Am. J. Trop. Med. Hyg., 74(3), 2006, pp. 376–385

PARASITEMIA, ANEMIA, AND MALARIAL ANEMIA IN INFANTS AND YOUNG CHILDREN IN A RURAL HOLOENDEMIC *PLASMODIUM FALCIPARUM* TRANSMISSION AREA

JOHN MICHAEL ONG'ECHA, CHRISTOPHER C. KELLER, TOM WERE, COLLINS OUMA, RICHARD O. OTIENO, ZACHARY LANDIS-LEWIS, DANIEL OCHIEL, JAMIE L. SLINGLUFF, STEPHEN MOGERE, GEORGE A. OGONJI, ALLOYS S. ORAGO, JOHN M. VULULE, SANDRA S. KAPLAN, RICHARD D. DAY, AND DOUGLAS J. PERKINS*

University of Pittsburgh/KEMRI Laboratories of Parasitic and Viral Diseases, Kenya Medical Research Institute, Center for Vector Biology and Control Research, Kisumu, Kenya; Department of Infectious Diseases and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania; Siaya District Hospital, Ministry of Health, Siaya, Kenya; Department of Health Sciences, Kenyatta University, Nairobi, Kenya; Kenya Medical Research Institute, Center for Vector Biology and Control Research, Kisumu, Kenya; Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; Department of Biostatistics, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract. Malarial anemia (MA) is a multifactorial disease for which the complex etiological basis is only partially defined. The association of clinical, nutritional, demographic, and socioeconomic factors with parasitemia, anemia, and MA was determined for children presenting at a hospital in a holoendemic area of *Plasmodium falciparum* transmission in western Kenya. Parasitemia was not associated with malaria disease severity. In univariate logistic regression, fever was significantly associated with parasitemia, and wasting was associated with increased presentation of MA. Caretaker's level of education and occupation were significantly correlated with parasitemia, anemia, and MA. Housing structure was also significantly associated with parasitemia and anemia. Bed net use was protective against parasitemia but not anemia or MA. Multivariate logistic regression models demonstrated that fever, mother's occupation, and bed net use were associated with parasitemia. In the current study, none of the factors were associated with anemia or MA in the multivariate models.

INTRODUCTION

Human malaria, transmitted by female *Anopheles* mosquitoes, is a protozoan disease caused by one of four members of the genus *Plasmodium*: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae.*[1] Malaria is one of the leading causes of morbidity and mortality of infectious disease origin throughout the world, resulting in 300–500 million clinical cases per annum and approximately 2 million deaths.[2,3] More than 90% of malaria cases occur in sub-Saharan Africa, with immune-naïve individuals such as children less than 5 years of age bearing the highest disease burden.[3] The majority of malaria-related morbidity and mortality in African children is due to infection with *P. falciparum*, with the most severe clinical manifestations defined by one or more of the following: hyperparasitemia, hypoglycemia, cerebral malaria (CM), malarial anemia (MA), and respiratory distress. Among these disease sequelae, MA is responsible for the greatest amount of malaria-related morbidity and mortality.[2,3]

In holoendemic areas of *P. falciparum* transmission, such as western Kenya, malaria prevalence is 83% in children 1 to 4 years of age, with MA [hemoglobin (Hb) < 8.0 g/dL] being the most common clinical manifestation of malaria in this population and CM occurring only in rare cases.[4] The prevalence of anemia in children less than 5 years of age in western Kenya is between 60% and 90%.[4,5] Although MA can occur as a consequence of repeated cycles of invasion, replication, and ultimately bursting of red blood cells (RBCs) due to the parasite, this form of anemia frequently begins 1 or 2 days after the clinical onset of infection and resolves in approximately 7 days in patients receiving antimalarial treatment.[6] In addition to the direct destruction of RBCs by the parasite, nonparasitized RBCs are also destroyed through increased activity of the reticuloendothelial system. Loss of parasitized and nonparasitized RBCs through host-induced immune responses, in conjunction with immune-mediated suppression of erythropoiesis, result in MA.

Previous large-scale studies in Senegal, Ghana, Nigeria, and Kenya have offered important insight into the complex factors that govern the pathogenesis of MA.[4,5,7–9] The severity of MA is multifactorial and influenced by hemoglobinopathies, nutritional deficiencies, and coinfection with bacteremia and HIV.[10–12] Additional variables such as sociodemographic factors influence the prevalence and outcomes of MA.[13–16] Building upon knowledge gained from previous investigations,[4,5,7–9] we designed a hospital-based study to address the complex etiologies of MA in infants and young children in a holoendemic area of malaria transmission: Siaya District, Nyanza Province, western Kenya. The current manuscript describes the study location and cross-sectional analyses of the child risk factors and sociodemographic indicators associated with parasitemia, anemia, and MA upon presentation at hospital during the first year of the activities (August 2003 to August 2004).

MATERIALS AND METHODS

Study area. The ongoing studies are conducted in Siaya District, Nyanza Province, western Kenya, and the surrounding community (Figure 1). Siaya District has an equatorial climate 1,140 to 1,430 m above sea level. The southern part of the district is relatively dry compared with the northern and eastern parts, with annual rainfall between 800 to 2,000 mm that drains into Lake Victoria. The average annual temperatures range from 15°C to 30°C.[17]

The population is ~500,000 people, with 81,304 children less than 5 years of age. The annual population growth rate is 0.9%,[18] while infant and under-5-years mortality rates are 176

* Address correspondence to Douglas J. Perkins, Department of Infectious Diseases and Microbiology, University of Pittsburgh, 603 Parran Hall, 130 DeSoto St., Pittsburgh, PA 15261. E-mail: djp@pitt.edu

