

RISK FACTORS FOR PLACENTAL MALARIA AND ITS EFFECT ON PREGNANCY OUTCOME IN YAOUNDE, CAMEROON

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Abstract. Between 1996 and 2001, the prevalence of placental malaria in pregnant women living in Yaounde, Cameroon and its effect on pregnancy outcome were evaluated with respect to gravidity and maternal age. Results showed that 19.9% of the women had placental malaria at delivery. After adjusting for relevant covariates, the major risk factor for placental malaria was an age < 25 years old. Placental malaria significantly increased the prevalence of anemia in women regardless of gravidity or age. In addition, the mean infant birth weight was lower and the percentage of pre-term deliveries (PTDs) and low birth weight (LBW) babies were higher in primigravidae and women < 20 years of age who had placental malaria. However, in a multivariate regression model taking relevant covariates into consideration, the major risk factor for PTDs was maternal anemia, and maternal anemia as well as first and second pregnancies were important risk factors for LBW babies.

INTRODUCTION

In sub-Saharan Africa, *Plasmodium falciparum* infections are more common in pregnant women than in non-pregnant women.^{1,2} A number of factors influence the prevalence of malaria in pregnant women, including maternal age, gravidity, use of prophylaxis, nutrition, host genetics, level of anti-parasite immunity, as well as parasite genetics and transmission rates. Accordingly, the epidemiology of *P. falciparum* in pregnant women differs significantly throughout Africa.

Most epidemiologic studies conducted in malaria-endemic countries have found that primigravidae are more susceptible to malaria than multigravidae.^{1,2} Therefore, pregnant women must acquire a form of pregnancy-associated immunity during their first pregnancies that helps protect them during subsequent pregnancies.^{2–4} Young women of child-bearing age may also be more susceptible than older women to malaria because they are still in the process of acquiring natural immunity to malaria.^{5–7} The relative importance of pregnancy-associated and age-dependent immunity may differ among pregnant women living in different environments.

Plasmodium falciparum-infected erythrocytes frequently sequester in the intervillous space of the placenta and cause pathologic alterations.^{8–10} Placental malaria has been associated with a significant decrease in infant birth weight, especially in primigravidae.^{2,9,11–14} It has also been identified as a risk factor for low birth weight (LBW) babies.^{11–16} Low birth weight can be caused by intrauterine growth retardation and pre-term deliveries (PTDs). The importance of placental malaria in PTDs is unclear, since some investigators have reported that *P. falciparum* infections increase the risk of PTD,^{17–19} but others have found it is not a significant risk factor in multivariate models.^{18,19} Since infants born to mothers with placental malaria may be at an increased risk of anemia,²⁰ increased malaria prevalence rates,²¹ and mortality²² during their first year of life, assessment of risk factors for placental malaria in different environmental settings is important.

The aim of the current study was to determine the prevalence of placental malaria in women residing in the city of Yaounde, Cameroon and evaluate its effect on pregnancy outcome, including maternal anemia, infant birth weight,

LBW babies, and PTD in relation to gravidity and maternal age. Malaria transmission in Yaounde is perennial with inoculation rates estimated to be 13 infectious bites per person per year.²³ During the study period (1996–2001), the health policy recommended chloroquine (CQ) prophylaxis. Although CQ resistance existed, results reported in 1995 found that CQ prophylaxis was still effective in increasing infant birth weight and lowering the percentage of LBW babies.¹⁴ However, the clinical failure rate for CQ in adults between 1994 and 1999 was estimated to be 41.1%.²⁴ The seroprevalence of human immunodeficiency virus was relatively low in Yaounde (4.2%, 95% confidence interval [CI] = 3.6–4.8%) from 1994 to 1996.²⁵ Thus, the epidemiology of placental malaria in Yaounde may be different from that in other areas of Africa.

MATERIALS AND METHODS

Study site and design. The study was conducted between 1996 and 2001 in Yaounde, Cameroon. Women were consecutively recruited at the Biyem Assi District Hospital where care is provided for low income women living in the adjacent area and the Central Maternity Hospital, a referral hospital that provides services for a diverse group of women. The study was reviewed and approved by the National Ethical Committee in Cameroon and the Institutional Review Board of Georgetown University.

The purpose of the study was explained to each woman and those giving informed consent were enrolled. A questionnaire was used to record information on the woman's age, number of pregnancies, obstetric history, and use of chemoprophylaxis during pregnancy. Information on the newborn was also recorded including infant birth weight. Length of gestation was estimated based on date of last menstrual period, and assessment of the newborn at delivery. Infants born between 28 and 37 weeks were considered premature. Singletons weighing less than 2,500 grams were LBW babies. A total of 1,944 women participated. Paucigravidae are women with 1–2 pregnancies, multigravidae those with ≥ 3 pregnancies, and grand multigravidae have ≥ 5 pregnancies.

