Sub-optimal Intermittent Preventive Treatment in pregnancy (IPTp) is associated with an increased risk of submicroscopic *P. falciparum* infection in pregnant women: a prospective cohort study in Benin

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Summary: In a cohort study, increased numbers of *P. falciparum* infections were found in pregnant women with a sub-optimal number of IPTp-SP doses, and in those whose first dose was late. The timing of IPTP-SP primarily affects the submicroscopic infections.

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Abstract

Background: Harmful maternal and neonatal health outcomes result from malaria in pregnancy, the prevention of which primarily relies on intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP). WHO recommends IPTp-SP in sub-Saharan Africa, but implementation is highly heterogeneous and often sub-optimal in terms of the number of doses and their timing. In this study, we assessed the impact of this heterogeneity on malaria in pregnancy, mainly with respect to submicroscopic *Plasmodium falciparum* infections.

Methods: We used data from 273 Beninese women followed throughout pregnancy. Screening for *P. falciparum* infections, using both microscopy- and polymerase chain reaction (PCR) -based methods, was performed monthly, and information on IPTp-SP dose was collected. Gestational age was estimated by repeated ultrasound scans. Using a negative binomial model, we investigated the effect of IPTp-SP doses and timing, after 17 weeks of gestation, on the number of *P. falciparum* infections, focusing on submicroscopic infections detectable only by PCR.

Results: At least two IPTp-SP doses were taken by 77.3% of the women. The median gestational age at first IPTp-SP dose was 22 weeks. A late first IPTp-SP dose (>21.2 weeks) was marginally associated with an increased number of *P. falciparum* infections (adjusted incidence rate ratio [aIRR] =1.3; p=0.098). The number of IPTp-SP doses was not associated with the number of submicroscopic infections (aIRR=1.2, p=0.543).

Conclusion: A late first IPTp-SP dose fail to provide optimal protection against *P. falciparum*, especially submicroscopic infections. This highlights the need for a new antimalarial drug for IPTp that could be taken early in pregnancy.

Keywords: Submicroscopic *P. falciparum* infection, pregnancy, intermittent preventive treatment, prospective cohort, sub-Saharan Africa.

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Introduction

In 2018, around 11 million women were exposed in sub-Saharan Africa (SSA) to malaria in pregnancy (MiP) [1], the adverse effects of which have been well documented [2–6]. To protect pregnant women from MiP in areas of stable malaria transmission the WHO recommends combined strategies including intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) [7–9]. IPTp-SP has succeeded in reducing malaria-related mortality and morbidity [10].

IPTp-SP is recommended for all pregnant women (infected or not), although contra-indicated in the first trimester because of possible teratogenic effects. It comprises repeated administrations one-month apart, of a curative dose of SP starting at the beginning of the second trimester of pregnancy (13 weeks of gestation (wg)) and continuing until delivery. In 2004, WHO recommended two doses of IPTp-SP during pregnancy to be taken at antenatal care (ANC) visits. It was revised eight years later to at least three recommended doses [7]. Since its implementation, there is substantial evidence that IPTp-SP helps to prevent the adverse effects of MiP [11, 12]. At the initiation of our study in Benin in 2014, the national guidelines recommended two doses of IPTp-SP after the 16th wg [13, 14]. This was modified in 2016 to recommend three doses [15].

Despite this clear policy, IPTp-SP implementation is highly heterogeneous across SSA. Thus IPTp-SP coverage varies greatly between countries and districts [13]. In 2017-2018, across different Benin districts, for example, coverage of at least two doses varies from 21.7% in Borgou to 55.6% in Mono with an average of 33.4% for the whole country [15]. Furthermore, pregnant women's gestational age (GA) at first ANC visit varies greatly, leading to variations in both timing and number of IPTp-SP doses. In West African countries like Benin, there is little data on IPTp-SP heterogeneity in terms of timing and number of doses and only few studies in SSA have assessed this question [16]. Such heterogeneity probably has consequences on women's protection against MiP, and is thus an important factor to assess. Moreover, the high prevalence of carriage of submicroscopic *P. falciparum* infections

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[17], especially in pregnancy [18], contributes to the transmission of malaria [19] and increases the risk of infections during pregnancy [20]. Investigating the impact of the heterogeneity of IPTp-SP implementation on the occurrence of submicroscopic infections during pregnancy is thus essential.

The "REtard de Croissance Intra-uterin et PALudisme (RECIPAL)" study, a prospective cohort with the follow-up of women from early pregnancy to delivery in Benin allowed to study in detail IPTp-SP implementation (number of doses and timing) and its impact on *P. falciparum* infections, both microscopic and submicroscopic, throughout pregnancy.