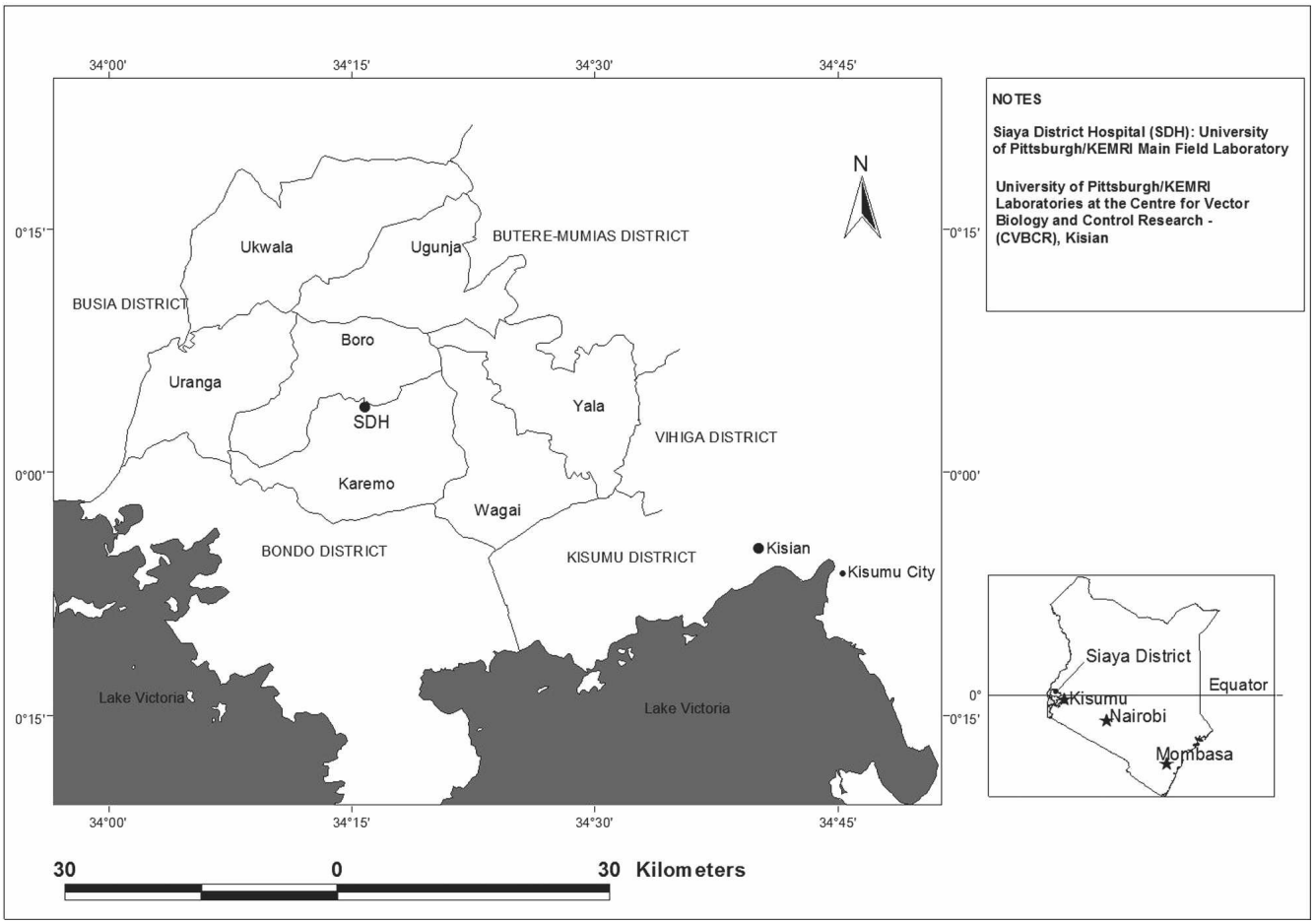

FIGURE 1. Location of the study area in western Kenya. Children enrolled in the study are recruited from the Siaya District in western Kenya. The University of Pittsburgh/Kenya Medical Research Institute (KEMRI) laboratories are located at the Siaya District Hospital (SDH) and at the Center for Vector Biology and Control Research (CVBCR) in Kisian.

of 1,000 (17.6%) and 257 of 1,000 (25.7%), respectively.[19] Malaria is the primary cause of childhood morbidity and mortality. The mosquito vectors in this area are *Anopheles gambiae* s.s., *Anopheles arabiensis,* and *Anopheles funestus.*[20] Falciparum malaria transmission is holoendemic with residents receiving 100–300 infective mosquito bites per annum.[4] The most intense malaria transmission occurs during the seasonal rainfalls in April to August and November to January.[21]

Inhabitants of the study area are predominantly of the Luo ethnic tribe (>96%), with the population being culturally homogeneous.[4] Although marriage is exogamous and polygamy is practiced, there are large numbers of women-led households. The poverty level is estimated at 58.02%.[17]

Clinical facilities. The ongoing studies are based at the pediatric ward of the Siaya District Hospital (SDH), in Siaya District, Nyanza Province, western Kenya, and the surrounding community. SDH is a 260-bed hospital with a 60-bed pediatric unit and is the only major hospital serving the Siaya District. The University of Pittsburgh/KEMRI clinical laboratories are located directly adjacent to the pediatric ward, while the molecular-based laboratories are located 67 km away at the Center for Vector Biology and Control Research in Kisian (Figure 1). The pediatric unit admits ~8 patients per day, which doubles during peak malaria transmission seasons. The average length of stay on the ward is between 2 to 3 days. Total admissions to the pediatric unit for the year 2003 were 3,538 children with a hospital mortality of approximately 10% (364 children).[18] The four leading diseases accounting for the majority of in-patient morbidity at the SDH for the year 2003 were malaria (31.6%), anemia (10.4%), pneumonia (8.6%), and diarrheal diseases (5.8%).[18] Earlier studies reported that a third of all pediatric admissions to SDH had Hb levels of <5.0 g/dL that accounted for half of all pediatric hospital deaths.[22] About 12% of children aged less than 5 years old were reported to die at home within 8 weeks after hospital admission, 32% being malaria-associated cases.[23]

Study design and participants. Children of both sexes aged 0 to 3 years visiting the hospital with symptoms of malaria were eligible for enrollment. After the parent/guardian of the child consented to participate in the study, a questionnaire was used to collect relevant demographic and clinical information. Because we were interested in enrolling children with minimal prior exposure to malaria, first hospital contacts were selected for enrollment. As such, the majority of children recruited into the study were less than 1 year of age at the time of enrollment. If the child had previously been hospitalized in his or her lifetime or there was reported antimalarial use within the past 2 weeks, he or she was not eligible

for participation in the study. This enrollment strategy was used because malaria is the primary cause of pediatric hospitalization in this area and prior antimalarial use can affect the immunologic and outcome variables that will be assessed at a later date. Children with CM, an exceedingly rare event in this high-transmission setting, were excluded from enrollment.

Heel/finger-prick blood (<100 μL) was used to determine parasitemia and Hb status. Based on the results of Hb and parasitemia status, children were placed into one of the six case definitions listed in Table 1. Healthy controls were recruited from the Maternal/Child Health Clinic at SDH during presentation at hospital for childhood vaccinations. Not all children in the non-SMA groups were hospitalized even though they presented at hospital for management of childhood illnesses. Anemia was defined as Hb < 11.0 g/dL, a standard definition for children less than 5 years of age in developing nations.[24] Definitions of mild, moderate, and severe anemia were based on previous large-scale studies examining the distribution of Hb concentrations in children less than 5 years of age in western Kenya and Northern Ghana.[4,5,7–9]

Children were treated according to Ministry of Health, Kenya (MOH) guidelines, which included the use of Coartem (Artemether and Lumefantrin combination) for uncomplicated malaria, while intravenous quinine was used to treat severe clinical malaria. Supportive care including hematinics and blood transfusions were administered according to the MOH guidelines. Human subjects approval for the investigations was obtained from the University of Pittsburgh and KEMRI, and the clinical protocol was approved by the National Institutes of Health.

Hematological measurements. Peripheral blood smears were prepared and stained with Giemsa reagent and examined under oil immersion for malaria parasites. Asexual malaria parasites were counted against 300 leukocytes and parasite densities estimated assuming a count of 8,000 white blood cells per microliter of blood. Screening for Hb levels was performed on heel/finger-prick blood using a HemoCue system (HemoCue AB, Angelholm, Sweden). For additional laboratory evaluations, peripheral blood was collected into Vacutainer tubes containing EDTA as an anticoagulant, and Vacutainer tubes without anticoagulants for plasma and serum measurements, respectively (Becton-Dickinson, San Diego, CA). Complete blood counts were performed with a Beckman Coulter AcT diff2 (Beckman-Coulter Corporation, Miami, FL).