Collection and processing of samples. After delivery, a 5-mL sample of heparinized maternal venous blood was collected and a sample of placental blood was obtained using the pool-biopsy methods.²⁶ Briefly, a 5 cm × 5 cm × 5 cm piece of the placenta was excised allowing maternal intervillous blood to accumulate at the site. Blood was collected using heparin and stored on ice until used. In addition, a piece of placental tissue was retained for parasitologic studies.

Parasitologic and hematologic studies. Samples were transported to the Biotechnology Center (Nkolbisson, Yaounde, Cameroon) here thick and thin blood films of maternal peripheral, and placental blood and impression smears of placental tissue were made. Slides were stained with Diff Quick (Baxter Scientific, McGraw Park, IL), coded, and examined by two technicians. Two hundred thick blood fields were examined. If parasites were found, 100–200 fields of the thin smear were examined and the percent parasitemia was calculated. Two thousand erythrocytes were screened for parasites on placental impression smears. A woman was considered to have placental malaria if parasites were found in either the placental blood or impression smear. Women were considered to be malaria positive if parasites were detected in either the peripheral blood or the placenta. Heparinized hematocrit tubes were filled with maternal peripheral blood, centrifuged, and the packed cell volume (PCV) was determined using standard methods. A woman was considered to be anemic if the PCV was less than 30%.

Statistical analysis. Data from the two hospitals were combined in the analysis since the study populations proved to be comparable for relevant covariates and outcomes. Sample sizes used in the analyses are provided in the tables. The Pearson chi-square test was used for between-group comparisons for the prevalence of parasitemia and anemia. The Wilcoxon signed rank test was used to compare the relationship between peripheral and placental parasitemias. A logistic regression model was used to determine risk factors associated with PTD and LBW. Descriptive and inferential statistical analyses were performed using SAS version 9.0 (SAS Institute, Cary, NC).

RESULTS

Study population. The age, gravidity, and pregnancy outcome of the participants are shown in Table 1. A significant decrease in the percentage of LBW babies with increasing

gravidity was seen ($P < 0.0001$). The prevalence of PTD also tended to decrease with gravidity ($P = 0.051$). Among the women, 76.3% reported taking chemoprophylaxis, with 36.6% reporting taking CQ, 32.4% pyrimethamine, 3.9% quinine salts, 1.2% proguanil, and 1.1% amodiaquine.

Malaria prevalence. Overall, 21.4% (405 of 1,895) of the women had malaria at the time of delivery, with 19.9% (371 of 1,866) having placental malaria. The prevalence of peripheral and placental malaria decreased with age (Figure 1). More than 30% of women ≤ 21 years old were infected with malaria (i.e., had parasites in either peripheral or placental blood). The prevalence decreased to 20% in women between 22 and 37 years old, and then to ≤ 6% in women ≥ 38 years old.

A gravidity-associated decrease in the prevalence of peripheral and placenta malaria was observed (Table 1), with placental parasites detected in 27.8% of primigravid compared with 15.6% of multigravid women ($P < 0.0001$). Among women with placental malaria, placental parasitemias ranged from < 0.001% to 82.6%, with 65% of the women having parasitemias between 0.1 and 10% (Figure 2).

As previously reported,²⁷ parasites were detected more frequently in placental than peripheral blood smears. Among malaria-positive women, 67.8% had parasites detected in both the peripheral placental blood, 26% had parasites detected in the placenta but not the peripheral blood, and only 6.2% had peripheral parasitemias without placental malaria. Based on detection of parasites in either peripheral or placental blood smears, 29.2% of the primigravidae, 23.4% of the secundigravidae, and 16.7% of the multigravidae were infected at delivery (Table 1).

Risk factors for placental malaria. Using univariate analysis, we identified being < 20 years old and 21–24 years of age (crude odds ratio [OR] = 2.4, 95% CI = 1.7–3.3 and 1.6, 95% CI = 1.2–2.1, respectively), a primigravidae (OR = 2.0, 95% CI = 1.4–2.9), and failing to take prophylaxis (OR = 1.3, 95% CI = 1.0–1.7) as significant risk factors for placental malaria (Table 2). However, after adjusting for other variables, only young women were determined to be at a higher risk of placental malaria ($P = 0.008$), with adjusted OR (95% CI) of 2.0 (1.3–3.0) for teenagers and 1.4 (1.0–1.9) for women in their early twenties. After adjusting for age and other covariates, gravidity ($P = 0.278$) and not taking prophylaxis ($P = 0.203$) were no longer significant.

