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Insecticide treated nets: impact on vector populations and relevance of initial intensity of transmission and pyrethroid resistance

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Insecticide treated bednets locate a deposit of a quick-acting insecticide of low human toxicity between a sleeper and host-seeking mosquitoes. Thus a chemical barrier is added to the often incomplete physical barrier provided by the net. Treated nets may be considered as mosquito traps baited by the odour of the sleeper. Trials in Assam, Tanzania and elsewhere have shown that when a whole community is provided with treated nets, so many mosquitoes of anthropophilic species are killed by contact with the nets that the density and/or sporozoite rate of the vector population is reduced. In order to gain this "mass" or community effect, in addition to widespread personal protection, and thus to achieve the full potential of the treated net method, a high per cent coverage of the community is needed. This suggests that organised free provision of treated nets, comparable to a house spraying programme, is likely to be more cost-effective than trying to market nets and insecticide to very poor rural people. In areas with high malaria transmission, where acquisition of immunity to malaria is very important, it has been argued that vector control (without vector eradication) could, in the long run, make the situation worse by preventing the normal build-up of immunity. However, our data from Tanzania do not support this idea—3–4 years after provision of nets (which are re-treated annually) young children are still showing clear health benefits; older children are not "paying" for this by showing worse impact of malaria. There is less malaria morbidity in a highland area where malaria transmission is about 15x less intense than in a nearby lowland area. The per cent impact of treated nets malaria morbidity in both areas was very similar. At present only pyrethroids are used for net treatment which suggested that emergence of pyrethroid resistance would have a disastrous effect. However, in West Africa, where there is now a high frequency of the *kdr* resistance gene in *Anopheles gambiae,* it is reported that treated nets continue to have a powerful impact on vector populations. In Tanzania, pyrethroid resistance has not been detected in malaria vectors, but it has emerged in bedbugs after seven years use of treated nets.

Key words Insecticide treated bednets – malaria transmission – permethrin resistance – vector control

Effect of nets and insecticide treatment of them on vector populations and on malaria incidence

Insecticide treated nets provide both a physical and a chemical barrier against vector mosquitoes. Table 1 shows three measures of malaria incidence in one of the first trials of treated nets in India, carried out in Assam[1]. There was a rise in malaria incidence, relative to the baseline year, in villages which lacked nets due to the withdrawal of DDT spraying. The physical barrier of untreated nets was enough to prevent this, but only by the use of pyrethroid (deltamethrin) treatment of the

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nets was a reduction in incidence achieved, relative to the baseline year.

Table 1. Data from Sonapur[1] Assam on per cent change in malaria parameters compared with the baseline year with treated or untreated nets or no nets

Nets	Slide +ve rate	Slide falciparum rate	Monthly parasite index
None	+64%**	+86%**	+178%**
Untreated	–3.5% ns	1.6% ns	18.1% ns
Deltamethrin treated	–68%***	–66%***	–80%***

Asterisks indicate singnificant changes relative to the baseline year.

In rural, lowland Tanzania most children are infected with *Plasmodium falciparum* most of the time. In order to measure incidence of infection we have to clear existing infections with the drug combination chlorproguanil-dapsone (which is effective where there is resistance to sulfadoxine-pyrimethamine) and then we monitor the children weekly for re-infection. Table 2 shows that with untreated nets the reduction of incidence of re-infection was only of borderline statistical significance. However, in villages with universal use of nets treated with either of two pyrethroids, incidence was highly significantly reduced[2]. We conclude that, under these conditions, the chemical barrier is more important than the physical barrier provided by the net.

