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IMPACT OF PERMETHRIN-TREATED BED NETS ON MALARIA, ANEMIA, AND GROWTH IN INFANTS IN AN AREA OF INTENSE PERENNIAL MALARIA TRANSMISSION IN WESTERN KENYA

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Abstract. As part of a community-based, group-randomized, controlled trial of insecticide-treated bed nets (ITNs) in an area with intense malaria transmission in western Kenya, a birth cohort (n = 833) was followed monthly until the age of 24 months to determine the potential beneficial and adverse effects of reduced malaria exposure during pregnancy and infancy. Malaria transmission and morbidity were comparable pre-intervention. The ITNs reduced malaria attack rates (force of infection) in infancy by 74%, and delayed the median time-to-first parasitemia (4.5 to 10.7 months; $P < 0.0001$). The incidence of both clinical malaria and moderate-severe anemia (hemoglobin level <7 g/dL) were reduced by 60% ($P < 0.001$ for both). Protective efficacy was greatest in infants less than three months old and similar in older infants and one-year-old children. Efficacy was lowest in the dry season. Infants from ITN villages experienced better height and weight gain. In areas of intense perennial malaria transmission, ITNs substantially reduce exposure to malaria and subsequent malaria-associated morbidity in children less than 24 months old. Reduced malaria exposure during infancy did not result, with continued ITN use, in increased malaria morbidity in one-year-old children.

INTRODUCTION

Insecticide-treated bed nets (ITNs) have been demonstrated to markedly reduce malaria morbidity and mortality over a wide range of malaria-endemic settings.[1] Little information is available from randomized controlled trials on the effects of ITNs in settings with intense and perennial malaria transmission,[1] such as in our study site in western Kenya, where the burden of malaria is predominantly in pregnancy and in very young children 2–16 months old.[2]

We show elsewhere in this supplement that ITNs reduce both the number of malaria transmitting mosquitoes by as much as 90%,[3] and adverse effects of malaria in pregnancy, resulting in improved birth weights.[4] Previous reports by others[5,6] have indicated a further beneficial impact on weight gain with continued ITN use during infancy, likely reflecting improved overall health from reduced episodes of clinical malaria.[7] One of the previous studies, conducted in an area with low-to-moderate and seasonal malaria transmission in The Gambia, also assessed the impact on linear growth in children, but found none. Infants were not included in this study.[8] In areas of intense malaria transmission, the periods of maximum vulnerability to malaria and maximum annual growth velocity coincide. It is thus possible, but remains to be determined, that protection from malaria morbidity by ITNs in the first year of life could potentially result in improved linear growth, a benefit that may be sustained beyond the period of highest malaria susceptibility.

Although ITNs have the potential to impart considerable infant health benefit, any intervention that substantially reduces exposure to malaria may also have undesirable consequences. During the first few months of life infants are partially protected from symptomatic malaria through a combination of reduced exposure to mosquito biting, physiologic (e.g., fetal hemoglobin) and immunologic factors, including the transfer of maternal IgG antibodies and possibly sensitization of the fetus *in utero*.[9,10] Although the mechanisms of these immunologic factors are incompletely understood,[9] they do suggest that maternal or fetal exposure to malaria during pregnancy may contribute to immune protection in early infancy. While ITNs reduced malaria-associated morbidity in pregnancy,[4] they also reduced maternal antibody responses to liver and blood stage antigens and the transfer of these antibodies to the newborn, potentially affecting passive immune protection in young infants.[11]

Similarly, a marked decrease in exposure to malaria early in life may result in a delay in the acquisition of clinical immunity against malaria, which in the long term could result in increased clinical disease and possibly malaria-associated deaths in these children at older ages.[12,13] This is a particular concern in areas of intense malaria transmission, where children experience approximately 10–11 blood-stage infections before the age of one year.[14] It is unclear whether a reduction in this high attack rate will prolong the period of vulnerability beyond 16 months, or may actually result in better antibody responses to malaria blood-stage antigens, possibly resulting from less, but more efficient, immune stimulation, as is suggested by our previous immuno-epidemiologic cohort studies and from our bed net cross-sectional surveys.[15,16]

To address the risks and benefits of bed net use before birth and during infancy, we have followed a cohort of pregnant women and their infants as part of the Asembo Bay Cohort Project; a study of the epidemiology of malaria in pregnancy and the acquisition of natural immunity to malaria in children less than five years of age.[17] We report here the impact of bed nets on the dynamics of malaria parasitemia and subsequent malaria morbidity, severe anemia, and growth in the first two years of life. This study also provided the opportunity to evaluate several determinants of ITN efficacy in this young age group.

MATERIALS AND METHODS

Study site and population. This study was conducted within the context of a large community-based, group-randomized,

controlled trial designed to assess the impact of bed nets on mortality in children less than five years of age.[18] The mortality surveillance of the bed net trial was conducted in both Asembo and Gem in an area of 500 km^2 in Bondo and Siaya districts of western Kenya.[19] The Asembo Bay Cohort Project was conducted in an area of 70 km^2 (18,000 people) in the southernmost part of Asembo on the shores of Lake Victoria. Full details of the study site have been described elsewhere.[20,21] Briefly, the area has intense perennial malaria transmission (60–300 infective bites per person annually), with *Anopheles gambiae* as the predominant malaria vector.[22] Malaria transmission occurs throughout the year, with peaks during May through July, and October through November. The point prevalence of *Plasmodium falciparum* parasitemia in children less than five years old ranges between 60% and 80%, depending on the season.[17] More than 95% of these infections are due to *P. falciparum*, and almost all of the remainder is due to *P. malariae*. Infections with *P. ovale* are rare.[17] High-grade chloroquinine resistance is widespread,[23] and parasitologic treatment failure by day 7 with sulfadoxine-pyrimethamine was approximately 20% by the start of the ITN study (ter Kuile FO, unpublished data). Malnutrition is common; 30% of children less than five years old are stunted and 20% are underweight.[24] Infant and under-five mortality are very high: 176 and 257 per 1,000 live births, respectively.[25]

