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Risk factors associated with *Plasmodium falciparum* infections among pregnant women attending their first antenatal care visit in Burkina Faso

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Abstract: Background: Malaria during pregnancy has adverse effects on both mothers and their offspring. To tackle this, the WHO proposed rapid case management and preventive measures delivered through ANC channels. However, a large proportion of pregnant women remain unprotected due to no or late attendance. In this context, our study aimed to assess the prevalence of malaria and associated risk factors in pregnant women receiving ANC for the first time. **Methods**: A total of 418 pregnant women who attended an ANC clinic in Nanoro, Burkina Faso were recruited. Venous blood (2 mL) was collected for study-associated investigations. Malaria microscopy was used as gold standard and hemoglobin was measured using Hemocue®. Data analysis was performed using R Studio interface version 4.3.1. **Results**: The overall malaria prevalence was 20.4% (95% CI 18.5–22.4%). The geometric mean of parasite density was 442 parasites/µL (95% CI 380–515). In

the univariate analysis, lower educational level, younger age, and lower parity were significant risk factors for malaria, while older age (⩾25 years, OR 0.57, 95% CI 0.41–0.79) and multiparity (OR 0.50, 95% CI 0.33–0.74) were associated with a reduced risk of malaria parasitemia. Only 32/118 (27.1%) of the infections were associated with overt clinical symptoms such as fever. The prevalence of anemia (hemoglobin < 11.0 g/dL) was 60.8%. Malaria was significantly associated with increased odds of developing anemia. **Conclusion**: One out of five pregnant women attending their first ANC clinic visit in this high-transmission area had *Plasmodium falciparum* infection. Most infections were below 1000 asexual stage parasites/μL, without signs and symptoms suggestive of malaria, but were associated with anemia. There is a need to increase early ANC clinic attendance so mothers fully benefit from the existing malaria prevention strategies and to prevent unfavorable maternal and fetal birth outcomes in this population.

Keywords: malaria in pregnancy; ANC visit; signs and symptoms; anemia; risk factors; Burkina Faso

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Background

Malaria remains a public health threat, with 249 million malaria cases reported in 2022, representing an increase of 5 million cases compared to 2021 [1]. According to the World Health Organization (WHO), the African region bears 95% of the worlds burden of malaria cases, with children under five years and pregnant women being the groups at highest risk [2]. In sub-Saharan Africa, 35.4 million pregnancies were recorded in 2022, of which 12.7 million (36%) pregnancies were exposed to malaria [1]. Malaria in pregnancy (MiP) is responsible for adverse effects both on mothers and their offspring, and these include maternal anemia, pre-term delivery, abortion, stillbirth and low-birth weight (LBW) [3,4]. Without pregnancy-specific intervention in African endemic areas, MiP would have resulted in an additional 521,000 neonates with low-birth weight given the current coverage levels of the main preventive drug-based strategy implemented among pregnant women, i.e., intermittent preventive treatment during pregnancy (IPTp) [1]. In non-endemic areas, *falciparum* malaria in pregnancy is more likely to lead to overt clinical symptomatology and even severe complications such as cerebral malaria than in the non-pregnant population. In contrast, parasitemic pregnant women are rarely symptomatic, and death from malaria is extremely unusual due to the progressive and relatively rapid acquisition of significant levels of anti-malarial immunity in areas of stable transmission [5]. The risk of malaria and morbidity is higher among primiparous women, adolescents and those co-infected with human immunodeficiency virus (HIV), and malaria-related maternal deaths are estimated to account each year for up to a quarter of all maternal deaths in high-transmission settings [6]. To prevent these adverse effects in areas with moderate-to-high transmission of *Plasmodium falciparum* (*P. falciparum*), the WHO recommends a series of interventions. This relies on the promotion and use of long-lasting insecticide-treated nets (LLINs), together with the administration of intermittent preventive treatment

with sulfadoxine-pyrimethamine (IPTp-SP) during pregnancy and appropriate case management through prompt and effective diagnosis and treatment of malaria in pregnant women [7]. However, all of these interventions are delivered through antenatal care (ANC) channels, leaving a great proportion of pregnant women unprotected as a significant proportion may not attend or may delay their initial ANC visits. Malaria occurring early in pregnancy is associated with a higher risk of LBW [8,9]. In Burkina Faso, the permanent secretary for malaria elimination (former National Malaria Control Program, NMCP) recommends for each pregnant woman at least four ANC visits with a minimum of three doses of IPT-SP before delivery [10]. Pregnant women then need to start receiving ANC in early pregnancy to fully benefit from these preventive measures. The WHO has recommended surveillance as an additional tool to control malaria and to monitor trends over time in endemic countries [11]. Groups at risk of malaria should then be identified and resources be allocated for malaria control in high-malaria-burden countries like Burkina Faso [12]. This would require the availability of reliable statistics on the prevalence and risk factors of malaria in pregnancy across the country. In Burkina Faso, several studies on malaria in pregnancy have been conducted to assess the efficacy of anti-malarial drugs during pregnancy but studies characterizing the profile of pregnant women at their first ANC visit are scarce [13]. Characterizing pregnant women attending ANC clinics for the first time can help customize interventions for this specific group. This study was carried out to determine the prevalence of malaria in pregnant women attending ANC clinics for the first time and the associated risk factors.

