Title

Effects of Malaria in the First Trimester of Pregnancy on Poor Maternal and Birth Outcomes in Benin

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Using data from a Beninese preconceptional cohort, malaria in the 1st trimester of pregnancy had a direct and negative effect on maternal anaemia late in pregnancy. Repeated infections starting in the 1st trimester tended to increase the low birthweight risk.

Abstract

Background. In sub-Saharan Africa, malaria in the first half of pregnancy is harmful for both the mother and her fœtus. However, malaria in the 1st trimester of pregnancy, when women are usually not protected against malaria, has been little investigated. For the first time, we assessed the effects of malaria in the 1st trimester on maternal and birth outcomes using a preconceptional study design.

Methods. From June 2014 to March 2017, 1214 women of reproductive age were recruited and followed monthly until 411 became pregnant. Pregnant women were then followed from 5-6 weeks of gestation until delivery. Path analysis was used to assess the direct effect (i.e., not mediated by malaria in the 2nd or 3rd trimester) of malaria in the 1st trimester on maternal anaemia and poor birth outcomes. The cumulative effect of infections during pregnancy on the same outcomes was also evaluated.

Results. The prevalence of malaria infection in the 1st trimester was 21.8%. Malaria in the 1st trimester was significantly associated with maternal anaemia in the 3rd trimester (adjusted odds ratio [aOR]: 2.25, 95% CI 1.11, 4.55). While we did not evidence any direct effect of 1st trimester malaria infections on birth outcomes, their association with infections later in pregnancy tended to increase the risk of low birthweight.

Conclusions. Malaria infections in the 1st trimester were highly prevalent and have deleterious effects on maternal anaemia. They highlight the need for additional preventive measures starting in early pregnancy, or even before conception.

Key words: Malaria infection, first trimester, maternal anaemia, poor birth outcomes, preconceptional cohort, Africa

Introduction

Over 125 million pregnancies are exposed to malaria each year, with sub-Saharan Africa (SSA) accounting for 25% of this total burden [1]. While the overall consequences of malaria in pregnancy on maternal and birth outcomes have been well documented [2], the influence of the timing, particularly the effect of infections in the 1st trimester, remains under-investigated. However, this period may be critical for the fœtus, since parasite sequestration into the placenta may occur as early as 8 weeks of gestation (wg) [3], with subsequent alterations of placental development and function [4–7]. Furthermore, women are not, or insufficiently, protected against malaria during this period since the recommended strategies–Intermittent Preventive Treatment (IPTp) with sulfadoxine-pyrimethamine (SP) and long-lasting insecticide treated nets (LLITNs) [8]−are usually provided beginning in the 2nd trimester.

Most studies have demonstrated malaria's deleterious effects in the first half of pregnancy on maternal and perinatal outcomes [9–12]. Only a few of these assessed malaria in the 1st trimester specifically [13–17], and some have methodological limitations. A study in the largest cohort of women in Southeast Asia found malaria in the 1st trimester of pregnancy to be strongly associated with miscarriage [14], but not with other poor birth outcomes [15].

To assess the effect of malaria in the 1st trimester on maternal and birth outcomes, a prospective cohort of women followed from preconception to delivery was established as part of the "REtard de Croissance Intra-utérin et PALudisme" (RECIPAL). We evaluated the effect of malaria infections in the 1st trimester on preterm birth (PTB), small-birthweight-for-gestational age (SGA), low birthweight (LBW) and maternal anaemia in the 3rd trimester of pregnancy.

Methods

Ethics Statement

This study was approved by the Ethics Committee of the "Institut des Sciences Biomédicales Appliquées" and the Ministry of Health in Benin. Before recruitment, the study was explained in the local language to each woman, and her voluntary consent was obtained.

Study design

The study methodology and sample size calculation have been widely described elsewhere [18]. Briefly, women of reproductive age (WRA) were recruited at the community level and followed monthly for a maximum of 24 months until becoming pregnant. The subsample of women who became pregnant was then followed up monthly at study health facilities from early pregnancy to delivery. The study started in June 2014, and follow-up was completed in August 2017. It was conducted in the districts of Sô-Ava and Abomey-Calavi, South Benin, where malaria is hyperendemic [19], and *P. falciparum* is the most common species.

Preconceptional follow-up

Demographic and socioeconomic characteristics, as well as reproductive history and anthropometric measurements, were collected at enrolment. Malaria screening using a thick blood smear (TBS) and haemoglobin (Hb) level determination were performed once at enrolment. Women were then visited at home monthly, where the first day of last menstrual period (LMP) was recorded and a urinary pregnancy test was performed.

Gestational follow-up

As soon as the pregnancy was confirmed, clinical, obstetrical, and anthropometric data were collected monthly until delivery. Pregnant women received a new LLITN at their first antenatal care (ANC) visit; its use was recorded at subsequent visits. Each month, malaria screening was performed using a TBS; proteinuria, glycosuria and urinary infection were

detected using a urine dipstick test. Besides, women were encouraged to attend the maternity clinic outside the scheduled visits if symptomatic. A TBS and a rapid diagnostic test (*P. falciparum* + pan rapid test SD Bioline Ag®, IDA foundation, the Netherlands; Biosynex®, France) were performed in case of fever or symptoms suggestive of malaria. A venous blood sample was collected in the 1st and 3rd trimesters for Hb determination. The first ultrasound scan (US) for dating the pregnancy was performed between 9−13 wg. The final gestational age (GA) estimation was based either on LMP or first US following INTERGROWTH-21st methodology [20]. TBS and rapid diagnostic test (RDT) were performed in cases of fever or malaria-like symptoms.

Newborns were weighed within 1 hour after birth on an electronic digital scale with an accuracy of 2g (SECA, Germany). Maternal, placental and cord blood was screened for malaria using TBS.

Women with uncomplicated malaria were treated with oral quinine in the 1st trimester and artemether-lumefantrine in the 2nd and 3rd trimesters. Those with severe malaria received intravenous artesunate until oral medication could be tolerated. Anaemic pregnant women were either treated with oral ferrous sulfate or transfused, depending on the severity. As recommended, IPTp administration was scheduled from the 2nd trimester onward. All medications for acute diseases during pregnancy were paid for by the project.