Nutritional status. The nutritional status of the children was also determined during their enrollment and whenever they presented to the hospital during the follow-up visits. Weight, height, head, and mid upper-arm circumference (MUAC) measurements were taken for use in the determination of whether the children were wasted, stunted, or underweight, based on Z-scores < −2. The weight measurements were taken using a Salter scale to the nearest 0.1 kg for children over 10 kg and to the nearest 0.01 kg for children under 10 kg. Height measurements were made to the nearest 0.1 cm in a recumbent position for children under 2 years of age and in the standing position for children greater than 2 years of age. Height-for-age (stunting), weight-for-height (wasting), and weight-for-age (underweight) Z-scores were calculated using growth reference curves developed by the National Center for Health Statistics (NCHS) using Epi Info 6.04 (CDC, Atlanta, GA).

Data management. A computer-based health information system was developed in partnership with Baobab Health Partnership, Inc. (Pittsburgh, PA) to establish an Electronic Medical Records (EMR) system to support the research activities. All information for the questionnaires related to the demographic and clinical data were electronically recorded with the EMR system. Five touch-screen workstations located on the pediatric ward were connected to a server through an Ethernet network. All data entered into the networked system were analyzed for consistency by separate individuals in the United States and Kenya, and any discrepancies identified were sent back to the field site for validation and/or correction. Hard copies for all of the questionnaires and laboratory data were also compared against data entered into the EMR system.

Statistical analyses. All statistical analyses were performed using Minitab (Minitab Release 13.32, Minitab Inc.). Kruskal-Wallis tests were used for multiple group comparisons. Those groups that were significantly different by Kruskal-Wallis tests were then compared by Mann-Whitney *U* tests for pairwise analyses (statistical significance set at $P \leq 0.05$, corrected for multiple comparisons). Differences between proportions were determined using χ^2 analyses. Logistic regression, controlling for age and gender, was used to assess determinants

TABLE 1
Case definitions for the study groups

Categories	Definition upon recruitment into the study
Healthy controls (HC)	Children with a malaria-negative smear for *P. falciparum* parasitemia, Hb ≥ 11.0 g/dL, free of fever or diarrhea for the past 14 days, and no prior hospitalizations.
Hospitalized controls (HosC)	Children with a malaria-negative smear for *P. falciparum* parasitemia and Hb < 11.0 g/dL that presented at hospital for clinical management of nonmalarial diseases.
Uncomplicated malaria (UM)	Children with a malaria-positive smear for *P. falciparum* parasitemia (of any density), absence of anemia (i.e., Hb > 11.0 g/dL), and free from the symptoms of severe malaria, such as hypoglycemia.
Mild malarial anemia (M*l*MA)	Children with a malaria-positive smear for *P. falciparum* parasitemia (of any density), Hb of 8.0–10.9 g/dL, and free from the symptoms of severe malaria, such as hypoglycemia.
Moderate malarial anemia (M*d*MA)	Children with a malaria-positive smear for *P. falciparum* parasitemia (of any density), Hb of 6.1–7.9 g/dL, and free from the symptoms of severe malaria such as hypoglycemia.
Severe malarial anemia (SMA)	Children with a malaria-positive smear for asexual *P. falciparum* parasitemia (of any density) and Hb ≤ 6.0 g/dL. Children with cerebral malaria, a rare event in high transmission settings, were excluded from the study.

of parasitemia, anemia, or MA (statistical significance set at $P \leq 0.05$). Determinants found to be correlated at the 90% confidence level ($P \leq 0.10$) in the univariate analysis were included in multivariate logistic regression models for parasitemia, anemia, or MA controlled for age and gender. For identifying factors associated parasitemia, any density of malaria parasitemia was included in the analyses, because concomitant parasitemia levels are not predictive of malaria disease severity in this area.[5] Determination of factors associated with anemia were based on Hb < 11.0 g/dL (in the presence or absence of malaria parasitemia), while the definition of MA was Hb < 8.0 g/dL with any density of malaria parasitemia, as this categorization identifies those children with the greatest degree of malaria-related morbidity and mortality.[25]

RESULTS

Description of study population. By the end of the first year of the study (August 2003 to August 2004), 374 children were enrolled. The demographic characteristics for 371 (99.2%) of the children with complete data were defined by one of the six following clinical definitions: healthy control (HC, $N = 29$), hospital controls (HosC, $N = 63$), uncomplicated malaria (UM, $N = 18$), mild malarial anemia (M*l*MA, $N = 106$), moderate malarial anemia (M*d*MA, $N = 90$), and severe malarial anemia (SMA, $N = 65$) (Table 1). The mean age of the children upon enrollment was 11.6 ± 0.3 months (mean ± SEM), and 61.2% (227 of 371) of the children were less than 12 months of age. Although children in the SMA group were younger relative to the other groups, the difference in age between all of the groups was not significantly different ($P = 0.07$; Table 2). The enrolled population was composed of 200 males (53.9%) and 171 females (46.1%) with 99% of the children belonging to the Luo ethnic group. The proportion of males and females in the different categories was not significantly different ($P = 0.20$; Table 2). The mean axillary temperatures (°C) between the groups were significantly different ($P < 0.001$; Table 2), and relative to the HC group were significantly elevated in the UM, M*l*MA, M*d*MA, and SMA groups ($P < 0.05$ for all groups, corrected for multiple comparisons; Table 2).

Presentation of anemia and parasitemia. The primary variables for recruitment into the study were Hb concentrations and peripheral parasitemia prior to antimalarial treatment and/or transfusion. The means of Hb concentrations for the different groups were HC (11.76 ± 0.12, SEM), HosC (8.94 ± 0.22), UM (11.51 ± 0.14), M*l*MA (9.11 ± 0.08), M*d*MA (6.94 ± 0.06), and SMA (5.02 ± 0.09; Table 2). The density of parasitemia (parasites/μL) was highest in the UM group (49,697 ± 10,457) and lowest in the SMA group (26,070 ± 4,179), however, parasite densities between the UM, M*l*MA, M*d*MA, and SMA groups were not significantly different ($P = 0.17$; Table 2). Geometric mean parasitemias in the malaria positive children were UM (23,771), M*l*MA (12,858), M*d*MA (15,493), and SMA (12,142; Table 2). The proportion of children with high-density parasitemia (≥ 10,000 parasite/μL) was not significantly different between the groups ($P = 0.35$; Table 2).

Nutritional status. To investigate the association between nutritional status and malaria disease severity, height-for-age (stunting), weight-for-height (wasting), and weight-for-age (under-weight) Z-scores were calculated for all children. Overall, 17.1% (61 of 357) were stunted, 15.4% (55 of 357) were wasted, and 25.8% (92 of 357) of the children were underweight. Analysis of the nutritional indices revealed that there were no significant differences between the groups with respect to stunting ($P = 0.64$), wasting ($P = 0.15$), and underweight ($P = 0.71$; Table 2). However, children with the most severe disease (i.e., the M*d*MA and the SMA groups) had the highest proportion of stunted children (Table 2). The proportion of wasted children was highest in the HosC and SMA groups and was approximately threefold lower in the HC and UM groups (Table 2). The proportion of underweight children was highest in the M*d*MA group and lowest in the UM group (Table 2).

