Malaria during pregnancy and newborns outcome in an unstable transmission area in Brazil: a population-based record linkage study

Jamille Gregório Dombrowski, BSc[a], Rodrigo Medeiros de Souza, PhD[b], Natércia Regina Mendes Silva, BSc[a], André Barateiro, MSc[a], Sabrina Epiphanio, PhD[c], Lígia Antunes Gonçalves, PhD[a,#,*], Cláudio Romero Farias Marinho, PhD[a,#,*]

[a] Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

[b] Multidisciplinary Center, Federal University of Acre, Acre, Brazil

[c] Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

[#] These authors are joint senior authors on this work.

[*] Corresponding authors:

Cláudio Romero Farias Marinho, PhD

e-mail: marinho@usp.br

Lígia Antunes Gonçalves, PhD

e-mail: lig.antunes.goncalves@gmail.com

[*] Address correspondence to Cláudio R. F. Marinho

University of Sao Paulo - USP; Av. Prof. Lineu Prestes, 1374; São Paulo - SP – Brazil - 05508-900;

Fone: +55(11)30917989 (Laboratory); +55(11)30917417 (Fax)

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ABSTRACT

Background: Malaria during pregnancy is one of the major causes of mortality in tropical regions, causing maternal anemia, intrauterine growth retardation, preterm birth, and low birth weight (LBW). The integration of the information systems is crucial to assess the dimension of gestational malaria in a wide and useful way, to improve decision making and maternal-child health.

Methods and Findings: An observational population-based study acquired information retrospectively from all live births that occurred between 2006 and 2014 in Cruzeiro do Sul (Acre, Brazil). Social and clinical data of the mother and newborn was extracted from the Information System of Live Births. Malaria episodes information was obtained from the Brazilian Epidemiological Surveillance Information System Malaria. A deterministic record linkage was performed to assess malaria impact on pregnancy. The studied population presented a malaria incidence of 8.9%, of which 63.9% infected by *Plasmodium (P.) vivax*. Reduction of newborns birth weight at term (small for gestational age (SGA) and LBW) has been found associated with *P. vivax* infection during pregnancy (SGA - OR 1.24, 95% CI 1.02-1.52, p=0.035; term LBW - OR 1.39, 95% CI 1.03-1.88, p=0.033). Additionally, *P. falciparum* infection during pregnancy has been found to be associated with preterm births (OR 1.54, 95% CI 1.09-2.18, p=0.016), which is related with late preterm births (OR 1.59, 95% CI 1.11-2.27, p=0.011).

Conclusions: Despite the decrease of malaria cases during the evaluated period, we present evidence of the deleterious effects of gestational malaria in a low transmission area in the Amazonian region. Regardless of *Plasmodium* species, malaria during pregnancy poses a risk for newborns birth weight reduction, highlighting the impact that *P. vivax* has on the fetus.

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INTRODUCTION

Malaria is a severe and potentially fatal parasitic disease that constitutes a major public health issue, being one of the greatest causes of mortality in tropical regions. Pregnant women are particularly vulnerable to malaria infection and are estimated that 125 million women are at risk of malaria in pregnancy each year [1]. Malaria can be devastating for both mother and fetus, leading up to 10,000 maternal and 75,000 to 200,000 child deaths each year [2]. Maternal malaria presents a significant impact on the neonates, being the primary cause of abortion, stillbirth, premature delivery, fetal death, low birth weight (LBW) and fetal/child development retardation in malaria-endemic countries [2].

LBW reflects an intra-uterine growth retardation (IUGR) and preterm delivery, which are compelling indicators of infant morbidity [2–5]. LBW has been linked to infant mortality and poor cognitive development, and the occurrence of non-communicable diseases later in life [5,6]. In fact, LBW in newborns due to malaria is related with up to 100,000 infant deaths each year in endemic countries [7,8]. These adverse birth outcomes have been extensively associated with *P. falciparum* infection during pregnancy. In contrast to *P. falciparum*, the *P. vivax* burden in pregnancy is less well described, and have been described as having less impact in the newborn [2,9]. Though, recent studies have presented the two species as similar threats to the mother and fetus [10]. Despite the efforts to reduce malaria the prevalence of these adverse birth outcomes remains high.

Therefore, it is crucial to have an efficient epidemiological surveillance of malaria during pregnancy. The linkage of two or more health public surveillance record databases with shared variables presents an important and effective strategy to plan preventive measures. Currently, most of the malaria-endemic countries have malaria public surveillance record databases since it is compulsory notification disease. This will contribute to the identification of epidemics and areas most affected. Thus, allowing to direct and intensify the control and preventive measures to the

affected communities, and reduce negative birth outcomes [11]. In fact, due to the potential assemble with other information systems it can be recognized as an important tool for research [12,13,14].

In 2003, the Brazilian Epidemiological Surveillance Information System (SIVEP)-Malaria was implemented to systematize the flow and quality of the information on malaria. This system gathers information on malaria morbidity according to gender, age, *Plasmodium* species, site of residence, the probable site of infection, treatment, and pregnancy status [15]. Another essential Brazilian information system of national coverage is the Information System of Live Births (SINASC), implemented in 1990. This system collects and systematizes information on maternal, pregnancy, delivery and newborns data [16,17]. Linkage of record databases is still scarcely used in Brazil, despite being an easy to perform technique with low operational cost. Here we present the first study that evaluates the association between gestational malaria and adverse birth outcomes in the Brazilian Amazonian region for nine years (2006-2014), using information obtained through the linkage of SINASC and SIVEP-Malaria.

METHODS

Study design and data collection

This is a population-based observational study developed in the city of Cruzeiro do Sul - Acre (Brazil), located in the Brazilian Amazonian region (7°37'51''S, 72°40'12''W) (Fig 1). Cruzeiro do Sul has an estimated population of 82,075 inhabitants and an average of 1,650 births per year [18]. Together with Porto Velho and Manaus, the three cities are responsible for 21.9% of the malaria cases notified in Brazil [19]. It is a high transmission risk city, with an annual parasitic incidence of 214 cases per 1,000 inhabitants, with the prevalence of *P. vivax* infection [19]. The universe of the studied population was composed of all live newborns delivered by women living in the city, between January 2006 and December 2014. The information regarding the mother, newborn and delivery was extracted from SINASC, and the information on the malaria episodes and parasite species was obtained from the SIVEP-Malaria. By knowing that the primary health care is provided free of cost in Brazil and that the information systems of the Ministry of Health (MoH) offer wide coverage, we can presume that these datasets are reliable.

Figure 1. Map showing the geographic location of Cruzeiro do Sul, Acre State, Brazilian Amazon. Cruzeiro do Sul has an estimated population of 82,075 inhabitants. The map also indicates Rio Branco, the capital of Acre state.

Ethical considerations

According to the Resolution nº 196/96 of the Brazilian National Health Committee, ethical clearance was provided by the committees for research of the University of São Paulo and the Federal University of Acre (Plataforma Brasil, CAAE: 03930812.8.0000.5467 and 03930812.8.3001.5010, respectively). The authors have agreed to maintain the confidentiality of the

data collected from the medical records and databases, by signing the Term of Commitment for the Use of Data from Medical Records.

Exclusion criteria

In this study, the SINASC database was considered the reference. Before performing the record linkage, curation was performed, and newborns with double entries, lack of information on birth weight, presenting congenital diseases or twins were excluded. Upon the linkage of SINASC with SIVEP-Malaria database, newborns with less than 22 weeks (miscarriage) or with no information on the gestational age at birth were excluded (Fig 2).

Figure 2. Flowchart detailing exclusion criteria applied to the evaluation of the enrolled maternal-child pairs. Mixed infection – *P. vivax*- and *P. falciparum*-infection occurring at the same time and/or at different times during pregnancy.

Screening of malaria infection

In Brazil, whenever individual show suspicious malaria symptoms, it is tested by qualified endemic agents that monitor micro-regions. The gold standard method for malaria diagnosis is the thin and thick blood smear, which is screened by trained microscopists from the System of Epidemiological Surveillance of the MoH, and further revised by senior experts, to confirm the results. Infections were categorized per parasite species: *P. falciparum*, *P. vivax,* or mixed infections. All women who had malaria during pregnancy were treated with antimalarial drugs under medical prescription, according to the Brazilian MoH guidelines.