To further explore the relationship between age and gravidity, the prevalence of placental malaria in 852 mothers < 25

TABLE 1
Description of the women, pregnancy outcomes, and malaria status*

	All women	Number of pregnancies			P
		1	2	≥3	
No. of women	1,898	534	398	966	
Mean ± age, years	25.8 ± 5.9	21.4 ± 4.1	24.0 ± 4.4	29.1 ± 5.3	< 0.0001†
Delivery outcome					
% Pre-term	20.3	23.2	21.9	18.1	0.051‡
% LBW	16.4	20.7	19.4	12.6	< 0.0001‡
Malaria status					
% Peripheral blood positive	15.9	21.8	17.7	12.3	< 0.0001‡
% Placental blood positive	19.9	27.8	20.8	15.6	< 0.0001‡
Total % malaria positive§	21.4	29.2	23.4	16.7	< 0.0001‡

* LBW = low birth weight.

† Wilcoxon rank-sum test.

‡ Chi-square test.

§ Parasites were detected in the peripheral and/or placental blood.

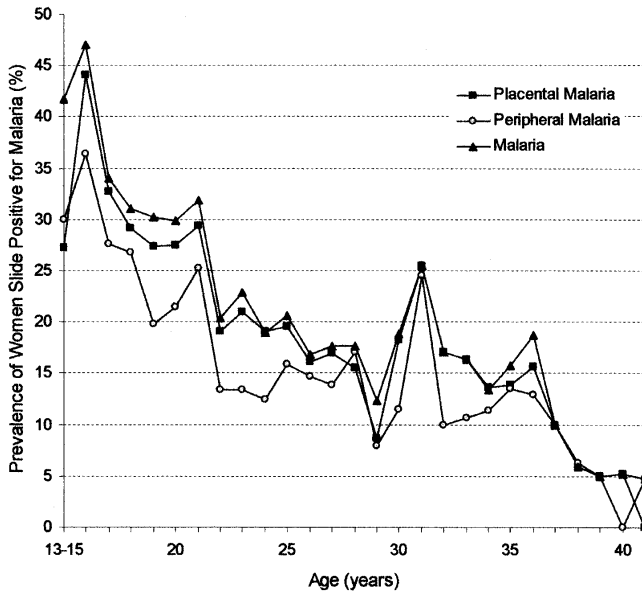


FIGURE 1. Distribution of *Plasmodium falciparum* malaria in 1,499 women residing in Yaounde, Cameroon. Individual plots represent the percentage of women with parasites detected by microscopy in the placenta (■) and the peripheral blood (○), and the total, i.e., present in the peripheral blood and/or the placenta (▲).

years of age was analyzed. A significant difference in the prevalence of placental malaria was found between primigravidae (29.2%), secundigravidae (25.3%), and multigravidae (18.9%) ($P = 0.03$, by chi-square test). However, among the 1,016 women ≥ 25 years old, the difference in prevalence of placental malaria by gravidity was not statistically significant (primigravidae = 20.2%, secundigravidae = 15.2%, and multigravidae = 15.0%; ($P = 0.417$). Thus, mothers < 25 years old, but not those ≥ 25 years old, with 1–2 pregnancies were at the highest risk of placental malaria.

Effect of placental malaria on maternal anemia and pregnancy outcomes. Maternal anemia and placental malaria were associated in all gravidity and age groups (Table 3). Placental malaria also had an effect on babies born to primigravidae and young mothers. Primigravidae with placental malaria gave birth to infants with lower mean birth weights ($P < 0.0001$) and were more likely to have LBW babies ($P < 0.001$) and PTD ($P < 0.0001$) (Table 3). Likewise, women < 25 years of age with placental malaria had infants with significantly lower mean birth weights ($P = 0.001$) than older women, while women < 20 years old gave birth to a higher proportion of LBW babies ($P = 0.012$) and had more malaria-associated PTDs ($P = 0.002$). Clearly, placental malaria is a significant risk factor for poor pregnancy outcomes among primigravidae and younger women.

Risk factors for LBW and PTD babies. Since LBW and premature newborns are at an increased risk of morbidity and mortality during the first year of life,²⁸ risk factors for these two conditions were assessed. Using univariate analysis, we observed that risk factors associated with LBW babies included maternal age ($P < 0.0001$), gravidity ($P = 0.0001$), placental malaria ($P = 0.01$), and maternal anemia (Table 4). However, after adjusting for relevant covariates in a multivariate logistic model, only an age < 20 years (adjusted OR = 2.1, 95% CI = 1.2–3.5), primigravidae (OR = 2.0, 95% CI =

1.1–3.5), secundigravidae (OR = 2.5, 95% CI = 1.5–4.3), and anemia (OR = 2.4, 95% CI = 1.8–3.4) were identified as risk factors.