Laboratory procedures

The Lambaréné technique was used to quantify parasitaemia, with an estimated detection threshold of 5 parasites/µL. Slides were read by 2 qualified microscopists [21]. Hb level was measured with a HemoCue®.

Statistical analysis

Our main objective was to evaluate how the timing of malaria infection during pregnancy affected maternal and birth outcomes. The primary outcomes were: LBW (birthweight <2500 g), PTB (GA at birth <37 wg), SGA (birthweight <10th percentile for GA using INTERGROWTH-21st charts [22]), and maternal anaemia in the third trimester (Hb concentration ≤110 g/L). The secondary outcome was a composite of PTB, LBW, SGA, or stillbirth.

Malaria infection was defined as either a positive TBS or a positive RDT. Women's exposure was analyzed in two ways: (i) the occurrence of at least of one malaria infection in each trimester of pregnancy (≤14 wg, 15-27 wg, and ≥28 wg, for the 1^{st}, 2^{nd} and 3^{rd} trimester, respectively); and (ii) a composite variable including both the timing and number of malaria infections during pregnancy (women not infected during the entire pregnancy, women infected at least once in the 1^{st} trimester but not later on, women infected both in the 1^{st} trimester and in the 2^{nd} or 3^{rd} trimester, and women infected at least once in the 2^{nd} or 3^{rd} trimester but not in the 1^{st} trimester).

First, we studied the association between each maternal and birth outcome and the occurrence of malaria in the 1^{st}, 2^{nd}, and 3^{rd} trimesters using path analysis regressions [23,24]. Path analysis allowed us to take into account the chronology of malaria infections during pregnancy in order to assess both the direct and indirect effect (i.e., mediated by malaria in the 2^{nd} and 3^{rd} trimester) of malaria in the 1^{st} trimester. The potential confounding factors considered included maternal sociodemographic, medical conditions and obstetrical complications during pregnancy, nutritional status before and during pregnancy, number of ANC visits, number of IPTp intakes and use of LLITN, and rainy season at delivery. Women were classified as underweight (body mass index (BMI) before conception <18.5 kg/m²), normal weight (BMI between 18.5-24.9 kg/m²), overweight (BMI between 25-29.9 kg/m²) or obese (BMI ≥30 kg/m²). Gestational weight gain was considered normal when between 12.5-

18 kg, 11.5-16 kg, 7-11.5 kg and 5-9 kg in underweight, normal, overweight and obese women, respectively [25]. Variables were eliminated step-by-step using the backward selection procedure. Only variables whose *P* value was less than 0.05 were retained.

Secondly, we assessed the cumulative effect of malaria infections during pregnancy using the composite variable. The proportion of maternal and birth outcomes was compared between the four groups of women using chi2 (Fisher's exact) test.

Stata version 13 for Windows (Stata Corp., College Station, TX) was used for all statistical analyses.

Results

The flowchart (Figure 1) shows that 1214 WRAs were recruited: 411 (33.8%) became pregnant, 359 (29.6%) completed the preconceptional follow-up without conceiving, and 444 (36.6%) did not complete it. Of the 411 pregnant women, 273 (66.4%) completed the follow-up until delivery; most of the remaining women had either a miscarriage (17.5%) or withdrew their consent (11.2%). The median GA at miscarriage diagnosis was 7 wg. Maternal age, gravidity, education, socioeconomic status, pre-pregnancy BMI, and malaria and anaemia status before conception were similar between women who completed (n=273) and those who did not complete (n=138) follow-up until delivery (Table 1).

Women's characteristics and malaria infection before and during pregnancy

The median duration of follow-up before conception was 3.9 months (Interquartile range, 1.77-7.49). The mean age was 26.8 years and 21.3% were primi- or secundigravidae. More than half of women (57.2%) were anemic before conception and 32.4% had an abnormal BMI. The prevalence of malaria infection before conception was 6.3%.

The median GA at the 1st ANC visit was 6.4 wg (range, 2.4-19.3). Women benefited from a mean of 8.9 scheduled ANC visits; 58% were anaemic in the 3rd trimester of pregnancy. The proportion of women with at least one malaria infection, including both scheduled and unscheduled visits, was 43.1%; 22.1% of these infections were symptomatic. Forty women (14.7%) had two or more malaria infections during pregnancy. The geometric mean parasite density among infected women was 757 parasites/µL (range: 12–138,600). Women were more likely to be infected with malaria in early pregnancy than before conception (Figure 2). Malaria infection was more prevalent during the 1st trimester than in the 2nd and the 3rd trimesters (21.8% *vs.* 17.7%, and 14.6%, respectively). The risk of malaria infection decreased steadily from the 1st trimester to the end of pregnancy, with a more pronounced decrease from the middle of the 3rd trimester (Figure 2; score test for trend of odds, P=0.002). The proportion of women with malaria infection in the 1st trimester only, malaria infection in the 1st trimester and in the 2nd or 3rd trimester, and infection in the 2nd or 3rd trimester only, were 12.7%, 9.2%, and 21.5%, respectively. Placental malaria was detected in 6.4% of women.

During pregnancy, more than 97% of women declared having slept under an ITN the night before the visit; 62.9% and 13.9% of women received two and three doses of SP-IPTp, respectively. The 1st, 2nd and 3rd dose of SP-IPT were administered at a mean of 23.3 (± 4.8) wg, 29.9 (± 4.9) wg, and 34.4 (± 3.7) wg, respectively.

The prevalence of PTB, SGA, and LBW were 8.9%, 20.4% and 9%, respectively. The stillbirth rate was 19.5 per 1000 live births. Overall, 29.7% of newborns presented at least one poor birth outcome (Table 2).