Definitions and gestational age estimation

LBW was defined as birth weight < 2500 grams (g). WHO child growth standards were used to classify the small for gestational age newborns, weight ≤ 10^{th} centile (boys ≤ 2758 g, girls ≤ 2678g). The very preterm birth was defined as birth between ≥28 and <32 weeks' gestation; late preterm birth was defined as birth between ≥32 and <37 weeks' gestation, and total preterm birth was defined as birth <37 weeks' gestation. The gestational age was established by the woman's last menstrual period and, when possible, adjusted by ultrasound during antenatal visit care. In SINASC database, gestational age is categorized as follows: less than 22 weeks' gestation, 22-27 weeks, 28-31 weeks, 32-36 weeks, 37-41 weeks, and 42 weeks or more.

Record linkage strategy

The record linkage was performed by using the RecLink III software through the deterministic method (manual search). For the data preprocessing, standardization of both databases was performed by withdrawing accentuations, extra spaces, special characters, and prepositions. After, databases were unified only by two shared variables that presented the appropriate fulfillment. Each year of the record linkage corresponds to one year of the SINASC (containing births records) assembled with two years of the SIVEP-Malaria, to identify all malaria cases presented by women during pregnancy. The linked database gathered the variables from SINASC (mother age, gestational age at delivery, parity, number of antenatal visits, birth weight, and type of birth), with variables from SIVEP-Malaria (infection by *Plasmodium* spp. (yes / no) and parasite species).

Statistical analysis

Data were extracted into Microsoft Excel, and Stata 14.2 and GraphPad Prism software were used for statistical analyses. We used descriptive statistics to assess the distribution of all continuous (means and standard deviation [SD] or median and interquartile ranges [IQR]), and categorical (frequencies and percentages) variables. Differences between groups were evaluated using Mann-

158 Whitney U-tests, accordingly. Categorical data and proportions were analyzed using chi-square
159 tests. Every p values were 2-sided at a significance level of <0.05. To assess the association
160 between malaria and birth weight reduction or prematurity, adjusted odds ratios (OR) with 95%
161 confidence intervals (CI) were estimated using a multivariate logistic regression approach. These
162 models included infections by malaria (yes / no), maternal age (≥ 18 years old / ≤ 17 years old),
163 gravidity (primigravida / multigravida), and years of formal education (≥ 4 years / ≤ 3 years) as
164 explanatory variables, and birth weight [≤ 10^{th} centile] (yes / no) or LBW (yes / no) as response
165 variables. The first category for each explanatory variable was considered as reference [20].
166
167 **Role of the funding source**
168 The funders of this study played no part in the study design, data collection, data analysis, data
169 interpretation, or writing of the manuscript. The corresponding author had full access to all the data
170 in the study and had final responsibility for the decision to submit for publication.

171 **RESULTS**

172 **Study Population and Baseline Characteristics**

173 Between January 2006 and December 2014, 16,444 births occurred in Cruzeiro do Sul (Acre) with a
174 total of 3,021 malaria cases notified during pregnancy. After applying the exclusion criteria, 14,487
175 maternal-child pairs remained for further analysis (Fig 2). Table 1 shows maternal characteristics
176 according to infection status (detailed by year in the S1 Table). To highlight that: circa 35% of
177 women were primigravida; above 40% had at least 8 years of formal education (despite the high
178 proportion of no-schooling women); and more than 70% had a minimum of four antenatal visits
179 (Table 1). Nevertheless, it was possible to observe that there were no major differences between
180 non-infected and infected mothers. Malaria incidence in the studied population was 8.9%, with *P.*
181 *vivax* contributing to 63.9% of the cases (Fig 2). Time series of malaria cases in pregnant women
182 allowed to detect three epidemic peaks along the studied period, one in 2007 with more than 500
183 cases, and other two in 2010 and 2013 (Fig 3A and S2 Table). Interestingly, the significant
184 reduction of cases from 2007 to 2008 coincides with the introduction of artemisinin combined
185 therapy in Brazil [15]. Though, *P. falciparum* infections represented on average more than 30% of
186 cases reported during pregnancy, in the assessed years (S2 Table).
187

Table 1. Baseline characteristics of mothers at delivery.

Characteristics	Non-infected (N=13,204)	Malaria [a] (N=1,283)	*P. vivax* (N=820)	*P. falciparum* (N=350)	Mixed (N=113)
Maternal age (years), mean (SD) [b]	24.3 ± 6.4	23.3 ± 6.0	23.3 ± 5.9	24.0 ± 6.3	21.9 ± 5.8
Primigravida, no. (%) [c]	4798 (36.3)	439 (34.2)	299 (36.5)	98 (28.0)	42 (37.2)
Male newborns, no. (%)	6860 (52.0)	675 (52.6)	425 (51.8)	189 (54.0)	61 (54.0)
Gestational age, no. (%)					
22-27 weeks	15 (0.1)	3 (0.2)	3 (0.4)	0	0
28-31 weeks	69 (0.5)	12 (0.9)	9 (1.1)	2 (0.6)	1 (0.9)
32-36 weeks	884 (6.7)	97 (7.6)	52 (6.3)	35 (10.0)	10 (8.9)
37 weeks or more	12236 (92.7)	1171 (91.3)	756 (92.2)	313 (89.4)	102 (90.2)
Years of formal education, no. (%) [d,f]					
No formal education	1894 (14.5)	215 (16.9)	117 (14.4)	75 (21.7)	23 (20.5)
1-3 years	1204 (9.2)	120 (9.5)	73 (9.0)	35 (10.1)	12 (10.7)
4-7 years	2866 (22.0)	338 (26.6)	219 (27.0)	90 (26.1)	29 (25.9)
8-11 years	5041 (38.6)	467 (36.8)	308 (37.9)	122 (35.4)	37 (33.0)
12 or more	1990 (15.2)	122 (9.6)	91 (11.2)	21 (6.1)	10 (8.9)
Antenatal care visit, mean (SD) [e,f]					
None	1295 (9.9)	86 (6.7)	39 (4.8)	39 (11.1)	8 (7.1)
1-3 visits	2100 (16.0)	211 (16.5)	130 (16.0)	65 (18.6)	16 (14.3)
4-6 visits	4004 (30.5)	417 (32.7)	281 (34.5)	109 (31.1)	27 (24.1)
7 or more	5676 (43.2)	560 (43.9)	363 (44.6)	136 (38.9)	61 (54.5)

N, number of individuals; SD, standard deviation; no., number of events.

[a] Malaria group consists of total pregnant women who had an infection (*P. falciparum* and *P. vivax* or both).

[b] Maternal age was recorded in 13,202 non-infected pregnant women.

[c] Information generated based on the number of live births and deaths, reported by the mother.

[d] Years of formal education were recorded in 13,054 non-infected and 1,269 infected pregnant women (812 *P. vivax* and 345 *P. falciparum*).

[e] Antenatal visits were recorded in 13,138 non-infected and 1,276 infected pregnant women (814 *P. vivax*).

[f] Theses variables have ignored values.

Figure 3. Time-series of gestational malaria cases between 2006-2014. (A) Number of gestational malaria cases per species, (B) mean birth weight of newborns from non-infected and infected women during pregnancy.

Association of gestational malaria with reduction of the newborns' birth weight

The analysis of the newborns birth weight across the nine years period, allowed to observe a significant reduction in the mean weight of babies born from women that had malaria during pregnancy (Fig 3B, Table 2, and S3 Table). Newborns from *P. falciparum*-infected mothers presented a more prominent difference of approximately 150 g ($p<0.0001$) when compared to newborns from non-infected women (Table 2). Notably, the comparison of each group by year evidenced that newborns from *P. vivax*-infected mothers showed higher weight reduction when compared with non-infected (S3 Table). These differences can be explained by the higher prevalence of newborns with LBW among *P. vivax*-infected women (term LBW: NI 4.8%, Pv 6.5%, $p=0.031$; all LBW: NI 6.8%, Pv 8.9%, $p=0.020$) (Table 2 and S4 Table). Although this prevalence occurred throughout the assessed years, it was more evident in 2006 and 2013 (S4 Table).

Table 2. Clinical outcomes of newborns at birth.

