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Incidences of asymptomatic *Plasmodium vivax* infection and its association with anaemia during pregnancy in malaria endemic population of Hazaribag, Jharkhand, India

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ABSTRACT

The escalating burden of malaria in pregnancy (MiP) is of public health concern across the globe, in view of the pathogenesis, epidemiology, clinical sequelae, prevention and treatment of malaria; all have unique features during pregnancy. The combinatorial impact of malaria in pregnancy includes effect of parasitic infection, anaemia, abortions, low birth weight, still births, and maternal mortality further perplexed the situation of prompt diagnosis, treatment and preventive strategies. This prompted us to estimate the population at risk of MIP in Hazaribag, Jharkhand, a malaria-endemic state in central-east India. Cross-sectional evaluation between September 2021 and December 2022 at antenatal clinics (ANC) and delivery units (DU) were performed at Sadar hospital, Hazaribag. Malaria was screened by Giemsa-stained blood smear and/or rapid diagnostic test using peripheral blood. Anaemia was defined as haemoglobin concentration. Pre-tested questionnaires were used to gather socio-demographic, clinical and obstetrical data. 1865 pregnant women were enrolled at the antenatal clinics and 934 at the delivery units. The prevalence of malaria during pregnancy was 4.8% and 3.7% at ANC and DU, and there were 11.4% malaria in women without pregnancy. Interestingly, majority (73%) were infected with *P.vivax*, and over 67% of the pregnant women were asymptomatic malaria. Anaemia was prevalent in 78% of ANC attendee as compared to 67% in DU, whereas, severe anaemia was 11.8% as compared to 6.3%. Anaemia was significantly associated with malaria; however, severe anaemia was more common among women with parasitaemia. In multivariate analyses, asymptomatic malaria increased over four times the likelihood of having anaemia. In view of sizable prevalence of malaria and in pregnancy with significantly associated anaemia and parasitemia suggests early diagnosis regardless of symptoms and comprehensive drug regime should be offered to pregnant women in association with existing measures in clinical spectrum of infection, delivery and its outcome.

Key Words - MIP, Asymptomatic, *P.vivax*, Jharkhand, Anaemia

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INTRODUCTION

Tropical malaria, which is caused by the protozoan parasites *Plasmodium falciparum* and *Plasmodium vivax*, is responsible for 478 million clinical cases (Snow *et al.*, 2005) and 0.5 to 2 million deaths

annually (Sachs, 2002). *Plasmodium vivax*, the most widespread parasite causing human malaria, is responsible for an estimated 123-367 million infections annually and is the major cause of malaria in most of Asia and Latin America (Baird, 2007; Hay *et al.,* 2004). Although *P. vivax* infection is commonly considered to be much more benign than *Plasmodium falciparum* infection, historical evidence suggests significant mortality associated with *P. vivax* malaria in the pre-antimalarial era (Dobson, 1994), and death caused by *P. vivax* malaria has been increasingly recognized over the past few years (Baird, 2007; Barcus *et al.,* 2007; Anstey and Price, 2007).

The emergence and spread of drug resistance to commonly used chemotherapeutics are major factors contributing to this increasing burden and most of the mortality and morbidity is borne by children and pregnant women. Pregnant women and their infants are susceptible to common and preventable infectious diseases including malaria but are woefully left unscreened and untreated. According to an estimate, approximately 125 million pregnant women worldwide are exposed to the risks of malaria in pregnancy (MiP) each year, resulting in 200,000 infant deaths (Dellicour *et al.*, 2010; Steketee *et al.*, 2001). Every year, in India, 28 million pregnancies take place with 67,000 maternal deaths (Registrar General of India. Sample Registration System. Special Bulletin on Maternal Mortality in India. 2004-06), 1 million women left with chronic ill health, and 1 million neonatal deaths (UNICEF, 2009). Pregnancy is an event of immunologic tolerance, whereby a woman accepts the implantation of the fetal allograft in her uterus and a unique period for vulnerability to malaria infection.

Pregnant women with low levels of pre-existing immunity are especially vulnerable to the most severe complications of malaria during pregnancy. These complications include cerebral malaria, severe anaemia, miscarriage, intrauterine fetal death, premature birth, stillbirth, and both maternal and infant mortality (Steketee *et al.*, 2001; Cot and Deloron, 2003). In regions where malaria is endemic, pregnant women are more susceptible to *Plasmodium* infections than non-pregnant women. While the most serious outcomes are typically seen in first-time mothers (primigravidae) (Rogerson *et al.,* 2007; Menendez, 2006), in areas with low or unstable transmission, women of all pregnancy orders are at similar risk (Menendez, 2006). Compared to non-pregnant women, pregnant women are three times more likely to develop severe malaria, with mortality rates from such cases reaching up to 50% (Monif, 2004; WHO, 2006).

Despite the severe and potentially fatal consequences of malaria during pregnancy for the mother, fetus, and newborn, these harmful effects are largely preventable through available interventions (Desai *et al.,* 2007) or timely and appropriate treatment following early and accurate diagnosis (Menendez *et al.,* 2007; Gamble *et al.,* 2007; Ter Kuile and Steketee, 2007). Since malaria infection during pregnancy is often asymptomatic, the most widely used control strategy is Intermittent Preventive Treatment in pregnancy (IPTp). This approach aims to clear existing infections at the time of administration and provide short-term prophylaxis to prevent new infections. However, growing concerns about widespread resistance to commonly used antimalarial drugs (Bardaji *et al.,* 2012) have highlighted the urgent need for alternative and more effective interventions. Diagnosing malaria during pregnancy is particularly challenging due to various factors, including the immunological changes across different stages of pregnancy, increased vulnerability to severe disease, obstetric complications, and the sequestration of parasites in the spleen and placenta. These challenges are often compounded by varying clinical presentations and different forms of anaemia. Consequently, the development of prompt and reliable diagnostic tools remains a critical focus of malaria in pregnancy (MiP) research.

