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Molecular Evidence of Submicroscopic Malaria Parasitaemia among Asymptomatic Populations in Jos, Plateau State, Nigeria

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Abstract

Despite ongoing malaria control efforts, the disease remains a major cause of morbidity and mortality in Nigeria. As transmission declines in many endemic settings, asymptomatic and submicroscopic infections increasingly constitute a hidden reservoir that sustains malaria transmission. These infections often escape detection by routine diagnostic methods yet remain epidemiologically significant, particularly in elimination-focused programmes. This study assessed the prevalence of asymptomatic and submicroscopic malaria parasitaemia among individuals attending selected health facilities in Jos, Plateau State, Nigeria.

A cross-sectional survey was conducted among 400 apparently healthy individuals across five selected health facilities in Jos, Plateau State, Nigeria. Participants aged ≥6 months who exhibited no clinical signs or symptoms of malaria were enrolled. Malaria parasitaemia was confirmed by Giemsa-stained microscopy, submicroscopic *Plasmodium* infection by 18S mRNA using nested PCR. Out of 400 participants screened, 78 (19.5%) tested positive for malaria by microscopy, with prevalence varying across health facilities from 11.2% to 30.0%. Males had a significantly higher infection prevalence (26.0%) compared to females (15.7%). Molecular analysis revealed a substantially higher burden of infection: among 120 microscopy-negative samples analysed by nPCR, 74 (61.7%) were positive, indicating a high prevalence of submicroscopic malaria parasitaemia. Although submicroscopic infections did not differ significantly across demographic groups ($p > 0.05$), the highest prevalence was observed among pregnant women (64.5%) and children (66.7%). *Plasmodium falciparum* was the predominant species (55.9%), followed by *P. ovale* (16.5%), *P. malariae* (6.5%), and *P. vivax* (5.3%). Asymptomatic and submicroscopic malaria infections constitute a substantial hidden reservoir in Jos, Nigeria. Reliance solely on routine diagnostic methods may lead to significant underestimation of malaria prevalence. Integrating sensitive molecular diagnostics into surveillance systems is critical for effective malaria elimination in low-to-moderate-transmission settings.

Keywords: Submicroscopic Malaria; Asymptomatic Malaria; PCR; Malaria Elimination; Nigeria

1. Introduction

Malaria remains a major public health challenge globally, with sub-Saharan Africa still accounting for the majority of cases and deaths [1]. Despite substantial progress achieved through vector control interventions and effective antimalarial therapies, transmission persists in many endemic regions. Malaria continues to pose a significant public health problem in Nigeria, with an estimated 68 million cases and 194,000 deaths recorded in 2021 [2]. The country bears the heaviest malaria burden globally, contributing nearly 27% of total malaria cases worldwide [3]. In Nigeria, malaria control efforts face additional obstacles, including drug and insecticide resistance, inadequate surveillance

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systems, and insufficient healthcare infrastructure, which collectively impede effective case management, vector control, and disease prevention [3]. Among these challenges, asymptomatic malaria represents one of the most critical yet frequently overlooked barriers to elimination [4].

In many endemic settings, the clinical spectrum of malaria extends beyond symptomatic disease to include asymptomatic infections, were individuals harbour *Plasmodium* parasites without overt clinical symptoms. The World Health Organization (WHO) defines asymptomatic malaria as the presence of asexual malaria parasites in the blood in the absence of any clinical signs or symptoms of disease [5]. It refers to the existence of malaria parasitaemia of any density in the blood without causing noticeable symptoms in the individual, particularly if they have not recently received antimalarial treatment [6]. While, Submicroscopic malaria (SMM) refers to low-density *Plasmodium* infections that can only be detected through molecular methods [7].

Asymptomatic carriers constitute a significant reservoir of infection and play a pivotal role in sustaining malaria transmission in the community. In some of these communities, asymptomatic malaria parasite carriers represent a persistent pool for maintaining the life cycle and transmission of the *Plasmodium* species by the anopheline vector [8]. A recent systematic meta-analysis in Nigeria estimated the pooled prevalence of asymptomatic malaria at approximately 33 %, with higher rates among children and variations by demographic subgroups, underscoring the epidemiological significance of undiagnosed parasitaemia [4].

