

# Prevalence of *Plasmodium falciparum* Submicroscopic Infection and Pregnancy Outcomes in Congolese Women at Delivery

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**Abstract:** Suphodoxine-Pyrimethamine (SP) acts by inhibiting *P. falciparum* replication, therefore, long term use of SP in malarial endemic settings as intermittent preventive treatment (IPT) during pregnancy might lead to increased risk of submicroscopic parasitaemia. The present study aimed to determine the prevalence of submicroscopic *P. falciparum* infection in women at delivery from southern Brazzaville, where IPT-SP has been implemented since 2004, and investigate the relationship between the submicroscopic parasitaemia and pregnancy outcomes. This descriptive cross-sectional study was carried out from March 2014 to April 2015 with 281 women randomly recruited at delivery at a Health Centre in southern Brazzaville, Republic of Congo. Matched peripheral, placental, and cord blood collected from each women with malaria negative thick smears, were used for the diagnostic of *P. falciparum* submicroscopic infection by nested-polymerase chain reaction (nPCR) targeting the multi-copy 18 S ribosomal ARN gene. The prevalence of *P. falciparum* submicroscopic infection was 31.7%, 36.37%, and 12.9%, when using the peripheral, placental, and cord blood respectively. The submicroscopic *P. falciparum* was slightly associated with increased risk of maternal anaemia (adjusted odds ratio [OR] 1.33, 95% CI 0.82-2.17). No significant association was found between the submicroscopic parasitaemia and low birth weight, or preterm delivery. The data suggest that the prevalence of submicroscopic *P. falciparum* infection is high in women at delivery in Brazzaville; and might increase the risk for maternal anaemia. The infection had no impact on the prevalence of low birth weight and preterm delivery in this study setting.

**Keywords:** *Plasmodium falciparum*, Submicroscopic Infection, Pregnant Women, Republic of Congo

## 1. Introduction

Pregnant women, especially at their first pregnancy, are at high risk of severe malaria due to *P. falciparum*, a major cause of mother offspring morbidity and mortality in Africa [1]. In 2019, about 11 million pregnant in sub-Saharan were exposed to *P. falciparum* infection during pregnancy, resulting in 872 000 low birth weight babies [2]. Among the four principal *Plasmodium* spp (*P. falciparum*, *P. vivax*, *P.*

*malariae*, and *P. ovale*) known to infect human beings, *P. falciparum* is a major contributor of the malaria burden in sub-Saharan Africa [2]. In pregnant women, *P. falciparum*-infected erythrocytes (IE) can bind to chondroitin sulfate A (CSA) expressed on syncytiotrophoblasts lining the intervillous space of the placenta via VAR2CSA-type PfEMP1 [3, 4]. This condition causes the sequestration of the parasite in the placental tissue known as placental malaria (PM), which increases the risk for poor pregnancy outcomes

such as abortion, preterm delivery, low birth weight, and maternal anaemia [5-8]. Pregnancy associated malaria (PAM) is also a risk factor for neonatal and infant mortality [8, 9]. About 25% of pregnant women from malaria endemic areas may have placental tissue infected by *P. falciparum* at the time of delivery [6].

To prevent the general burden of PAM, measures, such as long-lasting insecticide-treated nets (LLIN) have been adopted [2]. Moreover, the Intermittent Preventive Treatment with sulfadoxine-pyrimethamine (IPT-SP) has been implemented in most countries in sub-Saharan Africa since 2004 [10]. According to World Health Organization (WHO) recommendations, each woman from the stable malaria transmission areas should take a dose of SP at each antenatal care visit, starting in the second semester of pregnancy. Compared to women who do not use IPT-SP during pregnancy, women taking 3 doses of SP have been shown to have higher maternal hemoglobin level, fewer cases of placental malaria and low birth weight babies [11]. Since SP inhibit folic acid synthesis in malaria parasite, which is required for parasite replication, the mass administration and long term use of SP as intermittent preventive treatment might lead to increased risk of submicroscopic malaria parasitaemia, which also is common in asymptomatic malaria infection.

Malaria infections in pregnancy, even asymptomatic and submicroscopic, could be harmful to the mother, fetus, and newborn health [9, 12]. Furthermore, a previous study demonstrated that these submicroscopic infections represent an important reservoir of gametocytes, which can contribute to sustaining malaria transmission [13]. In sub-Saharan Africa, the prevalence of *P. falciparum* infection is higher in primigravidae than multigravid women, and most of the malaria infections become asymptomatic in multigravid women, due in part to anti-disease immunity acquired from previous subsequent exposures [6]. With considerable efforts and progress made in malaria control over the past decades (adoption of artemisinin-based combination therapy, use of IPT-SP, mass distribution of long-lasting insecticide-treated mosquito nets), many endemic countries are moving toward malaria elimination and might be exposed to high risk of submicroscopic infection.