Maternal anemia was also identified as a significant risk factor for PTD, with an OR of 2.3 (95% CI = 1.7–3.1) before and 2.2 (95% CI = 1.6–3.0) after adjusting for age, gravidity, and placental malaria (Table 4). Univariate analysis also identified maternal age, paucigravidae, and placental malaria as significant risk factors for PTD. However, after adjusting for the covariates, only women in the age groups < 20, 20–24, and ≥ 30 years old were at a higher risk of having PTD than women 25–29 years old ($P = 0.002$, by likelihood ratio [LR] test). The effect of gravidity and placental malaria were not significant after adjusting for other covariates, ($P = 0.566$ and $P = 0.650$, respectively, by LR test).

DISCUSSION

The epidemiology of placental malaria in Yaounde, Cameroon is similar to that in other African countries, but it also has unique characteristics. Many studies have found the prevalence of placental malaria to be higher in primigravidae than multigravidae.^{2,3} In the current study, primigravidae and secundigravidae were more likely to have placental malaria, but only if they were < 25 years old ($P = 0.03$). Paucigravidae ≥ 25 years old were not at a significant increased risk of placental malaria compared with multigravidae ($P = 0.417$). Overall, after adjusting for confounding variables, age was determined to be a major risk factor for placental malaria in this population ($P = 0.008$), whereas gravidity was not ($P = 0.278$) (Table 2). Thus, younger but not older first-time mothers were more likely to have placental malaria. These results are interesting and suggest the relative importance of pregnancy-associated and naturally-acquired immunity may differ as women in Yaounde become older. That is, development of pregnancy-associated immunity, e.g., production of antibodies that inhibit the adherence of placental parasites to chondroitin sulfate A (CSA),⁴ may be very important in women < 25 years of age who have lower levels of acquired immunity.

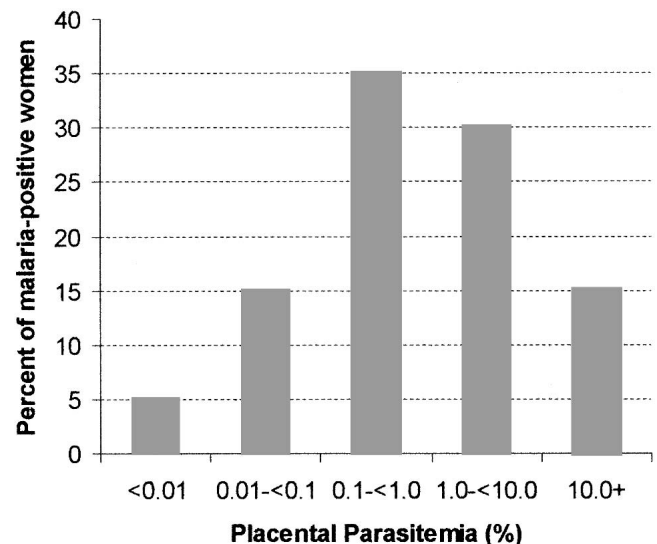


FIGURE 2. Distribution of parasitemia in the placenta based on 371 women with placental malaria.

TABLE 2
Risk factors associated with placental malaria in singleton deliveries*

Risk factors	Prevalence of placental malaria (n = 1,740)			Placental parasitemia (n = 371) Median (Q1–Q3)
	% with placental malaria	Crude OR (95% CI)	Adjusted OR† (95% CI)	
Age, years				
≤ 20	29.4	2.4 (1.7–3.3)	2.0 (1.3–3.0)	0.77 (0.12–3.5)
20–24	22.9	1.6 (1.2–2.1)	1.4 (1.0–1.9)	0.96 (0.3–3.1)
25–29	16.1	Referent	Referent	0.60 (0.1–3.8)
≥ 30	15.5	0.9 (0.6–1.3)	1.0 (0.7–1.4)	0.68 (0.2–3.8)
<i>P</i>	< 0.0001		0.008	0.809‡
Gravidity				
1	27.2	2.0 (1.4–2.9)	1.5 (1.0–2.2)	0.7 (0.1–3.9)
2	20.9	1.4 (0.9–2.0)	1.2 (0.8–1.8)	1.37 (0.2–5.6)
3	15.4	Referent	Referent	0.51 (0.1–2.5)
4	18.8	1.1 (0.7–1.8)	1.2 (0.8–1.9)	0.89 (0.2–2.8)
≥ 5	13.7	0.8 (0.6–1.3)	1.0 (0.6–1.6)	0.60 (0.1–2.7)
<i>P</i>	< 0.0001		0.278	0.217‡
Chemoprophylaxis				
Yes	18.5	Referent	Referent	0.62 (0.1–3.0)
No	23.8	1.3 (1.0–1.7)	1.2 (0.9–1.6)	1.01 (0.4–6.2)
<i>P</i>	< 0.05		0.203	0.307‡

* OR = odds ratio; CI = confidence interval; Q1–Q3 = first to third quartiles (25–75th percentiles).
† Estimated by a multivariate logistic regression model including all the risk factors in the table plus seasonality.
‡ Estimated by the Wilcoxon rank sum or Kruskal-Wallis tests.