Conventional diagnostic techniques commonly used in malaria surveillance, such as light microscopy and rapid diagnostic tests (RDTs), are limited by their detection thresholds, often failing to identify low-density infections. This limitation results in an underestimation of true parasite prevalence, particularly among clinically healthy individuals. In contrast, molecular assays such as Polymerase Chain Reaction (PCR) offer superior sensitivity and can uncover submicroscopic malaria infections that are undetectable by standard field diagnostics, but are nonetheless capable of contributing to transmission. Evidence from suburban populations in Nigeria has demonstrated that PCR methods detect additional infections missed by microscopy and RDTs, revealing submicroscopic parasite carriage in a substantial proportion of asymptomatic individuals [9].

Sub-microscopic infections are present across different settings and populations [8]. Several reports in low transmission settings have also suggested higher proportions of sub-microscopic infections, when compared to microscopically detectable infections particularly in settings where recent malaria control efforts have been successful [10]. The contribution of submicroscopic parasitaemia to malaria transmission has been increasingly recognized in the scientific literature. Studies indicate that individuals with low-density infections, although often asymptomatic, can carry gametocytes that infect mosquitoes and thereby sustain onward transmission cycles. The human infectious reservoir thus includes not only clinically apparent infections but also submicroscopic carriers that evade routine detection yet contribute meaningfully to transmission persistence [11].

Despite the recognized importance of this hidden reservoir, data on the prevalence and molecular characteristics of submicroscopic malaria among asymptomatic populations in Jos, Plateau State remain sparse. Plateau State, with its heterogeneous transmission dynamics influenced by altitude and seasonal rainfall patterns, represents a unique epidemiological setting within Nigeria. Molecular detection of submicroscopic parasitaemia in this region, is therefore critical for accurate estimation of the parasite reservoir, refinement of malaria control strategies and progress toward elimination goals.

2. Materials and Methods

2.1. Study Area

The study was conducted in Jos, Plateau State, located in North-central Nigeria. Jos is characterized by a temperate climate with distinct wet (May–October) and dry (November–April) seasons. The region lies between latitude 9°52'N and longitude 8°54'E, at an elevation of approximately 1,200 meters above sea level, creating favorable conditions for malaria transmission year-round. Five health facilities were selected to represent different locations and populations: Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital (PSSH), Vom Christian Hospital (VCH), Bingham University Teaching Hospital (BHUTH), and Comprehensive Health Centre, Dadin Kowa (CHC).

