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Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets

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Abstract

Background: Highland areas with naturally less intense malaria transmission may provide models of how lowland areas might become if transmission was permanently reduced by sustained vector control. It has been argued that vector control should not be attempted in areas of intense transmission.

Methods: Mosquitoes were sampled with light traps, pyrethrum spray and window exit traps. They were tested by ELISA for sporozoites. Incidence of malaria infection was measured by clearing existing infections from children with chlorproguanil-dapsone and then taking weekly blood samples. Prevalence of malaria infection and fever, anaemia and splenomegaly were measured in children of different age groups. All these measurements were made in highland and lowland areas of Tanzania before and after provision of bednets treated with alphacypermethrin.

Results: Entomological inoculation rates (EIR) were about 17 times greater in a lowland than a highland area, but incidence of infection only differed by about 2.5 times. Malaria morbidity was significantly less prevalent in the highlands than the lowlands. Treated nets in the highlands and lowlands led to 69–75% reduction in EIR. Malaria morbidity showed significant decline in younger children at both altitudes after introduction of treated nets. In children aged 6–12 the decline was only significant in the highlands

Conclusions: There was no evidence that the health benefits to young children due to the nets in the lowlands were "paid for" by poorer health later in life. Our data support the idea of universal provision of treated nets, not a focus on areas of natural hypo-endemicity.

Background

The policy of the Roll Back Malaria programme of WHO has put strong emphasis on the introduction of insecticide treated nets throughout Africa [1]. However, this is contrary to the view common until quite recently, and is still held by some [2,3], that any attempts at vector control should be focused on hypo-endemic areas e.g. in highlands. It is suggested that, in areas of stable malaria with intense transmission in lowland Africa, attempts to control malaria by intervention against vectors would be futile, or even counter-productive. It is considered that, in such areas, reducing transmission would interfere with normal build-up of immunity so that early reduction in morbidity would be "paid for" by postponement of morbidity until a later age, so that the lifetime burden of malaria would not decline and might actually increase. Furthermore, reduction in transmission might convert stable malaria to epidemic malaria, which might exact a higher death toll.

The existing data on insecticide-treated nets are mostly based on short-term trials [4], but Maxwell *et al* [5] have reported encouraging results in eight Tanzanian villages which have had community-wide use of nets (re-treated annually) for up to 4 years. Another approach to settling the issue of the effects of long-term exposure to either intense, or more moderate, transmission is to study malaria morbidity in different age groups in nearby areas which differ naturally and semi-permanently in vector density, due to a difference in altitude. Ellman *et al* [6] compared malaria morbidity in the East Usambara mountains of Tanzania with that in culturally similar people in the nearby lowlands, which have much greater densities of vectors. They found markedly less morbidity in the highlands and a clear decline of prevalence of morbidity with age at both altitudes. However, children were only studied up to 5 years of age which, it could be argued, was not enough to completely exclude the delay of morbidity into early adolescence, reported by Trape and Rogier [2].

This paper reports a study, in the same highland and lowland areas where Ellman *et al* [6] worked, which compared the areas by entomological measures of transmission, incidence of *Plasmodium falciparum* infection after clearing existing infections and malaria morbidity up to the age of 12 years. It also reports on the impact of introducing insecticide-treated nets in both areas to determine whether better results would be obtained by focusing anti-vector measures on areas of lower initial rates of malaria transmission.

Clearance for the studies was granted by the ethical committees of the Tanzanian National Institute for Medical Research and the London School of Hygiene & Tropical Medicine.

Methods

Twenty one hamlets (*vitongoji* in Ki-Swahili, i.e. sub-communities of politically defined villages (*vijiji*)) were included in the study which fell into distinct categories of 11 highland hamlets (altitudes 784 to 1148 metres) and 10 lowland hamlets (199 to 300 metres). In the first (baseline) year, the vector population, incidence of infections and measures of morbidity were studied in the two areas without any vector control. In the second year, bednets treated with 20 mg alphacypermethrin/m^2 of netting were provided free of charge to protect all beds and sleeping mats in five of the highland and five of the lowland hamlets. In some hamlets the aqueous mix of insecticide was provided by appropriate dilution of Fendona SC and, in others, of granular Fastac. Bioassays were carried out on treated nets by wrapping them round wire frames and inserting wild collected *Anopheles funestus* inside and observing either (a) % mortality 24 hrs after a 3 minute exposure, or (b) the median time for knock-down with continuous exposure [7]. The nets were not re-treated until 15 months after the first treatment, i.e. after the end of the observations on the vector populations reported in this paper.