Plasmodium falciparum malaria during pregnancy is a well-established cause of maternal and fetal morbidity and mortality. Although *P. vivax* infection

has received less attention, it is increasingly recognized as a significant contributor to maternal anaemia and low birth weight (Duffy *et al.,* 2001; Singh *et al.,* 1999; Nosten *et al.,* 1999), particularly in regions where both species frequently coexist. Of the approximately 50 million pregnancies occurring each year in malaria-endemic countries, nearly half take place in areas where *P. vivax* is prevalent. While *P. vivax* infection during pregnancy has been acknowledged for many years (Duffy *et al.,* 2001), its specific impact has only been systematically assessed in recent studies. Research from Thailand and India has shown that pregnant women infected with *P. vivax* are more likely to be anaemic and to deliver infants with lower birth weights compared to uninfected women. However, these effects tend to be less severe than those linked to *P. falciparum* infection. In both studies (Nosten *et al.,* 1999; Singh *et al.,* 1999), *P. vivax* infection was most frequently observed during first pregnancies, with prevalence peaking early in the second trimester.

Limited and earlier studies on malaria in pregnancy (MiP) in India have highlighted the significant role malaria plays in contributing to maternal and neonatal morbidity and mortality (Singh *et al.,* 1999; Singh *et al.*, 1998; Brooks *et al.*, 2008; Singh *et al.,* 1995). Preliminary findings, primarily from central India, suggest that both *Plasmodium falciparum* and *P. vivax* infections are associated with adverse pregnancy outcomes. However, these studies largely focused on symptomatic pregnant women infected with *P. vivax* (Singh *et al.,* 1999; Singh *et al.,* 1995), leaving significant gaps in understanding the broader impact of vivax malaria during pregnancy. Notably, there is a scarcity of data from Jharkhand-an understudied, tribal-dominated region with perennial malaria transmission-where malaria remains widespread and is responsible for a substantial number of annual deaths, second only to Odisha in India, according to recent reports (Dhingra *et al.,* 2010; Hussain *et al.,* 2011). This underscores the critical need for comprehensive investigations in the region, particularly concerning the pathology of malaria during pregnancy. As noted in previous research (Singh *et al.,* 1999), malaria infection during pregnancy is linked to an increased risk of neonatal and infant mortality, further emphasizing the urgency of focused research in high-burden areas like Jharkhand.

Given the limited data on asymptomatic and *Plasmodium vivax* infections during pregnancy in India, this study was undertaken with the objective of better estimating the burden of malaria in pregnancy (MiP), determining the prevalence of asymptomatic malaria, and assessing the relative contribution of *P. falciparum* and *P. vivax* infections during pregnancy and at delivery. To the best of our knowledge, such a comprehensive epidemiological and clinical profile has not previously been investigated in pregnant women from Hazaribag, Jharkhand-an underrepresented, malaria-endemic region of India. Notably, this study represents the first attempt to evaluate the interaction between anaemia, pregnancy, and asymptomatic malaria, stratified by clinical presentation, in an adult population residing in a perennial transmission zone characterized by co-dominance of *P. vivax* and *P. falciparum.* Conducted in Hazaribag, located in the eastern Indian state of Jharkhand, the study ultimately aims to inform and support the development of evidence-based policies to reduce the burden of MiP in this high-risk region.

METHODS

Study Sites/design and Population

This study was based on cross-sectional surveys conducted at three healthcare units: antenatal care (ANC) clinics, delivery units (DU), and the inpatient antepartum ward of Sadar Hospital in Hazaribag district, Jharkhand, India (Figure 1). Jharkhand, located in eastern India, is a malaria-endemic state with a significant tribal population. Over the past three years, the state has recorded an average Slide Positivity Rate (SPR) of 6.8% among symptomatic individuals, with *Plasmodium falciparum* accounting for 44% of cases (National Vector Borne Disease Control Programme, 2007). Malaria remains a major public health challenge in this region, particularly in tribal-dominated areas.

According to the Government of India's 2005 Draft National Policy on Tribals, the central-eastern belt-which includes Jharkhand- contributes approximately 15-20% of all malaria cases in the country. In 2009 alone, Jharkhand reported 230,686 malaria cases, with *P. falciparum* responsible for 39.53% (Jharkhand, 2009). Hazaribag district, where the present study was conducted, is recognized as a malaria-endemic area within this high-burden state and was selected for its representative nature of stable malaria transmission in tribal settings.

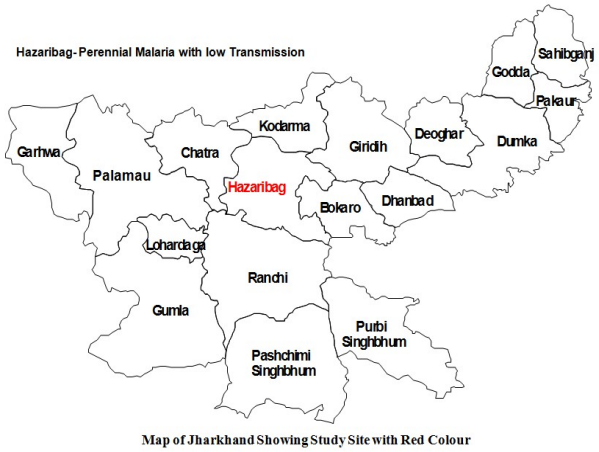

Figure 1- Map of Jharkhand Showing Study Site

Hazaribag district, with a total population of 1,734,005 according to the 2011 census, was selected for this study as it represents a rural-cum-semi-urban region with low but perennial malaria transmission. Over the past three years, Hazaribag recorded an average Slide Positivity Rate (SPR) of 7.3% among symptomatic individuals, with *Plasmodium falciparum* accounting for 14% of cases (Jharkhand, 2008; State Malaria Control Program Annual Report, Ranchi, Jharkhand, Directorate of Health Services). The population is socio-culturally diverse, comprising tribal communities, Scheduled Castes, Scheduled Tribes, and other caste groups, characterized by unique social stratification and notable gender disparities.

Situated in the tropical zone, the district experiences an average annual rainfall of 1234.5 mm and has geo-climatic and ecological conditions favorable for year-round malaria transmission. According to the District Level Household and Facility Survey (DLHS-3) conducted between December 2007 and April 2008, 56% of women had at least one antenatal clinic (ANC) visit, while only 18% delivered in health facilities-59% in urban areas and just 13% in rural settings (International Institute for Population Sciences, 2009).

Sadar Hospital, the primary district hospital serving Hazaribag's predominantly rural population, features a dedicated obstetric unit with 40 beds. Between 2010 and 2013, it recorded a high volume of annual deliveries, ranging from approximately 4,800 to 5,500. The hospital also managed a substantial number of ANC visits during the same period, averaging between 5,200 and 6,600 per year.

