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# Subclinical malaria among pregnant women living in refugee settlements in Northern Uganda

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***Abstract***

**Background:** Malaria during pregnancy contributes to significant perinatal morbidity and mortality, accounting for almost 25% of global maternal mortality. However, the epidemiology and risk factors for subclinical malaria among pregnant women living in refugee settlements is poorly understood.

**Objective:** To determine the prevalence and predictors of subclinical malaria among pregnant women in refugee settlements in Northern Uganda.

**Design:** We conducted a multi-center, cross-sectional study.

**Methods:** The study was conducted between April and June 2023 and involved pregnant women aged 18–45 years attending routine antenatal care (ANC) at three health facilities serving refugee communities in Adjumani district, Uganda. We collected sociodemographic, environmental, maternal, and obstetric factors using a structured questionnaire. Both CareStart Malaria HRP-2/pLDH (Pf/Pan) combo rapid diagnostic test (RDT) and blood smear microscopy with 3% Giemsa staining were simultaneously performed on samples from each patient. Logistic regression analysis identified factors independently associated with subclinical malaria, reported as adjusted odds ratios (aORs) and 95% confidence intervals (CIs).

**Results:** We enrolled 304 pregnant women, with a mean age of 25 years. In total, 68.8% ($n = 209$) had lived in the settlement for over 12 months, 25.7% ($n = 78$) were primigravida, and 1.0% ($n = 3$) were living with HIV. Malaria prevalence was 5.3% ($n = 16$) by RDT and 3.2% ($n = 10$; seven *Plasmodium falciparum* and three *P. malariae*) by microscopy. Only 4 (25.0%) of the RDT-positive cases were also positive by microscopy (Cohen's kappa: 0.278—Fair agreement). All participants were asymptomatic. Factors associated with higher odds of subclinical malaria included primiparity (aOR: 2.79, 95% CI: 1.25–6.25, $p = 0.013$), ⩾4 ANC visits (aOR: 2.41, 95% CI: 1.34–4.34, $p = 0.003$), and residence in the settlement for less than 12 months (aOR: 2.54, 95% CI: 2.0–3.22, $p < 0.001$). Living in the settlement for over 3 years, being primigravida, and being married were associated with 68%, 50%, and 68% lower odds of subclinical malaria, respectively (aOR: 0.32, 95% CI: 0.13–0.79, $p = 0.014$; aOR: 0.50, 95% CI: 1.22–5.52, $p = 0.016$; aOR: 0.32, 95% CI: 0.13–0.78, $p = 0.012$).

**Conclusion:** Our study reveals the high prevalence of subclinical malaria among pregnant women in refugee settlements, particularly among primiparous women and recent arrivals. The poor agreement between RDT and microscopy suggests the need for dual screening in asymptomatic pregnant women.

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## Plain language summary

### Asymptomatic malaria in pregnant women living in refugee settlements in Northern Uganda

Malaria during pregnancy is a major health issue, causing many deaths worldwide. In this study, we aimed to determine the burden and factors that increase the risk of asymptomatic malaria among pregnant women in refugee settlements in Northern Uganda. Using diagnostic methods such as rapid diagnostic tests (RDTs) and microscopy, we tested 304 pregnant women at three health centers serving the refugee communities in Adjumani District between April and June 2023. We found that 5.3% of the women had malaria according to the RDT, and 3.2% had it according to microscopy. Women who were pregnant for the first time, had frequent antenatal visits, or had lived in the camp for less than a year were more likely to have asymptomatic malaria. This study highlights the need for regular malaria testing, even if pregnant women show no symptoms, to better improve their health in refugee settlements.