Effect of malaria on maternal and birth outcomes

Placental malaria was significantly associated with LBW (aOR: 5.29; 95% CI, 1.42–19.7). Moreover, the prevalence of LBW was significantly higher among women with several malaria infections during pregnancy compared to non-infected women (30% vs. 10.8%, P=0.01).

Using path models adjusted for potential confounders, we showed a significant direct effect of malaria in the 1st trimester on maternal anaemia in the 3rd trimester (aOR: 2.25; 95% CI, 1.11–4.55) (Table 3). We did not evidence any direct effect of malaria in the 1st trimester on PTB, SGA or LBW. We also did not detect any direct effect of malaria in either the 2nd or 3rd trimester except for SGA, which was unexpectedly lower in women infected in the 3rd trimester of pregnancy.

Figure 3 presents the crude association between malarial infections by timing and number during pregnancy and adverse pregnancy outcomes. We observed that the proportion of PTB, SGA, LBW, poor birth outcome, and maternal anaemia was highest among women with several infections starting in the 1st trimester. This trend was significant for LBW (Fisher exact test, P=0.002).

Others factors associated with maternal and birth outcomes

Low maternal age, residence in the Sô-Ava district, being illiterate, low socioeconomic status, low pre-pregnancy BMI, low gestational weight gain, short stature, short birth interval, primi- or secundigravidity, and low number of IPTp doses were also significantly associated with a higher risk of poor maternal and birth outcomes (Supplementary Table S1, S2).

Discussion

To our knowledge, this is the first study to assess the effect of malaria in the 1st trimester on maternal and birth outcomes in SSA using a specific study design. Tracking women prior to

conception allowed us to detect the earliest malaria infections during pregnancy. Also, GA could be estimated by early ultrasound scan to accurately determine the timing of malaria infections. Moreover, unlike previous studies, we used path analysis to assess the direct effect of malaria in the 1st trimester on pregnancy outcomes, independently of its indirect effect mediated by malaria in the 2nd and 3rd trimesters.

We confirmed that microscopic malaria was highly prevalent in the 1st trimester compared to the preconception period, with most infections occurring before 6 wg [26]. The proportion of women infected with malaria was highest in the 1st trimester and decreased as the pregnancy progressed. This result has been reported elsewhere in SSA [27,28] and Southeast Asia [15]. In our study, the decrease in malaria prevalence from the middle of the 2nd trimester is likely explained by the administration of IPTp. It is noteworthy that this decrease started far earlier than IPTp administration. One explanation is the high proportion of LLITN use from the first ANC visit. In a previous analysis, we had shown that LLITN use in the 1st trimester was associated with a decreased risk of malaria infection [29]. Another explanation is that monthly malaria screening and immediate treatment of infected women probably contributed to the reduction of malaria prevalence throughout pregnancy. Finally, we cannot exclude the possibility that women could better control pregnancy-associated parasites as the pregnancy evolved [7,30].

We found a significant direct effect of malaria in the 1st trimester on maternal anaemia in the 3rd trimester. This result agrees with a previous study in the same area, which showed a higher risk of anaemia at delivery in women infected with malaria before 4 months of pregnancy [10]. Women infected both in the 1st trimester and later in pregnancy had the highest risk of anaemia in late pregnancy, suggesting a cumulative effect throughout pregnancy.

Our hypothesis that malaria in the 1st trimester had an independent effect on birth outcomes was based on previous studies that reported a higher risk of LBW and foetal growth restriction [9–12], as well as impaired placentation [31,32], in women infected before 4-5 months of pregnancy. Few studies have specifically assessed the effect of malaria in the 1st trimester of pregnancy, and these found no association between malarial infections in the 1st trimester and SGA, PTB or LBW [14–17], although one [14] showed a strong association with miscarriage. However, in most studies women were recruited late in the 1st trimester or early in the 2nd trimester with potential misclassifications (categorizing women infected in early pregnancy as non-infected). Additionally, women attending their first ANC visit early may have had particular characteristics such as a high education level or economic status [33,34], leading to selection bias.

In our study, we did not find evidence for any direct effect of microscopic malaria in the 1st trimester on poor birth outcomes. However, these infections appeared to contribute to the cumulative effect of malaria during pregnancy on birth outcomes. Indeed, women infected both in the 1st trimester and in the 2nd or 3rd trimester had a significantly higher risk of LBW compared to women infected in the 2nd and 3rd trimester only. This result agrees with previous findings emphasizing the cumulative effect of microscopic malaria infections [35,36].

Our study presents some limitations that should be considered. First, the analysis included only 20 (7.3%) primigravidae, who are the most likely to have poor birth outcomes related to malaria. Secondly, we recorded a high number of miscarriages, which partly contributed to the cohort attrition. At the end, birth outcomes were evaluated in only 66% of women, leading to a probable lack of power for the final analyses. Besides, we cannot exclude a survivor bias due to the exclusion of women with a miscarriage from the analysis, although preliminary results do not seem to suggest any effect of early malaria on miscarriage. Finally, regular screening and treatment of infected women may have contributed to reducing both the

exposure of women to malaria and the prevalence of malaria infections in early pregnancy. Immediate treatment of—usually undetected—asymptomatic infections, which represented nearly 80% of all infections, is likely to have attenuated the observed effect of 1st trimester infections on maternal and birth outcomes and biased our results toward the null hypothesis.

During RECIPAL follow-up, women were also screened monthly for submicroscopic infections using Polymerase Chain Reaction which have been suggested to be associated with adverse pregnancy outcomes [37–39]. Additional analyses are currently ongoing to assess the effect of submicroscopic infections, particularly those occurring in the 1st trimester.

IPTp with SP and LLITN are efficacious strategies to prevent malaria in pregnancy, but remain under implemented. In this study, only 14% of women received the three IPTp doses recommended in Benin and IPTp was generally administered late during pregnancy. Our results suggest a cumulative effect of malaria infections starting in the 1st trimester on pregnancy outcomes. These results argue in favour of starting preventive strategies against malaria from the very beginning of pregnancy. The assessment of new safe drugs that could be administered in the 1st trimester is warranted. Also, preconceptional strategies such as vaccination against VAR2CSA-parasites [40] or drug-related strategies administered before conception significant for reducing the prevalence of malaria infections in the 1st trimester.

