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# IMPACT OF PERMETHRIN-TREATED BED NETS ON MALARIA AND ALL-CAUSE MORBIDITY IN YOUNG CHILDREN IN AN AREA OF INTENSE PERENNIAL MALARIA TRANSMISSION IN WESTERN KENYA: CROSS-SECTIONAL SURVEY

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*Abstract.* Information on the impact of insecticide (permethrin)-treated bed nets (ITNs) from randomized controlled trials in areas of intense perennial malaria transmission is limited. As part of a large-scale, community-based, group-randomized controlled trial of the effect of ITNs on childhood mortality in a holoendemic area in western Kenya, we conducted three cross-sectional surveys in 60 villages to assess the impact of ITNs on morbidity in 1,890 children less than three years old. Children in ITN and control villages were comparable pre-intervention, but after the introduction of ITNs, children in intervention villages were less likely to have recently experienced illness requiring treatment (protective efficacy [95% confidence intervals] = 15% [1–26%]), have an enlarged spleen (32% [20–43%]), be parasitemic (19% [11–27%]), have clinical malaria (44% [6–66%]), have moderately severe anemia (hemoglobin level < 7.0 g/dL; 39% [18–54%]), or have a pruritic body rash, presumably from reduced nuisance insect bites (38% [24–50%]). Use of ITNs was also associated with significantly higher mean weight-for-age Z-scores and mid-upper arm circumferences. There was no evidence, however, that ITNs reduced the risk of helminth infections, diarrhea, or upper or lower respiratory tract infections. The ITNs substantially reduced malaria-associated morbidity and improved weight gain in young children in this area of intense perennial malaria transmission.

## INTRODUCTION

Results from previous trials have shown insecticide (permethrin)-treated bed nets (ITNs) and curtains reduce mortality in children less than five years old by 18% in sub-Saharan Africa.[1–4] However, it is unclear whether ITNs also reduce mortality in areas with intense, year-round transmission, where the burden of malaria is in very young children and severe malaria-related anemia is a primary cause of death. Two recently completed studies address this question: a case-control study conducted as part of a social marketing program in Tanzania,[5] and a group-randomized controlled trial conducted in western Kenya, the results of which are presented elsewhere in this supplement.[6]

Although mortality reduction is the ultimate goal of malaria control, data addressing the effect of ITNs on all-cause, and disease-specific morbidity, is required to better understand and estimate their true impact on the malaria burden in a community, and to determine their cost-effectiveness. Despite an extensive database on the effect of treated bed nets and curtains on malaria infection and morbidity, little information is available from randomized controlled trials in settings with intense perennial malaria transmission. A Cochrane review identified 65 bed net and curtain studies,[7] only one of which was a randomized controlled trial conducted in an area (in western Kenya) with intense perennial transmission,[8] but that study involved subjects of all ages. Since the Cochrane review, one additional randomized controlled trial has been conducted in southern Tanzania in an area with similar extreme transmission pressure and involved children 5–24 months of age.[9] Although the number of young children involved in these two trials were limited, the results suggested that ITNs reduce malaria infection (parasitemia) rates by 17–30% and increase hemoglobin levels. The impact on clinical malaria (with fever) was equivocal since no reduction was found in the Tanzanian study,[9] while the study in western Kenya reported a reduction of 38% in febrile episodes in all age groups, but results for young children were not reported separately.[8] Three additional non-randomized controlled studies in pre-school children from these same study sites and a similar area with intense transmission found ITN-associated reductions in febrile episodes of 60% and greater.[10–12]

Within the context of the large-scale, group-randomized controlled trial of ITNs in western Kenya in an area of intense, perennial malaria transmission,[6] we conducted a series of cross-sectional surveys in children less than three years of age to measure the impact of ITNs on all-cause and malaria-specific morbidity, and nutritional indices. We also evaluated whether the impact on morbidity translated into a tangible reduction in household expenditure for child health care. The results of this economic analysis are presented elsewhere in this supplement.[13]

## MATERIALS AND METHODS

**Study area and population.** The ITN trial was conducted in Rarieda Division (Asembo) in Bondo district (until 1999, part of Siaya district) and in Gem (Siaya district) on the shores of Lake Victoria in Nyanza Province of western Kenya. Mortality surveillance was conducted in both Asembo and Gem, comprising an area of 500 $km^2$. Morbidity surveillance was conducted in Asembo only (200 $km^2$), with a population of approximately 55,000 people living in 79 villages. Asembo was comprised of a cohort area (19 villages) located in the southernmost area of Asembo and a non-cohort area (the remaining 60 villages).[14] The cross-sectional surveys were conducted in the non-cohort area (130 $km^2$), where no longitudinal monitoring took place. According to the bi-annual census conducted as part of the ITN project, approximately

3,300 children less than three years of age lived in this area (mid-point estimate).

The study population has been described in detail elsewhere.[15,16] Briefly, the population is ethnically homogeneous; more than 95% are members of the Luo tribe. Except for market centers, the population of each village is highly dispersed, since the majority of the inhabitants are subsistence farmers who live in family compounds surrounded by their fields. There are two rainy seasons: the long rains from March to May and the short rains from October to December. Malaria is holoendemic and transmission occurs throughout the year. The number of infective bites ranges between 60 and 300 per person per year. Approximately 98% of the malaria infections are due to *Plasmodium falciparum*; 90% are only *P. falciparum,* 7% are mixed with *P. malariae*, and 1% are mixed with *P. ovale*. Single infections with *P. malariae* or *P. ovale* make up the remainder. Three mosquito species transmit nearly all of the malaria, with *Anopheles gambiae* and *An. funestus* responsible for more than 90% of the transmission and *An. arabiensis* responsible for most of the rest. Both *An. gambiae* and *An. funestus* bite primarily indoors late at night and their biting is diminished by use of ITNs.[17] Cholera and bacillary dysentery are endemic in this area. Malnutrition is also an important health problem; more than 30% of the children 6–59 months old are stunted.[18] Mortality in children less than five years of age in the study area was assessed prior to the ITN trial in 1992–1996; it was estimated that 25% of children do not reach their fifth birthday. Mortality in children less than one year of age is particularly high, with an estimated infant mortality rate of 176/1,000.[19]

**Study design.** Following randomization, ITNs (Siamdutch Mosquito Netting Co., Bangkok, Thailand) pre-impregnated with a target dose of 0.5g of permethrin/$m^2$ of netting were distributed to half of the villages in Asembo by January 1997 (ITN villages). The control villages received ITNs in early 1999 after the two-year intervention period.[16] The ITNs were re-treated biannually by the project. The coverage was 1.46 persons per ITN. Direct observation studies showed that adherence (persons sleeping under ITNs) throughout the two-year intervention was 72% overall, and 66% in children less than five years old.[20]

**Cross-sectional surveys.** Between October 1996 and December 1998, three cross-sectional surveys were conducted in Asembo to determine the impact of ITNs on all cause and malaria specific morbidity in children less than three years of age. The first cross-sectional survey (survey 0) represented the baseline and was conducted in October–November 1996, just prior to the distribution of ITNs. The second and third surveys (surveys 1 and 2) were conducted in February–March and November–December 1998, 14 and 22 months, respectively, after the introduction of ITNs.

