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Burden of Malaria during Pregnancy at the Time of IPTp/SP Implementation in Gabon

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Abstract. The new recommendations to prevent malaria in pregnant women have recently been implemented in Gabon. There is little information on the pregnancy indicators that are useful for their evaluation. A cross-sectional study for the assessment of the prevalence of peripheral, placental, and cord malaria and anemia among delivering women was performed at the largest public hospital of Gabon. Malaria prevalence was 34.4%, 53.6%, and 18.2% for maternal peripheral, placental, and cord blood respectively, with no difference between primigravidae and multigravidae. Submicroscopic infections were frequent and concerned all the positive cord samples. Maternal peripheral, late placental, and cord infections were all associated with a reduced mean birth weight in primigravidae ($P = 0.02$). Anemia prevalence was 53%, low birth rate was 13%, and prematurity was 25%. The use of intermittent preventive treatment with sulfadoxine-pyrimethamine (greater than or equal to one dose) combined with bed net was associated with a reduction in infection only in multigravidae and with a reduced risk of maternal anemia.

INTRODUCTION

In highly endemic malaria regions where adult women have acquired premunition, *Plasmodium falciparum* infection during pregnancy is often asymptomatic. Placental infection frequently occurs, despite the absence of parasites in peripheral blood, and results in the accumulation of *P. falciparum*-infected erythrocytes in the intervillous space. This effect is a key feature of placental malaria (PM) and is caused by the cytoadherence of infected erythrocytes to placenta-specific receptors.[1] Clinical consequences of pregnancy-associated malaria include maternal anemia, low birth weight (LBW) for newborns, pre-term delivery, and increased perinatal morbidity.[2,3] Pregnant women residing in malaria-endemic regions are targeted for antimalarial prophylaxis. The use of insecticide-treated bed nets and 2–3 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp/SP) during the second and third trimester for all pregnant women is recommended.[4] Studies performed in stable transmission areas have shown that this intervention is safe and effective.[5–7] In 2003, the Gabonese Ministry of Health adopted the IPTp/SP protocol, and it was subsequently implemented in 2005.[8] It has already been shown that this implementation was followed by a reduction of maternal *P. falciparum* infection.[9] However, few data were available concerning placental malaria and its consequences in Gabon. This study aimed to assess the prevalence of maternal, placental, and cord-blood *P. falciparum* infection and its association with pregnancy outcome in a population of women living in Gabon.

MATERIALS AND METHODS

Study site. This observational cross-sectional study was conducted from September 2005 to January 2006 in Libreville, the capital city of Gabon. The population of Gabon is estimated at 1.5 million with ~40% of the population living in Libreville. In this area, malaria is endemic, predominantly caused by *P. falciparum*, and a stable perennial mode of transmission. Data on the efficacy of sulfadoxine-pyrimethamine in children showed a therapeutic efficacy rate of 88.4% at day 28 in 2005.[10]

This study was reviewed and approved by the Gabonese Ministry of Health.

Data collection. As part of a study assessing the sensitivity and specificity of a rapid diagnostic test for the diagnosis of PM at delivery, data were collected from delivering pregnant women in the obstetric department of the largest public hospital of Libreville (Center Hospitalier de Libreville), which had signed informed consent. The women were mainly primigravidae, which are known to be at increased risk of malaria. They were clinically examined by a nurse and the research physician. The following data were recorded in an observation file: sociodemographics, parity, treatment of fever using antimalarial drugs during the current pregnancy, bed net and IPTp/SP use. Because of the absence of the number of SP doses received by the women in the antenatal-care visit (ANC) card, these data were considered as absent or present and were recorded as "at least one dose IPTp/SP." Gestational age was determined by the date of the last menstrual period and confirmed by the morphometric measurement of the uterus. Newborns were weighed immediately at delivery using a hanging scale.

Peripheral, placental, and cord-blood samples were obtained from women who had an uncomplicated pregnancy and gave birth through the vaginal canal. Peripheral blood was collected by venipuncture, placental blood was collected by incising the cleaned maternal surface of the placenta and aspirating the blood welling from the incision with a sterile syringe (immediately at the delivery), and cord blood was taken directly from the umbilical vein.

Malaria diagnosis. Malaria diagnosis was performed using three different methods: microscopy, *P. falciparum* histidin-rich protein-2 (HRP-2) detection, and polymerase chain reaction (PCR).

Giemsa-stained thick blood films from peripheral, placental, and cord blood were examined by two trained microscopists; 15 μL of blood were spread on a fixed area (1.8 cm^2), and the entire area was read.[11] Parasitaemia was expressed as the number of asexual forms of *P. falciparum* per microliter (p/μL). Ten percent of all slides were randomly selected and

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reread by a third microscopist for a quality-control procedure. The film was considered to be negative if there were no asexual forms of the parasite identified in the entire slide. In placental films, the presence of leukocyte-associated haemozoin was also recorded.

The HRP-2 detection was done by a rapid immunochromatographic test, the Binax Now Malaria dipstick test (Binax Inc., Scarborough, ME), directly at the delivery. Each woman with a positive test received an antimalarial drug according to the national policy.

Within a few hours of collection, blood samples (2 mL) were centrifuged to separate the pellet containing the packed erythrocytes from the plasma, and then, they were frozen at −80°C. After DNA extraction (QIAmp, Qiagen, BmbH, Hilden, Germany), nested PCR for the genotyping of the merozoïte-surface protein-2 (MSP2) of *P. falciparum* was performed according to Ntoumi and others.[12]

Haemoglobin measurements. Haemoglobin (Hb) concentrations were estimated using a Coulter counter (SKTS, Beckman Coulter Corporation, Brea, CA).