***Keywords:*** antenatal care, asymptomatic malaria, maternal health, Northern Uganda, refugee settlement

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## Introduction

Malaria during pregnancy is a significant public health concern, with negative outcomes for both the pregnant woman and her fetus or newborn child.[1] Malaria disproportionately affects vulnerable groups of people, such as refugees in sub-Saharan Africa.[2] In 2022, the World Health Organization (WHO) estimated that there were approximately 249 million malaria cases in endemic regions, reflecting an increase of about 5 million cases from 2021.[3] Moreover, in 2022, the WHO African Region accounted for approximately 93.6% of all reported malaria cases and 95.4% of malaria-related deaths.[3] Uganda contributed significantly to the global burden of malaria in 2022, accounting for 5.1% of malaria cases and 2.9% of malaria deaths worldwide.[3] According to the World Malaria Report 2023, an estimated 36% of pregnancies (12.7 million) in Africa were exposed to malaria infection in 2022, with a malaria prevalence of 27% in East and Southern Africa estimated at 27%.[3] The Uganda Monthly Malaria Surveillance Status Report from July 2023 by the Ministry of Health reported an incidence of 28 malaria cases per 1000 people, with 1,269,082 cases diagnosed in total, and the Acholi region contributing about 13% of all malaria cases.[4] In addition, 23.1% of the 174,684 pregnant women attending their first antenatal care (ANC) visit tested positive for malaria.[4] In Northwestern Uganda, malaria prevalence among pregnant women was estimated to be 26.1% based on rapid diagnostic testing (RDT).[5] However, to date, no study has empirically measured subclinical malaria in pregnant women living in refugee camps in Northern Uganda.

Subclinical malaria, characterized by the absence of symptoms, often goes undiagnosed due to a lack of observable clinical signs.[6] This undiagnosed state leads to chronic low-grade inflammation and an increased risk of anemia caused by persistent hemolysis, along with an increased susceptibility to invasive bacterial infections due to neutrophil dysfunction.[6–10] Studies across various regions have reported the prevalence of subclinical malaria ranging from 5% in Southeast Asia to 12.2% along the Myanmar–China border, with notably high rates of about 9.0% among asymptomatic immigrants in Sweden, predominantly from Uganda.[11,12] Furthermore, the subclinical malaria prevalence in pregnant women has been reported to be approximately 24%, 77.6%, and 40.6% in Ethiopia, Nigeria, and Malawi, respectively.[13–15] Notably, subclinical malaria may easily be missed by conventional light microscopy and

rapid diagnostic techniques.[12] For instance, in a study conducted by Huang et al.,[12] light microscopy missed approximately 86.5% of subclinical malaria cases. Despite the absence of an apparent illness, subclinical malaria still exerts detrimental effects on maternal and fetal health, such as severe anemia.[16]

Several factors have been implicated in the association between malaria and pregnancy, including being in the first trimester due to the changes in the immune response during early pregnancy, and late initiation of ANC which is associated with missed opportunities for malaria preventive interventions.[5,17,18] Furthermore, seeking obstetric care from traditional birth attendants during pregnancy causes missing of malaria preventive measures coupled with delayed diagnosis and treatment of malaria.[19] Other risk factors include use of open water sources, poor socioeconomic status, and lack of insecticide-treated bed nets.[20] Malaria during pregnancy has far-reaching effects, including maternal anemia, placental malaria, congenital malaria, low birth weight, preterm delivery, intrauterine growth restriction, and increased infant and maternal mortality.[21,22] In addition to the adverse effects of clinical malaria, the insidious presence of subclinical malaria further compounds the challenges faced by pregnant women, especially those residing in vulnerable settings, such as refugee settlements.[23]

There is a paucity of data on the prevalence of malaria among pregnant refugee women in Northern Uganda. However, pregnant women living in refugee settlements face unique challenges, which may exacerbate their vulnerability to subclinical malaria. Factors such as inadequate access to healthcare, poor living conditions, limited resources for malaria prevention and treatment, and disruption of regular ANC services due to displacement contribute to the heightened risk of malaria transmission and its adverse consequences in this population. Despite multiple preventive measures such as vector control and intermittent preventive treatment against malaria for pregnant women as recommended by the WHO and reinforced by the Ministry of Health, malaria persists and continues to claim the lives of pregnant women. In this study, we aimed to determine the prevalence and predictors of subclinical malaria among pregnant women in refugee settlements in Northern Uganda.