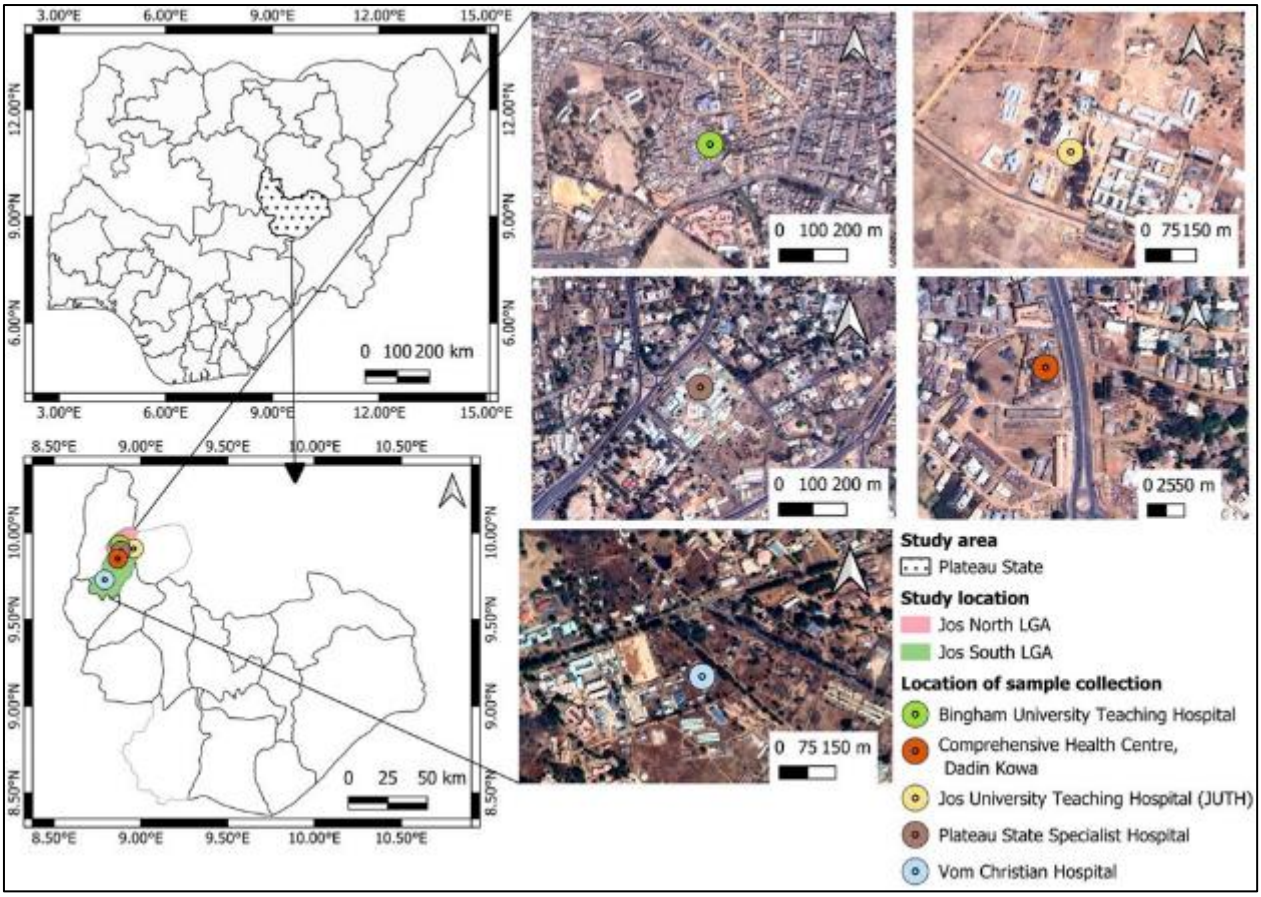

Figure 1 Map of Nigeria showing Plateau State and Location of the Sampled Hospitals (Generated using QGIS version 3.40)

2.2. Sample Size Determination

The sample size for this study was determined using the Rao soft® sample size calculator, a widely accepted tool for estimating adequate sample sizes for surveys [12]. A confidence level of 95% and 5% margin of error was used as parameters. This gave a minimum sample size (n) of 384, this was increased to 400 in order to have an even distribution of samples collected across all the selected health facilities.

2.3. Study Design and Population

A cross-sectional study was conducted between January and December, encompassing both dry and wet seasons. Four hundred (400) asymptomatic individuals attending outpatient clinics were recruited.

Inclusion Criteria

Individuals aged six months and above (≥6 months) were considered for inclusion in the survey. The study population included children, pregnant women and adults (non-pregnant women and males) with axillary temperature ≤ 37.5°c, who were not exhibiting any clinical signs or symptoms of malaria at the time of recruitment and who had not received any form of malaria treatment within two weeks prior to data collection.

Exclusion Criteria

Individuals that did not meet the above criteria were excluded from the study.

2.4. Microscopy

About five milliliters (mL) of venous blood sample was collected from each participant using sterile disposable syringes and poured into Ethylenediaminetetraacetic acid (EDTA) tubes. Part of the blood sample was used to prepare thick and thin films for detection and identification of malaria parasites, by Giemsa-stain microscopy using standard methods [13]. The remaining blood samples was then preserved for molecular analysis.