**Participant recruitment and study design.** The study was designed to have 80% power to detect a 25% difference between ITN and control villages in the prevalence of moderately severe anemia (hemoglobin level < 7.0 g/dL) in surveys 1 and 2 combined, with 95% confidence, assuming a design effect of 1.25 and a prevalence of 25% in the control group.

*Design survey 0.* A random cluster-sample design was used for survey 0, with village as the cluster unit.[21] Prior to randomization, data on the number of children less than five years old was available from the ITN census procedure. In addition, the location of each village and household was available from a global positioning system–generated map.[22] Villages were then selected by random sampling proportional to size and stratified by randomization status and by geographic region, such that selected villages were equally distributed over the study area. A total of 27 of 60 villages were included in survey 0. In each village, a fixed number of index children less than five years old were randomly selected using the computerized list. Any siblings in the household less than 12 months old were also included in the survey. Thus, survey 0 was designed to oversample infants. Ten teams consisting of three members each visited the selected children within their homes, where all questionnaires were completed and measurements and samples taken.

*Design survey 1 and survey 2.* The design of cross-sectional surveys 1 and 2 was different from survey 0. These surveys were conducted in all 60 villages and used a simple random sampling method with households as the sampling unit. Prior to survey 1, 60% of all households (regardless of the age of the occupants) were randomized to cross-sectional surveys 1 and 2. This design ensured that one household and their occupants could only contribute once, and young children who were not yet born at the time of randomization could be included. Caregivers of the randomly selected households were invited to bring all children less than three (survey 1) or five years (survey 2) to a central location in the village on a preset day. Only data from children less than three years of age were eligible for inclusion in the analysis of ITN efficacy. Caregivers were also asked to bring a fresh (< 24 hours old) stool sample for each child in containers provided by the study. Each survey took 20 working days, and three villages were surveyed per day by three different teams consisting of 13 staff members each, including a clinical officer. A three-day training workshop for all team members was held prior to each survey to standardize assessment methodology. In each team, two staff members and the supervisor were trained by one of the investigators to take anthropometrics measurements using standard training guidelines.[23] To minimize observer or instrumental bias, teams rotated daily between intervention and control villages.

**Procedures and data collection.** *Household characteristics.* A structured questionnaire was used for each household to record indicators of socioeconomic status and educational status of the caregiver and head of household. The choice of indicators for the assessment of household socioeconomic status was based on a previous study conducted prior to the intervention period.[13]

*Demographic and clinical data.* The ages of the children were transcribed from census records and vaccination cards (if available) following verbal verification with the caretaker. If a written record of the date of birth was not available, the verbal information from the caregiver was used. The year and month of birth could be determined for all children. For those children with an unknown day of birth, the 15th day of the month was used. Anthropometric measurements were recorded and a finger prick blood sample (250–500 μL) was taken for determination of the hemoglobin concentration and the presence of malaria parasites. Each child was examined by the clinical officer, and the results recorded on standard forms. All sick children were treated as needed free of charge. All febrile children (axillary temperature ≥ 37.5°C) received a presumptive single dose of sulfadoxine-pyrimethamine (SP), regardless of the presence or absence of malaria para-

sitemia. Children with asymptomatic parasitemias ≥ 5,000/μL received SP the next day, after the malaria smear results were known. Children with hemoglobin concentrations < 11.0 g/dL were treated with SP and iron supplementation. Children with a severe illness were referred for further evaluation and treated free of charge at the local mission hospital.

*Laboratory methods.* Hemoglobin was measured by a portable photometer in the field (HemoCue AB, Angelholm, Sweden). Blood samples were stored in cooler boxes and transported every day to the Centers for Disease Control and Prevention/Kenya Medical Research Institute laboratories in Kisian. A full blood count, including repeat hemoglobin, was determined the same afternoon using a Coulter counter (Coulter, Hialeah, FL). Hemoglobin concentrations assessed by Coulter counter are used in this analysis. If a Coulter counter reading was not available, the value assessed by HemoCue in the field was used instead. Blood slides prepared in the field were stained with Giemsa in the laboratory and examined for the presence of malaria parasites. The total number of asexual parasites and gametocytes was determined per 500 white blood cells, and are expressed as the number per microliter, assuming a total white blood cell count of 8,000/μL. Slides were considered negative if no asexual parasites were found in 200 high-power ocular fields of the thick blood smear. Species diagnosis was made using the thin blood film. In surveys 1 and 2, a stool sample was microscopically examined for helminth infection using a modification of the formol-ether and ethyl acetate by concentration technique and by Kato-Katz methods.[24,25]

**Definitions.** Anemia and moderately severe anemia were defined as hemoglobin concentrations < 11.0 g/dL and <7.0 g/dL, respectively. Malaria parasitemia was defined as any asexual parasitemia detected on a thick or thin blood smear. Clinical malaria was defined as a documented axillary temperature ≥ 37.5°C in the presence of malaria parasitemia (any species) above an age- dependent fever threshold parasite density (0–5 months old = 1,500/mm$^3$, 6–11 months old = 6,000/mm$^3$, and 12–35 months old = 7,000/mm$^3$).[15] Moderately severe malarial anemia was defined as a hemoglobin level < 7.0 g/dL in the presence of any asexual malaria parasitemia. Helminth infection was defined as the presence of hookworm, *Ascaris lumbricoides*, *Trichuris trichiura,* and *Strongyloides stercoralis,* or *Schistosoma mansoni*. Anthropometric measurements included height-for-age (HAZ), weight-for-age (WAZ), and weight-for-length (WHZ) Z-scores and were calculated using reference data from the U.S.-based National Centers for Health Statistics and the World Health Organization,[26] using the software package Epi-Info version 2000 (Centers for Disease Control and Prevention, Atlanta, GA). Children were classified as stunted, underweight, or wasted if the HAZ, WAZ, and WHZ Z-score was < −2, respectively. The impact of ITNs on nutritional parameters was assessed only in children 3–35 months old because nutritional status in the first few months of life is more reflective of an *in utero* experience than an *ex utero* one, and children in the first three months of life are relatively unaffected by stunting or underweight. Splenomegaly was defined as any palpable spleen.