Table 2. Effect of untreated or treated nets on incidence of reinfection in Tanzanian children after clearing of existing infections with chlorproguanil-dapsone[2]

Nets	Probability of reinfection per child week (%)	Significance of differences
No nets	21.7	
Untreated nets	14.6	$p = 0.06$
Lambdacyhalothrin nets	3.7)	$p < 0.001$
Alphacyperthmethrin nets	4.2)	

We consider that the great importance of the pyrethroid deposit on the net arises because the body odour of the sleepers attracts human-seeking (anthropophilic) mosquitoes to make repeated contact with the nets and many are killed. Thus, with widespread usage of treated nets, mosquito mortality would be so high that one could expect a reduction in the mean mosquito age and hence a major reduction in the proportion living long enough for *Plasmodium* to develop to the sporozoite stage. Mean mosquito physiological age can be measured by the Polovodova method[3] using skilful dissection to observe the mean number of ovarian dilatations, as well as the sporozoite rate, in large mosquito samples. T.J.Wilkes carried out such dissections on *An. gambiae* from traditional and from more modern Tanzanian villages[4]. He found that, even before intervention, the mean physiological age and sporozoite rate were less in the latter type of village (Tables 3 & 4),

Table 3. Data of T.J. Wilkes on ovarian age grade (Polovodova method) before and after introduction of permethrin treated nets or DDT spraying in Tanzanian villages[4]

Village	Before intervention	Intervention	After intervention
Mng'aza (mud & thatch)	1.255 (n = 1533)	Permethrin nets	0.792 (n = 1195)
Mindu (mud & thatch)	1.229 (n = 506)	DDT	0.400 (n = 95)
Mlingano (brick & iron)	0.764 (n = 292)	Permethrin nets	0.518 (n = 160)

Table 4. Sporozoite rates before and after introduction of permethrin treated nets or DDT spraying in Tanzanian villages[4]

Village	Before intervention	Intervention	After intervention
Mng'aza (mud & thatch)	6.1% (n = 1344)	Permethrin nets	2.3% (n = 2181)
Mindu (mud & thatch)	7.9% (n = 379)	DDT	2.5% (n = 201)
Mlingano (brick & iron)	4.9% (n = 217)	Permethrin nets	0.6% (n = 311)

presumably because the iron-roofed houses become hotter at mid-day and thus less conducive for mosquito survival. In both types of villages DDT house spraying or introduction of nets treated with a pyrethroid (permethrin) reduced both mean age and sporozoite rate compared with that of the pre-intervention level in the village concerned.

The relative importance of personal protection and the mass effect of treated nets and the arguments for free provision of treated nets

In Assam, the relative importance of the personal protection of the individual sleeper and the community-wide, "mass" effect of treated nets on the vector populations was assessed by landing catches on human "baits" either provided with no net, or a partially raised net, with or without pyrethroid treatment[1]. For the highly anthropophilic species and main local vector, *An. minimus,* as well as for other anopheline species, there were significant effects of the pyrethroid deposit in reducing the likelihood of a mosquito finding its way into a net, as shown by the fact that the catch was significantly reduced if the "bait" lay under a treated rather than an untreated net (Table 5). However, when catches in villages with no nets, untreated nets or treated nets were compared, only for *An. minimus* was a significant difference seen between these villages. We interpret this inter-species difference as indicating that only for members of a highly anthropophilic species is there a major risk of death from contact with a treated net–other species would greatly reduce this risk by taking many of their blood meals from animals. With *An. mimimus* there was an unexpected difference between the mosquito catch in villages without nets or with untreated nets. This might suggest that, for this anthropophilic species, the universal use of untreated nets was making it so difficult for mosquitoes to find blood meals that some mosquitoes were dying from starvation.

Table 5. Data[1] on individual and community effects of nets on human landing catches of *Anopheles* species in Sonapur, Assam

Anopheles species	Individual/ Whole village	No net	Untreated net	Deltamethrin net
minimus	Individual	4.4^{a}	3.9^{a}	0.3^{b}
minimus	Whole village	5.5^{a}	2.8^{b}	0.3^{c}
Others	Individual	15.7^{a}	9.1^{b}	1.7^{c}
	Whole village	6.5^{a}	7.0^{a}	6.5^{a}

Data in same row with different superscripts differ significantly.