Characteristics	Non-infected (N=13,204)	Malaria [a] (N=1,283)	p value [b]	*P. vivax* (N=820)	p value [b]	*P. falciparum* (N=350)	p value [b]	Mixed (N=113)	p value [b]
All birth weight (g)			<0.0001		<0.0001		<0.0001		0.0002
Mean (SD)	3200.1 ± 514.7	3090.1 ± 524.2		3118.9 ± 532.4		3049.9 ± 506.7		3005.8 ± 503.9	
Median (IQR)	3215.0 (2900.0-3530.0)	3100.0 (2785.0-3420.0)		3132.5 (2800.0-3447.5)		3030.0 (2780.0-3350.0)		3095.0 (2690.0-3330.0)	
Term birth weight (g) [c]			<0.0001		<0.0001		<0.0001		0.004
Mean (SD)	3236.1 ± 482.2	3139.6 ± 471.6		3159.5 ± 486.0		3106.4 ± 447.9		3094.4 ± 426.9	
Median (IQR)	3240.0 (2940.0-3550.0)	3130.0 (2840.0-3430.0)		3150.0 (2842.5-3452.5)		3060.0 (2850.0-3380.0)		3155.0 (2770.0-3348.0)	
All low birth weight, no. (%)	896 (6.8)	120 (9.4)	0.001	73 (8.9)	0.020	31 (8.9)	0.130	16 (14.2)	0.002
Term low birth weight, no. (%) [c]	581 (4.8)	74 (6.3)	0.017	49 (6.5)	0.031	17 (5.4)	0.575	8 (7.8)	0.144
Prematurity, no. (%)	968 (7.3)	112 (8.7)	0.069	64 (7.8)	0.614	37 (10.6)	0.022	11 (9.7)	0.330
Very preterm birth, no. (%)	69 (7.1)	12 (10.7)	0.058	9 (14.1)	0.032	2 (5.4)	0.901	1 (9.1)	0.596
Late preterm birth, no. (%)	884 (91.3)	97 (86.6)	0.239	52 (81.3)	0.694	35 (94.6)	0.015	10 (90.9)	0.362
Very preterm birth weight (g)			0.868		0.863		0.627		0.638
Mean (SD)	2036.3 ± 722.2	2032.3 ± 625.5		1978.9 ± 692.0		2112.5 ± 576.3		2353.0	
Median (IQR)	1880.0 (1460.0-2575.0)	2045.0 (1532.5-2547.5)		1915.0 (1360.0-2575.0)		2112.5 (1705.0-2520.0)		2353.0	
Late preterm birth weight (g)			0.093		0.838		0.164		0.0009
Mean (SD)	2814.1 ± 621.6	2683.1 ± 677.2		2839.0 ± 645.6		2598.6 ± 703.5		2167.5 ± 444.1	
Median (IQR)	2840.0 (2422.5-3232.5)	2720.0 (2240.0-3100.0)		2797.5 (2397.5-3267.5)		2765.0 (2320.0-3075.0)		2225.0 (1860.0-2500.0)	

N, number of individuals; no., number of events; SD, standard deviation; IQR, interquartile range.

[a] Malaria group consists of total pregnant women who had an infection (*P. falciparum* and *P. vivax* or both).

[b] Statistical tests were applied according to the type of variable (Mann-Whitney or Chi-square).

[c] Term indicates all babies born at 37 weeks' gestation or later.

Further, multivariate logistic regression analysis disclosed the association of malaria with the likelihood of occurring newborns small for gestational age (SGA) at term (weight ≤ 10th centile, boys ≤ 2758g and girls ≤ 2658g) (Odds ratio [OR] 1.23, 95%, confidence interval [CI] 1.05-1.45, p=0.013), which relates to *P. vivax* infection (OR 1.24, 95% CI 1.02-1.52, p=0.035) (Fig 4). Moreover, LBW at term was significantly increased in newborns from malaria-infected mothers, (OR 1.34, 95% CI 1.04-1.72, p=0.024), which was evidenced when mothers were infected by *P. vivax* (OR 1.39, 95% CI 1.03-1.88, p=0.033) (Fig 4). Additionally, segregation by gravidity showed that newborns at term from both primigravida and multigravida presented reduced birth weight when mothers had malaria during pregnancy, irrespective of species (Fig 5A-B, and S5 Table). Nevertheless, newborns from primigravida showed a more prominent birth weight reduction upon infection (Fig 5B, and S5 Table).

Figure 4. Forest plot of the Odds Ratio for weight reduction in newborns from women infected during pregnancy compared to babies from non-infected women, according to *Plasmodium* species. Each model adjusting for maternal age, parity and years of formal education (less than 4 years); mixed infection (*P. vivax* and *P. falciparum*-infection). p values were estimated through logistic regression methods. n, number of events; N, total number in each group; CI, confidence interval; SGA, small for gestational age; LBW, low birth weight.

Figure 5. Impact of malaria on birth weight at term according to gravidity. Tukey boxplots show the gravidity effect on the weight of newborns from malaria-infected women (A), and on newborns from women infected according with *Plasmodium* species (B). The bottom and the top of the box are the first and third quartiles, the line inside the box is the median, and the whiskers represent the lowest and the highest data within 1.5 IQR of the first and upper quartiles. The line indicates the cut-off of low birth weight. Differences between each group were examined with

Mann-Whitney or Kruskal-Wallis test with a Dunn's post hoc test. (A) P - NI x Infec ($p<0.0001$); M - NI x Infec ($p<0.0001$); NI- P x M ($p<0.0001$); and Infec - P x M ($p=0.0004$). (B) P - NI x Pv ($p=0.0001$); NI x Pf ($p=0.0003$); M - NI x Pv ($p=0.0009$); NI x Pf ($p<0.0001$); NI x Mix ($p=0.003$); Pv x Pf ($p=0.025$); Pv - P x M ($p=0.0009$). P, primigravida; M, multigravida; NI, non-infected pregnant women; Infec, infected pregnant women; Pv, *P. vivax*-infection; Pf, *P. falciparum*-infection; Mix, mixed-infection.

P. falciparum infection during pregnancy increases preterm births

The assembly of databases unveiled increased prematurity among babies born from *P. falciparum*-infected women during pregnancy (Table 2). Prematurity prevalence increased around 3% when women were infected with *P. falciparum*, and the association was evidenced by multivariate logistic regression analysis (OR 1.54, 95% CI 1.09-2.18, p=0.016), which corresponded with late preterm births (OR 1.59, 95% CI 1.11-2.27, p=0.011) (Fig 6). Moreover, *P. vivax* infections were related to very preterm births in women with malaria during pregnancy (OR 2.09, 95% CI 1.04-4.20, p=0.039) (Fig 6).

Together, these results demonstrate that linkage of national record databases is a valuable research tool, which disclosed adverse neonatal outcomes upon malaria infection during pregnancy in Brazil.

Figure 6. Forest plot of the Odds Ratio for prematurity in newborns from women infected during pregnancy compared to babies from non-infected women, according to *Plasmodium* species. Each model adjusting for maternal age, parity and years of formal education (less than 4 years). Mixed infection (*P. vivax* and *P. falciparum*-infection). p values were estimated through logistic regression methods. n, number of events; N, total number in each group; CI, confidence interval.

DISCUSSION

Malaria during pregnancy is known as an important risk factor for miscarriage, stillbirth, LBW and maternal anemia [8,21–23]. Nevertheless, little is known about malaria in pregnancy in the Americas endemic regions, where it predominates *P. vivax* infections. This work is the first to assess the effect of gestational malaria in Brazil through the linkage of national databases of the Brazilian Ministry of Health, SINASC and SIVEP-Malaria. Interestingly, despite the decrease in the number of gestational malaria cases through the studied period (2006-2014), the impact of malaria during pregnancy is still evident. The reduction of the mean of the weight was maintained throughout the years and the higher prevalence of preterm births among newborns from women that presented malaria during pregnancy.

The number of studies estimating the real frequency of malaria in pregnant women is still limited, both in Brazil and in other regions of the Americas, which are considered low transmission areas [2]. The prevalence (8.9%) of malaria during pregnancy in our study was similar to the findings of a multi-centric study that enrolled data from the Americas (Guatemala, Colombia, and Brazil) between 2008-2011, and another study performed in Urubá (Colombia) between 2005-2009 [24,25]. Though, the prevalence is higher in relation to reports from Iquitos (6.6%) (Peru), and from other Brazilian cities, such as Manaus (6.1%) and Coari (4.3%) in the Amazonas state, and Rio Branco (1.4%) in Acre state [26–29]. The discrepancies may encompass differential study designs and endemicity of studied areas.