**Data management and statistical methods.** Data forms collected in the field were checked, coded, and entered using Clarion™ software (Topspeed/Soft Velocity, Pompano Beach, FL). Data were cleaned using range and internal consistency checks. All bivariate confidence intervals (CIs) and *P* values in Tables 1 and 2 were estimated taking the cluster design into account using SUDAAN software (version 8.0, SAS callable version; Research Triangle Institute, Research

TABLE 1

Baseline clinical and laboratory characteristics of 889 children <36 months old enrolled in survey 0 from 14 subsequent insectide-treated bed net (ITN) (n = 440) and 13 subsequent control villages (n = 449) in October–November 1996

| | ITN | Control | Crude *P* and PR (95% CI)* |
|---|---|---|---|
| Age in months, median (range) | 12.8 (0.3, 35.9) | 12.0 (0.1, 35.9) | 0.82 |
| No. of males (%) | 217 (49.3) | 204 (45.4) | 0.32, 1.09 (0.92–1.28) |
| History | | | |
| Any illness, no. (%) | 235 (54.4) | 255 (57.3) | 0.51, 0.95 (0.81, 1.11) |
| Acute malaria, no. (%) | 77 (17.8) | 72 (16.2) | 0.68, 1.10 (0.68, 1.77) |
| Diarrhea, no. (%) | 112 (25.8) | 127 (28.5) | 0.51, 0.90 (0.66, 1.23) |
| Respiratory tract problems, no. (%) | 45 (10.4) | 46 (10.3) | 0.99, 1.00 (0.59, 1.72) |
| Itchy body rash, no. (%) | 97 (22.2) | 113 (25.2) | 0.35, 0.88 (0.67, 1.16) |
| Sought health care, no. (%) | 251 (57.4) | 273 (61.2) | 0.58, 0.94 (0.75, 1.18) |
| Used malaria medicine, no. (%) | 123 (28.3) | 145 (32.6) | 0.38, 0.87 (0.63, 1.20) |
| Used any medicine, no. (%) | 261 (60.0) | 292 (65.6) | 0.18, 0.91 (0.80, 1.04) |
| Physical examination | | | |
| Axillary temperature ≥37.5°C, no. (%) | 28 (6.8) | 32 (7.7) | 0.68, 0.88 (0.46, 1.68) |
| MUAC-for-age Z-score†‡ Mean (95% CI) | −1.20 (−1.45, −0.95) | −1.13 (−1.34, −0.92) | 0.62 |
| Weight-for-height Z-score‡ mean (95% CI) | −0.33 (−0.55, −0.11) | −0.03 (−0.13, 0.06) | 0.01 |
| Weight-for-age Z-score‡ mean (95%) | −1.03 (−1.24, −0.82) | −0.88 (−1.07, −0.70) | 0.25 |
| Height-for-age Z-score,‡ mean (95% CI) | −1.22 (−1.40, −1.04) | −1.31 (−1.54, −1.07) | 0.54 |
| Laboratory characteristics | | | |
| Parasitemic, no. (%) | 310 (70.8) | 310 (69.0) | 0.72, 1.03 (0.89, 1.18) |
| High-density parasitemia, no. (%) | 99 (22.5) | 93 (20.7) | 0.58, 1.09 (0.81, 1.46) |
| Clinical malaria, no. (%) | 16 (3.9) | 16 (3.9) | 0.99; 1.00 (0.46, 2.21) |
| Gametocytemic, no. (%) | 54 (12.3) | 77 (17.1) | 0.04; 0.72 (0.52, 0.99) |
| Hemoglobin level in g/dL, mean (95% CI) | 8.0 (7.5, 8.4) | 8.3 (8.0, 8.6) | 0.28 |
| Hemoglobin < 7.0 g/dL, no. (%) | 149 (34.2) | 113 (25.2) | 0.07, 1.35 (0.97, 1.88) |

* PR = prevalence ratio with 95% confidence intervals (CIs). All *P* values and CIs were determined taking village clustering into account.
† MUAC = mid upper arm circumference.
‡ Children 3–35 months old.

TABLE 2
Characteristics of 1,890 children <36 months old enrolled in surveys 1 and 2*

| | ITN (n = 978) | Control (n = 912) | Crude *P* and PR (95% CI)† |
|---|---|---|---|
| Cross-sectional survey | | | |
| 1) Feb–Mar 1998, no. (%) | 501 (51.2) | 479 (52.5) | |
| 2) Nov–Dec 1998, no. (%) | 477 (48.8) | 433 (47.5) | 0.66 |
| Age in months, mean (95% CI) | 17.1 (16.5–17.8) | 18.1 (17.5–18.8) | 0.03 |
| No. of males (%) | 494 (50.5) | 450 (49.3) | 0.66, 1.02 (0.92–1.14) |
| Elevation < 1,250 meters, no. (%) | 540 (55.2) | 463 (50.8) | 0.71, 1.09 (0.69–1.70) |
| Always lived in area, no. (%) | 916 (93.7) | 827 (90.7) | 0.06, 1.03 (1.00–1.07) |
| More than 3 children <5 years old in household, no. (%)† | 112 (12.0) | 118 (13.8) | 0.35, 0.87 (0.65–1.17) |
| Total years of education of head of household and caretaker < population median, no. (%)† | 451 (48.5) | 421 (49.7) | 0.70, 0.98 (0.87–1.10) |
| SES index score < median, no. (%)† | 458 (49.0) | 417 (48.8) | 0.95, 1.00 (0.86–1.16) |
| Subsistence farming, no. (%)† | 667 (71.4) | 575 (67.3) | 0.25, 1.06 (0.96–1.17) |
| Unskilled laborer, no. (%)† | 163 (17.5) | 158 (18.5) | 0.65, 0.94 (0.73–1.22) |

* ITN = insecticide-treated bed net; SES = socioeconomic status.
† PR = prevalence ratio with 95% confidence intervals (CIs). All *P* values and CIs were determined taking village clustering into account.
† Data available from 1,788 of 1,890.