TABLE 2
Clinical and child characteristics of study participants

	HC	HosC	UM	M*l*MA	M*d*MA	SMA	*P*
No. of subjects	29	63	18	106	90	65	–
Demographic factors							
Age, mo	10.37 (1.39)	12.00 (0.99)	12.58 (1.77)	12.49 (0.63)	11.70 (0.59)	9.76 (0.62)	0.07*
Sex							
Male, *n* (%)	12 (41)	37 (59)	10 (56)	65 (61)	41 (46)	35 (54)	0.20†
Female, *n* (%)	17 (59)	26 (41)	8 (44)	41 (39)	49 (54)	30 (46)	
Clinical factors							
Temperature, °C	36.90 (0.17)	37.30 (0.13)	37.99 (0.30)	37.62 (0.11)	38.01 (0.18)	37.53 (0.12)	<0.001*
Hemoglobin, g/dL	11.76 (0.12)	8.94 (0.22)	11.51 (0.14)	9.11 (0.08)	6.94 (0.06)	5.02 (0.09)	<0.001*
Parasitemia, per μL	0	0	49,697 (10,457)	31,931 (3,879)	30,468 (3,708)	26,070 (4.179)	0.17‡
Geometric mean parasitemia, per μL	0	0	23,771	12,858	15,493	12,142	–
High-density parasitemia; ≥10,000 parasites/μL, *n* (%)	0 (0)	0 (0)	15 (83.30)	65 (61.32)	58 (64.40)	40 (61.54)	0.35§
Nutritional factors							
Stunting, *n* (%)	5 (17.2)	7 (12.1)	2 (14.3)	15 (15.0)	20 (22.2)	12 (20.3)	0.64†
Wasting, *n* (%)	2 (6.9)	12 (20.7)	1 (6.3)	17 (17.0)	9 (10.0)	14 (21.9)	0.15†
Underweight, *n* (%)	7 (24.1)	14 (24.1)	3 (18.8)	23 (23.0)	29 (32.3)	16 (26.6)	0.71†

Values represent mean (SEM) unless otherwise noted.
* Multiple group comparisons were analyzed by Kruskal-Wallis tests.
† Chi-square analysis was performed for multiple group comparisons of proportions.
‡ Multiple group comparisons were analyzed by Kruskal-Wallis tests and included all parasitemic groups.
§ Chi-square analysis was performed for multiple group comparisons of proportions and included all parasitemic groups.

Sociodemographic indicators. *Parental educational level.* A questionnaire was administered to the parents/guardians of the children upon enrollment to assess sociodemographic factors associated with MA. The formal educational level of the parents/guardians was classified as no education (none), 7 years of education (primary), 11 years of education (secondary), 13 years of education (high), and training after 13 years of education (tertiary). Analyses of the parent/guardian educational levels revealed that 3% had none, 81% had primary, 11% had secondary, 3% had high, and 2% had tertiary levels of education. To determine the association of education level with MA, the education level was divided into less than primary (i.e., no education or primary) and greater than primary (i.e., secondary, high, and tertiary education). When stratified according to disease severity, there was a significant difference in parent/guardian educational levels ($P < 0.001$; Table 3).

Parental occupational status. The occupation status of the parents/guardians of the children was categorized based on the occupation of the head of the household and for the mothers of the participants if the mother was *not* the head of household. The majority of the children (90%, 243 of 374) were brought to the hospital by their mothers. The main occupation of the head of household was farming (33.9%, 121 of 357), tradesman/craftsman (36.1%, 129 of 357), professional (9.5%, 34 of 357), businessman (11.8%, 42 of 357), or others (25.0%, 7 of 28). Relative to farmer, head of household occupation of tradesman, professional, businessman, and other was significantly different between the groups ($P < 0.01$, $P < 0.001$, $P = 0.04$, and $P = 0.01$, respectively; Table 3).

Subclassification of the data according to the mother's occupation illustrated that 40.6% (140 of 345) of the mothers were farmers, 29.0% (100 of 345) were housewives, and 30.4% (105 of 345) did not fall into these categories. Relative to farmer, mother's occupation of housewife and other was significantly different between the groups ($P < 0.001$ and $P < 0.001$, respectively; Table 3).

Housing structures. Determination of the housing structures was based on the type of wall (i.e., brick or mud) and roofing material (i.e., iron-sheets or grass-thatched) used in construction of the house. The majority of houses (72.3%, 266 of 368) had mud walls and 27.7% (102 of 368) of the houses were constructed from bricks. Approximately half of the houses (50.5%, 186 of 368) had grass-thatched roofs, while 48.6% (179 of 368) contained iron-sheet roofs and 0.9% (3 of 368) had roofs constructed from other materials (i.e., asbestos or tin). In addition, the type of windows in the houses was also determined, which was categorized as screened windows (54.9%, 202 of 368), no windows (37.8%, 139 of 368), or unscreened windows (7.3%, 27 of 368). Wall, roof, and window type were significantly different between the groups ($P < 0.001$, $P < 0.001$, and $P = 0.02$, respectively; Table 3).

Mosquito control methods. Assessment of the different methods used for mosquito control revealed that more than half (54.7%, 203 of 371) of the households did not use any method of mosquito control. Thirty-five percent (35.0%, 130 of 371) of the households reported bed net usage, 10.2% (38 of 371) burned mosquito coils, and 0.8% (3 of 371) used insecticide sprays. There was no other mosquito control measure reported being used in the households. Between the

