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

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Abstract. A group-randomized controlled trial of insecticide (permethrin)-treated bed nets (ITNs) was conducted in an area of high perennial malaria transmission in western Kenya to test the effect of ITNs on all-cause mortality in children 1–59 months of age. Child deaths were monitored over a two-year period by biannual household census in Asembo (1997–1998) and in Gem (1998–1999). Overall, 1,722 deaths occurred in children 1–59 months followed for 35,932 child-years. Crude mortality rates/1,000 child-years were 51.9 versus 43.9 in control and ITN villages in children 1–59 months old. The protective efficacy (PE) (95% confidence interval) adjusted for age, study year, study site, and season was 16% (6–25%). Corresponding figures in 1–11- and 12–59-month-old children in control and ITN villages were 133.3 versus 102.3, PE = 23% (11–34%) and 31.1 versus 28.7, PE = 7% (-6–19%). The numbers of lives saved/1,000 child-years were 8, 31, and 2 for the groups 1–59, 1–11, and 12–59 months old, respectively. Stratified analysis by time to insecticide re-treatment showed that the PE of ITNs re-treated per study protocol (every six months) was 20% (10–29%), overall and 26% (12–37%) and 14% (−1–26%) in 1–11- and 12–59-month-old children, respectively. ITNs prevent approximately one in four infant deaths in areas of intense perennial malaria transmission, but their efficacy is compromised if re-treatment is delayed beyond six months.

INTRODUCTION

Randomized controlled trials in different malaria transmission settings have shown insecticide-treated bed nets (ITNs) and curtains to be effective in reducing all cause mortality in children less than five years of age.[1–4] Overall, these trials reduced all cause mortality in children less than five years old by 17%, and were estimated to have saved six lives per 1,000 children protected per year.[5] Mortality reduction in these trials appeared to be associated with malaria transmission pressure, with a lower efficacy detected at sites with the highest transmission (greater than 100 infective bites per person a year), compared with sites experiencing lower transmission (less than 10–30 infective bites per person per year). The role of transmission pressure in modifying the efficacy of insecticide-treated materials has been much debated.[6–8] However, when efficacy was measured in terms of absolute lives saved, there was no apparent correlation with transmission pressure.[5,6] Although the lowest efficacy observed during the trials was observed in Burkina Faso, the site with the highest transmission pressure, this was the only trial that used permethrin-treated curtains, rather than bed nets.[4] Furthermore, this trial observed high efficacy in only the first study year (26%), while no efficacy was seen in the second year. In parallel, the efficacy of ITNs in The Gambia, the site with the lowest transmission pressure, was similar (25%), but this estimate was based upon first-year observations only.[1] Thus, it was unclear from the previous ITN and curtain trials whether there was indeed a relationship between efficacy and transmission pressure, whether the apparent differences in efficacy were coincidental, or whether they were consequent to variations in study design, population characteristics, or other unrecognized factors.

Implementation of ITN programs has begun in a number of countries in sub-Saharan Africa, although uncertainty remains of their impact in areas with the most intense perennial malaria transmission where the number of infective bites remains high throughout the year (100–500 per year). This high transmission pressure places the bulk of malaria-associated mortality in a relatively narrow time-window in the first 2–18 months of life,[9] as older children who survive this period have typically acquired sufficient clinical immunity to be protected from severe malaria.[10,11] There was no evidence from the previous randomized controlled trials that ITNs would reduce this burden specifically in the very young.

Information has more recently become available on the potential impact of ITNs on mortality in children less than five years old in areas with intense malaria transmission.[12] A large-scale, social marketing project in Tanzania estimated a 27% increase in survival of children using purchased ITNs. However, direct comparison against results from the previous randomized controlled trials was unfeasible because the study used a case-control design. We present the results of a large-scale, randomized, controlled trial of ITNs conducted in an area with intense, perennial malaria transmission in western Kenya and report on their effect over a two-year period in reducing mortality in children 1–59 months of age. Factors affecting the protective efficacy (PE) of ITNs are described.