Triangle Park, NC). Dichotomous health outcomes were modeled to estimate the effect of ITNs using data from surveys 1 and 2 (Figures 1 and 2). All models were created using log-binomial regression in the Proc Genmod procedure within Statistical Analysis System (SAS version 8.02; (SAS Institute, Cary, NC).[27,28] The village based cluster randomization was taken into account by using an exchangeable correlation structure for observations obtained from residents within one village. Age was included in each model. Because of the rapid physiologic changes in hemoglobin levels in the first few months of life, narrow age categories were used (0–2, 3–5, 6–11, 12–17, 18–23, and 24–35 months). The interaction term ITN × survey number was not significant in the model for moderately severe anemia ($P = 0.33$) or the other models for the secondary malaria indices. Results were therefore pooled for surveys 1 and 2. However, cross-sectional survey number was included as a covariate to control for seasonal differences between surveys and possible methodologic differences between surveys (difference in instruments used and staff). In addition, the sex of the child and the wealth index were included as covariates in linear regression analysis of anthropometric measures. Other variables considered for inclusion were household elevation and distance to the lake shore, nearest borehole, nearest clinic, years of education and primary occupation of the caretaker and head of household, duration of residency in study area, helminth infections, mean rainfall 30–90 days prior to the survey date, and the mean daily temperature in the 10–30-day period prior to survey. These covariates were significant determinants, but not confounders of the relationships of interest, and had no or minimal impact on the precision of the point estimates and so were excluded from final models. Protective efficacy was calculated as 100 × (1 - adjusted prevalence ratio)%. The protective efficacy of ITNs by age group was compared by including an ITN × age interaction term in the final model, with age as a categorical variable (0–5, 6–11, 12–23, and 24–35 months) (Figure 2). The *P* value of the interaction term was used to assess whether effect modification by age was statistically significant. Linear regression models (Proc REGRESS in SUDAAN) were used to determine the mean difference in continuous end points (e.g., hemoglobin concentrations and nutritional Z-scores) between ITN and control villages, with the same covariates as the models for dichotomous end points, and a robust variance estimation correcting for village-based cluster randomization. Adjusted means were obtained from least squares mean estimates. All comparisons between ITN and control villages were on an intention-to-treat basis. For all statistical tests a two-sided *P* value < 0.05 was considered significant.

**Ethical clearance and informed consent.** The ITN project was reviewed and approved by the institutional review boards of the Kenya Medical Research Institute (Nairobi, Kenya) and the Centers for Disease Control and Prevention (Atlanta, GA). Written informed consent was obtained from caregivers for each individual participant.

## RESULTS

A total of 2,779 children less than 36 months of age were seen, including 889 children in cross sectional survey 0, 980 in survey 1, and 910 in survey 2. This represented 97% of randomized households with children less than three years old.

**Characteristics of the study population.** *Survey 0.* The characteristics of the 889 children less than three years of age enrolled in the pre-intervention survey 0 are shown in Table 1. The median age was 12.1 months. An acute illness in the previous two weeks for which treatment was sought was reported in more than half (56%) of the children. The overall prevalence of malaria parasitemia was 70%; most of these infections were asymptomatic (91%). Only 4% had fever with a parasite density above the age-dependent fever threshold.[29] *Plasmodium falciparum* accounted for 86.1% of the infections, and almost all of the remaining were due to mixed infections of *P. falciparum* with either *P. malariae* (10.7%) or *P. ovale* (1.5%). Infections with only *P. malariae* were rare (1.8%) and those with *P. ovale* were not observed. Almost all children had hemoglobin levels less than 11.0 g/dL (90%) and 30% had moderately severe anemia (hemoglobin level < 7.0 g/dL). Malaria was strongly associated with moderately severe anemia, and 1.78 times more likely (95% CI = 1.42–2.23) in parasitemic children compared with parasite-negative children.

The children were comparable before the intervention, with the exception that children living in villages randomized to receive ITNs had lower mean weight-for-height scores ($P =$

