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Original article

Prevalence of *Plasmodium* Infection Among Migrant Populations in Tripoli, Libya: A Cross-Sectional Study

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A B S T R A C T

Libya, a malaria-free country since 1973, faces reintroduction risks from migrants traversing endemic regions and living in substandard Libyan conditions. A cross-sectional study (February–July 2025) screened 100 adult migrants (94% male, mean age 28.7) in Tripoli, primarily from Nigeria (54%) and Sudan (38%), residing ≤3 years in Libya. Using RDT, microscopy, and PCR, plus surveys, overall malaria prevalence was 1.0% (1/100), with full diagnostic concordance. The single case was an asymptomatic 25-year-old Sudanese male with P. falciparum (residency 6-12 months). The history of malaria infection was higher in Sudanese (35.0%) vs. Nigerians (24.6%), primarily occurring in youth. Recent arrivals (<1 year) reported more symptoms (47.5% vs. 26.2%). Critically, 82% of symptomatic participants had not received any treatment. Despite low prevalence, the asymptomatic carrier highlights treatment gaps and heightened risk among recent migrants.

Introduction

Malaria constitutes a life-threatening febrile illness caused by protozoan parasites of the Plasmodium genus: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*, transmitted by the bite of infected female *Anopheles* ssp. mosquitoes [1,2]. Among Plasmodium species, *P. falciparum* shows the highest virulence and is responsible for the majority of global malaria mortality, particularly in sub-Saharan Africa, where it is the most prevalent [2, 3]. Libya occupies a strategic position along the Mediterranean coast, functioning as a key transit hub for international migrants and refugees—primarily from sub-Saharan Africa and Asia—on their journey toward Europe [4,5].

Since the 2011 uprising, a long period of political and economic instability has significantly degraded Libya's public health infrastructure and limited its capacity for effective disease surveillance [6,7]. Simultaneously, substantial migration flows traverse regions of high malaria endemicity (e.g., Niger, Chad, Sudan) [8] prior to arrival in Libya, where an estimated 570,000 migrants and refugees were present in 2021 [9]. This population frequently endures conditions of heightened vulnerability within detention centers or informal settlements, characterized by finite access to healthcare services, unsuitable sanitation, and lack of vector control interventions such as insecticide-treated nets (ITNs) [10, 11]. Policies enacted by the European Union (EU) aimed at containing irregular migration have worsened these fragile living situations and associated health risks, including potential malaria exposure [12, 13]. Therefore, this study aimed to determine the prevalence of Plasmodium infection among migrants and to evaluate the sensitivity and specificity of the Rapid Diagnostic Test (RDT) by comparing it with microscopic examination of malaria blood films and Polymerase Chain Reaction (PCR).

Methods

Study Design, Setting, and Population

This descriptive cross-sectional study was conducted at the reference lab of parasitology and vector-borne disease in National Centre for Disease Control (NCDC), while samples were obtained from the Reference Medical Laboratory of Tripoli, Libya, in the period of February and July 2025. This serves as Libya's primary center for mandatory pre-residency viral screening for all workers, whether they are Libyans or migrants. A randomized sample of 100 adult legal migrants (>18 years) participated in the study according to the following criteria: country of origin from malaria-endemic regions, arrival date to Libya, and history of antimalarial treatment. Exclusion criteria include pregnancy and severe comorbidities. A written informed consent was signed by all participants before enrollment.

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Data Collection
Demographic, clinical, and behavioral data were collected using a structured questionnaire form administered by the research team; the form was in Arabic, English, and French. The instrument captured seven key variables: age, gender, geographic origin, duration of Libyan residence, history of prior malaria infection, self-observed malaria symptoms (fever, chills, or headache) within the past six months, and treatment-seeking behavior information. Pretesting established acceptable instrument reliability (Cronbach's α = 0.78).

Biological Sample Collection and Handling
Venous blood samples (3 mL) were collected from each participant into K_2EDTA vacutainers (Becton Dickinson, USA). Samples were maintained at 4°C and transported immediately to the NCDC for processing, within two hours of collection.

Laboratory Diagnostics
Rapid Diagnostic Testing (RDT)
The WHO-prequalified SD BIOLINE Malaria Ag P.f/Pan RDT (Standard Diagnostics, Yongin, South Korea) was used following the manufacturer's protocol. Results were interpreted by the team after 30 minutes based on the presence of Control (C), P. falciparum-specific Histidine Rich Protein II (T1: HRP-II), and Pan-specific Plasmodium lactate dehydrogenase (T2: pLDH) lines. Manufacturer reports indicate the test characteristics include sensitivities/specificities of 99.7%/99.5% for P. falciparum (HRP-II) and 95.5%/99.5% for pan-malarial (pLDH) detection.