MATERIALS AND METHODS

Study site and population. An overview of the study site and population has been reported in detail elsewhere.[13,14] The initial study site of Asembo, covering an area of 200 km², is on the shore of Lake Victoria approximately 50 km from Kisumu town. Asembo Bay, where a mother-child birth cohort study has been conducted,[15] and Saradidi, where a community-based health development project took place,[16] are

both within the Asembo trial site. The second trial site of Gem, covering an area of 300 km[2], lies north but contiguous with Asembo, where a small-scale ITN and curtain study previously took place.[17] The study population in both sites is predominantly Luo. Villages are highly dispersed; inhabitants are mostly subsistence farmers who live in family compounds surrounded by their fields. Additional income is generated from fishing in Lake Victoria and marketing food and grain.[18] Baseline studies revealed a low level of ITN use (<5%), and of those using ITNs, preference is given to adults and visitors to protect against nuisance mosquitoes.[19]

Rainfall occurs year round, with the heaviest rains falling in March and April, and a second lighter rainy period in November and December. Total annual rainfall averages approximately 1,400 mm/year, but a poor lighter rainy period in 1998 resulted in an annual total of 1,064 mm.[13] Malaria is holoendemic in the region and estimates of entomologic inoculation rates range between 60 and 300 infectious bites per person per year.[20] The primary vectors in this region are *Anopheles gambiae* Giles and *An. funestus* Giles. *Plasmodium falciparum* is the principal species of human malaria in the region. Parasite prevalence studies indicated that 70–80% of those less than five years old were infected at any time, with little seasonal variation.[15]

Study design. The study design was consistent with the previous randomized controlled trials to enable comparison of outcomes among sites. Cluster randomization was used, with village as the cluster unit. Detailed accounts of the study site and design are given elsewhere.[13,14] The study was initially designed to detect a 30% reduction in all cause mortality in children 1–59 months old, with 90% power, in a population of 60,000. Demographic surveillance was established during 1996 in Asembo where the trial commenced. After distribution of ITNs in Asembo, final results from the trial in Burkina Faso suggested the efficacy of curtains and ITNs in areas of high transmission might be substantially lower than 30%,[4] implying that the sample size in Asembo might be insufficient. The trial site was therefore expanded to Gem, increasing the total population in the trial to 125,000 people, with approximately 18,500 children 1–59 months of age. After expansion, the study was estimated to have 90% power to detect a 17.5% difference in mortality in children 1–59 months old, given a study period of two years in each site, loss to follow-up of 5% per year, a design effect of 20%, and a ratio of intervention to control of 1.

Randomization and allocation of ITNs. Prior to the trial, government officials, opinion leaders, and the community were informed about the proposed study.[13] Modes of communication included public meetings (*baraza*), Luo and English language leaflets, field staff workshops, participatory educational theatre, school competitions, message calendars, and songs produced by traditional birth attendants. Randomization of villages took place by public lottery at celebration days launching the project. Forty of 79 villages in Asembo and 73 of 142 villages in Gem were randomized to receive ITNs. The ITNs were distributed in the fourth quarters of 1996 and 1997, in Asembo and Gem, respectively. A total of 45,667 polyester, 100-denier, 156-mesh ITNs, (Siamdutch Mosquito Netting Co., Ltd., Bangkok, Thailand), were distributed to cover all sleeping spaces of the intervention population (approximating 61,000 persons). This provided an initial coverage ratio of 1.34 persons per ITN. Quarterly monitoring of adherence with ITN use during intervention estimated a coverage ratio of 1.46 persons per ITN during the trial, and an adherence rate (persons directly observed to be sleeping under ITNs) of 66% in children less than five years old.[21] Bed nets were pre-treated with a target dose of 0.5 g of permethrin/m^{2} of netting. We aimed to re-treat nets every six months with the same target dose, using permethrin in the form of Peripel® 55% emulsifiable concentrate (AgrEvo, Berlin, Germany). Both insecticide and nets were imported duty-free via the U.S. Embassy in Nairobi. The terrorist bombing of the Embassy in August 1998 resulted in delays in net re-treatment in some villages in 1998 and 1999. Start dates for the randomized controlled trial were January 1, 1997 and January 1, 1998 in Asembo and Gem, respectively, and completion dates were December 31, 1998 and December 31, 1999, respectively. Participants in control villages in Asembo and Gem received ITNs after closure of the trial.

