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Suboptimal Intermittent Preventive Treatment in Pregnancy (IPTp) is Associated With an Increased Risk of Submicroscopic *Plasmodium falciparum* Infection in Pregnant Women: A Prospective Cohort Study in Benin

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Background. Harmful maternal and neonatal health outcomes result from malaria in pregnancy, the prevention of which primarily relies on intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP). The World Health Organization recommends IPTp-SP in sub-Saharan Africa, but implementation is highly heterogeneous and often suboptimal 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-based and polymerase chain reaction (PCR)–based methods, was performed monthly, and information on IPTp-SP doses 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 2 IPTp-SP doses were taken by 77.3% of the women. The median gestational age at the 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; $P = .098$). The number of IPTp-SP doses was not associated with the number of submicroscopic infections (aIRR = 1.2, $P = .543$).

Conclusions. A late first IPTp-SP dose failed 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.

In 2018, around 11 million women in sub-Saharan Africa (SSA) were exposed 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 World Health Organization (WHO) recommends combined strategies, including intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (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 it is contra-indicated in the first trimester because of possible teratogenic effects. It comprises repeated administrations 1 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, the WHO recommended 2 doses of IPTp-SP during pregnancy to be taken at antenatal care (ANC) visits. The recommendation was revised 8 years later to at least 3 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 2 doses of IPTp-SP after the 16th wg [13, 14]. This was modified in 2016 to recommend 3 doses [15].

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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 2 doses varied 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 the first ANC visit varies greatly, leading to variations in both timing and numbers of IPTp-SP doses. In West African countries like Benin, the data are limited on IPTp-SP heterogeneity in terms of timing and number of doses, and only a 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 [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 that follows up with women from early pregnancy to delivery in Benin, allowed us to study in detail both 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 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 polymerase chain reaction (PCR) assays that were performed after the follow-up. Additionally, a TBS and a rapid diagnostic test (RDT) were performed in cases of acute illness, for rapid diagnosis and treatment. There were 5 ultrasound scans completed, with the first conducted between 9 and 14 wg, ensuring accurate estimates of the 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.

Laboratory Procedures

Parasitemia was (1) quantified by the Lambaréné technique, with a detection threshold estimated at 5 parasites/μL [22, 23]; and (2) tested by real-time quantitative PCR that targeted the 18S ribosomal DNA [24, 25]. A negative control with no DNA template was run in all reactions. The RDT used was Pf + pan rapid test SD Bioline Ag (IDA Foundation, Netherlands; BioSynex, France) for an 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 effects of IPTp-SP use on the number of (1) both microscopic and submicroscopic and (2) 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 protection against *P. falciparum* infections [16]. As shown in Figure 1, different scenarios involving these 2 variables show either the potential impact of the number of IPTp-SP doses (first 2 scenarios) or the impact of a delay between the onset of pregnancy and intake of the first dose (last 2 scenarios) on the protective effect of IPTp-SP during pregnancy, knowing in addition that the 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* 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 of either an early or 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).

Plasmodium falciparum infection was defined, at each visit, as negative if all tests (TBS, PCR, and RDT, when applicable) were negative, submicroscopic if TBS (and RDT, when applicable) was 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

