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REDUCTION OF MALARIA DURING PREGNANCY BY PERMETHRIN-TREATED BED NETS IN AN AREA OF INTENSE PERENNIAL MALARIA TRANSMISSION IN WESTERN KENYA

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Abstract. The impact of insecticide (permethrin)-treated bed nets (ITNs) on malaria in pregnancy was studied in a rural area in western Kenya with intense perennial malaria transmission. All households in 40 of 79 villages were randomized to receive ITNs by January 1997. The ITNs were distributed in control villages two years later. Complete data on birth outcome were available on 2,754 (89.6%) of 3,072 deliveries. Women (n = 780) were followed monthly throughout pregnancy in 19 of 79 villages. Among gravidae 1–4, ITNs were associated with reductions of 38% (95% confidence interval [CI] = 17–54%) in the incidence of malaria parasitemia and 47% (95% CI = 6–71%) in the incidence of severe malarial anemia (hemoglobin level < 8 g/dL with parasitemia) during pregnancy. At the time of delivery, mean hemoglobin levels were 0.6 g/dL (95% CI = 0.01–1.2 g/dL) higher, the prevalence of placental or maternal malaria was reduced by 35% (95% CI = 20–47%), and the prevalence of low birth weight was reduced by 28% (95% CI = 2–47%) in gravidae 1–4 from ITN villages. No beneficial impact was observed in gravidae five or higher. In areas of intense perennial malaria transmission, permethrin-treated bed nets reduce the adverse effect of malaria during the first four pregnancies.

INTRODUCTION

Each year in sub-Saharan Africa, where 80–90% of the world's malaria cases occur, approximately 19–24 million women are at risk for malaria and its adverse consequences during pregnancy.[1,2] In areas with stable malaria transmission, which represents most of sub-Saharan Africa, the vast majority of infections with *Plasmodium falciparum* in pregnancy remain asymptomatic, undetected and untreated.[3,4] The major impact of malaria during pregnancy in these regions is caused by persistent or recurrent, predominantly low-grade, sometimes sub-patent[5–7] parasitemia, resulting in maternal anemia and a reduced birth weight.[8] Primigravidae and secundigravidae are most at risk, but in areas with moderate-to-intense transmission, or a high prevalence of infection with human immunodeficiency virus (HIV), women of higher gravidity are also affected.[3,9–13]

The spread of chloroquine resistance and the low adherence to antimalarial prophylaxis during pregnancy has led to the reconsideration of the role of antimalarial chemoprophylaxis in controlling malaria in pregnancy.[14] Intermittent preventive treatment with sulfadoxine-pyrimethamine (SP) has increasingly been used in sub-Saharan Africa[15–19] and is currently recommended as the national policy in Kenya.[20] However, resistance to SP is steadily increasing in some areas in sub-Saharan Africa, and the available arsenal of alternative tools for malaria control in pregnancy is very limited.[12,14,21] One of the most promising of these tools is insecticide-treated bed nets (ITNs), which have been shown to reduce the number of infective mosquito bites by 70–90% in a variety of ecologic settings, and to reduce all cause mortality among young children by 16–33%.[22,23–26] Four previous randomized-controlled trials have been conducted to determine the impact of ITNs in pregnancy, covering a wide spectrum of malaria endemicity ranging from unstable-low to high and markedly seasonal malaria transmission. In these studies ITNs significantly reduced malaria parasitemia and maternal anemia and increased birth weight, in areas with the lowest and most seasonal transmission (Thailand and The Gambia),[27,28] but no impact was observed in areas with more intense transmission (coastal Kenya, and Ghana).[5,29] Thus, although the data are limited, it has been hypothesized that the impact of ITNs decreases with increasing intensity of malaria transmission.[5]

No data are available from randomized controlled trials in areas with extremely high, year-round malaria transmission. We report here the results of the efficacy of ITNs on maternal malaria and anemia and adverse outcomes in pregnancy in an area with intense perennial malaria transmission in western Kenya.

MATERIALS AND METHODS

Study site and population. The study was conducted in Rarieda Division (locally known as Asembo), Siaya District, in western Kenya. Full details of the study site have been described elsewhere.[30,31] Briefly, the area is located on the shores of Lake Victoria and has intense perennial malaria transmission (60–300 infected bites per person annually).[32] The predominant malaria vector is *Anopheles gambiae*. Malaria transmission occurs throughout the year, with peaks during May through July, and October through November. Previous studies have indicated that 60–80% of children less than five years old are parasitemic at any time.[33,34] More than 95% of these infections are due to *Plasmodium falciparum*, and almost all of the remainder is *P. malariae*. Infections with *P. ovale* are rare.[33]

Within the Asembo area, most inhabitants live within 5 km of a fixed health care facility. There are 16 such facilities, of which five routinely provide antenatal care. A previous study in neighboring Kisumu district indicated that antenatal clinic attendance is high, with more than 90% of pregnant women visiting antenatal care clinics at least twice during pregnancy (Parise M and others, unpublished data). Antimalarial chemoprophylaxis during pregnancy was not routinely avail-

able at antenatal care clinics during the period of this study. The Kenyan Ministry of Health introduced national guidelines for the control of malaria in pregnancy, which included the use of intermittent preventive treatment with SP in 1999, after this study was completed.[4]

Study design and randomization. This study was conducted within the context of a large community based group randomized controlled trial designed to assess the impact of ITNs on mortality in children less than five years of age.[35] The study area for the mortality trial consisted of two main surveillance sites: Asembo and Gem.[26] The current study of the impact of ITNs in pregnancy was conducted in Asembo only, which has an area of 200 km^2 and a population of 55,000 people living in 79 villages. Asembo was subdivided into two distinct areas: the non-cohort area (60 contiguous villages) and the cohort area (the remaining 19 villages), which is the study site of the Asembo Bay Cohort Project.[30,36]

Half of the villages were randomly assigned to the intervention group and each household in these villages received ITNs during the fourth quarter of 1996.[26] Households in control villages received ITNs in April 1999 after the trial was completed. Large households received multiple ITNs according to bed space measurements and baseline demographic data, providing an intervention ITN coverage ratio of 1.5 persons per ITN.[37] At distribution, bed nets were pretreated with permethrin (Siamdutch Mosquito Netting Co., Bangkok, Thailand), and re-treated biannually by the study team to maintain a target dose of 500 mg of permethrin/m^2.

Asembo Bay Cohort Project (19 villages). Pregnant women in the cohort area were recruited as part of the Asembo Bay Cohort Project; an ongoing immuno-epidemiologic study of malaria in pregnancy and the acquisition of natural immunity in children aged less than 5 years.[30,36] Four of the 15 original cohort villages were much larger than the remaining 11 and were split into two, giving a total of 19 village clusters,[30] henceforth referred to as villages. Pregnant women of all parities were identified through monthly census by trained village monitors or trained traditional birth attendants residing in the same village. Two village monitors and two study birth attendants were trained per village. At the recruitment visit, a questionnaire was completed with data on demographic characteristics, obstetrical history, illness and treatment during the current pregnancy, education, and socioeconomic status.[38] Enrolled women were examined by the study birth attendants and maternal weight, height, mid-upper-arm circumference, date of last menstrual period, and fundal height were recorded. A capillary blood sample (250–500 μL) was drawn for determination of hemoglobin levels (1992–1996) or hematocrit (1997–1999), hemoglobin S phenotype (using hemoglobin electrophoresis), malaria thick and thin blood smears, and immunologic and molecular biologic assays.

The village monitor and traditional birth attendant visited each study participant at monthly intervals until delivery. During each visit, a morbidity questionnaire was completed, the mother was weighed to the nearest kilogram (120 kg; Salter, Smethwick, United Kingdom), mid-upper-arm circumference (MUAC) was measured (MUAC insertional tapes; UNICEF, Copenhagen, Denmark), and data were copied from the antenatal clinic cards. A capillary blood (finger stick) specimen for determination of hemoglobin levels or hematocrit and malaria smears was also collected monthly, or at any time that the pregnant woman reported illness between scheduled visits.