Conflict of interest.

None declared

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Authors contributions.

A.M, M.C, and V.B conceived and designed the study. A.M and V.B analyzed the data. A.M., E.Y., G.A., G.C., M.A., B.V., D.S., N.F., A.G., Y.MP, N.FF., D.D., and V.B. contributed reagents/materials/analysis tools. A.M., M.C., and V.B. drafted and finalized the manuscript. The final manuscript was read and approved by all authors.

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Figure legends

Figure 1. Study profile

* Study completion: follow-up from enrolment until the end of the study (24-month follow-up without pregnancy for women recruited before December 2014 or monthly follow-up without pregnancy for women recruited between December 2014 and December 2016), excluding consent withdrawal, migration and lost to follow-up.

Figure 2. Variation in risk estimates (odds) of malaria throughout the pregnancy. Risk of malaria infection (solid line) and its 95% confidence interval (dash lines).

Abbreviation: BC, before conception.

Figure 3. Prevalence of poor maternal and birth outcomes according to both the timing and number of microscopic malaria infections during pregnancy. Women infected at least once in the 1st trimester but not later on (12.7%, 33/260), women infected both in the 1st trimester and in the 2nd or 3rd trimester (9.3%, 24/260), women infected at least once in the 2nd or 3rd trimester but not in the 1st trimester (21.5%, 56/260), women without malaria during the whole pregnancy (56.5%, 147/260). The statistical significance of differences between groups was determined using the non-parametric fisher exact test.; * Significant association ($P \leq 0.05$)

Table 1. General characteristics of pregnant women of RECIPAL cohort, Southern Benin, 2014-2017

Characteristics		Pregnant women with complete follow-up (n=273)	Pregnant women with incomplete follow-up (n=138)	P value[$]
		Mean[#] (± SD) or %	Mean[#] (± SD) or %	
Age (years)	All participants	26.8 (± 4.9)	26.7 (± 5.3)	0.95
	< 23 y	20.2%	23.9%	0.56
	23-30 y	60.4%	55.1%	
	> 30 y	19.4%	21.0%	
Ethnic group	Toffin	74.3%	70.3%	0.65
	Fon	7.7%	8.0%	
	Aïzo	12.9%	17.4%	
	Others [a]	5.1%	4.3%	
Education	Illiterate	71.4%	68.8%	0.65
Socioeconomic status*	Low	34.8%	32.6%	0.43
	Middle	38.5%	44.9%	
	High	26.7%	22.5%	
Gravidity	1	7.4%	9.4%	0.16
	2	13.9%	20.3%	
	≥3	78.7%	70.3%	
ITN possession	Yes	97.1%	95.7%	0.57
Pre-pregnancy BMI (kg/m²)	All participants	22.8 (± 4.2)	23.5 (± 4.7)	0.14
	< 18.5	9.2%	10.1%	0.14
	18.5-25	67.6%	58.0%	
	≥ 25	23.2%	31.9%	
Anaemia before conception	Yes	57.2%	48.5%	0.12
Median (range) gestational age at the first ANC visit (weeks) [b]	All participants	6.4 (2.4-19.3)	6.2 (2.3-15.4)	0.42
Number of ANC visits during pregnancy [c]	All participants	8.9 (± 1.8)	-	-
Number of unscheduled ANC visits	All participants	2.1 (± 1.3)	-	-

Table 1. General characteristics of pregnant women of RECIPAL cohort, Southern Benin, 2014-2017 (Continued)

Characteristics		Pregnant women with complete follow-up (n=273) Mean[#] (± SD) or %	Pregnant women with incomplete follow-up (n=138) Mean[#] (± SD) or %	P value[$]
Number of IPTp doses	All participants	1.8 (± 0.7)	-	-
	0	3.7%	-	-
	1	19.5%	-	-
	2	62.9%	-	-
	≥ 3	13.9%	-	-
HIV status	Positive	1.5%	1.4%	0.87
Anaemia in the 3rd trimester of pregnancy	Yes	58.1%	-	-
Anaemia during pregnancy	≥ 1 episode(s)	69.5%	-	-
Gestational weight gain[†]	Adequate	28.7%		
	Lower than recommended	62.1%		
	Higher than recommended	9.2%		
Gestational hypertension	≥ 1 episode(s)	2.6%	-	-
Short stature (height < 155 cm)	Yes	27.9%	31.2%	0.49
Malaria infection before conception	Yes	6.3%	5.1%	0.82
Malaria infection during pregnancy	≥ 1 episode(s)	43.1%	-	-
	1st trimester	21.8%	-	-
	2nd trimester	17.7%	-	-
	3rd trimester	14.6%	-	-
Clinical malaria infection during pregnancy [d]	≥ 1 episode(s)	22.1%	-	-
Placental malaria infection	Yes	6.4%	-	-

Abbreviations: SD, standard deviation; IQR, interquartile range; ITN, insecticide-treated bed net; BMI, body mass index; ANC visit, antenatal care visit.
$ Student's t-test and $\chi 2$ test were used for comparing continuous and categorical variables, respectively
# Arithmetic mean
* Socioeconomic status was approximated using a synthetic score combining occupation and ownership of assets, which was then categorized according to the tertiles.
† Gestational weight gain was considered as adequate when the total weight gain during pregnancy was between 12.5-18 kg, 11.5-16 kg, 7-11.5 kg and 5-9 kg in underweight women (pre-pregnancy BMI < 18.5 kg/m²), normal weight women (pre-pregnancy BMI between 18.5 and 24.9 kg/m²), overweight women (pre-pregnancy BMI between 25.0 and 29.9 kg/m²) and obese women (pre-pregnancy BMI ≥30 kg/m²), respectively. Above and under these ranges, it was considered as "higher than recommended" and "lower than recommended", respectively (IOM guidelines, 2009)
(a) Other ethnic groups: Yoruba, Adja, Goun, Ahoussa, Cotafon, Mahi, Sahoue; **(b)** Nonparametric equality-of-medians test was used for comparison. Gestational age was estimated using ultrasound scan or last menstrual period; **(c)** Including both scheduled and unscheduled visits; **(d)** Positive thick blood smear or rapid diagnostic test with an axillary temperature ≥ 37.5°C or history of fever in the last 24 hours