Prematurity is one of the adverse effects commonly observed in malaria during pregnancy [6,30,31]. Usually, it correlates with infections occurring during the third trimester of pregnancy and contributes to increasing the number of newborns with LBW, which is more likely to be observed in low transmission areas [2,32]. In fact, our data show that *P. falciparum* infections during pregnancy are responsible for a high proportion of preterm births, mainly late preterm births (≥32 and <37 weeks

of gestation). However, it was not possible to correlate the time of infection with the gestational trimester.

Newborns reduced weight at birth either classified as LBW or SGA, is an important predictive marker of neonatal and child survival, and can result from two basic factors: intrauterine growth restriction and preterm births [33,31]. In gestational malaria, birth weight reduction is the main adverse outcome observed in studies involving *P. falciparum* infections [2,30,34,35]. In our observations, malaria infection during pregnancy represents a critical morbidity that impacts newborns' weight. The records show that malaria in pregnant women increases the number of babies born at term with SGA by 19.3% and LBW by 28.6% (SGA – NI 14.0%, Malaria 16.7%; term LBW – NI 4.9%, Malaria 6.3%). Strikingly, *P. vivax* infection during pregnancy represents the higher odds for the occurrence of birth weight reduction (SGA - OR 1.24, 95% CI 1.02-1.52, p=0.035; term LBW - OR 1.39, 95% CI 1.03-1.88, p=0.033). The absence of the association between *P. falciparum* infections and the occurrence of SGA or LBW newborns can be related with the restricted pool of variant genes of the Amazonian parasite, which can explain the mild outcomes observed in the Americas, substantially different from other endemic regions in the world [36]. Nevertheless, our data corroborate some findings in Southeast Asia from Moore *et al.* that show that *P. vivax* infection is associated with SGA and *P. falciparum* infection with late preterm, although we could not correlate with time of infection [34].

The reduction of newborns birth weight is multifactorial, and it can be related to social-economic, environmental, nutritional, and clinic factors during pregnancy. However, in this study, it was not possible to assess other risk factors, once these variables were absent in the databases used. Of note, it is important to highlight that it is impossible to compare this study with other carried out in Africa. There, *P. falciparum* infections are predominant and, in general, the health systems that diagnose and treat malaria have several limitations, summed up with the high rate of co-infection with other diseases, such as HIV and tuberculosis. The Brazilian Amazonian region has a health

care system with effective strategies to control, diagnose, and treat malaria, despite being a low transmission area with predominance of *P. vivax* infections. These characteristics make our findings even more interesting, as we observed a substantial impact of infection during pregnancy in newborns.

In Brazil, malaria is a mandatory notification disease, and SIVEP-Malaria is essential to plan health interventions that enable effective control and preventive strategies to eradicate the disease. For pregnant women, the early diagnosis is essential to prevent adverse outcomes. In 2014, it was enforced, by Brazilian MoH, a malaria routine screen during antenatal care and at delivery, in women living in Brazilian Amazonian region states [37]. This initiative brought important benefits for both mother and fetus, enabling early treatment and preventing gestational adverse outcomes.

This work present potential limitations. First, the databases used have only two shared variables with adequate fulfillment, and this hampered the identification of all women. Therefore, the number of pregnant women with malaria can be underestimated. Second, the reduction of birth weight has different etiologies. Although we used important exclusion criteria, it was not possible to identify through SINASC women presenting other infections, such as TORCHs, as well as, nutritional or other risk factors.

In conclusion, this work allowed us to observe through a time-lapse study, the effect of gestational malaria on newborns birth weight in a region considered of low transmission and with *P. vivax* infections predominance. During the evaluated period (2006-2014), malaria infections continue to be an important risk factor for prematurity and reduction of newborns' birth weight, despite the decline in the number of cases reported in the region. We have shown that the SINASC and the SIVEP-Malaria databases linkage allow to estimate the extent of malaria adverse effects, which permit to improve information and further plan interventions. These findings reinforce the urgent need for health programs and actions to prevent and protect pregnant women against the consequences of malaria, especially during the antenatal care.

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Contributors

JGD, LAG, and CRFM designed the study and were involved in data acquisition and scientific input. JGD, RMS, NRMS, AB, SE, LAG, and CRFM contributed to the analysis and interpretation of data. JGD, LAG, and CRFM wrote the manuscript. CRFM and SE were the main funders of this work. CRFM had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors reviewed and approved the final version of this manuscript.

Declaration of interests

All authors declare no competing interests.

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SUPPORTING INFORMATION CAPTIONS

S1 Table. Characteristics of mothers and newborns per year in Cruzeiro do Sul, 2006-2014.

Data from the System Information of Live Births provided by the Cruzeiro do Sul Municipal Secretariat of Health. [a] Information generated based on the number of live births and deaths reported by the mother. [b] There are other groups with ignored values.

S2 Table. Trends in malaria infection during pregnancy in Cruzeiro do Sul, 2006-2014.

N, number of malaria cases. Data are N or N (%). Values correspond to the total number of malaria episodes reported between 2006 and 2014.

S3 Table. Description of the birth weight of newborns from Non-Infected and Infected pregnant women per year.

N, number of individuals; IQR, interquartile range. [a] Malaria group consists of total pregnant women who had an infection (*P. falciparum*, *P. vivax*, and Mixed infections). [b] Differences between Non-Infected and the other groups were evaluated using Mann-Whitney rank sum tests.

S4 Table. Description of term low birth weight newborns from Non-Infected and Infected pregnant women per year.

N, number of individuals; no., number of newborns with low birth weight. [a] Malaria group consists of total pregnant women who had an infection (*P. falciparum*, *P. vivax*, and Mixed infections). [b] Differences between Non-Infected and Infected groups were evaluated using Chi-square tests.

S5 Table. Description of birth weight of term newborns from Non-Infected and Infected pregnant women per gravidity.

N, number of individuals; SD, standard deviation; IQR, interquartile range. [a] Malaria group consists of total pregnant women who had an infection (*P. falciparum*, *P. vivax*, and Mixed infections). [b] Differences between each group were examined using Mann-Whitney or Kruskal-Wallis test with Dunn post hoc test. [c] p=0.025 for *P. vivax* versus *P. falciparum* groups.