2.5. Molecular Analysis

Genomic DNA was extracted using Zimo Quick-DNA™ Miniprep Extraction Kit following manufacturers protocol and stored at −20°C.The parasite genomic DNA was amplified using the following set of oligonucleotide primers.

Table 1 Set of Primers used for PCR Amplification and Sequencing of *Plasmodium* Genes

	Primer Name	Primer Sequence (5' to 3')	Amplicons (bp)	Target Gene	Reference
Plasmodium genus	rPLU5	CCTGTTGTTGCCTTAAACTTC	1100	18S rRNA	Johnston *et al.,* 2006
	rPLUS6	TTAAAATTGTTGCAGTTAAAACG			
P. falciparum	rFAL1	TTAAACTGGTTTGGGAAAACCAAATATATT	205		
	rFAL2	ACACAATGAACTCAATCATGACTACCCGTC			
P. vivax	rVIV1	CGCTTCTAGCTTAATCCACATAACTGATAC	120		
	rVIV2	ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA			
P. ovale	rOVA1	ATCTCTTTTGCTATTTTTTAGTATTGGAGA	800		
	rOVA2	GGAAAAGGACACATTAATTGTATCCTAGTG			
P. malariae	rMAL1	ATAACATAGTTGTACGTTAAGAATAACCGC	144		
	rMAL2	AAAATTCCCATGCATAAAAAATTATACAAA			

Two-step nested PCR targeting genus (18S rRNA) and species-specific genes was used. This was carried out in a 25 µL reaction volume using FIREPOL® Master Mix and 0.5 µM primers. PCR's reaction was carried out on a MiniAmp™ Thermal Cycler (Applied Biosystems). Species-specific annealing conditions were 60°C/55°C for all *Plasmodium* species except for *P. ovale* that had a lower annealing temperature of 45°C. Products were ran on 1.5% agarose gel and sequenced using Applied Biosystems 3500 [14]. Samples negative by microscopy but positive by PCR were classified as submicroscopic infections.

2.6. Data Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 20 software. Prevalence estimates were calculated for microscopy and PCR. Associations between asymptomatic and submicroscopic malaria parasitaemia and demographic variables, were assessed using chi-square tests, with significance established at $p < 0.05$.

3. Results

3.1. Prevalence of Asymptomatic Malaria Infection across Selected Health Facilities

Out of the 400 asymptomatic participants examined, 78 (19.5%) were positive for malaria parasites by microscopy. Prevalence varied across health facilities, with the highest recorded at Vom Christian Hospital (30.0%) and the lowest at Jos University Teaching Hospital (11.2%). However, the prevalence of asymptomatic malaria infection did not vary significantly ($p > 0.05$) across all the hospitals sampled as contained in (Table 2).

Table 2 Overall Prevalence of Asymptomatic Malaria Infection Across Some Selected Health Facilities in Jos, Plateau State, Nigeria confirmed by Microscopy

Location	No. examined	No. infected	Percentage infected (%)
BHUTH	80	16	20.0
CHC, Dadin Kowa	80	14	17.5
JUTH	80	9	11.2
PSSH	80	15	18.8
VCH	80	24	30.0
Total	400	78	19.5

Note: BHUTH = Bingham University Teaching Hospital = CHC, Dadin Kowa (Comprehensive Health Center, Dadin Kowa); JUTH=Jos University Teaching Hospital; PSSH = Plateau State Specialist Hospital; VCH = Vom Christian Hospital

χ^2=9.333, DF=4, p=0.053

3.2. Prevalence of Asymptomatic Malaria Infection by Category

The sampled population was grouped into demographic category (Figure 1). Across these categories, asymptomatic malaria infection although did not differ significantly (p>0.05), however, adult males accounted for the highest burden of the infection (25%) followed by adult females (21%). In the children and pregnant women, the prevalence recorded was 21% and 11% respectively.