High proportions of submicroscopic *P. falciparum* infections have been reported in non-pregnant adults in Sub-Saharan Africa. However, there is a paucity of published data in pregnant women. A previous study reporting the prevalence of *P. falciparum* submicroscopic infection in pregnant women in the Republic of Congo used PCR assay based on the detection of the central region of a single copy of *msp-2* gene, which would have led to the underestimation of the infection prevalence [14]. Moreover, the relationship between submicroscopic malaria infection in malaria asymptomatic pregnant women and pregnancy outcomes was not investigated in that previous study. The present study sought to determine the prevalence of submicroscopic *P. falciparum* infection in women at delivery in Brazzaville using nPCR assay targeting the multi-copy 18S ribosomal ARN gene, and investigate its relationship with pregnancy

outcomes. The results from the study could help to update the data on malaria burden in pregnant women in the Republic of Congo.

## 2. Materials and Methods

### 2.1. Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of the Congolese Foundation for Medical Research, Republic of Congo (ethics Clearance N° 001 / CEI / FCRM / 2012). Participation in the study was voluntary with written informed consent from each woman or parents (or guardians) for participants less than 18 years old. As the motivation, microscopic malaria diagnostic test was performed for each woman at the time of enrollment and positive results were reported to the physician for prescription of treatment according to the national policy.

### 2.2. Study Site

This descriptive cross-sectional study was carried out from March 2014 to April 2015 at the Madibou integrated health center, located in the southern part of Brazzaville, in the Republic of Congo. The capital, Brazzaville, is located along the Congo River, in the south of the country. Since the country is located on the Equator, the climate is consistently humid year-round, with the average day temperature of 25°C and night between 16 and 21°C [15]. The average yearly rainfall ranges from 1100 mm in the south to over 2000 mm in the central and north parts of the country. The rainy season which lasts 9 months, has two rainfall maxima: one in March-May and another in September–November [15]. The malaria transmission in Brazzaville is perennial with *P. falciparum* as the predominant malaria parasite and *Anopheles gambiae* as the main mosquito vector.

### 2.3. Study Population and Sample Collection

A total of 370 women aged 16-39 years with no history of clinical malaria in the last two weeks before the delivery and fever, 48 h before the delivery or at the time of sample collection, were randomly recruited [14, 16]. A portion of the blood was used to prepare thick and thin smears for microscopy and to measure hemoglobin levels. The remaining blood portion was stored at -80°C for nPCR assay.

### 2.4. Diagnosis of Malaria and Determination of Hemoglobin Levels

In order to exclude all women with microscopic malaria infection, Thick and thin blood smears, were prepared for each peripheral, placental and cord blood sample using Giemsa-Wright stain and read by two skilled microscopists to determine the presence of malaria parasites. If parasites were not detected after examining 200 microscopic fields of thick smears, the individual was considered malaria negative. Hemoglobin levels in maternal peripheral blood were determined using a hematologic analyzer. Maternal anaemia was defined as

hemoglobin level less than 11 g/dL of whole blood.

### 2.5. DNA Extraction and Detection of *P. falciparum* Submicroscopic Infection

All microscopic negative peripheral, placental, and cord blood samples were used for diagnosis of *P. falciparum* submicroscopic infection by a nested polymerase chain reaction (nPCR) assay. Briefly, total genomic DNA was extracted from 200  $\mu$ L whole blood using QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany). DNA amplifications were performed in a Master Cycler X50a Eppendorf AG, Hamburg, Germany) using primers specific to the multi-copy 18S rRNA plasmodial gene. For the first step of the nested PCR, a 18 $\mu$ L PCR reaction mix consisting of 12.5  $\mu$ L of nuclease-free water, 2.5  $\mu$ L of 10X PCR buffer, 1.25  $\mu$ L of 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ L of 10 mM dNTPs, 5U 0.25  $\mu$ L of Taq DNA polymerase / $\mu$ L, and 0.5  $\mu$ L each of 10  $\mu$ M genus-specific forward and reverse primers (rPLU5: 5'-CCTGTTGTTGCCTTAAACTTC-3' and rPLU6: 5'-TTAAAATTGTTGCAGTTAAACG-3') was prepared for 2  $\mu$ L DNA extract. The amplification conditions included a pre-denaturation at 94°C for 4 min, 35 cycles of 30s denaturation at 94°C, 1 min annealing at 55°C, and 1min extension at 72°C. Then, a final extension cycle was performed at 72°C for 4 min. The nest-2 PCR reactions used 1 $\mu$ L of next-1 amplicons and *P. falciparum*-specific primers (rFAL1: 5'-TTAAACTGGTTTGGGAAAACCAATATATT3' and rFAL2: 5'-ACACAATGAACTCAATCATGACTACCCGTC-3') [36, 37] with the following conditions: 4min pre-denaturation at 94°C, 35 cycles of 30s denaturation at 94°C, 1 min annealing at 55°C and 1 minute extension at 72°C for, and 4 min final extension cycle at 72°C. Positive and negative controls were included in the reactions to avoid false-positive and false-negative results. Five microliters of each of PCR amplicons were loaded onto a 1.5% agarose gel, stained with Syber Green, separated by electrophoresis and visualized under UV rays (BIORAD Gel doc™ EZ Imager, USA).