Figure 1

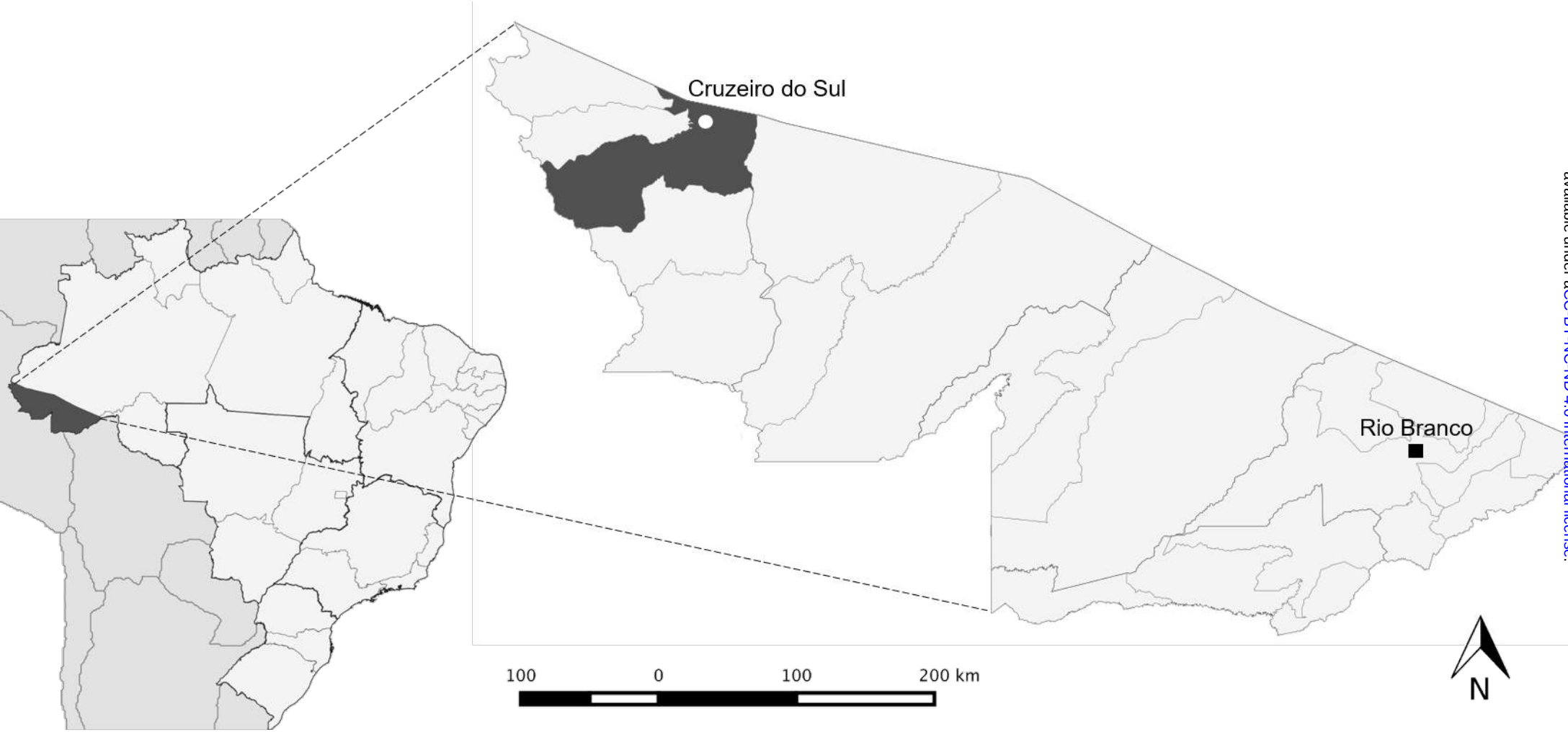


Figure 2

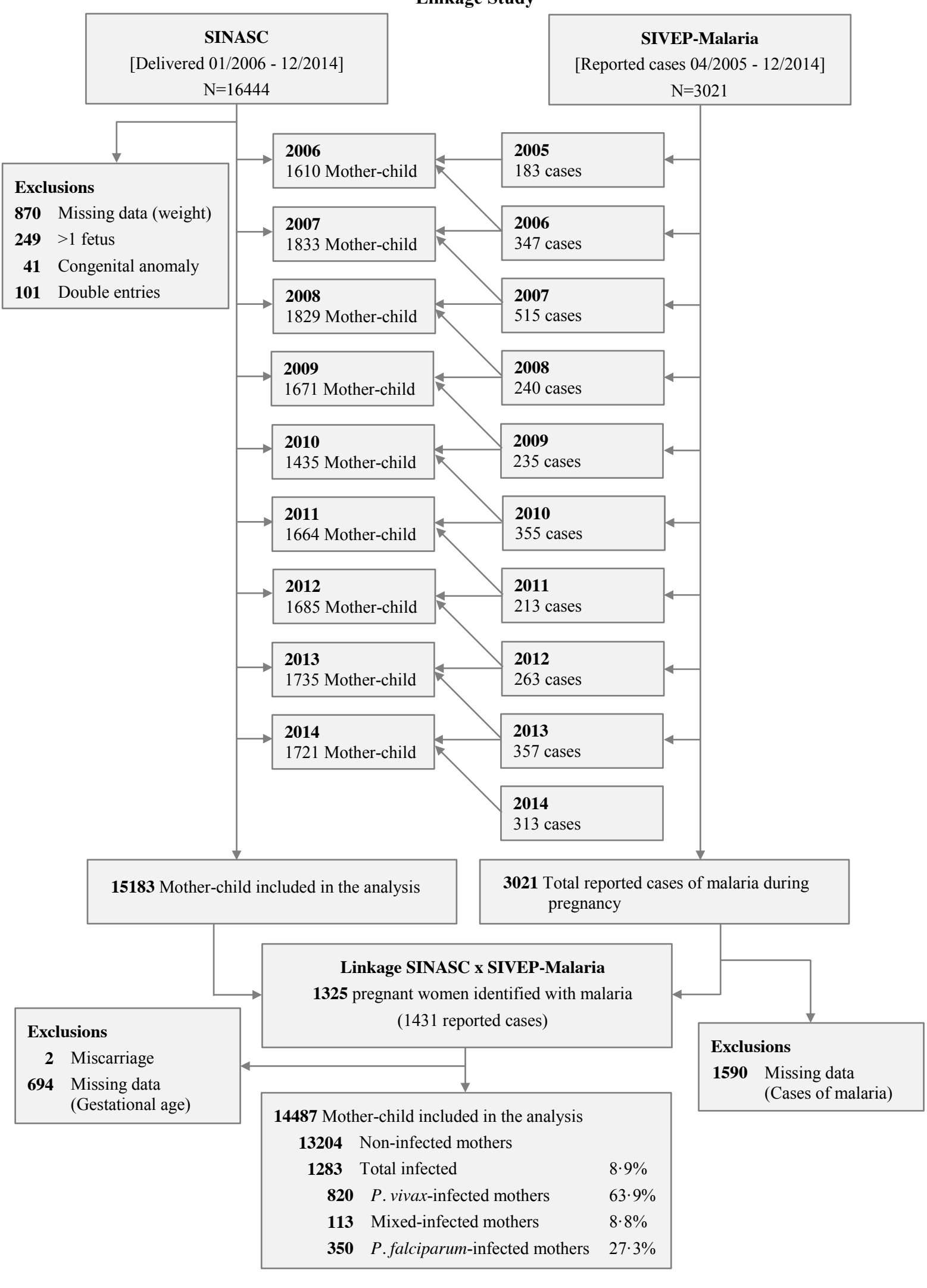


Figure 3

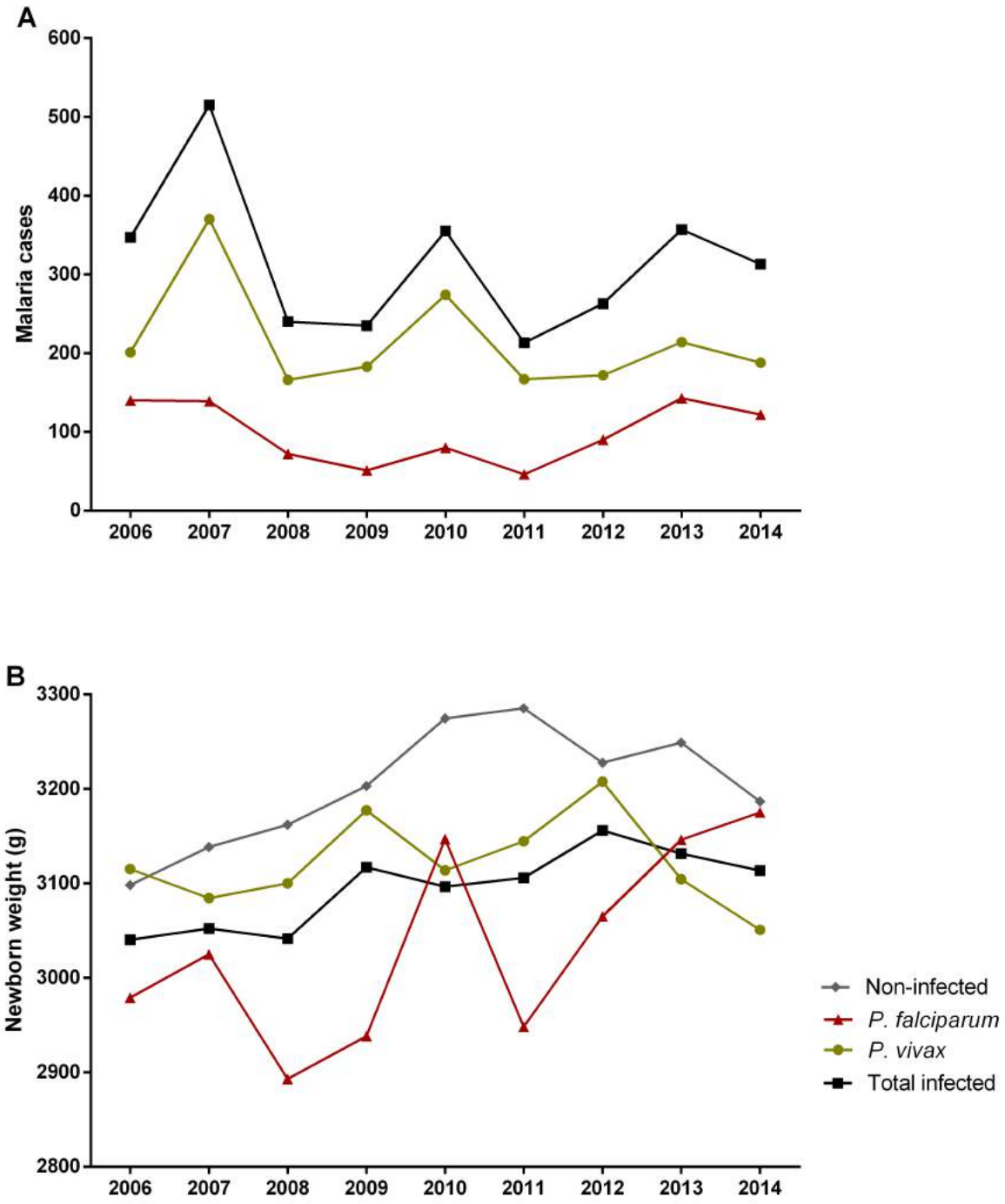


Figure 4

Adverse events	n/N	Prevalence (%)	Unadjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Term SGA (≤10th centile)	1874/13407	14·0				
Malaria	195/1171	16·7	1·26 (1·07-1·48)	0·006	1·23 (1·05-1·45)	0·013
P. vivax	124/756	16·4	1·23 (1·01-1·51)	0·039	1·24 (1·02-1·52)	0·035
Mixed	18/102	17·7	1·35 (0·81-2·25)	0·253	1·24 (0·74-2·08)	0·424
P. falciparum	53/313	16·9	1·28 (0·95-1·73)	0·105	1·21 (0·89-1·64)	0·224
Term low birth weight (< 2500g)	655/13407	4·9				
Malaria	74/1171	6·3	1·35 (1·05-1·74)	0·018	1·34 (1·04-1·72)	0·024