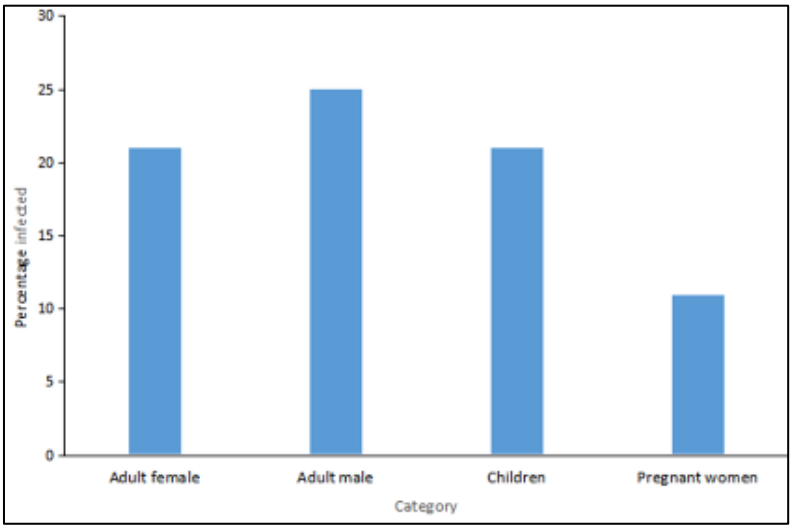

Figure 1 Prevalence of Asymptomatic Malaria Infection by Category

χ^2=6.816, DF=3, p<0.078

3.3. Prevalence of Asymptomatic Malaria Infection in Relation to Sex and Age of Respondents

The prevalence of asymptomatic malaria was significantly higher in males (26.0%) than in females (15.7%) (p = 0.012) (Table 3). Across age groups, infection was highest among children aged 1–5 years (20.6%) and 11–20 years (20.5%), with no significant difference across age groups (p > 0.05) (Table 4).

Table 3 Prevalence of Asymptomatic Malaria Infection in Relation to Sex of Studied Participants confirmed by Microscopy

Sex	No. examined	No. infected	Percentage infected (%)
Female	254	40	15.7
Male	146	38	26.0
Total	400	78	19.5

χ^2=6.241, DF=1, p=0.012

Table 4 Prevalence of Asymptomatic Malaria Infection in Relation to Age Group of Studied Participants confirmed by Microscopy

Age group	No. examined	No. infected	Percentage infected (%)
1-5 years	34	7	20.6
6-10 years	24	4	16.7
11-20 years	73	15	20.5
21-30 years	141	28	19.9