Pregnant women who had parasitemia and a documented fever or a history of fever within the previous 48 hours were treated with SP by the study team. Women with hemoglobin concentrations <8 g/dL were given ferrous sulfate supplementation and women with hemoglobin concentrations <5 g/dL were referred to the local hospital for further evaluation and treatment.

Delivery. Most (>80%) deliveries in this setting take place at home or at the home of the village-based traditional birth attendant. The study birth attendant recorded the details of labor, infant and placenta weights, and sex of the newborn on standardized study forms. If a delivery took place at home in the absence of the study traditional birth attendant, the mother was instructed to keep the placenta. The traditional birth attendant visited the household within 24 hours after delivery to record the details of labor retrospectively. Placental and cord blood thick and thin blood smears and a 5-mL cord blood sample were taken for serology. At the same time, a blood sample was taken from the mother for maternal peripheral malaria smears, hematocrit, and antimalarial serology. In addition, each mother-newborn pair was visited at home by a specially trained study supervisor within a target period of 24 hours (maximum = 96 hours) after delivery to determine the weight, height, and mid-upper-arm circumference and to estimate the gestational age of the newborn.[39] Undressed newborns were weighed to the nearest 10 grams using a 10-kg hanging weighing scale (235 10 S; Salter, Smethwick, United Kingdom). Recumbent length was measured to the nearest 0.1 cm, using a horizontal measuring board with sliding foot piece.[40] Measurements were taken twice and the mean value was computed prior to data analysis. Adult and newborn weighing scales were checked and calibrated monthly by specially trained staff using standard weights. Similar data were obtained for deliveries that took place in the local health facilities.

Procedures for the remaining (non-cohort) study area (60 villages). Delivery data (only) were gathered in the 60 contiguous villages in the rest of Asembo by copying data on a daily basis from routine antenatal care records, including standard antenatal and demographic parameters, as well as birth outcome (abortion, stillborn, or live born). Maternal, placental, and cord blood malaria smears were taken at the time of delivery, but blood samples for hematocrit and malaria serology were not collected because no cold-chain infrastructure was in place to store and transport these samples. Birth weight and gestational age of the newborn were assessed using the same methods as in the 19 cohort villages.

Laboratory analysis. All laboratory assays were processed at the research laboratories in Kisian, Kenya. 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 assumed 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. Placental and cord blood smears were stained and examined in the same way as peripheral blood smears, and parasite densities were calculated as for peripheral smears. Between 1992 and 1996, hemoglobin concentrations were measured using the HemoCue system (Hemocue, Angelholm, Sweden). From 1997 onwards, capil-

lary tubes containing whole blood obtained by finger prick were centrifuged at 10,000 cycles/second for three minutes to determine the packed cell volume (hematocrit). All hematocrit values were divided by a factor of three and are presented as hemoglobin values for consistency with the 1992–1996 data.

Definitions. Malaria parasitemia was defined as asexual blood stage malaria parasites of any *Plasmodium* species detectable on a thick blood smear. Clinical malaria was defined as any parasitemia associated with an axillary temperature ≥37.5°C. Anemia was defined as a hemoglobin concentration <11 g/dL, severe anemia as a hemoglobin concentration <8 g/dL, and severe malarial anemia as a hemoglobin concentration <8 g/dL in the presence of malaria parasitemia.

Each delivery was defined to have occurred in the rainy, post-rainy, or dry season using rainfall data collected in the study area. This was done by ranking the dataset into three equal groups based on the mean daily rainfall during the six months period prior to the date of delivery (i.e., during the second and third trimester of pregnancy).

The educational status of the pregnant women was also ranked and categorized as low, medium, or high. Similarly socioeconomic status was graded as low, medium, and high according to the rank position of the computed wealth index based on type of house and ownership of livestock, radios, bicycles, and sofas.[38]

Approximately 15% of birth weights were taken 24–96 hours after delivery, and were corrected for the physiologic fall in birth weight occurring in the first days following delivery. Birth weights taken 24–48 hours and 48–96 hours after delivery were corrected by a factor of +2% and +4%, respectively, to obtain the estimated weight at birth.[28,41] All analysis presented involving birth weights refer to the corrected birth weights. Low birth weight was defined as a corrected birth weight <2,500 grams. Preterm was defined as a delivery occurring before 37 weeks gestation. Weight for gestational age at birth was used to categorize infants as having normal or subnormal growth *in utero* (small-for-gestational age). Small-for-gestational age was defined as a birth weight below the 10th percentile of a distribution of birth-weight-for-gestational-age derived from a sex-specific, multi-racial, United States–based reference population for single births.[42,43] Deliveries with no evidence of cardiac or respiratory effort were classified as fetal loss. No attempt was made to differentiate between abortions and stillbirths because of the uncertainty of the gestational age assessment based on the expected date of delivery and assessment of the fundal height. Grand multigravida was defined as gravida five or higher.

Statistical analysis. The study was designed to detect a 25% reduction in the prevalence of adverse birth outcome defined as low-birth weight, pre-term or small-for-gestational age deliveries among primigravidae and secundigravidae with 80% power and 95% confidence allowing for a design effect of 1.2 and assuming that data on birth outcome could be collected within 96 hours following birth from at least 80% of the deliveries. The proportion of adverse birth outcomes in the control group was based on data collected from more than 1,200 births as part of the Asembo Bay Cohort Project conducted between 1992 and 1996.

All pregnancies that were in the second or third trimester when the study started on January 1, 1997 (defined as a delivery date before July 12, 1997) were excluded *a priori* from analysis of birth outcome to allow for a sufficient time period of ITN coverage per pregnancy to impact on intra-uterine growth retardation and gestational age. Deliveries occurring after April 1, 1999 (the date of distribution of ITNs to control villages) were also excluded.

The incidence rate of malaria and anemia was defined as the number of women who developed parasitemia or anemia during follow-up, divided by the number women-months contributed by that group. A woman could only contribute a single event and did not contribute to the denominator after that first event had occurred. All observations occurring after April 4, 1997 contributed to the numerator and denominator. Thus, observations occurring in the first 13.3 weeks (one trimester) of the study were excluded from the analysis of the impact on malaria and anemia during pregnancy to allow for a sufficient period of ITN use.

Analysis was based on intention-to-treat. Two sided P values <0.05 were considered statistically significant. The impact of ITNs on the various incidence rate estimates was expressed as the protective efficacy, estimated as $100 \times (1 - \text{hazard ratio})\%$. The hazard ratios were obtained by fitting a Cox Proportional Hazards Model (Proc survival, SUDAAN release 8; Research Triangle Institute, Research Triangle Park, NC), with gravidity, age, season, and year of study as time-independent covariates. A robust variance estimation corrected for the village based cluster randomization. Linear regression models (Proc REGRESS, SUDAAN) were used to determine the mean difference between ITN and control villages in birth weight, gestational age and delivery hemoglobin. Study area (cohort versus non-cohort area), gravidity, maternal stature (mid-upper-arm circumference and height), maternal age, and sex of the newborn were all significant ($P < 0.05$) determinants of birth outcome and included as co-variates in the final model. Season was also included because of its unequal distribution between the two intervention groups ($P = 0.03$) and its association with birth weight ($P = 0.057$). Again, a robust variance estimation was used to correct for the village based cluster randomization. Adjusted means were obtained from least squares mean estimates using the LSMEANS output group in this procedure. The impact of ITNs on the prevalence data at delivery was assessed using Poisson regression in Proc Genmod, a procedure within Statistical Analysis System (version 8.0; SAS Institute, Cary, NC), using the same co-variates as the linear regression models. Impact is expressed as the protective efficacy, estimated as $100 \times (1 - \text{prevalence ratio})\%$. The cluster randomization was taken into account by using an exchangeable correlation structure for observations obtained from residents within one village. Separate models were created for gravidae 1–4 and grand multigravidae because previous analysis of data collected in the period 1992–1996 had shown malaria to have a statistically significant impact on birth weight in the first four pregnancies, but not in higher pregnancy order (ter Kuile FO and others, unpublished data). Furthermore, multivariate analysis of birth outcome showed statistically significant effect modification (interaction) by these two gravida groups on the effect of ITNs on birth outcome.