Study design and randomization. Half of the villages in Asembo were randomly assigned to the intervention group and each household in these villages received ITNs, covering all bed spaces, during the fourth quarter of 1996, providing a coverage ratio of 1.5 persons per ITN.[26] Households in control villages received ITNs in April 1999 after the mortality trial was completed. Four of the 15 original villages in the Asembo Bay Cohort Project[20] were much larger than the remaining 11 and were split into two at the beginning of the ITN project, giving a total of 19 village clusters, henceforth referred to as villages. At distribution, bed nets were pretreated with permethrin (Siamdutch Mosquito Netting Co., Bangkok, Thailand), and re-treated twice a year by the study team to maintain a target dose of 500 mg of permethrin/m^2.[19]

Asembo Bay Cohort Project. The infants in this cohort were born to women of all parities identified through monthly census by trained village monitors and/or trained traditional birth attendants residing in the same village. All resident pregnant women and their newborns were eligible for enrollment. Full details of recruitment procedures and follow-up of pregnant women and their newborns have been described elsewhere.[4] Briefly, between June 1992 and April 1999, pregnant women were enrolled and followed at monthly intervals, until the final month of gestation, when weekly visits were conducted. A traditional birth attendant monitored the deliveries and completed a data form that described details of each birth event. At the time of delivery, birth weight and gestational age[27] were determined and blood samples obtained from the mother, placenta and cord for determination of malaria parasitemia, hemoglobin concentrations, and malaria serology. Seven and 14 days after birth, and every two weeks thereafter, each newborn child was visited by a village monitor until the end of the study, the child's second birthday, or until the child's death, whichever came first. If children moved out of the cohort study area for six or more months, they were dropped from the study (from 1997 onwards). Children born in 1992–1996 were followed until their fifth birthday, but only observations in the first two years of life have been included in this analysis. At each visit, a standard morbidity questionnaire was administered and the axillary temperature was recorded. At every other visit (i.e., every four weeks), a finger or heel prick blood sample (250–500 μL) was taken for determination of hemoglobin (Hb) concentrations or packed cell volume (hematocrit) and the presence of malaria parasites. Anthropometric measurements were also performed at this time. Children with non-severe symptomatic malaria (axillary temperature ≥37.5°C with any malaria parasitemia) or high density infections (≥5,000/mm^3 [1997 onwards]) detected at follow-up visits, or when the child was ill in between routine visits, received a supervised dose of sulfadoxine-pyrimethamine. All children with severe malaria, with hemoglobin concentrations less than 5 g/dL (hematocrit <15%) or with any other severe disease requiring hospitalization were referred immediately to the local mission hospital for further management free of charge.

The sample size was based on the number of deliveries required to detect a 25% reduction in the prevalence of adverse birth outcome defined as described elsewhere.[4]

Laboratory analysis. All laboratory assays were processed at the Kenya Medical Research Institute/Centers for Disease Control and Prevention laboratories in Kisian, 40 km from the field site. Thick and thin blood smears were stained with Giemsa and examined for parasites. Parasite densities were counted against 300 leukocytes and expressed per mm^3 of blood using an estimated leukocyte count of 8,000/mm^3. Slides were considered negative if no asexual parasites were found in 200 high-power ocular fields of the thick smear. Between 1992 and 1996, hemoglobin concentrations were measured using the HemoCue system (HemoCue, Angelholm, Sweden). From 1997 onwards, heparinized capillary tubes containing whole blood obtained by finger prick were centrifuged at 10,000 cycles/second for three minutes to determine the hematocrit. All hematocrit values have been divided by a factor of three and are presented as hemoglobin values for consistency with the 1992–1996 data. Hemoglobin electrophoresis was conducted prospectively for all enrolled children born during the intervention phase to determine hemoglobin phenotype. The hemoglobin A and S genes for children born between 1992 and 1996 were typed retrospectively using a polymerase chain reaction method as described by Wu and others.[28]

Definitions. Malaria parasitemia was defined as asexual blood stage malaria parasites of any *Plasmodium* species and density detected on a thick blood smear. High-density parasitemia was defined as malaria parasitemia (any species) above an age-dependent threshold parasite density (0–5 months = 1,500/mm^3; 6–11 months = 6,000/mm^3; and 12–35 months = 7,000/mm^3).[17] Symptomatic malaria was defined as a documented axillary temperature ≥37.5°C in the presence of any malaria parasitemia. Symptomatic high-density malaria was defined as a documented axillary temperature ≥37.5°C in the presence of high-density parasitemia.[17] Moderately severe and severe anemia were defined as hemoglobin concentrations <7 g/dL and 5 g/dL, respectively, and moderately severe and severe malarial anemia were defined as hemoglobin concentrations <7 g/dL and 5 g/dL, respectively, in the presence of any malaria parasitemia. Force of infection was defined as the attack rate of malaria parasitemia per day per child obtained from a reversible catalytic model (see also

Data analysis). The educational status of the caretaker was ranked and grouped in terciles based on the total years of education and categorized as low, medium, high, or missing. Socioeconomic status was divided into percentiles for bivariate analysis and in modeling as a categorical variable as low, medium, high (terciles), or missing, according to the rank position of the computed wealth index based on type of house and ownership of livestock, radios, bicycles, and sofas.[29] Each observation was defined to have occurred in the rainy, post-rainy, or dry season using equal rank groups based on rainfall data collected in the study area in the period 30–90 days before the observation. Wasted, underweight, and stunted were defined as having at least two consecutive Z-score values <–2 below the reference median Z-scores for weight-for-length, weight-for-age, and length-for-age, respectively. Nutritional Z-scores were calculated using Epi-Info, version 2000 (Centers for Disease Control and Prevention, Atlanta, GA).