## Methods

### *Study design*

We conducted a multi-center, cross-sectional study between April and June 2023.

### *Study setting*

We randomly selected three out of twelve health facilities serving refugee communities in Adjumani district: Mungula Health Center (HC) IV, Ayiri HC III, and Pagirinya HC III. These facilities serve both the refugees and the host population. The selected health facilities are located in high-density refugee settlements in Adjumani district, chosen for their accessibility, frequent utilization by pregnant women, and the diverse demographic composition of the populations they serve. The ANC clinics in all the study sites run from Monday to Friday every week, offering ANC services to about 20–40 mothers every clinic day. The services offered at the ANC include health education, complete blood count, malaria screening, HIV testing, intermittent preventive treatment, deworming, and immunization.

The Acholi region in Northern Uganda, which includes Adjumani district, is among the highest malaria transmission areas in Uganda, with malaria being endemic throughout the year. According to the July 2023 Malaria Surveillance Status Report by the Ministry of Health, Uganda, Acholi contributed 13% of the country's total malaria cases, with a regional incidence of 16% and 9% of all malaria-related hospital admissions.[4] While the prevalence of malaria among pregnant women in northwestern Uganda, a neighboring region, is estimated to be about 26%, specific data on malaria prevalence in Adjumani and its refugee settlements are lacking.[5] However, the high endemicity in surrounding areas suggests a substantial malaria burden among pregnant women within these settlements.

### *Sample size and participants*

With an estimated prevalence of malaria among pregnant women in Uganda of 26.1%,[5] precision of 5%, and a two-tailed *Z*-statistics of 1.96 at 95% confidence interval (CI), a sample size of 296 participants was calculated using the Kish–Leslie formula.[24] We enrolled pregnant women aged 18 years or older who were willing and competent

to provide informed written consent regardless of gestational age or gravidity. We excluded pregnant women with symptoms of malaria, those who declined participation in the study, and pregnant women under 18 years of age. Trained research assistants consecutively enrolled eligible participants until the required sample size was reached. Questionnaires were administered in several languages, including the local language (*Ma'di*), Acholi, English, and Arabic, with the help of trained translators. Data collection was supervised by two investigators (FB and WK), and data entry was continuously monitored by SL for completeness using KoboTool Box. The data collection tool was piloted in Mungula HC IV before deployment as a way of quality assurance. In cases of discordant results, a third clinical laboratory technologist was consulted to review the slides and provide a final determination.

### Study measurements

*Participants' characteristics.* Data were collected through face-to-face interviews administered by trained research assistants on maternal characteristics such as age, gravidity, education level, occupation, marital status, HIV status, diet, gestational age, smoking and alcohol use, and the number of ANC visits during the current pregnancy. The study variables included in the questionnaire were guided by previous studies in Uganda.[5,13–15] The HIV status of the mothers was self-reported and confirmed using an ANC card. Gestational age was estimated from the first day of the last normal menstrual period using Naegele's formula.[25]

*Complete blood count.* A study nurse drew 4 mL of blood, and samples were analyzed using the HumaCount 3D Hematology System (Wiesbaden, Germany) at the Mungula HCIV laboratory. Anemia in pregnancy was defined to the WHO classification as Hb $<$ 11 g/dL and further classified into mild (Hb 10.0 – 10.9 g/dL), moderate (Hb 7.0 – 9.9 g/dL) and severe (Hb $<$ 7.0 g/dL. A mean corpuscular volume of $<$76 femtolitres was considered microcytic, 76–100 femtolitres normocytic and $>$100 femtolitres macrocytic.