TABLE 3
Socioeconomic characteristics of study participants

	HC	HosC	UM	M/MA	M*d*MA	SMA	*P*
Education and occupation							
Caretaker education							
(<primary)	21 (77.8)	39 (66.1)	13 (86.7)	84 (85.7)	79 (92.9)	56 (91.8)	<0.001
(>primary)	6 (22.2)	20 (33.9)	2 (13.3)	14 (14.3)	6 (7.1)	5 (8.2)	
Head of household occupation							
Farmer	21 (77.8)	12 (20.0)	5 (27.8)	30 (29.4)	33 (37.5)	34 (55.7)	
Professional	1 (3.6)	13 (21.7)	2 (11.1)	13 (12.7)	4 (4.5)	1 (1.6)	<0.001*
Businessman	4 (14.3)	10 (16.7)	3 (16.7)	13 (12.7)	4 (4.5)	8 (13.2)	0.04*
Tradesman	14 (50.0)	14 (23.3)	6 (33.3)	39 (38.3)	44 (50.0)	12 (19.7)	<0.01*
Other	2 (7.1)	11 (18.3)	2 (11.1)	7 (6.9)	3 (3.5)	6 (9.8)	0.01*
Mother's occupation							
Farmer	8 (27.6)	10 (17.2)	8 (44.4)	37 (37.4)	45 (55.6)	32 (53.4)	
Housewife	14 (48.3)	19 (32.8)	7 (38.9)	33 (33.3)	16 (19.7)	11 (18.3)	<0.001*
Other	7 (24.1)	29 (50.0)	3 (16.7)	29 (29.3)	20 (24.7)	17 (28.3)	<0.001*
House type							
Wall type							
Brick	13 (44.8)	30 (49.2)	8 (44.4)	26 (24.8)	17 (19.1)	8 (12.3)	<0.001
Mud	16 (55.2)	32 (50.8)	10 (55.6)	79 (75.2)	72 (80.9)	57 (87.7)	
Roof type							
Iron	22 (75.9)	36 (60.0)	10 (55.6)	52 (50.0)	31 (34.8)	28 (43.1)	<0.001
Grass	7 (24.1)	24 (40.0)	8 (44.4)	52 (50.0)	58 (65.2)	37 (56.9)	
Window type							
Unscreened	1 (3.4)	4 (6.5)	1 (5.6)	9 (8.6)	7 (7.9)	5 (7.6)	0.44†
Screened	22 (75.9)	42 (67.7)	9 (50.0)	57 (54.3)	42 (47.2)	30 (46.2)	<0.001†
None	6 (20.7)	16 (25.8)	8 (44.4)	39 (37.1)	40 (44.9)	30 (46.2)	
Mosquito control measures							
Bed net use	11 (37.9)	40 (63.5)	4 (22.2)	37 (34.9)	22 (24.4)	16 (24.6)	0.02†
Mosquito coil use	1 (3.5)	5 (7.9)	0 (0)	12 (11.3)	12 (13.3)	8 (12.3)	0.39†
None	17 (58.6)	18 (28.6)	14 (77.8)	57 (53.8)	56 (62.3)	41 (63.1)	

Values represent *n* (%). Chi-square analysis was performed for multiple group comparisons of proportions.
* Statistical significance relative to "Farmer."
† Statistical significance relative to "None."

groups, bed net use was significantly different ($P = 0.02$), however, mosquito coil use was not significantly different ($P = 0.39$; Table 3).

Child factors associated with parasitemia, anemia, and MA. To determine the ability of child factors and socioeconomic indicators to predict parasitemia, anemia, or MA, univariate and multivariate analyses were performed. For assessing the factors associated with parasitemia (any density) or anemia (Hb < 11.0 g/dL, with or without parasitemia), all children were included in the analyses. Determination of the factors that predict MA included all children with any density peripheral parasitemia ($N = 279$) in the analyses.

Analyses of clinical and child factors revealed that gender was not associated with parasitemia ($P = 0.89$) or anemia ($P = 0.30$) but was of borderline significance with MA ($P = 0.06$), with males being at greater risk (Table 4). An elevated axillary temperature (>37.5°C) was associated with an increased risk of parasitemia ($P < 0.001$), but not with anemia ($P = 0.34$) or MA ($P = 0.97$; Table 4). Elevated temperature was associated with parasitemia in the multivariate model ($P < 0.01$; see Table 6). Stunting (height-for-age), wasting (weight-for-height), and underweight (weight-for-age) were not associated with parasitemia ($P = 0.35$, $P = 0.82$, and $P = 0.61$, respectively) or anemia ($P = 0.97$, $P = 0.11$, and $P = 0.79$, respectively; Table 4). Stunting and underweight were not associated with MA ($P = 0.20$ and $P = 0.52$, respectively), however, wasting was significantly associated with MA in the univariate analysis ($P = 0.04$; Table 4). Wasting was not associated with MA in the multivariate model ($P = 0.68$; see Table 6).

Sociodemographic indicators associated with parasitemia, anemia, and malarial anemia. *Parental educational level and occupational status.* Table 5 shows the socioeconomic risk factors associated independently with malaria parasitemia, anemia, or MA by univariate analysis, and Table 6 shows the same factors after multivariate analysis. To examine the association of caretaker education level with parasitemia, anemia, or MA, the education levels were dichotomized into less than or equal to primary level (83.7%, 292 of 349) and greater than primary level (16.3%, 57 of 349). In a univariate analysis, education level greater than primary was protective from parasitemia ($P < 0.001$) and MA ($P = 0.03$), but not anemia ($P = 0.54$; Table 5). Education level was not significantly associated with parasitemia or MA in their respective multivariate models ($P = 0.09$ and $P = 0.16$, respectively; Table 6).

Relative to farmer, a head of household occupation of professional, businessman, or other was protective from parasitemia in a univariate analysis ($P < 0.01$, $P = 0.02$, and $P < 0.01$, respectively; Table 5); however, the head of household occupation was not significantly associated with protection from parasitemia in a multivariate analysis ($P = 0.83$; Table 6). Head of household occupation was not significantly associated with protection from anemia (Table 5). In a univariate analysis, a head of household occupation of professional and businessman were protective from MA ($P = 0.04$ and $P < 0.01$, respectively; Table 5); however, head of household occupation was not significant in the multivariate model ($P = 0.18$; Table 6).

For mother's occupation, housewives and other occupations were associated with protection from parasitemia in a univariate analysis ($P < 0.001$ and $P < 0.001$, respectively; Table 5), and in the multivariate model ($P = 0.03$; Table 6). Relative to farmers, housewives were associated with protection from anemia ($P = 0.04$) and MA ($P < 0.01$) in a univariate analysis (Table 5) but not in the multivariate models ($P = 0.98$ and $P = 0.47$, respectively; Table 6).

Housing structures. Univariate analysis of housing structure illustrated that wall type (brick versus mud) was associated with protection from childhood parasitemia ($P < 0.001$) and anemia ($P < 0.01$; Table 5); however, there was no association of wall type with parasitemia ($P = 0.09$) or anemia ($P = 0.27$) in the multivariate models (Table 6). Brick walls were not protective for MA in a univariate or multivariate analysis ($P = 0.07$ and $P = 0.11$, respectively; Table 5 and Table 6).

Roof type (iron versus grass-thatched) was protective for parasitemia ($P < 0.01$) and anemia ($P < 0.01$) in a univariate analysis (Table 5) but not in the multivariate models for parasitemia ($P = 0.93$) or anemia ($P = 0.10$; Table 6). Roof type was not associated with MA in the univariate analysis ($P = 0.14$; Table 5).

In assessing the impact of window type on predisposition to malaria infection, screened windows were not protective versus unscreened windows for parasitemia ($P = 0.17$), anemia ($P = 0.31$), or MA ($P = 0.94$; Table 5). Screened windows were protective versus having no windows for parasitemia ($P < 0.01$) but not for anemia ($P = 0.16$) or MA ($P = 0.55$; Table 5). In the multivariate model for parasitemia, windows were categorized into screened (54.9%, 202 of 368) and unscreened/none (included both unscreened and none, 45.1%, 166 of 368). Window type was not protective for parasitemia in the multivariate model ($P = 0.83$; Table 6).