Methods

Study design

The RECIPAL study was conducted from June 2014 to August 2017 in southern Benin and was designed as a preconception cohort [21]. Women of reproductive age were first enrolled and followed-up monthly until they became pregnant. The pregnant women were then followed up at monthly ANC visits. During these visits, clinical, obstetrical and malaria infection screening data (by thick blood smear [TBS]) were collected. Dried blood spots were also collected monthly for PCR assays that were performed after the follow-up. Additionally, a TBS and a rapid diagnostic test (RDT) were performed in case of acute illness for rapid diagnosis and treatment. Five ultrasound scans were completed, the first conducted between 9 and 14 wg, ensuring accurate estimates of GA. IPTp-SP administrations at the health center were reported from ANC medical records of pregnant women. Infected women (detected by TBS or RDT, when applicable) were given appropriate antimalarial treatment according to the national guidelines applicable at the time of the study.

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Laboratory procedures

Parasitaemia was i) quantified by the Lambaréné technique with a detection threshold estimated at 5 parasites/µL [22, 23] and ii) tested by real-time quantitative PCR that targeted the 18S rDNA [24, 25]. A negative control with no DNA template was ran in all reactions. The RDT used was Pf+pan rapid test SD Bioline Ag (IDA Foundation, Netherlands; BioSynex, France) for immediate diagnosis [21].

Ethics Statement

The Ethics Committee of the "Institut des Sciences Biomédicales Appliquées" and the Ministry of Health in Benin approved this study. Before enrolment, the study was explained to each participant in the local language, and her voluntary informed consent was obtained. All treatments administered for acute illnesses during pregnancy were paid for by the project.

Statistical analysis

We used a negative binomial model to assess the effect of IPTp-SP use on the number of (i) both microscopic and submicroscopic and (ii) submicroscopic *P. falciparum* infections occurring during pregnancy with an adjustment for the confounding variables. The assumption supporting the analyses was that not only the number of IPTp-SP doses, but also their timing (especially the first administration), could play an important role in the protection against *P. falciparum* infections, [16]. As shown Figure 1, different scenarios involving these two variables show either the potential impact of the number of IPTp-SP doses (first two scenarios) or the impact of the delay between the onset of pregnancy and intake of the first dose (last two scenarios) on the protective effect of IPTp-SP during pregnancy, knowing in addition that timing and number of doses are correlated (the later a woman receives her first dose, the lower the possible number of doses).

Because IPTp-SP was recommended after 16 wg in Benin at the start of RECIPAL, we assumed that the first IPTp-SP dose should theoretically have been administered to all pregnant women from 17 wg. Our outcome variable was thus the number of *P. falciparum*

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infections detected between 17 wg and delivery. In order to estimate the impact of the timing of the first IPTp-SP dose, a binary variable « early/late first dose of IPTp-SP » was defined according to the first quartile of the distribution of the women's GA at first dose (around 21.2 wg).

P. falciparum infection was defined, at each visit, as follows: "negative" if all tests (TBS, PCR, and RDT when applicable) were negative, "submicroscopic" if TBS (and RDT when applicable) were negative whereas PCR was positive, and "microscopic" if TBS or RDT (when applicable) was positive. Then, to assess the impact of IPTp-SP use on MiP, the number of *P. falciparum* infections occurring during the pregnancy was defined as the sum of *P. falciparum* infections (either submicroscopic or microscopic) at all the visits for each pregnant woman. In a second step, we restricted the sample to the women who did not present any microscopic infections during their pregnancy (i.e. either negative or submicroscopically infected at all visits) to evaluate the IPTp-SP effect on submicroscopic infections.

We defined two IPTp-SP-related explanatory variables: i) the number of doses received ("0-1" vs "2-3") over the period of theoretical IPTp-SP protection during pregnancy (from 17 wg to delivery) and ii) the timing of the first dose ("before 21.2 wg" vs "after 21.2 wg").

The potential confounding factors included maternal socio-demographic characteristics; *P. falciparum* infection during the first trimester of pregnancy (≤14 wg) defined as "negative" at each visit, "submicroscopic" if only submicroscopic infections were diagnosed during the trimester and "microscopic" if at least one microscopic infection was detected during the trimester; use of Insecticide Treated bed Nets (Yes/No); gravidity and the season at delivery. To take into account the different number of ANC visits for the detection of *P. falciparum* infections for each woman during the follow-up, the log of the number of visits was used as an offset in the model.

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Variables were eliminated by a step-by-step backward selection procedure. We retained variables with a p-value less than 0.1. Statistical analyses were carried out with STATA software version 13.1.

Results

Of the 273 pregnant women, 10 (3.7%) did not receive any IPTp-SP. One, two and three doses of IPTp-SP were taken by, respectively, 19%, 63% and 14% of the women throughout pregnancy.

Pregnant women's characteristics

Table 1 shows that maternal age, gestational rank and marital status were similar between women as a function of the number of IPTp-SP doses (0-1 versus 2-3). Women were on average 26 years old. Women with at least two doses had more ANC visits after the 17th wg, were more educated, and had a lower average number of infections than the ones with 0-1 IPTp-SP dose during pregnancy (2.7 vs 3.2).

Figure 2 shows the proportion of *P. falciparum* infections during pregnancy before and after the first IPTp-SP dose according to the number of doses. Whatever the period of gestation, the proportion of submicroscopic infections was always higher than microscopic infections (chi2 test, all p<0.001). We observed a higher proportion of *P. falciparum* infections (both microscopic and submicroscopic) in pregnant women who had one dose of IPTp-SP (32.7%) compared to those with at least two (13.9%) (p<0.001).