*Anthropometry.* Weight was measured with minimal clothing and without shoes using a digital bathroom weighing scale (SECA-Germany), while mid–upper arm circumference (MUAC) was measured on women's nondominant hand (arm) at the midpoint between the olecranon and acromion processes. Non-stretchable MUAC tape with the correct tension (not too loose or too tight) was used, and the values were recorded to the nearest 0.1 cm. Measurements were performed twice, and the average values were used for analysis. A MUAC of 22vcm or less ($<$23 cm) was considered undernutrition, and 23 cm or more ($>$22 cm) was considered normal nutrition.

*Malaria test.* For each study participant, 4 mL of venous blood was collected in an Ethylenediaminetetraacetic acid (EDTA tube). Both CareStart Pf/Pan combo tests and thick blood smear microscopic examinations were simultaneously performed on the samples obtained from each patient. Thick blood smears were prepared. Each slide was then stained with a 3% Giemsa solution for 45 min. All blood smears were examined microscopically using an oil-immersion lens at 1000× magnification. For positive smears, the number of parasites was counted against 200 white blood cells (WBCs) in thick smears or against 500 WBCs for low-density infections. Parasite density was calculated by assuming 8000 WBCs per μL. All the slides were examined independently by two experienced clinical laboratory technologists licensed by the Ministry of Health, who were blinded to the RDT results.

The RDT kit used was the CareStart Malaria HRP-2/pLDH (Pf/Pan) combo test (Access Bio, Somerset, New Jersey, USA). This kit is a three-band RDT targeting HRP-2 and pan-pLDH. The test kits were stored at room temperature, and the expiration date was checked before use. Tests were performed according to the manufacturer's directions. The CareStart tests were labeled with a patient identification number and date, and the results were recorded after 15 min. A unique pan-pLDH line was found when an individual is infected with one or more non-falciparum species. A test result without a control line was considered invalid and the sample was retested.

### Operational definition

Subclinical malaria was defined as the presence of Plasmodium parasites detected by either microscopy or RDT in the absence of clinical symptoms.

### Statistical analysis

Data were downloaded from KoboCollect and exported to Microsoft Excel for analysis in Stata

version 15.0 (College Station, TX, USA). Categorical variables were expressed as frequencies and percentages. Parametric data were summarized as mean and standard deviation (mean ± SD), and non-parametric data as median and interquartile range (IQR). A multivariable logistic regression model was used to assess the independent predictors of malaria in pregnancy. All variables with a $p$-value $< 0.2$, as determined by the bivariable analysis and known to be associated with malaria infection, were fitted into the multivariate logistic regression model. We adjusted for potential confounders, such as age, parity, gestational age, and comorbidities. The adjusted odds ratio (aOR) and 95% CI were used to report the strength of association. Statistical significance was set at $p < 0.05$.

### *Ethical considerations*

The study protocol was approved by the Gulu University Research and Ethics Committee (GUREC-2022-450), with additional administrative approvals obtained from the Office of the Prime Minister (Ref# RDA/1B/081/23), Medical Teams International (MTI), and study sites. Written informed consent was obtained from all study participants. The consent process was conducted in a language understood by the participants, with trained interpreters available when needed. Confidentiality was strictly maintained throughout the study. Personal identifiers were replaced with unique identification codes, and all data were securely stored with access restricted to investigators only. To mitigate any potential coercion, it was made clear that participation in the study was independent of the receipt of any services, and the consent process emphasized that participation was entirely voluntary. The principles of human subject research outlined in the *Declaration of Helsinki* were strictly adhered to. All participants were provided with a copy of results of their complete blood count, stool analysis, and malaria tests, and those with positive findings were provided with appropriate care in routine clinical settings.