P. vivax	49/756	6·5	1·39 (1·03-1·88)	0·032	1·39 (1·03-1·88)	0·033
Mixed	8/102	7.8	1.71 (0.83-3.53)	0.149	1.57 (0.76-3.27)	0.226
P. falciparum	17/313	5·4	1·15 (0·70-1·89)	0·576	1·13 (0·69-1·86)	0·630
All low birth weight (< 2500g)	1016/14487	7·0				
Malaria	120/1283	9·4	1·42 (1·16-1·73)	0·001	1·40 (1·15-1·71)	0·001
P. vivax	73/820	8·9	1·34 (1·05-1·72)	0·021	1·34 (1·04-1·72)	0·024
Mixed	16/113	14·2	2·27 (1·33-3·86)	0·003	2·12 (1·24-3·62)	0·006
P. falciparum	31/350	8·9	1·34 (0·92-1·94)	0·131	1·33 (0·91-1·93)	0·141

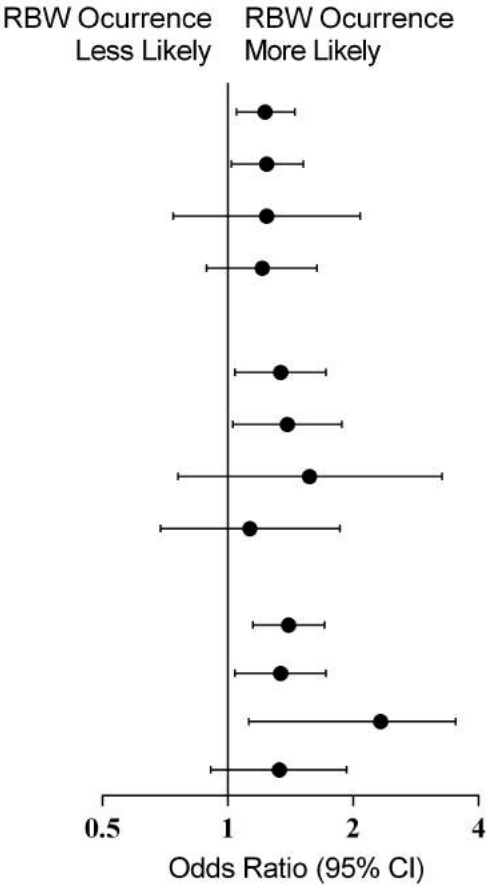


Figure 5

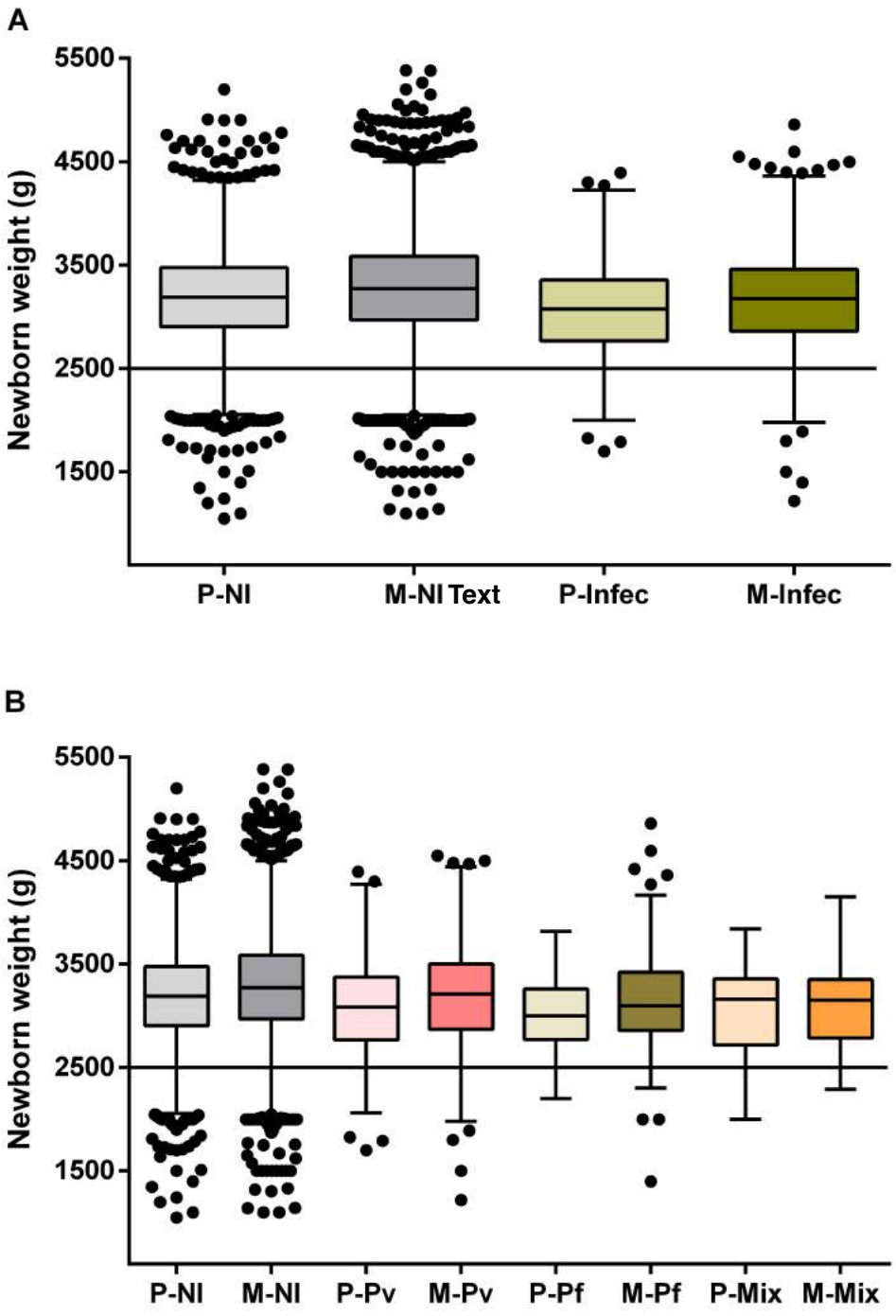
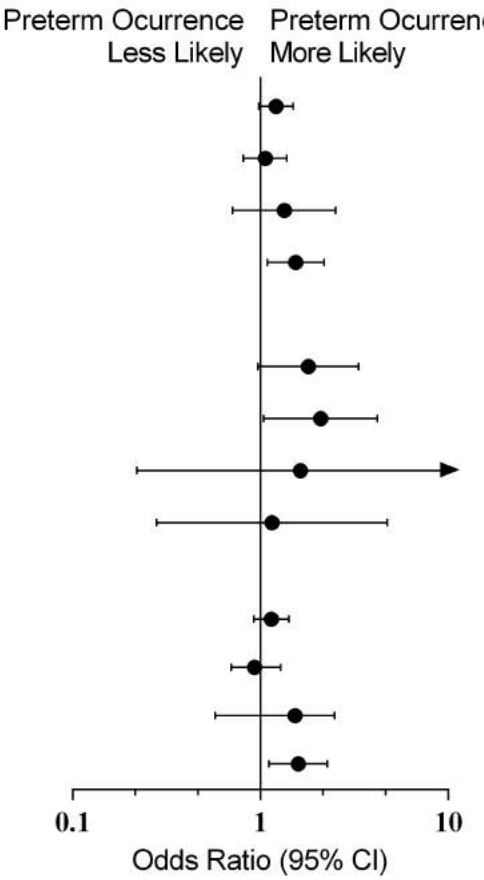


