J., Petrarca, V. & Byass, P. (in press c) Ability of *Anopheles gambiae* mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. *Journal of Tropical Medicine and Hygiene* **94**.

Lines, J.D., Myamba, J. & Curtis, C.F. (1987) Experimental hut trials of permethrin-impregnated mosquito nets and curtains against malaria vectors in Tanzania. *Medical and Veterinary Entomology* **1**, 37–51.

Miller, J.E. (1990) Laboratory and field studies of insecticide impregnated fibres for mosquito control. 336 pp. PhD Thesis, University of London.

Miller, J.E., Lindsay, S.W. & Armstrong, J.R.M. (in press) Experimental hut trials of bednets impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia. *Medical and Veterinary Entomology* **5**.

Payne, C.D. (1986) *The GLIM system release 3.77 Manual*. 183 pp. Oxford, Numerical Algorithms Group.

Rapley, R.E. (1961) Notes on the construction of experimental huts. *Bulletin of the World Health Organisation* **24**, 659–663.

Rozendaal, J.A. (1989) Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Diseases Bulletin* (Supplement) **86**, 1–41.

Rozendaal, J.A., Voorham, J., Van Hoof, J.P.M. & Oostburg, B.F.J. (1989) Efficacy of mosquito nets with permethrin in Suriname. *Medical and Veterinary Entomology* **3**, 353–365.

Service, M. (1976) *Mosquito ecology: field sampling methods.* 583 pp. London, Applied Science Publishers.

Smith, A. (1965) A verandah-trap hut for studying the house frequenting habits of mosquitoes and for assessing insecticides. I. A description of the verandah-hut and of studies of the egress of *Anopheles gambiae* (Giles) and *Mansonia uniformis* (Theo.) from an untreated hut. *Bulletin of Entomological Research* **56**, 161–165.

Snow, R.W., Jawara, M. & Greenwood, B.M. (1987) Observations on *Anopheles gambiae* Giles *s.l.* during a trial of permethrin treated bednets in The Gambia. *Bulletin of Entomological Research* **77**, 279–286.

Snow, R.W., Lindsay, S.W., Hayes, R.J. & Greenwood, B.M. (1988) Permethrin-treated bed nets (mosquito nets) prevent malaria in Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 838–842.

Wells, D., Grayson, B.T. & Lagner, E. (1986) Vapour pressure of permethrin. *Pesticide Science* **17**, 473–476.

WHO World Health Organisation (1989) *The use of impregnated bednets and other materials for vector-borne disease control.* WHO mimeographed document (WHO/VBC/89.981). 45 pp. Geneva, World Health Organization.

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