31-40 years	75	15	20.0
41 years and above	53	9	17.0
Total	400	78	19.5

χ^2= 0.437, df=5, p=0.994

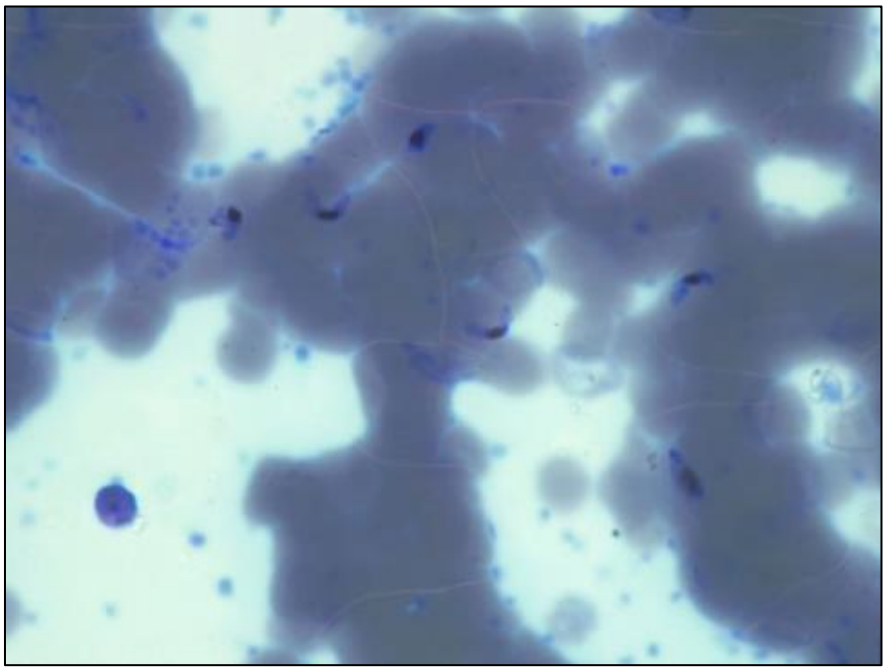

Figure 2 Thin film showing *Plasmodium falciparum* Gametocytes Detected in Blood Sample of a Child with Asymptomatic Malaria (x100 magnification)

3.4. Prevalence of Submicroscopic Malaria Infections in Some Selected Health Facilities within Jos, Plateau State.

Out of 120 microscopy-negative blood samples tested via PCR, a total of 74 (61.7 %) were positive for submicroscopic malaria. Site level heterogeneity was also evident, as the highest prevalence was recorded in Plateau State Specialist Hospital (87.5%), while the least was recorded in Vom Christian Hospital (20.8%) as seen in (Table 5). Across the demographic categories, submicroscopic malaria infection although did not differ significantly (p>0.05), however, pregnant women (64.5%) and children (66.67%) carried the highest burden. In adult female and male subjects, the prevalence was 60.0% and 55.2% respectively, being slightly higher in the adult females (Figure 2).

Table 5 Prevalence of Submicroscopic Malaria Infection Across Some Health Facilities in Jos Plateau State confirmed by PCR

Location	No. examined	No. infected	Percentage infected (%)
BHUTH	24	17	70.8
CHC, Dadin Kowa	24	14	58.3
JUTH	24	17	70.8
PSSH	24	21	87.5
VCH	24	5	20.8
Total	120	74	61.7

Note: BHUTH = Bingham University Teaching Hospital = CHC, Dadin Kowa (Comprehensive Health Center, Dadin Kowa); JUTH=Jos University Teaching Hospital; PSSH = Plateau State Specialist Hospital; VCH = Vim Christian Hospital.