## Results

### *Sociodemographic characteristics of the participants*

A total of 304 women were recruited for the study. The mean age of participants was 25.4 years with a standard deviation of 4.9 years. Many participants (48.4%, $n = 147$) were in the age group of 25–34 years, and (93.8%, $n = 285$) were married (Table 1). The largest proportion of study participants had primary education (61.8%, $n = 188$), followed by secondary education (22.0%, $n = 67$). Regarding source of income, the majority (85.9%, $n = 261$) reported that they had no source of income, and almost all (97.0%, $n = 295$) had an average monthly household income of less than UGX 100,000 (27 USD). More than one-third of the study participants (39.7%, $n = 118$) had spent between 1 and 3 years at the camp (Table 1).

### *Obstetric and medical characteristics of the participants*

Approximately 35.5% ($n = 108$) of the participants had parity of 2–4. Less than one-tenth (5.6%, $n = 17$) reported a history of abortion. Regarding ANC attendance, most of the participants (64.5%, $n = 196$) had attended ANC less than four times. Almost all participants (99.0%, $n = 300$) were HIV-negative, and more than a quarter of the participants (28.0%, $n = 85$) had anemia (hemoglobin level $< 10.5$ g/dL), (Table 2).

### *Prevalence of malaria*

Of the 304 asymptomatic participants enrolled in the study, 7.2% ($n = 22$) were confirmed to have subclinical malaria; 5.3% ($n = 16$) by RDT and 3.2% ($n = 10$; seven *P. falciparum* and three *P. malariae*) by microscopy. Only 4 (18.2%) of 22 cases were positive for malaria by both RDT and microscopy (Cohen's kappa: 0.278—Fair agreement).

### *Factors associated with malaria among pregnant women living in refugee camps in Northern Uganda*

Attendance of ⩾4 ANC visits (aOR: 1.99, 95% CI: 1.64–2.42, $p < 0.001$), and living in the refugee settlement for <12 months (aOR: 1.80, 95% CI: 1.18–2.75, $p < 0.001$) were associated with higher odds of having malaria (Table 3).

Women who had lived in the refugee settlement for more than 3 years (aOR: 0.32, 95% CI: 0.13–0.79, $p = 0.014$), those who were primigravida (aOR: 0.50, 95% CI: 1.22–5.52, $p = 0.016$), and married (aOR: 0.32, 95% CI: 0.13–0.78, $p = 0.012$) had lower odds of having malaria (Table 3).

**Table 1.** Sociodemographic characteristics of the participants.

| Characteristic ($n = 304$) | Frequency ($n$) | Percentage (%) |
|---|---|---|
| Study site | | |
| Adjumani refugee camp | 54 | 17.8 |
| Mungula Health Center IV | 101 | 33.2 |
| Pagirinya Health Center III | 149 | 49.0 |
| Mother's age (years), mean ± SD; 25.42 ± 4.85 years | | |
| 18–24 | 139 | 45.7 |
| 25–34 | 147 | 48.4 |
| ⩾35 | 18 | 5.9 |
| Highest education level | | |
| Informal | 29 | 9.5 |
| Primary | 188 | 61.8 |
| Secondary | 67 | 22.0 |
| Tertiary | 20 | 6.6 |
| Marital status | | |
| Married | 285 | 93.8 |
| Separated | 14 | 4.6 |
| Widowed | 5 | 1.6 |
| Source of income | | |
| Business | 38 | 12.5 |
| Casual laborer | 1 | 0.3 |
| Formal employment | 4 | 1.3 |
| I have no source of income | 261 | 85.9 |
| Estimated personal monthly income (UGX), Median (IQR); 70,000 (55,000–70,000) (19 (15–19 USD)) | | |
| <100,000 (27 USD) | 295 | 97.0 |
| 100,000–500,000 (27–135 USD) | 8 | 2.6 |
| >500,000 (135 USD) | 1 | 0.3 |
| Time at the camp | | |
| Less than 1 year | 88 | 29.6 |
| 1 to 3 years | 118 | 39.7 |
| >3 years | 91 | 30.6 |