Evaluation of the efficacy of ITNs on child mortality. Surveillance methods conformed to guidelines set by the World Health Organization for the previous randomized controlled trials. A community-based demographic and health surveillance site was established in Asembo and Gem to enumerate children in the study site and monitor deaths through biannual household census. Studies evaluating child morbidity, entomology, weather, behavior, and health economics were conducted in Asembo only.[14] The household census, conducted by traditional birth attendants in their village of residence, monitored migration, births, and deaths. Standardized forms were used to document information on all participants. Households were revisited approximately every six months, using computer-generated printout forms containing demographic details of the household documented from the previous census. New enrollment forms were completed for new migrants and births during census follow-up. Deaths of children were validated by follow-up to confirm that the deaths had occurred, and that the age at death and reported deaths were of children resident in the study area.

Prior to distribution of ITNs, a preliminary census in August 1996 estimated a total of 8,576 children resided in Asembo, of whom 4,195 (48.9%) lived in villages designated to receive ITNs. The baseline census detected 294 deaths in children less than five years old in the preceding six months, of whom 146 (49.7%) were children who had lived in villages due to receive ITNs, with no significant difference between pre-intervention and control villages ($P = 0.802$). Vital registration, conducted independently of the census, detected 242 deaths in children less than five years old in the preceding six-month period, of whom an equal proportion (123, 50.8%; $P = 0.558$) were from villages due to receive ITNs. Thus, there was no evidence to suggest mortality rates pre-intervention differed significantly between treatment groups.

Eligibility criteria. Analysis was restricted to children who lived in the study site for more than one month and who were between 28 days and 59 months old. The following exclusion criteria were used to standardize deaths and person-time:

Deaths were excluded if a child died on or before the first day of the study; was stillborn; died within 28 days of birth; died within 30 days of migration into the study area and had no earlier person-time; contributed no person-time for a reason other than those listed; or had a data record with one or more conflicting critical variables that could not be resolved.

Person-time was excluded if the child had a date of migra-

tion into the study area plus 30 days that was after the last day of the study (December 31, 1998 in Asembo and December 31, 1999 in Gem; thus, no person-time was generated); died within 28 days of birth (death within the study period); had a date of birth plus 28 days that was after the last day of the study; left within 28 days of birth and did not return; died within 30 days of migration into the study area and had no earlier person-time; turned five years old before the arrival date plus 30 days and had no earlier person-time; contributed no person time for a reason other than those listed; and or had a data record with one or more conflicting critical variables that could not be resolved.

Data management and statistical analysis. Forms were checked and coded at the central field office, and forwarded to the Kenya Medical Research Institute/Centers for Disease Control and Prevention office in Kisian for data entry. Data entry was performed using custom designed (Clarion™ software; Topspeed/Soft Velocity, Pompano Beach, FL) data entry screens. Validation was performed by running logical checks and error listings. Analysis was performed using Statistical Analysis System software (SAS system for Windows, Release 8.02; SAS Institute, Cary, NC).

Crude village-based analysis was first performed using Proc Genmod (SAS Institute) to fit a Poisson regression model, with the number of deaths in a village as the outcome variable and the log base 10 of the person-time in that village as an offset to adjust for any differences in the amount of person-time in the intervention and control villages. Person time calculation and the estimation of mortality rates by randomization status, age, and year of death for this analysis did not need to take clustering into account since this analysis was done at the village level rather than at the individual level.