Overall, the median GA at first IPTp-SP dose was 22.6 wg. Median GA was respectively 27.6, 22.6 and 20.4 wg for women who had one, two or three IPTp-SP doses during pregnancy. Regardless of the number of doses (Figure 3), at least 70% of women had their first IPTp-SP dose administered after 17 wg (right-hand graphs). However, most women had their previous visit at a GA at which they were eligible for IPTp-SP but did not receive it

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(left-hand graphs). Indeed, 75% of the women who had one IPTp-SP dose could have received it earlier.

Factors contributing to the number of P. falciparum infections

In Table 2 (final multivariate model), a low number of doses and a late first dose of IPTp-SP were associated with a higher number of *P. falciparum* infections after 17 wg (p=0.009 and p=0.098, respectively). In the 173 women who received two IPTp-SP doses, a late first IPTp-SP dose was also positively associated with a higher number of *P. falciparum* infections (p=0.084, Table 3).

We then focused the analysis on the women with no microscopic infection after 17 wg. These women were similar to those who had at least one microscopic infection according to gravidity, sociodemographic characteristics, number of ANC visits after 17 wg (on average 5) and the timing of the first dose. In contrast, most of them (83.4%) had at least two IPTp-SP doses during pregnancy vs 69% in pregnant women who harbored at least one microscopic infection (Table 4). In this subsample, the proportion of women with at least one submicroscopic infection after 17 wg was 52.9% (83/157), the remaining being uninfected at all visits.

In this sub-sample, the same factors contributed to the number of submicroscopic infections except, the number of IPTp-SP doses (tables 5 and 6). In addition, the association between a late GA at first IPTp-SP dose (> 21.2 wg) and an increased number of submicroscopic infections seemed to be more pronounced than in the total sample.

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Discussion

Many clinical trials have demonstrated the efficacy of IPTp-SP in MiP in Africa. Most studies [9, 15, 26, 27] have described the impact of the number of doses but very few have described the impact of the timing of IPTp-SP [16], especially with respect to submicroscopic infections. We showed that a sub-optimal number of IPTp-SP doses, as well as a delay in administration of the first dose of SP are associated with an increased risk of *P. falciparum* infection, particularly submicroscopic, during pregnancy.

The proportion of women with at least two IPTp-SP doses (77.3%) was higher than the coverage officially reported in the study area based on the Demographic and Health Survey (39.2% in 2017-2018) [15]. This may be partially due to the regular visits planned in thisstudy. The proportion of pregnant women who received 3 doses (13%) was below the WHO report estimations for the whole SSA (18% in 2015 and 19% in 2016) [10]. In Benin, the official recommendation of three IPTp-SP doses was implemented in 2016, near the end of the RECIPAL study, which probably explains (at least partially) this low proportion. Of important note, although the two-dose recommendation was implemented 10 years ago in Benin, there were still 25% of pregnant women who received no or only one dose, in a study with on average 8 ANC visits and monitoring since the first trimester. We observed also that women received on average their first dose much later than recommended by the Benin national guidelines (median 22.6 wg vs 16 wg as recommended) [15]. Such a delay could imply that women did not attend ANC visits early enough to have their first dose on time, but our data contradict this assumption, showing that the vast majority of women (around 75%) attended an ANC visit at a time (GA) at which they were indeed eligible to receive the first dose, but were not given the drug. This raises the question of the health staff's understanding of and adherence to the IPTp-SP policy. Several reasons are reported in the literature concerning inappropriate administration of the first IPTp-SP dose the most plausible argument being stock-outs [28, 29]. During the study, there were indeed some SP stock-out issues, but in such situation, SP was provided to the women by the study, then

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this could not adequately explain such a late first dose. Thus, sub-optimal IPTp-SP administration is a reality and unfortunately a common occurrence.

We assessed the consequences of this sub-optimal administration on the protection of women against MiP. We confirmed that a low number of IPTp-SP doses was significantly associated with a higher risk of *P. falciparum* infection during pregnancy; which is consistent with the literature [9, 15, 26, 27]. In addition, the late timing of the first dose was associated with an increased risk of infection, independently of the number of doses. A delayed first dose (as well as a low number of doses), shortening the duration of the period of protection against infection, leaves women more vulnerable to MiP [16]. Additionally, our results have shown a non-negligible proportion of women infected during their pregnancy, even in those who received two or three doses of IPTp. One possible hypothesis could be the resistance of the parasites to SP, but in Benin the majority of mutants contain triple or quadruple mutations which do not seem to cause a lack of efficacy of SP [34]. Resistance to SP therefore does not seem to be the major hypothesis explaining this.