Definitions. According to their age, women were classified as < 20 (young), 20–24, and ≥ 25 years old (older). History of possible malaria infection (HPMI) was defined as any episode of fever that occurred during the current pregnancy that was treated with any antimalarial drug. A positive blood sample with at least one of the three methods described above was considered infected. Based on placental thick-film microscopy, the stage of placental infection was categorized as follows: early (only parasites visible), late (both parasites and pigment visible), resolved (only haemozoin), and none or absent (neither parasite nor pigment visible).[13] Women with Hb between 10.9 and 8 g/dL and Hb < 8 g/dL were considered to have low and moderate to severe anemia, respectively. LBW was defined as a birth weight < 2500 g, and prematurity for gestational age was < 37 weeks.

Statistical analysis. Data were entered twice and cleaned using Epi info (version 6.04b, CDC, Atlanta, GA). Stata version 10 (Stata Corp., College Station, TX) was used for descriptive and comparative analysis. A control of data entry was performed before the statistical analysis. Medians with interquartile ranges (IQR) of parasite densities among the microscopically infected women are presented. Continuous variables were compared using the Mann-Whitney *U* test or the Kruskall Wallis test for the comparison of distributions. Differences between proportions were tested with the χ^2 or Fischer's exact tests. Age, fever, HPMI, bed net use, IPTp-SP use, and parity were each examined in a univariate analysis to identify predictors of maternal or placental infection, LBW, and maternal anemia. Those predictors that were significant at a *P* value < 0.20 were included in a multivariate logistic regression model using backward selection. The odds ratio, either crude (OR) or adjusted (aOR) for confounders, with 95% confidence intervals (95% CI) are presented.

RESULTS

Study population and use of malaria prophylaxis. Characteristics of the 203 delivering women who met the inclusion criteria and their newborns are shown in Table 1. Primigravidae accounted for 77% of the women. The mean age was 22.7 (±5.1) years, and multigravidae was significantly higher (26.5 ± 6.0 years) compared with primigravidae (21.5 ± 3.3 years; $P < 0.01$); 73% of all the women were < 25 years old. The mean gestational age assessed in 185 women was 38.7 (±2.9) weeks in multigravidae and 37.8 (±3.2) weeks in primigravidae; one quarter of all women delivered prematurely (Table 1). The proportion of pregnant women who reported sleeping under a bed net was 37% (*N* = 76). At least one dose of IPTp/SP was taken by 83 (41%) of 203 enrolled women, and 34 (17%) reported concomitant bed net use. Fever was rare (5%) and present only in primigravidae women. Mean birth weight was 3,022 g (±494 g) and slightly higher in primigravidae (3,046 ± 505 g) than in multigravidae (2,939.3 ± 451 g). Thirteen percent of the singleton newborns had LBW. Hb level was measured in 120 primigravidae; the mean Hb concentration was 10.7 (±1.8) g/dL. A total of 56 (47%) delivering women were not anemic, 54 (45%) had low anemia, and 10 (8%) had moderate to severe anemia.

Young age (< 20 years) was associated with higher risk of premature delivery (Table 2A–B). Pregnant women with moderate to severe anemia were younger (19.0 ± 3.3 years old) than those with normal Hb level (21.6 ± 3.3 years old; $P = 0.02$) or with low anemia (21.3 ± 3.3 years old; $P = 0.04$). After multivariate analysis, younger age and maternal anemia remained independently associated with premature delivery (Table 2B).

There was a reduced risk of maternal anemia in the IPTp/SP plus bed net group (Table 2A–B). Additionally, there was a higher mean Hb level in this group (11.6 ± 1.3 g/dL) compared with women without malaria prophylaxis (10.5 ± 1.2 g/dL; $P = 0.02$).

An HPMI occurred more frequently in women without malaria preventive measures during pregnancy (Table 2A).

Maternal malaria. *P. falciparum* was the only malaria species identified. Peripheral infection was detected in 12.4% (*N* = 25/202), 13.3% (*N* = 27/203), and 27.6% (*N* = 56/203) of samples by microscopy, HRP-2 test, and PCR, respectively. In the group of infected women, the parasite load was higher in primigravidae (median of parasitaemia = 44 [IQR = 23–868] p/µL) than multigravidae (median of parasitaemia = 16 [IQR = 13–239] p/µL) women ($P = 0.03$).

Irrespective of the diagnostic method used, the global prevalence of maternal *P. falciparum* infection was of 34.5% (*N* = 70/203), and there was no difference between primigravidae

TABLE 1
Characteristics of the studied women

	Number	%
Age (years)		
< 20	60	30
20–24	88	43
≥ 25	55	27
Parity		
Primiparous	157	77
Multiparous	46	23
Gestational age < 37 weeks*	46	25
IPTp/SP use	49	24
Bed net use	42	21
IPTp/SP plus bed net use	34	14
HPMI†	22	11
Fever at delivery‡	8	5
Maternal anemia§	64	53
Low birth weight	27	13
Total	203	

* *N* = 185.
† History of possible malaria infection, *N* = 202.
‡ *N* = 177.
§ Hb level measured in only 120 primigravidae.