Survival analysis using individual-level person-time was conducted using Proc PHREG (SAS Institute) to fit a Cox proportional hazards model with time-dependent covariates (covariates included were rainfall, temperature, site, study year, age group, and calendar year). A robust sandwich estimate was used to correct the covariance matrix for clustering at the village level (Proc PHREG, version 8.02; SAS Institute). A counting process method was used to allow children to move in and out of the survival analysis risk set as well as control for time-dependent covariates.[22] Age was included in the model as a time-dependent covariate. Thus, the model accounted for time under ITNs for each age group and age at enrollment. No effect modification ($P = 0.7437$) was observed between sites in 1998 (the only year that both sites contributed data concurrently); thus data from the two sites were pooled. Subsequent models used pooled data while still adjusting mortality estimates for site. Study year × ITN interactions were significant. To test whether this effect reflected sequence (first year of study versus second year of study) or characteristics of particular years, we evaluated rain × ITN and temperature × ITN interactions, but these were not significant, and were dropped from the model. However, rainfall and ambient temperate were kept in the model as main-effect covariates to adjust for season. Subsequent examination of the data showed that most of the year effect was associated with delayed re-treatment with insecticide in late 1998 and 1999. A final model was therefore created to include the effect of time since re-treatment of nets with insecticide. As the goal stated in the study protocol was to re-treat nets every six months, the breakpoint for a promptly treated versus late

treated net was set as six months, defined as 180 days. In this model, the exposure variable was dichotomized as promptly treated nets (defined as those treated within six months of previous treatment) or late treated nets (those treated more than six months after last treatment); the comparison group was no net (defined by residence in a control village). In this model, year × ITN interactions were not significant and were dropped; the effect of late re-treatment was significant and was incorporated in the final model.

All analyses between ITN and control villages were on an intention-to-treat basis. Statistics are presented as the relative risk or hazard ratio (HR) and their 95% confidence intervals (CIs). For all statistical tests a two-sided P value < 0.05 was considered significant. Protective efficacy of ITNs was calculated using the adjusted hazard ratio estimates from Cox proportional hazards models as $100 \times (1 - HR)\%$.

Definition of mortality rates. Mortality rates per 1,000 person-years were calculated. The post-neonatal mortality rate was defined as the rate of death in infants 28–365 days old (henceforth referred to as infants 1–11 months old) per 1,000 contributing person-time within that age group. The early child mortality rate was defined as the rate of death in children between 12 and 59 months of age per 1,000 children contributing person-time within that age group. The overall mortality rate was calculated for children between the ages of 28 days to 59 months (henceforth referred to as 1–59 months old) per 1000 children contributing person-time within that age group.

Ethical clearance. The ITN trial 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).

RESULTS

Between January 1, 1997 and December 31, 1999, 1,722 deaths were reported and verified in children 1–59 months of age, in a population followed for 35,932 child-years. Of these, 940 (54.6%) and 782 (45.4%) were from control and ITN villages, respectively. The distribution of deaths by age in months indicates that the majority of deaths in children less than five years old occurred early in the first year of life (Figure 1). Fifty-three percent of deaths in children 1–59 months of age from control villages occurred in children 1–11 months old, with most deaths occurring between the ages of two and seven months. Distribution of death rates by calendar month illustrates a rough association with rainfall, particularly in deaths of children living in control villages (Figure 2). Following a lag time of 1–2 months, death rates peaked after the rains in 1997 and 1998.