Data analysis. *Incidence of morbidity.* The age-specific incidence of several morbidity parameters, identified at either the two weekly (clinical assessment) or four weekly (malaria smear and anemia) routine follow-up visits, or in-between scheduled visits, were obtained by dividing the number of episodes fulfilling the case definition by the total number of days at risk for the appropriate age group. The days at risk were obtained by dividing the observation period into time intervals of approximately two weeks (clinical assessment) or four weeks (malaria smears and anemia) corresponding to either the number of days in-between routine home visits, or until an extra visit was made because of an episode fulfilling the case definition, whichever came first. Only intervals of less than 21 days (fever assessment) or 35 days (malaria smears and anemia) were included (i.e., a two or four weekly routine visit could be no more than six days late).[14] Each time interval was assigned to the appropriate age group based on the age of the child at the midpoint of each interval. Observations were included in the time at risk analysis from birth until the child had reached the age of 24 months, was lost to follow-up, or until the date of death, or the end of the study period (December 31, 1996 for the pre-intervention cohort and March 31, 1999 for intervention cohort), whichever came first. All episodes and time at risk, occurring within 28 days (malaria and all cause morbidity) or 84 days (i.e., 3×4 weeks) (anemia) after each episode, were excluded from the analysis.[30]

Force of infection (instantaneous attack rates). Because the standard estimates of infection rates underestimate the true acquisition of malaria infection, the force of infection was also approximated for each intervention group as the acquisition rate (h) per child per day using a reversible catalytic model, which allows for the possibility that a child both gained and cleared (in the absence of treatment) an infection in a given time interval, as described in detail by others.[31] All episodes and time at risk after each treated episode (sulfadoxine-pyrimethamine) were excluded for a 28-day period from the analysis.

Time to first event. An analysis of determinants of the time and risk of first infection was modeled using Cox-proportional hazards regression models with age independent co-variables (maternal, household, and birth characteristics, including season of birth). Median time to first infection was obtained from Kaplan-Meier survival analysis. Children who missed more than one follow-up visit (interval of 35 days or greater) were considered censored at the age of the last visit (status unknown after that age).

Statistical analysis. All analyses were based on intention-to-treat, and took the cluster design at the village level into account. SUDAAN release 8 (Research Triangle Institute, Research Triangle Park, NC), Statistical Analysis System (SAS 8.2; SAS Institute, Cary, NC), and STATA (Intercooled version 7; Stata Corp., College Station, TX) software packages were used.

Data collected before the start of the ITN study, i.e., between June 1992 and December 31, 1996, were used to compare the distribution of the endpoints and characteristics prior to the introduction of ITNs. Data collected between January 1, 1997 and April 1, 1999 were used to assess the impact of ITNs. The statistical significance of the differences in the incidence of morbidity measures between (subsequent) ITN and control villages was tested using Poisson regression with the number of days at risk as the offset. The magnitude of the effect of ITNs was expressed as the protective efficacy and estimated as $100 \times (1 - \text{adjusted rate ratio})\%$ or $100 \times (1 - \text{adjusted hazard ratio})$. All Poisson or Cox-proportional hazard regression models allowed for the effect of age by including age as categorical variables (as 0–2, 3–5, 6–8, 9–11, 12–15, and 16–23 months unless indicated otherwise), and also included sickle hemoglobin phenotype (HbAS, HbAA, HbSS, or missing) rainfall and gravidity. Years of education of the caretakers were unequally divided between ITN and control villages and associated with some of the morbidity endpoints independent of maternal age. Educational status, ranked in three categories based on reported years of schooling was, therefore, included as covariate in all subsequent models to adjust for any potential confounding.

To compare the differences between the mean nutritional score in the ITN and control groups, a repeated measure analysis in SAS proc mixed, using maximum likelihood estimation and a compound symmetry covariance structure to account for the within village clustering, was implemented. To account for any misspecification in the covariance structure a robust (empirical) variance estimator was used. Because of a difference in mean length-for-age Z-scores by randomization status before the introduction of ITNs, we also assessed the impact of ITNs using a historical comparison by adding an interaction term for the time-effect and randomization-group to the models. The time-effect indicated whether the observation was before or after January 1, 1997 (the start of the ITN study). Two sided P values <0.05 were considered statistically significant.

Ethical clearance. The ITN study and the Asembo Bay Cohort Project were approved by the institutional review boards of the Kenya Medical Research Institute (Nairobi, Kenya) and the Centers for Disease Control and Prevention (Atlanta, GA). Informed consent was obtained from all caretakers after explanation of the study procedures in the local language.

RESULTS

In the pre-intervention period, 1,501 single live births were enrolled at birth or within 14 days thereafter, and a further 860 during the intervention period (Figure 1). Of these, 1,451 (96.7%) pre-intervention and 831 (96.6%) during interven-