TABLE 2A
Relations between maternal and neonatal characteristics as well as age, IPTp/SP use, and bed net use

	Age (years)				Malaria prophylaxis				
	< 20	20–24	25	*P*	IPTp/Sp	Bed net use	IPTp/Bed net use	No prophylaxis	*P*
	(*N* = 60)	(*N* = 88)	(*N* = 55)		(*N* = 49)	(*N* = 42)	(*N* = 34)	(*N* = 78)	
Primiparous	88%	84%	55%	< 0.01	27%	23%	16%	34%	0.08
Multiparous	12%	16%	45%	< 0.01	15%	13%	20%	52%	0.08
Gestational age, mean	38	39	39	0.20	39	39	39	39	0.50
HPMI*	8%	10%	15%	0.55	4%†	7%†	12%	17%	0.12
Premature delivery	41%	17%	19%	< 0.01	29%	22%	22%	25%	0.84
Maternal anemia	65%	49%	49%	0.17	54%	59%	33%†	63%	0.15
LBW	13%	9%	20%	0.17	14%	9%	12%	15%	0.82
Maternal malaria	23%	31%	42%	0.40	37%	26%†	15%†	46%	< 0.01
Placental malaria	47%	51%	51%	0.85	49%	48%	38%†	56%	0.35
Cord-blood infection	13%	17%	26%	0.22	16%	21%	3%†	24%	0.05

*History of possible malaria infection during pregnancy.
†$P < 0.01$ for comparison with the group without malaria prophylaxis.

(33.1%; N = 52/157) and multigravidae (39.1%; N = 18/46) women ($P = 0.45$). Among the 14 samples considered infected with the absence of MSP2 gene detection, all were HRP-2 positive, and three were positive by microscopy (parasitaemia was below 18/μL).

HPMI during pregnancy was associated with a higher risk of peripheral maternal infection. Delivering women in the bed net (26%) and the IPTp/SP plus bed net (15%) groups were less frequently infected in peripheral blood compared with those who did not use malaria-prevention measures during pregnancy (46%) (Table 2A). Univariate analysis indicated that HPMI and bed net use alone or combined with IPTp/SP decreased the risk of maternal parasitaemia (Tables 2A–B).

Among primigravidae women, all febrile women had peripheral parasitaemia (Table 3A). Maternal *P. falciparum* infection was also related to a HPMI that was reported by 19% of the infected primigravidae compared with only 5% of the uninfected ones ($P < 0.01$). Two more associations were found with maternal *P. falciparum* infection: first, there was a trend toward a reduced proportion of those infected who took SP and slept under bed nets, and secondly, the infected primigravidae had newborns with a lower mean birth weight (202.3 g reduction; $P = 0.02$). The proportion of infected primigravidae women who used any malaria-prevention measure during pregnancy was significantly higher (59%) than that of infected multigravidae women (16%; $P < 0.01$) (Table 3A).

Among the multigravidae women, none of those in the IPTp/SP plus bed net group had peripheral infection. Reported use of malaria prophylaxis was associated with a reduced risk of peripheral malaria in univariate analysis (Table 3B).

Maternal malaria was not a risk factor for anemia, premature delivery, or LBW in univariate and multivariate analysis.

The median maternal parasite density was higher among the youngest women (523 [21–1316] p/μL in women < 20 years old versus 26 [18–60] p/μL in women 18–24 years old and 11 [12–368] p/μL in older women; $P = 0.02$). Table 4 shows that maternal parasite densities were highest in women who had HPMI, anemia, premature babies, or children with LBW.

Placental malaria. Overall 53.6% (N = 109/203) of women examined had placental malaria (PM) detected either by microscopy (presence of infected erythrocytes = 23.4% [N = 46/197] or presence of haemozoin = 27.9% [N = 55/197]) and/or HRP-2 test (13.3%; N = 27/203) and/or MSP2 genotyping (29.1%; N = 59/203). Among the 50 samples considered infected despite a negative result in PCR, 35 had only pigment detected by microscopy and 15 had detectable low parasitaemia (< 14 p/μL); among these 15 samples, 13 were also positive with HRP-2 testing. Prevalence of PM did not vary between primigravidae (54.8%; N = 86/157) and multigravidae (50.0%; N = 23/26) women and was not associated with either age or maternal anemia in primigravidae women. Occurrence of premature delivery and LBW was not directly related to the presence of placental infection (Table 3A).

Median placental parasitaemia was lower in the IPTp/SP and IPTp/bed net groups and higher in groups of women with HPMI or LBW children (Table 4).

In primigravidae women, HPMI during pregnancy was the only risk factor for PM; 14 of 15 primigravidae who reported treatment of fever with an antimalarial drug had PM at delivery (Tables 3A–B).

Concerning multigravidae women, the use of IPTp/SP alone or combined with bed net was a protective factor for PM (Table 3B); among the 15 women who slept under a bed net during the current pregnancy, only 4 (26.6%) had PM ($P = 0.04$).

The presence of pigmented leukocytes in the placenta was associated with a lower gestational age at delivery (37.7 ± 1.3 weeks versus 39.2 ± 1.6 weeks in the group of women without malaria pigment in their placenta; $P < 0.01$).

TABLE 2B
Univariate and multivariate logistic regression analysis of factors associated with pregnancy outcome in all the studied population

	Univariate analysis		Multivariate analysis	
Factors	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
HPMI				
IPTp/SP	0.3 (0.06–1.3)	0.08	0.3 (0.03–2.1)	0.2
Prematurity				
Age < 20 years	2.8 (1.4–5.5)	< 0.01	3.9 (1.5–10.0)	< 0.01
maternal anemia	3.2 (1.5–6.9)	< 0.01	2.9 (1.1–7.3)	< 0.01
Maternal anemia				
Age < 20 years	2.1 (0.9–4.7)	0.06	1.6 (0.7–3.7)	0.3
IPTp/bed net use	0.4 (0.1–0.9)	0.03	0.4 (0.1–0.9)	0.04
Maternal malaria				
HPMI	3.0 (1.2–7.2)	0.02	2.3 (1.2–7.7)	0.02
Bed net use	0.3 (0.2–0.9)	0.03	0.4 (0.1–1.4)	0.1
IPTp/bed net use	0.3 (0.1–0.8)	< 0.01	0.7 (0.2–2.1)	0.2
Placental malaria				
IPTp/bed net use	0.3 (0.3–1.2)	0.1	1.4 (0.5–3.9)	0.5
LBW				
Age = 20–24 years	0.5 (0.2–1.2)	0.09	1.9 (0.8–2.1)	0.7