Methods

Study area

This study was conducted by the Clinical Research Unit of Nanoro in the health district of Nanoro, located 85 Km from Ouagadougou, the capital city of Burkina Faso. The Nanoro health district is one of the seven districts of the center-west region of Burkina Faso. The literacy rate of the population is about 23% [14]. There has been a health and demographic surveillance system (HDSS) implemented since 2009, with 65,500 individuals being routinely under surveillance [15]. The HDSS regularly collects information related to socioeconomic status, household characteristics, living conditions and health conditions. Malaria is the main cause of health facility attendance, with *P. falciparum* being the predominant species [16]. Malaria transmission is highly seasonal and overlaps with the rainy season (July–October), with a peak of transmission in September–October. Pregnant women receive insecticide-treated nets (ITNs) free of charge as soon as they complete two ANC visits or immediately after delivery, while children receive theirs at each vaccination campaign.

Study design, procedures and data collection

A cross-sectional study was conducted in four centers of the Nanoro health district (Nanoro, Soaw, Pella and Kindi) from December 2020 to March 2021 (Figure 1). The aim of the study was explained and all pregnant women attending their routine ANC visit for the first time regardless of their gestational age were asked to consent to participate in the study. For illiterate participants, the informed consent process involved a detailed verbal explanation of the studys purpose, procedures, risks and benefits in the language of the participant. The nurse ensured that each participant had the opportunity to ask questions and receive clarifications. A neutral, literate witness, who was not part of the study team, was present throughout to verify that the participants rights and understanding are respected. If the participant agreed to join the study, they provided consent using a thumbprint, while the witness signed the consent form to confirm that the process was conducted appropriately. At inclusion and after the physical examination, a standardized questionnaire was administered to assess the socio-demographic characteristics, obstetric history, socioeconomic status

(SES), anti-malarial drug use and use of other malaria vector preventive measures. For prompt management, all pregnant women with a positive malaria (rapid diagnostic test or microscopy) result were treated following the national policy recommendation with artemether-lumefantrine (standard adult dose) if they were in the second and third trimester of pregnancy, while quinine was used for those in the first trimester. Anemia was treated following national guidelines with daily oral ferrous sulfate (200 mg) and folic acid (0.25 mg) for one month. All patients with confirmed malaria and/or anemia were treated free of charge.