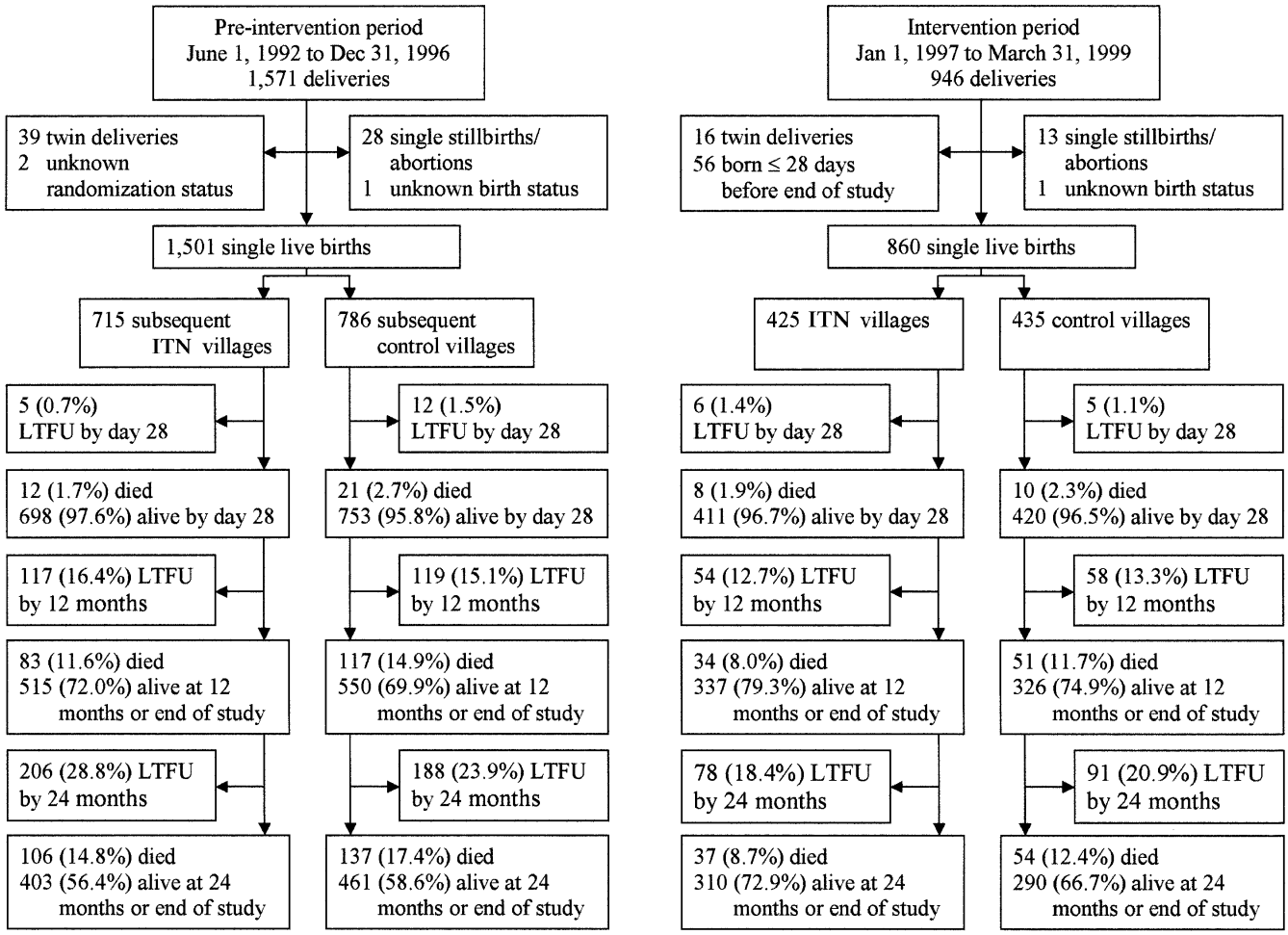

FIGURE 1. Trial profile. LTFU = lost to follow-up, ITN = insecticide-treated bed net.

tion contributed at least 28 days follow-up time and were included in the analysis (Table 1).

During the intervention period, 691 (80.3%) (ITN = 81.6%, control = 79.1%) of the newborns were followed until they reached the age of 24 months, the end of the study period, or until the time of death, whichever came first. These proportions were 87.0% (ITN = 87.3%, control = 86.7%) for 12 months of follow-up. For the pre-intervention period, these were 73.8% by 24 months (ITN = 71.2%, control = 76.1%) and 84.2% by 12 months (ITN = 83.6%, control = 84.9%). The overall median duration of follow-up was 494 days (pre-intervention) and 269 days (during intervention), which reflected the higher fraction that could potentially reach the age of 24 months before the end of the study during the five-year pre-intervention study than in the 27-months intervention study. The birth characteristics of the children who were lost to follow-up during the intervention phase were not statistically different from the successfully followed children and those who died. However, comparison of the maternal characteristics indicated that infants who were lost to follow-up were more likely to be born to mothers of young age, who were single, were primigravidae, and of lower socioeconomic status ($P < 0.05$ for all).

Characteristics of the study sample. During the intervention period, children from ITN and control villages were born to households with equal socioeconomical status, but the caretakers from ITN villages had more years of education ($P = 0.01$) (Table 1). Prompted questioning showed that 94.7% of the infants from households in the ITN villages (1997 onwards) were reported to sleep under an ITN on a regular basis, of whom 99.7% (i.e., 94.4% of the intervention group) reportedly used the study net. Only 8.4% of the households in the control villages reported using an ITN (1997 onwards), of whom 26.5% reported using the treated study ITN (i.e. 2.2% of the control group).

Malaria parasitology. The overall crude median time to first infection was 3.1 months in the pre-intervention period (pre-ITN = 3.3, pre-control = 3.0), and 10.7 (ITN) versus 4.5 (control) months during the intervention period (Figure 2). In the pre-intervention period, the estimates of the daily attack (h) rate, obtained from the catalytic conversion model (force of infection) was 0.0233 (95% confidence interval [CI] = 0.0221–0.0245). Results were similar in pre-ITN and pre-control villages: (0.0218 [95% CI = 0.0200–0.0233] and 0.0249 [95% CI = 0.0231–0.0267], respectively). During the intervention period, h was 69% lower in ITN than control villages (0.0041 [95% CI = 0.0033–0.0048]) versus 0.0133 [95% CI = 0.0116–0.0151], respectively). The reduction in h from the force of infection model containing only infants was 74%.

Age-adjusted geometric mean (95% CI) parasite densities

TABLE 1

Maternal, household, and birth characteristics of 1,451 children enrolled in the birth cohort before the bed net study and 831 children during the bed net study period*