TABLE 3A
P. falciparum infection and pregnancy outcome among primigravidae and multigravidae women

	Primigravidae				Multigravidae			
	N	Infected	Uninfected	*P*	N	Infected	Uninfected	*P*
Peripheral infection		*N* = 52/157	*N* = 105/157			*N* = 18/46	*N* = 28/46	
Fever	8	16%	0%	< 0.01	0	0%	0%	–
HPMI*	15	19%	5%	< 0.01	7	18%	14%	0.76
IPTp	42	31%	24%	0.35	7	11%	18%	0.84
Bed net	36	19%	25%	0.44	6	5%	18%	0.84
IPTPp/bed net use	25	9%	19%	0.13	9	0%	32%	0.02
Mean Hb	120	10.2 g/dL	10.9 g/dL	0.18	–	–	–	–
Maternal anemia	120	58%	51%	0.70	–	–	–	–
Prematurity	141	29%	28%	0.97	44	11%	15%	0.68
Mean birth weight	157	2,909 g	3,112 g	0.02	46	2,973 g	2,917 g	0.37
LBW	157	17%	9%	0.16	46	17%	18%	0.92
Placental infection		*N* = 86/157	*N* = 78/157			*N* = 23/46	*N* = 23/46	
HPMI	15	18%	1%	< 0.01	7	19%	12%	0.54
IPTp	42	28%†	26%	0.72	7	9%†	21%	0.50
Bed net	36	22%	24%	0.38	6	14%	13%	0.70
IPTPp/bed net use	25	15%	17%	0.56	9	4%	34%	0.02
Mean Hb	120	10.7 g/dL	10.7 g/dL	0.96	–	–	–	–
Maternal anemia	120	51%	43%	0.47	–	–	–	–
Prematurity	141	25%	32%	0.34	44	15%	13%	0.68
Mean birth weight	157	3,037 g	3,056 g	0.85	46	2,915 g	2,961 g	0.74
LBW	157	15%	9%	0.23	46	18%	17%	0.89
Cord-blood infection		*N* = 27/157	*N* = 130/157			*N* = 10/46	*N* = 16/46	
HPMI	15	25.9%	6.1%	0.02	7	22%	14%	0.54
IPTp	42	18%	28%	0.25	6	30%	11%	0.55
IPTPp/bed net use	25	9.%	19%	0.13	9	0%	25%	0.24
Mean birth weight	157	2,855 g	3,085 g	0.02	46	3,042 g	2,931 g	0.43

*History of possible malaria infection during pregnancy.
†Difference between infected primigravidae and infected multigravidae statistically significant with $P < 0.05$.

Placental infection was classified in the 197 women whose placenta was tested using the three techniques as early (13.1%; *N* = 26), late (10.1%; *N* = 20), resolved (17.7%; *N* = 35), submicroscopic (14.1%; *N* = 28), and absent (44.4%; *N* = 88) (Table 5). This distribution did not differ according to parity and gestational age. Detection of infected erythrocytes and haemozoin by microscopy revealed a PM prevalence of 40.9% (81 women). Among the 35 women with resolved placental infection, only 2 women were HRP-2 positive.

HPMI was a risk factor for late (27.3%; OR = 6.5; 95% CI = 2.2–17.3; $P < 0.01$) and submicroscopic (22.3%; OR = 4.3; 95% CI = 1.1–15.5; $P = 0.01$) placental infection (Table 5).

There was a trend toward an association between frequent resolved or absent infections ($P = 0.09$) and less prevalent early infection ($P = 0.03$) with reported malaria prophylaxis use (Table 5). These associations were more pronounced in multigravidae women. Among those who had no PM, only 16% did not report using any prophylaxis ($P < 0.01$), and none of those with PM who used a bed net and/or IPTp/SP during pregnancy had an early infection ($P < 0.01$). Only one of nine multigravidae from the IPTp plus bed net group had a placental infection (i.e., a resolved infection).

Women with late PM had lower mean Hb concentration ($P = 0.04$) and a 315-g reduction in mean birth weight ($P = 0.03$) (Table 5). There was a strong association between the presence of placental submicroscopic infection and cord infection ($P < 0.01$).

Cord-blood infection. Infection of cord blood was submicroscopic and only detected by PCR in 18.2% (*N* = 37) of the newborns. Data analysis in the group of primigravidae women showed an association of cord infection with a HPMI and with absence of IPTp/SP use (aOR = 3.02; 95% CI = 1.17–8.17; $P = 0.02$). Maternal infection (OR = 9.2; 95% CI = 4.1–21.08; $P < 0.01$) and placental infection (OR = 11.9; 95% CI = 4.1–25.1;

TABLE 3B
Analysis of risk factors for pregnancy outcome associated with *P. falciparum* infection in primigravidae and multigravidae women