However, women ≥ 25 years old in Yaounde may have obtained adequate immunity following repeated exposures to eliminate their parasites and thus are less dependent on anti-cytoadherent antibodies. Recently, we reported that antibodies to antigens other than those preventing cytoadherence of parasites to CSA are important in reducing placental malaria.²⁹ Women in Yaounde with anti-cytoadherent antibodies had lower placental parasitemias (i.e., few parasites in the placenta), but women who lacked antibodies to the carboxy-terminal region of merozoite surface protein 1 (MSP₁₋₁₉) were at a significant increased risk of placental malaria.²⁹ Differences in levels of acquired immunity may help explain the epidemiologic pattern in Yaounde. However, the relationship between age and gravidity is complicated by socioeconomic factors since older, first-time mothers may also be better educated and more conscientious about prenatal care. Further studies on the role of naturally acquired and gravidity-associated immunity are needed to aid in the development of vaccines for preventing placental malaria.

The strong effect of age may be due, in part, to Yaounde being an urban environment. Previously, we compared the prevalence of malaria during the course of pregnancy in women living in Yaounde and a nearby rural village.³⁰ After adjusting for age, gravidity, prophylaxis, trimester of pregnancy, and seasonality, both gravidity and age were identified as risk factors in the village, but only age was a factor in the city.³⁰ Several recent studies conducted in other cities have also found age to be a critical factor in susceptibility to malaria in pregnant women.^{31,32} In Blantyre, Malawi, Rogerson and others screened 4,764 women during pregnancy and reported that age and season were more important than gravidity as predictors of infection.³¹ In Libreville, Gabon, Bouyou-Akotet and others found that among primigravidae and secundigravidae, being < 20 years of age was associated with enhanced susceptibility (adjusted OR 1.7, 95% CI = 1.1–2.85, *P* < 0.01).³² The effect of age, however, may not be restricted to urban settings. In a cross-sectional study of 686 pregnant women in rural Manhica, Mozambique, Saute and

TABLE 3
Effect of placental malaria on maternal anemia and infant outcome in singleton deliveries*

	Placental malaria	Number of pregnancies			Maternal age (years)		
		1	2	≥ 3	< 20	20–24	≥ 25
No. of women	Positive	125	73	132	64	120	144
	Negative	336	277	719	154	403	768
% women with anemia	Positive	31.6	28.8	29.8	31.8	32.7	26.5
	Negative	15.7	16.7	15.3	17.7	16.0	15.3
	<i>P</i> †	0.0003	0.027	< 0.0001	0.026	< 0.0001	0.002
Mean ± SD infant birth weight, grams	Positive	2,642 ± 727	2,846 ± 778	3,055 ± 636	2,443 ± 807	2,864 ± 655	3,095 ± 590
	Negative	2,934 ± 691	2,970 ± 786	3,115 ± 740	2,807 ± 789	3,017 ± 717	3,195 ± 633
	<i>P</i> ‡	< 0.0001	0.216	0.309	0.001	0.05	0.09
% LBW babies	Positive	28.0	20.6	9.9	35.9	18.3	13.3
	Negative	14.7	18.9	11.1	19.7	14.0	12.5
	<i>P</i> †	0.001	0.753	0.689	0.012	0.248	0.791
% women with PTD	Positive	32.8	23.3	14.4	40.6	26.7	13.9
	Negative	16.4	20.9	17.4	20.1	19.6	16.8
	<i>P</i> †	0.0001	0.664	0.400	0.002	0.097	0.397

* LBW = low birth weight; PTD = pre-term delivery.
† Estimated by chi-square test.
‡ Estimated by Student's *t*-test.

TABLE 4
Risk factors associated with LBW and PTD babies among singleton deliveries*