	Pre-intervention period April 1992–December 1996			During intervention period January 1997–March 1999		
	ITN (n = 698)	Control (n = 753)	*P* or PR (95% CI)	ITN (n = 411)	Control (n = 420)	*P* or PR (95% CI)
Maternal characteristics						
Age in years, mean (SE)	26.9 (0.31)	27.4 (0.39)	0.32	25.6 (0.51)	26.3 (0.33)	0.23
Gravida during pregnancy, median (quartiles)	3.6 (1.8–5.8)	4.1 (2.1–6.5)	0.002	3.1 (1.6–5.4)	3.8 (1.6–6.3)	0.06
Height in cm, mean (SE)	163.0 (0.40)	163.1 (0.36)	0.93	163.7 (0.30)	163.5 (0.39)	0.63
Maternal parasitemia at delivery, No. (%)	249 (37.3)	244 (33.4)	1.12 (1.01–1.24)	62 (17.5)	77 (21.5)	0.82 (0.62–1.07)
Socioeconomic rank score, median (quartiles)	49.6 (24.3–73.2)	48.2 (24.0–75.0)	0.90	51.8 (28.6–70.8)	45.2 (19.1–76.6)	0.30
Years of schooling, median (quartiles)	6.5 (5.1–7.5)	6.3 (4.6–7.3)	0.03	6.9 (5.1–7.9)	6.4 (4.2–7.5)	0.01
Birth characteristics						
Gestational age in weeks at delivery, mean (SE)	38.7 (0.02)	38.8 (0.03)	0.03	39.8 (0.05)	39.5 (0.15)	0.06
Birth weight in kg, mean (SE)	3.09 (0.02)	3.13 (0.02)	0.16	3.19 (0.02)	3.15 (0.03)	0.33
Males, No. (%)	348 (49.9)	381 (50.6)	0.99 (0.88–1.10)	202 (49.2)	209 (49.8)	0.99 (0.87–1.12)
Cord blood parasitemia, No. (%)				7 (2.0)	5 (1.5)	1.41 (0.51–3.88)
Cord blood hemoglobin in g/dL, mean (SE)				14.8 (0.20)	15.3 (0.16)	0.13
Placental malaria, No. (%)	181 (32.4)	160 (27.9)	1.16 (0.99–1.37)	48 (14.0)	57 (16.5)	0.85 (0.60–1.20)
Season at delivery						
Driest, No. (%)	248 (35.5)	235 (31.2)		133 (32.4)	143 (34.1)	
Intermediate, No. (%)	214 (30.7)	265 (35.2)		138 (33.6)	141 (33.6)	
Wettest, No. (%)	236 (33.8)	253 (33.6)	0.02	140 (34.1)	136 (32.4)	0.75
Hemoglobin S type						
AA, No. (%)	450 (80.8)	435 (75.7)		232 (74.4)	230 (79.0)	
AS, No. (%)	87 (15.6)	120 (20.9)		77 (24.7)	58 (20.0)	
SS, No. (%)	20 (3.6)	20 (3.5)	0.04	3 (1.0)	3 (1.0)	0.19

* PR = prevalence ratio; CI = confidence interval; ITN = insecticide-treated bed nets.

were lower in parasitemic children from ITN compared with children from control villages: 1,514 (1,208–1,897) versus 2,692 (2,351–3,082), respectively; $P < 0.0001$. Analysis by age groups showed that this ITN effect was most marked in the infants less than three months of age: 246 (143–422) versus 1,000 (697–1,435), $P = 0.0006$. The incidence of high-density infections was similar in intervention and control villages pre-intervention, and was reduced 74% in ITN villages during the intervention period (Table 2).

Morbidity. The ITNs were associated with marked reductions in the incidence of symptomatic malaria and severe anemia, while there were no reductions in the incidence of non-malaria fevers (Table 2). Several potential determinants of ITN efficacy in preventing symptomatic malaria and moderately severe anemia were evaluated by adding two-way interaction terms to the models. These included age, hemoglobin S phenotype, study year, season, and duration of bed net use in pregnancy. The efficacy of ITNs in children 12–15 or 16–24 months old was similar to that of infants 3–11 months old in all models (Figure 3). The greatest reduction was seen in the children less than three months of age, although this was not significantly different from older children. Models evaluating ITN protective efficacy for symptomatic malaria and moderately severe anemia with other interaction terms indicated similar effects in the first and second study year (1997 versus 1998 plus 1999; $P > 0.5$ for both), but significant effect modification by season ($P = 0.02$). The ITNs were most effective in reducing the incidence of symptomatic malaria in the rainy and post-rainy season (protective efficacy = 61% [95% CI = 43–73%]) compared with the dry season (protective efficacy = 30% [95% CI = −16–58%]). There was no significant effect modification by season for the anemia endpoints. The efficacy was similar in children born before and after July 5 1997, indicating that ITNs were equally effective in infants born to mothers protected by ITNs throughout pregnancy or at least the entire second and third trimesters of pregnancy, as opposed to pregnancies, which were unprotected or only partially protected during the second or third trimester.

Nutritional parameters. The mean Z-scores for the standard summary nutritional parameters decreased from the age of three months onwards (Figure 4). There was a difference, albeit not statistically significant, in favor of subsequent ITN villages in mean length-for-age Z-score before the introduction of ITNs (difference = 0.17; $P = 0.08$). After the introduction of ITNs, the magnitude of this contemporaneous difference increased (0.36; $P = 0.01$) (Table 3). Because of the borderline statistically significant difference pre-intervention, the effect of ITNs was also assessed using a historical comparison. The P value of the time × randomization status interaction term for the effect on length-for-age Z-scores was 0.15 (Table 3). This indicates that the improvement (+0.11) in mean Z-scores over time (pre-intevention versus during intervention) in ITN villages was not statistically significantly different from the simultaneous decrease in mean Z-scores (−0.8) in control villages. The weight-for-length and weight-for-age Z-scores were comparable pre-intervention, but

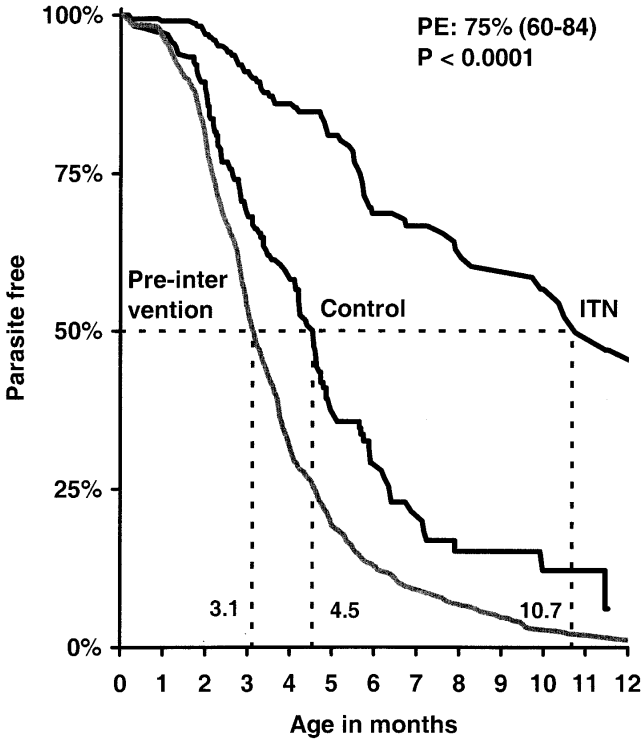

FIGURE 2. Time to first parasitemia. ITN = insecticide-treated bed net; PE = protective efficacy. Value in parenthesis is the 95% confidence interval.

weight-for-age Z-scores were significantly higher in ITN than control villages during the intervention period. Unlike the difference in length-for-age Z-scores, which was sustained in one-year-old children, this difference in weight-for-age Z-scores was only evident in infancy (Figure 4).