	Primigravidae				Multigravidae			
Factors	OR (95% CI)	*P*	aOR (95% CI)	*P*	OR (95% CI)	*P*	aOR (95% CI)	*P*
Associated with peripheral infection								
HPMI	4.8 (1.5–15.3)	< 0.01	2.0 (1.4–9.9)	0.02	1.2 (0.2–6.2)	0.8	0.8 (0.1–5.8)	0.8
IPTp	1.3 (0.6–2.8)	0.4	1.6 (0.5–4.7)	0.4	0.6 (0.1–1.0)	0.06	0.3 (0.05–2.3)	0.3
Bed net	0.7 (0.3–1.6)	0.4	0.5 (0.1–1.9)	0.3	0.3 (0.03–1.2)	0.07	0.2 (0.01–2.5)	0.2
IPTPp/bed net use	0.4 (0.1–1.3)	0.1	0.8 (0.2–3.0)	0.7	–	–	–	–
Associated with placental malaria								
HPMI	16.6 (2–234)	< 0.01	13.3 (1.4–136.1)	0.02	1.6 (0.3–8.0)	0.6	3.3 (0.3–33.4)	0.3
IPTp	1.1 (0.6–2.3)	0.7	1.7 (0.6–4.8)	0.3	0.4 (0.06–2.3)	0.2	0.1 (0.01–1.4)	0.09
IPTPp/bed net use	0.9 (0.4–2.1)	0.8	1.6 (0.5–5.1)	0.4	0.1 (0.01–1.0)	0.08	0.02 (0.001–0.4)	< 0.01
Associated with cord-blood infection								
HPMI	5.3 (1.7–17.0)	< 0.01	12.0 (1.8–79.8)	0.01	1.6 (0.2–9.7)	0.6	10.6 (0.5–235)	0.1
IPTPp/bed net use	0.2 (0.02–1.3)	0.05	1.3 (0.2–7.9)	0.7	2.0 (0.3–13.3)	0.4	16.1 (0.7–384)	0.08

TABLE 4
Distribution of median parasite densities according to the presence of some studied variables

Variable	Parasitaemia (p/μL)			P
Maternal parasitaemia				
HPMI	264 (25–1400)	No HPMI	17 (11–62)	0.10
Anemic	1480 (372–1840)	Non-anemic	24 (13–53)	< 0.01
Prematurity	1640 (748–1880)	Normal delivery	16 (13–48)	0.02
LBW	485 (23–1920)	Normal birthweight	18 (13–58)	0.09
Placental parasitaemia				
IPTp	12 (12–17)	No prophylaxis	76 (34–145)	< 0.01
IPTp/bed net	15 (12–21)	No prophylaxis	76 (34–145)	0.02
HPMI	109 (23–296)	No HPMI	15 (13–18)	0.04
LBW	48 (13–742)	Normal birthweight	17 (12–16)	0.06

$P < 0.01$) were risk factors for cord-blood infection, and the odds ratio was higher when considering only submicroscopic infections (data not shown). Neonates with submicroscopic cord infection had a mean birth weight reduced to 230 g in primigravidae women only ($P = 0.02$) (Table 3).

DISCUSSION

At the time of implementation of the World Health Organization (WHO) recommendations for malaria prevention in Gabon, it was essential to better document indicators of malaria in pregnancy. In this observational study performed in Libreville, an urban area, delivering women had high prevalence of maternal and placental malaria and anemia.

Malaria diagnosis was based on a combination of different methods, namely microscopy, malaria rapid-diagnostic test, and PCR, to allow active and sensitive detection; the conventional parasite detection by microscopy alone is associated with an underestimation of malaria prevalence.[13,14] The method used for microscopic detection of *P. falciparum* asexual forms, different from the conventional thick-film microscopic examination, allowed a better estimation of the parasitaemia. A precise volume of blood (15 μL) was spread on the slide and that one was read entirely before the report of the results. This could explain the slight discrepancy between PCR and microscopy results. Although the PCR methods are able to detect parasite DNA in the case of microscopic and subpatent parasitaemia, sensitivity is known to be dependent of the parasite density, parasite viability, and markers used for genotyping.[15–18] Furthermore, absence of parasite DNA amplification in the presence of microscopic parasitaemia is not uncommon, and the limit of PCR detection is reported to be between 0.01/μL to 500/μL.[15,17–20] It is important to notice that 95% of the microscope positive samples that were negative by PCR had low parasitaemia (< 18/μL) and were HRP-2 positive, suggesting an old infection with probably a large number of non-viable parasites detected by microscopy. Nevertheless, PCR remains more repeatable, less subjective, and more sensitive than microscopy for low-grade parasitaemia. Our results highlight the need for using both PCR and microscopy for active parasite detection in areas where parasite densities are low.

Prevalence of microscopic maternal infection (12.4%) is lower compared with that reported at the first antenatal care visit (57%) in pregnant women from Libreville in 1997.[21] But when also considering submicroscopic infections, this prevalence jumped to 34.5%. The same range of maternal *P. falciparum* malaria prevalence (31%) was found in Lambaréné, a city located 237 km south of Libreville.[22] Submicroscopic infections seem to be more frequent than microscopic infections in Gabon, probably because of improved case management and use of preventive strategies that contribute to better control of parasitaemia.