Ethical clearance. The ITN study and the Asembo Bay Cohort Project were reviewed and approved by the institutional review boards of the Kenya Medical Research Institute (Nairobi, Kenya) and the Centers for Disease Control and Pre-

vention (Atlanta, GA). Informed consent was obtained from all women after explanation of the study procedures in the local language.

RESULTS

Between the start of the Asembo Bay Cohort project in 1992 and December 31, 1996, data were available from 1,557 single pregnancies contributed by 1,184 pregnant women, who were followed during pregnancy prior to the introduction of ITNs. Between January 1, 1997 (start of the ITN study) and April 1, 1999, 3,170 single pregnancies were enrolled, of whom 911 resided in the 19 cohort villages where longitudinal follow-up took place. Of these, 780 pregnancies occurred after April 4, 1997 and were eligible for the analysis of the impact of ITNs on malaria and anemia during pregnancy. Overall, 2,754 single pregnancies were eligible from all 79 villages for the analysis of birth outcome data (Figure 1). Prompted questioning showed that 85.9% of the women in the ITN villages reported sleeping under an ITN on a regular basis, of which 99.1% (i.e., 85.1% of the intervention group) reported using the green (i.e., insecticide-treated) study net. Only 3.7% of the women in the control villages reported using an ITN, of whom 22.5% reported using the treated study bed net (i.e., 0.8% of the control group).

Follow-up data during pregnancy (19 villages). Of the 780 women followed between 1997 and 1999, 381 lived in ITN villages and 399 lived in the control villages. The baseline characteristics of the 1,184 women enrolled between 1992 and 1996 were comparable for all characteristics between the pre-ITN and pre-control villages (data not shown). The baseline characteristics of the 780 women enrolled between 1997 and 1999 are shown in Table 1.

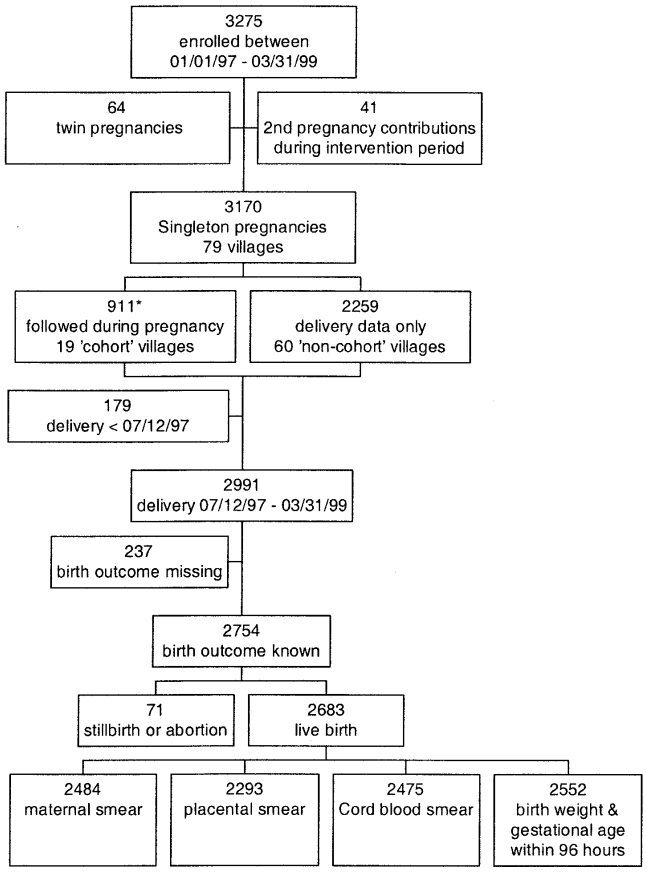

FIGURE 1. Trial profile. Data were collected from 780 of 911 women after April 4, 1997. These women were eligible for analysis of the impact of insecticide-treated bed nets during pregnancy.

The incidence of malaria parasitemia, parasite densities, clinical malaria, and overall morbidity (febrile episodes) before (1992–1996) and after the introduction of ITNs (1997–1999) is summarized in Table 2. Prior to the introduction of ITNs, there were no differences in the incidence risk and rate of malaria parasitemia and anemia between women in the ITN and control villages with the exception of malarial anemia, which occurred at a higher rate in grand multigravidae from the pre-intervention villages compared to those from the pre-control villages. However, after Jan 1997, gravidae 1–4 in the ITN villages had less parasitemia and anemia, whereas there were no significant differences in gravidae 5 or greater (Table 2).

Delivery data (79 villages). The birth outcome was unknown for 237 (7.9%) of the 2,991 single deliveries occurring between July 12, 1997 and April 1, 1999 (Figure 1). This was equally divided between ITN and control villages. Women with missing birth outcome data were more likely to be primigravid (31.6% versus 22.4%; relative risk [RR] = 1.41, 95% confidence interval [CI] = 1.11–1.80), to deliver in the rainy season (47.7% versus 33.3%; RR = 1.43, 95% CI= 1.10–1.86), and to be shorter (mean height = 160.3 cm versus 161.7 cm; $P = 0.01$).

Data on birth outcome were available from the remaining 2,754 (92.1%) women (Figure 1). They were equally divided among intervention and control villages (50.0% each) and their characteristics were comparable for all parameters, with the exception that gravidae 1–4 in the ITN villages were more likely to be pregnant during or shortly following the rainy season (peak malaria season) (Table 3). All subsequent multivariate analysis of birth outcome was therefore adjusted, among other co-variates, for season. Although alcohol use was not common in the study population, grand multigravidae in ITN villages were more likely to consume alcohol during their pregnancy, but this was not associated with birth outcome, and was not a confounder. Complete data on the child's sex, gestational age, and birth weight was available from 2,552 (95.1%) of the 2,683 live births (Figure 1). Of the 2,552 births 84.6% and 97.7% of the birth weights and 78.9% and 96.6% of the gestational ages were measured within 24 and 48 hours, respectively. The remaining measurements were taken between 48 and 96 hours. These times were equally distributed among the ITN and control group.

Malaria and anemia at the time of delivery. Peripheral smear, cord blood smear, and placental smear results were not available for 9.8%, 10.1%, and 16.7% of the women at delivery (Figure 1). This was equally distributed among ITN and control villages.

Similarly to malaria parasitemia during pregnancy, women from the ITN villages were less likely to be parasitemic (either peripheral [maternal] or placental malaria) at the time of delivery than those living in the control villages (19.6% versus 26.1%, protective efficacy = 25%; 95% CI = 12–35%). The beneficial impact was apparent in all pregnancies but only statistically significant in the first four pregnancies (25.3% versus 33.8%, protective efficacy = 25%; 95% CI = 13–36% compared with 10.4% versus 14.1%, protective efficacy =

TABLE 1
Baseline characteristics of 780 pregnant women who contributed to the comparison of malaria and anemia during pregnancy*