DISCUSSION

In this birth cohort, we monitored parasitologic, hematologic, clinical, and nutritional parameters in infants who were protected by ITNs and compared these to infants in control villages in an area of intense perennial malaria transmission. This study offered the opportunity to determine the potential risks and benefits of ITN use, before and after birth, on infant health, and evaluate potential determinants of ITN efficacy in children less than two years of age.

The ITNs were found to reduce the force of infection in infancy by 74%, resulting in a delay in time to first infection from 4.5 to 10.7 months. The clinical benefits of this reduced exposure were found to be substantial; the incidence of symptomatic high-density malaria, as well as moderately severe anemia, was reduced by 60%. These efficacy estimates on malaria morbidity are higher than those found in our separate cross-sectional surveys conducted simultaneously in 60 adjacent study villages (44% and 39%, respectively),[32] reflecting the younger age group in this birth cohort and the assessment of incidence instead of prevalence. Our estimate of protective efficacy for all cause severe anemia is remarkably similar to that obtained from the social-marketing program of ITN in Ifakara Tanzania (63%), which also included children less than two years old.[33] Spatial analyses presented in a companion report in this supplement suggest that the true efficacy of ITNs may be even higher because the high ITN coverage resulted in an area wide reduction in malaria transmission, which in turn reduced malaria-associated morbidity in young children from control households located within 300 meters of an ITN village (community or mass effect).[34] In addition to reduced rainfall in 1998 compared with 1992–1996,[21] the community effect also partly explains the lower morbidity and longer time to first infection in the control villages during the intervention period compared with pre-intervention (4.5 versus 3.0 months).

The efficacy of ITNs was greatest in the rainy season and the months following the rains, when malaria transmission is highest, and lowest in the dry season, consistent with our passive surveillance studies.[35] There was no indication that the efficacy was lower in the second study year, consistent with our mortality analysis,[18] but different from the results of our passive surveillance monitoring[35] and some of the previous mortality trials.[36]

Similar to reports by others,[5,6,8] mid-upper-arm circumfer-

TABLE 2

Crude incidence per 1,000 child-months of malaria, anemia, and non-malaria indices: pre-intervention (baseline) and intervention period*

	Pre-intervention period June 1992–December 1996				Intervention period January 1997–March 1999				
	ITN	Control	Adjusted RR (95% CI)	*P*	ITN	Control	Adjusted RR (95% CI)	Adjusted PE (95% CI)	*P*
Any parasitemia	733	772	1.09 (0.88–1.35)	0.45	134	408	0.28 (0.20–0.38)	72 (62–80)	<0.0001
High-density parasitemia†	328	330	1.04 (0.91–1.19)	0.58	56	202	0.26 (0.19–0.36)	74 (64–81)	<0.0001
Symptomatic malaria‡	249	231	1.17 (0.97–1.40)	0.10	34	75	0.48 (0.33–0.70)	52 (30–67)	0.0001
Symptomatic high-density malaria	160	144	1.09 (0.89–1.34)	0.42	22	53	0.40 (0.28–0.58)	60 (42–72)	<0.0001
Any fever (regardless of parasitemia)	300	274	1.07 (0.88–1.30)	0.48	76	98	0.74 (0.52–1.06)	0.26 (–6–48)	0.10
Non-malaria fevers (aparasitemic)	50	43	1.17 (0.87–1.56)	0.30	38	26	1.48 (0.99–2.22)	–48 (–222–1)	0.06
Upper respiratory tract infection	722	718	1.17 (0.85–1.59)	0.33	578	513	0.89 (0.48–1.66)	11 (–66–52)	0.72
Moderate-severe anemia (Hb <7 g/dL)	103	108	0.95 (0.86–1.06)	0.38	36	84	0.40 (0.26–0.63)	60 (37–74)	<0.0001
Severe anemia (Hb <5 g/dL)	14.3	14.2	1.00 (0.74–1.36)	0.99	8.8	19.8	0.44 (0.27–0.71)	56 (29–73)	0.0007
Moderate-severe malarial anemia§	89	92	0.94 (0.83–1.07)	0.33	14	52	0.33 (0.18–0.61)	67 (39–82)	0.0004
Severe malarial anemia¶	11.7	11.4	1.09 (0.82–1.46)	0.55	3.8	12.1	0.37 (0.17–0.81)	63 (19–83)	0.01

* ITN = insecticide-treated bed net; RR = rate ratio; PE = protective efficacy; CI = confidence interval. The PE, 95% CI, and associated *P* values are adjusted for sickle cell hemoglobin, gravida status, maternal education, season, and child's age.
† Defined as an age-dependent density above fever threshold (see Materials and Methods).
‡ Defined as any parasitemia with fever.
§ Hemoglobin level <7 g/dL plus parasitemia.
¶ Hemoglobin level <5 g/dL plus parasitemia.