Intention to treat analysis showed that the crude mortality rates in children from control and intervention villages were 51.9 and 43.9 per 1,000 child-years, respectively. The result of the crude (village-based) estimates of the relative risk obtained from Poisson regression models were identical to the hazard ratios obtained from Cox proportional hazard modeling of individual person-time when we controlled for age, study site, study year, rainfall, and temperature. The overall reduction in mortality was 16% and was statically significant (Table 1). Mortality rates in the 1–11-month-old age group, were 102.3 and 133.3 per 1,000 in intervention and control
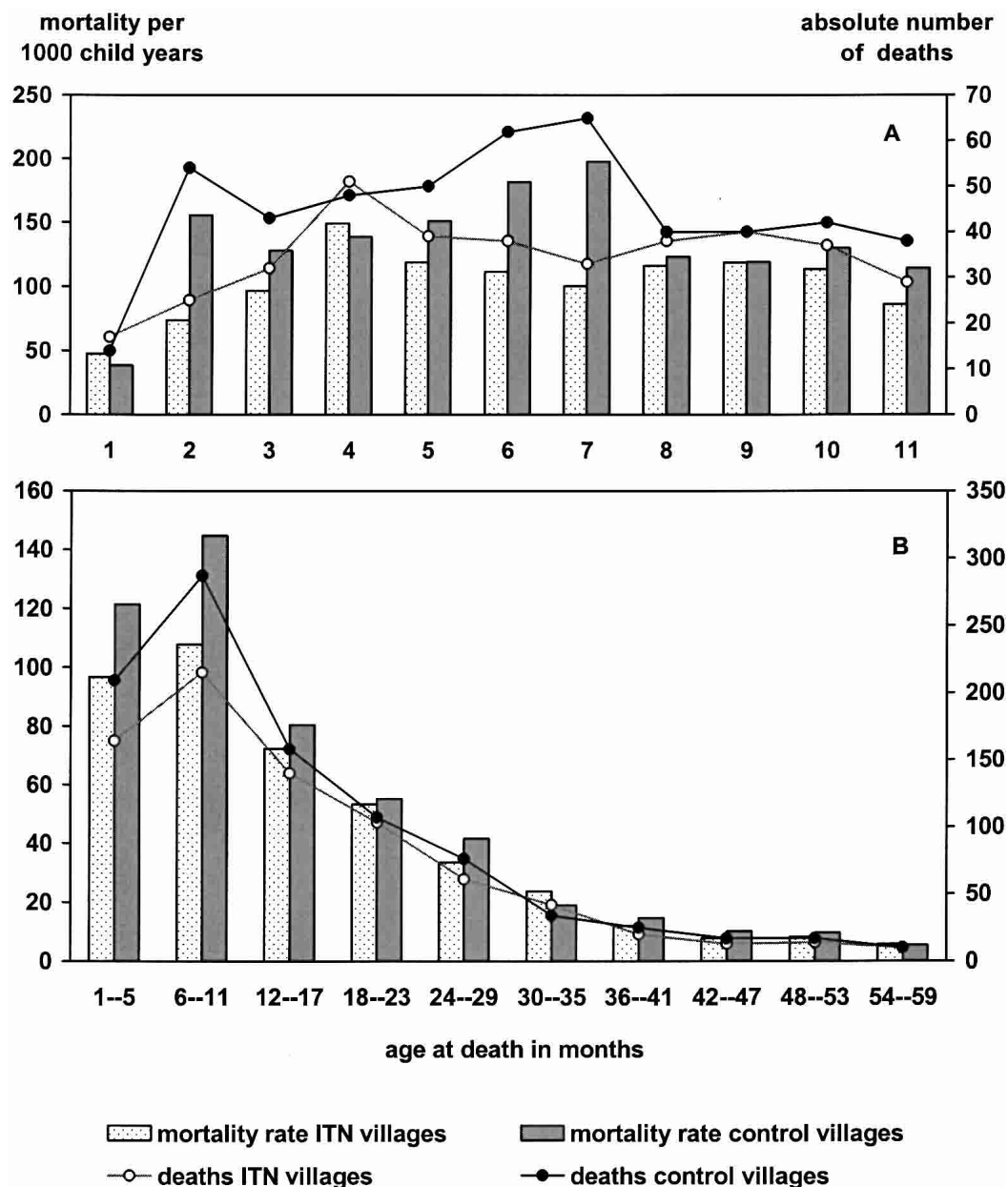
FIGURE 1.   Mortality rates of children during the insecticide-treated bed net (ITN) trial in western Kenya. **A**, Mortality rate in infants 1–11 months of age. **B**, Mortality rate in children 1–59 months of age.

villages, respectively. The adjusted PE of ITNs in infants was 23% compared with 7% in children 12–59 months old. In all, eight lives were saved per year for every 1,000 children 1–59 months old protected. For children 1–11 months old, 31 lives were saved per 1,000 protected, while for children 12–59 months of age, two lives were saved per 1,000 per year.