Screening and enrollment

The study comprised two components, with participant recruitment targeting all pregnant women presenting at antenatal care (ANC) units and delivery units (DU) ward.

For the ANC component, pregnant women aged $\geq$17 years who reported for routine care at the study site were screened and enrolled, provided they were willing and gave informed consent. In the DU component, women aged $\geq$18 years presenting for delivery were enrolled upon providing written informed consent. For the inpatient component, pregnant women admitted with a diagnosis of malaria, anaemia, or febrile illnesses of unknown origin were screened. Those with a confirmed malaria-related diagnosis were enrolled after obtaining informed consent.

At each point of contact, all women underwent clinical assessment, peripheral blood smear examination for malaria parasites, and measurement of axillary body temperature prior to enrollment. The detailed enrollment strategy, sampling procedures, and classification into broad clinical groups are illustrated in the schematic flow chart shown in Figure 2.

ANC procedures

Trained study personnel conducted interviews with enrolled participants to collect data on socio-demographic characteristics (e.g., date of birth, socio-economic status, literacy), reproductive

history including gravidity, recent history of fever, use of anti-malarial medications, and preventive measures against malaria. A comprehensive physical examination was performed for each participant, including assessment of gestational age-estimated by palpation of the uterine fundus in conjunction with the date of the last menstrual period-along with measurement of axillary temperature using a digital thermometer and evaluation of other vital signs.

Peripheral venous blood samples (3-5 ml) were collected from all participants for malaria blood film preparation, rapid diagnostic testing (RDT), haemoglobin measurement, and other biochemical and molecular analyses. Women who tested positive on the RDT or were found to be anaemic were referred immediately to a hospital physician for appropriate treatment. Additionally, hospital staffs were notified of any parasitaemic individuals identified through microscopy to ensure timely clinical management.

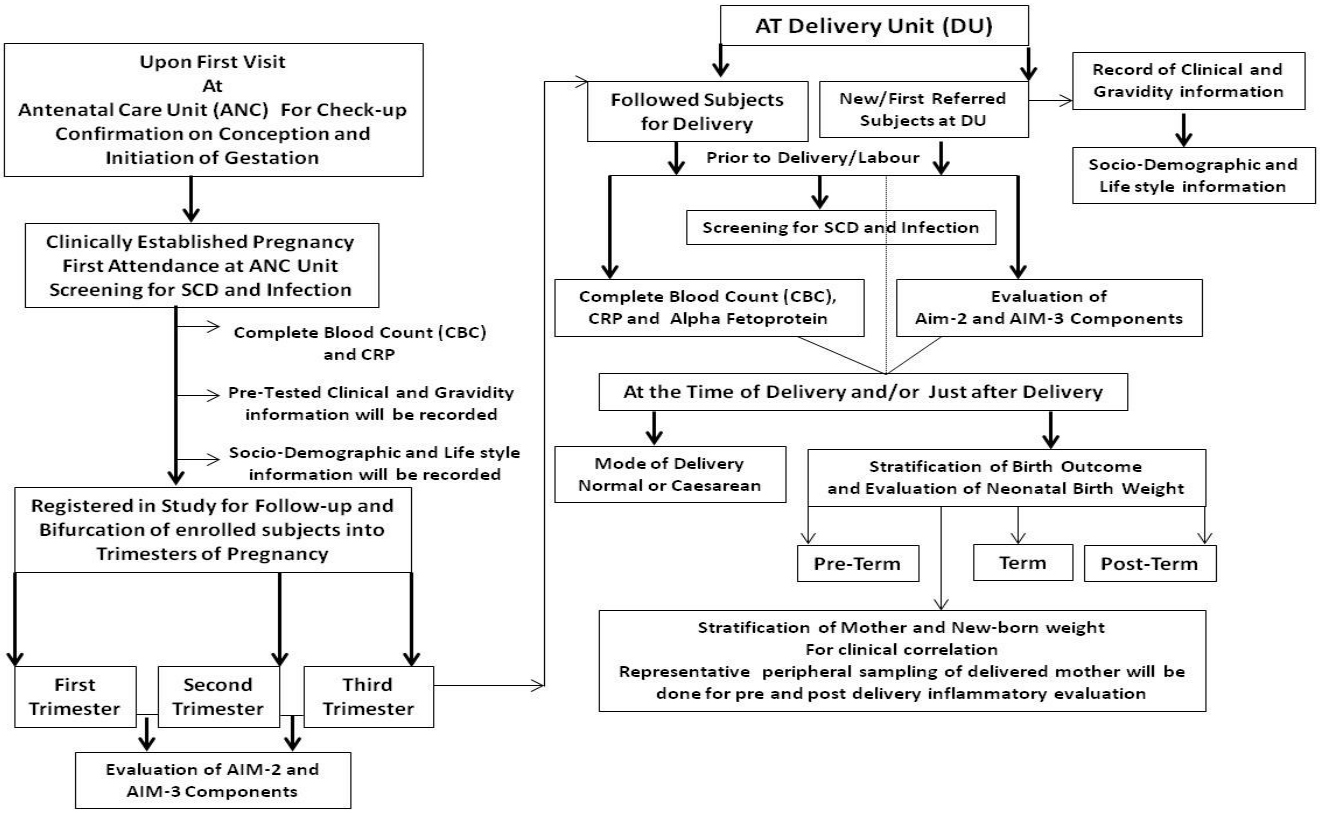

Figure 2- Schematic Flow Chart Summarizing the Sampling Strategy and Groups

DU procedures

Pregnant women enrolled at the delivery units (DUs) were interviewed to collect information on socio-demographic and anthropometric characteristics, obstetric complications, history of fever and anti-malarial drug use during pregnancy, use of anti-malarial preventive measures, birth outcomes, and mode of delivery. After delivery, peripheral venous blood samples (3-5 ml) were collected for malaria blood film preparation, rapid diagnostic testing (RDT), haemoglobin measurement, and additional biochemical and molecular analyses. Women who tested positive by RDT or blood smear were referred promptly for treatment.

In addition to assessing malaria prevalence among women at the DUs, clinical and demographic data and samples were also stratified by mode of delivery-normal, cesarean, and stillbirth-as well as by birth outcomes, including preterm, term, and post-term deliveries. These detailed results are presented in Table 1.