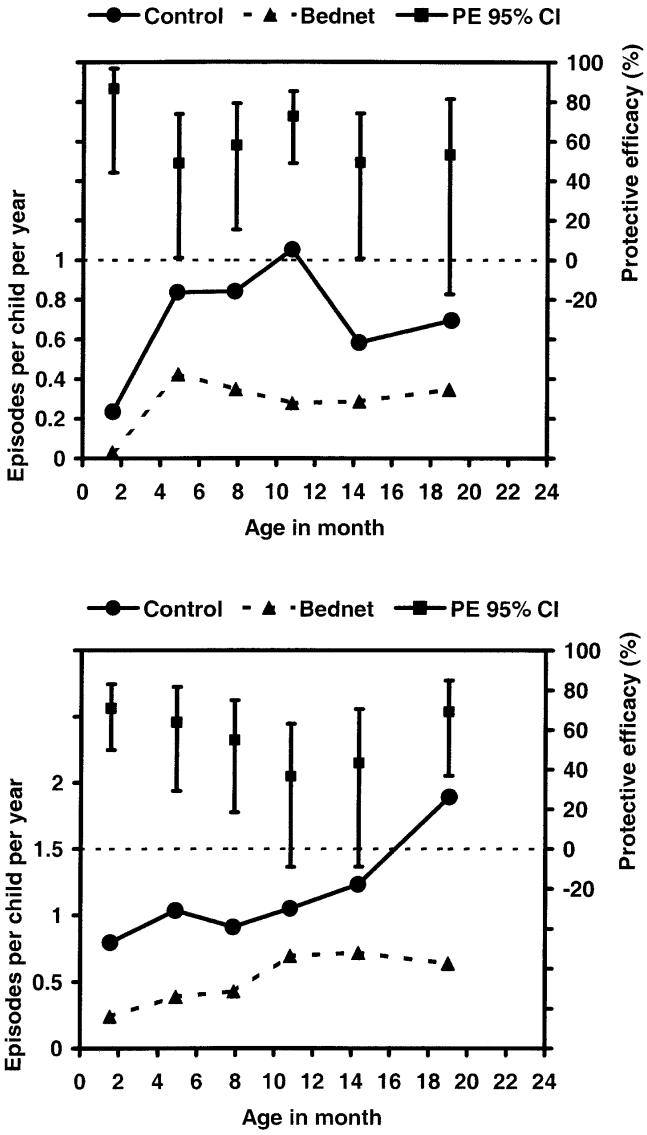

FIGURE 3. Incidence of high-density symptomatic malaria (**top graph**) and moderate-severe anemia (hemoglobin level < 7 g/dL) (**bottom graph**) by age and randomization status. PE = protective efficacy: 100 × (1 – hazard ratio ITN/control); ITN = insecticide-treated bed net; CI = confidence interval.

ence and weight-for-age Z-scores were better in children from intervention villages. The weight gain benefit was only apparent in infancy. In addition, a significant difference in length-for-age Z-scores was apparent (mean difference in Z-score = 0.36). Although this difference was statistically significant *(P* = 0.01), it could not be concluded with certainty that this was associated with the introduction of ITNs because of an existing difference, albeit smaller and not statistically significant, in length-for-age Z-scores pre-intervention (mean difference in Z-score = 0.17; $P = 0.08$). Of interest is that unlike weight gain, the beneficial effect on linear growth was maintained in the second year of life. Thus, ITN use in infancy, the period of maximum annual growth velocity in life, could potentially result in sustained benefits, since the opportunity for catch-up growth later in life is limited for children who remain in the same unfavorable environment, as is the case in most parts of the developing world.[37,38]

The frequency of non-malaria-related illnesses, including the incidence of mild upper respiratory symptoms and ear and eye infections, remained unaffected by ITNs. A higher incidence of non-malaria fevers was observed in ITN villages ($P = 0.06$) during the intervention period. This is unlikely to reflect replacement morbidity by other diseases following the reduction in malaria, but more likely reflects the unexplained 50% decrease in the incidence of non-malaria fevers in the control villages from pre to post-intervention, whereas in ITN villages it remained the same.

We reported elsewhere that ITN use in pregnancy reduced maternal IgG antibody concentrations in the cord blood of newborns against blood-stage antigens (merozoite surface protein-1).[11] These maternal antibodies may provide important passive immune protection against malaria in the first few months of life.[9] Our current epidemiologic observations suggest that, with continued ITN use by the newborn, this does not result in increased risks of blood stage infection or clinical malaria in the first few months of life. Indeed, the efficacy of ITNs appeared greatest in the infants less than three months old. The protective efficacy estimates were greater than 70% for all malaria morbidity parameters in this age group.

Although ITNs had the greatest impact in the youngest infants, they were also found to be protective in children between 16 and 24 months of age. The efficacy in this older age group was the same as in children 3–11 or 12–15 months of age. Although these findings do not exclude the possibility that decreased exposure to malaria early in life may result in delayed acquisition of immunity against malaria,[12,13,39] they do suggest that, with continued ITN use, this does not result in increased clinical disease in one-year-old children. This is consistent with the finding from our cross-sectional surveys that reduced exposure to malaria does not compromise, but may improve humoral immune responses in young children to well-characterized pre-erythrocytic and erythrocytic malaria vaccine candidate antigens.[16]

It is important to note the limitations of these findings. They only apply to children who continue using properly re-treated ITNs after infancy, but tell us little what will happen if children stop using ITNs on a regular basis post-infancy. Furthermore, the observation time contributed by children in the 16–24-months-old age group was limited, as well as the precision of the corresponding efficacy estimates (Figure 3). This was because only 30% of the cohort was born in time to reach the age of 16 months before the end of the study, and 25% of them had died (12%) or defaulted (13%) by 12 months. Furthermore, our observation time was restricted to 24 months and a shift in morbidity to older children can thus not be excluded. Further investigations with larger numbers and longer bed net use and follow-up (four years) are under way to validate and extend these findings.