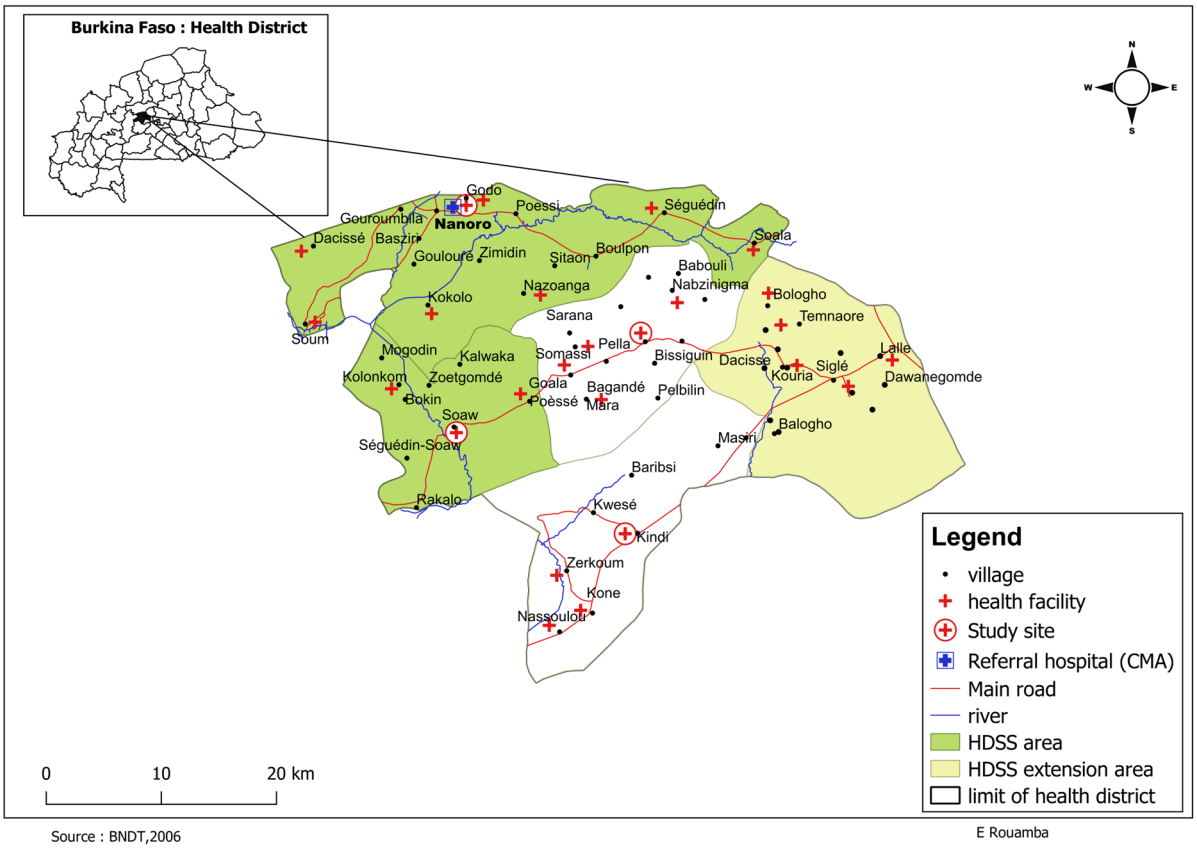

Figure 1: A map of the Nanoro health district showing the geographical location of the four study sites/health centers.

A map depicting the four health centers where the study was conducted, along with the locations of nearby rivers and distances between villages, is presented.

Laboratory investigations

For every pregnant woman recruited for the study, a blood sample was collected at inclusion by a trained phlebotomist using sterile venipuncture, allowing us to conduct the following tests:

Malaria rapid diagnosis tests (RDTs)

Histidine-rich protein-2-based RDTs are recommended in Burkina Faso for malaria investigation. The CareStart^{TM} Malaria Pf (HRP2) Ag RDT (ACCESSBIO, Somerset, NJ, USA) was used for the rapid screening and management of the study participants in the study. All tests were performed according to the manufacturers instructions.

Malaria microscopy: Malaria slides were prepared using 7 μL and 2 μL of blood for the thick and thin smears, respectively. The smears were stained with Giemsa diluted at 10% in phosphate-buffered water (pH 7.2). An independent double-reading system was used. Only malaria microscopists graded as expert or reference by the NICD EQA (National Institute for Communicable Diseases External Quality Assessment) survey were allowed to read and report results for the study. The asexual parasites were counted against 200 leukocytes in the thick blood film and the number of parasites per μL was estimated using a white blood cell (WBC) count of 8,000. A third independent reading was performed in cases of discrepancy between the two readers (positive/negative, more than two-fold difference for parasite densities ⩾400/μL, or more than log10 for those <400/μL) [17]. The mean of the two closest results was chosen as the final result.

Hemoglobin level: The hemoglobin level was measured with *HemoCue Hb 301* (*Danaher Corporation) to determine the presence or absence of anemia.*

Sample size determination and sampling technique

Using a single population proportion formula with 43% prevalence, a 95% confidence level, a 5% margin of error, and a 10% non-response rate, the total sample size was determined to be 418 to evaluate the prevalence of malaria at the first visit during pregnancy.

Statistical methods and definitions

Double data entry and data cleaning were conducted using REDCap. For categorical variables, descriptive statistics were computed using proportions, whereas quantitative variables were computed using the mean and standard deviation. Pearsons chi-square test was used to compare proportions, while Students and Kruskall–Wallis tests were used to compare means.

The univariate and multiple logistic regression models were used to identify risk factors for malaria and anemia during the first ANC visits. The statistical analysis was conducted using the R Studio interface of R software version 4.3.1 (16 June 2023). A *p*-value less than 5% was considered statistically significant. Malaria was defined as the presence of any level of *P. falciparum* parasitemia in the presence of accompanying signs and symptoms suggestive of malaria. The level of parasitemia was classified as low (1–199 parasites/μL), medium (200–999 parasites/μL) and high (>1000 parasites/μL). According to WHO guidelines [18], anemia was defined as a hemoglobin concentration <11.0 g/dL, and sub-divided as severe (<7.0 g/dL), moderate (7.0–9.9 g/dL) and mild (10.0–10.9 g/dL).

Ethics

The study protocol was approved by the Institutional Review Board of the Institut de Recherche en Science de la Santé/Direction Régionale de lOuest (IRSS-DRO), Burkina Faso (N/REF. A15-2020 CEIRES).