	Gravidae 1–4			Grand multigravidae		
	ITN (n = 234)	Control (n = 229)	*P* or RR (95% CI)	ITN (n = 147)	Control (n = 170)	*P* or RR (95% CI)
Age in years, mean (SE)	21.7 (0.3)	21.1 (0.1)	0.027	31.3 (0.5)	32.1 (0.4)	0.240
Marital status¶						
Married, no. (%)	175 (75.4)	179 (79.6)		139 (94.6)	157 (92.9)	
Never been married, no. (%)	50 (21.6)	44 (19.6)		2 (1.4)	1 (0.6)	
Widowed, no. (%)	6 (2.6)	62 (0.9)		5 (3.4)	11 (6.5)	
Divorced/separated, no. (%)	1 (0.4)	0 (0)	0.470	1 (0.7)	0 (0.0)	0.400
Socioeconomic rank score, median (quartiles)	46 (25–70)	43 (17–75)	0.625	59 (33–76)	52 (26–78)	0.603
Years of schooling, median (quartiles)	8 (6–9)	8 (6–8)	0.395	7 (4–8)	7 (4–7)	0.808
Gestational age on enrollment, mean (SE)	25.5 (1.0)	24.7 (0.9)	0.559	22.0 (1.1)	24.0 (0.9)	0.153
Gravida, median (quartiles)	2 (1–3)	2 (1–3)	0.523	6 (6–8)	7 (6–8)	0.100
Previous stillborn/abortion, no. (%)†	13 (5.6)	18 (7.9)	0.71 (0.35–1.41)	37 (25.2)	47 (27.7)	0.91 (0.63–1.31)
Previous child death, no. (%)†‡	25 (16.8)	21 (14.2)	1.18 (0.64–2.18)	79 (55.6)	93 (58.1)	0.96 (0.78–1.18)
Season, second-third trimester¶						
Driest, no. (%)	69 (29.5)	89 (38.9)		55 (37.4)	47 (27.7)	
Intermediate, no. (%)	82 (35.0)	71 (31.0)		54 (36.7)	53 (31.2)	
Wettest, no. (%)	83 (35.5)	69 (30.1)	0.31	38 (25.9)	70 (41.2)	0.04
Height in cm, mean (SE)	163.6 (0.35)	163.1 (0.27)	0.285	164.4 (0.38)	163.8 (0.59)	0.44
Mid-upper-arm circumference in cm, mean (SE)	25.4 (0.15)	24.8 (0.14)	0.007	26.2 (0.23)	25.5 (0.24)	0.053
Tetanus toxoid during pregnancy, no. (%)	82 (41.2)	75 (36.4)	1.13 (0.76–1.68)	28 (20.9)	51 (34.2)	0.61 (0.47–0.80)
Attended antenatal care clinics, no. (%)	84 (42.2)	75 (36.4)	1.16 (0.78–1.72)	31 (23.1)	51 (34.2)	0.68 (0.50–0.92)
Smoked during pregnancy, no. (%)	2 (0.9)	1 (0.4)	1.96 (0.18–21.84)	3 (2.1)	5 (3.0)	0.68 (0.23–2.05)
Alcohol use during pregnancy, no. (%)	14 (6.0)	10 (4.4)	1.36 (0.64–2.91)	19 (13.0)	9 (5.4)	2.40 (1.00–5.74)
Hemoglobin S phenotype¶						
AA, no. (%)	130 (72.2)	122 (76.3)		95 (70.9)	111 (76.6)	
AS, no. (%)	50 (27.8)	38 (23.8)		38 (28.4)	34 (23.5)	
SS, no. (%)	0 (0)	0 (0)	0.80	0 (0.0)	0 (0.0)	0.48

* RR = relative risk; CI = confidence interval; ITN = insecticide-treated bed nets.
† Multigravidae only.
‡ Includes death among children who were born alive and died before the start of the insecticide-treated bed net study (January 1, 1997).
¶ Column percentage.

26%; 95% CI = –7–48%) in grand multigravidae. This pattern was very similar for both placental and peripheral parasitemia (Figure 2). There was little influence of season. Although the beneficial impact was greatest in the rainy season (all gravidae: protective efficacy = 26%; 95% CI = 2–43%), it was also seen in the dry season (protective efficacy = 21%; 95% CI = 1–36%). Maternal and placental geometric mean parasite densities in parasitemic women were identical in ITN and control villages ($P = 0.82$ and $P = 0.91$, respectively). Infants in the ITN villages born to both gravidae 1–4 as well as grand multigravidae (Figure 2) were less likely to have cord blood parasitemia (2.8% versus 4.1%), although the differ-

TABLE 2
Incidence per 1,000 person-months of malaria and anemia during pregnancy: pre-intervention (baseline) and during intervention period

	Gravidae 1–4					Grand multigravidae				
	N	ITN	Control	HR (95% CI)*	*P**	N	ITN	Control	HR (95% CI)*	*P**
Pre-intervention period (1992–1996)										
Any parasitemia	678	449	417	1.06 (0.94–1.19)	0.33	506	229	221	1.05 (0.85–1.30)	0.64
High-density parasitemia†	678	288	263	1.06 (0.92–1.23)	0.39	506	109	91	1.23 (0.88–1.71)	0.21
Clinical malaria‡	662	21	24	0.87 (0.45–1.69)	0.67	499	15	7	1.83 (0.56–5.95)	0.29
Hemoglobin <11 g/dL	387	688	615	1.12 (0.97–1.30)	0.11	328	522	542	0.99 (0.87–1.13)	0.89
Hemoglobin <8 g/dL	387	146	138	1.04 (0.64–1.67)	0.88	328	87	85	0.91 (0.49–1.69)	0.76
Malarial anemia§	387	344	334	1.08 (0.85–1.36)	0.51	328	183	128	1.55 (1.05–2.29)	0.03
Severe malaria anemia¶	387	106	81	1.33 (0.71–2.50)	0.35	328	26	16	1.75 (0.61–5.01)	0.28
During the intervention period (1997–1999)										
Any parasitemia	463	169	284	0.62 (0.46–0.83)	0.003	317	81	105	0.83 (0.51–1.37)	0.45
High-density parasitemia†	463	111	174	0.67 (0.48–0.93)	0.02	317	36	47	0.88 (0.42–1.83)	0.71
Clinical malaria‡	463	5	7	0.72 (0.19–2.78)	0.62	317	2	0		
Hemoglobin <11 g/dL	451	554	703	0.79 (0.65–0.96)	0.02	313	610	629	1.00 (0.86–1.18)	0.97
Hemoglobin <8 g/dL	451	57	88	0.70 (0.42–1.16)	0.15	313	62	49	1.24 (0.69–2.24)	0.45
Malarial anemia§	451	120	215	0.56 (0.39–0.80)	0.003	313	59	68	1.01 (0.58–1.76)	0.96
Severe malarial anemia¶	451	20	37	0.53 (0.29–0.94)	0.03	313	11	6	1.27 (0.22–7.34)	0.77

ITN = insecticide-treated bed nets.
* Hazard ratio (HR) and 95% confidence interval (CI) and associated *P* values are adjusted for gravida status, age, season, and year of study using the survival analysis procedures in SUDAAN release 8.0.
† Defined as >250/mm^3.
‡ Defined as any parasitemia with fever.
§ Hemoglobin concentration <11 g/dL plus parasitemia.
¶ Hemoglobin concentration <8 g/dL plus parasitemia.

TABLE 3
Baseline characteristics of 2,754 pregnant women who contributed to the comparison of birth outcome*