The sample included in this cohort represented approximately 84% of all births that occurred during the intervention period in these 19 villages. Approximately 3.6% of the approached households with pregnant women refused to participate, but no information was available from the remaining non-participants. They likely include recent arrivals and also young unmarried teenage primigravidae, who are more mobile than multigravidae in this rural community, and often move temporarily to other family households for the perinatal

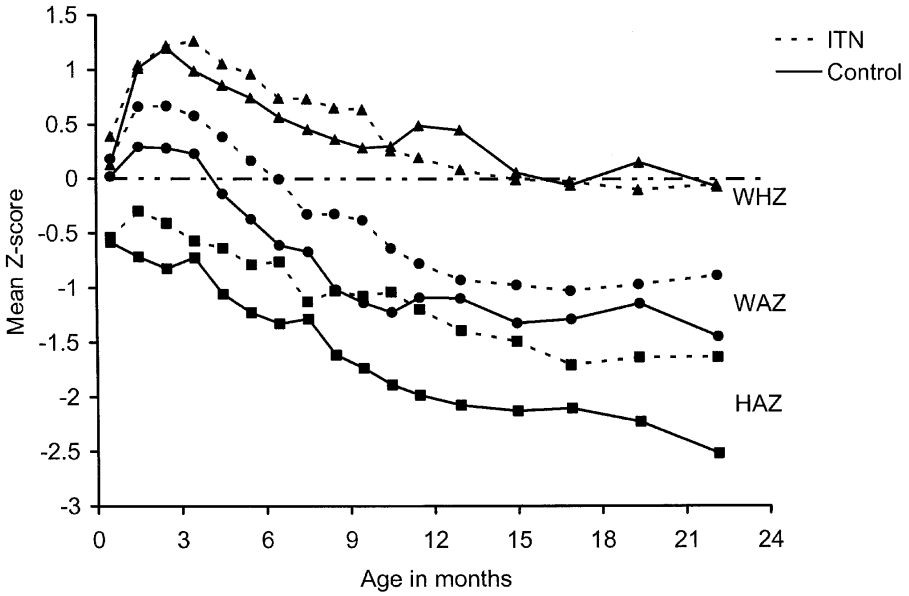

FIGURE 4. Mean nutritional Z-scores, by age and randomization status during the intervention phase. The x-axis values represent the mean age for each age category for each point estimate. ITN = insecticide-treated bed net; WHZ = weight-for-length Z-score; WAZ = weight-for-age Z-score; HAZ = length-for-age Z-score.

period (Alaii JA, unpublished data). For the same reasons, enrolled infants of young mothers were the most likely to be lost-to-follow-up, but the default rate was equally divided between ITN and control villages. Mothers of newborns from intervention villages had more years of education ($P = 0.01$), but this potential source of bias was adjusted for in multivariate analysis. Furthermore, malaria transmission and malaria morbidity in the four years prior to the start of the ITN study were comparable in the ITN and control villages, and the observed benefit was greatest for the malaria-specific parameters and absent for non-malaria illness. We believe therefore that these results are valid and representative for areas with intense perennial malaria transmission and high coverage of continued bed net use.

We conclude that in this area of intense perennial malaria transmission, ITNs not only reduce malaria and its adverse effects in gravidae 1–4,[4] but also reduce malaria-associated morbidity in their newborns by 60% and improve growth, regardless of birth order. The ITNs were equally as effective in the second year of life as in infancy. Thus, although follow-up was limited to 24 months, there was no evidence that, with continued use of properly re-treated bed nets, a 74% reduction in the force of infection in infancy was associated with an increased risk of malaria morbidity in one-year old children. These results suggest that the use of ITNs by pregnant women and their newborns can result in substantial health benefits which outweigh any potential adverse effects in areas of intense malaria transmission.

TABLE 3
Impact of ITNs on mean Z-scores of nutritional parameters*

	Pre-intervention period April 1992–December 1996				Interventional period January 1997–March 1999				Change over time in ITN versus control villages	
	ITN mean	Control mean	Mean difference 95% CI	*P*	ITN mean	Control mean	Mean difference (95% CI)	*P*	Mean† difference	*P*†
0–23 months										
WHZ	0.56	0.63	–0.07 (–0.33–0.19)	0.60	0.41	0.36	0.05 (–0.27–0.36)	0.76	0.12 (–0.26–0.50)	0.55
HAZ	–0.51	–1.68	0.17 (–0.01–0.34)	0.08	–1.40	–1.76	0.36 (0.10–0.62)	0.01	0.19 (–0.06–0.44)	0.15
WAZ	–0.57	–0.63	0.07 (–0.16–0.30)	0.58	–0.59	–0.89	0.31 (0.05–0.56)	0.03	0.24 (0.03–0.46)	0.04
MAZ	NA	NA			–1.04	–1.38	0.35 (0.08–0.61)	0.02		
0–11 months										
WHZ	0.83	0.89	–0.05 (–0.30–0.20)	0.68	0.73	0.63	0.10 (–0.21–0.41)	0.53	0.16 (–0.22–0.53)	0.43
HAZ	–1.03	–1.19	0.16 (–0.002–0.32)	0.07	–0.96	–1.30	0.34 (0.08–0.60)	0.02	0.18 (–0.08–0.45)	0.19
WAZ	–0.01	–0.02	0.01 (–0.22–0.24)	0.48	–0.15	–0.49	0.35 (0.11–0.59)	0.01	0.34 (0.05–0.48)	0.03
MAZ	NA	NA			–0.75	–1.04	0.29 (0.02–0.56)	0.05		
12–23 months										
WHZ	0.24	0.31	–0.07 (0.36–0.23)	0.65	–0.06	0.13	–0.19 (–0.67–0.28)	0.44	–0.13 (–0.65–0.40)	0.64
HAZ	–1.90	–2.12	0.22 (–0.06–0.50)	0.15	–1.64	–2.09	0.45 (0.05–0.85)	0.04	0.23 (–0.11–0.58)	0.20
WAZ	–0.96	–1.04	0.09 (–0.20–0.37)	0.57	–1.03	–1.17	0.14 (–0.35–0.62)	0.59	0.05 (–0.40–0.50)	0.83
MAZ	NA	NA			–0.97	–1.39	0.41 (0.13–0.70)	0.01		

* ITN = insecticide-treated bed net; CI = confidence interval; WHZ = weight-for-length Z-score; HAZ = length-for-age Z-score; WAZ = weight-for-age Z-score; MAZ = mid-upper-arm circumference-for-age Z-score; 6–24 months of age only; NA = not asssessed.
† Difference in change in mean Z-score over time in ITN versus control villages and associated *P* value.

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