Results

Baseline characteristics of study population

Overall, 418 pregnant women attending the maternity clinic for their first ANC visit were included in the study. The mean age in years was 26 (SD = 6.59), with more than three-quarters (316/418) of the participants having an age ranging from 19 to 34 years. Younger participants were defined as aged 16 to 18 years and accounted for 10.5% (44/418) of the study population. Approximately 70% of participants had no formal education, while only 10% had completed primary school and 20% had

reached secondary school or higher (Table 1). Almost 80% of the study participants were in the second trimester of pregnancy during their first ANC visit, while less than 15% of participants were in the first trimester. More than 60% were multigravidae, with secundigravidae and primigravidae representing 13.4% and 26.1%, respectively (Table 1). About 82.5% (345/418) of participants reported possessing an insecticide-treated net. Less than 20% (81/418) reported having clinical signs and symptoms suggestive of malaria. The main signs/symptoms were headaches at 55.6% (45/81), tiredness at 39.5% (32/81) and dizziness at 24.7% (20/81). Only 9.9% (8/81) had fever.

Table 1: Baseline characteristics of study population. N = 418.

Characteristic	Frequency, n (%)
Age (years), mean (SD)	26.0 (6.59)
Age category	
[16,18]	44 (10.5)
(18,34]	316 (75.6)
(34,45]	58 (13.9)
Educational level	
None	292 (69.9)
Primary	38 (9.1)
Secondary and over	88 (21.1)
Gestational age (trimester)	
1st trimester	58 (13.9)
2nd trimester	332 (79.4)
3rd trimester	28 (6.7)
Gravidae	
Primigravidae	109 (26.1)
Secundigravidae	56 (13.4)
Multigravidae	253 (60.5)
LLIN possession (Yes)	345 (82.5)
Clinical signs (Yes)	81 (19.4)

SD: standard deviation; %: percentage; LLIN: long-lasting insecticidal net. All data are presented as number and frequency n (%) unless stated otherwise.

Malaria prevalence and parasite density

Using microscopy as the gold standard, the overall malaria prevalence was 28.2% (95% CI 24.1–32.7%) (Table 2). A high prevalence of malaria at 52.3% (95% CI 37.9–66.2) was found in participants aged 16 to 18 years and decreased to 26.6% (95% CI 22.0–31.7) and 19% (95% CI 10.9–30.8), respectively, for participants aged 19 to 34 and $\geqslant$ 35 years (Table 2). The prevalence of malaria was 39.5% (95% CI 29.6–50.4) among participants presenting with fever or other signs/symptoms suggestive of malaria, compared to 25.5% (95% CI 21.1–30.4) in participants without malaria-related signs or symptoms. The overall parasite density ranged from 48 to 17715 parasites/µL (data not shown) with a median of 759 (222–2811), and more than 75% of the infected pregnant women had a parasite density above 222 parasites/µL. Younger participants were infected with higher parasite densities at 2662 parasites/µL (95% CI 1709–4147) compared to elder participants, with 211 parasites/µL (95% CI 107–415). In addition, primigravidae were infected with higher parasite densities at 2208 parasites/µL (95% CI 1627–2997) compared to multigravidae, at 275 parasites/µL (95% CI 199–382). Both differences were statistically significant (p<0,05). According to species detection using microscopy, all infections were due to *P. falciparum,* and other species such as *P. malariae, P. ovale or P. vivax* were not found using microscopy as the gold standard.

Table 2: Clinical and obstetrical characteristics and parasite density among malaria-positive participants.

Parameters	Frequency		Parasite Density, Geometric Mean, (95% CI)	*P* Value
	n/N	% (95% CI)		
Overall	118/418	28.2 (24.1–32.7)	790 (602–1036)	
Age category (years)				
[16,18]	23/44	52.3 (37.9–66.2)	2662 (1709–4147)	
(18,34]	83/316	26.6 (22.0–31.7)	673 (493–918)	0.0001
(34,45]	11/58	19 (10.9–30.8)	211 (107–415)	
Gestational age (trimester)				
1st trimester	14/58	24.1 (13.9–37.2)	1242 (385–4007)	
2nd trimester	94/332	28.3 (23.5–33.5)	945 (690–1295)	0.0525
3rd trimester	10/28	35.7 (18.6–55.9)	451 (253–804)	
Gravidity				
Primigravidae	48/109	44.0 (34.5–53.9)	2208 (1627–2997)	
Secundigravidae	21/56	37.5 (24.9–51.5)	876 (462–1667)	0.0001
Multigravidae	49/253	19.4 (14.7–24.8)	275 (199–382)	
Clinical signs				
Symptomatic	32/81	39.5 (29.6–50.4)	723 (517–1010)	0.3135
Asymptomatic	86/337	25.5 (21.1–30.4)	1001 (636–1577)	

CI: confidence interval.