Microscopic Examination
Thick and thin blood films were prepared according to WHO standardized protocols. Slides were examined microscopically under 100× oil immersion. Parasite density (parasites/μL), species identification based on morphological characteristics of the parasite, and staging were recorded. Discordant results (defined as ≥1 difference in species identification or parasite density category) underwent adjudication by a senior reference parasitologist.

Molecular Detection of Plasmodium falciparum
Genomic DNA was extracted from 200 μL of whole blood using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Following the manufacturer's instructions, conventional PCR amplification targeted the P. falciparum plasmepsin 4 gene (pfpm4) using the Genesig® Standard Kit (Primerdesign Ltd, Chandler's Ford, UK). Thermocycling conditions (T100™ Thermal Cycler, Bio-Rad, USA) comprised initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 10 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 90 seconds; and a final extension at 72°C for 10 minutes. Amplification products were resolved by electrophoresis on 1% (w/v) agarose gels (1× TAE buffer), stained with SYBR® Gold (Thermo Fisher Scientific, USA), and visualized under UV transillumination. A positive result was confirmed by the presence of the expected 205 bp.

Statistical Analysis
Results were calculated as the ratio between positive samples and all tested samples. Chi-square and Fisher's exact tests were used to evaluate if the collected data was statistically significant ($p \leq 0.05$). All data were analyzed statistically using the Statistical Package for Social Sciences program (SPSS) version 26.0 software.

Results
Demographic and Migration Characteristics
The study included 100 participants (94% male, 6% female); the age range of participants was between 16 and 65 years, while the mean of the participants' age was 28.7 years. The highest number of participants were from Nigeria (54%), and the smallest number were from Bangladesh and Ghana (1%), as distributed in table 1.

Table 1. Demographic Overview (n=100).

Variable	Category	Count	%
Gender	Male	94	94.0%
	Female	6	6.0%
Country	Nigeria	54	54.0%
	Sudan	38	38.0%
	Chad	4	4.0%

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	Mali	2	2.0%
	Ghana	1	1.0%
	Bangladesh	1	1.0%
Age	16-25 years	63	63.0%
	26-40 years	31	31.0%
	>40 years	6	6.0%

Malaria History and Clinical Status

The current study demonstrated a low historical burden of malaria, with 75% acknowledging no previous infection. Among 75 participants, 15% had experienced one infection and 10% multiple episodes. Despite 25% presenting with malaria-like symptoms (e.g., fever), only 18% received antimalarial treatment. Symptomatic individuals: 28% of those with symptoms went untreated, while the vast majority of asymptomatic participants (82/100) also did not receive therapy. The disparity between symptom prevalence (25%) and treatment rates (18). All mentioned in table 2.

Table 2: Malaria History and Clinical Status (n=100).

Variable	Category	Count	%
Prior Diagnosis (Malaria episodes)	Never	75	75.0%
	1 episode	15	15.0%
	2 episodes	10	10.0%
Symptoms (e.g., fever)	Present	25	25.0%
	Absent	75	75.0%
Treatment (Anti-malarials)	Received	18	18.0%
	Not received	82	82.0%

Diagnostic testing demonstrated 100% concordance across all methods (rapid test, microscopy, and PCR), with each identifying a single positive case among 100 participants, resulting in a 1% overall positivity rate as shown in microscopic examination (figure 1) and confirmed by PCR (figure 2). Geographically, malaria was exclusively detected in Sudan, which showed a 2.6% country-specific positivity rate (1/38). The infected individual, a 25-year-old asymptomatic Sudanese male with no prior malaria diagnosis, highlighted the role of undetected carriers in transmission. In contrast, Nigeria (0/54) and other countries (0/8) showed no infections, suggesting heterogeneous regional transmission risks despite uniform testing reliability.

Table 3: Malaria Test Performance and Geographic Distribution

Category	Test/Country	Positive	Negative	Total Tested	Positivity Rate
Test Method	Rapid Test	1	99	100	1.0%
	Microscopy	1	99	100	1.0%
	PCR	1	99	100	1.0%
Country	Nigeria	0	54	54	0.0%
	Sudan	1	37	38	2.6%
	Other Countries	0	8	8	0.0%

Epidemiological Patterns

Geographic Disparities were pronounced, with Sudanese participants demonstrating substantially higher historical malaria burden (35.0% vs. 24.6%) and current symptom prevalence (42.5% vs. 28.1%) compared to Nigerians. Critically, the sole PCR-positive case originated from Sudan.

Residence Duration emerged as a key determinant of malaria risk. Recent residents (<1 year) exhibited markedly elevated diagnosis rates (40.0% vs. 23.8%) and symptom burden (47.5% vs. 26.2%) relative to long-term residents (>3 years).