Mosquito populations were monitored in each hamlet by CDC light traps set beside occupied, untreated bednets [8] monthly in each of eight "sentinel" bedrooms per hamlet. The anophelines caught by these indoor traps were found by morphological examination to consist almost entirely of *Anopheles gambiae s.l.*, *An. funestus* and *Anopheles marshallii s.l.*, all of which have been incriminated as vectors in this area [9]. The heads and thoraces of these species were tested by ELISA for *P. falciparum* circumsporozoite protein (CSP) [10]. In the second year of the study, resting mosquitoes were collected by pyrethrum spray catch monthly in five bedrooms per lowland hamlet and in window exit-traps on these bedrooms. In the netted hamlets these collections were made in bedrooms with treated bednets. Anophelines were classified as bloodfed (or semi-gravid) or unfed and the total numbers and the numbers of bloodfed individuals were compared in the netted and untreated villages. After adjusting for the differences in the hamlet populations of anophelines found by the light traps, the ratio of the numbers of fed mosquitoes was used as a measure of the protection from biting conferred to the individual net users by the treated nets.

In the wet season (April-June) of the baseline year, incidence of infection was tested in four lowland and four highland hamlets after clearing existing infections with chlorproguanil-dapsone (lap-dap), as in several previous studies [11–13]. Thick bloodfilms were taken from 60 children per hamlet each week for eight weeks after lap-dap treatment. The results were expressed as numbers converting from parasite negativity to positivity per child week at risk.

Morbidity was monitored monthly by requesting the attendance of 50 children from each hamlet at monitoring sessions. The children's names for each session were picked at random from our census lists, after excluding those who had attended in the previous month. The following parameters were measured on each child attending: (a) malaria parasite presence and density per 200 w.b.c. in thick blood films, (b) core body temperature as measured with a Thermoscan thermometer placed first in

Table 1: Bioassays with wild collected *An. funestus* on domestically used nets which had been treated with each of the formulations of alphacypermethrin

Formulation	Months since treatment	No. nets tested	Mean no. washes reported	% mortality 24 h after 3 min exposure (no. mosq.)	KT50 (secs) (95% confidence limits)
Fastac granules	6	7	2.3	97.7% (88)	no data
	15	10	2.7	99.3% (154)	517(390,643)
	Retreated	9	---	100% (77)	385 (306,464)
Fendona SC	6	8	1.6	100% (117)	no data
	15	8	3.5	100% (87)	375 (296,454)

one ear and then the other, (c) haemoglobin density measured with a Hemocue machine, (d) weight and (e) presence and grade of splenomegaly on the Hackett scale, as assessed by palpation by an experienced medical aide. The age of each child was determined, where available, from the hospital birth registration or MCH card or, if it was not available, by questioning the parent or guardian. They were also questioned about occurrence of fever episodes in the child in the previous two days.

Children found positive for infection were given antimalarial treatment according to Tanzanian guidelines.

Results and Discussion

Entomological data

The granular Fastac formulation of alpahcypermethrin was found preferable to the concentrated Fendona SC liquid for use in the field because, in the event of spillage on to the skin, the granules could be easily brushed off but the concentrated liquid needs to be carefully washed off. With both formulations, bioassays involving 3 minutes exposure and 24 hrs holding yielded mortalities very close to 100% even after 15 months domestic use and about 3 washes (Table 1). The mean median times for knockdown 15 months after net treatment suggested that the nets treated with Fendona had higher insecticidal activity than those treated once with Fastac and that re-treatment boosted insecticidal activity of the latter. However, the 95% confidence limits of the means overlapped, so no definite conclusion can be drawn about apparent differences in insecticidal activity.

Table 2 shows the data from CDC light trap catches in rooms with untreated bednets in the four quarters of each year of the study. Data are also presented on the proportion of mosquitoes found CSP positive; there were few mosquitoes for testing in the dry seasons and no obvious seasonal trend in CSP rates, so the CSP data are presented for each whole year. The hamlets are grouped into those assigned in the second year in the highlands and in the lowlands to receive treated bednets and those assigned not to receive them. The following points are evident:-

- Anopheline densities were more than 12 times greater in the lowlands than the highlands.