### 2.6. Statistical Analysis

Graph Pad Prism (version 6.0.1) and XLSTAT (version 2011.2.08) softwares were used for the statistical analyses. Continuous variables were reported as means  $\pm$  standard deviations (SD) or medians with interquartile range (IQR). Differences between groups were compared using unpaired Student's t test, analysis of variance or Mann-Whitney Rank Sum test, while categorical variables were reported as percentages and were compared using Fisher's exact test or Chi Square test. Multiple logistic regression analyses were performed where the submicroscopic infection status were entered in the model as dependent variable and age, gravidity, using bed nets and IPT-SP as independent variables. The submicroscopic parasitaemia itself was entered as independent variable in the models when anaemia, preterm delivery, and low birth weight were the dependent variables. ORs and 95% CI were calculated, two-sided P values <0.05

were considered statistically significant.

## 3. Results

### 3.1. Study Population

The characteristics of women in this study are summarized in Table 1. Overall, 370 women were randomly recruited among whom 281 were negative to microscopic malaria infection and were then used for the investigation of submicroscopic infection. The median age of women was 25 years. Of the 281, 145 (51.6%) women had the age range of 21 to 30 years while 63 (22.4%) women were less than 21 years old, and 73 (26.0%) over 30 years old.

The mean gravidity and parity of women were 3.7 and 2.7 respectively, and 115 (40.9%) women were paucigravidae while 166 (59.1%) women were multigravidae respectively. The median maternal hemoglobin level was 11.20 g/dL with interquartile range of 10.0 to 12.50 g/dL. Similarly, a wide range in gestational age was found (median: 38 weeks with interquartile range of 37 to 39 weeks). The mean baby birth weight was 3103 g. The proportion of women using bed nets was 65.1% and the mean number of IPT-SP doses taken per women was 1.8. However, 107 (38.1%) women did not take any or took a single dose of SP while 174 (61.9%) women took 2 doses or 3 doses of the drug.

Table 1. Characteristic of the study population.

Variables	Women at delivery (n=281)
Age in years (median with interquartile ranges)	25 (21; 31)
$\leq 20$ years, n (%)	63 (22.4%)
21-30 years, n (%)	145 (51.6%)
$> 30$ years, n (%)	73 (26.0%)
Parity (mean $\pm$ SD)	2.7 $\pm$ 1.5
Gravidity (mean $\pm$ SD)	3.1 $\pm$ 1.8
Paucigravidae (1 or 2 gravidies), n (%)	115 (40.9%)
Multigravidae ( $\geq 3$ gravidies), n (%)	166 (59.1%)
Maternal hemoglobin in (g/dL) (median with interquartile ranges)	11.20 (10.0-12.50)
Gestational age (median with interquartile ranges)	38 (37-39)
Baby birth weight in g (mean $\pm$ SD)	3103 $\pm$ 470
IPT-SP usage	
Number of Doses of SP (mean $\pm$ SD)	1.8 $\pm$ 1.0
0-1 Dose of SP, n (%)	107 (38.1%)
2-3 Doses of SP, n (%)	174 (61.9%)
Women using ITNs, n (%)	183 (65.1%)