Table 1-Baseline characteristics of pregnant women attending antenatal and delivery units

Characteristics	Antenatal clinics n=1271	Delivery units n=870
	N, (%)	N, (%)
Age (Years)		
<20	166(13.1)	109(12.5)
20-34	983(77.4)	708(81.4)
≥35	122(9.5)	53(6.1)
Prior pregnancies		
Primigravid	423(33.3)	338(38.38)
Secundigravid	578(45.5)	209(24.1)
Multigravid*	270(21.2)	323(37.1)
Gestational age at enrollment (weeks)**		
<20 weeks	567(44.6)	n/a
20-36 weeks	641(50.4)	57(6.5)
≥37 weeks	63(5)	813(93.5)
Caste		
Schedule caste	169(13.3)	93(10.7)
General caste	428(33.7)	307(35.3)
Other backward caste	311(24.5)	219(25.2)
Scheduled tribe	363(28.5)	251(28.8)
Education		
No formal schooling	357(28.1)	321(36.9)
Attended school any length of time	914(71.9)	549(28.8)
Socioeconomic characteristics		
Owns TV	567(44.6)	387(44.5)
Owns bicycle	1173(92.2)	687(78.9)
Owns house	958(75.4)	643(73.9)
Owns refrigerator	123(9.6)	83(905)
Roof material		
Mud	622(48.9)	513(58.9)
Corrugated iron/asbestos sheet	242(19)	182(20.9)
cement/concrete	329(25.8)	107(12.3)
Other	78(6.1)	68(7.8)
Wall material		
Mud/sand/dung	673(52.9)	478(54.9)
Mud bricks	127(9.9)	93(10.7)
Cement bricks	419(32.9)	267(30.7)
Other	52(4.1)	32(3.7)
Primary cooking fuel		
wood	619(48.7)	387(44.5)
charcoal	437(34.4)	279(32.1)
Gas	153(12.1)	136(15.6)
Other	62(4.9)	68(7.8)
Mode of delivery among pregnant women		
Normal	n/a	586(67.3)
Caesarean	n/a	179(20.6)
Still Birth	n/a	105(12.1)
Birth Outcome		
Pre-Term Delivery(≤36 weeks)	n/a	129(14.8)
Term Delivery (31-41 weeks)	n/a	623(71.6)
Post-Term Delivery (after 41 weeks)	n/a	118(13.5)

†Numbers may not add to sample size secondary to missing data.
* Defined as 3 or more pregnancies
** For ANC enrollees, gestational age assessed by fundal height. For DU enrollees, gestational age was assessed by Ballard score.

Laboratory procedures

Thick and thin blood smears prepared from peripheral blood samples of ANC and DU participants were stained with Giemsa and examined under high-power microscopy. Parasite density was quantified by counting the number of asexual parasite forms per 200 leukocytes, assuming a standard leukocyte count of 8,000 leukocytes/?l of blood (Trape, 1985). The thin smear was specifically used for Plasmodium species identification. All slides were independently cross-checked by trained technical staff following stringent diagnostic criteria to confirm Plasmodium infection. Additionally, the commercial First Response Malaria pLDH/HRP2 combo rapid diagnostic test (RDT) kits (Premier Medical Corporation, Mumbai, India) were employed as per the manufacturer's instructions to screen pregnant women for malaria.

Haemoglobin Concentration

Hemoglobin (Hb) levels were measured during the initial ANC and DU visits. Hb concentrations were determined from peripheral blood samples using a portable HemoCue hemoglobinometer (HemoCue AB, Ängelholm, Sweden), following the manufacturer's instructions. The Hb values were recorded on the study questionnaire and verified by a laboratory technician. Women were classified as anemic if their Hb was below 11 g/dL. Further classification identified moderate to severe anemia with Hb levels below 8 g/dL and 7 g/dL, respectively, as the primary outcome. Those with Hb levels of 9 g/dL or higher were categorized as mildly anemic or non-anemic, based on the WHO anemia classification (McLean *et al.,* 2012).

Study definitions

Malaria was defined by the presence of asexual blood stages of *Plasmodium* parasites in peripheral blood or a positive rapid diagnostic test (RDT), regardless of the species or symptoms. Symptomatic malaria infection was identified when there was a history of fever within the past week or a temperature of $\geq$37.5°C, along with the presence of asexual forms of *P. falciparum* or *P. vivax* detected on a thick blood smear or a positive

RDT. Severe malaria was characterized by a malaria episode accompanied by any of the following conditions: cerebral malaria, severe anemia, renal failure, pulmonary edema, hypoglycemia, shock, spontaneous bleeding, or repeated convulsions. Maternal height and weight were measured during the first ANC and DU visits, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. A low BMI was defined as less than 22.0 kg/m². A documented fever was considered an axillary temperature of $\geq$37.5°C.

Ethics Statement and Subject Consent

All human blood samples used in this study were collected after obtaining consent from the study participants under protocols activities approved by the Institutional Ethics Committee (IEC) of the Vinoba Bhave University, Hazaribag, Jharkhand and human ethical guidelines as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Govt. of India. All study participants provided informed consent. The protocol is approved from IEC, VBU having memo no. VBU/R/888/2012 dated 05-06-2012.

Data management and analysis

All clinical, demographic, and anthropometric data were thoroughly reviewed for accuracy, and any inconsistencies were resolved prior to analysis. The data were entered into MS Excel, and statistical analyses were conducted using SPSS version 16 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, Inc., CA, USA). For comparing means between two groups, the Student's t-test was applied to normally distributed data, while non-parametric tests (Mann-Whitney U) were used when the data did not follow a normal distribution. Categorical variables are reported as frequency counts (percentages) and compared using either the chi-square test or Fisher's exact test, as appropriate. Continuous variables are presented as means (± standard error) and compared using t-tests or analysis of variance (ANOVA), depending on the context. Because most participants were unsure of their exact birthdates, ages were categorized into ranges based on their estimates. Risk factors for *P. falciparum* or *P. vivax* parasitemia were first assessed through univariate analysis, followed by multivariate analysis to adjust for significant predictors. Statistical significance was set at a p-value of less than 0.05.