Our focus on the subsample of women with no microscopic infections is particularly valuable as they would be classified "negative" by the usually diagnostic methods (TBS or RDT). Interestingly, we found that most of them (53%) had at least one submicroscopic infection after 17 wg, making these women a potential reservoir of malaria transmission [19] with an increased risk of adverse consequences [18, 30, 31]. Our results suggested that a higher number of IPTp-SP doses was less efficient in clearing submicroscopic infections than microscopic infections. In addition, taking the first dose comparatively early seems important for prevention of the occurrence of both submicroscopic and microscopic infections. As the study period overlapped with the period during which the Beninese national policy was revised to apply the new WHO strategy (3 doses or more), it would clearly be interesting to strengthen our results by other studies with the current recommendations of IPTp-SP3+.

Furthermore, we also found a substantial impact of *P. falciparum* infections (submicroscopic and microscopic) detected in the first trimester (a known critical and non-

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optimally protected period [20, 32, 33]) on the number of *P. falciparum* infections occurring after 17 wg, independently of IPTp-SP administration. An explanation could be that those women may be the most exposed during the overall pregnancy. This result suggests that a preventive measure starting early in pregnancy or even before pregnancy such as malaria vaccine would be of particular public health relevance. Thus, initiatives like IPTp community delivery (by community health workers) may be an interesting option for improving early IPTp administration [34, 35]. Later, it could lead to an easier access to IPTp in the community with a drug safe in the first trimester [36].

Our study has limitations. First, our conclusions about the sub-optimal IPTp-SP implementation may not be generalizable. However, such sub-optimal levels of IPTp-SP implementation have been reported in multiple Benin districts [15] as well as in several other SSA countries [37–39]. This suggests that sub-optimal administration, in terms of number of doses as well as adequate timing (the two being linked) is probably not just a local but could also be national or even regional issue. The monthly monitoring conducted as part of the study protocol increased the number of ANC visits compared to real-life settings, and could thus be considered as a second limitation since it could have impacted on IPTp-SP implementation. However, in this case in real-life, the gaps in IPTp-SP implementation may be more pronounced than in this study with a likely higher impact on MiP.

Our study has also several strengths. The follow-up of women since the early pregnancy allowed the collection of prospective data related to IPTp-SP administration (number and timing of doses) and avoided the memory bias inherent to retrospective studies. The study design also allowed for the detection of first trimester malaria infections, revealing their impact on the occurrence of infections from the second trimester onwards despite IPTp-SP. Moreover, accurate GA dating substantially increased the fidelity of our results. Finally, diagnosis by PCR allowed to assess the impact of IPTp-SP administration on the reservoir of submicroscopic infections, which remain untreated routinely, as usually asymptomatic.

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We highlighted the association between a sub-optimal IPTp-SP administration and the increased risk of MiP. An important result is that a late first dose of IPTp-SP decreases the prophylactic effect of IPTp-SP and precludes the full clearance of *P. falciparum* infections (both submicroscopic and microscopic) during pregnancy. It is therefore urgent, as a public health target, to increase access to early IPTp administration, preferably with a drug tolerated during the first trimester of pregnancy. The major challenge will be to reach pregnant women in SSA early in the first months of pregnancy, a time when they are generally reluctant to attend ANC visit.

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Notes

Authors' contributions:

Study conception and design: M.A., A.M., M.C., and V.B. (Principal Investigator)/Data collection: M.A., E.Y., N.F., D.S., B.V. and V.B./Statistical analyses: C.P.A.H. and G.C./Manuscript writing: C.P.A.H., V.B., M.C., and G.C./Biology and molecular analyses: N.T.N., A.M., D.S., B.V. and N.F. /All authors read and approved the final manuscript.

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Potential conflict of Interest:

No conflict declared. All authors report no potential conflict of interest. All authors have submitted the ICMJE Form of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Abbreviations

ANC: Antenatal care

IPTp: Intermittent Preventive Treatment in pregnancy

IPTp-SP: Intermittent Preventive Treatment in pregnancy with sulfadoxine-pyriméthamine

MiP: Malaria in Pregnancy

PCR: Polymerase Chain Reaction

P. falciparum : *Plasmodium falciparum*

qPCR : quantitative Polymerase Chain Reaction

RECIPAL: REtard de Croissance Intra-uterin et PALudisme

SP: Sulfadoxine-Pyriméthamine

SSA: Sub-Saharan Africa

TBS: Thick Blood Smear

Wg: Week of Gestation

WHO: World Health Organization

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Figure legends

Figure 1: Example of four hypothetical scenarios illustrating the different possible impacts of number of doses and timing of IPTP-SP on the pregnant women's protection against P. falciparum infection

Legend:

We assumed that the theoretical protection period of the pregnant women started from the 17th wg. Scenarios 1 and 2 consider two pregnant women taking respectively three and one doses of IPTP-SP, with the first dose at the same time. The effect of IPTP-SP remains high for the first woman, but decreases over time for the second one, showing the likely relationship between the number of doses and the protective effect during pregnancy. Scenarios 3 and 4 show two pregnant women with the same number of IPTP-SP doses but a different timing for the first dose. Scenario 4 (late first dose) implies a longer unprotected period between 17w.g. and the first dose, and therefore a greater susceptibility to P. falciparum infection, illustrating the possible relationship between the timing of the first dose and the protection of women against P. falciparum infections.