- Anopheline densities peaked in April-June, the main rainy season.

- *An. marshallii s.l.* formed a considerable proportion of the total indoor anopheline populations in the highlands, especially between July and December; in the same months *An. funestus* was the majority species at both altitudes; *An. gambiae s.l.* dominated the anopheline populations in the main rainy season, when anopheline populations were largest, especially in the lowlands. The sibling species of the *An. gambiae s.l.* were not identifed during this study as they are generally found to be all *An. gambiae s.s.* [14] in this area except for some *An. arabiensis* which were found after very heavy El Nino associated rainfall [9].

- The CSP (sporozoite) rate appeared to be slightly (but not significantly) greater in the lowlands than the highlands.

- In the first year the means of the population densities and CSP rates, and hence the EIR values, for each of the groups of hamlets in the highlands and the lowlands showed that these groups were comparable.

- In the second year, anopheline population densities were less at each altitude even where no nets were introduced, presumably for climatic reasons; the CSP rates did not change in these groups of villages without nets in the second year.

- Where treated nets were introduced, the anopheline densities showed significant reductions compared with the untreated contemporary controls in 6 out of 8 of the comparisons at the same season and altitude. In the other 2 cases the differences were in the expected direction but were not significant.

Table 2: Data leading to estimates of entomological inoculation rates in highlands and lowlands with and without treated nets

	HIGHLAND						LOWLAND					
Hamlet:-	MI, M2, M3, M6, M8, M9			M4, M5, M7, M10 M11			B1, B3, B5, B6, B8			B2, B4, B7, B9, B10		
	Geom Mean	% Af	% Am	Geom Mean	% Af	% Am	Geom Mean	% Af	% Am	Geom Mean	% Af	% Am
Oct-Dec'98	1.65^{a}	60	20	1.60^{a}	43	45	10.40^{b}	62	6	13.0^{b}	36	4
Jan-Mar'99	1.57^{a}	59	10	1.26^{a}	45	16	31.47^{b}	7	0	31.59^{b}	4	0
Apr-Jun'99	3.65^{a}	30	6	3.96^{a}	35	7	47.70^{b}	11	1	67.49^{b}	8	0
Jul-Aug'99	1.27^{a}	63	22	0.67^{a}	38	35	6.09^{b}	55	11	7.52^{b}	39	2
Total on 4 trap nights	8.14			7.49			95.66			119.60		
Bites/pers/year^{j}	1114			1025			13093			16370		
%CSP +ve (no. tested) [15]	3.66% (709)			3.97% (478)			4.33% (2681)			4.49% (1381)		
EIR/year	40.1			37.4			566.9			735.0		
	NO NETS			NETS IN			NO NETS			NETS IN		
Oct-Dec'99	0.95^{a}	50	29	0.26^{b} s;f [0.11 0.34]	18	79	1.12^{a}	55	19	0.31^{b} s;f [0.35 0.28]	60	13
Jan-Mar'00	0.52^{a}	75	4	0.10^{b} s;f [0; 0.12]	20	40	1.53^{C}	26	0	0.40^{a} s;f [0.50 0.35]	11	0
Apr-Jun'00	1.19^{a}	49	8	0.28^{b} s;f [0.34 0.24]	33	37	11.76^{c}	19	1	7.29^{c} s;f [5.52 8.76]	12	0
Jul-Aug'00	0.31^{a}	44	28	0.21^{a} s;f [0.17 0.24]	27	27	9.99^{b}	68	3	2.04^{c} s;f [1.59 2.51]	58	0
Total on 4 trap nights	2.06			0.85			24.40			10.04		
Bites/pers./year^{j}	282			116			3339			1374		
%CSP +ve (no. tested) [16]	3.68% (380)^{a}			2.74% (73)^{a}			4.45% (629)^{a}			2.73% (916)^{a} s:f [2.4; 3.0%]		
EIR/year	10.4			3.2			148.6			37.5		
Reduction in EIR	69.2%						74.8%					