Mosquito control methods. Use of bed nets was associated with protection from parasitemia ($P < 0.001$) but not anemia ($P = 0.61$) or MA ($P = 0.13$; Table 5). In the multivariate model for parasitemia, bed net use was protective ($P < 0.01$; Table 6). Mosquito coil use was not associated with parasitemia ($P = 0.18$), anemia ($P = 0.09$), or MA ($P = 0.36$; Table

TABLE 4

Univariate analysis of clinical and child risk factors associated with parasitemia, anemia, or malarial anemia

	Parasitemia			Anemia			Malarial anemia		
	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Sex (male vs. female)	0.97	0.60–1.55	0.89	0.72	0.39–1.33	0.30	1.59	0.99–2.57	0.06
Axillary temperature ≥37.5°C	2.40	1.46–3.93	<0.001	1.36	0.73–2.52	0.34	1.01	0.62–1.64	0.97
Stunting	1.40	0.69–2.83	0.35	0.98	0.40–2.39	0.97	1.52	0.80–2.91	0.20
Wasting	0.93	0.48–1.80	0.82	2.66	0.79–8.93	0.11	2.00	1.01–3.96	0.04
Underweight	1.16	0.65–2.09	0.61	1.11	0.51–2.44	0.79	1.79	0.99–3.23	0.52

Associations of child risk factors with parasitemia, anemia (Hb ≤ 11.0 g/dL, in the absence or presence of parasitemia), or malarial anemia (Hb ≤ 8.0 g/dL, in the presence of parasitemia) were determined by univariate analysis.

TABLE 5
Univariate analysis of sociodemographic risk factors associated with malaria parasitemia, anemia, or malarial anemia

	Parasitemia			Anemia			Malarial anemia		
	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Education and occupation									
Caretaker education (>primary)	0.30	0.17–0.55	<0.001	0.77	0.33–1.78	0.54	0.42	0.19–0.93	0.03
Head of household									
Professional vs. farmer	0.25	0.10–0.59	<0.01	1.11	0.29–4.27	0.88	0.40	0.17–0.96	0.04
Businessman vs. farmer	0.36	0.16–0.82	0.02	0.53	0.19–1.47	0.22	0.20	0.07–0.61	<0.01
Tradesman vs. farmer	0.66	0.35–1.27	0.22	0.61	0.28–1.31	0.19	0.65	0.37–1.16	0.15
Other vs. farmer	0.26	0.11–0.61	<0.01	0.71	0.21–2.41	0.58	0.63	0.22–1.78	0.38
Mothers									
Housewife vs. farmer	0.30	0.16–0.57	<0.001	0.47	0.23–0.97	0.04	0.40	0.22–0.75	<0.01
Other vs. farmer	0.28	0.15–0.53	<0.001	1.19	0.52–2.76	0.68	0.71	0.39–1.30	0.27
House type									
Wall type (brick vs. mud)	0.29	0.17–0.48	<0.001	0.40	0.21–0.76	<0.01	0.58	0.33–1.04	0.07
Roof type (iron vs. grass)	0.43	0.27–0.70	<0.01	0.41	0.22–0.78	<0.01	0.71	0.45–1.12	0.14
Window type									
Screened vs. unscreened	0.49	0.18–1.35	0.17	0.46	0.10–2.06	0.31	1.04	0.40–2.69	0.94
Screened vs. none	0.41	0.24–0.70	<0.01	0.62	0.32–1.21	0.16	0.75	0.29–1.92	0.55
Mosquito control measures									
Bed net use	0.31	0.19–0.51	<0.001	1.19	0.61–2.30	0.61	0.66	0.39–1.13	0.13
Mosquito coil use	1.85	0.75–4.59	0.18	5.79	0.77–43.29	0.09	1.43	0.66–3.09	0.36

Associations of sociodemographic factors with parasitemia, anemia (Hb ≤ 11.0 g/dL, in the absence or presence of parasitemia), or malarial anemia (Hb ≤ 8.0 g/dL, in the presence of parasitemia) were determined by univariate analysis.

5), and was not associated with anemia in the multivariate model ($P = 0.13$; Table 6).

DISCUSSION

Childhood MA is one of the leading causes of morbidity and mortality in endemic areas of *P. falciparum* transmission, particularly in regions of holoendemicity.[5,26,27] Improved understanding of the etiological factors that govern disease severity will aid in the rational design of appropriate management strategies. Because anemia likely represents a continuum of disease that does not fall into discrete categories with distinctly associated clinical features, the current study includes children with varying degrees of MA (i.e., from mild to severe MA).

Children enrolled in the study were predominantly (>99%) from the Luo ethnic group. The homogeneity of the current population should be advantageous for conducting host genetic investigations to identify factors that condition the outcomes and susceptibility to SMA. The study population evaluated here consisted of more males than females, as was previously reported for other hospital-based studies in malaria endemic areas.[14,15,28] The reason for the higher number of male than female children may be due to household-level gender bias,[14,29] suggesting that hospital-based sampling may not reflect the overall malaria disease burden within the community. However, a recent community-based study of severe anemia in northern Ghana reported higher rates of anemia in male than female children.[30] In the current study, males also had an increased risk of developing MA that approached significance ($P = 0.06$).

TABLE 6
Multivariate analysis of risk factors associated with malaria parasitemia, anemia, or malarial anemia

	Parasitemia			Anemia			Malarial anemia		
	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Child risk factors									
Axillary temperature ≥37.5°C	2.26	1.27–4.03	<0.01		ND*			ND*	
Nutritional factors									
Wasting		ND*			ND*		1.17	0.55–2.48	0.68
Education and occupation									
Caretaker education (>primary)	0.53	0.26–1.09	0.09		ND*		0.54	0.22–1.28	0.16
Head of household									
Other vs. farmer	1.09	0.49–2.42	0.83		ND*		0.64	0.33–1.24	0.18
Mothers									
Other vs. farmer	0.42	0.19–0.92	0.03	0.99	0.49–2.00	0.98	0.79	0.42–1.49	0.47
House type									
Wall type (brick vs. mud)	0.51	0.24–1.10	0.09	0.64	0.28–1.42	0.27	0.57	0.29–1.12	0.11
Roof type (iron vs. grass)	0.97	0.47–2.01	0.93	0.50	0.22–1.13	0.10		ND*	
Window type (screened vs. other)	1.08	0.54–2.17	0.83		ND*			ND*	
Mosquito control measures									
Bed net use	0.43	0.24–0.77	<0.01		ND*			ND*	
Mosquito coil use		ND*		4.71	0.62–35.80	0.13		ND*	

* ND, not determined in the multivariate model due to non-significance of the risk factor at the univariate level.

Previous results illustrate that fever is not predictive of malaria disease severity in African children presenting at hospital.[31] Although fever was associated with parasitemia in the univariate and multivariate analyses, there was no correlation with either anemia or MA. The current study also demonstrates that malaria parasitemia upon presentation at the hospital is not associated with disease severity in infants and young children residing in areas with high malaria transmission intensity. This observation is supported by several studies illustrating that the risk of developing MA is associated with a history of malaria parasitemia but is not correlated with the concurrent parasitemia.[5,15] Thus, caretakers may be able to identify the signs and symptoms of malaria parasitemia (i.e., fever) and seek treatment at hospital, while the signs and symptoms of anemia may not be as apparent.