Malaria in relation to age and gravidity

The prevalence of malaria was highest in primi- and secundigravidae aged < 25 years and lowest in multigravidae aged ⩾ 25 years, at 43.4% (95% CI: 35.8–51.4) and 19.2% (95% CI: 14.5–25.1) ($p < 0.001$), respectively. Among pregnant women aged < 25 years old, the prevalence was statistically lower in the multigravidae compared with the primi- and secundigravida (Figure 2).

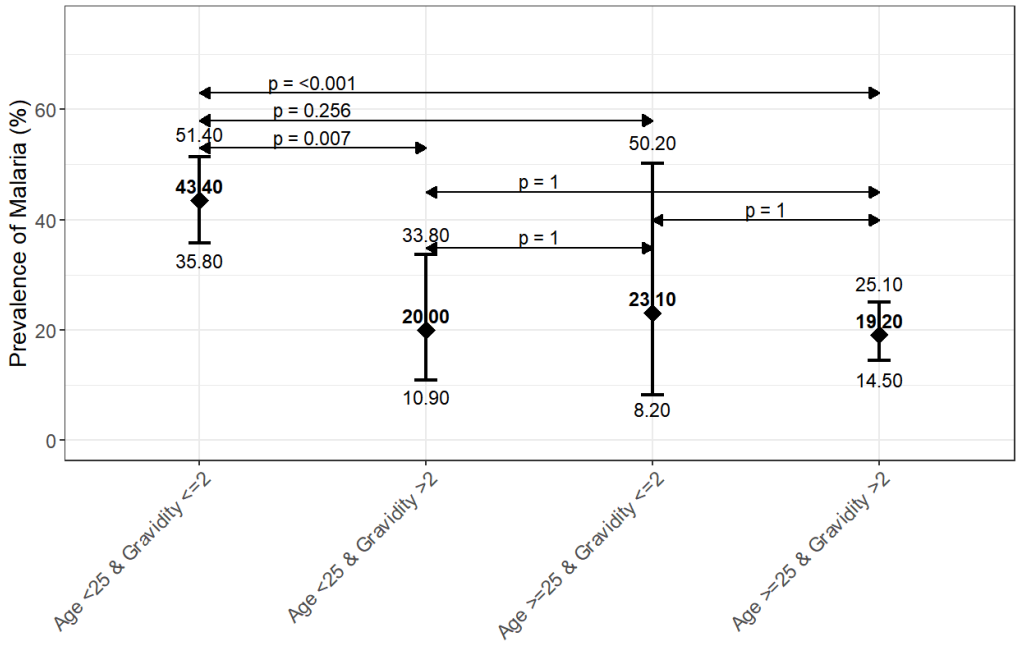

Figure 2: Prevalence of malaria parasitemia in relation to age and gravidity.

Factors associated with malaria parasitemia

In the univariate analysis, educational level (secondary and over) and the presence of symptoms were identified as being associated with high odds for malaria parasitemia (Table 3). In the univariate analysis, pregnant women aged under 25 during their first or second pregnancy had 3.22-times higher odds of being infected with malaria. This probability persisted after the multivariate analysis and remained statistically significant (aOR = 3.10, 95% CI: 1.62–6.02). Similarly, those who were single had statistically increased odds of having malaria (aOR = 7.30, 95% CI: 1.21–51.18), with a wide confidence interval due to the small sample size. However, pregnant women in their first trimester of pregnancy had lower odds (aOR = 0.25, 95% CI: 0.07–0.86) of experiencing malaria compared to pregnant women in their third trimester.

Table 3: Risk factors for malaria based on microscopy results.

Factors	Malaria		uOR	*p* Value	aOR	*p* Value
	Negative	Positive				
Educational_level						
None	220 (73.3)	72 (61.0)	1	-	1	-
Primary	25 (8.3)	13 (11.0)	1.59 (0.75–3.22)	0.208	1.16 (0.45–2.79)	0.753
Secondary and over	55 (18.3)	33 (28.0)	1.83 (1.10–3.04)	0.019	0.75 (0.32–1.70)	0.499
Age_Gravidity						
Age $\geqslant$ 25 and Gravidity $\geqslant$ 2	168 (56.0)	40 (33.9)	1	-	1	-
Age < 25 and Gravidity $\leqslant$ 2	86 (28.7)	66 (55.9)	3.22 (2.02–5.19)	<0.001	3.10 (1.62–6.02)	0.001
Age < 25 and Gravidity $\geqslant$ 2	36 (12.0)	9 (7.6)	1.05 (0.44–2.28)	0.906	1.47 (0.59–3.39)	0.383
Age $\geqslant$ 25 and Gravidity $\leqslant$ 2	10 (3.3)	3 (2.5)	1.26 (0.27–4.34)	0.734	1.49 (0.31–5.64)	0.576
Gestational Age (trimester)						
3rd trimester	18 (6.0)	10 (8.5)	1	-	1	-
1st trimester	44 (14.7)	14 (11.9)	0.57 (0.21–1.55)	0.265	0.25 (0.07–0.86)	0.031
2nd trimester	238 (79.3)	94 (79.7)	0.71 (0.32–1.65)	0.408	0.50 (0.20–1.31)	0.147
LLIN use						
Yes	201 (67.0)	69 (58.5)	1	-	1	-
No	56 (18.7)	19 (16.1)	0.99 (0.54–1.76)	0.969	0.94 (0.49–1.88)	0.923
Unknown	43 (14.3)	30 (25.4)	2.03 (1.18–3.48)	0.010	1.61 (0.87–2.96)	0.124
Clinical_signs						
No	251 (83.7)	86 (72.9)	1	-	1	-
Yes	49 (16.3)	32 (27.1)	1.91 (1.14–3.16)	0.013	1.84 (0.97–3.44)	0.056