Results of analysis of ITN efficacy stratified by insecticide re-treatment time as per study protocol (<6 months since last treatment) are shown in Table 2. Using no nets as the comparison group, we observed that the adjusted individual-based estimate of the overall protective efficacy of promptly re-treated ITNs was 20% (95% CI = 10–29%). The effect of promptly treated ITNs was marked in infants 1–11 months of age: PE = 26% (95% CI = 12–37%). A lower impact, which was not statistically significant, was seen in children 12–59

months old (PE = 14%; 95% CI = −1–26%). However, the difference in protective efficacy of promptly treated nets between these two age groups was not significant (*P* = 0.16). When ITN re-treatment was delayed beyond six months, estimates of efficacy were not significant in any age group. Based on the crude estimate of 133.3 deaths per 1,000 infants 1–11 months old in the control population, and using the adjusted individual-based estimate of 26% reduction in deaths in that age group, we estimate that 35 infant deaths per 1,000 protected would be saved if ITNs were used and re-treated according to the standard protocol (Table 2).

DISCUSSION

In this paper, we describe the effect of permethrin-treated bed nets on child mortality in an area of high and perennial
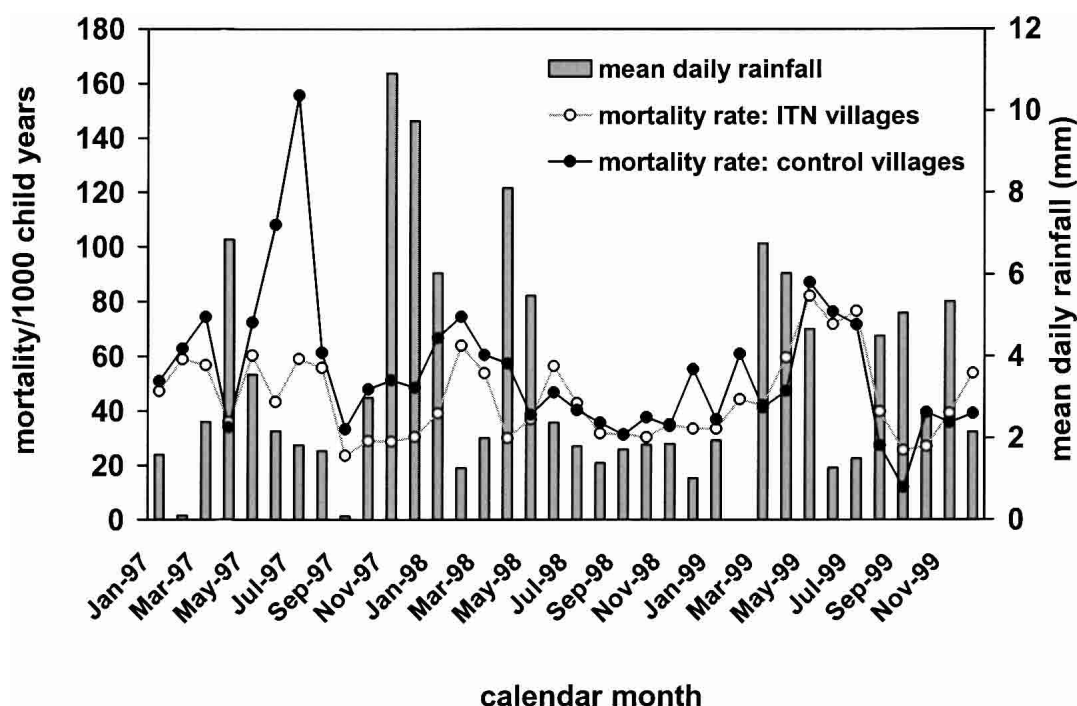
FIGURE 2. Seasonal trends in less than five years old (1–59 months old) child mortality during the insecticide-treated bed net (ITN) trial in western Kenya.

malaria transmission in western Kenya. In previous controlled trials, the smallest protective efficacy was recorded in Burkina Faso (reduction of 15% in children 6–59 months old, with confidence limits overlapping zero),[4] an area with very

high but seasonal malaria transmission. The strongest efficacy (30% reduction in children 1–59 months old) was reported from coastal Kenya where malaria transmission is both seasonal and approximately one-tenth as intense as that observed at the Burkina Faso study site.[3] Recent findings from a case-control study in Tanzania, where the transmission intensity and pattern are similar to that in our study site in western Kenya, detected a reduction of 27% in the mortality of 1–59-month-old children based on data generated from a social marketing project.[12] However, as with any case-control study, control of confounding is problematic. Our randomized controlled trial confirms that ITNs reduce all-cause child mortality in children in this age group by 16% (95% CI = 6–25%) in this area of intense perennial malaria transmission. Our confidence limits overlap with the social marketing trial, and with the rates generated from all other randomized controlled trials, irrespective of malaria transmission intensity.