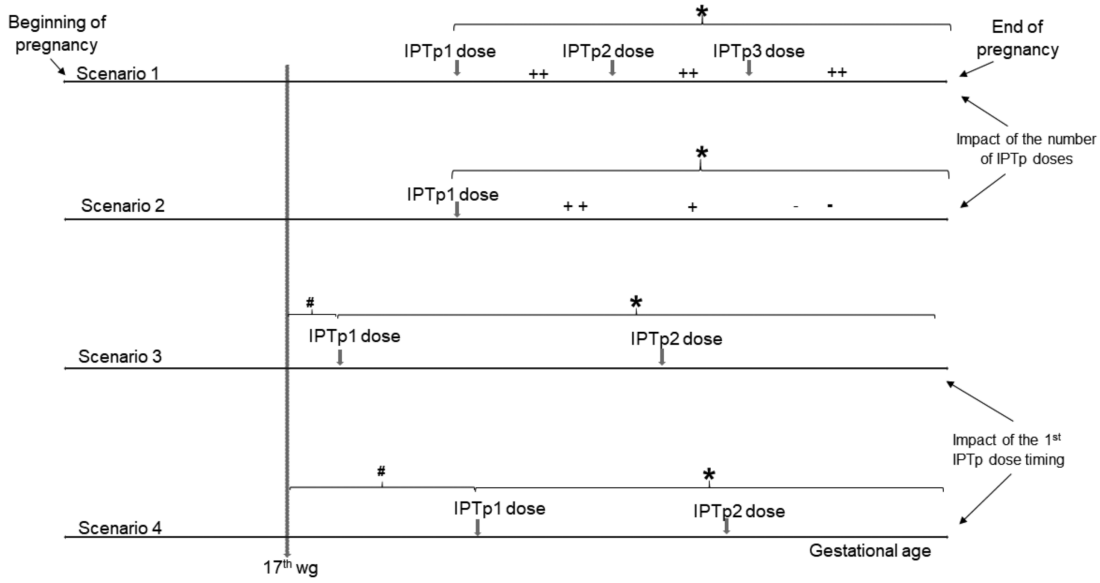

Figure 1. Example of 4 hypothetical scenarios illustrating the different possible impacts of the number of doses and timing of IPTp-SP on pregnant women's protection against *Plasmodium falciparum* infection. We assumed that the theoretical protection period of the pregnant women started from the 17th week of gestation. Scenarios 1 and 2 consider 2 pregnant women taking 3 and 1 doses, respectively, 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 dose, showing the likely relationship between the number of doses and the protective effect during pregnancy. Scenarios 3 and 4 show 2 pregnant women with the same number of IPTp-SP doses but with different timing for the first dose. Scenario 4 (late first dose) implies a longer unprotected period between 17 weeks of gestation and the first dose and, therefore, 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. The * indicates the theoretical maximum period of IPTp-SP doses coverage; # indicates the unprotected period; and ++ and - indicate the evolution of the IPTp-SP effect on the protection of pregnant women against infection with *P. falciparum*. Abbreviations: IPTp, intermittent preventive treatment in pregnancy; SP, sulfadoxine-pyrimethamine.

step, we restricted the sample to the women who did not present any microscopic infections during their pregnancy (ie, either negative or submicroscopically infected at all visits), to evaluate the IPTp-SP effect on submicroscopic infections.

We defined 2 IPTp-SP–related explanatory variables: (1) 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 (2) the timing of the first dose (before 21.2 wg vs after 21.2 wg).

The potential confounding factors included maternal sociodemographic characteristics; *P. falciparum* infection status during the first trimester of pregnancy (≤14 wg), defined as "negative" if negative at each visit, "submicroscopic" if only submicroscopic infections were diagnosed during the trimester, and "microscopic" if at least 1 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 specific 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.

Variables were eliminated by a step-by-step backward selection procedure. We retained variables with a P value less than .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. Throughout pregnancy, 1, 2, and 3 doses of IPTp-SP were taken by 19%, 63%, and 14% of the women, respectively.

Pregnant Women's Characteristics

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

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 that of microscopic infections (chi-square test; all P values < .001). We observed a higher proportion of *P. falciparum* infections (both microscopic and submicroscopic) in pregnant women who had 1 dose of IPTp-SP (32.7%) as compared to those with at least 2 doses (13.9%; P < .001).

Overall, the median GA at the first IPTp-SP dose was 22.6 wg. The median GAs were 27.6, 22.6, and 20.4 wg for women who had 1, 2, or 3 IPTp-SP doses, respectively, during

Table 1. General Characteristics of the 273 Pregnant Women Included in the Analysis

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 (95% CI)	Mean or proportion (95% CI)	
Age, years		26.2 (25.1–27.4)	26.9 (26.2–27.6)	.34
Gestational rank	<3 pregnancies	33.9 (22.7–46.8)	40.3 (33.8–47.1)	.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)	.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)	.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)	.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)	.42
	After 17 wg	4.9 (4.8–5.2)	5.2 (5.1–5.4)	.001
Proportion of women with at least 1 *P. falciparum* infection (microscopic + submicroscopic) during pregnancy[a]		83.9%	69.2%	.13
Number of *P. falciparum* infections (microscopic + submicroscopic) during pregnancy[b]	3.2 (2.6–3.7)	2.7 (2.5–3.0)	.07	

Data are comparing those who had 0 or 1 IPTp dose (n = 62) versus 2 or 3 IPTp doses (n = 211) during the pregnancy. RECIPAL cohort, 2014–2017, Benin. *P* values correspond to the *t*-test and chi-square tests for continuous and categorical variables, respectively.