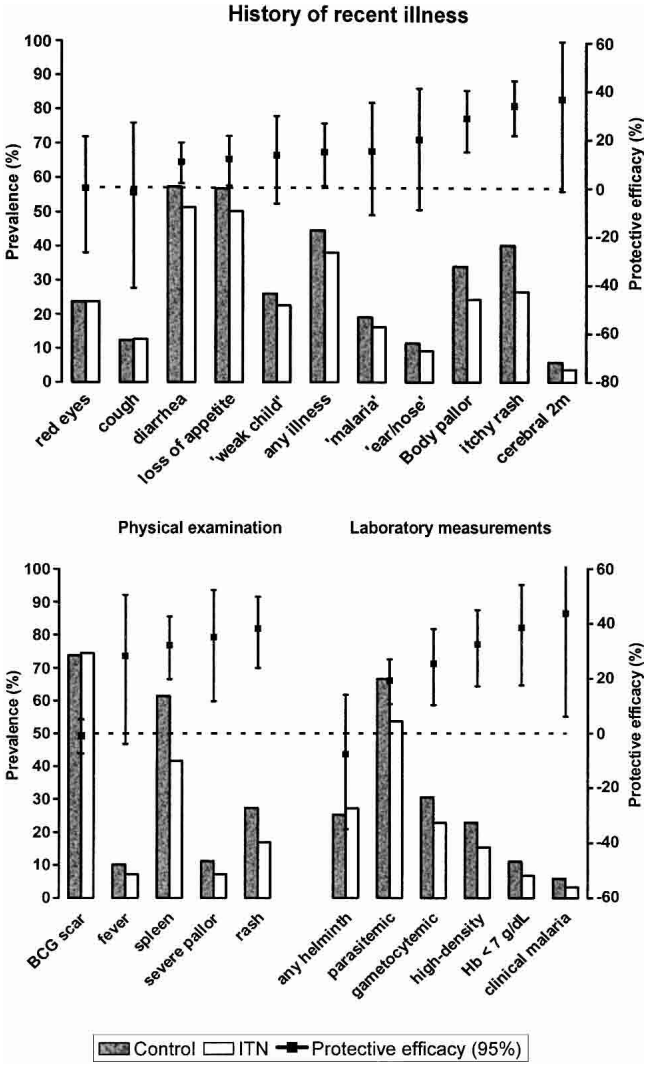


FIGURE 1. Impact of insecticide-treated bed nets (ITN) on symptoms, signs, and laboratory findings in 1,890 children less than 36 months of age. The prevalence (**left y-axis**) and protective efficacy (**right y-axis**) were estimated using binomial regression analysis, taking age and survey number into account. Significant differences are illustrated by the lack of overlap of the 95% confidence intervals of the estimated protective efficacy (**error bars**) with the horizontal dashed line. Cerebral 2m = serious illness with coma or convulsions as reported by the caretaker; BCG = bacilli Calmette-Guérin; parasitemic = presence of any asexual malaria parasitemia (any species); high-density = parasite density above an age-dependent threshold density (0–5 months old = 1,500/mm$^3$; 6–11 months old = 6,000/mm$^3$; 12–35 months old =7,000/mm$^3$);[15] Hb = hemoglobin.

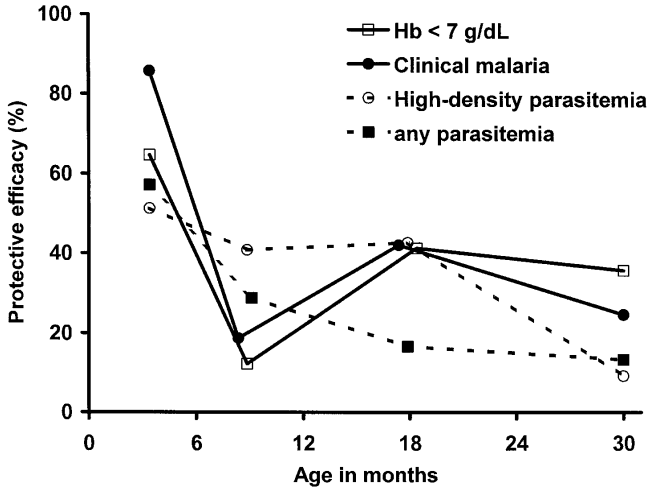


FIGURE 2. Protective efficacy of insecticide-treated bed nets by age. Hb = hemoglobin.

0.01) and were more likely to have moderately severe anemia ($P = 0.07$). Children in the control villages were more likely to carry gametocytes ($P = 0.04$). The prevalences of malaria parasitemia, high-density parasitemia, clinical malaria, and severe-to-moderate malarial anemia were identical. *Plasmodium* species distribution was also similar between subsequent ITN and control villages ($P = 0.92$).

*Surveys 1 and 2.* The demographic and socioeconomic characteristics of the children enrolled in surveys 1 and 2 are shown in Table 2. The mean age was 17.6 months. Whereas the age of the children from intervention and control villages in survey 0 was comparable (Table 1), the children in the control villages in survey 1, and particularly in survey 2, were older than the children in the intervention villages (mean [95% CI] difference in survey 1 = 0.49 [–0.83–1.80] months, $P = 0.47$ and survey 2 = 1.58 [0.34–2.81] months, $P = 0.02$). The finding that the age difference between control and intervention villages increased with time is consistent with the greater beneficial impact of ITNs on infant mortality compared with mortality among older children.[16] Of the children in the control villages 2.6% had an ITN in the house.

**Impact of ITNs on morbidity.** The impact of ITNs as assessed in surveys 1 and 2 is summarized in Figure 1. Overall, 45% of the children living in the control villages had experienced an acute illness requiring treatment in the two weeks before the survey, and this cumulative prevalence was 14.6% (95% CI = 0.8–26.4) lower in the ITN villages, illustrating that ITN use had a small but statistically significant impact on reported all-cause morbidity.

There was a clear impact on anemia and other malaria-specific indices. The mean hemoglobin level was 0.5 g/dL (95% CI = 0.2–0.8 g/dL) higher in children living in ITN compared with those in control villages (mean = 10.0 g/dL versus 9.5 g/dL; $P = 0.0005$). The prevalence of moderately severe anemia (hemoglobin level < 7.0 g/dL) and severe malarial anemia was reduced by 39% (95% CI = 18–54%) and 45% (95% CI = 5–68%), respectively. The prevalence of malaria (any species) was reduced by 19% (95% CI = 11–27%). The protective efficacy for pure or mixed infections with *P. ovale* (72%, 95% CI = 4–92%) or *P. malariae* (66%, 95% CI = 54–75%) was much greater than that for pure or mixed infections with *P. falciparum* (19%, 95% CI = 10–26%). Overall geometric mean parasite densities were slightly lower in the bed net group (1,643/μL, 95% CI = 1,333–2,025/μL), but were not significantly different from children in the control group (2,036/μL, 95% CI = 1,726–2,401/μL, $P = 0.12$). Gametocytemia was 26% (95% CI = 10–38%) less prevalent in the ITN group, but geometric mean gametocyte densities were comparable (ITN = 30.2 versus control = 29.0; $P = 0.61$). The prevalence of clinical malaria was 44% (95% CI = 6–66%) lower in ITN villages. Children 0–6 months of age benefited most from ITN use

(Figure 2). The *P* values for the ITN × age interaction term in Figure 2 were $P = 0.005$, $P = 0.11$, $P = 0.20$, and $P = 0.29$ for any parasitemia, clinical malaria, high-density parasitemia, and hemoglobin level < 7.0 g/dL, respectively.

The greatest beneficial impact on non-malaria-related illnesses was on body rash. Children living in intervention villages were 38.2% (95% CI = 23.9–49.8%) less likely to have evidence of (an insect bite) rash on their extremities on physical inspection. There was no evidence that ITNs had any beneficial impact on reported eye, ear, and upper or lower respiratory tract infections and helminth infections. Nevertheless, the proportion of children with reported acute diarrhea was significantly lower in the ITN group, as well as the proportion of children reported to have vomited or have loss of appetite in the previous two weeks (Figure 1). Children 3–59 months of age from ITN villages had significantly higher mean weight-for-age and mid upper arm circumference (MUAC)-for-age Z-scores (Table 3).