*: Theoretical maximum period of IPTP-SP doses coverage; #: Unprotected period; + + - : Evolution of the IPTP-SP' effect on the protection of pregnant women against infection with *P. falciparum* infections

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Figure 2: Proportion of *P. falciparum* with or without infection before and after the first IPTp dose for the 273 pregnant women, RECIPAL 2014-2017, Benin

P: p-value corresponding to the chi-square test

Figure 3: Distribution of the gestational age, at the visit preceding the first IPTP-SP dose and at the visit of the first IPTP-SP dose, according to the total number of the IPTP-SP dose per women during pregnancy, RECIPAL 2014-2017 (N=263), Benin

Legend:

The numbers at the right of the 17th wg correspond to (i) the proportion of visits where pregnant women were eligible to receive IPTP-SP but did not (left-hand graphs) and (ii) the proportion of pregnant women who received their first IPTP-SP dose after the 17th wg.

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Table 1: General characteristics of the 273 pregnant women included in the analysis comparing those who had 0 or 1 IPTp (n=62) versus 2 or 3 IPTp (n=211) during the pregnancy. RECIPAL cohort, 2014-2017, Benin

Characteristics		Pregnant women who received 0 or 1 IPTp dose (N= 62)	Pregnant women who received 2 or 3 IPTp doses (N=211)	p-value
		Mean or proportion (CI 95 %)	Mean or proportion (CI 95 %)	
Age (years)		26.2 [25.1-27.4]	26.9 [26.2-27.6]	0.34
Gestational rank	< 3 pregnancies	33.9 [22.7-46.8]	40.3 [33.8-47.1]	0.36
	>= 3 pregnancies	66.1 [53.2-77.0]	59.7 [52.9-66.2]	
Marital status	Cohabitation	8.1 [3.3-18.3]	6.2 [3.6-10.4]	0.59
	Married	91.9 [81.6-96.7]	93.8 [89.6-96.4]	
Ethnicity	Toffin	83.9 [72.2-91.2]	71.1 [64.5-76.8]	0.04
	Others	16.1 [8.7-27.8]	28.9 [23.1-35.4]	
Education level	Illiterate	80.6 [68.5-88.8]	68.7 [62.1-74.6]	0.07
	Literate	19.3 [11.1-31.4]	31.3 [25.3-37.9]	
Number of ANC visits	Between 17 and 21 wg	2.5 [2.0-2.9]	2.7 [2.4-2.9]	0.42
	After 17 wg	4.9 [4.8-5.2]	5.2 [5.1-5.4]	0.001
Proportion of women with at least one *P. falciparum* infections (microscopic+ submicroscopic) during pregnancy¥		83.9%	69.2%	0.13
Number of *P. falciparum* infections (microscopic+ submicroscopic) during pregnancy*		3.2 [2.6-3.7]	2.7 [2.5-3.0]	0.07

Abbreviations: IPTp, intermittent preventive treatment in pregnancy; N, number of pregnant women; RECIPAL, Retard de croissance intra-utérin et paludisme; ANC, antenatal consultation; CI, confidence interval; wg, weeks of gestation; p-values correspond to the t-test and chi-square tests for respectively continuous and categorical variables.

¥: Proportion of women with at least one *P. falciparum* infections (microscopic+ submicroscopic) detected at scheduled and emergency ANC visits during pregnancy

*: Number of *P. falciparum* infections for women with at least one infection during pregnancy

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Table 2: Factors associated with the number of *P. falciparum* infections (microscopic+submicroscopic) after the 17th week of gestation (Multivariate negative binomial model, N=273), RECIPAL 2014-2017, Benin

Variables		Number of *P. falciparum* infection(s)			
		N	aIRR	CI 95%	p-value
Microscopic *P. falciparum* infection at the first trimester of pregnancy		55	1.52	[1.07-2.15]	0.019
Submicroscopic *P. falciparum* infection at the first trimester of pregnancy		79	1.75	[1.28-2.38]	<0.001
Total number of IPTp doses during the pregnancy	2&3	211	1		
	0&1	62	1.50	[1.11-2.03]	0.009
Ethnicity	Toffin	202	1.62	[1.12-2.33]	0.010
Timing (wg) of the first IPTp intake	≤ 21.2	68	1		
	>21.2	205	1.34	[0.95-1.88]	0.098

Abbreviations: N, number of pregnant women; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; aIRR, adjusted incidence rate ratio; wg, weeks of gestation.

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Table 3: Factors associated with the number of *P. falciparum* infections (microscopic+submicroscopic) after the 17th week of gestation for women receiving 2 IPTp doses during the pregnancy (Negative binomial model, N=173), RECIPAL 2014-2017, Benin

Variables		Number of *P. falciparum* infection(s)			
		N	aIRR	CI 95%	p-value
Microscopic *P. falciparum* at the first trimester of pregnancy		35	1.99	[1.28-3.11]	0.002
Submicroscopic *P. falciparum* infection at the first trimester of pregnancy		51	1.99	[1.32-2.99]	0.001
Ethnicity	Toffin	125	1.91	[1.20-3.04]	0.007
Timing of the first IPTp dose	≤ 21.2 wg	40	1		
	>21.2 wg	133	1.48	[0.95-2.32]	0.084

Abbreviations: N, number of pregnant women; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; aIRR, adjusted incidence rate ratio; IPTp, intermittent preventive treatment in pregnancy; wg, weeks of gestation.