Table 2. Characteristics at birth of the 273 newborns* included in the analysis. RECIPAL cohort, Southern Benin, 2014-2017

Characteristics		**Mean[#] (± SD) or %**
Gender	Male	52.9%
Stillbirth	Per 1000 live births	19.5
Preterm birth (< 37 weeks)[£]	Yes	8.9%
Small birthweight for gestational age[‡ †]	Yes	20.4%
Birthweight (g)[‡]		3028.7 (± 414.2)
	< 2500	9.0%
Birth length (cm)		48.3 (± 2.6)
Birth head circumference (cm)		34.0 (± 1.5)
Positive thick blood smear in cord blood	Yes	0.9%
Poor birth outcome[‡ §]	Yes	29.7%

* Low birthweight and small birthweight for gestational age (N=256), preterm birth (N=268), poor birth outcome (N=273)
# Arithmetic mean.
£ Twins included
‡ Stillbirths and twins were excluded for estimating the prevalence of low birthweight and small birthweight for gestational age.
† Small birthweight for gestational age: < 10th percentile of birthweight for gestational age using INTERGROWTH-21st charts.
§ Stillbirth, preterm birth, small birthweight for gestational age or low birthweight.

Table 3. Effect of malaria according to the timing of infections during pregnancy on maternal anaemia and poor birth outcomes. Multivariate analysis using path analysis*

		Univariate analysis			Multivariate analysis		
Microscopic malaria infection	**% of maternal anaemia[&#]**	OR	95% CI	*P*	aOR	95% CI	*P*
1st trimester of pregnancy							
No (N= 204)	54.9	1			1		
Yes (N= 58)	74.1	2.35	(1.23, 4.51)	0.01	2.25	(1.11, 4.55)	0.02
2nd trimester of pregnancy							
No (N = 220)	57.3	1			1		
Yes (N = 47)	63.8	1.32	(0.69, 2.53)	0.41	1.01	(0.49, 2.03)	0.99
3rd trimester of pregnancy							
No (N = 227)	56.8	1			1		
Yes (N = 38)	71.1	1.86	(0.88, 3.94)	0.10	1.44	(0.65, 3.19)	0.37
Microscopic malaria infection	**% of preterm birth[†]**	OR	95% CI	*P*	aOR	95% CI	*P*
1st trimester of pregnancy							
No (N= 204)	8.3	1			1		
Yes (N= 58)	10.3	1.27	(0.48, 3.38)	0.63	0.93	(0.34, 2.56)	0.90
2nd trimester of pregnancy							
No (N= 220)	9.1	1			1		
Yes (N= 47)	8.5	0.93	(0.30, 2.86)	0.90	0.77	(0.24, 2.46)	0.66
3rd trimester of pregnancy							
No (N= 227)	9.2	1			1		
Yes (N=38)	7.9	0.84	(0.24, 2.97)	0.79	0.60	(0.16, 2.21)	0.44
Microscopic malaria infection	**% of SGA[‡]**	OR	95% CI	*P*	aOR	95% CI	*P*
1st trimester of pregnancy							
No (N= 196)	21	1			1		
Yes (N= 54)	18.5	0.85	(0.40, 1.84)	0.67	0.72	(0.31, 1.66)	0.43
2nd trimester of pregnancy							
No (N= 212)	19.4	1			1		
Yes (N= 43)	25.6	1.42	(0.66, 3.96)	0.36	1.35	(0.58, 3.1)	0.48
3rd trimester of pregnancy							
No (N= 215)	22.8	1			1		
Yes (N= 38)	8.1	0.30	(0.09, 1.01)	0.06	0.24	(0.06, 0.86)	0.03

Table 3. Effect of malaria according to the timing of infections during pregnancy on maternal anaemia and poor birth outcomes. Multivariate analysis using path analysis* (Continued)

		Univariate analysis			Multivariate analysis		
Microscopic malaria infection	**% of low birthweight§**	OR	95% CI	*P*	aOR	95% CI	*P*
1st trimester of pregnancy							
No (N= 196)	8.7	1			1		
Yes (N= 54)	11.1	1.30	(0.49, 3.50)	0.59	1.10	(0.38, 3.0)	0.90
2nd trimester of pregnancy							
No (N= 212)	9.0	1			1		
Yes (N= 43)	9.3	1.03	(0.33, 3.21)	0.95	0.78	(0.24, 2.57)	0.69
3rd trimester of pregnancy							
No (N= 215)	9.3	1			1		
Yes (N= 38)	8.1	0.86	(0.24, 3.05)	0.82	0.65	(0.17, 2.43)	0.52
Microscopic malaria infection	**% of poor birth outcome£$**	OR	95% CI	*P*	aOR	95% CI	*P*
1st trimester of pregnancy							
No (N= 208)	21.6	1			1		
Yes (N= 58)	21.1	0.97	(0.50, 1.87)	0.93	0.83	(0.41, 1.68)	0.61
2nd trimester of pregnancy							
No (N= 223)	15.8	1			1		
Yes (N= 48)	20.5	1.38	(0.70, 2.71)	0.35	1.24	(0.61, 2.56)	0.55
3rd trimester of pregnancy							
No (N= 228)	17.0	1			1		
Yes (N= 39)	9.2	0.49	(0.20, 1.18)	0.11	0.44	(0.18, 1.09)	0.08

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; SGA, small birthweight for gestational age

* Influence of the timing of malaria infections was assessed on 256 pregnant women for low birthweight and SGA, on 268 pregnant women for preterm birth and maternal anaemia and on 273 pregnant women for poor birth outcome. The multivariate analysis was adjusted for the number of antenatal care visits using an "offset option". Reference class was absence of malaria infection.