Geometric means of light trap catches in rooms with untreated nets in highland and lowland villages in the four seasons of the years before and after introduction of alphacypermethrin treated nets into half of the villages. From these data and data on sporozoite (CSP) rates determined by ELISA, entomological inoculation rates per person per year are calculated. Means in the same row with the same superscript do not differ significantly (t test, Kruskall Wallis or χ^2); ^{j}calculated from mean catch on 4 nights multiplied by 365/4 × 1.5 [8]; the species composition of the anopheline vector population is indicated in terms of the percentage that were *An. funestus (Af)* and *An. marshallii s.l. (Am):* the remainder were *An. gambiae s.l.* [Data in square brackets marked by s;f indicate light trap catches and CSP rates in villages with nets treated respectively with the SC or the Fastac (granular) formulations of alphacypermethrin]

- The apparent impact of the SC formulation of alphacypermethrin was greater than that of the Fastac formulation in 5 out of 8 of the comparisons at the same season and altitude. In the other 3 cases the difference was in favour of Fastac and thus no convincing difference between the impact of the two formulations was demonstrated.

- The CSP rates appeared to be reduced due to the introduction of nets, but the effect did not reach statistical significance with the data pooled over all seasons as shown in Table 2. However, if account was taken of possible heterogeneity in CSP rate by altitude and season and a Mantel Haenszel χ^2 was applied to the CSP rates stratified by altitude and season, the CSP rates were found significantly less in hamlets with nets than in those without them [16].

- Converting the trap catches to estimates of bites per person [8] and multiplying by the CSP rates gave estimates of the entomological inoculation rate per year; these differed about 17 fold between lowland and highland and showed 69–75% reduction as a result of the "mass" (or community) effect on the vector populations due to introducing treated nets at each altitude.

Table 3 shows the much reduced numbers of blood-fed anophelines in, and exiting from, lowland bedrooms with treated nets compared with corresponding catches in lowland villages with no nets. We must draw attention to two sources of error in these data. One is that the exit trap did not cover all possible mosquito escape routes and, if we missed many of the exiting mosquitoes, that would have exaggerated the difference in feeding rates, with or without treated nets since, as shown in Table 3, there was a

Table 3: Data leading to estimates of the personal protection against biting provided by the treated nets.

Lowland hamlets:-	B1, B3, B5, B6, B8 (no nets)	B2, B4, B7, B9, B10 (treated nets)
No. of collections	103	82
All anophelines resting indoors	4.21	0.276
All anophelines in exit traps	1.36	0.578
Total caught	5.57	0.854
Fed & resting indoors	3.44	0.219
Fed & in exit trap	0.71	0.236
Total fed	4.15	0.455
% fed	74.5%	53.3%
Light trap catches per night in rooms with untreated nets in same months	7.76	3.24
Blood fed catch/light trap catch	0.534	0.140
Reduction in biting due to personal protection from treated nets	73.8%	

Geometric mean catches of all anophelines, and of those found blood fed, by pyrethrum spraying to collect mosquitoes resting indoors in bedrooms and by window exit traps in/on bedrooms with treated nets in treated lowland hamlets or in rooms without nets in untreated hamlets in January–July 2000. Also included are the light trap catches in rooms with untreated nets over the same months, as a measure of the vector populations of these hamlets, and hence a calculation of the % of the reduction in biting which is attributable to the personal protection due to the treated nets.

larger difference in the feeding rate in those resting indoors than in those exiting. On the other hand, some of the fed mosquitoes may have entered to rest after feeding outside the house and this would have led to underestimation of the protection provided by the nets. The latter source of error could be avoided by molecular matching [17] of all mosquito blood meals with the blood of the sleepers under the nets in the same houses. We have not had the resources to undertake this yet. Meanwhile, we note that these two types of error would have tended to cancel out and we consider that further analysis of these data is worthwhile. The number of blood-fed mosquitoes was about 89% less (0.455 versus 4.15) in the treated rooms compared with the untreated rooms in untreated hamlets. Part of this difference is undoubtedly because of the differences in mosquito population densities of the treated and untreated hamlets due to the mass or community effect. This effect was corrected for by dividing by the contemporary light trap catches (Table 2) which, in all hamlets, were in rooms with untreated nets. The results lead to an estimate that the personal protection effect of the treated nets, on its own, gives a 73.8% reduction in biting. The scale of this effect was very similar to the 69–75% reduction in EIR due to the mass effect as demonstrated in Table 2. We conclude from this that applying treated nets in such a way as to exclude a mass effect, would reduce the total benefit to the individual net user by about half, compared to the full potential of treated nets when there is comprehensive coverage, as in the present trial. The relative importance of the mass, compared with the personal protection, effect was less than in two of our previous studies (data of both tabulated in [5]). However, all three studies demonstrated the need for a distribution system which ensures a high percentage coverage with effectively treated nets so as to maximise mosquito killing in the community.