Table 6 shows data on the comparison in Tanzania of the "mass" and the personal protection effects[5]. The former was monitored from light-trap catches in rooms with untreated nets situated in villages, either with no nets or with wide-spread use of treated nets. Both the number of *An. gambiae* caught and their sporozoite

Table 6. Mass effect and personal protection of treated nets[5]

	Village vector pop*	Sporozoite rate (%)	EIR	% reduction due to mass effect
Villages				
Netted	1374	2.73	37.5	74.7
Control	3339	4.45	148.6	
	Blood fed**	**Village vector pop**	**% biting**	**% reduction due to personal protection**
Rooms				
Netted	166	1374	12.1	73.7
In control villages	1514	3339	45.3	

* From light traps; **From spray and exit trap catches.

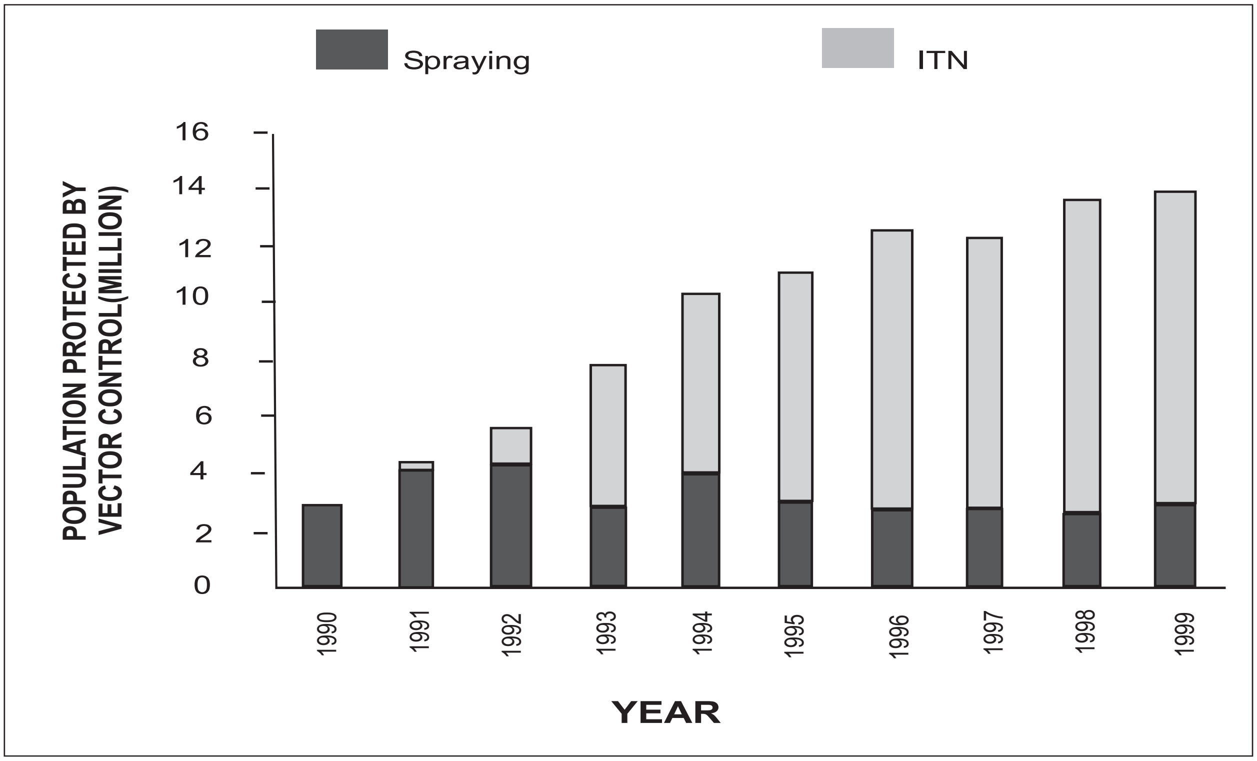

Fig. 1: Population (millions) in Vietnam protected by house spraying or by insecticide treated nets (ITN)