The baseline census suggested that mortality rates pre-intervention were similar between the subsequent ITN and

TABLE 1

Crude (village-based) mortality rates and multivariate survival analysis of insecticide-treated bed net (ITN) efficacy in western Kenya, by age

	1–11 months	12–59 months	1–59 months*
Crude village-based analysis (ITN)			
Deaths/person-years	376/3,677	406/14,163	782/17,833
Mortality/1,000 person-years	102.3	28.7	43.9
Control			
Deaths/person-years	492/3,691	448/14,413	940/18,099
Mortality/1,000 person-years	133.3	31.1	51.9
Relative risk†	0.77	0.92	0.84
95% confidence intervals	0.67–0.88	0.80–1.05	0.77–0.93
P value†	0.0001	0.2275	0.0005
Cox-proportional hazard model			
Adjusted hazard ratio‡	0.77	0.93	0.84
95% confidence intervals	0.66–0.89	0.81–1.06	0.75–0.94
Protective efficacy§	23%	7%	16%
95% confidence intervals	(11–34)	(−6–19)	(6–25)
Lives saved per 1,000 child-years§	31	2	8
95% confidence intervals	(14–45)	(−2–6)	(3–13)

* Insecticide-treated netting: 8,805 children; control: 8,947 children (January 1, 1998).
† Poisson regression.
‡ Hazard ratio (HR) adjusted for age, study year, study site, and season.
§ Protective efficacy = 100 × (1 − HR)%, lives saved/1,000 child-years = (1 − HR) × crude mortality in control villages.

TABLE 2

Age-specific reductions in child mortality, expressed as the protective efficacy, stratified by time to retreatment

Time since last bed net re treatment age category (months)	Protective efficacy* (95% confidence intervals)	Lives saved/1,000 child-years† (95% confidence intervals)
<6 months		
1–11	26 (12–37)	35 (16.4–50)
12–59	14 (−1–26)	4 (0–8)
1–59	20 (10–29)	10 (5–15)
≥6 months		
1–11	17 (−5–34)	22 (−7–45)
12–59	−8 (−34–13)	−2 (−10–4)
1–59	5 (−11–19)	3 (−5–10)

* Protective efficacy estimate of promptly and delayed re-treated insecticide-treated nettings compared to no nets. Estimates represent 100 × (1 − hazard ratio)%. Hazard ratios (HRs) were adjusted for age, study year, study site, and season.
† (1 − HR) × crude mortality in control villages.

control villages. Our passive surveillance system in 13 peripheral health facilities in Asembo also showed that availability of drugs and access to healthcare was the same for intervention and control villages, and this is thus unlikely to have resulted in bias or explain our findings.[23]

Our trial detected the greatest protective effect in post-neonatal infants 1–11 months old (PE = 23% [95% CI = 11–34%]) compared with a non-statistically significant reduction of 7% (95% CI = −6–19%) in older children. Because more than half of all recorded deaths in children 1–59 months old occurred in the post-neonatal infant period, this difference in ITN efficacy by age strata translates into a particularly striking difference in absolute number of lives saved: 8 per 1,000 children per year overall (1–59 months old), but 31 per 1,000 infants 1–11 months old, and approximately 8 per 1,000 children 12–59 months old (i.e., 2 per 1,000 child-years). Thus, although infants 1–11 months old contributed just over 20% of the overall person-time in this study, more than three-fourths of all deaths prevented occurred in this age group. These findings have important implications for public health programs. Other studies described in the companion manuscripts in this series illustrate the marked impact of ITNs on malaria-associated anemia in pregnant women and adverse birth outcome, in particular low birth weight,[24] as well as the beneficial effect on improved child hemoglobin levels, improved growth in young children,[25] and reduced frequency of sick child visits to peripheral health facilities.[23] This suggests that in areas of intense malaria transmission mother-infant pairs benefit most from ITN use and should be the main target group of malaria control programs.