Figure 6

Adverse events	n/N	Prevalence (%)	Unadjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Prematurity	1080/14487	7·5				
Malaria	112/1283	8·7	1·21 (0·99-1·48)	0·069	1·21 (0·98-1·49)	0·071
P. vivax	64/820	7·8	1·07 (0·82-1·39)	0·614	1·06 (0·81-1·38)	0·667
Mixed	11/113	9·7	1·36 (0·73-2·55)	0·332	1·34 (0·71-2·51)	0·363
P. falciparum	37/350	10·6	1·49 (1·06-2·11)	0·023	1·54 (1·09-2·18)	0·016
Very preterm birth	81/14487	0·6				
Malaria	12/1283	0·9	1·78 (0·97-3·33)	0·062	1·80 (0·97-3·34)	0·061
P. vivax	9/820	1·1	2·11 (1·05-4·25)	0·036	2·09 (1·04-4·20)	0·039
Mixed	1/113	0·9	1·70 (0·23-12·35)	0·600	1·63 (0·22-11·88)	0·631
P. falciparum	2/350	0·6	1·09 (0·27-4·48)	0·901	1·15 (0·28-4·73)	0·844
Late preterm birth	981/14487	6·8				
Malaria	97/1283	7·6	1·14 (0·92-1·42)	0·239	1·14 (0·92-1·42)	0·246
P. vivax	52/820	6·3	0·94 (0·71-1·26)	0·694	0·93 (0·70-1·28)	0·645
Mixed	10/113	8·9	1·35 (0·70-2·60)	0·364	1·33 (0·69-2·57)	0·391
P. falciparum	35/350	10·0	1·55 (1·09-2·21)	0·016	1·59 (1·11-2·27)	0·011



S1 Table. Characteristics of mothers and newborns per year in Cruzeiro do Sul, 2006-2014.

Characteristics	2006 (N=1594)	2007 (N=1795)	2008 (N=1748)	2009 (N=1625)	2010 (N=1382)	2011 (N=1510)	2012 (N=1553)	2013 (N=1620)	2014 (N=1660)
Age, years, mean (SD)	23.6 (6.1)	23.7 (6.1)	24.4 (6.3)	24.5 (6.3)	24.3 (6.3)	24.3 (6.5)	24.1 (6.4)	24.3 (6.4)	24.4 (6.6)
Primigravida, no. (%) [a]	551 (34.6)	481 (26.8)	534 (30.6)	530 (32.6)	520 (37.6)	644 (42.7)	632 (40.7)	659 (40.7)	688 (41.5)
Gestational age, no. (%)									
22-27 weeks	1 (0.1)	5 (0.3)	3 (0.2)	-	-	5 (0.3)	-	1 (0.1)	3 (0.2)
28-31 weeks	5 (0.3)	2 (0.1)	7 (0.4)	2 (0.1)	11 (0.8)	22 (1.5)	16 (1.0)	9 (0.5)	7 (0.4)
32-36 weeks	27 (1.7)	34 (1.9)	55 (3.1)	68 (4.2)	64 (4.6)	157 (10.4)	199 (12.8)	164 (10.1)	213 (12.8)
37 weeks or more	1561 (97.9)	1754 (97.7)	1683 (96.3)	1555 (95.7)	1307 (94.6)	1326 (87.8)	1338 (86.2)	1446 (89.3)	1437 (86.6)
Marital status, no. (%) [b]									
Single	1408 (89.1)	1503 (84.3)	1390 (81.0)	1329 (83.3)	1021 (75.3)	516 (34.8)	414 (26.8)	378 (23.6)	369 (22.3)
Married or cohabiting	163 (10.3)	257 (14.4)	310 (18.1)	252 (15.8)	327 (24.1)	960 (64.6)	1120 (72.6)	1199 (74.9)	1272 (76.8)
Years of formal education, no. (%) [b]									
No education	616 (38.8)	622 (34.9)	248 (14.4)	202 (12.7)	130 (9.5)	86 (5.8)	66 (4.3)	77 (4.8)	62 (3.8)
1-3 years	120 (7.6)	135 (7.6)	282 (16.4)	239 (15.0)	170 (12.5)	168 (11.2)	92 (6.0)	63 (3.9)	55 (3.4)
4-7 years	226 (14.2)	264 (14.8)	487 (28.3)	469 (29.4)	383 (28.1)	390 (26.1)	371 (24.0)	317 (19.8)	297 (18.1)
8-11 years	402 (25.3)	295 (16.6)	483 (28.1)	477 (29.9)	461 (33.9)	675 (45.2)	815 (52.7)	914 (57.1)	986 (60.2)
12 or more	222 (14.0)	461 (25.9)	219 (12.7)	206 (12.9)	217 (15.9)	170 (11.4)	196 (12.7)	206 (12.9)	215 (13.1)
Antenatal visits, no. (%) [b]									
None	245 (15.6)	233 (13.1)	295 (16.9)	241 (15.0)	118 (8.6)	101 (6.7)	57 (3.7)	57 (3.5)	34 (2.0)
1-3 visits	147 (9.4)	132 (7.4)	332 (19.1)	399 (24.8)	366 (26.7)	285 (18.9)	235 (15.1)	213 (13.2)	202 (12.2)
4-6 visits	216 (13.7)	191 (10.8)	583 (33.5)	568 (35.4)	552 (40.2)	621 (41.1)	591 (38.1)	556 (34.3)	543 (32.7)
7 or more	960 (61.1)	1213 (68.3)	523 (30.0)	380 (23.7)	314 (22.9)	502 (33.3)	669 (43.1)	794 (49.0)	881 (53.1)
Caesarean section, no. (%)	317 (19.9)	418 (23.3)	469 (26.9)	509 (31.3)	543 (39.3)	525 (34.8)	623 (40.1)	744 (45.9)	752 (45.3)
Birth weight (g), mean (SD)									
Mean (SD)	3093.3 (523.6)	3128.3 (529.0)	3150.8 (506.7)	3197.3 (509.6)	3256.2 (524.0)	3271.7 (503.7)	3222.7 (515.0)	3237.5 (486.2)	3180.8 (521.9)
Median (IQR)	3100.0 (2700.0-3450.0)	3450.0 (2770.0-3480.0)	3170.0 (2850.0-3500.0)	3220.0 (2900.0-3515.0)	3270.0 (2950.0-3590.0)	3275.0 (2990.0-3585.0)	3225.0 (2930.0-3560.0)	3235.0 (2955.0-3537.5)	3210.0 (2907.5-3500.0)
Low birth weight, no. (%)	123 (7.7)	134 (7.5)	126 (7.2)	111 (6.8)	96 (7.0)	80 (5.3)	114 (7.3)	95 (5.9)	137 (8.3)
Very low birth weight, no. (%)	6 (0.4)	11 (0.6)	10 (0.6)	4 (0.3)	4 (0.3)	5 (0.3)	7 (0.5)	7 (0.4)	17 (1.0)

Data from the System Information of Live Births provided by the Cruzeiro do Sul Municipal Secretariat of Health.