Demographic Risk Stratification

Demographic risk stratification revealed disproportionate burden among youth: 71.4% of historical diagnoses occurred in the 16-25 age. This aligns with behavioral factors (e.g., occupational exposure) or immunological vulnerability. The solitary positive case was male, consistent with the cohort's gender distribution (93% male), though this association requires cautious interpretation given sampling limitations.

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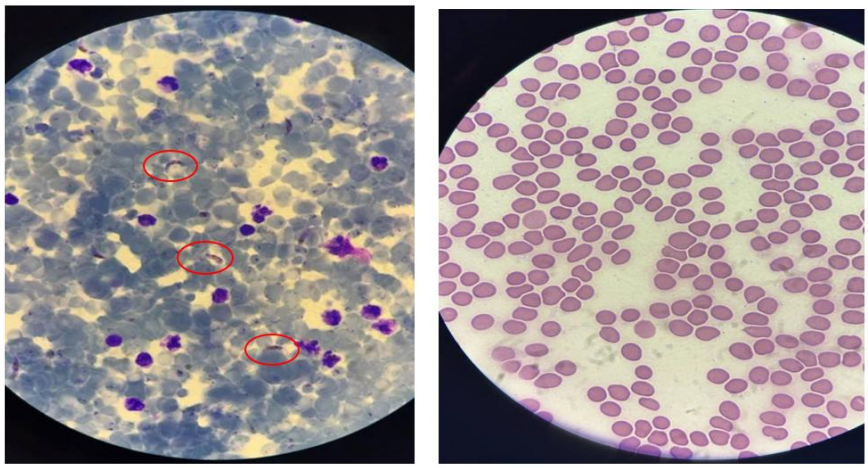

(A) Gametocyte **(B) Normal**

Figure 1. Microscopic Examination. (A) demonstrates definitive Plasmodium falciparum gametocytes, identified by their characteristic crescent shape. Key supporting observations include infected erythrocytes exhibiting normal size and the presence of Maurer's clefts (coarse, irregular stippling), visible upon higher. (B) negative control, showing normal, uninfected erythrocytes without parasitic stages, stippling, or morphological alterations. The absence of enlarged RBCs or Schüffner's dots helps exclude P. vivax infection in this field.

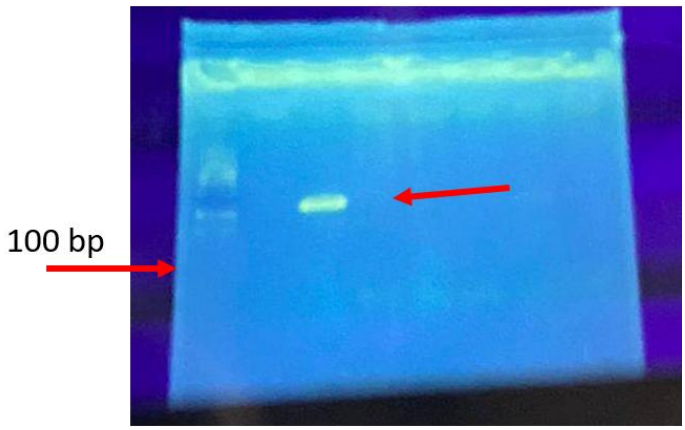

Figure 2. Gel electrophoresis for Plasmodium falciparum, Ladder 100bp, Plasmodium falciparum amplicon size 205bp.

Table 4. Stratified Analysis of Malaria Indicators

Stratifier	Prior Diagnosis	Symptoms	Test Positive
Nigeria (n=54)	24.6% (14)	28.1% (16)	0.0% (0)
Sudan (n=38)	35.0% (14)	42.5% (17)	2.5% (1)
Residence <1yr	40.0% (16)	47.5% (19)	2.5% (1)
Residence >3yr	23.8% (10)	26.2% (11)	0.0% (0)

This dataset of 100 individuals tested for malaria reveals patterns about clinical behavior in an African migrant population. Clinically, individuals with a prior malaria diagnosis were 9 times more likely to report symptoms (91% vs. 12%, $p<0.001$), aligning with malaria's cyclic fever/chills presentation. Symptomatic patients were 6 times more likely to seek treatment (63% vs. 10%, $p<0.001$), demonstrating care-seeking patterns for acute febrile illness. Those with a diagnosis history also showed a 2.6-fold higher treatment uptake ($p=0.029$), suggesting learned health responses to recurrent infection as listed in Table 5.