χ^2=25.523, DF=4, p<0.001

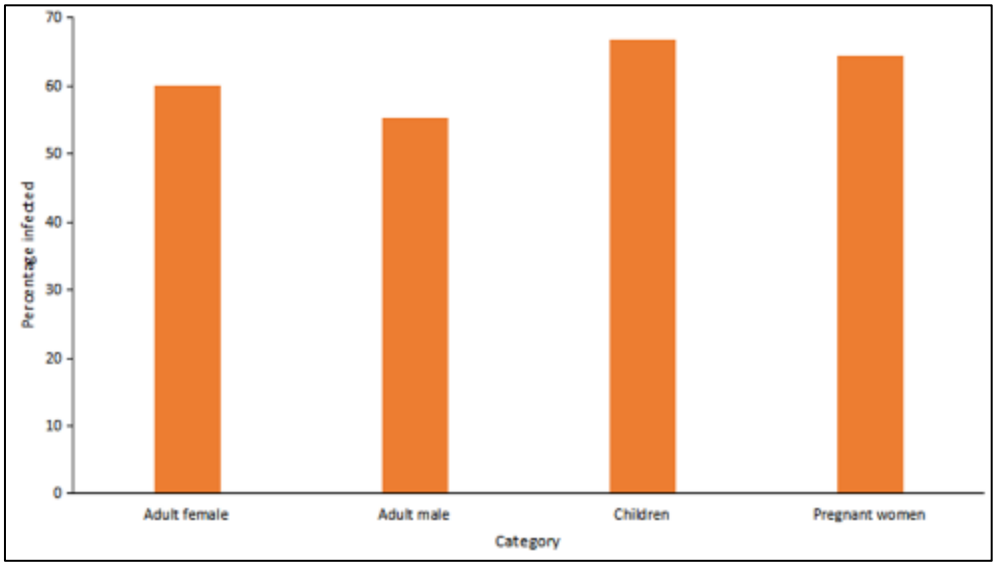

Figure 3 Prevalence of Submicroscopic Malaria Infection by Category

χ^2=0.976, DF=3, p<0.807

3.5. *Plasmodium* Species Composition across the Selected Health Facilities

The results in this study showed that, *Plasmodium falciparum* was the most predominant species, accounting for 95 cases (55.88%), with the highest frequency recorded at Jos University Teaching Hospital (JUTH) (14.71%) and Bingham University Teaching Hospital (BHUTH) (14.12%). *Plasmodium ovale* was the second most detected species, found in 28 individuals (16.47%), with the highest occurrence at the CHC, Dadin kowa (5.29%), indicating a relatively significant presence of relapsing malaria types. *Plasmodium malariae* was found in 11 cases (6.47%), distributed across all health facilities except PSSH, while *Plasmodium vivax*, which is generally uncommon in West Africa due to the high prevalence of Duffy-negative blood types, was detected in 9 cases (5.29%) exclusively in BHUTH and CHC Dadin kowa. Notably, PSSH showed a distinct pattern by having no *P. vivax* or *P. malariae* cases, but still recording 7 *P. ovale* cases (4.12%), as shown in Table 6.

Table 6 *Plasmodium* Species Composition in Asymptomatic and Submicroscopic Malaria Infections Across the Selected Health Facilities in Jos Plateau State confirmed by PCR

Health Facilities	*P. falciparum*	*P. vivax*	*P. malariae*	*P. ovale*
BHUTH	24(14.12)	8(4.71)	6(3.53)	8(4.71)
JUTH	25(14.71)	0(0.00)	3(1.76)	3(1.76)
PSSH	21(12.35)	0(0.00)	0(0.00)	7(4.12)
CHC	16(9.41)	1(0.59)	1(0.59)	9(5.29)
VCH	9(5.29)	0(0.00)	1(0.59)	1(0.59)
Total	95(55.88)	9(5.29)	11(6.47)	28(16.47)

Key= BHUTH=Bingham University Teaching Hospital, JUTH= Jos University Teaching Hospital, PSSH= Plateau State Specialist Hospital, CHC= Comprehensive Health Center, Dadin Kowa Jos, VCH= Vom Christian Hospital.

4. Discussion

This study provides robust molecular evidence that asymptomatic and submicroscopic malaria infections constitute a substantial hidden reservoir in Jos, Plateau State. The marked disparity between microscopy and PCR detection highlights the limitations of routine diagnostic methods in low-to-moderate transmission settings. Although malaria prevalence did not differ significantly across age groups, higher infection rates were observed among children aged 1–

5 years and adolescents aged 11–20 years. This pattern is consistent with established evidence indicating increased vulnerability among younger age groups due to incomplete acquisition of protective immunity [2, 15]. Similar age-related trends have been reported across Nigeria and other endemic regions [16-18], although contrasting findings have also been documented, with higher prevalence among young adults in certain facility-based studies [19]. These variations likely reflect differences in transmission intensity, immunity profiles, healthcare-seeking behaviour, and study design.

Malaria prevalence was significantly higher among males than females, consistent with reports from Nigeria and other African settings [17, 20, 21]. This disparity is often attributed to greater male involvement in outdoor and night-time activities, including farming, fishing, construction, and social gatherings, which increase exposure to mosquito vectors [22]. Additional explanations include lower health-seeking behaviour among males, which may result in prolonged untreated infections and higher detection rates in cross-sectional surveys [23]. Furthermore, emerging evidence suggests that females may clear asymptomatic *Plasmodium* infections more rapidly due to sex-related differences in immune response [24]. Nonetheless, other studies have reported higher malaria burden among females or no significant sex-based differences, reflecting the context-specific influence of behavioural, biological, and health system factors [25].