## DISCUSSION

This study confirmed the high prevalence of malaria (70%) and moderately severe anemia (hemoglobin level < 7.0 g/dL; 30%) in this area before the introduction of ITNs. More than 60% of caretakers reported having sought health care for an acute illness in the two-week period prior to the date of the survey, illustrating the very high overall disease burden in this age group in this community. Our results indicate that ITNs substantially reduced malaria and malaria-associated morbidity, particularly in young infants, and had the greatest impact on the most malaria-specific indices.

This study recruited a randomly selected sample representing approximately 57% of the population of children less than three years of age. Non-response during the study was very low (< 3%). The prevalence of malaria parasitemia, high-density infections, and clinical malaria were identical before the start of the intervention, as were the other determinants of the key outcome variables of interest. Therefore, we believe that the conclusions are valid for the study population.

This study adds valuable information to the limited data on the impact of ITNs in areas with the most intense malaria transmission. Our findings of a 19% reduction in *P. falciparum* prevalence and 0.5 g/dL increase in mean hemoglobin concentration are almost identical to that reported from the smaller randomized controlled trial conducted in an area with similar intense transmission in Tanzania, and from a previous trial in our own study area.[8,9] Unlike the Tanzanian study,[9] we found a substantial reduction (by 44%) in the prevalence of clinical malaria; the most specific indicator for malaria-associated disease (fever plus parasite density above an age-specific threshold).[29] This was consistent with the reduction of 38% reported in the earlier ITN and curtain study conducted in 1988 in the same location in western Kenya. However, that study did not separately report data for young children.[8] All three of these randomized controlled studies showed that parasite densities were only marginally affected. Of note, the reductions in anemia and clinical malaria observed in the current study are similar to that reported from controlled trials of ITNs in areas with stable, but less intense or more seasonal malaria transmission.[7]

TABLE 3

Impact of insecticide (permethrin)-treated bed nets (ITNs) on mean Z-scores of nutritional parameters

| | Randomization group | | Mean difference (95% CI)* | *P** |
|---|---|---|---|---|
| | ITN | Control | | |
| Weight-for-height† | –0.34 | –0.45 | 0.11 (–0.03–0.26) | 0.13 |
| Height-for-age† | –1.06 | –1.21 | 0.15 (–0.04–0.34) | 0.14 |
| Weight-for-age† | –0.96 | –1.12 | 0.16 (0.01–0.31) | 0.04 |
| MUAC-for-age‡ | –1.05 | –1.25 | 0.20 (0.06–0.34) | 0.008 |

* Mean Z-scores and mean difference in Z-scores and associated *P* values are adjusted for cross-sectional survey, age, sex, and wealth index score using the regression procedure in SUDAAN release 8. CI = confidence interval.

† Children 3–35 months old.

‡ MUAC = mid upper arm circumference; 6–35 months (World Health Organization/National Centers for Health Statistics reference population for children <6 months old not available at time of study).

The protective estimates reported are markedly lower than that reported by the three non-randomized or quasi-randomized controlled studies conducted in intense transmission settings in Tanzania and western Kenya.[10–12] All three of these studies reported reductions of greater than 60% in febrile episodes and 45–62% reductions in parasitemia, and also greater improvements in mean hemoglobin values. The greater efficacy in the earlier study in western Kenya may partly be explained by the significantly higher *P. falciparum* prevalence at the beginning of the study in the two control villages compared with the two ITN villages.[12] Furthermore, two of the three studies[11,12] assessed protective efficacy based on the incidence of new infections following curative antimalarial treatment, which, in highly endemic areas, is a more sensitive indicator of the impact of interventions than use of prevalence. The investigators in the social marketing study in southwestern Tanzania also note that despite efforts to control for it, residual confounding, as well as a younger and more anemic study population, may explain the higher estimates in their study.[10] Nevertheless, despite these differences in study design and estimates of the magnitude of effect, all of the aforementioned studies, as well as this one, show a substantial impact afforded by ITN use on malaria-associated morbidity in areas with intense perennial transmission.

Of interest was the substantially greater reduction in the prevalence of *P. ovale* and *P. malariae* infections (from 15% to 5%, protective efficacy = 66%) than in the *P. falciparum* malaria prevalence (protective efficacy = 19%). This has been reported previously for *P. malariae* in a study from Burkina Faso, where the prevalence of microscopically detectable *P. malariae* was higher (30%) than in our site in western Kenya (10–15%).[30] It was postulated that the differential impact between *P. malariae* and *P. falciparum* can be explained by the impact of ITNs on mosquito life expectancy,[31] thus reducing the success rate of transmission of *P. malariae*, which is known to have a longer sporogenic cycle than *P. falciparum*.[30]

Importantly, the reduction in malaria morbidity was also associated with improvements in overall health, as reflected in the improved weight gain and greater MUAC values. The improved weight gain was not associated with a significant reduction in the proportion of children classified as underweight. Analysis of the distribution curves for the MUAC and weight-for-age Z-scores illustrated that the increase in arm circumference and weight was a generalized phenomenon, resulting in an upward shift of the entire distribution curve. Thus, the beneficial impact on protein energy nutritional status was not restricted to children at risk, but also included

children not classified as malnourished (i.e., with Z-scores >= -2). Our findings are consistent with the results from our birth cohort study,[32] two other randomized insecticide-treated ITN trials in The Gambia and Kenya,[1,28] and one non-randomized controlled study from the Tanzanian coast.[33] They add further evidence to the importance of malaria as a preventable cause of malnutrition over a wide range of epidemiologic transmission settings.