& Maternal anaemia in the 3rd trimester of pregnancy was defined as a haemoglobin level < 110 g/L ; it was assessed at a mean of 33 weeks of gestation (range 24.2-40.4)

Adjusted for maternal age, education, residence area, gravidity, household density, birth interval, number of antenatal care visits, anaemia in the 1st trimester of pregnancy and socioeconomic status.

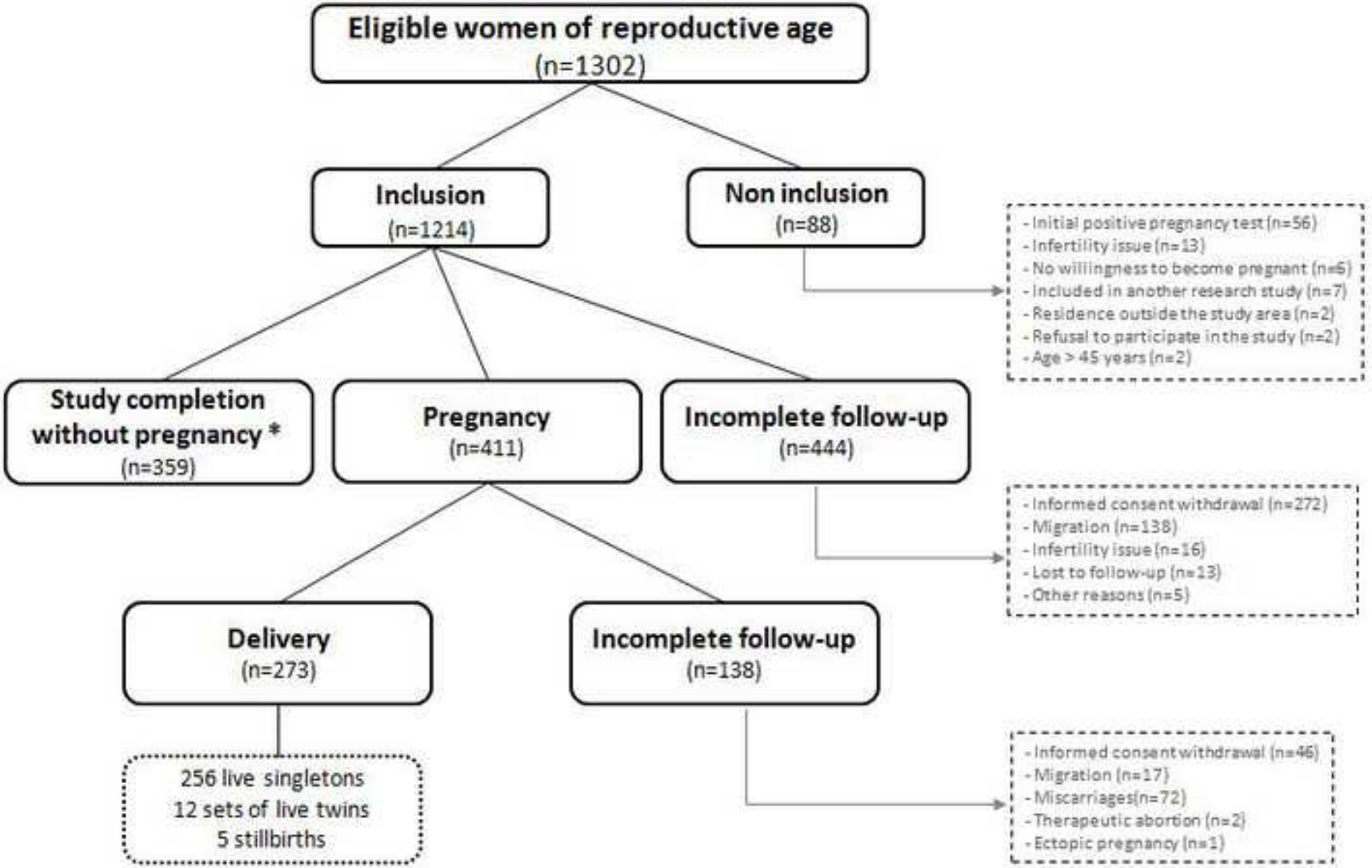
† Adjusted for residence area, socioeconomic status, maternal nutritional status, urinary infection and number of IPTp intakes.

‡ Adjusted for residence area, maternal age, marital status, education, maternal short stature, birth interval, maternal anaemia and number of antenatal care visits.

§ Adjusted for maternal age, gravidity, birth interval, pre-pregnancy body mass index, newborn's sex and number of antenatal care visits

£ Adjusted for residence area, maternal age, birth interval, maternal short stature, maternal anaemia, number of IPTp doses and number of antenatal care visits

$ Stillbirth, preterm birth, SGA (using INTERGROWTH-21st charts) or low birthweight.