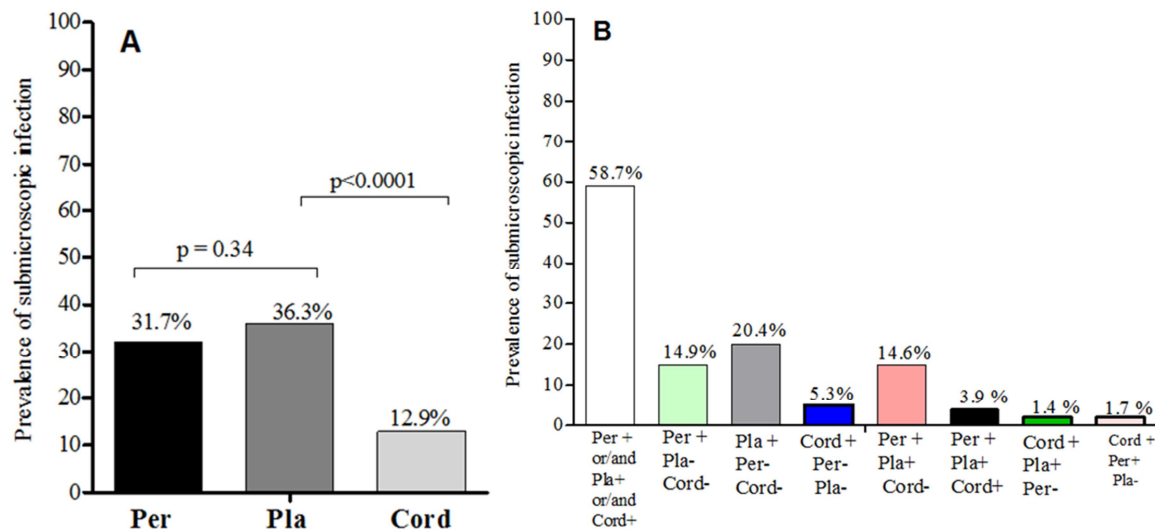
IPT-SP: intermittent preventive treatment using sulfadoxine-pyrimethamine, ITNs: insecticide treated bed nets.

### 3.2. Prevalence of Submicroscopic *Plasmodium falciparum* Infection in Malaria Asymptomatic Pregnant Women at Delivery

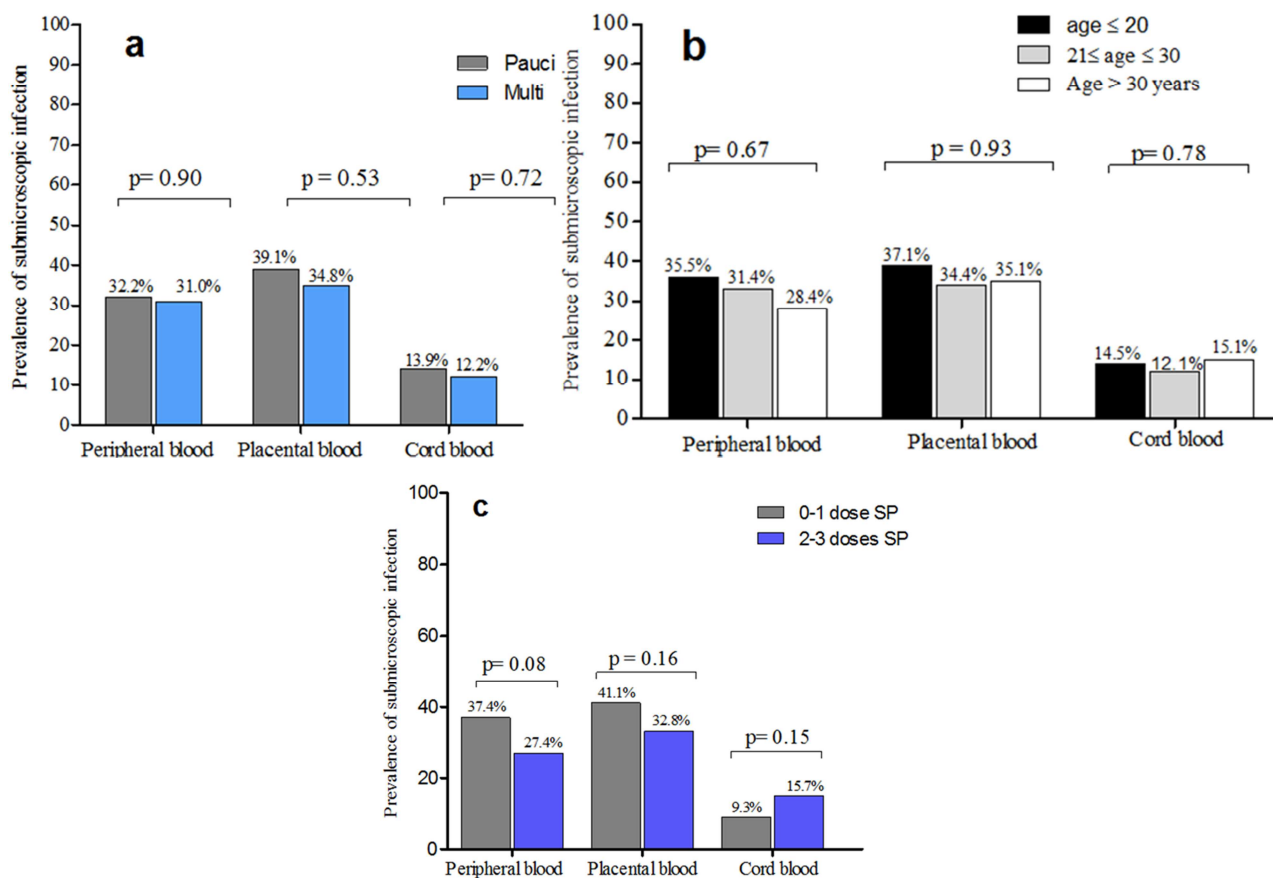
Among the 281 women enrolled, 31.7% were tested positive for *P. falciparum* submicroscopic infection when using peripheral blood while 36.4% and 12.9% of women were positive for the infection when placental blood and cord blood respectively were used (Figure 1A). However, the dynamic of infection across the three blood compartment was