RESULTS

Recruitment and Enrollment

Between September 2021 and December 2022, 1,890 pregnant women were screened during antenatal care (ANC) visits. Of these, 1,746 were willing to hear the study protocol, 1,271 consented and provided peripheral samples, while 475 declined participation. In parallel, 444 non-pregnant women consented to peripheral sampling. The final study population included 1,271 pregnant women and 444 non-pregnant women. Pregnant women were further stratified by trimester: 135 in the first, 492 in the second, and 644 in the third. The non-pregnant cohort comprised 227 malaria-positive and 217 malaria-negative women. In delivery units (DU), an additional 870 pregnant women were screened and enrolled.

Antenatal Clinics (ANC)

Most ANC participants were aged 18-38 years and had some formal education (Table 1). Nearly all were Hindi-speaking (97.6%) and non-smokers (98.7%). A majority owned their homes (75.4%) and were engaged in household work (76.7%), while 12.3% were involved in farming. The median number of ANC visits was one (range: 0-9), and 33.3% were primigravidae. Slightly more than half attended ANC during the second or third trimester, while 44.6% presented before 20 weeks' gestation. Only 46.3% reported iron/folate supplementation and 33.2% took multivitamins. Bed nets were commonly present in households, but insecticide-treated nets (ITNs) were rare (Table 2). Nine women reported taking malaria prophylaxis; however, seven could not identify the drug, while two reported chloroquine use.

Malaria prevalence in ANC attendees was 5.4% (68/1271; Table 3). Blood smears were positive in 4.3% of women, with an additional 1.1% detected by rapid diagnostic tests (RDTs). The mean parasite density among smear-positive women was 63,236

asexual forms/μl (range: 600-489,000). Species distribution showed Plasmodium falciparum in 4.4%, P. vivax in 86.8%, and mixed infections in 8.8%. Rural residence was strongly associated with parasitaemia (OR 4.32, 95% CI 1.67-9.46). Primigravidae and secundigravidae were also at higher risk compared to multigravidae (OR 4.75, 95% CI 1.23-11.58). A history of fever within the prior week or fever at enrollment was associated with parasitaemia (4.2% vs. 2.3%, p=0.02). Importantly, 70.6% (48/68) of malaria-positive women were asymptomatic. Most infections occurred between July and January, peaking during the monsoon season (August-October). Multivariate analysis confirmed that primigravidity, recent fever, and rural residence were independent predictors of parasitaemia (Table 4).

Table 2: Use of malaria prevention measures by pregnant women attending antenatal clinics and delivery units

Prevention measures utilized	Antenatal clinics n=1271 N (%)	Delivery units n=870 N (%)
Bed net in household	937(73.7)	643(73.9)
Insecticide-treated bed net in household	43(3.3)	21(2.4)
Sleeps under net most nights	873(68.6)	503(57.8)
Taken malaria prophylaxis in pregnancy	9(0.7)	3(0.3)

Table 3: Parasitaemia, reported fever, and anaemia among pregnant women attending antenatal clinics and delivery units

	Antenatal clinics n=1271 N (%)	Delivery units n=870 N (%)
Peripheral Parasitaemia		
Overall	68(5.4)	37(4.3)
Falciparum	3(0.23)	2(0.22)
Vivax	59(4.6)	32(3.67)
Mixed	6(0.47)	3(0.34)
By gravidity		
Primigravid	21/423(4.9)	11/338(3.2)
Secundigravid	38/578(6.6)	15/209(7.1)
Multigravid**	9/270(3.3)	11/323(3.4)
Report of fever within 1 week	167(13.1)	93(10.6)
Anaemia	1093(86)	626(72)
Severe anaemia	148/1093(13.6)	49/626(7.8)

Table-4 Factors associated with peripheral parasitemia during malaria in pregnancy (MIP) attending antenatal clinic (ANC) and delivery unit (DU) at Sadar Hospital, Hazaribag, Jharkhand, using univariate and multivariate analysis

Factors at ANC	Peripheral Parasitemia % (Positive/Total)	Adjusted OR (95%CI)	P	Adjusted OR (95%CI)	P
1st/2nd pregnancies	6.3 (64/1001)	4.45 (2.32 - 9.61)	0.0001	4.23 (2.15-8.42)	0.0001
3rd or greater pregnancies	1.4 (4/270)	1		1	
Age < 20	7.2 (12/166)	1.43 (0.34 - 3.76)	0.052	1.31 (0.26 - 2.84)	0.076
Age ≥ 20	5.0 (56/1105)	1		1	
Fever within past week	16.1 (27/167)	4.42 (3.64 - 8.21)	0.002	4.62(3.73-9.83)	0.001
No fever within past week	3.7 (41/1104)	1		1	
Bednet use*	7.6 (42/563)	1.12 (0.27 - 2.47)	0.072	1.37 (0.48 - 3.24)	0.084
No bednet use	6.7 (26/374)	1			
Rural	7.1 (61/857)	4.21 (1.53 - 5.21)	0.003	4.36(2.48-7.32)	0.0001
No Rural	1.7 (7/414)	1		1	
Tribal caste	6.3 (23/363)	1.26 (0.64 - 2.96)	0.054	1.42 (0.81 - 3.75)	0.12
No Tribal caste	4.9 (45/908)	1		1	
No formal education	6.1 (22/357)	1.22 (0.42 - 2.46)	0.065	1.34 (0.68 - 3.92)	0.084
Formal education	5.0 (46/914)	1		1	
Factors at DU					
1st/2nd pregnancies	5.8 (32/547)	3.9 (0.97 - 11.56)	0.004	3.62 (0.94-7.83)	0.001
3rd or greater pregnancies	1.5 (5/323)	1		1	
Age < 20	8.2 (28/109)	2.32 (1.32 - 9.37)	0.062	2.47 (1.17- 10.63)	0.14
Age ≥ 20	3.6 (9/761)	1		1	
Fever within past week	13.9 (13/93)	4.47 (1.25 - 12.42)	0.0001	4.43 (1.38 - 11.57)	0.0001
No fever within past week	3.1 (24/777)	1		1	
Bednet use*	5.3 (27/503)	1.97 (0.83 - 7.62)	0.084	1.62 (0.58 - 6.39)	0.27
No bednet use	2.7 (10/367)	1		1	
Rural	7.1 (29/405)	4.22 (0.41 - 4.51)	0.003	3.87 (0.78-13.62)	0.0001
No Rural	1.7 (8/465)	1		1	
Tribal caste	5.5 (14/251)	1.51 (0.56 - 3.92)	0.053	1.74 (0.83 - 5.38)	0.59
No Tribal caste	3.7 (23/619)	1		1	
No formal education	5.3 (17/321)	1.46 (1.23 - 3.17)	0.57	1.62 (0.87 - 4.63)	0.21
Formal education	3.6 (20/549)	1		1	

* ITN use was not evaluated in this model since these were very rarely used as well as quite lesser awareness about ITN among women.