**Table 2.** Obstetric and medical characteristics of the participants.

| Characteristic (*n* = 304) | Frequency (*n*) | Percentage (%) |
|---|---|---|
| Gravidity, median (IQR) 2(1–3) | | |
| Primigravida | 78 | 25.7 |
| 2–4 | 186 | 61.2 |
| >4 | 40 | 13.2 |
| Parity, median (IQR) 1(0–2) | | |
| 0 | 86 | 28.3 |
| 1 | 92 | 30.3 |
| 2–4 | 108 | 35.5 |
| >4 | 18 | 5.9 |
| History of abortion | | |
| No | 287 | 94.4 |
| Yes | 17 | 5.6 |
| Number of ANC visits | | |
| <4 | 196 | 64.5 |
| ⩾4 | 108 | 35.5 |
| Has gastritis/Ulcers | | |
| No | 262 | 86.2 |
| Yes | 42 | 13.8 |
| HIV status | | |
| Negative | 300 | 99.0 |
| Positive | 3 | 1.0 |
| Human Papillomavirus (HPV) infection or cervical cancer | | |
| No | 298 | 98.3 |
| Yes | 5 | 1.7 |
| Diabetes mellitus history | | |
| No | 299 | 98.7 |
| Yes | 4 | 1.3 |
| Dewormed in the last 6 months | | |
| No | 274 | 90.1 |
| Yes | 30 | 9.9 |
| Has anemia | | |
| No | 219 | 72.0 |
| Yes | 85 | 28.0 |

IQR, interquartile range.

**Table 3.** Factors associated with malaria among pregnant women living in refugee camps in Northern Uganda.

| Variable | COR | 95% CI | *p* Value | AOR | 95% CI | *p* Value |
|---|---|---|---|---|---|---|
| Age | | | | | | |
| 18–24 | 1.00 | | | 1.00 | | |
| 25–34 | 0.94 | (0.24–3.67) | 0.930 | 0.82 | (0.12–5.74) | 0.842 |
| ⩾35 | — | — | — | — | — | — |
| Highest education level | | | | | | |
| None/Primary | 1.00 | | | 1.00 | | |
| Secondary/Tertiary | 1.47 | (0.41–5.31) | 0.558 | 0.95 | (0.12–7.45) | 0.963 |
| Marital status | | | | | | |
| Married | 0.25 | (0.20–0.32) | <0.001 | 0.32 | (0.13–0.78) | 0.012 |
| Single | 1.00 | | | 1.00 | | |
| Time at the camp | | | | | | |
| Less than 1 year | 1.73 | (1.08–2.77) | 0.022 | 1.80 | (1.18–2.75) | 0.007 |
| 1–3 years | 1.00 | | | 1.00 | | |
| >3 years | 0.27 | (0.14–0.53) | <0.001 | 0.32 | (0.13–0.79) | 0.014 |
| Parity | | | | | | |
| 0 | 1.05 | (0.32–3.40) | 0.939 | 0.50 | (1.22–5.52) | 0.016 |
| 1 | 2.31 | (0.77–6.94) | 0.136 | 1.29 | (0.72–2.33) | 0.383 |
| 2–4 | 1.00 | | | 1.00 | | |
| >4 | — | — | — | — | — | — |
| Number of ANC visits | | | | | | |
| <4 | 1.00 | | | 1.00 | | |
| ⩾4 | 1.56 | (1.09–2.26) | 0.017 | 1.99 | (1.64–2.43) | <0.001 |
| Anemia | | | | | | |
| No | 1.00 | | | 1.00 | | |
| Yes | 0.39 | (0.01–1.61) | 0.097 | 0.41 | (0.12–1.39) | 0.153 |

AOR, adjusted odds ratio; COR, crude odds ratio; CI, conference interval; 1, Reference category.