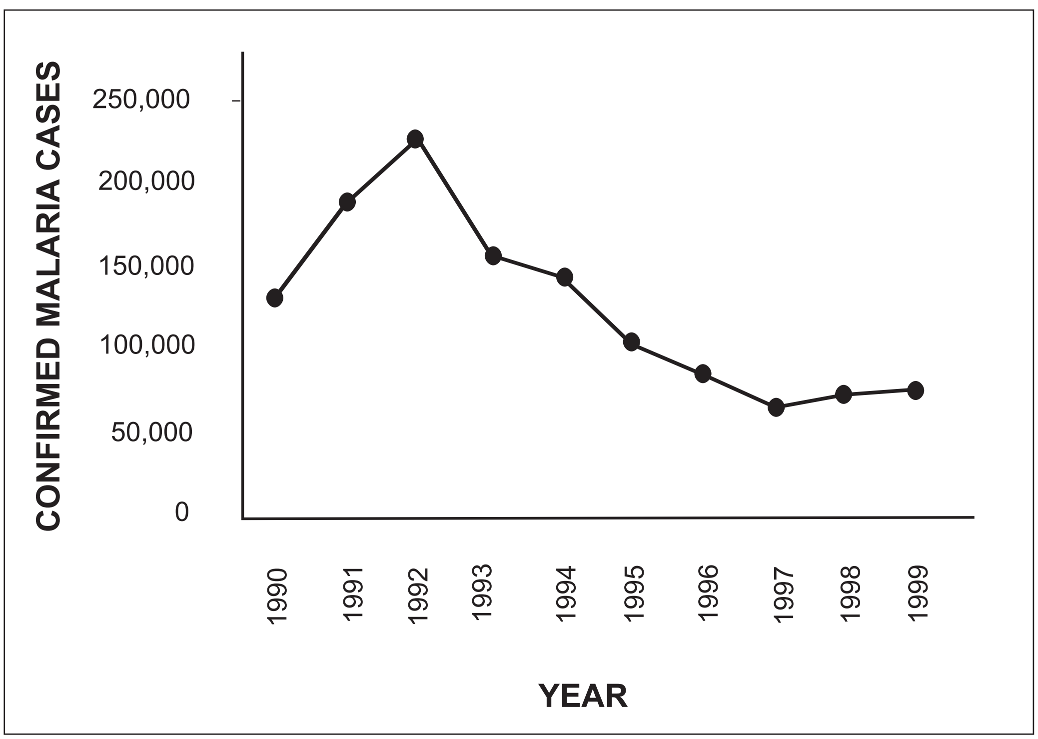

Fig. 2: Malaria cases in Vietnam in 1990’s

rate were reduced in the latter villages, so that the overall effect on the entomological inoculation rate (EIR—the number of infective bites per person per year) was a reduction by 74.7%. Catches of blood fed mosquitoes resting in rooms with treated nets in the treated villages (as well as in window traps set to catch mosquitoes exiting from these rooms) were much reduced compared with rooms without nets in villages without nets. Part of this effect can be explained by the reduction in mosquito populations already demonstrated in the netted villages. When one corrects for that effect, the personal protection effect alone in reducing the chances that a mosquito would succeed in biting through a treated net was found, in this trial, to be a 73.7% (very similar in scale to the mass effect). We conclude that, if percentage coverage of the beds in a community was too low to achieve an appreciable mass effect, about half of the potential benefit of the method would be lost even for those people who *did* have nets (in some of our other trials the relative importance of the mass effect was even greater than in the data summarised in Table 6).

Thus it is in everyone's interest to make sure that as many people as possible in a community are using treated nets–if the poor are left without treated nets they will infect mosquitoes with *Plasmodium* which can later be transmitted to the more affluent. We, therefore, disagree[6] with the quite widely held view (e.g. WHO)[7] that children should only be protected from malaria if their parents can be persuaded to pay for nets and insecticide. In our experience the productivity is extremely high of teams who go to villages and, by arrangement with the local authorities, and provide nets, and/or the insecticide to treat them, for everyone in the village in one or two day's work[8]. We can do the job much more quickly, comprehensively and equitably than those trying to market nets, because our team is not encumbered by having to handle money or to decide who deserves a subsidised net and who does not; also we generally do not have to provide storage space for nets because we can arrange for delivery from the factory just in time for the net distribution day.