Delivery Units (DU)

Similar demographic patterns were observed in the DU cohort: most participants were aged 20-36 years, formally educated, non-smokers (100%), and Hindi-speaking (97.2%) (Table 1). Most owned their homes (73.9%) and were primarily engaged in household work (84.3%). The median number of ANC visits was three (range: 0-9). Nearly two-thirds were primigravidae or secundigravidae. Bed nets were common, though ITN ownership remained rare (Table 2). Only three women reported malaria prophylaxis, none of whom could identify the drug.

Peripheral parasitaemia was detected in 4.3% of DU participants. Among these, *P. falciparum* accounted for 5.4% (2/37), *P. vivax* for 86.5% (32/37), and mixed infections for 8.1% (3/37). The mean parasite density was 16,395 asexual forms/μl (range: 870-65,000). Primigravidae had significantly higher parasite densities than multigravidae (36,600 ± 9,743 vs. 7,532 ± 4,623 asexual forms/μl, p=0.002). Asymptomatic infection predominated (75.7%, 28/37), while 24.3% were symptomatic. Parasitaemia was significantly associated with fever at enrollment (36.4% vs. 9.2%, p=0.005). Rural residence was again a significant risk factor (OR 3.46, 95% CI 1.29-10.4, p=0.03). Gravidity also influenced infection risk, with primigravidae and secundigravidae more likely to be parasitaemic (OR 4.23, 95% CI 1.97-23.2, p=0.04). Most infections occurred between July and September. Anaemia was strongly associated with parasitaemia: 83.7% of infected women were anaemic compared to 47.6% of uninfected (p=0.004), and severe anaemia was more common among infected women (5.7% vs. 2.6%, p=0.02).

Table 5 - Prevalence range of haemoglobin levels in stratified groups during malaria in pregnancy and control groups at ANC and DU.

Group of Subjects Haemoglobin Range (g/dl.)	Healthy Women Mean±SE (95%CI)	MIP at ANC Mean±SE (95%CI)	Women with Malaria Mean±SE (95%CI)	MIP at DU Mean±SE (95%CI)	*P.vivax* Infected Subjects at ANC Mean±SE (95%CI)	*P.vivax* Infected Subjects at DU Mean±SE (95%CI)
≥11.5-14	12.3±0.1 (12.1-12.6)	12.1±0.1 (12.2-12.8)	12.2±0.08 (12-12.4)	12.2±0.2 (11.4-13)	12.5±0.1 (12.2-12.8)	12.4±0.1 (12.1-12.7)
≥10.3-≤11.4	11.2±0.06 (11-11.3)	10.4±0.1 (10.1-10.6)	11.2±0.08 (11.1-11.4)	11.3±0.09 (11.1-11.5)	10.8±0.1 (10.5-11)	11.3±0.1 (11-11.5)
≥8.4-≤10.2	10.2±0.07 (10-10.3)	9.6±0.1 (9.4-9.8)	9.8±0.06 (9.7-9.9)	9.6±0.07 (9.3-9.9)	9.6±0.07 (9.4-9.7)	9.4±0.08 (9.2-9.6)
≤8.3	8.2±0.1 (7.8-8.5)	7.9±0.1 (7.6-8.2)	8.2±0.1 (7.7-8.6)	8.4±0.1 (8-8.8)	7±0.3 (6.2-7.8)	7.8.5±0.3 (6.3-9.2)

Table 6 - Frequency and status of anaemia in malaria during pregnancy among stratified cases at ANC and DU.

Group of Subjects Classified Anaemia	Healthy Women N (%) [95%CI] N=68	Malaria in Pregnancy At ANC* N (%) [95%CI] N=68	Women with Malaria N (%) [95%CI] N=68	Malaria in Pregnancy At DU* N (%) [95%CI] N=37	*Overall Malaria in Pregnancy N (%) [95%CI] N=105
Non-anaemic	18(26.4) [12.1-12.6]	13(19.1) [12.4-12.8]	13(19.1) [12-12.4]	4(10.8) [11.4-13]	17(16.1) [12.3-12.7]
Mild anaemia	15(22) [11-11.3]	14(20.5) [10.1-10.6]	14(20.5) [11.1-11.4]	13(35.1) [11.1-11.5]	27(25.7) [10.6-11.1]
Moderate anaemia	31(45.5) [10-10.3]	27(39.7) [9.4-9.8]	37(54.4) [9.7-9.9]	13(35.1) [9.3-9.9]	40(38.1) [9.4-9.7]
Severe anaemia	4(5.8) [7.8-8.5]	14(20.5) [7.6-8.2]	4(5.8) [7.7-8.6]	7(18.9) [8-8.8]	21(20) [7.8-8.3]
Overall Anaemia (%)	73.6	81	81	89	84

Association between Pregnancy, asymptomatic *P. vivax* and Haemoglobin

Anaemia, the most common haematological consequence of malaria, was widespread. Jharkhand reports among the highest prevalence of anaemia in India, particularly among women with low education, tribal affiliation, or low socioeconomic status. In the study cohort, anaemia was prevalent in both ANC and DU participants (Table 5). Severe anaemia was significantly more common among women with parasitaemia (p=0.001). The overall mean haemoglobin concentration was 9.7 ± 1.3 g/dl. Anaemia prevalence was 84% (95% CI: 43.53-97) in ANC participants and 83% (95% CI: 41.27-84.18) in DU participants (Table 6). Multivariate analysis showed that asymptomatic malaria increased the odds of anaemia by more than fivefold (aOR 5.64, 95% CI: 1.56-14.75, p=0.002). Anaemia was significantly associated with malaria (p=0.02); however, severe anaemia was more common among women with parasitemia (p=0.001). More than two-thirds of the DU participants were anaemic whereas 16.3% had severe anaemia. Of these ANC and DU participants, the prevalence of mild, moderate and severe anaemia are shown in Table 3. Further, mean haemoglobin concentration was 9.7 ± 1.3 g/dl and the prevalence of anaemia in ANC was 84% (95% CI: 43.53-97) as compared to 83% (95% CI: 41.27-84.18) in DU. In multivariate analyses, asymptomatic malaria increased over five times the likelihood of having anaemia (aOR 5.64 95% CI: 1.56-14.75; p=0.002)

DISCUSSION

The estimate of malaria in pregnancy continues to be grave concern for community reproductive health care management across the globe including India, up to the level of pacifying the concept of healthy mother and healthy baby of National Family Welfare Programme. In fact, situation is much more aggravated in developing countries like India, where poverty, illiteracy, geographical diversity, socio-economic disparities and multiple pregnancies take their toll of mother's health.