## Discussion

In this study, we determined the prevalence and predictors of subclinical malaria among pregnant women in refugee settlements in Northern Uganda. We found that the prevalence of subclinical malaria was 7.2% ($n=22$), with 5.3% ($n=16$) confirmed by RDT and 3.2% ($n=10$; 7 *P. falciparum* and 3 *P. malariae*) confirmed by microscopy. Only 4 (18.2%) of the 22 cases were positive for malaria by both RDT and microscopy. Our findings align with previous research indicating that RDTs tend to detect more subclinical malaria cases compared to

microscopy.[12,26,27] This heightened sensitivity of RDTs can be attributed to their ability to detect low-density infections, which are typical in subclinical malaria cases.[28–31] Moreover, it is worth noting that light microscopy, while a commonly used technique, relies heavily on the skill of the user and the quality of the staining reagents.[32,33] It typically requires an estimated range of 50–500 parasites per microliter of blood, although experienced users can detect malaria parasites at concentrations as low as 10 parasites per microliter.[32,33] The observed fair agreement between RDT and microscopy results, as indicated by a Cohen's kappa value of 0.278, reflects the inherent limitations and differences between these diagnostic methods. Malaria RDTs, especially those detecting HRP2 antigens, have been shown to have an increased tendency to yield false positives due to residual antigens from past infections.[34] Furthermore, Polymerase Chain Reaction tests (PCRs) assays have been reported to offer even greater sensitivity and specificity in detecting malaria parasites, capable of detecting concentrations as low as one to five parasites per microliter of blood.[35–37] However, PCR is a more expensive diagnostic method and was not used in the present study.

Our findings are consistent with studies conducted in various countries, reporting relatively similar prevalence rates of subclinical malaria, estimated at 7% in Congo,[38] 8.2% in Southern Ethiopia,[39] 8.3% in Southern Laos,[40] and 9.1% in North-Shoa, Ethiopia.[41] However, studies in West Guji Zone, Ethiopia, Oyo State, and Ogun State, Nigeria reported higher prevalence rates of 24.1%, 25.9%, and 49.6%, respectively.[13,42,43] Conversely, studies conducted in Bangladesh, Colombia, India, and Oromia region, Ethiopia, reported a relatively lower prevalence of subclinical malaria of 3.6%, 4.2% 5.4%, and 5.79%, respectively.[44–47] These variations highlight the considerable differences in subclinical malaria prevalence across different populations, possibly due to differences in malaria control strategies, sample size, altitude, and temperatures in the study areas.

The high prevalence of subclinical malaria observed in our study could be attributed to the fact that our study area is one of the high malaria transmission areas in Uganda.[48] In malaria-endemic areas, individuals frequently exposed to malaria develop protective immunity, which renders Plasmodium infection asymptomatic.[49,50] Additionally, subclinical malaria could also be linked to mutations in *pfkelch13* genes after intravenous artesunate treatment, which were observed in the study area by Balikagala et al.[51] These mutations are associated with prolonged half-lives in parasite.[51] However, in the current study, assessment of mutations in *pfkelch13* genes was not performed.

This study revealed a statistically significant difference between the RDT and the microscopy results. Twelve malaria cases were missed by microscopy but detected by the RDT, and six cases were missed by the RDT but detected by microscopy. Similar observations have been reported in previous studies that used RDT and microscopy for malaria diagnosis.[52,53] The difference between the RDT and microscopy results in our study could be linked to the assumption that some of the study participants may have been treated within the past 2 weeks for malaria infection. A study by Leslie et al.[54] discovered that microscopy had a lower operational sensitivity for the detection of malaria parasites than RDT. The difference between the RDT and microscopy results could also be associated with low parasitemia in the tested samples.[53,54]

Our study showed that married women had 68% less odds of having malaria than those who were single. This finding is consistent with previous studies that revealed marital status as a demographic factor significantly associated with malaria infection.[53,55] This finding could be supported by the postulation that married women receive more care and social support from their partners, including preventive healthcare such as providing reminders on medication and providing support in the proper utilization of treated mosquito nets, among others, unlike unmarried women.[55] In this study, we also found that primigravidity (first-time pregnancy) was associated with lower odds of malaria than was multigravidity. This is contrary to findings from previous studies, which indicated that women who were primigravida had higher odds of having a malaria infection.[43,56] This could be due to the improved prenatal care and malaria prevention measures targeted specifically at first-time mothers in the refugee centers in Northern Uganda showing that enhanced interventions and education for primigravid women effectively reduces their risk of malaria infection.