CI: confidence interval; uOR: unadjusted odds ratio; aOR: adjusted odds ratio.

Prevalence of anemia among pregnant women

Overall, 61% of the participants were anemic (Table 4), of which 26.7% (95% CI 22.5–31.2%) had mild anemia, 31.7% (95% CI 27.3–36.4%) were moderate and 2.4% (95% CI 1.2–4.4 %) suffered from severe anemia. The mean hemoglobin rate in pregnant women with malaria was 9.92 (SD = 1.52), and it was 10.71 (SD = 1.56) in the non-malaria-infected women. There were mild (27.4% vs. 26.4%), moderate (46.2% vs. 26.1%) and severe (2.6% vs. 2.3%) anemic and malaria-infected pregnant women compared to non-malaria-infected women. This difference was statistically significant ($p < 0.001$; Table 4).

Table 4: Prevalence of anemia among pregnant women according to malaria status. n = 418.

Anemia Status	Overall	Malaria Status		*p* Value
		Negative	Positive	
Severe (<7.0 g/dL)	10 (2.4)	7 (2.3)	3 (2.6)	<0.001
Moderate (7.0–9.9 g/dL)	132 (31.7)	78 (26.1)	54 (46.2)	
Mild (10.0–10.9 g/dL)	111 (26.7)	79 (26.4)	32 (27.4)	
Non-anemic (⩾11.0 g/dL)	163 (39.2)	135 (45.2)	28 (23.9)	

Data are presented as number and proportion (n, (%)); Hb level was considered normal if Hb > 11.0 g/dL and sub-divided as severe (<7.0 g/dL), moderate (7.0–9.9) and mild (10.0–10.9 g/dL).

Factors associated with anemia among pregnant women

In the univariate analysis, marital status (in couple but unmarried), profession (student) and malaria positivity were identified as being associated with higher odds of anemia, whereas the trimester of pregnancy (first) was identified as being associated with lower odds of anemia. In the multivariate analysis, having malaria (aOR = 2.18, 95% CI: 1.24–3.95) and being a student (aOR = 7.31, 95% CI: 1.36–67.12) were significantly associated with higher odds of anemia (Table 5).

Table 5: Risk factors for anemia among study participants, n = 416.

Factors	Anemia		uOR	*p* Value	aOR	*p* Value
	No	Yes				
Age_Gravidity						
Age >= 25 and Gravidity >= 2	85 (52.1)	121 (47.8)	1	-	1	-
Age < 25 and Gravidity <= 2	49 (30.1)	103 (40.7)	1.48 (0.95–2.30)	0.082	1.17 (0.63–2.20)	0.622
Age < 25 and Gravidity >= 2	25 (15.3)	20 (7.9)	0.56 (0.29–1.07)	0.082	0.51 (0.24–1.05)	0.070
Age >= 25 and Gravidity <= 2	4 (2.5)	9 (3.6)	1.58 (0.50–5.99)	0.458	1.44 (0.41–5.90)	0.582
Educational level						
None	119 (73.0)	171 (67.6)	1	-	1	-
Primary	14 (8.6)	24 (9.5)	1.19 (0.60–2.46)	0.621	0.84 (0.36–1.98)	0.688
Secondary and over	30 (18.4)	58 (22.9)	1.35 (0.82–2.24)	0.244	0.65 (0.30–1.40)	0.272
Marital status						
Married	97 (59.5)	117 (46.2)	1	-	1	-
In couple but unmarried	64 (39.3)	127 (50.2)	1.65 (1.10–2.47)	0.016	1.23 (0.74–2.03)	0.430
Single	2 (1.2)	9 (3.6)	3.73 (0.93–24.85)	0.097	1.33 (0.13–32.24)	0.827
Profession						
Housewife	138 (84.7)	186 (73.5)	1	-	1	-
Farmer	9 (5.5)	16 (6.3)	1.32 (0.58–3.20)	0.521	1.13 (0.46–2.91)	0.793
Student	3 (1.8)	21 (8.3)	5.19 (1.75–22.30)	0.009	7.31 (1.36–67.12)	0.038
Public/private worker	13 (8.0)	30 (11.9)	1.71 (0.88–3.51)	0.125	2.40 (1.02–6.17)	0.054
Gestational Age (trimester)						
3rd trimester	11 (6.7)	17 (6.7)	1	-	1	-
1st trimester	38 (23.3)	19 (7.5)	0.32 (0.12–0.81)	0.018	0.40 (0.12–1.23)	0.114
2nd trimester	114 (69.9)	217 (85.8)	1.23 (0.54–2.69)	0.606	1.50 (0.60–3.68)	0.378
LLIN use						
Yes	106 (65.0)	164 (64.8)	1	-	1	-
No	34 (20.9)	40 (15.8)	0.76 (0.45–1.28)	0.300	0.90 (0.49–1.65)	0.722
Unknown	23 (14.1)	49 (19.4)	1.38 (0.80–2.43)	0.256	1.54 (0.81–3.00)	0.193