very complex. In fact, 14.9% of women were exclusively positive in peripheral blood, 20.4% in placental blood, and 5.3% in cord blood. A proportion of 14.6% of women had infection in both peripheral and placental blood but remained non-infected in cord blood. About 1.4% of women were infected in both placental and cord blood with no infection in

peripheral blood. Also, 1.7% of women were infected in both peripheral and cord blood but remained non-infected in placental blood. Only 3.9% of women were positive for all of the three blood compartment (peripheral; placental and cord blood) and 58.4% of women were positive for at least one of the blood compartment (Figure 1B).



**Figure 1.** Prevalence of *P. falciparum* submicroscopic infection in peripheral, placental, and cord blood of women at delivery. Fisher exact test was used for the comparison of proportions. Per +: *P. falciparum* submicroscopic infection in the peripheral blood, Pla+: *P. falciparum* submicroscopic infection in the placental blood, Cord +: *P. falciparum* submicroscopic infection in the cord blood.



**Figure 2.** Prevalence of *P. falciparum* submicroscopic in asymptomatic women as the function of parity (a), age (b), and IPT-SP usage (c). Fisher exact test or Khi square test was used for the comparison of proportions. SP: sulfadoxine-pyrimethamine, Pauci: Paucigravid women (women with 1 or 2 gravidities), multi: multigravid women (women with more than 2 gravidities).

### 3.3. Prevalence of Submicroscopic *P. falciparum* Infection in Relation with Gravidity, Age, and IPT-SP Usage

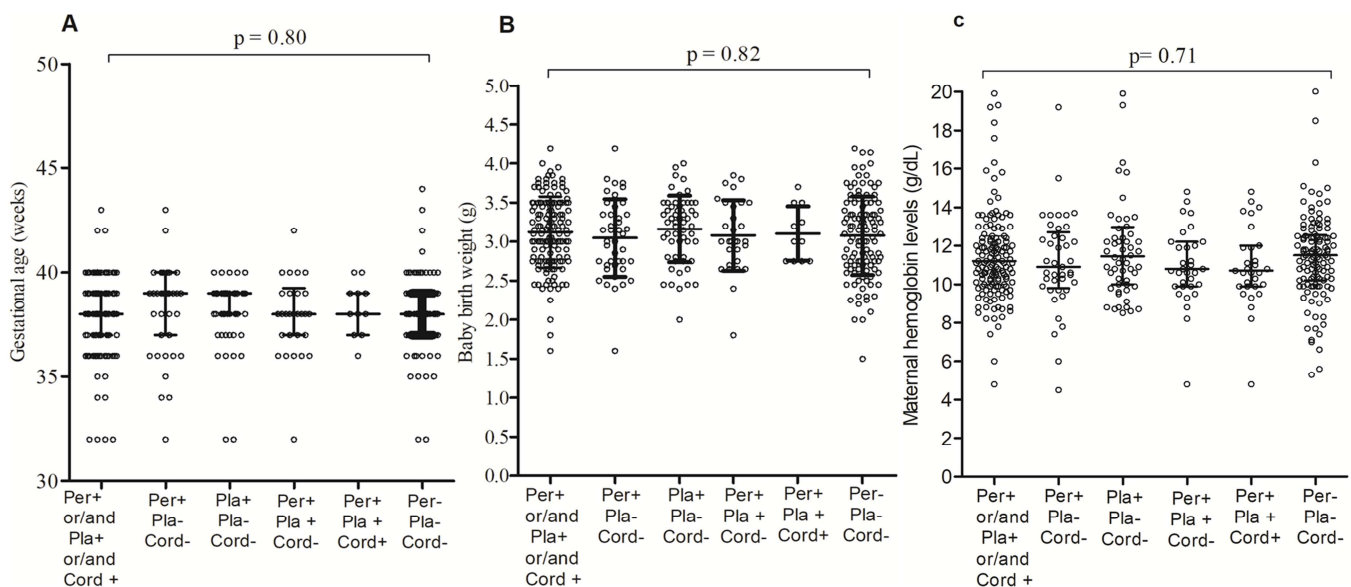
The prevalence of submicroscopic *P. falciparum* infection was analyzed in the present study in relation with women gravidity, age, and IPT-SP usage (Figure 2, Table 2). No significant difference was found between paucigravid and multigravid women in terms of the prevalence of the infection in peripheral blood (32.2%, and 31.0% respectively;  $p=0.90$ ), in placental blood (39.1%, and 34.8% respectively;  $p=0.53$ ) and in cord blood (13.9%, and 12.2% respectively;  $p=0.72$ ) (Figure 2A). Similarly, no significant difference was found between women less than 21 years old, women with age range of 21 to 30 years and over 30 years old women in terms of the prevalence of submicroscopic infection in the