Fig. 1 shows data on population coverage by the national programme in Vietnam which provides indoor residual spraying or insecticide treatment of nets (ITN) free of charge[9]. As the net treatment programme has built-up during the 1990s, so that more than 10 million people are now provided for, the national malaria incidence has declined remarkably (Fig. 2). Malaria deaths have dropped almost to zero during this period; this is at least partly due to adoption as first line antimalarial drugs of compounds with improved effectiveness against multi-drug resistant *P. falciparum*.

Data on factory treated nets which are claimed to be more wash-resistant than conventionally treated nets

The need to re-treat nets in the field may eventually be removed if factory applied insecticide deposits can be made reliably wash-resistant. To evaluate one of these products, we and our colleagues, S. Magesa and M. Kayedi, carried out two series of bioassays involving observation of the median time in contact with the nets before mosquitoes were knocked down (Table 7). In one series, the factory product was found not significantly more wash-resistant than nets conventionally treated with alpha-cypermethrin. In the second series with nets treated in the factory on a different occasion, a distinct advantage for the factory product was found. It would seem that quality control of this product was

Table 7. Bioassays (median time for knock-down) of Permanets which are sold as carrying a wash-resistant insecticide deposit-compared with conventionally treated net

	Permanets bought in 2000	Alphacypermethrin treated nets
Unwashed	441 sec	399 sec
After 20 washes	1289 sec	1252 sec
Data of M. Kayedi	Permanets bought in 2001	K-O tab treated nets
After 15 washes	633 sec	982 sec

not adequate in 2000 when the first batch was purchased.

Effects of longer term use of treated nets on malaria morbidity

It has been established from many trials that treated nets significantly reduce morbidity and mortality due to malaria in the year or two after they are introduced[10]. However, it has been argued that in intensely malarious areas that the longer term effects might be counter-productive because reduction (without elimination) of malaria transmission might interfere with the normal acquisition of anti-malaria immunity and might postpone malaria infections to later in childhood when the effects might be more serious[11]. There is some evidence for lower levels and less diversity of antibodies where there has been prolonged use of treated nets[12]. To investigate the possibility that this might lead to more malaria morbidity, we have followed up the children three and four years after introduction of nets into Tanzanian villages, the nets being re-treated annually. Table 8 shows data, for infants aged less than two years on fever associated with high malaria parasitaemia and on anaemia (defined as < 8 g Hb/dl). Comparing villages without nets and with nets which had been in use for 3–4 years, the nets were clearly beneficial[13]. Within the netted villages, infants with little or no personal protection, because they lacked their own nets or had badly torn nets, still benefited from the mass effect of their neighbours' nets (Table 8). For children aged 6–12 years, there was much less malaria morbidity and little or no evident benefit from the nets (Table 9). However, it is important to emphasise that there was no sign that they were "paying for" the reduced malaria transmission which they experienced earlier in childhood by showing worse morbidity later in life. The data for children aged 2–5 years are not shown here, but were intermediate between those shown in Tables 8 and 9[13].

Effects of treated nets in areas differing in initial transmission intensity

Partly arising from the above described fears about the adverse effects of vector control on build-up of immunity, it has been considered that, in areas of intense transmission, vector control should not even be attempted, but that this should be reserved for areas of more moderate transmission—in highlands[14]. We have investigated this idea in nearby areas in northeast Tanzania[5] at 200 and at 1000 m. Vector biting was far less intense in the cooler highlands. Though there was not much difference detected in this trial in sporozoite rates in the control villages without the interventions at the two altitudes, overall the EIR differed about 15 fold between the two areas (Table 10). The mass effects of introduction of treated nets at both altitudes reduced the EIR by 70–75%, as a joint effect of reduction of numbers and sporozoite rates.