Risk factor	Low birth weight (n = 1,708)			Premature deliveries (n = 1,720)		
	% LBW	Crude OR (95% CI)	Adjusted OR† (95% CI)	% PTD	Crude OR (95% CI)	Adjusted OR† (95% CI)
Age (years)						
≤ 20	24.1	2.7 (1.8–4.1)	2.1 (1.2–3.5)	26.1	2.2 (1.5–3.2)	2.4 (1.5–4.0)
20–24	15.3	1.6 (1.1–2.2)	1.2 (0.8–1.8)	21.6	1.7 (1.3–2.3)	1.7 (1.2–2.5)
25–29	10.4	Referent	Referent	13.8	Referent	Referent
≥ 30	16.4	1.7 (1.2–2.5)	2.0 (1.3–3.2)	20.6	1.6 (1.1–2.3)	1.6 (1.1–2.4)
<i>P</i> ‡	< 0.0001		0.002	0.0002		0.002
Gravidity						
1	18.5	2.5 (1.5–4.0)	2.0 (1.1–3.5)	21.3	1.5 (1.0–2.2)	1.1 (0.7–1.7)
2	19.2	2.6 (1.6–4.3)	2.5 (1.5–4.3)	21.6	1.5 (1.0–2.3)	1.4 (0.9–2.2)
3	8.3	Referent	Referent	15.4	Referent	Referent
4	13.0	1.7 (0.9–3.0)	1.5 (0.8–2.9)	17.6	1.2 (0.7–1.9)	1.2 (0.7–2.1)
≥ 5	11.9	1.5 (0.9–2.5)	1.1 (0.6–2.0)	17.8	1.2 (0.8–1.8)	1.1 (0.7–1.9)
<i>P</i> ‡	0.0001		0.003	0.189		0.566
Placental malaria						
Positive	19.4	1.5 (1.1–2.1)	1.0 (0.7–1.5)	23.6	1.4 (1.1–1.9)	1.1 (0.8–1.5)
Negative	13.5	Referent	Referent	17.8	Referent	Referent
<i>P</i> ‡	0.01		0.819	0.02		0.650
Maternal anemia						
Yes	26.1	2.5 (1.8–3.4)	2.4 (1.8–3.4)	30.9	2.3 (1.7–3.1)	2.2 (1.6–3.0)
No	12.5	Referent	Referent	16.3	Referent	Referent
<i>P</i> ‡	< 0.0001		< 0.0001	< 0.0001		< 0.0001

* LBW = low birth weight; PTD = pre-term delivery; OR = odds ratio; CI = confidence interval.

† The adjusted ORs were obtained by including all covariates in the table in the multivariate logistic model. The interaction term between age and parity was not significant (*P* 0.165 for LBW and 0.946 for PTD) and thus was excluded from the model.

‡ The univariate *P* value was estimated using a chi-square test and the *P* value for the adjusted OR was estimated by a type-3 likelihood ratio test in the above multivariate logistic model.

others found using multivariate analysis that age, but not parity, was associated with an increased risk of microscopic parasitemia.³³ A study of women in rural and urban areas of Bandiagara, Mali found that living in rural areas and young age, rather than parity, were important risk factors for malaria in pregnant women.³⁴ Taken together, these results show that teenagers are at a greater risk of malaria, regardless of gravidity, in a number of African communities.

When the study was initiated in 1996, CQ prophylaxis was recommended for pregnant women. In 2000, Ringwald and others reported that the therapeutic failure rate of CQ in adults in Yaounde between 1994 and 1999 was 41.1%, and parasites were only successfully eliminated from the peripheral blood by day 14 in 50.7% of the cases.²⁴ It is therefore not surprising that only 36.6% of the women took CQ. Approximately 32.4% of the women reported taking pyrimethamine, 24.9% failed to take prophylaxis, and 6% reported taking drugs not traditionally used for prophylaxis. In the late 1990s, increasing drug resistance not only to CQ, but also to pyrimethamine, proguanil, and probably amodiaquine were reported in Yaounde.^{35–37} Accordingly, intermittent preventive treatment with sulfadoxine-pyrimethamine in pregnant women has been adopted, but sulfadoxine-pyrimethamine-resistant strains are present in Yaounde,³⁵ making the efficacy of the new policy uncertain.

Malaria was a risk factor for maternal anemia in essentially all Cameroonian women (Tables 3 and 4). Placental malaria also had a significant impact on pregnancy outcomes in primigravidae and young women (Tables 3 and 4). Placental malaria reduced mean infant birth weight by 289 grams in primigravidae, by 364 grams in mothers < 20 years old, and by 153 grams in women 20–24 years old (Table 3). It also essentially doubled the percentage of LBW infants and PTDs in these women (Table 3). However, many factors can contribute to

LBW babies and PTDs. Thus, general risk factors for LBW and PTD were assessed (Table 4). Using multivariate analysis, we observed that maternal anemia was associated with PTD and maternal anemia and the first and second births were identified as risk factors for LBW infants. Although placental malaria was identified as a risk factor by univariate analysis for both LBW and PTD, after adjusting for age, gravidity, chemoprophylaxis, and history of PTD, placental malaria was not found to be a significant risk factor for either adverse outcome (Table 4). The results for PTD are similar to those reported by Sullivan and others in Malawi, who found placental parasitemia was a risk for PTD (crude OR = 2.4), but after adjusting for covariates, the risk became non-significant.¹⁸ Using univariate and multivariate analysis, Steketee and others reported similar results for Malawian women.¹⁹ Thus, placental malaria may increase the risk for LBW and PTD, especially in young paucigravid women, but numerous factors contribute to these adverse outcomes in addition to malaria.