peripheral blood (35.5%, 31.4%, and 28.4% respectively;  $p=0.67$ ), placental blood (37.1%, 34.4%, and 35.1% respectively;  $p=0.93$ ) and cord blood infection (14.5%, 12.1%, and 15.1% respectively;  $p=0.78$ ) (Figure 2B). Although non statistically significant, the prevalence of peripheral blood submicroscopic infection was slightly lower in women who took at least 2 doses of SP (27.4%) compared to those who took no dose of SP or a single dose of the drug (37.4%) ( $p=0.08$ ) (Figure 2C). The prevalence of submicroscopic infection in the placental blood were 41.1%, and 32.8% for women who took no dose or a single dose of SP, and those who took 2-3 doses of the drug, while in cord blood, the prevalence of the infection was 9.3%, and 15.7% for women who took the respective doses of SP (Figure 2C).

**Table 2.** Factors associated with *P. falciparum* submicroscopic infection in women at delivery.

	N	n (%) submicroscopic infection	Non-adjusted Odds ratio (CI. 95%)	Adjusted Odds ratio (CI. 95%)	p values
Mother age:					
14-20 years	62	34 (54.8)	Reference	Reference	
21-30 years	146	79 (54.1)	0.97 (0.53-1.76)	0.87 (0.43-1.76)	0.71
>30 years	73	37 (50.7)	0.84 (0.41-1.45)	0.75 (0.32-1.79)	0.52
Gravidity:					
1- 2	115	60 (52.2)	Reference	Reference	
$\geq 3$	166	90 (54.2)	1.084 (0.67-1.75)	1.17 (0.64-2.17)	0.60
IPT-SP usage					
0-1 dose	107	65 (60.7)	Reference	Reference	
2-3 doses	174	85 (48.9)	0.62 (0.38-1.01)	0.62 (0.37-1.02)	0.06
ITNs usage:					
Yes	183	100 (54.6)	Reference	Reference	
No	98	50 (51.0)	1.16 (0.71-1.89)	1.20 (0.72-1.97)	0.48

IPT-SP: intermittent preventive treatment using sulfadoxine-pyrimethamine, CI: confident interval, ITNs: insecticide treated bed nets. P values for adjusted Odds ratio are shown



**Figure 3.** Comparison of baby birth weight (a), gestational age (b) and hemoglobin level (c) between women with and without *P. falciparum* submicroscopic infection at delivery. Krustal wallis or ANOVA tests was used for comparison between groups. Per +: *P. falciparum* submicroscopic infection in the peripheral blood, Pla+: *P. falciparum* submicroscopic infection in the placental blood, Cord +: *P. falciparum* submicroscopic infection in the cord blood.

### 3.4. Relationship Between Submicroscopic *Plasmodium falciparum* Infection and Pregnancy Outcomes

The gestational age of *P. falciparum* submicroscopic negative-women was not significantly different to that of women having the infection in both peripheral and placental blood compartments, women exclusively infected in peripheral blood or placental blood, and women having the infection in at least one of the blood compartment (Figure 3A). Similarly, the baby birth weight and maternal hemoglobin levels were not significantly different between non-infected groups and respective infection groups (Figure 3B and C). Furthermore, the percentage of preterm delivery, low birth weight and anaemia were not significantly different

between the non-infected group of women and groups of women having infection in both peripheral and placental blood compartment, or in the groups of women exclusively infected in placental blood or peripheral blood, as well as those infected in at least one of the blood compartment (Table 3). However, the prevalence of anaemic women was slightly higher in infected groups of women compared to non-infected group (Table 3). In addition, no association was found between the infection and preterm delivery, and baby birth weight; excepting the borderline positive association observed between the submicroscopic *P. falciparum* infection and the prevalence of anaemia (adjusted odds ratio [OR] 1.33, 95% CI 0.82-2.17) (Table 4).