	Gravidae 1–4			Grand multigravidae		
	ITN (n = 868)	Control (n = 839)	*P* or RR (95% CI)	ITN (n = 509)	Control (n = 538)	*P* or RR (95% CI)
Source cohort area, no. (%)	226 (26.0)	239 (28.5)	0.94 (0.51–1.75)	144 (28.3)	157 (29.2)	0.98 (0.53–1.81)
Age in years, mean (SE)	21.5 (0.15)	21.3 (0.13)	0.20	32.0 (0.33)	31.7 (0.31)	0.56
Marital status¶						
Married, no. (%)	586 (67.7)	586 (70.3)		478 (93.9)	500 (93.5)	
Never been married, no. (%)	247 (28.5)	227 (27.2)		3 (0.6)	2 (0.4)	
Widowed, no. (%)	15 (1.7)	11 (1.3)		24 (4.7)	27 (5.1)	
Divorced/separated, no. (%)	18 (2.1)	10 (1.2)	0.73	4 (0.8)	6 (1.1)	0.92
Socioeconomic rank score, median (quartiles)†	49 (26–71)	42 (18–75)	0.52	58 (32–76)	55 (29–80)	0.88
Years of schooling, median (quartiles)†	8 (7–8)	8 (6–8)	0.25	7 (4–8)	7 (5–7)	0.85
Gestational age on enrollment, mean (SE)†	26.2 (0.39)	26.0 (0.39)	0.72	24.3 (0.50)	25.2 (0.41)	0.20
Gravida, median (quartiles)	2 (1–3)	2 (1–3)	0.91	7 (6–8)	7 (6–8)	0.44
Previous stillbirth/abortion, no. (%)‡	50 (8.0)	54 (8.8)	0.91 (0.60–1.39)	116 (22.8)	120 (22.3)	1.02 (0.78–1.34)
Previous child death, no. (%)‡§	142 (26.3)	126 (24.0)	1.10 (0.86–1.40)	354 (70.2)	385 (73.10	0.96 (0.7–1.06)
Season, second-third trimester¶						
Driest, no. (%)	274 (31.6)	279 (33.3)		182 (35.8)	183 (34.0)	
Intermediate, no. (%)	274 (31.6)	306 (36.5)		177 (34.8)	164 (30.5)	
Wettest, no. (%)	320 (36.9)	254 (30.3)	0.03	150 (29.5)	191 935.5)	0.12
Height in cm, mean (SE)	161.8 (0.51)	160.9 (0.42)	0.19	162.5 (0.52)	161.8 (0.52)	0.32
Mid-upper-arm circumference in cm, mean (SE)	25.8 (0.18)	25.8 (0.20)	0.99	26.6 (0.20)	26.5 (0.21)	0.90
Tetanus toxoid during pregnancy, no. (%)	470 (60.0)	430 (57.0)	1.05 (0.89–1.26)	231 (50.4)	252 (51.6)	0.98 (0.76–1.25)
Attended antenatal care clinics, no. (%)	530 (67.7)	489 (64.8)	1.05 (0.88–1.24)	275 (60.0)	300 (61.5)	0.98 (0.78–1.23)
Smoked during pregnancy, no. (%)	8 (0.9)	4 (0.5)	1.94 (0.62–6.02)	12 (2.4)	11 (2.1)	1.15 (0.50–2.61)
Alcohol use during pregnancy, no. (%)	34 (3.9)	31 (3.7)	1.06 (0.58–1.95)	48 (9.5)	20 (3.8)	2.52 (1.37–4.64)
Hemoglobin S phenotype¶						
AA, no. (%)	216 (74.2)	215 (77.3)		171 (74.0)	165 (76.7)	
AS, no. (%)	74 (25.4)	63 (22.7)		57 (24.7)	50 (23.3)	
SS, no. (%)	1 (0.3)	0 (0.0)	0.45	3 (1.3)	0 (0)	0.19

ITN = insecticide-treated bed nets.
* RR = relative risk; CI = confidence interval.
† Cohort study only.
‡ Multigravidae only.
§ Includes deaths among children who were born alive and died before the start of the bed net study (January 1, 1997).
¶ Column percentage.

ence was not statistically significant (protective efficacy = 32%; 95% CI = -7–56, P =0.09) (Figure 2).

Hemoglobin levels were determined at the same time as the placental, cord blood, and peripheral smears (i.e., within 24 hours after delivery) from women in the cohort area. Mean hemoglobin levels were significantly higher in gravidae 1–4 who lived in ITN villages (11.1 versus 10.5 g/dL, mean difference = 0.6; 95% CI = 0.01–1.2, P = 0.0497). However, this did not correspond to a significantly lower prevalence of severe anemia (hemoglobin concentration <8 g/dL): ITN = 9.4% versus control = 11.8%, protective efficacy = 20%; 95% CI = –37–54%. There was no apparent beneficial effect in the grand multigravidae (mean hemoglobin concentration of the ITN = 10.3 versus control = 10.7 g/dL; P = 0.28).

Stillbirths and abortion. Of the recorded births among gravidae 1–4, 50 (2.9%) resulted in fetal loss. This was 2.3% in the ITN group and 3.3% in the control group (protective efficacy = 31%; 95% CI = –16–59%, P = 0.17). The prevalence of fetal loss in gravidae 5 and greater was similar among women from ITN (2.2%) and control villages (2.1%).

Birth weight and gestational age. The adjusted mean (95% CI) birth weight was 77.6 (34.0–121.2) grams higher in the first four pregnancies in women living in ITN villages (3,088 grams) compared with the control villages (3,011 grams) (P = 0.0008). There was no difference in gravidae 5 and greater (Figure 3). The mean gestational age overall was 39.8 (ITN) and 39.7 (control) weeks (mean difference = 0.14 weeks; 95% CI = –0.02–0.31 weeks, P = 0.096). This small difference was similar in gravidae 1–4 (0.15 weeks; 95% CI = -0.05–0.32 weeks, P = 0.15) and in grand multigravidae (0.16 weeks; 95% CI = –0.03–0.35 weeks, P = 0.11). The prevalence of low birth weight, preterm births, and small-for-gestational age infants was consistently lower in the ITN group in gravidae 1–4, but not the grand multigravidae (Figure 2). Overall, 24.1% versus 32.1% newborns from gravidae 1–4 in ITN villages were either aborted, stillborn, or born with low birth weight, preterm, or small-for-gestational age (protective efficacy = 25%; 95% CI = 13–35%, P < 0.0001), whereas there was no beneficial impact in grand multigravidae (18.3% versus 15.3%, protective efficacy = –19%; 95% CI = –63—12%, P = 0.26). The beneficial impact on any of the adverse birth outcomes in gravidae 1–4 was equally evident across the seasons.

Self-reported ITN use. The overall proportion of self-reported ITN use in the intervention villages was 85%. Univariate analyis showed that this was strongly associated with age, marital status, and gravida status. Use of ITNs was lowest among primigravid women (71%), those less than 21 years old (74%), single (71%), or widowed, divorced, or separated (80%), and highest in grand multigravidae (96%), those 30 years of age and older (97%), and among married women (89%). The use of ITNs was also slightly higher in the women enrolled in the cohort study (who were visited at home by study staff on a monthly basis) compared with those living in