We did not find that ITN use was associated with a reduced risk of other childhood diseases. Although children in the ITN villages had less febrile episodes and mild gastrointestinal complaints (loss of appetite/vomiting), this was mostly associated with a reduction in clinical malaria episodes, and there was no reduction in aparasitemic febrile episodes. The risk of eye and upper or lower respiratory infections were similar among children in the ITN villages and control villages. There was a small (10%; $P = 0.02$) difference in the cumulative prevalence of all-cause diarrhea as reported by the caregivers; however, the same difference, although not statistically significant, was already present before the introduction of ITNs. Although mild diarrhea or softening of stool is associated with acute malaria,[34] care should be taken not to attribute the observed difference to the introduction of ITNs. The greatest impact on non-malaria-related morbidity was on pruritic body rash, which was found in 27% of children in the control villages and 17% in the ITN villages, a 38% reduction. This presumably reflects the reduction in mosquito densities and thus mosquito bites, as well as other insects such as scabies mites (*Sorarcoptes scabiei*), bedbugs (*Cimex hemipterus*), and other nuisance anthropods.[35–37] Reduced nuisance from insects may be a key additional benefit of ITNs, which contribute to their widespread acceptance and adherence by communities.[36]

To our knowledge, this is the first group-randomized controlled trial of ITNs conducted in areas with intense perennial malaria transmission using communities as the unit of randomization (in this case villages). The difference between individual versus community ITN use is now recognized to be important. Spatial analyses of the impact of ITNs on morbidity and mortality has indicated that the effect of ITNs is not only due to individual protection, but with sufficient coverage, there is an overall suppression of the mosquito population, resulting in a community or mass effect of the insecticide that reduces malaria transmission.[17,38–40] The current ITN study also showed that both mosquito abundance and the proportion of mosquitoes infected with sporozoites were reduced in compounds lacking ITNs, but located close to compounds with ITNs.[41] In a separate analysis presented elsewhere, we show that this beneficial community effect on the mosquito population extends to malaria-related morbidity in young children living within 300 meters of an intervention village.[22] This community effect on morbidity was substantial and benefited at least one-fourth of the population from the control villages. Adjustment for the community effect increased the estimate of the protective efficacy by ITNs on morbidity by 7% (clinical malaria) to 20% (hemoglobin levels). We conclude that ITNs are a valuable contribution towards child health in areas of intense perennial malaria transmission.

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## REFERENCES

1. D'Alessandro U, Olaleye BO, McGuire W, Langerock P, Bennett S, Aikins MK, Thomson MC, Cham MK, Cham BA, Greenwood BM, 1995. Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet 345:* 479–483.
2. Binka FN, Kubaje A, Adjuik M, Williams LA, Lengeler C, Maude GH, Armah GE, Kajihara B, Adiamah JH, Smith PG, 1996. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Trop Med Int Health 1:* 147–154.
3. Nevill CG, Some ES, Mung'ala VO, Mutemi W, New L, Marsh K, Lengeler C, Snow RW, 1996. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Trop Med Int Health 1:* 139–146.
4. Habluetzel A, Diallo DA, Esposito F, Lamizana L, Pagnoni F, Lengeler C, Traore C, Cousens SN, 1997. Do insecticide-treated curtains reduce all-cause child mortality in Burkina Faso? *Trop Med Int Health 2:* 855–862.
5. Schellenberg JR, Abdulla S, Nathan R, Mukasa O, Marchant TJ, Kikumbih N, Mushi AK, Mponda H, Minja H, Mshinda H, Tanner M, Lengeler C, 2001. Effect of large-scale social marketing of insecticide-treated nets on child survival in rural Tanzania. *Lancet 357:* 1241–1247.
6. Phillips-Howard PA, Nahlen BL, Kolczak MS, Hightower AW, ter Kuile FO, Alaii JA, Gimnig JE, Arudo J, Vulule JM, Odhacha A, Kachur SP, Schoute E, Rosen DH, Sexton JD, Oloo AJ, Hawley WA, 2003. Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 23–29.
7. Lengeler C, 2000. Insecticide-treated bednets and curtains for preventing malaria. *Cochrane Database Syst Rev 320:* CD000363.
8. Sexton JD, Ruebush TK 2nd, Brandling-Bennett AD, Breman JG, Roberts JM, Odera JS, Were JB, 1990. Permethrin-impregnated curtains and bed-nets prevent malaria in western Kenya. *Am J Trop Med Hyg 43:* 11–18.
9. Fraser-Hurt N, Felger I, Edoh D, Steiger S, Mashaka M, Masanja H, Smith T, Mbena F, Beck HP, 1999. Effect of insecticide-