The prevalence of stunting observed in this study (17.1%) was lower than that reported in eastern Kenya (39%), Asembo Bay, western Kenya (24.8% and 30%), and Nyanza province, western Kenya (33%).[32–34] These community-based studies observed that stunting increased with age and peaked at ages 12–23 months[33] and 18–23 months.[34] The low prevalence of stunting observed in our study population may, therefore, be attributed to the younger age of the study population in which more than 60% of the children were less than 1 year of age. The prevalence of underweight children in the study was comparable to those reported previously.[32,34] However, the prevalence of wasting, particularly in the HosC and SMA groups, was higher than that reported in previous studies.[32–34] Because wasting is considered a manifestation of acute malnutrition, whereas stunting represents long-term malnutrition,[35,36] the high prevalence of wasted children in the HosC and SMA groups suggests that acute nutritional deficiencies may predispose children to more severe anemia. This is further illustrated by the significant association of wasting with MA in the univariate analysis.

Several studies have demonstrated associations between socioeconomic factors and malaria parasitemia and/or anemia.[13–16] In agreement with previous studies in a similar area of western Kenya,[4] the majority of caretakers in the current study had <8 years education, which was predictive of parasitemia and MA, but not anemia. However, the multivariate models for parasitemia and MA illustrated that caretaker education level was not a significant predictive factor. Head of household and mother's occupation as a farmer was associated with increased parasitemia and MA but not anemia. Analyses of these variables in the multivariate models revealed that only mother's occupation was significantly associated with increased risk of parasitemia. This observation may reflect the fact that mothers who are farmers spend more time away from their children while working in the fields than those with other occupations.

A recent study in western Kenya observed that brick-walled houses are 9 times more expensive than mud-walled houses, and iron-sheet roofs were 2.5 times more expensive than grass-thatched roofs.[37] In univariate analyses, children residing in houses with brick walls or iron-sheet roofs were protected from parasitemia and anemia, supporting the hypothesis that relative wealth is an important variable for the prevalence of malaria and anemia. However, house structure was not associated with parasitemia or anemia in the multivariate models. These results are consistent with previous studies illustrating that sociodemographic indicators are not strong predictors of childhood anemia and MA.[13,38]

Use of bed nets has been reported to reduce malaria-related morbidity and mortality.[39–41] Consistent with these reports, bed net use was associated with protection from parasitemia in both univariate and multivariate analyses but was not predictive of either anemia or MA. It is important to note that information about whether the nets were appropriately treated with insecticides was not available. However, the fact that bed net use was strongly associated with protection against parasitemia, but not anemia or MA, provides support for the notion that differences in the host-immune response to *P. falciparum* are important determinants of disease severity to acute malaria. Because the influence of important host cofactors in the development of MA (e.g., hemoglobinopathies, coinfection, etc.) were not included in our current models, these factors will need to be examined in future analyses.

Results presented here in 374 children have identified the most prominent factors associated with parasitemia, anemia, and MA. It is possible that inclusion of more study participants would yield statistically significant results for some of the measures reported here that are currently non-significant. For example, "use of mosquito coils" as shown in Table 6 has a large odds ratio (4.71), but remains statistically non-significant ($P = 0.13$), indicating a relatively small effect size for this particular independent variable. We, therefore, propose that it is important to assess the general effect size of specific independent variables based on such a comparison of the final odds ratio to level of statistical significance.

Taken together, results presented here illustrate that bed net use, temperature, and mother's occupation are predictive of childhood parasitemia. However, none of the childhood factors or sociodemographic indicators were able to predict either anemia or MA. Although it is currently unclear if results presented here in the hospital-based study are representative of parasitemia and MA within the community, by recruiting children at hospital, we have likely identified those children at greatest risk for malaria-associated morbidity and mortality. Additional investigations within the community and studies aimed at determining the role of host and parasite factors in the development and outcomes of MA may aid in reducing this significant public health burden.

Received March 15, 2005. Accepted for publication June 30, 2005.

Acknowledgments: We sincerely thank all the parents, guardians, and children who participated in this study. We are grateful to all the University of Pittsburgh-KEMRI staff; Clifford Obuya, David Ounah, Agneta Awuor, and Jackline Okumu for excellent technical support; Oscar Odunga, Florence Awuor, Josephine Osore, Perez Siambe, and Solace Amboga for clinical support; and Amos K'Ogal and Emma Moenga for data management support. We also thank the Siaya District Hospital staff and management for their support during this study. We are grateful to Dr. Davy Koech, Director of Kenya Medical Research Institute (KEMRI), for approving this manuscript for publication. Portions of this work were presented at the 53rd Annual Meeting of the American Society for Tropical Medicine and Hygiene (ASTMH), November 7–11 2004, Miami Beach, Florida (Abstract no. 715).

Financial support: This work was supported by grants from the National Institutes of Health [AI51305-02 (D.J.P.) and TW05884-02 (D.J.P.)].

Authors' addresses: John Michael Ong'echa, Collins Ouma, and Richard O. Otieno, University of Pittsburgh/KEMRI Laboratories of Parasitic and Viral Diseases, Kenya Medical Research Institute, Cen-

tre for Vector Biology and Control Research, P.O. Box 1578, Kisumu, Kenya. Tom Were and Alloys S. Orago, Department of Health Sciences, Kenyatta University, P.O. Box 43844, Nairobi, Kenya. George A. Ogonji, Ministry of Health, Siaya District Hospital, P.O. Box 144, Siaya, Kenya. John M. Vulule, Kenya Medical Research Institute, Centre for Vector Biology and Control Research, P.O. Box 1578, Kisumu, Kenya. Sandra S. Kaplan, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA. Richard D. Day, Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA. Christopher C. Keller, Zachary Landis-Lewis, Daniel Ochiel, Jamie L. Slingluff, and Douglas J. Perkins, Department of Infectious Diseases and Microbiology, University of Pittsburgh, 603 Parran Hall, 130 DeSoto St., Pittsburgh, PA 15261, Telephone: 412-624-5894, Fax: 412-624-4953, E-mail: djp@pitt.edu.

Reprint requests: Douglas J. Perkins, University of Pittsburgh Graduate School of Public Health, Department of Infectious Diseases and Microbiology, 603 Parran Hall, 130 DeSoto St., Pittsburgh, PA 15261.