Abbreviations: ANC, antenatal consultation; CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; *P. falciparum*, *Plasmodium falciparum*; RECIPAL, Retard de Croissance Intra-utérin et Paludisme study; wg, weeks of gestation.

[a]Proportion of women with at least 1 *P. falciparum* infection (microscopic + submicroscopic) detected at scheduled and emergency ANC visits during pregnancy.

[b]Number of *P. falciparum* infections for women with at least 1 infection during pregnancy.

pregnancy. Regardless of the number of doses (Figure 3), at least 70% of women had their first IPTp-SP dose administered after 17 wg (Figure 3B). However, most women had their previous visit at a GA at which they were eligible for IPTp-SP but did not receive it (Figure 3A). Indeed, 75% of the women who had 1 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 higher numbers of *P. falciparum* infections after 17 wg ($P = .009$ and $P = .098$, respectively). In the 173 women who received 2 IPTp-SP doses, a late first IPTp-SP dose was also positively associated with a higher number of *P. falciparum* infections ($P = .084$; Table 3).

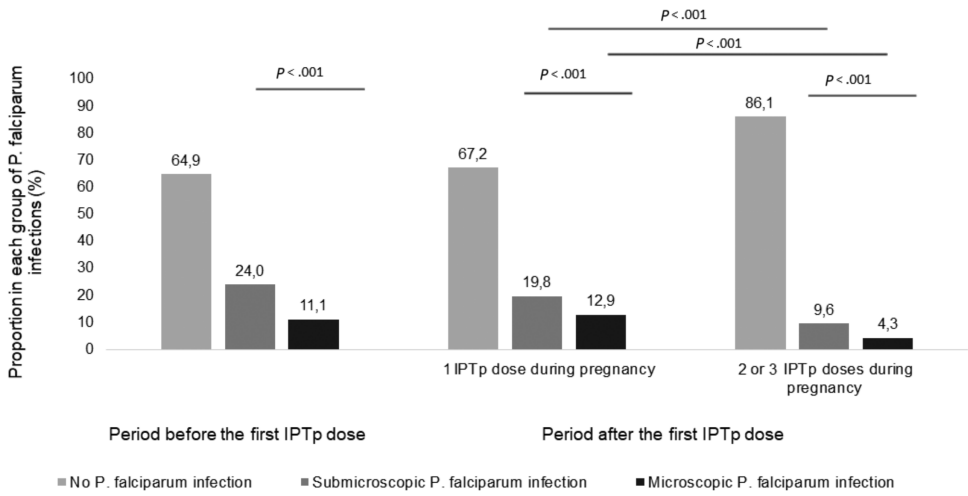

Figure 2. Proportions of *Plasmodium falciparum* with or without infection before and after the first IPTp dose for the 273 pregnant women, RECIPAL 2014–2017, Benin. The *P* values were calculated using the chi-square test. Abbreviations: IPTp, intermittent preventive treatment in pregnancy; RECIPAL, Retard de Croissance Intra-uterin et Paludisme study.