In summary, in the city of Yaounde, approximately one of every five women had placental malaria at delivery, with 45% having parasite densities > 1.0% (Figure 2). Young women ≤ 25 years of age at their first or second pregnancies were at the highest risk of placental malaria, which had a significant effect on the birth weight of their babies. However, numerous factors, especially maternal anemia, contribute to the risk of PTD and LBW infants in this population. Thus, the epidemiology of placental malaria in Yaounde appears to be different from that in other areas of Africa.

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REFERENCES

1. Bray RS, Anderson MJ, 1984. Falciparum malaria and pregnancy. *Trans R Soc Trop Med Hyg* 73: 427-431.
2. McGregor IA, 1984. Epidemiology, malaria, and pregnancy. *Am J Trop Med Hyg* 33: 517-525.
3. Brabin BJ, 1983. An analysis of malaria in pregnancy in Africa. *Bull World Health Organ* 61: 1005-1016.
4. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE, 1998. Maternal antibodies block malaria. *Nature* 395: 851-852.
5. Shi YP, Sayed U, Qari SH, Roberts JM, Udhayakumar V, Oloo AJ, Hawley WA, Kaslow DC, Nahlen BL, Lal AA, 1996. Natural immune response to the C-terminal 19-kilodalton domain of *Plasmodium falciparum* merozoite surface protein 1. *Infect Immun* 64: 2716-2723.
6. Oouvray C, Theisen M, Rogier C, Trape JF, Jepsen S, Druilhe P, 2000. Cytophilic immunoglobulin responses to *Plasmodium falciparum* glutamate-rich protein are correlated with protection against clinical malaria in Dielmo, Senegal. *Infect Immun* 68: 2617-2620.
7. Johnson AH, Leke RG, Mendell NR, Shon D, Suh YJ, Bombankolo D, Tchinda V, Kouontchou S, Thuita LW, van der Wel AM, Thomas A, Stowers A, Saul A, Zhou A, Taylor DW, Quakyi IA, 2004. HLA class II alleles influence levels of antibodies to the *Plasmodium falciparum* asexual-stage apical membrane antigen 1 but not to merozoite surface antigen 2 and merozoite surface protein 1. *Infect Immun* 72: 2762-2771.
8. Walter PR, Garin Y, Blot P, 1982. Placental pathologic changes in malaria. A histologic and ultrastructural study. *Am J Pathol* 109: 330-342.
9. Bulmer JN, Rasheed FN, Morrison L, Francis N, Greenwood BM, 1993. Placental malaria. II. A semi-quantitative investigation of the pathological features. *Histopathology* 22: 219-225.
10. Ordi J, Ismail MR, Ventura PJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL, Menendez C, 1998. Massive chronic intervillitis of the placenta associated with malaria infection. *Am J Surg Pathol* 22: 1006-1011.
11. Watkinson M, Rushton DI, 1983. Plasmodial pigmentation of placenta and outcome of pregnancy in west African mothers. *BMJ* 287: 251-254.
12. McGregor IA, Wilson ME, Billewicz WZ, 1983. Malaria infection of the placenta in The Gambia, west Africa; its incidence and relationship to stillbirth, birthweight and placental weight. *Trans R Soc Trop Med Hyg* 77: 232-244.
13. Jelliffe EF, 1968. Low birth-weight and malarial infection of the placenta. *Bull World Health Organ* 38: 69-78.
14. Cot M, Le Hesran JY, Miallhes P, Esveld M, Etya'ale D, Breart G, 1995. Increase of birth weight following chloroquine chemoprophylaxis during the first pregnancy: results of a randomized trial in Cameroon. *Am J Trop Med Hyg* 53: 581-585.
15. Kasumba IN, Nalunkuma AJ, Mujuzi G, Kitaka FS, Byaruhanga R, Okong P, Egwang TG, 2000. Low birthweight associated with maternal anaemia and *Plasmodium falciparum* infection during pregnancy, in a peri-urban/urban area of low endemicity in Uganda. *Ann Trop Med Parasitol* 94: 7-13.
16. Okoko BJ, Ota MO, Yamuah LK, Idiong D, Mkpanam SN, Avieka A, Banya WA, Osinusi K, 2002. Influence of placental malaria infection on foetal outcome in the Gambia: twenty years after Ian McGregor. *J Health Popul Nutr* 20: 4-11.
17. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, Font F, Alonso PL, 2000. The impact of placental malaria on gestational age and birth weight. *J Infect Dis* 181: 1740-1745.
18. Sullivan AD, Nyirenda T, Cullinan T, Taylor T, Harlow SD, James SA, Meshnick SR, 1999. Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. *J Infect Dis* 179: 1580-1583.
19. Steketee RW, Wirima JJ, Hightower AW, Slutsker L, Heymann DL, Breman JG, 1996. The effect of malaria and malaria prevention in pregnancy on offspring birthweight, prematurity and intrauterine growth retardation in rural Malawi. *Am J Trop Med Hyg* 55: 33-41.
20. Brabin B, Maxwell S, Chimsuku L, Verhoeff F, van der Kaay HJ, Broadhead R, Kazembe P, Thomas A, 1993. A study of the consequences of malarial infection in pregnant women and their infants. *Parassitologia* 35 (Suppl): 9-11.
21. Le Hesran JY, Cot M, Personne P, Fievet N, Dubois B, Beyeme M, Boudin C, Deloron P, 1997. Maternal placental infection with *Plasmodium falciparum* and malaria morbidity during the first 2 years of life. *Am J Epidemiol* 146: 826-831.
22. Guyatt HL, Snow RW, 2001. Malaria in pregnancy as an indirect cause of infant mortality in sub-Saharan Africa. *Trans R Soc Trop Med Hyg* 95: 569-576.
23. Manga L, Robert V, Mess J, Desfontaine M, Carnevale P, 1992. Le paludisme urbain a Yaounde, Cameroon: l'etude entomologique dans deux quartiers centraux. *Mem Soc R Belg Entomol* 35: 155-162.
24. Ringwald P, Same Ekobo A, Keundjian A, Kedy Mangamba D, Basco LK, 2000. Chemoresistance of *P. falciparum* in urban areas of Yaounde, Cameroon. Part 1: Surveillance of *in vitro* and *in vivo* resistance of *Plasmodium falciparum* to chloroquine from 1994 to 1999 in Yaounde, Cameroon. *Trop Med Int Health* 5: 612-619.
25. Mbopi Keou FX, Mbu R, Mauclere P, Andela A, Tetanye E, Leke R, Chaouat G, Barre-Sinoussi F, Martin P, Belec L, 1998. Antenatal HIV prevalence in Yaounde, Cameroon. *Int J STD AIDS* 9: 400-402.
26. Suguitan AJL, Leke RGF, Fouda G, Zhou A, Thuita L, Metenou S, Fogako J, Megnekou R, Taylor DW, 2003. Changes in the level of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. *J Infect Dis* 188: 1074-1082.
27. 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.
28. McCormick MC, 1985. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* 312: 82-90.
29. Taylor DW, Zhou A, Marsillio LE, Thuita LW, Leke EB, Branch O, Gowda DC, Long C, Leke RF, 2004. Antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulfate A and to the C terminus of merozoite surface protein 1 correlate with reduced placental malaria in Cameroonian women. *Infect Immun* 72: 1603-1607.
30. Zhou A, Megnekou R, Leke R, Fogako J, Metenou S, Trock B, Taylor DW, Leke RF, 2002. Prevalence of *Plasmodium falciparum*