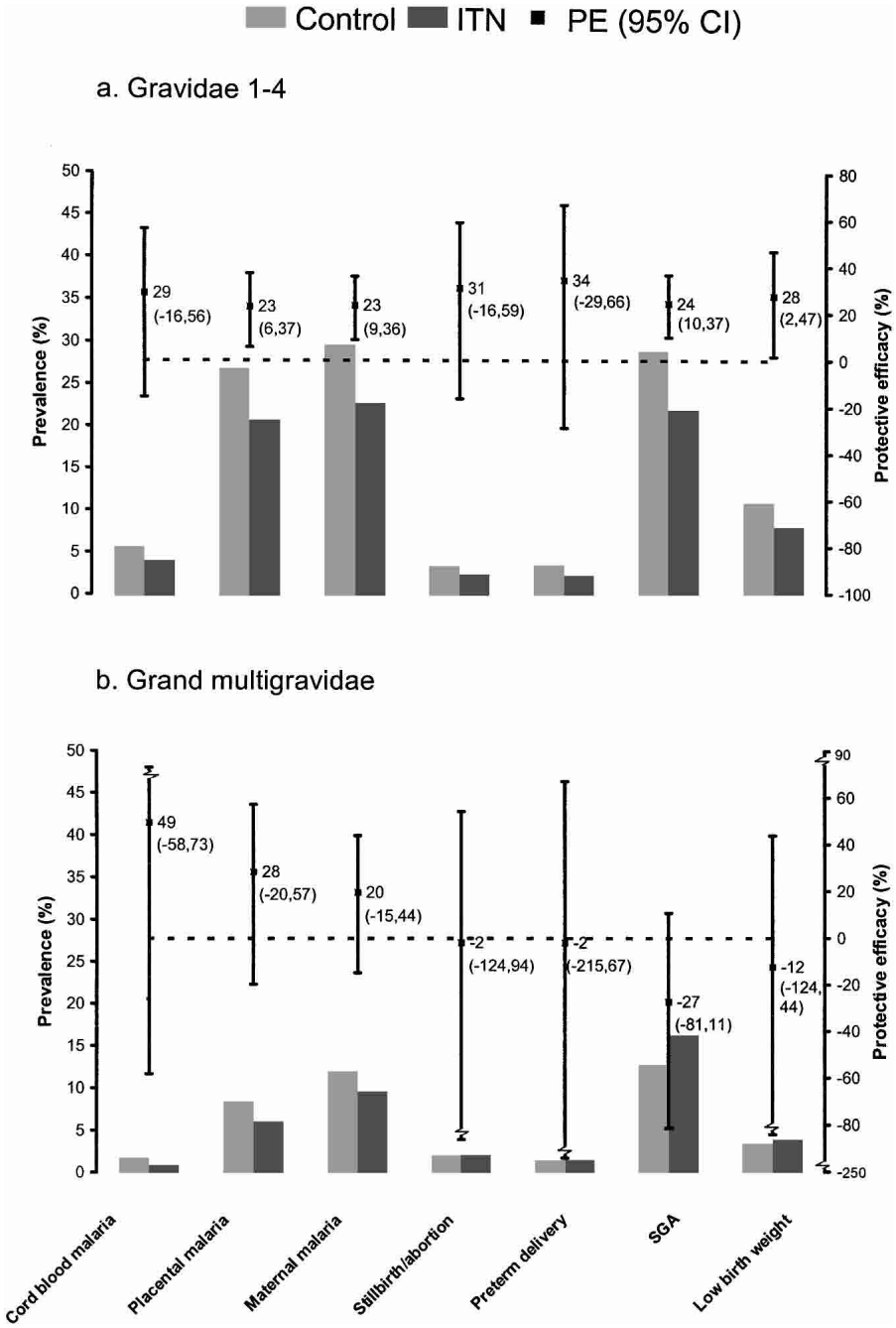

FIGURE 2. Adjusted prevalence and protective efficacy (PE) with 95% confidence intervals (CIs) for malaria at the time of delivery and for birth outcome in insecticide-treated bed net (ITN) villages and control villages among **a.**, gravidae 1–4 and **b.**, grand-multigravidae. SGA = small-for-gestational age.

the remaining non-cohort villages. There was no association with season (rainfall or temperature), socioeconomic status, years of education, or frequency of antenatal clinic attendance. Multivariate regression analysis showed that age, marital status, and enrollment in the cohort study were the three strongest predictors of ITN use (Table 4). Combined, young age (<21 years old) and being either single or widowed, accounted for 63% of the non-ITN users.

DISCUSSION

We have shown that in this area of intense perennial malaria transmission, where women on average may receive as many as 45–230 infective bites during 40 weeks gestation, malaria and anemia are common during pregnancy. Up to one-third of all infants are born preterm, intra-uterine growth retarded (small-for-gestational age), or with low birth weight. In the first four pregnancies, ITNs were associated with clinically significant reductions in parasitemia during pregnancy and at the time of delivery, and this resulted in improved maternal hemoglobin concentrations and a 28% reduction in the risk of low birth weight. Almost all of this beneficial effect on birth size was due to improved fetal growth, and only a small non-significant increase in duration of gestation was observed. There was no beneficial impact on birth outcome in

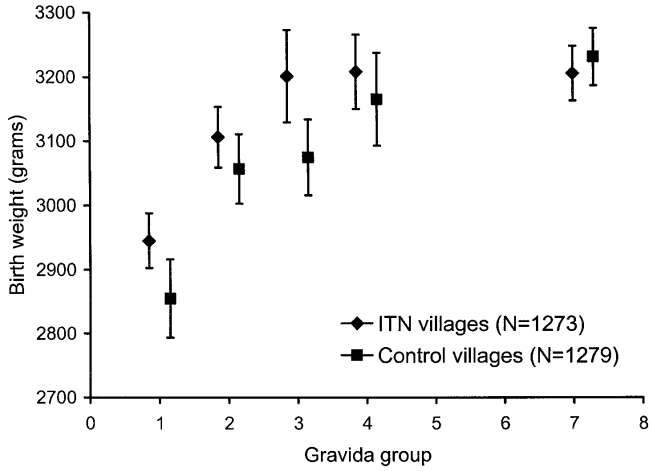

FIGURE 3. Mean (95% confidence interval) birth weight in grams by gravida in insecticide-treated bed net villages and control villages adjusted for maternal stature, delivery season, gravida, and newborn sex.

gravidae 5 or greater. Size at birth is an important indicator of fetal and neonatal health, and low birth weight in particular is associated with neonatal, post-neonatal, infant and child mortality, morbidity, and permanent deficits in growth, neurocognitive development, and performance.[42,44–46] Therefore, these results suggest that in settings with intense perennial malaria transmission, such as Asembo, the use of permethrin-treated bed nets may have substantial beneficial impact on public health.

Only one of the four previous randomized controlled trials on the impact of ITNs in pregnancy also found a beneficial impact on birth weight.[28] Could causes other than ITN use explain the observed differences in our study? This study was a non-blinded, group-randomized trial (cluster randomization), which because of the limited number of assignment units (villages) has a greater potential for bias than the more familiar clinical trial design based on randomization by individual.[47] Many factors apart from malaria are known to impact on birth weight and could have been a potential source of bias. However all known determinants, as well as other factors found to be associated with birth weight and gestational age in this study were controlled for in our multivariate analysis. One senior study team member determined gestational age and birth weight for every 4–6 villages, using a single weighing scale throughout the study, and none of them were exclusively assigned to either intervention or control villages. The inclusion of the variables for traditional birth attendant and weighing scale as covariates in the analysis did not suggest observer or instrumental bias.

There are several arguments in favor of a true impact of ITNs on birth outcome. First, the impact was greatest for the most malaria-specific parameters, i.e., malaria parasitemia and malarial anemia, and less for the non-specific and more distal indicators such as low birth weight. Second, this beneficial impact occurred only in gravidae 1–4, and not in gravidae 5 and higher. This is consistent with the previous findings from the Asembo Bay Cohort Study conducted in this same population between 1992 and 1996 (ter Kuile FO and others, unpublished data), as well as other studies in areas of intense malaria transmission, which indicate that the risk of malaria and its adverse consequences decreases with subsequent malaria-exposed pregnancies but can remain a significant problem in the first three to five pregnancies.[9–11,13] Third, the reduction in laboratory parameters such as maternal malaria and anemia were consistent with the impact on birth outcome, and laboratory personnel were unaware of the intervention status of the participants. Fourth, in the 19 cohort villages, there was no difference in birth weight or other birth outcomes between the subsequent ITN and intervention villages in the four years prior to the current study, and rates of malaria during pregnancy and placental malaria and maternal anemia were also similar, suggesting that the transmission and epidemiology of malaria was comparable in the subsequent ITN and control villages before the start of the ITN study.

The results of the current study do not support the hypothesis that in higher transmission settings, or where there are prolonged seasons of *P. falciparum* transmission, ITNs alone do not prevent the adverse effects of malaria in pregnancy.[5] Although the beneficial impact on maternal malaria and anemia in the current study was indeed smaller than that reported from the Thailand-Burma border[27] and The Gambia,[28] which have much lower malaria transmission, the effect was observed throughout the year, with little seasonal variation, whereas the impact on birth outcome in The Gambia was only present during the rainy (malaria) season.[28] Furthermore, the current study, in an area of high perennial transmission, indicates that women in their first four pregnancies benefited from ITN use (i.e., approximately two-thirds of the pregnant women), whereas in areas with lower malaria transmission the adverse effects of malaria seem to be confined mainly to primigravidae and secundigravidae (approximately one-third of the pregnant women).[8] Thus, although the relative impact in terms of protective efficacy (usually expressed as a percentage) may indeed decrease with increasing transmission pressure, the absolute effect, in terms of the total number of pregnancies with adverse outcome prevented, may be similar or greater in areas of intense transmission pressure. Nevertheless, the number of studies determining the impact of ITNs is still too few to draw firm conclusions about the relationship between impact of ITNs and transmission intensity.