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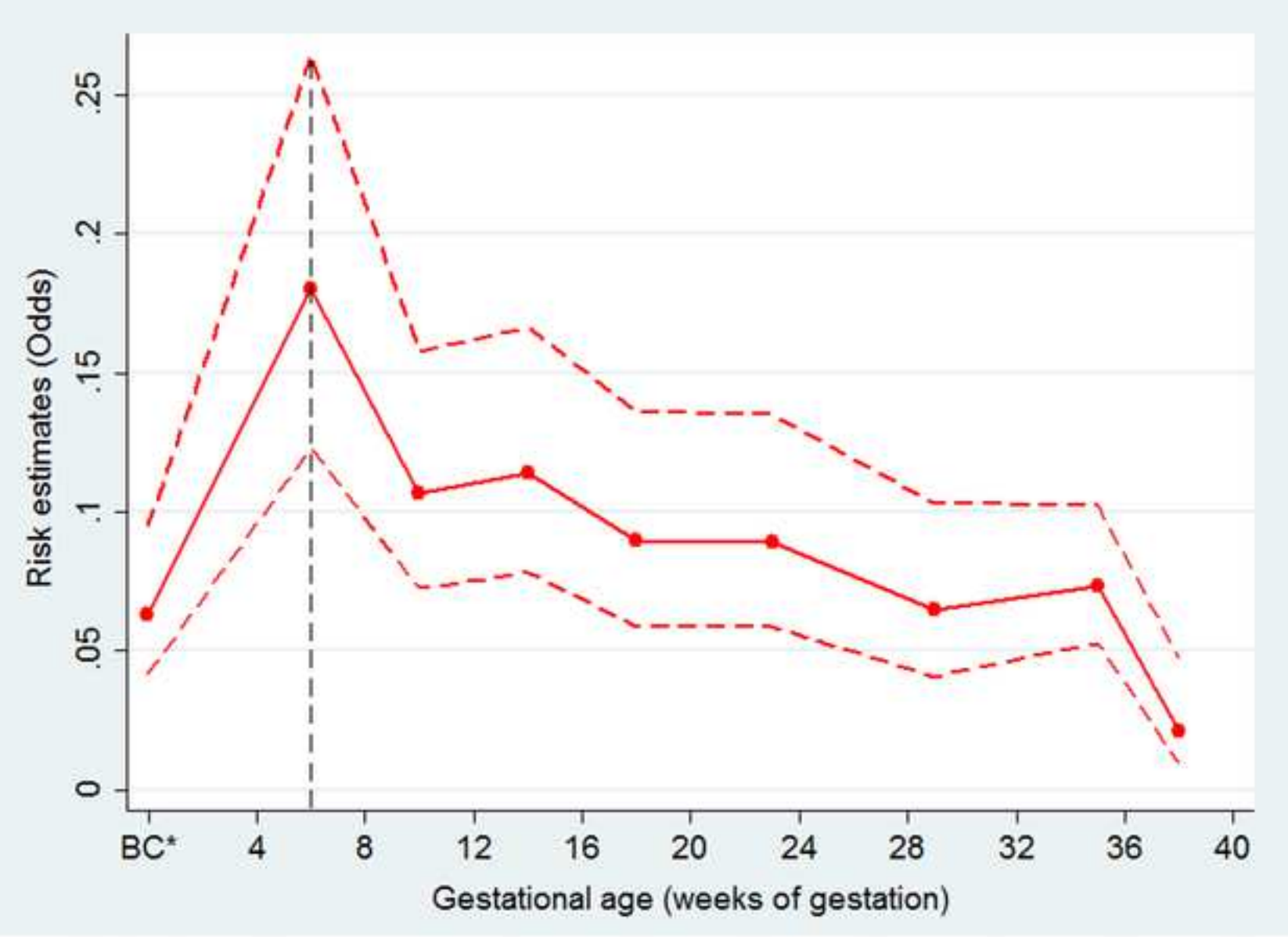
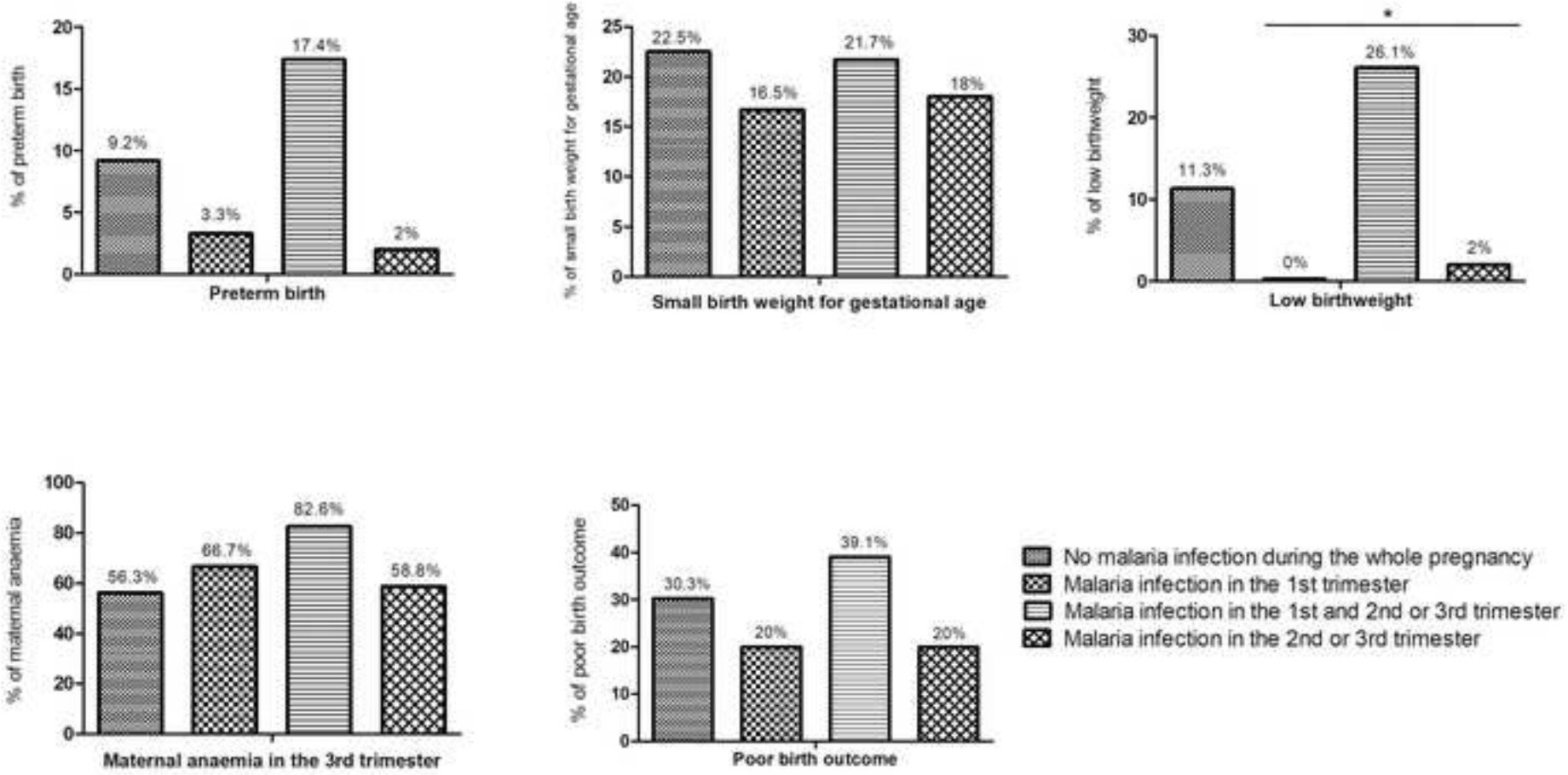