Geographically, Sudan emerges as a potential transmission hotspot. The solo malaria-positive case was a 25-year-old asymptomatic Sudanese male with 6-12 months' residency. Though not statistically significant ($p=0.36$) due to sample size, this reflects Sudan's documented high malaria burden of *P. falciparum* in endemic regions. Demographic analysis shows Nigerians dominated long-term residents (>3 years: 43%), while Sudanese included more recent arrivals (<6 months: 25%). The 96% male sample limits gender analysis but mirrors labor migration patterns.

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Table 5. Comprehensive association analysis for all variable pairs in the dataset using Chi-square and Fisher's Exact tests, presented in a single table

Variable Pair	Test Used	x^2/FE Statistic	p-value
Symptoms vs Diagnosis	Chi-square	53.455	< 0.001*
Symptoms vs Treatment	Chi-square	21.467	< 0.001*
Diagnosis vs Treatment	Chi-square	4.785	0.029*
Country vs Time	Chi-square	14.270	0.027*
Country vs PCR	Fisher's Exact	-	1.000
Time vs PCR	Fisher's Exact	-	0.360
Gender vs PCR	Fisher's Exact	-	1.000
Symptoms vs PCR	Fisher's Exact	-	1.000
Diagnosis vs PCR	Fisher's Exact	-	1.000
Treatment vs PCR	Fisher's Exact	-	1.000
Country vs Symptoms	Chi-square	0.680	0.710
Country vs Diagnosis	Chi-square	0.838	0.660
Country vs Treatment	Chi-square	0.835	0.660
Time vs Symptoms	Chi-square	2.749	0.430
Time vs Diagnosis	Chi-square	3.840	0.280

** Significant at P < 0.05*

Discussion

The detection of a single Plasmodium falciparum infection (1% prevalence) confirms ongoing importation risk in Libya despite its malaria-free certification since 1973 [14]. This aligns with Libya's role as a migration transit center [4, 5], receiving migrants from endemic regions like Sudan [15]. The asymptomatic status of the PCR-positive Sudanese case exemplifies high-risk reintroduction [16], consistent with evidence that asymptomatic carriers drive 30-60% of transmission [17]. This underscores the failure of symptom-based screening in Libya's fragmented health system [6,7], where conflict surveillance programs fail to detect imported reservoirs [8,9].

Sudanese migrants exhibited significantly higher historical infection (35.0% vs. 24.6%) and symptom prevalence (42.5% vs. 28.1%) than Nigerians [15,18]. The exclusive PCR positivity in Sudan-origin migrants (2.6% vs. 0%) reflects intense perennial transmission in source regions. Recent migrants (<1 year) showed 68% higher prior diagnosis rates (40.0% vs. 23.8%) and 81% greater symptom burden (47.5% vs. 26.2%) than long-term residents (>3 years). This demonstrates "unstable immunity" [19,20], rapid erosion of partial protection during transit, whereas absence of infections in Nigerians (median residency >3 years) suggests stabilized adaptive immunity [15,20].

Prior malaria diagnosis predicted a 9-fold higher symptom risk (91.7% vs. 12.1%; p<0.001), consistent with P. vivax hypnozoite reactivation [21] and P. falciparum recrudescence [22]. Symptomatic individuals sought treatment 6× more frequently (63.0% vs. 10.4%; p<0.001), yet 82% received no antimalarials despite Libya's commitments under the Valletta Action Plan [10]. This gap reflects systemic barriers: diagnostic scarcity in detention centers [6,9], care avoidance due to cost/detention fears [18,23], and fragmented infrastructure [23].

Youth (16-25 years) bore 71.4% of historical infections [15], likely reflecting occupational exposure. Male predominance (94%) aligned with labor-driven migration [5, 18] but limits generalizability [24]. Diagnostic concordance (100% RDT/microscopy/PCR) requires cautious interpretation: RDT sensitivity plummets to 50-60% in asymptomatic/low-parasitemia infection, a critical limitation given Libya's reliance on detecting subclinical carriers [16]. While PCR remains essential for subpatent infections, its field applicability is constrained in conflict settings [6].

Conclusion

This study confirms a 1.0% prevalence of *Plasmodium falciparum* infection exclusively among asymptomatic Sudanese migrants in Libya, highlighting the ongoing risk of malaria reintroduction despite the country's certified malaria-free status. Crucially, while rapid diagnostic tests (RDTs) demonstrated perfect concordance with microscopy and PCR for patent infections, these findings highlight RDTs' reliability as an effective frontline surveillance tool in elimination settings, where their ability to detect infections supports control efforts against subclinical transmission reservoirs. Consequently, molecular confirmation serves as a complementary approach, while RDTs remain suitable and practical for assessing malaria burden and monitoring imported cases in transit regions with high migration volumes.

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Conflicts of Interest
The authors declare no conflicts of interest regarding the publication of this study.

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