Table 8. Malaria morbidity in children aged < 2 years in Tanzanian villages where treatd nets had been used for 3.5 years or which had no nets[13]

	Villages without nets	Villages with treated nets		
		Children without nets	Children with torn nets	Children with intact nets
Malaria fever*	11.8%	6.8%	1.3%	1.8%
< 8 g Hb/dl	54.3%	21.2%	22.1%	19.8%

*Temp > 37.4 °C and/or reported fever with > 4000 parasites/µl.

Table 9. Malaria morbidity in children aged 6–12 years in Tanzanian villages where treatd nets had been used for 3.5 years or which had no nets[13]

	Villages without nets	Villages with treated nets		
		Children without nets	Children with torn nets	Children with intact nets
Malaria fever*	3.4%	3.7%	3.1%	3.7%
< 8 g Hb/dl	3.8%	3.0%	1.1%	2.0%

*Temp > 37.4°C and/or reported fever with > 4000 parasites/µl.

Table 10. Intensity of transmission by vectors in highland (1000 m) and lowland (2000 m) Tanzanian villages with or without nets[5]

	Highland		Lowland	
	No nets	Nets	No nets	Nets
Bites/person/yr	282	116	3339	1374
Per cent with sporozoites	3.68%	2.74%	4.45%	2.73%
EIR	10.4	3.2	148.6	37.5
% reduction due to nets	69.2%		74.8%	

Table 11 shows results pooled for all age groups of children <13 years old, in the highland and lowland villages with and without the nets[5]. In the highland villages without the nets, morbidity was markedly less than in lowland villages without nets[15], thus providing further evidence to set at rest fears of a paradoxical inverse relationship between intensity of transmission and level of morbidity. The treated nets had a beneficial effect in both areas, the benefit being by about 60% for both measures of morbidity in both areas. We are still analyzsing data on more severe consequences of malaria in these villages.

Pyrethroid resistance

There is evidence that in South Africa pyrethroid resistance in *An.funestus* built-up, with a consequent serious rise in malaria cases, after only five years of use of pyrethroids for house spraying[16]. These mosquitoes remained DDT susceptible and a reversion to DDT spraying has been associated with a dramatic improvement in the malaria situation.

Because at present only pyrethroids are used for net treatment, it was feared that evolution of pyrethroid resistance, where treated nets are widely used, would have as bad an effect as it had in sprayed villages in South Africa. However, current indications are that in areas of West Africa where there is a very high frequency of the *kdr* resistance gene, treated nets still have a highly significant effect on vector densities and sporozoite rates and on malaria incidence[17]. It is believed that this fortunate result comes about because the *kdr* carrying mosquitoes show abnormally low irritability by pyrethroids and therefore, remain in contact with treated nets long enough to pick up the relatively high dose of pyrethroid which is required to kill them.

In Tanzania we have found no pyrethroid resistance in *An. gambiae* or *An. funestus,* even where pyrethroids are used against cotton pests. However, recently we have found pyrethroid tolerance in *Cimex hemipterus* bedbugs in villages where pyrethroids have been in use for seven years and where the bedbug populations were eradicated when the treated nets were first introduced[18]. We are investigating non-pyrethroid alternatives or mixtures to try to kill off these bug populations whose continued presence might much reduce villagers' incentive to use and retreat their nets.

Conclusion

We remain on the look out for unexpected adverse side-effects of sustained use of treated nets in various

Table 11. Preliminary data on malaria morbidity in children in highland and lowland Tanzanian villages with or without treated nets[5]

	Highland			Lowland		
	No nets	Nets	% reduction	No nets	Nets	% reduction
Malaria fever	4.7 %	2.1 %	55 %	10.2 %	4.2 %	59 %
< 8 g Hb/dl	8.8 %	2.8 %	68 %	17.7 %	6.9 %	61 %

situations. However, we are now cautiously optimistic that one can advocate a major effort by the world community to provide treated nets for all malarious villages, with confidence that, whilst this will not eradicate malaria, it will provide a substantial and sustainable improvement.

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