- parum* infection in pregnant Cameroonian women. *Am J Trop Med Hyg* 67: 566–570.
31. Rogerson SJ, van den Broek NR, Chaluluka E, Qongwane C, Mhango CG, Molyneux ME, 2000. Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. *Am J Trop Med Hyg* 62: 335–340.
 32. Bouyou-Akotet MK, Ionete-Collard DE, Mabika-Manfoumbi M, Kendjo E, Matsiegui PB, Mavoungou E, Kombila M, 2003. Prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon. *Malar J* 2: 18.
 33. Saute F, Menendez C, Mayor A, Aponte J, Gomez-Olive X, Dgedge M, Alonso P, 2002. Malaria in pregnancy in rural Mozambique: the role of parity, submicroscopic and multiple *Plasmodium falciparum* infections. *Trop Med Int Health* 7: 19–28.
 34. Dicko A, Mantel C, Thera MA, Doumbia S, Diallo M, Diakite M, Sagara I, Doumbo OK, 2003. Risk factors for malaria infection and anemia for pregnant women in the Sahel area of Bandiagara, Mali. *Acta Trop* 89: 17–23.
 35. Basco LK, Same-Ekobo A, Ngane VF, Ndounga M, Metoh T, Ringwald P, Soula G, 2002. Therapeutic efficacy of sulfadoxine-pyrimethamine, amodiaquine and the sulfadoxine-pyrimethamine-amodiaquine combination against uncomplicated *Plasmodium falciparum* malaria in young children in Cameroon. *Bull World Health Organ*. 80: 538–545.
 36. Basco LK, 2002. Molecular epidemiology of malaria in Cameroon. XII. *In vitro* drug assays and molecular surveillance of chloroquine and proguanil resistance. *Am J Trop Med Hyg* 67: 383–387.
 37. Basco LK, 2003. Molecular epidemiology of malaria in Cameroon. XVI. Longitudinal surveillance of *in vitro* pyrimethamine resistance in *Plasmodium falciparum*. *Am J Trop Med Hyg* 69: 174–178.