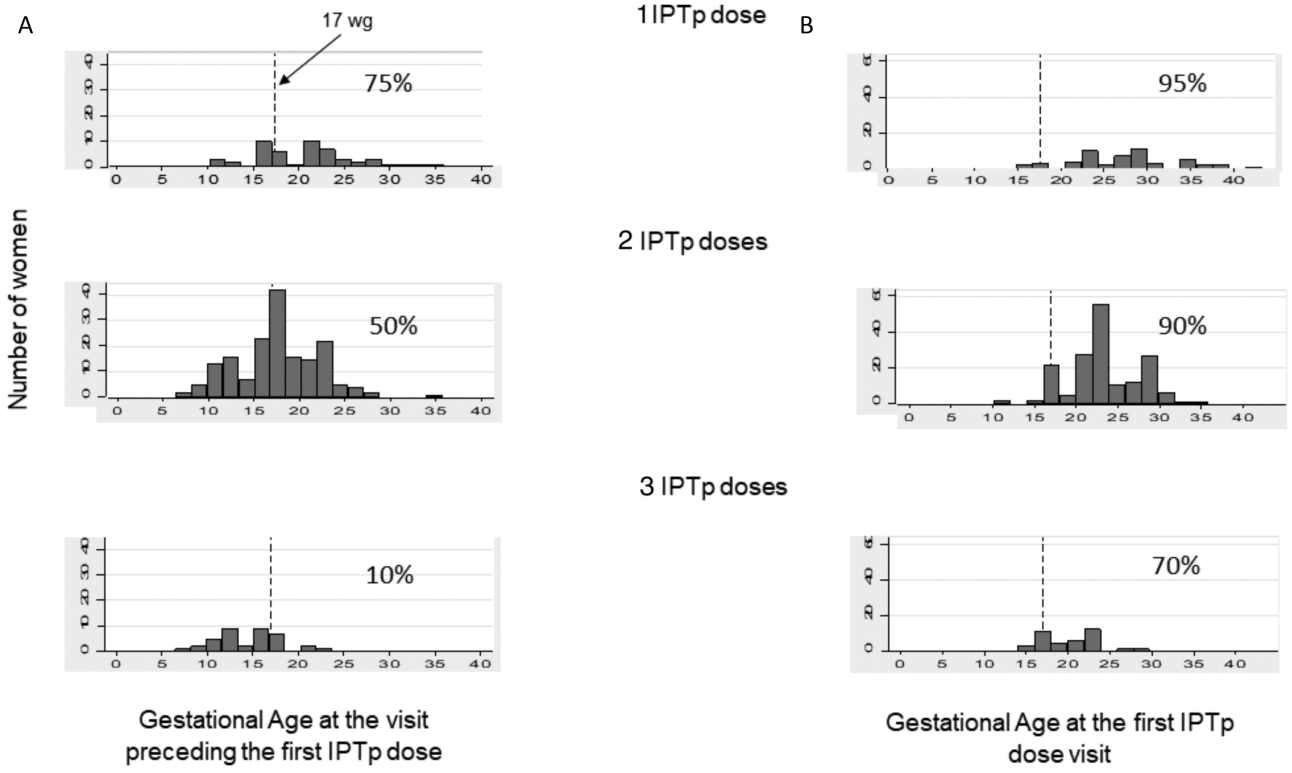

Figure 3. Distribution of the gestational age at the visit preceding the first IPTp-SP dose and at the visit of the first IPTp-SP dose, according to the total number of IPTp-SP doses per woman during pregnancy, RECIPAL 2014–2017 (n = 263), Benin. The numbers at the right of the 17th week of gestation correspond to (*A*) the proportion of visits where pregnant women were eligible to receive IPTp-SP but did not; and (*B*) the proportion of pregnant women who received their first IPTp-SP dose after the 17th week of gestation. Abbreviations: IPTp, intermittent preventive treatment in pregnancy; RECIPAL, Retard de Croissance Intra-uterin et Paludisme study; SP, sulfadoxine-pyrimethamine.

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 1 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 2 IPTp-SP doses during pregnancy, as compared to pregnant women who harbored at least 1 microscopic infection (69%; Table 4). In this subsample, the proportion of women with at least 1 submicroscopic infection after 17 wg was 52.9% (83/157), with the remaining women being uninfected at all visits.

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

Table 2. Factors Associated With the Number of *Plasmodium falciparum* Infections (Microscopic + Submicroscopic) After the 17th Week of Gestation

		Number of *P. falciparum* infection(s)			
Variables		n	aIRR	95% CI	*P* value
Microscopic *P. falciparum* infection in the first trimester of pregnancy		55	1.52	(1.07–2.15)	.019
Submicroscopic *P. falciparum* infection in the first trimester of pregnancy		79	1.75	(1.28–2.38)	<.001
Total number of IPTp doses during the pregnancy	2 and 3	211	1	…	
	0 and 1	62	1.50	(1.11–2.03)	.009
Ethnicity	Toffin	202	1.62	(1.12–2.33)	.010
Timing of the first IPTp intake, wg	≤21.2	68	1	…	
	>21.2	205	1.34	(.95–1.88)	.098

Data are from a multivariate negative binomial model (n = 273), RECIPAL 2014–2017, Benin.