Gestational age	BC	6	10	14	18	23	29	35	38
Cases	24	31	28	30	24	24	19	38	6
Controls	382	172	262	263	268	270	293	517	285
Odds	0.06	0.18	0.11	0.11	0.09	0.08	0.06	0.07	0.02
Lower odds	0.04	0.12	0.07	0.08	0.06	0.06	0.04	0.05	0.01
Upper odds	0.09	0.26	0.16	0.17	0.14	0.13	0.10	0.10	0.04
Odds ratio	1	2.87	1.70	1.81	1.42	1.41	1.93	1.17	0.33



Supplementary Table S1. Additional analysis. Logistic regression on maternal and sociodemographic factors associated with maternal anaemia in the 3rd trimester of pregnancy (N=273)

Factors		**% of maternal anaemia**[&]	Univariate analysis			Multivariate analysis[£]		
			OR	**95% CI**	***P***	**aOR**	**95% CI**	***P***
Maternal age (years)								
	23-30	50.9%	1			1		
	< 23	77.8%	**3.37**	**1.65-6.87**	**0.003**	**3.48**	**1.62-7.47**	**0.006**
	> 30	60.4%	1.47	0.78-2.76		1.37	0.68-3.74	
Residence area								
	Akassato	42.6%	1					
	Sô-Ava	63.5%	**2.34**	**1.34-4.09**	**0.003**			
Education level								
	Literate	48.1%	1			1		
	Illiterate	62.3%	**1.79**	**1.04-3.05**	**0.03**	**1.82**	**1.01-3.31**	**0.05**
Gravidity								
	Multigravidae	56.8%	1					
	Primigravidae	75.0%	2.28	0.80-6.45	0.12			
Birth interval								
	> 12 months (in multigravidae)	54.1%	1					
	0-12 months (in multigravidae)	56.2%	0.92	0.45-1.85	0.10			
	No previous pregnancy (primigravidae)	76.7%	2.56	1.05-6.23				
Socioeconomic status								
	High	50.0%	1					
	Middle	54.9%	1.21	0.66-2.23	**0.05**			
	Low	68.1%	**2.13**	**1.13-4.02**				
Household density*								
	< 5	52.5%	1					
	≥ 5	64.6%	**1.65**	**1.01-2.70**	**0.05**			
Anaemia in the 1st trimester of pregnancy								
	No	44.4%	1			1		
	Yes	75.0%	**3.75**	**2.21-6.36**	**<0.001**	**3.63**	**2.09-6.30**	**<0.001**

Supplementary Table S1 (continued)

Factors		% of maternal anaemia[&]	Univariate analysis			Multivariate analysis		
			OR	**95% CI**	***P***	**aOR**	**95% CI**	***P***
Malaria during pregnancy								
	No	50.9%	1			1		
	≥ 1 episode(s)	67.8%	**2.03**	**1.22-3.35**	**0.01**	**1.94**	**1.11-3.37**	**0.02**
Timing of malaria infections								
	No infection	54.4%	1					
	In the 1st trimester only	69.7%	1.92	0.86-4.33	**0.05**			
	In the 2nd or 3rd trimester only	59.3%	1.22	0.65-2.29				
	In the 1st trimester and 2nd or 3rd trimester	83.3%	**4.18**	**1.36-12.8**				

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval

£ Final model after backward selection procedure

* Household density: number of people living in the household

& Maternal anaemia in the 3rd trimester of pregnancy was defined as an haemoglobin level < 110 g/L

Supplementary Table S2. Additional analysis. Logistic regression on maternal and gestational factors associated with low birthweight (N=256)

Factors		**% of LBW**	Univariate analysis			Multivariate analysis		
			OR	**95% CI**	***P***	**aOR**	**95% CI**	***P***
Maternal age (years)	23-30	7.2%	1					
	< 23	17.0%	2.62	1.02-6.74	0.08			
	> 30	6.0%	0.82	0.22-3.05				
Gravidity	Multigravidae	8.1%	1					
	Primigravidae	20.0%	2.84	0.86-9.35	0.09			
Birth interval	> 12 months (in multigravidae)	6.8%	1			1		
	0-12 months (in multigravidae)	8.6%	1.28	0.35-4.76	**0.02**	1.24	0.25-6.09	**0.04**
	No previous pregnancy (primigravidae)	24.1%	**4.36**	**1.57-12.1**		**4.17**	**1.33-13.0**	
Pre-pregnancy BMI (kg/m²)	18.5-25	9.7%	1					
	≤ 18.5	18.2%	2.06	0.63-6.81	0.13			
	≥ 25	3.4%	0.33	0.07-1.48				
Gender	Male	6.6%	1					
	Female	26.7%	**5.12**	**1.44-18.1**	**0.01**			
Placental malaria	No	6.6%	1			1		
	Yes	26.7%	**5.12**	**1.44-18.1**	**0.01**	**5.29**	**1.42-19.7**	**0.01**
Timing of malaria infection	None	11.3%	1					
	In the 1st trimester only	-	-	-				
	In the 2nd or 3rd trimester only	2.0%	0.17	0.02-1.24	**0.02**			
	In the 1st trimester and 2nd or 3rd trimester	26.1%	**2.78**	**0.96-8.07**				

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; BMI, body mass index

- No case of LBW among women infected in the first trimester only