The microscopy-detected prevalence of asymptomatic malaria (19.5%) indicates a considerable burden of undiagnosed infection and is comparable to reports from other parts of Nigeria [9, 26]. A national meta-analysis reported pooled prevalence exceeding 30%, demonstrating substantial geographic and methodological heterogeneity [27, 28]. Notably, 61.7% of microscopy-negative samples were PCR-positive, confirming a high prevalence of submicroscopic infection and reinforcing evidence that routine diagnostics detect only a fraction of circulating infections [29, 30]. Variations in submicroscopic prevalence across facilities may reflect differences in demographic composition, prior antimalarial exposure, and local transmission dynamics [9, 28]. The higher burden observed among pregnant women and children is consistent with pregnancy-associated immunomodulation and immature immunity in early childhood [28, 29], emphasizing the epidemiological importance of these groups as reservoirs of infection.

Plasmodium falciparum was the predominant species identified in this study, accounting for 55.9% of all infections, in line with national and regional malaria epidemiology. This observation accords with the World Health Organization's 2023 malaria report, which affirms that *P. falciparum* remains the dominant malaria species across sub-Saharan Africa [3]. Nevertheless, a considerable proportion of infections in the present study were attributable to non-falciparum species. Specifically, *P. ovale*, *P. malariae*, and *P. vivax* accounted for 16.5%, 6.5%, and 5.3% of infections, respectively. This distribution is comparable to findings from a large longitudinal study conducted in the Democratic Republic of Congo, where *P. falciparum* constituted approximately 57.5% of infections, while *P. malariae* and *P. ovale* were detected at appreciable rates of 7.8% and 4.8%, respectively, although *P. vivax* was not identified in that setting [31]. In contrast, a PCR-based survey in Ibadan, southwestern Nigeria, reported markedly higher carriage rates of *P. malariae* (53%) and *P. ovale* (24%) among asymptomatic adolescents, with mixed infections comprising the majority of positive cases. In that study, co-infections involving *P. falciparum* and non-falciparum species occurred in over half of infected individuals, underscoring the limitations of microscopy alone in accurately capturing species diversity in Nigerian populations [32].

The identification of *P. vivax* infections in Jos is particularly noteworthy. Historically, West Africa has been characterised by a high prevalence of the Duffy-negative phenotype, which was believed to confer protection against *P. vivax* infection. However, emerging molecular evidence indicates that *P. vivax* can infect Duffy-negative individuals in Nigeria, challenging longstanding assumptions about its absence in the region [33]. Similar observations have been reported in Ethiopia and other parts of Central and West Africa, suggesting the possibility of alternative invasion mechanisms or evolving epidemiological patterns that permit the persistence of *P. vivax* in these settings.

Mixed-species infections were also common, particularly involving *P. falciparum* with *P. ovale* or *P. malariae*, consistent with reports from Nigeria and other endemic regions [31, 34, 35]. Such co-infections have important clinical and epidemiological implications, potentially influencing disease severity, treatment response, and transmission dynamics. Collectively, these findings indicate that non-falciparum species and submicroscopic infections contribute meaningfully to the hidden malaria reservoir in Jos and may affect surveillance accuracy and control strategies.

5. Conclusion

Asymptomatic and submicroscopic malaria parasitaemia represents a substantial and often overlooked reservoir of infection in Jos, Plateau State. The marked disparity between microscopy and molecular detection methods observed in this study underscores the limited sensitivity of routine diagnostic approaches in identifying low-density infections. This finding highlights the urgent need to incorporate more sensitive molecular diagnostic tools into malaria surveillance and control programmes, particularly in low-to-moderate transmission settings.

Addressing these hidden infections through targeted interventions is essential to interrupt ongoing transmission and reduce the silent reservoir sustaining malaria in the community. Strengthening surveillance systems to detect submicroscopic infections will significantly enhance control strategies and is critical to advancing toward malaria elimination in Nigeria.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Plateau State Ministry of Health and the research ethics committees of the participating health institutions.

Statement of informed consent

Written informed consent was obtained from all participants or their guardians prior to enrolment, and confidentiality was strictly maintained.

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