[a] Information generated based on the number of live births and deaths reported by the mother.

[b] There are other groups with ignored values.

S2 Table. Trends in malaria infection during pregnancy in Cruzeiro do Sul, 2006-2014.

Cases of malaria	2006	2007	2008	2009	2010	2011	2012	2013	2014
Overall	347	515	240	235	355	213	263	357	313
P. falciparum	140 (40.4%)	139 (27.0%)	72 (30.0%)	51 (21.7%)	80 (22.5%)	46 (21.6%)	90 (34.2%)	143 (40.1%)	122 (39.0%)
P. vivax	201 (57.9%)	370 (71.8%)	166 (69.2%)	183 (77.9%)	274 (77.2%)	167 (78.4%)	172 (65.4%)	214 (59.9%)	188 (60.0%)
Mixed	6 (1.7%)	6 (1.2%)	2 (0.8%)	1 (0.4%)	1 (0.3%)	0	1 (0.4%)	0	3 (1.0%)

N, number of malaria cases. Data are N or N (%). Values correspond to the total number of malaria episodes reported between 2006 and 2014.

S3 Table. Description of the birth weight of newborns from Non-Infected and Infected pregnant women per year.

	Non-infected		Malaria [a]		p value[b]	*P. vivax*		p value[b]	*P. falciparum*		p value[b]	Mixed		p value[b]
Year	N	Median (IQR)	N	Median (IQR)		N	Median (IQR)		N	Median (IQR)		N	Median (IQR)	
2006	1459	3100.0 (2700.0-3450.0)	135	3030.0 (2660.0-3370.0)	0.174	72	3135.0 (2770.0-3455.0)	0.071	51	3005.0 (2615.0-3300.0)	0.623	12	2827.5 (2612.5-3350.0)	0.259
2007	1581	3170.0 (2795.0-3500.0)	214	3077.5 (2690.0-3400.0)	0.047	147	3100.0 (2720.0-3445.0)	0.245	38	3017.5 (2640.0-3300.0)	0.816	29	3060.0 (2500.0-3340.0)	0.087
2008	1586	3190.0 (2860.0-3500.0)	162	3000.0 (2730.0-3390.0)	0.270	99	3100.0 (2770.0-3405.0)	0.521	45	2990.0 (2600.0-3165.0)	0.482	18	3055.0 (2770.0-3300.0)	0.338
2009	1517	3230.0 (2910.0-3530.0)	108	3117.5 (2850.0-3417.5)	0.559	83	3210.0 (2880.0-3460.0)	0.826	22	2900.0 (2750.0-3000.0)	0.664	3	2660.0 (2415.0-3200.0)	0.102
2010	1239	3300.0 (2970.0-3610.0)	143	3090.0 (2785.0-3385.0)	0.108	110	3095.0(2790.0-3430.0)	0.033	24	3185.0 (2995.0-3342.5)	0.362	9	2630.0 (2420.0-2920.0)	0.002
2011	1394	3287.5 (3010.0-3600.0)	116	3122.5 (2780.0-3420.0)	0.009	93	3150.0 (2800.0-3425.0)	0.008	18	2972.5 (2670.0-3245.0)	0.472	5	2880.0 (2760.0-3265.0)	0.133
2012	1442	3232.5 (2930.0-3560.0)	111	3100.0 (2845.0-3475.0)	0.107	73	3200.0 (2870.0-3480.0)	0.629	30	3030.0 (2745.0-3340.0)	0.463	8	3075.0 (2865.0-3190.0)	0.198
2013	1461	3245.0 (2975.0-3540.0)	159	3145.0 (2860.0-3520.0)	0.091	71	3210.0 (2810.0-3575.0)	0.181	74	3127.5 (2890.0-3475.0)	0.429	14	3270.0 (2920.0-3485.0)	0.772
2014	1525	3215.0 (2915.0-3510.0)	135	3160.0 (2855.0-3400.0)	0.087	72	3062.5 (2805.0-3395.0)	0.067	48	3205.0 (2957.5-3410.0)	0.932	48	3300.0 (3140.0-3470.0)	0.715

N, number of individuals; IQR, interquartile range.

[a] Malaria group consists of total pregnant women who had an infection (*P. falciparum*, *P. vivax,* and Mixed infections).

[b] Differences between Non-Infected and the other groups were evaluated using Mann-Whitney rank sum tests.

S4 Table. Description of term low birth weight newborns from Non-Infected and Infected pregnant women per year.

	Non-infected		Malaria [a]		p value[b]	*P. vivax*		p value[b]	*P. falciparum*		p value[b]	Mixed		p value[b]
Year	N	no. (%)	N	no. (%)		N	no. (%)		N	no. (%)		N	no. (%)	
2006	1429	82 (5.7)	132	13 (9.9)	0.059	72	9 (12.5)	0.019	49	3 (6.1)	0.910	11	1 (9.1)	0.635
2007	1551	91 (5.9)	203	16 (7.9)	0.259	141	10 (7.1)	0.557	37	3 (8.1)	0.568	25	3 (12.0)	0.199
2008	1528	81 (5.3)	155	10 (6.5)	0.546	97	6 (6.2)	0.707	40	4 (10.0)	0.195	18	0	0.316
2009	1449	80 (5.5)	106	4 (3.8)	0.442	82	2 (2.4)	0.228	22	1 (4.6)	0.842	2	1 (50.0)	0.006
2010	1175	57 (4.9)	132	7 (5.3)	0.820	105	5 (4.8)	0.967	19	0	0.325	8	2 (25.0)	0.009
2011	1229	32 (2.6)	97	5 (5.2)	0.142	77	3 (3.9)	0.496	15	1 (6.7)	0.330	5	1 (20.0)	0.016
2012	1247	53 (4.3)	91	5 (5.5)	0.574	63	5 (7.9)	0.165	22	0	0.323	6	0	0.606
2013	1306	38 (2.9)	140	9 (6.4)	0.026	60	4 (6.7)	0.099	67	5 (7.5)	0.037	13	0	0.533
2014	1322	67 (5.1)	115	5 (4.4)	0.734	59	5 (8.5)	0.249	42	0	0.135	14	0	0.387

N, number of individuals; no., number of newborns with low birth weight.

[a] Malaria group consists of total pregnant women who had an infection (*P. falciparum*, *P. vivax,* and Mixed infections).

[b] Differences between Non-Infected and Infected groups were evaluated using Chi-square tests.

S5 Table. Description of birth weight of term newborns from Non-Infected and Infected pregnant women per gravidity.

Birth weight (g)	**Non-infected (N=12,236)**	**Malaria [a] (N=1,171)**	**p value[b]**	***P. vivax* (N=756)**	**p value[b]**	***P. falciparum* (N=313)**	**p value[b]**	**Mixed (N=102)**	**p value[b]**
Primigravida									
Mean (SD)	3184.1 (454.8)	3072.1 (453.1)	<0.0001	3088.0 (478.0) [c]	0.0001	3021.6 (370.9)	0.0003	3070.1 (436.6)	0.104
Median (IQR)	3186.5 (2905.0-3475.0)	3075.0 (2769.0-3355.0)		3085.0 (2770.0-3370.0)		3000.0 (2769.0-3260.0)		3160.0 (2720.0-3348.0)	
Multigravida									
Mean (SD)	3265.1 (494.5)	3173.2 (477.2)	<0.0001	3199.0 (486.4) [c]	0.0009	3137.0 (469.5)	<0.0001	3108.2 (424.1)	0.003
Median (IQR)	3270.0 (2970.0-3585.0)	3175.0 (2860.0-3460.0)		3210.0 (2870.0-3500.0)		3095.0 (2860.0-3420.0)		3150.0 (2800.0-3340.0)	
p value (Primigravida x Multigravida)	<0.0001	0.0004		0.0009		0.083		0.862	

N, number of individuals; SD, standard deviation; IQR, interquartile range.

[a] Malaria group consists of total pregnant women who had an infection (*P. falciparum*, *P. vivax,* and Mixed infections).

[b] Differences between each group were examined using Mann-Whitney or Kruskal-Wallis test with Dunn post hoc test.

[c] p=0.025 for *P. vivax* versus *P. falciparum* groups.