Among the prominent findings of the present study, we found 5.4% and 4.3% malaria during pregnancy at ANC and DU unit, respectively, as compared to only 1.8% and 1.7% at ANC and DU unit, respectively, reported by Hamer *et al.* (2009) from the series of cross-sectional and multi-centric study in Jharkhand. However, our study design is slightly broader than the earlier investigation from Hamer *et al.* (2009) in terms of subject stratification, as we also took into account women with malaria without pregnancy and the prevalence of malaria was found to be 13.2%, which itself reflects the importance of the investigated region and population under malaria sensitive zone. However, our study lacks the difference of investigating placental malaria. The pondering difference in the prevalence of malaria during pregnancy between our investigations, though we have selected only one centre in one district, i.e. Hazaribag, Jharkhand, as compared to three centres from two districts i.e. Ranchi and Gumla of Jharkhand by Hamer *et al.* (2009), may be attributed to various other reasons but primarily linked to the selection of study sites. As Ranchi is an urbanized capital with lots of high-tech development in and around the city, local and buffering populations are much more educated, aware of practicing healthy lifestyle and various disease prevention strategies including malaria, high socio-economic status, excellent with a choice of health facility compared to the rest of the districts of Jharkhand state and most importantly less malarious than almost 20 other districts of Jharkhand as far as malarial epidemiology is concerned in the last ten years (Jharkhand, 2009). Thus, the site selected by Hamer *et al.* (2009) may not be the true representation of the malaria scenario or burden of malaria during pregnancy in Jharkhand but absolutely true as far as the outcome of the project is concerned.

Though, our results of higher prevalence of malaria in pregnancy are in accordance with the earlier observations (ranging from 1.7% to 20%) across India (Singh *et al.*, 1999; Singh *et al.*, 1998; Sholapurkar *et al.*, 1988; Hamer *et al.*, 2009). Most of these studies focused on pregnant women with

selective approach, tending towards screening for mostly febrile or those with a recent history of fever cases and thus may have had a selection bias towards expecting higher malaria rates. This approach, targeting malaria diagnostic and treatment for symptomatic pregnant women, is consistent with India's National Vector Borne Disease Control Programme guidelines (Ministry of Health and Family Welfare, 2008). In contrast, all pregnant women were evaluated in the current study regardless of classical symptoms and interestingly, we observed well over 70% of the pregnant women in ANC and DU had asymptomatic malaria during pregnancy, which suggests the region-specific intervention. The broader spectrum of screening strategies were in accordance with earlier investigation in this region (Hamer *et al.,* 2009), though our observations are notably varied from theirs as far as asymptomatic malaria during pregnancy is concerned.

The higher prevalence of malaria in women without pregnancy and with pregnancy, irrespective of ANC and DU attendees' location of residence i.e. rural, urban and semi-urban, suggests that Hazaribag and its buffering zone have perennial rate of malaria transmission. Therefore, population of all age groups including pregnant women are at potential risk of getting malaria infection even irrespective of transmission season, though peak was observed in post-monsoon season. Apart from this, there is significant lack of education, general awareness towards health issues, congenial environmental factors for vector growth and survival and most importantly sizable population lack access to vector control methods or limited access to antimalarial drugs. People residing below poverty line linking to malnutrition and anaemia may be plausible reasons for various opportunistic infectious diseases including malaria.

Interestingly, Insecticide residual spray (IRS) of homes, which is usually conducted by government agencies, was reported more in rural areas as compared to urban and semi-urban zones of Hazaribag, though its seasonal usage of IRS in those areas regarded as perennial transmission may suggest vector resistance and subsequent higher prevalence of disease. Our observations warrant the potential need to enhance the IRS and distribution of ITNs in and around the investigated district. Overall, there was significant burden of anaemia among women in Jharkhand and particularly during pregnancy (Hamer *et al.,* 2009). Our observations regarding anaemia are in accordance with the findings from other study in Jharkhand (Hamer *et al.,* 2009), across India (Das *et al.,* 1999; Singh *et al.,* 2013) and most relevant study by Nosten *et al.,* (1991) in which they demonstrated that women who had malaria at any time were more likely to be anaemic than women without malaria. Thus, regardless of transmission level and the level of pre-pregnancy immunity against malaria, maternal anaemia remains the most frequent adverse consequence of malaria during pregnancy (Menendez *et al.,* 1995).

The symptoms and complications of malaria in pregnancy vary according to malaria transmission intensity in the given geographical area, and the individual's level of acquired immunity. In low-transmission settings, where women of reproductive age have relatively little acquired immunity to malaria, MIP is associated with anaemia, an increased risk of severe malaria. This may lead to spontaneous abortion, stillbirth, prematurity and low birth weight (Schantz-Dunn and Nour, 2009; De Beaudrap *et al.,* 2013). In such settings, malaria affects all pregnant women, regardless of the number of times they have been pregnant. In pregnant women, additional sequestration of malaria infected erythrocytes occurs in the placenta. Pregnant women therefore suffer disproportionately from severe anaemia as a result of infection (Desai *et al.,* 2007). Our observation is also substantiated by the fact that the majority of malaria infections in pregnancy remain asymptomatic or pauci-symptomatic, yet are a major cause of severe maternal anaemia and low birth weight, especially in the first and second pregnancies (Nosten *et al.,* 1999; Hamer *et al.,* 2009). In areas with stable, but low transmission like our investigated area, and certainly in areas

with unstable and exceptionally low transmission, infections can become severe in all gravidae groups because most women of childbearing age in these regions have low levels of pre-pregnancy and pregnancy-specific protective immunity to malaria (Desai *et al.,* 2007).