The malaria parasites frequently sequester in the placenta, and this is a more appropriate place to evaluate malaria-prevention strategies, because it is directly linked to the newborn (the focus of the prevention). We considered placental malaria infection as one of the main parasitological indicators of infection in pregnancy. The global prevalence of placental infection (53.6%) was comparable to that observed in Ghana (59.4%), where microscopic detection of asexual form and malaria pigment, associated with HRP-2 testing and PCR, was used for the diagnosis.[13] Adegnika reported a lower prevalence using microscopy and PCR (31%) in Lambaréné, but haemozoïn detection was not performed.[22] The detection of pigmented leukocytes, although less sensitive than histological examination of the placenta because it neglects placental haemozoin deposition, allowed determination of late or resolved infections (cleared since at least 1 week as confirmed by the

TABLE 5
Distribution of the studied variables according to the stages of placental infection

	Stage of infection									
	Early (N = 26)		Late (N = 20)		Resolved (N = 35)		Submicroscopic (N = 28)		None (N = 88*)	
Outcomes (n)	n	%	n	%	n	%	n	%	n	%
Primiparous (155)	21	13.5	14	9.0	30	19.4	21	13.5	69	44.5
Multiparous (42)	5	11.9	6	14.3	5	11.9	7	16.7	19	45.2
IPTp (80)	9	11.2	6	7.5	16	20.0	10	12.5	39	48.8
Bed net use (74)	7	9.5	5	6.7	15	20.3	10	13.5	37	50.0
HPMI (22)	4	18.2	6	27.3	3	8.6	5	22.3	4	18.2
Prematurity (44)	8	18.2	5	11.4	7	15.9	5	11.4	19	43.2
Maternal anemia (64)	11	17.2	3	4.7	11	17.2	5	7.8	34	53.1
LBW (26)	3	11.5	6	23.1	3	11.5	4	15.4	10	38.5
Cord infection (37)	6	16.2	3	8.1	5	13.5	23	62.2	0	0.0
	Continuous outcomes									
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Haemoglobin (g/dL)	10.3	(1.7)	9.9	(1.6)	11.3	(1.6)	10.8	(1.8)	10.7	(2.3)
Birthweight (g)	3084.2	(466)	2832.0	(520)	3147.9	(487)	2961.4	(428)	3038.2	(507)

*Six samples were not tested with the three techniques.
SD = standard deviation; N = number.

absence of HRP-2 detection).[23] Late PM was associated with low Hb level and reduction of mean birth weight as reported in Ghanaian pregnant women.[13] This technique is easier, cheaper, and when coupled with parasite detection, was able to identify most of the placental infections (81/109). For epidemiological purposes, microscopic detection of infected erythrocytes and leukocytes associated with haemozoin can be used as an alternative method for the diagnosis of PM in endemic settings where placental histology is not routinely available.

Prevalence of cord infection, although always submicroscopic, was high (18.2%). This could be explained by the high frequency of submicroscopic placental infection that is now recognized as a predictor of submicroscopic cord infection.[24] In the present study, 62% of children with cord-blood infection were born from women with only submicroscopic PM.

Although this was not a randomized controlled study for the evaluation of the efficacy of malaria-preventive measures, analysis of the association of reported IPTp and/or bed net use with pregnancy outcome was performed. At the time of the study, free distribution of IPTp/SP and insecticide-treated bed nets to women attending ANC was ongoing in the country. However, less than one-half of the delivering women received at least one SP dose, and one-third used a bed net, indicating a low or irregular ANC attendance, especially when considering the WHO recommendation of four ANC visits. One year after the study, 83% and 57% of delivering women participating in the study performed in Libreville and Lambaréné had taken one and two doses of SP during pregnancy, respectively.[9] During this period in Gabon, only 70% of pregnant women attended two ANC during pregnancy, this proportion decreased to < 40% for four ANC (MK Bouyou-Akotet, unpublished data).[4] The global frequency of malaria infection was comparable between primigravidae and multigravidae. It is argued that with the use and spread of antimalarial preventive strategies, the susceptibility to malaria does not differ in pregnant women, regardless of parity.[25,26] But when taking into account the use of IPTp plus bed net, it is obvious that primigravidae remained at a higher risk to be infected. Indeed, in the IPTp/SP plus bed net group, parasitaemia and prevalence of maternal and placental *P. falciparum* infection were lower in multigravidae women. This could be explained by the association of their effect with the level of parasite-specific immunity that is known to be stronger in multigravidae compared with primigravidae. Another consideration is the impact of SP use on pregnancy outcomes depending on parity.[25–29] One SP dose is able to significantly reduce malaria prevalence in pregnant women, as observed in multigravidae women, and thus, higher dosing is needed for primigravidae women.[28–30] This is confirmed by the lowest proportion of maternal- (11%) and placental-infected (13%) multigravidae women who had taken at least one SP dose during pregnancy compared with primigravidae women from the same group who had maternal (40%) and placental (43%) infection. As opposed to Mbaye and others[27,31,32] in Gambia, a significant reduction of maternal and PM in multigravidae was achieved in the group using IPTp/SP plus bed net during pregnancy, which was reported by other authors.[27,31,32] Women who had taken IPTp/SP alone or combined with bed net use, particularly multigravidae, had less early placental infection. Similar results were observed in Mozambique and Malawi, confirming the ability of SP to cure recent infections and the necessity of giving a prophylactic dose near the end of term.[27,30,33,34]

Higher reduction (with strong odds ratios) of PM by IPTp/SP and/or bed net use and of LBW by IPTp in multigravidae compared with primigravidae have been observed.[24,26,30,32,33] Effort must be taken to improve access and use of insecticide-treated bed nets combined with recommended dosage of IPTp/SP.

HPMI was found to be the strongest predictor of malaria infection at delivery in primigravidae women.[28,34] Women in their first pregnancy seem to be unable to clear peripheral parasites, thereby increasing the risk of placental and cord infection at the end of the pregnancy. The risk of having HPMI is also reduced by IPTp/SP use.[28,34] Among women with HPMI, the proportion of those without IPTp/SP was two-fold higher compared with the ones in the IPTp/SP group.