treated bed nets on haemoglobin values, prevalence and multiplicity of infection with Plasmodium falciparum in a randomized controlled trial in Tanzania. *Trans R Soc Trop Med Hyg 93 (Suppl 1):* 47–51.
10. Abdulla S, Schellenberg JA, Nathan R, Mukasa O, Marchant T, Smith T, Tanner M, Lengeler C, 2001. Impact on malaria morbidity of a programme supplying insecticide treated nets in children aged under 2 years in Tanzania: community cross sectional study. *BMJ 322:* 270–273.
11. Premji Z, Lubega P, Hamisi Y, McHopa E, Minjas J, Checkley W, Shiff C, 1995. Changes in malaria associated morbidity in children using insecticide treated mosquito nets in the Bagamoyo district of coastal Tanzania. *Trop Med Parasitol 46:* 147–153.
12. Beach RF, Ruebush TK 2nd, Sexton JD, Bright PL, Hightower AW, Breman JG, Mount DL, Oloo AJ, 1993. Effectiveness of permethrin-impregnated bed nets and curtains for malaria control in a holoendemic area of western Kenya. *Am J Trop Med Hyg 49:* 290–300.
13. Meltzer MI, Terlouw DJ, Kolczak MS, Odhacha A, ter Kuile FO, Vulule JM, Alaii JA, Nahlen BL, Hawley WA, 2003. Phillips-Howard PA, The household-level economics of using permethrin-treated bednets to prevent malaria in children under 5 years of age. *Am J Trop Med Hyg 68 (Suppl 4):* 149–160.
14. Phillips-Howard PA, Nahlen BL, Alaii JA, ter Kuile FO, Gimnig JE, Terlouw DJ, Kachur SP, Hightower AW, Lal AA, Schoute E, Oloo AJ, Hawley WA, 2003. The efficacy of permethrin-treated bednets on child mortality and morbidity in western Kenya. I: development of infrastructure and description of study site. *Am J Trop Med Hyg 68 (Suppl 4):* 3–9.
15. Bloland PB, Ruebush TK, McCormick JB, Ayisi J, Boriga DA, Oloo AJ, Beach R, Hawley W, Lal A, Nahlen B, Udhayakumar V, Campbell CC, 1999. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission I. Description of study site, general methodology, and study population. *Am J Trop Med Hyg 60:* 635–640.
16. Phillips-Howard PA, ter Kuile FO, Nahlen BL, Alaii JA, Gimnig JE, Kolczak MS, Terlouw DJ, Kariuki SK, Shi YP, Kachur SP, Hightower AW, Vulule JM, Hawley WA, 2003. The efficacy of permethrin-treated bednets on child mortality and morbidity in western Kenya. II: Study design and methods. *Am J Trop Med Hyg 68 (Suppl 4):* 10–15.
17. Gimnig JE, Vulule JM, Lo TQ, Kamau L, Kolczak MS, Phillips-Howard PA, Mathenge EM, ter Kuile FO, Nahlen BL, Hightower AW, Hawley WA, 2003. Impact of permethrin-treated bednets on entomological indices in an area of intense year round malaria transmission. *Am J Trop Med Hyg 68 (Suppl 4):* 16–22.
18. Kwena AM, Terlouw DJ, de Vlas SJ, Phillips-Howard PA, Hawley WA, Friedman JF, Vulule JM, Nahlen BL, Sauerwein RW, ter Kuile FO, 2003. Prevalence and severity of malnutrition in pre-school children in a rural area in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 94–99.
19. McElroy PD, ter Kuile FO, Hightower AW, Hawley WA, Phillips-Howard PA, Oloo AJ, Lal AA, Nahlen BL, 2001. All-cause mortality among young children in western Kenya. VI: the Asembo Bay Cohort Project. *Am J Trop Med Hyg 64:* 18–27.
20. Alaii JA, Hawley WA, Kolczak MS, ter Kuile FO, Gimnig JE, Vulule JM, Odhacha A, Oloo AJ, Nahlen BL, Phillips-Howard PA, 2003. Factors affecting use of permethrin-treated bednets during a randomised-controlled trial in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 137–141.
21. Bindari-Hammad EL, Smith DL, 1992. Sampling for primary health care reviews. *Primary health care reviews: Guidelines and methods*. Geneva: World Health Organization, 153-179.
22. Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, Nahlen BL, Gimnig JE, Kariuki SK, Kolczak MS, Hightower AW, 2003. Community-wide effects of permethrin-treated bednets on child mortality and malaria morbidity in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 121–127.
23. United Nations, 1986. *How to weigh and measure children: Assessing the nutritional status of young children in household surveys*. New York: United Nations Department of Technical Cooperation for Development and Statistical Office.
24. Knight WB, Hiatt RA, Cline BL, Ritchie LS, 1976. A modification of the formol-ether concentration technique for increased sensitivity in detecting Schistosoma mansoni eggs. *Am J Trop Med Hyg 25:* 818–823.
25. Katz N, Chaves A, Pellegrino J, 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo 14:* 397–400.
26. Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM, 1979. Physical growth: National Center for Health Statistics percentiles. *Am J Clin Nutr 32:* 607–629.
27. Hardin J, Hilbe J, 2001. *Generalized Linear Models and Extensions*. Texas: Stata Press.
28. Skov T, Deddens J, Petersen MR, Endahl L, 1998. Prevalence proportion ratios: estimation and hypothesis testing. *Int J Epidemiol 27:* 91–95.
29. Bloland PB, Boriga DA, Ruebush TK, McCormick JB, Roberts JM, Oloo AJ, Hawley W, Lal A, Nahlen B, Campbell CC, 1999. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. *Am J Trop Med Hyg 60:* 641–648.
30. Habluetzel A, Cuzin N, Diallo DA, Nebie I, Belem S, Cousens SN, Esposito F, 1999. Insecticide-treated curtains reduce the prevalence and intensity of malaria infection in Burkina Faso. *Trop Med Int Health 4:* 557–564.
31. Magbity EB, Marbiah NT, Maude G, Curtis CF, Bradley DJ, Greenwood BM, Petersen E, Lines JD, 1997. Effects of community-wide use of lambdacyhalothrin-impregnated bednets on malaria vectors in rural Sierra Leone. *Med Vet Entomol 11:* 79–86.
32. ter Kuile FO, Terlouw DJ, Kariuki SK, Phillips-Howard PA, Mirel LB, Hawley WA, Friedman JF, Shi YP, Kolczak MS, Lal AA, Vulule JM, Nahlen BL, 2003. Impact of permethrin-treated bednets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 68–77.
33. Shiff C, Checkley W, Winch P, Premji Z, Minjas J, Lubega P, 1996. Changes in weight gain and anaemia attributable to malaria in Tanzanian children living under holoendemic conditions. *Trans R Soc Trop Med Hyg 90:* 262–265.
34. ter Kuile FO, Nosten F, Luxemburger C, Kyle D, Teja-Isavatharm P, Phaipun L, Price R, Chongsuphajaisiddhi T, White NJ, 1995. Mefloquine treatment of acute falciparum malaria: a prospective study of non-serious adverse effects in 3673 patients. *Bull World Health Organ 73:* 631–642.
35. Temu EA, Minjas JN, Shiff CJ, Majala A, 1999. Bedbug control by permethrin-impregnated bednets in Tanzania. *Med Vet Entomol 13:* 457–459.
36. Lindsay SW, Snow RW, Armstrong JR, Greenwood BM, 1989. Permethrin-impregnated bednets reduce nuisance arthropods in Gambian houses. *Med Vet Entomol 3:* 377–383.
37. Charlwood JD, Dagoro H, 1989. Collateral effects of bednets impregnated with permethrin against bedbugs (Cimicidae) in Papua New Guinea. *Trans R Soc Trop Med Hyg 83:* 261.
38. Howard SC, Omumbo J, Nevill C, Some ES, Donnelly CA, Snow RW, 2000. Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast. *Trans R Soc Trop Med Hyg 94:* 357–360.
39. Binka FN, Indome F, Smith T, 1998. Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural northern Ghana. *Am J Trop Med Hyg 59:* 80–85.
40. Ilboudo-Sanogo E, Cuzin-Ouattara N, Diallo DA, Cousens SN, Esposito F, Habluetzel A, Sanon S, Ouedraogo AP, 2001. Insecticide-treated materials, mosquito adaptation and mass effect: entomological observations after five years of vector control in Burkina Faso. *Trans R Soc Trop Med Hyg 95:* 353–360.
41. Gimnig JE, Kolczak MS, Hightower AW, Vulule JM, Schoute E, Kamau L, Phillips-Howard PA, ter Kuile FO, Nahlen BL, Hawley WA, 2003. Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 115–120.