Smith and others have emphasized that all-cause mortality in infants living in malaria-endemic areas is substantially higher than in children residing in areas of low malaria transmission pressure.[10] Data generated from our trial confirm previous reports of the very high post-neonatal and early childhood mortality in this area of rural western Kenya.[26] Our post-neonatal infant mortality rate in control villages was 133 per 1,000, which was very similar to the 148 per 1,000 detected in an earlier longitudinal cohort study.[26] However, both studies may have underestimated the true rates. In our ITN trial, deaths were detected by household census and may have underestimated babies born who died between censuses. In the cohort study, children were treated with sulfadoxine-pyrimethamine if febrile and parasitemic, and this treatment may have placed them at a survival advantage over the general population.

If we use ITN efficacy as a crude proxy for estimating the malaria-attributable fraction of mortality, we estimate that approximately one-fourth of the deaths in post-neonatal infants living in control villages were attributable to malaria. The proportion of deaths in children 12–59 months old that could be attributed to malaria was approximately half this (14% based on estimates from models with promptly re-treated ITNs). Thus, our data suggest that in this setting of high malaria transmission, one in four deaths in post-neonatal infants and one in seven to eight deaths in older children are a direct or indirect result of malaria-associated illness. These rates probably underestimate, rather than overestimate, the total burden of malaria in this population. As shown by Hawley and others, ITNs bestow a community-effect on unprotected children living within a 300-meter radius of homes with ITNs.[27] Controlling for this effect will increase estimates of

efficacy and therefore increase the estimate of the malaria-attributable fraction of mortality as well.

Several of the previous studies on insecticide-treated bed nets and curtains have shown a higher efficacy in the first compared with the second study year.[1,4] In Burkina Faso, treated curtains were highly effective in the first year, but no effect was observed in the second study year. With the exception of the Ghana bed net trial, other studies show similar but less marked trends. We also observed a significant reduction in ITN efficacy in the second year in our passive morbidity surveillance in Asembo, as described elsewhere,[23] but this was not confirmed in our individual-based mortality analysis. Although the village-based analysis and the initial multivariate survival analysis (adjusted for covariates) also indicated a reduced efficacy in the second year, further stratified analysis that took into account whether bed nets had been re-treated according to study protocol or not (every six months), suggested that almost all of the reduced efficacy in the second study year could be explained by late re-treatment of nets. The lowering of efficacy due to delayed re-treatment has not previously been evaluated during randomized controlled trials. The efficacy of promptly re-treated ITNs in children 1–11 months old was 26%, but this decreased to 17%, a 35% loss in efficacy, when re-treatment with permethrin was delayed beyond six months. Thus our intention to treat estimate of 23% is likely to underestimate the true efficacy of bed nets. Entomologic data generated during the trial biologically support this finding. Gimnig and others reported that the reduction in indoor resting densities of *An. gambiae s.l.* compared with control houses was only 16.9% ($P = 0.694$) if the prior insecticide treatment had occurred more than six months previously.[28] However, long delays in re-treatment had less effect on *An. funestus,* since a significant ($P = 0.001$) reduction of 84.2% was observed in houses with ITNs that had been re-treated at intervals of more than six months. Whether similar effects would be observed for other pyrethroids with longer residual effects is unknown.

Permethrin-treated bed nets are highly effective and prevent approximately one in four infant deaths in areas of intense perennial malaria transmission, but their efficacy is reduced if re-treatment with permethrin is delayed beyond six months. These findings support the hypothesis that the protective efficacy of ITNs is substantial in all malaria-endemic settings in sub-Saharan Africa, regardless of the intensity of malaria transmission. Monitoring morbidity and mortality in children less than five years of age continues in our two trial sites to determine if a reduction in malaria exposure for more than two years will result in a delay in the acquisition of immunity, and whether such effects will adversely affect child survival.

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