Adherence to ITN use was high overall (85%), particularly

TABLE 4

Multivariate analysis of determinants of non-adherence to insecticide-treated bed net use among pregnant women in intervention villages

	Does not use ITN %*	RR (95% CI)*	*P**
Age in years			
<21	23.3	8.74 (3.94–19.37)	<0.001
21–30	15.9	5.96 (2.81–12.65)	<0.001
>30	2.7	Reference	
Marital status			
Single†	20.8	1.57 (1.12–2.21)	0.009
Married	13.2	Reference	
Cohort participant			
Yes	11.6	0.72 (0.53–0.97)	0.03
No	16.1	Reference	

* Adjusted prevalence, relative risk (RR) with 95% confidence interval (CI) and corresponding *P* values for self-reported non-adherence to bed net use.
† Never been married, or widowed, divorced, or separated.

in older multigravid women (>95%), but lower in young teenage primigravidae (72%). These figures are likely to be an overestimate because this was based on self-reported use of ITNs using-prompted questioning. The lower adherence in the younger women portrays the characteristics associated with mobility in this rural community. Most young unmarried teenage primigravidae move to other family households for the duration of the pregnancy (Alaii JA, unpublished data). The lower adherence in this young age group who are most at risk of the adverse consequences of malaria, is consistent with that reported from the study in Ghana,[29] and reinforce the need to improve health education strategies for this high risk age group.

Our study has several important findings of relevance for control of malaria in pregnancy in areas of similar endemicity. Existing malaria control interventions in Africa predominantly reach women in their second half of pregnancy when they start attending antenatal clinics. Previous studies with intermittent preventive treatment with SP suggest that interventions in the second and third trimesters are sufficient to have a beneficial impact on birth outcome.[15,16,18] However, there is a dearth of information on the impact of malaria control in early pregnancy. The risk of peripheral malaria parasitemia is greatest in the first 20 weeks of gestation with malaria infection rates at delivery approximating the levels in the postnatal period and those seen in non-pregnant women.[8] Population-based ITN programs, such as ours, have the advantage of protecting women prior to, as well as throughout, pregnancy. Care should be taken before extrapolating the findings from the current study to predict the impact of ITNs when distributed in the second half of pregnancy as part of a pre-natal care package.

It is also noted that the beneficial effect observed in the current study was due to a combination of individual barrier protection by ITNs, and a general reduction in malaria transmission consequent to the observed community or mass effect of ITNs on vector populations and sporozoite rates.[48] It is likely that the mass killing effect on mosquito populations will have resulted in an underestimate of the impact of ITNs on malaria in pregnancy in the current study. In a separate analysis presented elsewhere, it is shown that the community effect results in a reduction in malaria-related morbidity in young children living in control households within 300 meters of an intervention village.[49] This beneficial effect was substantial and benefited at least 23% of the control population. Adjustment for the community effect increased the efficacy estimates on morbidity by 7% (clinical malaria) to 20% (hemoglobin levels).[49] Additional studies are required delineating the role of individual versus community effect and the impact and cost-effectiveness of malaria control programs in pregnancy that use single interventions versus programs that combine the benefits of intermittent preventive treatment with ITN use, distributed either as part of community-based programs or through antenatal clinics.

Our results are consistent with those recently reported from a non-randomized study of socially marketed ITNs in southern Tanzania, and a randomized controlled trial of ITN-use in the second and third trimesters among primigravidae and secundigravidae in a neighboring study site in western Kenya.[50,51] Both were conducted in areas with intense perennial malaria transmission. The study in Tanzania also found that a beneficial impact of ITNs extends beyond primigravidae and secundigravidae, and includes women of higher pregnancy order. These findings, together with the increasing evidence that in areas with intermediate and intense transmission the first four to five pregnancies are adversely affected by malaria, add further weight to the argument that pregnant women, regardless of their parity, should be targeted for protection against malaria in highly endemic areas. This is particularly true in areas with a high concomitant prevalence of HIV. Infection with HIV, even if asymptomatic, is known to impair a pregnant woman's ability to control malaria, especially among the older multigravidae,[52] to aggravate the adverse effects of malaria on maternal anemia and pregnancy outcome,[53] and to reduce the efficacy of intermittent preventive treatment.[16] Furthermore, if mothers continue to use the ITN post-partum, newborns, even from grand multigravidae, that share the sleeping space with their mothers will likely benefit from reduced malaria exposure in the first few months of life.[54] Lastly, targeting of all pregnancies, instead of a selected group of high-risk ones, will increase coverage and contribute to any community effect on malaria transmission,[48] and the likelihood of attaining the target of 60% coverage of high risk groups as stated in the Abuja declaration.[55]

The potential to benefit the pregnant woman and the growing fetus during the first four pregnancies, as well as, with continued use, mother and newborn in the post-partum period, and eventually the mother prior to, and during the next pregnancy, is a particularly attractive feature of ITNs. This study has shown that ITNs will result in improvement in the health of pregnant women and their newborns in areas with a high burden of malaria and HIV infection. Thus, ITNs can be added to the limited arsenal of available tools for the control of malaria in pregnancy in areas with intense malaria transmission in Africa south of the Sahara Desert.[56]