REFERENCES

1. White NJ, 1996. The treatment of malaria. *N Eng J Med 335:* 800–806.
2. Breman JG, Egan A, Keusch GT, 2001. The intolerable burden of malaria: a new look at the numbers. *Am J Trop Med Hyg 64:* iv–vii.
3. WHO, 2000. WHO Expert Committee on Malaria. *World Health Organ Tech Rep Ser 892:* 1–74.
4. 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.
5. 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.
6. Hillman RS, Finch CA, 1996. *Red Cell Manua*l. Philadelphia: F.A. Davis.
7. Achidi EA, Perlmann H, Salimonu LS, Perlmann P, Walker O, Asuzu MC, 1995. A longitudinal study of seroreactivities to *Plasmodium falciparum* antigens in Nigerian infants during their first year of life. *Acta Trop 59:* 173–183.
8. Akanmori BD, Afari EA, Sakatoku H, Nkrumah FK, 1995. A longitudinal study of malaria infection, morbidity and antibody titres in infants of a rural community in Ghana. *Trans R Soc Trop Med Hyg 89:* 560–561.
9. Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Brakimi K, Faye O, Druilhe P, Dasilva LP, 1994. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg 51:* 123–137.
10. Menendez C, Fleming AF, Alonso PL, 2000. Malaria-related anaemia. *Parasitol Today 16:* 469–476.
11. Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L, Tielsch JM, 2000. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. *J Nutr 130:* 1724–1733.
12. Nussenblatt V, Semba RD, 2002. Micronutrient malnutrition and the pathogenesis of malarial anemia. *Acta Trop 82:* 321–337.
13. Luckner D, Lell B, Greve B, Lehman LG, Schmidt-Ott RJ, Matousek P, Herbich K, Schmid D, Mba R, Kremsner PG, 1998. No influence of socioeconomic factors on severe malarial anaemia, hyperparasitaemia or reinfection. *Trans R Soc Trop Med Hyg 92:* 478–481.
14. Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, Acosta C, Schellenberg JA, Aponte JJ, Kimario J, Urassa H, Mshinda H, Tanner M, Alonso P, 1999. African children with malaria in an area of intense *Plasmodium falciparum* transmission: features on admission to the hospital and risk factors for death. *Am J Trop Med Hyg 61:* 431–438.
15. Villamor E, Fawzi WW, 2000. Vitamin A supplementation: implications for morbidity and mortality in children. *J Infect Dis 182 (Suppl 1):* S122–S133.
16. Kahigwa E, Schellenberg D, Sanz S, Aponte JJ, Wigayi J, Mshinda H, Alonso P, Menendez C, 2002. Risk factors for presentation to hospital with severe anaemia in Tanzanian children: a case-control study. *Trop Med Int Health 7:* 823–830.
17. Republic of Kenya, 2001. Siaya District Development Plan 2002-2008. Effective management for sustainable economic growth and poverty reduction. Ministry of Finance and Planning. Nairobi: Government Press.
18. Siaya District Health System Annual Bulletin, 2003. Effective use of information communication technology for health promotion. Isaac Onyango Oguma: Health Information System, Siaya, Kenya.
19. McElroy PD, ter Kuile FO, Hightower AW, Hawley WA, Phillips-Howard PA, Oloo AJ, Lal AA, Nahlen BL, 2001. All-cause mortality among young children in western Kenya. VI: the Asembo Bay Cohort Project. *Am J Trop Med Hyg 64:* 18–27.
20. Beach RF, Ruebush TK 2nd, Sexton JD, Bright PL, Hightower AW, Breman JG, Mount DL, Oloo AJ, 1993. Effectiveness of permethrin-impregnated bed nets and curtains for malaria control in a holoendemic area of western Kenya. *Am J Trop Med Hyg 49:* 290–300.
21. Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA, Perkins PV, Chumo DK, Koech DV, Whitmire RE, Roberts CR, Diggs CL, Hoffman SL, 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.
22. Lackritz EM, Campbell CC, Ruebush TK 2nd, Hightower AW, Wakube W, Steketee RW, Were JB, 1992. Effect of blood transfusion on survival among children in a Kenyan hospital. *Lancet 340:* 524–528.
23. Zucker JR, Lackritz EM, Ruebush TK 2nd, Hightower AW, Adungosi JE, Were JB, Metchock B, Patrick E, Campbell CC, 1996. Childhood mortality during and after hospitalization in western Kenya: effect of malaria treatment regimens. *Am J Trop Med Hyg 55:* 655–660.
24. Murray CJL, Lopez AD, World Health Organization., World Bank, 1996. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Cambridge, MA: Harvard School of Public Health.
25. Abdalla S, 1990. Hematopoiesis in human malaria. *Blood cells 16:* 401–416.
26. Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, Acosta CJ, Schellenberg DM, Galindo CM, Kimario J, Urassa H, Brabin B, Smith TA, Kitua AY, Tanner M, Alonso PL, 1997. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet 350:* 844–850.
27. Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, Weber MW, Pinder M, Nahlen B, Obonyo C, Newbold C, Gupta S, Marsh K, 1997. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet 349:* 1650–1654.
28. Koram KA, Owusu-Agyei S, Utz G, Binka FN, Baird JK, Hoffman SL, Nkrumah FK, 2000. Severe anemia in young children after high and low malaria transmission seasons in the Kassena-Nankana district of northern Ghana. *Am J Trop Med Hyg 62:* 670–674.
29. Slutsker L, Chitsulo L, Macheso A, Steketee RW, 1994. Treatment of malaria fever episodes among children in Malawi: results of a KAP survey. *Trop Med Parasitol 45:* 61–64.
30. Owusu-Agyei S, Fryauff DJ, Chandramohan D, Koram KA, Binka FN, Nkrumah FK, Utz GC, Hoffman SL, 2002. Characteristics of severe anemia and its association with malaria in young children of the Kassena-Nankana District of northern Ghana. *Am J Trop Med Hyg 67:* 371–377.
31. Smith T, Hurt N, Teuscher T, Tanner M, 1995. Is fever a good

sign for clinical malaria in surveys of endemic communities? *Am J Trop Med Hyg 52:* 306–310.
32. National Council for Population and Development, 1998. Kenya demographic and health survey 1998. Calverton, MD: Central Bureau of Statistics, Office of the Vice President and Ministry of Planning and National Development, Kenya and Macro International, Inc.
33. Verhoef H, West CE, Veenemans J, Beguin Y, Kok FJ, 2002. Stunting may determine the severity of malaria-associated anemia in African children. *Pediatrics 110:* e48.
34. Kwena AM, Terlouw DJ, de Vlas SJ, Phillips-Howard PA, Hawley WA, Friedman JF, Vulule JM, Nahlen BL, Sauerwein RW, ter Kuile FO, 2003. Prevalence and severity of malnutrition in pre-school children in a rural area of western Kenya. *Am J Trop Med Hyg 68:* 94–99.
35. Deen JL, Walraven GE, von Seidlein L, 2002. Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia. *J Trop Pediatr 48:* 78–83.
36. Caulfield LE, Richard SA, Black RE, 2004. Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. *Am J Trop Med Hyg 71:* 55–63.
37. 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:* 149–160.
38. Koram KA, Bennett S, Adiamah JH, Greenwood BM, 1995. Socio-economic determinants are not major risk factors for severe malaria in Gambian children. *Trans R Soc Trop Med Hyg 89:* 151–154.
39. D'Alessandro U, Olaleye BO, McGuire W, Thomson MC, Langerock P, Bennett S, Greenwood BM, 1995. A comparison of the efficacy of insecticide-treated and untreated bed nets in preventing malaria in Gambian children. *Trans R Soc Trop Med Hyg 89:* 596–598.
40. Abdulla S, Schellenberg JA, Nathan R, Mukasa O, Marchant T, Smith T, Tanner M, Lengeler C, 2001. Impact on malaria morbidity of a programme supplying insecticide treated nets in children aged under 2 years in Tanzania: community cross sectional study. *Bmj 322:* 270–273.
41. ter Kuile FO, Terlouw DJ, Phillips-Howard PA, Hawley WA, Friedman JF, Kolczak MS, Kariuki SK, Shi YP, Kwena AM, Vulule JM, Nahlen BL, 2003. Impact of permethrin-treated bed nets on malaria and all-cause morbidity in young children in an area of intense perennial malaria transmission in western Kenya: cross-sectional survey. *Am J Trop Med Hyg 68:* 100–107.