This study also showed that women who had attended at least four ANC visits had 2.41 times the odds of having malaria compared to those with <4 ANC visits. ANC attendance is often associated with better health outcomes during pregnancy, including reduced risk of malaria. However, women with higher frequencies of ANC attendance may include those with underlying health conditions or coinfections that increase their susceptibility to malaria. Furthermore, women who had lived in refugee settlements for more than 3 years had 68% less odds of having malaria compared to their counterparts. Studies have shown that acquired protective immunity against malaria is achieved in adults as a cumulative effect after repeated exposure in malaria-endemic areas.[57] Women who had resided in refugee settlements for more than 3 years could have had more exposure to malaria and less ability to seek healthcare, potentially leading to the development of immunity against malaria infection or have received aid, including malaria preventive measures, for a longer period. As a result, they were less likely to contract malaria than those who had lived in the settlement for less than 12 months.

This study has several limitations. The cross-sectional design prevents the establishment of causal relationships between selected exposures and subclinical malaria infection. Additionally, the study was conducted within a specific subgroup of the refugee population in Northern Uganda, which may limit the generalizability of the findings to other regions. We did not collect data on participants' previous history of malaria, which could have resulted in false-positive results due to the prolonged detection of PfHRP2 antigens after infection. Furthermore, the absence of molecular testing, such as PCR, reduced our ability to detect low-level parasitemia. The lack of follow-up also limited our ability to assess the progression and outcomes of subclinical malaria cases. Finally, while asymptomatic carriers were identified, their role in malaria transmission and potential long-term health impacts were not investigated. Future research should address these gaps by incorporating follow-up assessments, molecular testing, and exploring the impact of asymptomatic carriers.

## Conclusion

We highlight a high prevalence of subclinical malaria among pregnant women in refugee settlements, particularly among primiparous women and those who had lived in the settlement for a shorter duration. The high prevalence and low agreement between RDT and microscopy warrants routine malaria screening even in asymptomatic women, and the adopting of dual screening with both RDT and microscopy. Additionally, molecular testing such as PCR should be utilized in case of discordant results to improve diagnostic accuracy. Furthermore, developing policies to strengthen ANC services with comprehensive malaria prevention strategies in refugee settlements will significantly improve health outcomes in this population.

## Declarations

### *Ethics approval and consent to participate*

The study protocol was approved by the Gulu University Research and Ethics Committee (GUREC-2022-450), and additional administrative approvals were obtained from the Office of the Prime Minister (Ref# RDA/1B/081/23), Medical Teams International (MTI), and study sites. Written informed consent was obtained from all study participants. The principles of human subject research outlined in the Declaration of Helsinki were strictly adhered to. All participants were provided with a copy of their CBC, stool analysis, and malaria tests, and those with positive findings were provided with appropriate care in routine clinical settings by their ANC providers.

### *Consent for publication*

Not applicable

### *Author contributions*

**Winnie Kibone:** Conceptualization; Data curation; Investigation; Methodology; Project administration; Validation; Writing – original draft; Writing – review & editing.

**Felix Bongomin:** Conceptualization; Data curation; Investigation; Methodology; Resources; Supervision; Visualization; Writing – original draft; Writing – review & editing.

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**Rachel Beardsley:** Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing.

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### *Competing interests*

The authors declare that there is no conflict of interest.

### *Availability of data and materials*

All relevant data are within the manuscript and its supporting information files. Data are available upon reasonable request from the first author.

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