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REFERENCES

1. Guyatt HL, Snow RW, 2001. The epidemiology and burden of *Plasmodium falciparum*-related anemia among pregnant women in sub-Saharan Africa. *Am J Trop Med Hyg 64 (Suppl 1):* 1–106.
2. Steketee RW, Wirima JJ, Slutsker L, Heymann DL, Breman JG, 1996. The problem of malaria and malaria control in pregnancy in sub-Saharan Africa. *Am J Trop Med Hyg 55 (Suppl 1):* 2–7.
3. Desowitz RS, Alpers MP, 1992. Placental *Plasmodium falciparum* parasitaemia in East Sepik (Papua New Guinea) women of different parity: the apparent absence of acute effects on mother and foetus. *Ann Trop Med Parasitol 86:* 95–102.
4. Shulman CE, 1999. Malaria in pregnancy: its relevance to safe-motherhood programmes. *Ann Trop Med Parasitol 93 (Suppl 1):* S59–S66.
5. Shulman CE, Dorman EK, Talisuna AO, Lowe BS, Nevill C, Snow RW, Jilo H, Peshu N, Bulmer JN, Graham S, Marsh K, 1998. A community randomized controlled trial of insecticide-treated bednets for the prevention of malaria and anaemia among primigravid women on the Kenyan coast. *Trop Med Int Health 3:* 197–204.
6. Mockenhaupt FP, Rong B, Till H, Eggelte TA, Beck S, Gyasi-Sarpong C, Thompson WN, Bienzle U, 2000. Submicroscopic *Plasmodium falciparum* infections in pregnancy in Ghana. *Trop Med Int Health 5:* 167–173.
7. Leke RF, Djokam RR, Mbu R, Leke RJ, Fogako J, Megnekou R, Metenou S, Sama G, Zhou Y, Cadigan T, Parra M, Taylor DW, 1999. Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria. *J Clin Microbiol 37:* 2992–2996.
8. Brabin BJ, 1983. An analysis of malaria in pregnancy in Africa. *Bull World Health Organ 61:* 1005–1016.
9. Reinhardt MC, Ambroise-Thomas P, Cavallo-Serra R, Meylan C, Gautier R, 1978. Malaria at delivery in Abidjan. *Helv Paediatr Acta (Suppl 4):* 65–84.
10. Diagne N, Rogier C, Cisse B, Trape JF, 1997. Incidence of clinical malaria in pregnant women exposed to intense perennial transmission. *Trans R Soc Trop Med Hyg 91:* 166–170.
11. Shulman CE, Marshall T, Dorman EK, Bulmer JN, Cutts F, Peshu N, Marsh K, 2001. Malaria in pregnancy: adverse effects on haemoglobin levels and birthweight in primigravidae and multigravidae. *Trop Med Int Health 6:* 770–778.
12. Steketee RW, Mutabingwa TK, 1999. Malaria in pregnant women: research, epidemiology, policy and practice. *Ann Trop Med Parasitol 93 (Suppl 1):* S7–S9.
13. Beck S, Mockenhaupt FP, Bienzle U, Eggelte TA, Thompson WN, Stark K, 2001. Multiplicity of *Plasmodium falciparum* infection in pregnancy. *Am J Trop Med Hyg 65:* 631–636.
14. Robb A, 1999. Malaria and pregnancy: the implementation of interventions. *Ann Trop Med Parasitol 93 (Suppl 1):* S67–S70.
15. Schultz LJ, Steketee RW, Macheso A, Kazembe P, Chitsulo L, Wirima JJ, 1994. The efficacy of antimalarial regimens containing sulfadoxine-pyrimethamine and/or chloroquine in preventing peripheral and placental *Plasmodium falciparum* infection among pregnant women in Malawi. *Am J Trop Med Hyg 51:* 515–522.
16. Parise ME, Ayisi JG, Nahlen BL, Schultz LJ, Roberts JM, Misore A, Muga R, Oloo AJ, Steketee RW, 1998. Efficacy of sulfadoxine-pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. *Am J Trop Med Hyg 59:* 813–822.
17. Rogerson SJ, Chaluluka E, Kanjala M, Mkundika P, Mhango C, Molyneux ME, 2000. Intermittent sulfadoxine-pyrimethamine in pregnancy: effectiveness against malaria morbidity in Blantyre, Malawi, in 1997-99. *Trans R Soc Trop Med Hyg 94:* 549–553.
18. Shulman CE, Dorman EK, Cutts F, Kawuondo K, Bulmer JN, Peshu N, Marsh K, 1999. Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. *Lancet 353:* 632–636.
19. Verhoeff FH, Brabin BJ, Chimsuku L, Kazembe P, Russell WB, Broadhead RL, 1998. An evaluation of the effects of intermittent sulfadoxine-pyrimethamine treatment in pregnancy on parasite clearance and risk of low birthweight in rural Malawi. *Ann Trop Med Parasitol 92:* 141–150.
20. Kenya Ministry of Health, 1997. *Kenya National Policy Document for Malaria Control.* Nairobi: Kenya Ministry of Health.
21. McGready R, Nosten F, 1999. The Thai-Burmese border: drug studies of *Plasmodium falciparum* in pregnancy. *Ann Trop Med Parasitol 93 (Suppl 1):* S19–S23.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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 bed nets on child mortality and morbidity in western Kenya. II: Study design and methods. *Am J Trop Med Hyg 68 (Suppl 4):* 10–15.
27. Dolan G, ter Kuile FO, Jacoutot V, White NJ, Luxemburger C, Malankirii L, Chongsuphajaisiddhi T, Nosten F, 1993. Bed nets for the prevention of malaria and anaemia in pregnancy. *Trans R Soc Trop Med Hyg 87:* 620–626.
28. D'Alessandro U, Langerock P, Bennett S, Francis N, Cham K, Greenwood BM, 1996. The impact of a national impregnated bed net programme on the outcome of pregnancy in primigravidae in The Gambia. *Trans R Soc Trop Med Hyg 90:* 487–492.
29. Browne EN, Maude GH, Binka FN, 2001. The impact of insecticide-treated bednets on malaria and anaemia in pregnancy in Kassena-Nankana district, Ghana: a randomized controlled trial. *Trop Med Int Health 6:* 667–676.
30. 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.
31. 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 bed nets 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.
32. Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA, Perkins PV, Chumo DK, Koech DV, Whitmire RE, Roberts CR, 1994. *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am J Trop Med Hyg 50:* 529–536.
33. 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. De-

scriptive epidemiology of malaria infection and disease among children. *Am J Trop Med Hyg 60:* 641–648.
34. McElroy PD, Ter Kuile FO, Lal AA, Bloland PB, Hawley WA, Oloo AJ, Monto AS, Meshnick SR, Nahlen BL, 2000. Effect of *Plasmodium falciparum* parasitemia density on hemoglobin concentrations among full-term, normal birth weight children in western Kenya, IV. The Asembo Bay Cohort Project. *Am J Trop Med Hyg 62:* 504–512.
35. Phillips-Howard PA, Nahlen BL, Kolczak MS, Hightower AW, ter Kuile FO, Alaii JA, Gimnig JE, Arudo J, Vulule JM, Odacha 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.
36. McElroy PD, Lal AA, Hawley WA, Bloland PB, Kuile FO, Oloo AJ, Harlow SD, Lin X, Nahlen BL, 1999. Analysis of repeated hemoglobin measures in full-term, normal birth weight Kenyan children between birth and four years of age. III. The Asemobo Bay Cohort Project. *Am J Trop Med Hyg 61:* 932–940.
37. 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.
38. Meltzer MI, Terlouw DJ, Kolczak MS, Odhacha A, ter Kuile FO, Vulule JM, Alaii JA, Nahlen BL, Hawley WA, Phillips-Howard PA, 2003. The household-level economics of using permethrin-treated bed nets to prevent malaria in children less than five years of age. *Am J Trop Med Hyg 68 (Suppl 4):* 149–160.
39. Ballard JL, Novak KK, Driver M, 1979. A simplified score for assessment of fetal maturation of newly born infants. *J Pediatr 95:* 769–774.
40. United Nations, 1986. Summary procedures how to weigh and measure children. *Assessing the Nutritional Status of Young Children in Household Surveys*. New York: United Nations, 1–11.
41. Greenwood BM, Greenwood AM, Snow RW, Byass P, Bennett S, Hatib-N'Jie AB, 1989. The effects of malaria chemoprophylaxis given by traditional birth attendants on the course and outcome of pregnancy. *Trans R Soc Trop Med Hyg 83:* 589–594.
42. WHO, 1995. *Physical Status: The Use of and Interpretation of Anthropometry*. Geneva: World Health Organization.
43. Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris FD, Tashiro M, 1982. Fetal growth and perinatal viability in California. *Obstet Gynecol 59:* 624–632.
44. McCormick MC, 1985. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med 312:* 82–90.
45. Medicine/National Io, Sciences Ao, 1990. *Nutrition During Pregnancy*. Washington, DC: National Academy Press.
46. Teberg AJ, Walther FJ, Pena IC, 1988. Mortality, morbidity, and outcome of the small-for-gestational-age infant. *Semin Perinatol 12:* 84–94.
47. Murray DM, 1998. *Design and Analysis of Group-Randomized Trials*. New York: Oxford University Press.
48. 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.
49. 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 bed nets on child mortality and malaria morbidity in western Kenya. *Am J Trop Med Hyg 68 (Suppl 4):* 121–127.
50. Marchant T, Schellenberg JA, Edgar T, Nathan R, Abdulla S, Mukasa O, Mponda H, Lengeler C, 2002. Socially marketed insecticide-treated nets improve malaria and anaemia in pregnancy in southern Tanzania. *Trop Med Int Health 7:* 149–158.
51. Njagi JK, 2002. *The Effects of Sulfadoxine-Pyrimethamine Intermittent Treatment and Pyrethroid Impregnated Bed Nets on Malaria Morbidity in Pregnancy and Birth Weight in Bondo District, Kenya.* PhD thesis. Nairobi: Department of Medical Microbiology, Faculty of Medicine.University of Nairobi, 129.
52. Steketee RW, Wirima JJ, Bloland PB, Chilima B, Mermin JH, Chitsulo L, Breman JG, 1996. Impairment of a pregnant woman's acquired ability to limit *Plasmodium falciparum* by infection with human immunodeficiency virus type-1. *Am J Trop Med Hyg 55 (Suppl 1):* 42–49.
53. Ayisi JG, van Eijk AM, ter Kuile FO, Kolczak MS, Otieno JA, Misore AO, Kager PA, Steketee RW, Nahlen BL, 2003. The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya. *AIDS 17:* 585–594.
54. 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 bed nets 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.
55. World Health Organization, 2000. *The African Summit on Roll Back Malaria*. Geneva: World Health Organization.
56. Guyatt HL, Corlett SK, Robinson TP, Ochola SA, Snow RW, 2002. Malaria prevention in highland Kenya: indoor residual house-spraying vs. insecticide-treated bednets. *Trop Med Int Health 7:* 298–303.