Abbreviations: aIRR, adjusted incidence rate ratio; CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; *P. falciparum*, *Plasmodium falciparum*; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; wg, weeks of gestation.

Table 3. Factors Associated With the Number of *Plasmodium falciparum* Infections (Microscopic + Submicroscopic) After the 17th Week of Gestation for Women Receiving 2 Intermittent Preventive Treatment in Pregnancy Doses During the Pregnancy

		Number of *P. falciparum* infection(s)			
Variables		n	aIRR	95% CI	*P* value
Microscopic *P. falciparum* in the first trimester of pregnancy		35	1.99	(1.28–3.11)	.002
Submicroscopic *P. falciparum* infection in the first trimester of pregnancy		51	1.99	(1.32–2.99)	.001
Ethnicity	Toffin	125	1.91	(1.20–3.04)	.007
Timing of the first IPTp dose	≤21.2 wg	40	1	…	
	>21.2 wg	133	1.48	(.95–2.32)	.084

Data are from a negative binomial model (n = 173), RECIPAL, 2014–2017, Benin.

Abbreviations: aIRR, adjusted incidence rate ratio; CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; *P. falciparum*, *Plasmodium falciparum*; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; wg, weeks of gestation.

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 suboptimal number of IPTp-SP doses, as well as a delay in administration of the first dose of SP, are associated with increased risks of *P. falciparum* infections, particularly submicroscopic infections, during pregnancy.

The proportion of women with at least 2 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 this study. The proportion of pregnant women who received 3 doses (13%) was below the WHO report estimations for the whole of SSA (18% in 2015 and 19% in 2016) [10]. In Benin, the official recommendation of 3 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 2-dose recommendation was implemented 10 years ago in Benin, 25% of pregnant women still received no or only 1 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 the ANC visits early enough to have their first dose on time, but our data contradict this assumption, showing that the vast majority of

Table 4. Comparison of the Characteristics of Pregnant Women With or Without Microscopic *Plasmodium falciparum* Infection

		Pregnant women without microscopic *P. falciparum* infections during the pregnancy, n = 157	Pregnant women with at least 1 microscopic *P. falciparum* infection during the pregnancy, n = 116	*P* value[a]
Characteristics		Mean or proportion (95% CI)	Mean or proportion (95% CI)	
Age, years		27.3 (27.0–27.6)	26.1 (25.7–26.3)	.04
Ethnicity	Toffin	68.2 (60.4–75.0)	81.9 (73.7–88.0)	.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)	.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)	.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)	.26
	Literate	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)	.06
Total number of IPTp doses during the pregnancy	0 and 1	16.6 (11.5–23.3)	31.0 (23.2–40.1)	.005
	2 and 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)	.27
	>21.2 wg	72.6 (65.0–79.1)	78.4 (70.0–85.1)	

Data are from RECIPAL, 2014–2017, Benin (n = 273). There were 84.4% (98/116) women with at least 1 submicroscopic infection after 17 weeks of gestation in the group of women with at least 1 microscopic infection during pregnancy.

Abbreviations: ANC, antenatal care; CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; *P. falciparum*, *Plasmodium falciparum*; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; wg, weeks of gestation.

[a]The Student *t*-test and $\chi 2$ test were used for comparing continuous and categorical variables, respectively.

Table 5. Factors Associated With the Number of Submicroscopic *Plasmodium falciparum* Infections After the 17th Week of Gestation During the Pregnancy

Variables		n	Number of *P. falciparum* infection(s) aIRR	95% CI	*P* value
Submicroscopic *P. falciparum* infection in the first trimester of pregnancy		49	2.02	(1.23–3.31)	.005
Total number of IPTp doses during the pregnancy	2 and 3	131	1	…	
	0 and 1	26	1.22	(.64–2.34)	.543
Ethnicity	Toffin	107	1.76	(.96–3.24)	.068
Timing of the first IPTp dose	≤21.2 wg	43	1	…	
	>21.2 wg	114	1.72	(.94–3.14)	.081

Data are from a multivariate negative binomial model (n = 157). Data are from a subsample of pregnant women without microscopic *P. falciparum* infections from the 17th wg, RECIPAL, 2014–2017, Benin.