**Table 3.** Comparison of the prevalence of preterm delivery, baby low birth weight, and maternal anemia between women with and Without *P. falciparum* submicroscopic infection.

	Per, or/and pla, blood infected	No Infection	P	Per blood infected only	No infection	P	Pla blood infected Only	No infection	P	Per and pla blood infected	No infection	P
PTD	24/150 (16.0%)	19/131 (14.5%)	0.74	9/46 (19.6%)	19/131 (14.5%)	0.48	7/61 (11.5%)	19/131 (14.5%)	0.34	7/40 (17.5%)	19/131 (14.5%)	0.65
LBW	13/150 (8.7%)	13/131 (9.9%)	0.83	4/46 (8.7%)	13/131 (9.9%)	0.83	7/61 (11.5%)	13/131 (9.9%)	0.80	2/40 (5.0%)	13/131 (9.9%)	0.24
Anaemia	71/150 (47.3%)	52/131 (39.6%)	0.22	24/46 (52.2%)	52/131 (39.6%)	0.16	25/61 (41.0%)	52/131 (39.6%)	0.87	22/40 (55.0%)	52/131 (39.6%)	0.10

PTD: Preterm delivery (gestational age < 37weeks), LBW: Low birth weight (baby weight < 2.5 kg), Per blood: peripheral blood, Pla blood: placental blood

**Table 4.** Odds of the *P. falciparum* submicroscopic infection on the poor pregnancy outcomes.

	N	n (%) PTD	Adjusted Odds ratio (CI. 95%)	P values	n (%) LBW	Adjusted Odds ratio (CI. 95%)	P values	n (%) Anaemia	Adjusted Odds ratio (CI. 95%)	P values
Submicroscopic Infection <sup>a</sup>	150	24 (16.0)	1.11 (0.58-2.13)	0.72	13 (8.7)	0.87(0.38-1.97)	0.74	71 (47.3)	1.33 (0.82-2.17)	0.25
No infection	131	19 (14.5)	Reference		13 (9.9)	Reference		52 (39.6)	Reference	

Analyses were adjusted with age, gravidity, usage of intermittent preventive treatment using sulfadoxine-pyrimethamine (IPT-SP) and insecticide treated nets (ITNs. PTD: Preterm delivery (gestational age < 37weeks), Low birth weight (baby weight < 2.5 kg), CI: confident interval, a: in at least one of the peripheral, or placental blood compartment.

## 4. Discussion

The present study sought to determine the prevalence of submicroscopic *Plasmodium falciparum* infection in women at delivery in southern Brazzaville, and investigate its relationship with pregnancy outcomes. Although high proportions of *P. falciparum* submicroscopic infections have been reported in non-pregnant adults in Sub-Saharan Africa, little information with respect to pregnant women exist, especially concerning the relationship between submicroscopic malaria infection in pregnant women and pregnancy outcome. A previous study on the characterization of *P. falciparum* infection in the Republic of Congo reported 25.4%, 16.7%, and 9.4% as the prevalence of *P. falciparum* submicroscopic infection in the peripheral, placental, and cord blood in women at delivery [14]. However, the study used only the central region of *msp-2* gene as the PCR detection marker, which would have led to the underestimation of the infection prevalence. When using nPCR assay that targets the multi-copy 18 S ribosomal ARN

gene of *P. falciparum*, we obtained 31.7%, 36.37%, and 12.9% prevalence, for the peripheral, placental, and cord blood respectively. These findings confirm that nPCR assay is more sensitive for detection of *P. falciparum* submicroscopic infection compared to PCR assay targeting only the *msp-2* gene. Compared to 36% reported in a systematic review as the weighted mean of prevalence of *P. falciparum* submicroscopic infection in Africa [17], the prevalence of the infection in general was significantly higher in this study where about 58.7% of women were infected for at least one of the blood compartment at delivery. Previous studies carried out in unstable malaria transmission areas of Soudan reported higher prevalence of the submicroscopic malaria infection in the placental blood compared to peripheral and cord blood [12, 18]. The similar result was observed in the present study with the prevalence of *P. falciparum* submicroscopic infection being higher in the placental blood (36.3%) than the peripheral blood (31.7%) and cord blood (12.9%). Our findings contrast those obtained in previous studies from high malaria transmission area [19, 20] and that reported a higher prevalence of *P. falciparum*