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Table 4: Comparison of the characteristics of pregnant women with or without microscopic *P. falciparum* infection, N=273, RECIPAL 2014-2017, Benin

Characteristics		Pregnant women without microscopic *P. falciparum* infections during the pregnancy (N=157)	Pregnant women with at least one microscopic *P. falciparum* infection during the pregnancy (N=116)	p-value *
		Mean or proportion (CI 95 %)	Mean or proportion (CI 95 %)	
Age (years)		27.3 [27.0-27.6]	26.1 [25.7-26.3]	0.04
Ethnicity	Toffin	68.2 [60.4-75.0]	81.9 [73.7-88.0]	0.01
	Others	31.8 [24.9-39.6]	18.1 [12.0-26.3]	
Gravidity	< 3 pregnancies	36.9 [18.2-55.7]	41.4 [29.5-46.1]	0.46
	≥ 3 pregnancies	63.1 [44.3-81.8]	58.6 [53.9-70.5]	
Marital status	Cohabitation	6.4 [3.4-11.5]	6.9 [3.5-13.3]	0.86
	Married	93.6 [88.5-96.6]	93.1 [86.7-96.5]	
Education level	Illiterate	68.8 [61.0-75.6]	75.0 [66.2-82.1]	0.26
	Litterate	31.2 [24.4-39.0]	25.0 [17.9-33.8]	
Total number of ANC visits after 17 wg		5.0 [4.8-5.2]	5.3 [5.0-5.5]	0.06
Total number of IPTp doses during the pregnancy	0&1	16.6 [11.5-23.3]	31.0 [23.2-40.1]	0.005
	2&3	83.4 [76.7-88.5]	69.0 [59.8-76.8]	
Timing of the first IPTp dose	≤ 21.2 wg	27.4 [20.9-35.0]	21.6 [14.9-30.1]	0.27
	>21.2 wg	72.6 [65.0-79.1]	78.4 [70.0-85.1]	

Abbreviations: N, number of pregnant women; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; aIRR, ANC, antenatal care; wg, weeks of gestation.

*Student's t-test and χ2 test were used for comparing continuous and categorical variables, respectively.

Women with at least one submicroscopic infection after 17 weeks of gestation in the group of women with at least one microscopic infection during pregnancy were 84.4% (98/116).

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Table 5: Factors associated with the number of submicroscopic *P. falciparum* infections after the 17th week of gestation during the pregnancy (Multivariate negative binomial model, N=157). Sub-sample of pregnant women without microscopic *P. falciparum* infections from the 17th wg, RECIPAL 2014-2017, Benin

Variables		Number of *P. falciparum* infection(s)			
		N	aIRR	CI 95%	p-value
Submicroscopic *P. falciparum* infection at the first trimester of pregnancy		49	2.02	[1.23-3.31]	0.005
Total number of IPTp doses during the pregnancy	2&3	131	1		
	0&1	26	1.22	[0.64-2.34]	0.543
Ethnicity	Toffin	107	1.76	[0.96-3.24]	0.068
Timing of the first IPTp dose	≤ 21.2 wg	43	1		
	>21.2 wg	114	1.72	[0.94-3.14]	0.081

Abbreviations: wg, weeks of gestation; N, number of pregnant women; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; aIRR, adjusted incidence rate ratio; IPTP, intermittent preventive treatment in pregnancy.

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Table 6: Factors associated with the number of submicroscopic *P. falciparum* infections after the 17th week of gestation for women receiving 2 IPTp doses during the pregnancy (Negative binomial model, N=105). Sub-sample of pregnant women without microscopic *P. falciparum* infections from the 17th wg, RECIPAL 2014-2017, Benin

Variables		Number of *P. falciparum* infection(s)			
		N	aIRR	CI 95%	p-value
Submicroscopic *P. falciparum* at the first trimester of pregnancy		36	2.06	[1.10-3.87]	0.024
Ethnicity	Toffin	71	1.93	[0.90-4.13]	0.091
Timing of the first IPTp dose	≤21.2 wg	25	1		
	>21.2 wg	80	2.11	[0.90-4.94]	0.087

Abbreviations: N, number of pregnant women; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; aIRR, adjusted incidence rate ratio; IPTP, intermittent preventive treatment in pregnancy; wg, weeks of gestation.