Table 5: *Cont.*

Factors	Anemia		uOR	*p* Value	aOR	*p* Value
	No	Yes				
Clinical signs						
No	136 (83.4)	199 (78.7)	1	-	1	-
Yes	27 (16.6)	54 (21.3)	1.37 (0.83–2.30)	0.231	1.31 (0.71–2.48)	0.391
Microscopy						
Negative	135 (82.8)	164 (64.8)	1	-	1	-
Positive	28 (17.2)	89 (35.2)	2.62 (1.63–4.29)	<0.001	2.18 (1.24–3.95)	0.008

CI: confidence interval; uOR: unadjusted odds ratio; aOR: adjusted odds ratio.

Discussion

In this high-endemic malaria setting of Burkina Faso, a high prevalence of malaria-infected pregnant women was documented. This is worrying given the potential adverse effects of malaria during pregnancy [5,19–21]. This situation reveals a high level of malaria transmission in this region and a poor uptake of the preventive measures recommended due to late ANC visit attendance [10]. Consequently, a large proportion of pregnant women do not benefit from these preventive measures delivered through antenatal channels. They then carry malaria which will have detrimental consequences both for themselves but also for their offspring, in addition to serving as an important parasite reservoir by sustaining the malaria parasite transmission cycle [22]. In our context, although pregnant women and children under five receive treatment free of charge, cultural beliefs, practices and literacy levels may have contributed to this delayed attendance. Conducting anthropological studies could help uncover the underlying reasons for this delay, enabling the development of effective strategies to address it. The prevalence of malaria in this study was high compared to other studies conducted in neighboring districts. Indeed, Lingani et al. documented a lower prevalence (16.1%) in Yako, an urban area where preventive interventions may be more accessible and utilized effectively. Urban centers generally offer improved healthcare access and infrastructure, supporting greater ANC attendance and malaria prevention compared to rural areas [23]. This difference highlights the need for spatially adaptive intervention strategies, as advocated by the WHO, which recommends targeting high-transmission zones with intensified, tailored measures [24]. Similarly, a study by Yaro et al. in the same epidemiological area documented lower prevalence rates, likely due to its broader inclusion criteria, covering pregnant women at various ANC stages [25]. Many of these women may have already received IPTp-SP, which has been shown to improve pregnancy outcomes due to its efficacy in malaria prevention, and it could have reduced the overall malaria prevalence of the study participants [26,27]. Higher malaria prevalences during first ANC visits were found in various countries, with 31.8% in Zambia [28], 42.2% in Mali and 59.7% in Ghana [29]. However, it is worth noting that our study was conducted after the rainy season, when malaria transmission normally decreases. The decreased incidence found in our data may be partially explained by this seasonal context, since transmission rates are known to vary greatly between rainy and dry seasons. Nonetheless, the high malaria prevalence in our study is alarming and calls for early uptake of the preventive measures available. More than 80% of the study participants reported having an LLIN, but conversely, the prevalence of malaria-infected pregnant women was high in our study, suggesting that coverage alone may not fully capture effective bed net utilization or replacement practices. Family size may influence bed net access, as distribution ratios typically provide one LLIN per two family members, potentially leading to shortages and increased wear. In addition, lower literacy levels and socioeconomic constraints may hinder the effective use of these preventive strategies and further limit the ability of lower-income families to replace worn nets. Misuse, inconsistent use or poor net condition may reduce LLINs protective effect, along with issues related to outdoor exposure to mosquitoes due to household activities. In our case, the problem appears

to be more related to utilization gaps than to pyrethroid resistance, as LLINs have retained efficacy even in resistant settings [30,31]. While the permanent secretary for malaria elimination offers LLINs through sensitization campaigns, the ownership and likely utilization of the nets remain underexplored. In addition, outdoor mosquito biting remains a problem. This calls for social science studies to investigate in more detail the real state of LLIN utilization and the discrepancies between ownership and real-life utilization.