submicroscopic in the peripheral blood and not in the placental and cord blood. However, from the 281 women enrolled at delivery in this study, 1.4% were infected in placental and cord blood but remained non-infected in the peripheral blood. These findings underline the complexity and the dynamic of *P. falciparum* infection in pregnant women and this indicate that the non-detection of *P. falciparum* by PCR in the peripheral blood does not reflect the absence of the infection in the placenta or in the baby cord blood. This situation constitute a real challenge for the fight for malaria elimination, since only the peripheral blood is available for diagnosis during pregnancy, and studies from various transmission settings have shown that submicroscopic infections can be up to five times more common than microscopic infections during pregnancy [19, 21].

*P. falciparum* submicroscopic parasitaemia is common in asymptomatic malaria, and increased scientific evidences showed that in sub Saharan Africa, most of the malaria infections become asymptomatic in multigravid women, due in part to anti-disease anti-*VAR2CSA* antibody immunity acquired from previous exposures [22, 23]. Thus, the prevalence of submicroscopic infection in the present study was expected to be higher in multigravid women, but such was not the case. In fact, no significant difference was observed between paucigravid and multigravid women regarding the prevalence of the infection in peripheral, placental, and cord blood compartments. These findings are different to those reported previously by Mockenhaupt et al [22] in Ghanaian women during pregnancy showing the increase of submicroscopic infection rate with increased gravidity, but corroborate those of Walker-Abbey et al [20] and Fadlseed et al [24] who did not detect any relationship between the infection and gravidity among pregnant women from Cameroon and Soudan respectively. These conflicted data can be partially explained by the fact that in many of these studies, the analysis on the association between the prevalence of submicroscopic infection and gravidity was not adjusted with others variables such as malaria drugs usage, which has been shown to increase the frequency of submicroscopic infection [22].

Conversely to a previous studies showing the reduced prevalence of placental malaria in women at delivery with increasing age [6, 25], no significant relationship was found in this study between the prevalence of submicroscopic infection and women age. The similar observation was reported by Omar and collaborators in a previous study carried out in women at delivery in Soudan (24). The use of IPT-SP in many sub-Saharan Africa has been associated with reduced microscopic malaria infection episodes during pregnancy, and placental malaria at delivery [11]. This is in line with the borderline association of IPT usage with decreased malaria prevalence observed in this study (adjusted odds ratio [OR] 0.62, 95% CI 0.37-1.02). This finding suggests that IPT-SP might also have significant impact on *P. falciparum* submicroscopic infection.

It has been previously reported that malaria infections in

pregnancy, even asymptomatic and submicroscopic, could jeopardize the health of mother, fetus, and newborn [9, 12, 26]. In the present study, the submicroscopic *P. falciparum* had no significant impact on the baby weight, the maternal gestational age, excepting the borderline positive association observed between the submicroscopic *P. falciparum* infection and the prevalence of anaemia (adjusted odds ratio [OR] 1.33, 95% CI 0.82-2.17).

In the context of this study, the absence of the association between submicroscopic infection at delivery and poor pregnancy outcomes such as low birth weight might be explained by the fact that the majority of infections would have been contracted only few days or few weeks before the delivery, thus were not established long enough to have significant effects on the baby weight. In fact, conflicted data exist in term of the relationship between submicroscopic malaria infection and poor pregnancy outcomes with verities of studies showing no association [19, 20, 27], and few studies showing positive association [12, 22]. However it is evident that submicroscopic infections represent an important reservoir of gametocytes, which can contribute to sustaining malaria transmission [13]. Thus, the high prevalence of *P. falciparum* submicroscopic infection observed in this study in Congolese women at the delivery is worrisome and require awareness of authorities on the necessity to implement the sensitive method for the diagnosis of malaria infection during pregnancy.

## 5. Conclusion

These data revealed that the prevalence of submicroscopic *P. falciparum* infection is high in women at delivery in Brazzaville; and might increase the risk for maternal anaemia. The infection had no impact on the prevalence of low birth weight and preterm delivery in this study sitting.

## Authors' Contributions

FN supervised the overall study. JEM and NSGG were responsible for samples collection and analysis. JCD was involved in data interpretation and analysis. CV was responsible for data management and statistical analysis. All authors were involved in manuscript drafting. All authors read and approved the final manuscript.

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