Incidence of infection

Table 4 shows data on recurrence of infection of children in four lowland and four highland hamlets after clearing their existing infections with lap-dap. The observed mean rate of recurrence of infection per child week at risk was more than twice as great in the lowlands as the highlands. The hamlet with the highest recurrence rate in the highlands (M11) had a lower rate (0.327) than the hamlet (B8) with the lowest rate (0.385) in the lowlands. A significance test on the rates for the four highland versus the four lowland hamlets gave t (6 d.f.) = 4.53, $P < 0.01$.

Although the significance and direction of difference between the two altitudes was in the direction expected from the entomological data (Table 2), the scale of the difference in rate of recurrence of infection appeared smaller than would have been expected. However, a proper comparison requires that account be taken of the fact that there is occasional recrudescence of infection after lap-dap treatment. The best estimate for the rate of recrudescence, based on treated lowland children taken to stay at 1700 m where there was no transmission, was 0.013 per child week at risk [13]. This estimate did not differ significantly from one made by the more commonly used, but possibly less reliable, method of genotyping infections before and after drug treatment [18].

For infection *not* to recur requires that there is neither incidence of new infection from infective mosquito bites, nor recrudescence. The observed mean rate of recurrence of infection in the highlands of 0.220 implies a probability that recurrence will not occur of 0.780. On the reasonable

Table 4: Recurrence of infection in highland and lowland hamlets after clearing existing infections with Lap-Dap

Hamlet	Number of children becoming positive for infection	Number of child weeks at risk	Probability of becoming positive per week
HIGHLAND			
M7	24	144	0.167
M8	49	205	0.239
M9	51	325	0.157
M11	74	226	0.327
Highland totals	198	900	0.220
LOWLAND			
BI	61	138	0.442
B5	63	143	0.441
B7	37	88	0.420
B8	50	130	0.385
Lowland totals	211	143	0.423

assumption of independence of the processes of recrudescence and new infection:

(1-rate of new infection per child week) = 0.780/(1-0.013),

thus the rate of new infection can be calculated to have been 0.210 in the highlands. By the same method it was calculated to have been 0.415 in the lowlands.

These rates are based on weekly monitoring for infection and, in some cases, there could have been two or more separate new infection events in the same week; these would have been indistinguishable by our slide reading without molecular testing. Assuming independent occurrence of infection events there would be a Poisson distribution of weeks with 0,1,2,3... infections. Thus, if the probability of incidence of an infection is p, the proportion of child weeks with no infection would be $1/e^p$.

Therefore the above estimates, after correction for recrudescence, can be further corrected for multiple infection in the same week by calculating for the highlands that the probability of incidence of a new infection per week is:

ln (1/(1-0.210)) = 0.236; similarly the incidence in the lowlands = 0.536,

Note that without either of these corrections applied the estimates for the highlands and lowlands were 0.220 and 0.423, i.e. the corrections are of more than academic interest in the intense transmission conditions in the lowlands. Table 2 shows that in the months of April to June 1999 the mean light trap catch in the highlands was 3.80 per night, i.e. 26.6 per week, corresponding to a rate of biting per person per week of 39.9, assuming the same conversion factor from light trap catches to human biting as was found in the lowlands [8]. With the observed CSP rate for the highlands of 3.62%, this corresponds to an entomological inoculation rate of 1.45 CSP positive bites per person per week. Thus the estimated probability that a CSP positive bite in the highlands would lead to a patent infection was 0.236/1.45 = 0.163. Similarly, with the data from Table 2 for the same time period in the lowlands, there were 605 bites per person per week and 26.7 CSP positive bites per person per week. Thus, in the lowlands, the probability that a CSP positive bite would lead to a patent infection was only 0.536/26.7 = 0.020.