Anemia is considered a good indicator for maternal health. Its prevalence was high in primigravidae women, and it was associated with a higher parasite density. Uninfected women had a higher hemoglobin concentration (0.7 g/dL difference), but this difference was not statistically significant, probably because of the small sample size. IPTp combined with bed net use reduced the risk of maternal anemia, but even in the women who used these preventive strategies, the prevalence of anemia was still high. The impact of IPTp and/or bed net use on anemia depends on the dosing and is found to be generally low or absent.[7,25–27,31–34] Proper antenatal care that includes surveillance of the pregnancy and management of all risk factors for pregnancy outcomes is essential to improving Hb levels.[7,25,28]

Birth weight is a strong predictor of infant health and therefore, a good indicator for the control of malaria-preventive strategies. A mean birth-weight reduction of > 200 g was observed in the case of primigravidae women with maternal and cord-blood infection. This result is comparable with previous studies, and the relationship between cord infection and lower mean birth weight in primigravidae has been observed in Cameroon.[33–36] The absence of an association between PM and birth weight is probably caused by the small sample size of women with late and submicroscopic placental infections that were associated with a lower mean birth weight. Both are independent risk factors for LBW and mean birth weight reduction.[13,22]

Despite the small sample size, the lower and non-controlled dosing of SP, and the observational nature of the data presented, this overview of malaria-associated pregnancy indicators is useful for providing a baseline for assessing future programs and recommendations.

CONCLUSION

The burden of malaria and anemia among pregnant women is still high in Gabon. Submicroscopic infections, which have a negative impact on neonate outcomes, are frequent. Bed net use and IPTp/SP coverage, although low and insufficient, have some degree of positive impact on the frequency of the infection in multigravidae women and on mean birth weight in primigravidae women. Efforts must be made for a full and correct implementation of the recommended WHO strategies for malaria prevention during pregnancy throughout the country, and new assessment of malaria-associated pregnancy outcome in comparison with the presented data must be scheduled in the near future for the estimation of the impact of these strategies. Furthermore, better attendance to antenatal visits is

needed for the prevention and management of other causes of anemia, prematurity, and LBW.

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REFERENCES

1. Fried M, Duffy PE, 1998. Maternal malaria and parasite adhesion. *J Mol Med 76*: 162–171.
2. Uneke CJ, 2007. Impact of placental *Plasmodium falciparum* malaria on pregnancy and perinatal outcome in sub-Saharan Africa. II: effects of placental malaria on perinatal outcome; malaria and HIV. *Yale J Biol Med 80:* 95–103.
3. 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.
4. World Health Organization, 2004. *A Strategic Framework for Malaria Prevention and Control during Pregnancy in the African Region*. Brazzaville, Republic of Congo: WHO.
5. 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.
6. 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.
7. 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.
8. Ministère de Santé, 2003. Rapport de l'atelier national de consensus. Traitement Préventif Intermittent contre le Paludisme chez la Femme Enceinte. Programme National de Lutte Contre le Paludisme, Libreville, Gabon.
9. Ramharter M, Schuster K, Bouyou-Akotet MK, Adegnika AA, Schmits K, Mombo-Ngoma G, Agnandji ST, Nemeth J, Afène SN, Issifou S, Onnas IN, Kombila M, Kremsner PG, 2007. Malaria in pregnancy before and after the implementation of a national IPTp program in Gabon. *Am J Trop Med Hyg 77:* 418–422.
10. Nsimba B, Guiyedi V, Mabika-Mamfoumbi M, Mourou-Mbina JR, Ngoungou E, Bouyou-Akotet M, Loembet R, Durand R, Le Bras J, Kombila M, 2008. Sulphadoxine/pyrimethamine versus amodiaquine for treating uncomplicated childhood malaria in Gabon: a randomized trial to guide national policy. *Malar J 7:* 31–38.
11. Planche T, Krishna S, Kombila M, Engel K, Faucher JF, Ngou-Milama E, Kremsner PG, 2001. Comparison of methods for the rapid laboratory assessment of children with malaria. *Am J Trop Med Hyg 65:* 599–602.
12. Ntoumi F, Contamin H, Rogier C, Bonnefoy S, Trape JF, Mercereau-Puijalon O, 1995. Age-dependent carriage of multiple *Plasmodium falciparum* merozoite surface antigen-2 alleles in asymptomatic malaria infections. *Am J Trop Med Hyg 52:* 81–88.
13. Mockenhaupt FP, Bedu-Addo G, von Gaertner C, Boyé R, Fricke K, Hannibal I, Karakaya F, Schaller M, Ulmen U, Acquah PA, Dietz E, Eggelte TA, Bienzle U, 2006. Detection and clinical manifestation of placental malaria in southern Ghana. *Malar J 5:* 119–128.
14. Mayor A, Serra-Casas E, Bardají A, Sanz S, Puyol L, Cisteró P, Sigauque B, Mandomando I, Aponte JJ, Alonso PL, Menéndez C, 2009. Sub-microscopic infections and long-term recrudescence of *Plasmodium falciparum* in Mozambican pregnant women. *Malar J 8:* 9–18.
15. Färnert A, Arez AP, Babiker HA, Beck HP, Benito A, Björkman A, Bruce MC, Conway DJ, Day KP, Henning L, Mercereau-Puijalon O, Ranford-Cartwright LC, Rubio JM, Snounou G, Walliker D, Zwetyenga J, do Rosario VE, 2001. Genotyping of *Plasmodium falciparum* infections by PCR: a comparative multicentre study. *Trans R Soc Trop Med Hyg 95:* 225–232.
16. Jarra W, Snounou G, 1998. Only viable parasites are detected by PCR following clearance of rodent malarial infections by drug treatment or immune responses. *Infect Immun 66:* 3783–3787.
17. Beny A, Fabre R, Benoi-Vical F, Cassaing S, Magnaval JF, 2005. Contribution of PCR-based methods to diagnosis and management of imported malaria. *Med Trop 65:* 176–183.
18. Coleman RE, Sattabongkot J, Promstaporm S, Maneechai N, Tippayachai B, Kengluecha A, Rachapaew N, Zollner G, Miller RS, Vaughan JA, Thimasarn K, Khuntirat B, 2006. Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum*/*vivax* endemic area in Thailand. *Malar J 5:* 121–127.
19. Nicastri E, Bevilacqua N, Sañé Schepisi M, Paglia MG, Meschi S, Ame SM, Mohamed JA, Mangi S, Fumakule R, Di Caro A, Capobianchi MR, Kitua A, Molteni F, Racalbuto V, Ippolito G, 2009. Accuracy of malaria diagnosis by microscopy, rapid diagnostic test, and PCR methods and evidence of antimalarial overprescription in non-severe febrile patients in two Tanzanian hospitals. *Am J Trop Med Hyg 80:* 712–717.
20. Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, Bienzle U, 2002. Diagnosis of placental malaria. *J Clin Microbiol 40:* 306–308.
21. 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:* 18–24.
22. Adegnika AA, Verweij JJ, Agnandji ST, Chai SK, Breitling LP, Ramharter M, Frolich M, Issifou S, Kremsner PG, Yazdanbakhsh M, 2006. Microscopic and sub-microscopic *Plasmodium falciparum* infection, but not inflammation caused by infection, is associated with low birth weight. *Am J Trop Med Hyg 75:* 798–803.
23. Rogerson SJ, Mkundika P, Kanjala MK, 2003. Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology. *J Clin Microbiol 41:*1370-1374.
24. Malhotra I, Mungai P, Muchiri E, Kwiek JJ, Meshnick SR, King CL, 2006. Umbilical cord-blood infections with *Plasmodium falciparum* malaria are acquired antenatally in Kenya. *J Infect Dis 194:* 176–183.
25. Gies S, Coulibaly SO, Ouattara FT, D'Alessandro U, 2009. Individual efficacy of intermittent preventive treatment with sulfadoxine-pyrimethamine in primi- and secundigravidae in rural Burkina Faso: impact on parasitaemia, anaemia and birth weight. *Trop Med Int Health 14:* 174–182.
26. Njagi JK, Magnussen P, Estambale B, Ouma J, Mugo B, 2003. Prevention of anaemia in pregnancy using insecticide-treated bednets and sulfadoxine-pyrimethamine in a highly malarious area of Kenya: a randomized controlled trial. *Trans R Soc Trop Med Hyg 97:* 277–282.
27. Menéndez C, Bardají A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, Macete E, Berenguera A, David C, Dobaño C, Naniche D, Mayor A, Ordi J, Mandomando I, Aponte JJ, Mabunda S, Alonso PL, 2008. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. *Plos One 3:* 1–9.