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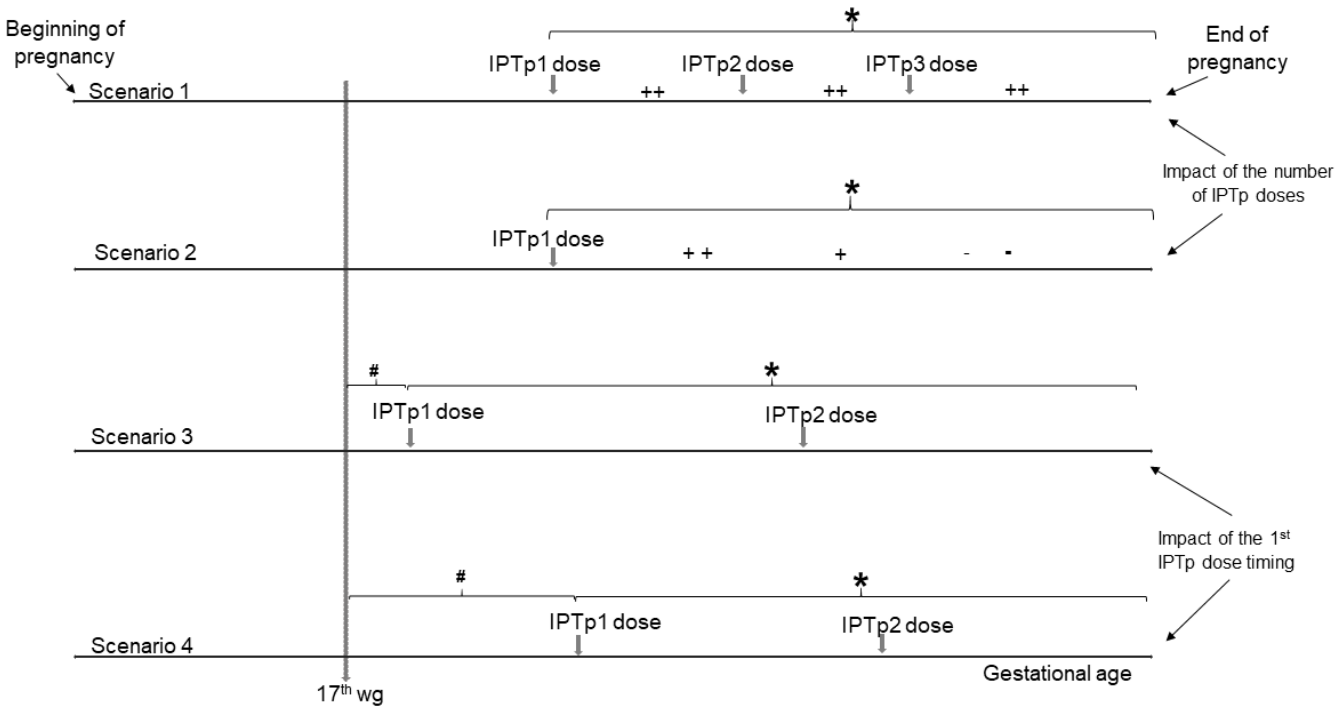

Figure 1: Example of four hypothetical scenarios illustrating the different possible impacts of number of doses and timing of IPTp-SP on the pregnant women's protection against *P. falciparum* infection

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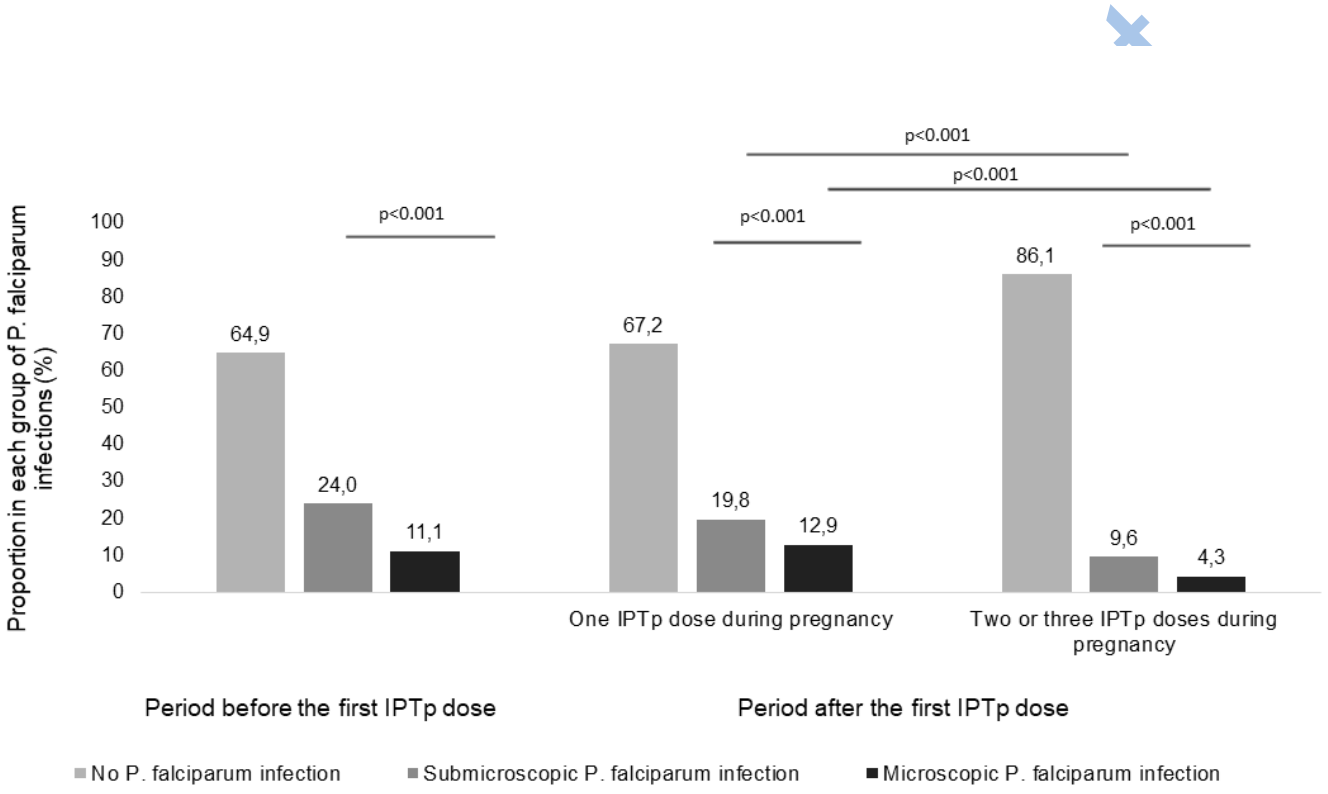