This is a further example of a phenomenon reported, for example, in [12,13] and [19] that, as the number of infective bites per unit time decreased (for natural reasons or due to vector control measures), so the probability that each bite would cause a patent infection tended to increase. This would seem to be a response of the immune system to less frequent inoculations of sporozoites [20].

Prevalence of malaria infection and mild malaria morbidity

Table 5 shows data by age group from monthly surveys in the pre-intervention year which agree with Ellman et al [6] in showing highly significantly greater prevalence of malaria infection and mild malaria morbidity in the lowlands than the highlands, up to age 5 years. This was also true for three of the parameters in the 6–12 age group

Table 5: Prevalence (with 95% confidence limits) by age group of malaria parasitaemia and three measures of mild malaria morbidity in highland and lowland hamlets from monthly surveys in each hamlet in the pre-intervention year

	Altitude	Age group		
		6 mo-2 yrs	2–5 yrs	6–12 yrs
% with malaria parasites	High	52.6 (49.0, 56.2)	65.4 (63.3, 67.5)	62.4 (60.4, 64.3)
	Low	78.9 (75.3, 82.6)	88.2 (86.5, 89.9)	80.0 (78.1, 81.8)
		[3.26***(2.32, 4.58)]	[4.21***(2.87, 6.18)]	[2.52***(1.94, 3.26)]
% with malaria fever[f]	High	4.8 (3.2, 6.3)	3.8 (3.0, 4.7)	1.4 (0.9, 1.9)
	Low	12.4 (9.4, 15.4)	5.8 (4.6, 7.1)	1.0 (0.5, 1.5)
		[2.93***(1.95, 4.40)]	[1.69**(1.16, 2.46)]	[0.66 n.s. (0.33, 1.34)]
% anaemic[w] (Hb<8 g/dl)	High	30.3 (26.9, 33.8)	9.5 (8.1, 10.8)	3.5 (2.7, 4.3)
	Low	49.4 (44.7, 54.1)	15.3 (13.3, 17.2)	6.6 (5.4, 7.8)
		[2.19***(1.67, 2.87)]	[1.74***(1.25, 2.44)]	[1.86**(1.19, 2.95)]
% with spleno-megaly	High	25.5 (21.9, 29.1)	39.6 (37.1, 42.0)	39.4 (37.2, 41.6)
	Low	63.7 (58.3, 69.0)	76.2 (73.5, 78.9)	57.1 (54.4, 59.8)
		[5.41***(3.47, 8.43)]	[4.87***(3.02, 7.86)]	[1.99*** (1.36, 2.90)]

[Shown in square parentheses are odds ratios[a] (with 95% confidence limits) for each parameter in the lowlands relative to values of 1.0 for the corresponding parameter in the highlands]. [a]odds ratios adjusted for non-independence of children in the same hamlet and in the same monthly survey [21]. [w]odds ratios of anaemia were additionally adjusted for the weight of the child, which was significantly associated with anaemia status for all age groups (P < 0.001). [f]as in [5] malaria fever was defined as temperature > 37.4C and/or fever reported by parent or guardian in previous 2 days with >4000 parasites/μl n.s. = non-significant, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

Table 6: Prevalence (with 95% confidence limits) by age group of malaria parasitaemia and three measures of mild malaria morbidity in highland and lowland hamlets from monthly surveys in hamlets with or without treated nets in the post-intervention year.