High prevalence of anaemia was observed and strongly correlated with asymptomatic P. vivax infection. This prevalence is similar to those reported by Brutus *et al.,* (2013) and Douglas *et al.,* (2012). Recent work has shown that in Papua New Guinea and Papua, Indonesia, mixed infection causes more severe haematological impairment than infection with either species alone (Genton *et al.,* 2008; Tjitra *et al.,* 2008; Poespoprodjo *et al.,* 2009). The impact of *Plasmodium vivax* infection on haemoglobin concentration varies from negligible to dramatic (Genton et al., 2008; Tjitra *et al.,* 2008; Kochar *et al.,* 2009; Alexandre *et al.,* 2010; Luxemburger *et al.,* 1996). The clinical consequences of the reduction in haemoglobin depend on the haemoglobin concentration prior to infection. Although the spectrum of anaemia seen with vivax infection is reasonably well documented, the clinical, developmental, and socioeconomic consequences are largely unknown. Population-based estimates of mortality in severely anaemic individuals with vivax malaria have not been established but recent studies from Latin America, New Guinea and the Indian subcontinent have identified deaths in patients with severe vivax anaemia (Barcus *et al.,* 2007; Tjitra *et al.,* 2008; Kochar *et al.*, 2009; Rodriguez-Morales *et al.,* 2009). Though authors did not establish the extent to which anaemia contributed to those deaths.

The very low rate of ownership of insecticide-treated bed nets (ITNs) and awareness suggests that this component of the enhanced malaria control programme (EMCP) has not effectively reached this vulnerable population, although it was encouraging to find that many households had bed nets and that they were used on a regular basis. However, our investigation suggests that approaches for ITN distribution and enhancing community awareness about the importance of their use need to be addressed as similarly observed and proposed by earlier investigation in adjacent region by Hamer *et al.,* (2009).

Despite the change in drug policy in 2008 in the studied state (Jharkhand), the availability and implementation of combination therapy i.e. Artesunate plus Sulfadoxine Pyrimethamine is a major concern. It has been well documented that chloroquine resistance has been rising in India (Lumb *et al.,* 2012; Mixson-Hayden *et al.,* 2010; Kumar *et al.,* 2007; Mittra *et al.,* 2006); this drug was recommended for malaria prophylaxis in pregnant women in high-risk areas as reported by Hamer *et al.* (2009), though it has been discontinued since recommendation. Presently, quinine sulphate was recommended for malaria prophylaxis in pregnant women in the investigated area irrespective of gestational age. Though this is partly in accordance with The Directorate of National Vector Borne Disease Control Programme (NVBDCP) and current WHO guidelines suggesting prophylaxis for trimester-based treatment of malaria during pregnancy as quinine for first trimester and subsequently ACTs in the second and third trimester of pregnancy (http://www.nvbdcp.gov.in/Doc/Diagnosis-Treatment-Malaria-2013.pdf). Since the intensity of transmission and the prevalence of malaria in pregnant women in Jharkhand are comparatively lesser than in many areas in sub-Saharan Africa. Notably, sulphadoxine-pyrimethamine was commonly used in Africa as intermittent preventive treatment of pregnant women (IPTp) (Menendez *et al.,* 2007), which may not be presently suggestive priority for Jharkhand to implement IPTp though may be considered as an alternative to the priority failure strategy. The top priority for Jharkhand should be on preventive measures like improved availability, awareness and uses of ITNs by pregnant women and well organised IRS system. In addition, we recommend much more stringent and frequent screening and diagnosis using conventional and RDTs irrespective of classical malaria symptoms to pregnant women in all the trimesters. Most importantly, in view of sizable

prevalence based on hospital study and potential risk for population at large in the investigated region, we are also suggestive of dedicated active and passive surveillance for MiP at the community level like regular malaria surveillance under India's NVBDCP. This strategy alone could potentially reduce the burden of MIP while limiting the potential for anti-malarial resistance to develop due to the widespread use of drugs for chemoprophylaxis. The present study shows two important findings: that the temporal and spatial distribution of asymptomatic infections differ from that of symptomatic disease and that *P. vivax* infection and pregnancy synergistically contribute to maternal anaemia in a low and perennial malaria transmission setting.

One major limitation of this study is that we could not be able to access the placental malaria due to limitation of our study design. Although the study was restricted to women delivering in the hospital, sizable number of (more than 60%) women give birth outside Sadar Hospital, Hazaribag.

Further, a longitudinal study instead of cross-sectional would have provided better estimate of MIP in this region and probably our study design may have given underestimate as compared to actual risk population. This has also been apprehended and suggested by Hamer *et al.* (2009). Despite these limitations, this study provides important data on the epidemiology and clinical implications of vivax malaria during pregnancy and delivering at Hazaribag district Sadar hospital. In spite of restricted and facility-based study, we preferentially covered marginalized, tribes and remote population of the investigated semi-urban cum rural district, Hazaribag. The majority of the districts and particularly malaria endemic districts in Jharkhand have similar geographical, socio-economic, demographic, literacy, basic amenities including health facility and awareness. Thus, our observation may be utilized for baseline information for further comprehensive and multi-centric study design; in strengthening MiP-associated preventive measures and screening methods within the state of Jharkhand.

CONCLUSION

As the global control and elimination of malaria progresses, *P. vivax* is set to become the dominant *Plasmodium* species, yet the health, developmental and socioeconomic consequences of *vivax* malaria and *vivax*-associated anaemia have received very little attention. This study reports a high prevalence of anaemia among pregnant women in the Hazaribag, Jharkhand and anaemia is strongly correlated to asymptomatic *P.vivax* infection. The results are quite indicative and emphasize the need to actively diagnose and treat malaria infection during ANC visit in the areas of perennial transmission. In view of the population at risk in this malaria-endemic region of India, there is a need to enhance ITN use and awareness for the prevention of MiP and distribution of ITNs at first ANC visit will be lucrative alternative. There should be a focus on improving case management of asymptomatic pregnant women, and evaluating the efficacy and effectiveness of the intermittent screening and treatment strategy. Further research is urgently needed to understand the nature and distribution of asymptomatic malaria infection serving as an important infected reservoir to continue malaria transmission. Our finding highlights the public health importance of integrated genus-wide malaria control strategies using diagnostic tests including RDTs and ensuring the availability of safe and effective drugs for the treatment of pregnant women in areas of *Plasmodium* co-endemicity.

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