Figure 2: Proportion of *P. falciparum* with and without infection before and after the first IPTp dose for the 273 pregnant women, RECIPAL 2014-2017, Benin

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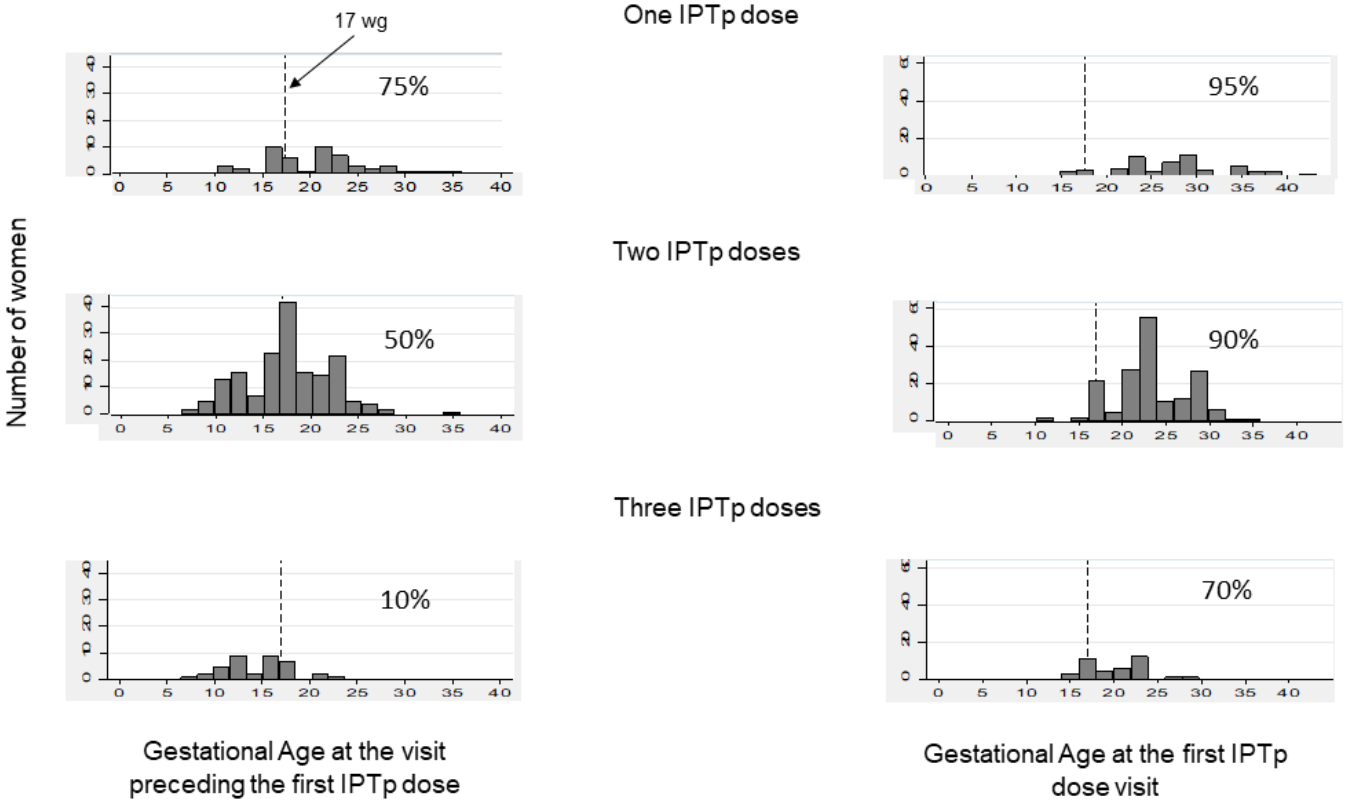

Figure 3: Distribution of gestational age, at the visit preceding the first IPTp dose and at the first IPTp dose visit, according to the total number of IPTp doses per woman during pregnancy, RECIPAL 2014-2017 (N=263), Benin

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