	Altitude	Treated Nets?	Age group		
			6 mo-2 yrs	2–5 yrs	6–12 yrs
% with malaria parasites	High	Yes	31.4(26.1,36.7)	44.3 (40.6, 48.0)	49.4 (46.3, 52.6)
		No	54.1(48.9,59.5)	73.0(69.9,76.1)	67.7 (65.0, 70.5)
			[2.60***(1.86, 3.64)]	[3.55***(1.95, 6.49)]	[2.14***(1.39, 3.28)]
	Low	Yes	63.1(56.2,69.9)	78.3 (74.9, 81.7)	80.6 (77.9 (83.3)
		No	82.9 (77.8, 87.9)	88.8(86.2,91.4)	83.3 (80.9, 85.8)
			[2.80***(1.70, 4.59)]	[2.21**(1.11, 3.68)]	[1.25 n.s.(0.70, 2.23)]
% with malaria fever[f]	High	Yes	2.3(0.6,4.1)	1.8(0.8,2.8)	0.4 (0.0, 0.8)
		No	7.4(4.6,10.2)	4.5(3.1,5.9)	1.2(0.6,1.9)
			[3.10**(1.27, 7.60)]	[2.70**(1.31, 5.57)]	[2.71* (1.06, 6.93)]
	Low	Yes	5.1 (2.0,8.3)	3.2(1.7,4.6)	1.3(0.6,2.1)
		No	14.8 (10.0, 19.6)	8.0 (5.7, 10.2)	1.0(0.4,1.7)
			[3.14***(1.96, 5.02)]	[2.56**(1.32, 4.97)]	[0.76 n.s.(0.26, 2.22)]
% anaemic[w] (<8 g Hb/dl)	High	Yes	3.9(1.6,6.2)	2.0 (0.9,3.0)	0.7(0.1,1.2)
		No	19.9(15.5,24.3)	4.3 (2.9, 5.8)	2.2(1.3,3.0)
			[5.86***(2.89, 11.87)]	[2.43**(1.31, 4.51)]	[2.94**(1.27, 6.82)]
	Low	Yes	15.4(10.3,20.6)	3.4(1.9,4.8)	2.1(1.1,3.1)
		No	37.5(31.0,44.0)	8.2 (5.9, 10.4)	4.2 (2.9, 5.5)
			[3.59***(2.01, 6.40)]	[2.75**(1.39, 5.43)]	[1.85 n.s.(0.83, 4.12)]
% with splenomegaly	High	Yes	12.4(8.6,16.1)	26.1(22.9,29.3)	33.3 (30.3, 36.3)
		No	39.2 (34.0, 44.5)	52.5 (49.0, 56.0)	52.9 (50.0, 55.9)
			[4.93***(2.51, 9.70)]	[3.23**(1.53, 6.84)]	[2.32**(1.21, 4.46)]
	Low	Yes	51.1(43.9,58.2)	70.8 (67.0, 74.6)	56.0 (52.5, 59.5)
		No	69.6 (63.4, 75.8)	75.4(71.8,79.0)	60.8(57.6,64.1)
			[2.18*(1.09,4.34)]	[1.26 n.s.(0.80, 1.99)]	[1.25 n.s.(0.88, 1.76)]

[Shown in square parentheses are odds ratios (with 95% confidence limits) in hamlets without nets relative to values of 1.0 in corresponding hamlets with nets] Footnotes as for Table 5.

which was not studied by Ellman et al [6]. For malaria fever, there was no significant difference between highland and lowland in the oldest age group, but there was no evidence that the benefit to the young children of the reduced transmission in the highlands was "paid for" by more malaria fever later in childhood. In fact there was far less malaria fever and anaemia in the older children than the younger ones.

Table 6 shows the impact of introducing treated nets in hamlets at both altitudes. For the youngest children this gave highly significant benefits in all respects measured, and the 95% confidence limits of the odds ratios in the highlands and lowlands overlapped. Thus there was no evidence that the benefit of the nets was any less in the initially intensely malarious lowlands than in the more moderate conditions in the highlands. In the 6–12 age group, significant benefits were seen in the highlands for all parameters measured. In this age group in the lowlands the benefits were not significant. However, the 95% confidence limits of the odds ratios for the two altitudes overlapped and, in no case, approached evidence for a disadvantage to older children of the intervention which reduced the intense transmission in the lowlands. The concentration of the benefit of treated nets among the younger children was also found 3–4 years after introduction of treated nets into nearby villages [5].

We are still working on data on acute malaria episodes from villages with and without nets as recorded at the District Hospital and via resident village reporters.

Conclusions

Our data on mild malaria morbidity is re-assuring in indicating that treated nets are about equally beneficial to young children, and not disadvantageous to older children, whether the initial transmission is intense or more moderate. Thus we consider that the present WHO policy of encouraging high coverage with effectively treated nets all over Africa is the correct one and there is no justification for focusing only on hypo-endemic highland areas.

Authors' contributions

Caroline Maxwell: organised the data collection in the field.

William Chambo, Mathew Mwalimu and Frank Magogo: led the field teams.

Ilona Carneiro: statistical analysis of malaria morbidity data.

Christopher Curtis: strategic planning of the trial and analysis of entomological and malaria incidence data.

Competing interests

None declared.

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