28. Sirima SB, Cotte AH, Konaté A, Moran AC, Asamoa K, Bougouma EC, Diarra A, Ouédraogo A, Parise ME, Newman RD, 2006. Malaria prevention during pregnancy: assessing the disease burden one year after implementing a program of intermittent preventive treatment in Koupela District, Burkina Faso. *Am J Trop Med Hyg 75:* 205–211.
29. Hommerich L, von Oertzen C, Bedu-Addo G, Holmberg V, Acquah PA, Eggelte TA, Bienzle U, Mockenhaupt FP, 2007. Decline of placental malaria in southern Ghana after the implementation of intermittent preventive treatment in pregnancy. *Malar J 6:* 144–151.
30. Mbonye AK, Bygbjerg I, Magnussen P, 2008. Intermittent preventive treatment of malaria in pregnancy: a community-based delivery system and its effect on parasitemia, anemia and low birth weight in Uganda. *Int J Infect Dis 12:* 22–29.
31. Mbaye A, Richardson K, Balajo B, Dunyo S, Shulman C, Milligan P, Greenwood B, Walraven G, 2006. A randomized, placebo-controlled trial of intermittent preventive treatment with sulphadoxine-pyrimethamine in Gambian multigravidae. *Trop Med Int Health 11:* 992–1002.
32. Kabanywanyi AM, Macarthur JR, Stolk WA, Habbema JD, Mshinda H, Bloland PB, Abdulla S, Kachur SP, 2008. Malaria in pregnant women in an area with sustained high coverage of insecticide-treated bed nets. *Malar J 7:* 133.
33. Msyamboza K, Senga E, Tetteh-Ashong E, Kazembe P, Brabin BJ, 2007. Estimation of effectiveness of interventions for malaria control in pregnancy using the screening method. *Int J Epidemiol 36:* 406–411.
34. van Eijk AM, Blokland IE, Slutsker L, Odhiambo F, Ayisi JG, Bles HM, Rosen DH, Adazu K, Lindblade KA, 2005. Use of intermittent preventive treatment for malaria in pregnancy in a rural area of western Kenya with high coverage of insecticide-treated bed nets. *Trop Med Int Health 10:* 1134–1140.
35. Falade CO, Yusuf BO, Fadero FF, Mokuolu OA, Hamer DH, Salako LA, 2007. Intermittent preventive treatment with sulphadoxine-pyrimethamine is effective in preventing maternal and placental malaria in Ibadan, south-western Nigeria. *Malar J 6:* 88–97.
36. Akum AE, Kuoh AJ, Minang JT, Achimbom BM, Ahmadou MJ, Troye-Blomberg M, 2005. The effect of maternal, umbilical cord and placental malaria parasitaemia on the birthweight of newborns from South-western Cameroon. *Acta Paediatr 94:* 917–923.