The overall geometric mean parasite density reported in the present study is similar to those reported by Yaro et al. (777.3/μL) [25] in Burkina Faso and Fana et al. in Nigeria (800/μL) [32], while Lingani et al. reported a slightly higher density in Yako [13]. Factors such as the level of immunity and the uptake of protective measures can explain these differences [33,34]. In addition, this difference can be attributed to the parasite density calculation method used (absolute count of white blood cells versus assumed 8000 WBCs per μL) as there is variation between the two methods [35]. Nonetheless, the same method was used by Lingani et al. [13]. Nearly 20% of pregnant women exhibited signs and symptoms indicative of malaria, with a positivity rate of 40%. A study in Cameroon observed a similar trend, as 21% of febrile pregnant women had a positivity rate of 65% [36]. Given that most pregnant women have asymptomatic presentation of malaria in endemic areas, it is recommended that all pregnant women attending ANC visits be screened for malaria regardless of their symptom status.

Our analysis also highlighted higher malaria susceptibility in younger women (particularly those aged 16–18) and primigravidae (40% of the infected women). In addition, the highest parasite densities were recorded in these groups. These results indicate that younger age (reduced exposure and hence limited immunity) contributes to higher susceptibility to *P. falciparum* infection in primigravidae compared to multigravidae [37]. Greater risks of infection and adverse outcomes in primigravidae have been attributed to the absence of pregnancy-specific malaria immunity to pregnancy-associated variant surface antigens (VSAPAM) that predispose pregnant women to increased malaria severity [38].

A high prevalence of anemia was found in our study. The same picture of high prevalence of anemia was seen in neighboring countries such as Ghana (70.0%) [39], Benin (67.7%) and Cote dIvoire (62.7%) [40], but a lower prevalence was found in southern African countries in a comparative study [41]. The difference in anemia frequency between these two parts of the continent is probably due to the intensity of malaria transmission [42,43] and the high co-existence of soil-infected helminths [44]. Anemia is known to be caused by malaria, especially *P. falciparum* infections which directly destroy red blood cells and can also impede the formation of new red blood cells by triggering an inflammatory response [45]. Chronic anemia can result from repeated malaria infections over time in areas with high transmission rates, particularly in susceptible populations like children and pregnant women [41]. Additionally, the high co-occurrence of soil-transmitted helminths further exacerbates the prevalence of anemia in West Africa, while conversely, Southern African regions typically report lower helminth infection rates due to different environmental factors, increased healthcare access and the implementation of deworming programs [46]. This lower burden of helminth infections likely contributes to a reduced anemia prevalence compared to West Africa. This is worrying as anemia combined with malaria can negatively impact the course of pregnancy. In the multivariate analysis, the factors associated with the presence of anemia among pregnant women were primigravidae (2.1 times compared to multigravidae), malaria parasites (2.2 times compared to those with negative results) and malaria symptoms (with the presence of symptoms being 1.3 times compared to the absence of symptoms). It is generally assumed that adherence to iron supplementation and ANC service utilization among pregnant women are typically poor in such settings, which are common problems in sub-Saharan Africa [47]. Surprisingly, pregnant women going to school were the most at risk of being anemic. This can be explained by the relatively low number of this subgroup in our study, which could amplify variations in anemia prevalence. However, anemia is known to be a multifactorial phenomenon, and other factors such as low iron or folate levels, as well as infections, genetic predispositions and overall health conditions that were not investigated in the present study, could be another explanation. Moreover, our study design did not allow us to follow pregnant women

from their first ANC visit through to delivery. While this approach could have provided deeper insights, it was not feasible within our budget constraints.

Conclusion

The prevalence of malaria during the first ANC visit was high, with one-quarter of women being infected. High parasite densities were found in pregnant women of younger ages. Our study suggests that younger age and lower parity are significant risk factors for malaria, while older and multiparous women are relatively more protected. In addition, a very high proportion of anemic pregnant women was reported, with malaria significantly increasing the odds of developing anemia. Altogether, this suggests that malaria control in Burkinabese pregnant women still faces several challenges, and that there is a need to reinforce malaria and anemia preventive measures through education and sensitization, with special emphasis on increasing early ANC visit attendance.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Ethical Approval: The Comité dEthique Institutionnel pour la Recherche en Santé approved the present study (N/Réf. A15-2020/CEIRES), and the study was conducted under good clinical practices. All participants were duly informed of the studys objectives and the protocol for sample collection. Written informed consent was obtained from each study participant. Participation was voluntary.

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