Abbreviations: aIRR, adjusted incidence rate ratio; CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; *P. falciparum*, *Plasmodium falciparum*; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; wg, weeks of gestation.

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, with the most plausible argument being stock-outs [28, 29]. During the study, there were indeed some SP stock-out issues, but in such situations, SP was provided to the women by the study, so this theory could not adequately explain such late first doses. Thus, suboptimal IPTp-SP administration is a reality and, unfortunately, a common occurrence.

We assessed the consequences of this suboptimal 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 show a nonnegligible proportion of women who were infected during pregnancy, even in those who received 2 or 3 doses of IPTp. A 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 [30]. Resistance to SP, therefore, does not seem to be the major cause.

Our focus on the subsample of women with no microscopic infections is particularly valuable, as they would be classified "negative" by the usual diagnostic methods (TBS or RDT). Interestingly, we found that most of them (53%) had at least 1 submicroscopic infection after 17 wg, making these women a potential reservoir of malaria transmission [19] and putting them at an increased risk of adverse consequences [18, 31, 32]. 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 conducting other studies with the current recommendation of 3+ doses of IPTp-SP.

Furthermore, we also found a substantial impact of *P. falciparum* infections (submicroscopic and microscopic) detected in the first trimester (a known critical and nonoptimally protected period [20, 33, 34]) on the number of *P. falciparum* infections

Table 6. Factors Associated With the Number of Submicroscopic *Plasmodium falciparum* Infections After the 17th Week of Gestation for Women Receiving 2 Intermittent Preventive Treatment in Pregnancy Doses During the Pregnancy

Variables		n	Number of *P. falciparum* infection(s) aIRR	95% CI	*P* value
Submicroscopic *P. falciparum* in the first trimester of pregnancy		36	2.06	(1.10–3.87)	.024
Ethnicity	Toffin	71	1.93	(.90–4.13)	.091
Timing of the first IPTp dose	≤21.2 wg	25	1	…	
	>21.2 wg	80	2.11	(.90–4.94)	.087

Data are from a negative binomial model (n = 105). Data are from a subsample of pregnant women without microscopic *P. falciparum* infections from the 17th wg, RECIPAL, 2014–2017, Benin.

Abbreviations: aIRR, adjusted incidence rate ratio; CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; *P. falciparum*, *Plasmodium falciparum*; RECIPAL, Retard de Croissance Intra-utérin et Paludisme; wg, weeks of gestation.

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 a malaria vaccine, would be of particular public health relevance. Thus, IPTp community delivery (by community health workers) may be an interesting option for improving early IPTp administration [30, 35]. Later, it could lead to an easier access to IPTp in the community, with a drug that is safe in the first trimester [36].

Our study has limitations. First, our conclusions about the suboptimal IPTp-SP implementation may not be generalizable. However, such suboptimal 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 suboptimal administration, in terms of both the number of doses and adequate timing (the 2 being linked) is probably not just a local issue, but could also be a national or even regional issue. The monthly monitoring conducted as part of the study protocol increased the number of ANC visits as compared to real-life settings, and could thus be considered as a second limitation, since it could have impacted IPTp-SP implementation. 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 early in 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 us to assess the impact of IPTp-SP administration on the reservoir of submicroscopic infections, which routinely remain untreated since they are usually asymptomatic.

We highlighted the association between suboptimal 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, at a time when they are generally reluctant to attend ANC visits.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. V. B., M. C., and G. C. contributed equally to the work. M. A., A. Massougbodji, M. C., and V. B. conceived of and designed the study. V. B. was the Principal Investigator. M. A., E. Y., N. F., D. S., B. V., and V. B. collected the data. C. P. A. H. and G. C. conducted the statistical analyses. C. P. A. H., V.B., M. C., and G. C. wrote the manuscript. N. T. N., A. Mama, D. S., B. V., and N. F. conducted the biology and molecular analyses. All authors read and approved the final manuscript.

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