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**Proposal:**

# Detections of Malaria Species distribution among Malaria Patients in Dongola Locality, Northern State, Sudan

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# 1. Introduction

## 1.1 Background

Malaria is a mosquito-borne protozoan disease caused by five *Plasmodium* species that infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. It remains endemic across tropical and subtropical Africa, Asia and South America. In 2020 an estimated 241 million cases and 627,000 deaths occurred in 85 endemic countries, 96% of the cases in Africa [1].

Sudan has the highest malaria incidence in the WHO Eastern Mediterranean Region, contributing about 56% of the Region's estimated cases, and reported cases nearly doubled between 2015 and 2019 even as the regional incidence fell [1]. Transmission follows a clear ecological gradient, from hyperendemic desert-fringe states to mesoendemic and hyperendemic areas in the east, south and west, and is shaped by rainfall, irrigation schemes, floods, conflict and population displacement [1].

Species composition matters clinically and programmatically. *P. falciparum* causes most severe disease; *P. vivax* forms dormant liver stages that cause relapses and requires different treatment; mixed infections can be mistreated if one species is missed. In Sudan, *P. falciparum* historically caused more than 95% of cases, but the Sudan Malaria Indicator Survey 2016 reported about 87.6% *P. falciparum* and 8.1% *P. vivax*, with *P. vivax* higher in urban areas (16.9%) than in rural areas (6.7%) and internally displaced persons camps (3.1%) [1]. A multi-state survey of suspected malaria cases found a *P. vivax* prevalence of 26.6% among malaria cases, varying significantly between states and by residence [2]. Hospital-based studies have likewise reported *P. vivax* in 5–27% of confirmed cases [3,4]. Other recent changes include the first report of the invasive vector *Anopheles stephensi* in Sudan in 2019, including in urban areas, and evidence of pfhrp2/3 gene deletions in Sudanese *P. falciparum* that can cause false-negative rapid diagnostic tests [1].

## 1.2 Statement of the problem

Malaria in Sudan is changing: *P. vivax* is increasing and spreading, urban transmission is emerging, and diagnostic tests that rely on a single antigen can miss non-falciparum infections [1,2]. Light microscopy remains the routine, WHO-recommended method for identifying species at the point of care, yet recent Sudanese hospital-based studies of species distribution relied on it alone in small samples of 100 and 300 patients [3,4]. Northern State, a low-transmission state on the River Nile, lacks any current species-level data among confirmed patients. Without it, clinicians and programme managers cannot be sure that first-line treatment, diagnostic choices and *P. vivax*-directed measures match the species that are actually circulating.

## 1.3 Justification

The study addresses an identified knowledge gap between current, species-specific data from malaria patients in Dongola Locality, Northern State, generated by quality-assured light microscopy. Its findings will

(i) show the real share of P. vivax, mixed and other non-falciparum infections, supporting treatment guidance

(ii) document the reliability of microscopic species identification through inter-reader agreement

(iii) identify groups (age, sex, residence, displacement, travel, Blood group) in which particular species concentrate, so that interventions can be targeted

(iv) provide a baseline against which the National Malaria Strategic Plan can be monitored in a setting affected by floods, urbanization, new vectors and recent population movement. The study is feasible, uses standard laboratory methods available in routine facilities

## 1.4 Research questions and hypothesis

## Research questions

- What is the distribution of Plasmodium species (P. falciparum, P. vivax, mixed and other) identified by light microscopy among confirmed malaria patients attending selected health facilities in Dongola Locality, Northern State?
- How well do two independent Medical Laboratories agree on species identification
- Is species type associated with age, sex, Blood group, residence, displacement status, recent travel, season or bed-net use?

## Hypothesis

Null hypothesis (H0): The distribution of Plasmodium species among malaria patients in Dongola Locality, Northern State does not differ by age group, sex, blood group, residence, travel history or season of presentation.

Alternative hypothesis (H1): The distribution of Plasmodium species differs by at least one of these characteristics.

General objective

To determine the distribution of Plasmodium species, identified by light microscopy, among malaria patients attending selected health facilities in Dongola Locality, Northern State, Sudan

## 1.5 Objectives

### General objective

To determine the distribution of *Plasmodium* species, identified by light microscopy, among malaria patients attending selected health facilities in Dongola Locality, Northern State, Sudan, between February and September 2027.

### Specific objectives

**1**. To estimate the proportions of P. falciparum, P. vivax, mixed and other Plasmodium infections among confirmed malaria patients by light microscopy.
2. To assess inter-reader agreement (Cohen's kappa) between the two Medical laboratory scientists on species identification, and the agreement between final microscopy species results
3. To describe species distribution by age group, sex, Blood group,residence, displacement status and month/season of presentation.
4. To determine factors associated with P. vivax infection compared with P. falciparum infection.
Methodology

## 1.6 Operational definitions of terms used

| Term | Operational definition in this study |
|---|---|
| Suspected malaria case | A patient presenting with fever (axillary temperature ≥37.5 °C) or history of fever in the preceding 48 hours, with or without chills, headache or sweating. |
| Confirmed malaria case | A suspected case with *Plasmodium* parasites seen on Giemsa-stained blood film (and/or positive rapid diagnostic test) at the facility, who is then enrolled. |
| Species by microscopy (reference) | Species reported by consensus of two blinded microscopists on thin film (third reader resolves discordance). This final result is the study's reference for species identification. |
| *P. falciparum* mono-infection / *P. vivax* mono-infection | Only that species seen on the thick and thin films. |
| Mixed infection | Two or more species seen in the same blood film (e.g., *P. falciparum* + *P. vivax*). |
| Other non-falciparum | *P. malariae* or *P. ovale* alone or in combination. |
| Discordant reading | Species result of reader 1 that differs from that of reader 2 for the same slide. |

| Term | Operational definition in this study |
|---|---|
| Parasite density | Asexual parasites per µL = parasites counted per 200 white blood cells × 8,000 ÷ 200 (assumed 8,000 WBC/µL). Low <1,000; moderate 1,000–9,999; high ≥10,000 parasites/µL. |
| Age groups | <5, 5–14, 15–24, 25–44 and ≥45 years. |
| Residence | Urban or rural according to the patient's home locality as reported and classified by the Northern State administrative definition (Dongola town versus surrounding villages). |
| Displaced person | A patient who reports having left their usual home because of conflict, flooding or other emergency in the last 12 months, or living in a camp/shelter. |
| Recent travel | Overnight stay outside Northern State within 4 weeks before illness onset; destination recorded. |
| Bed-net use | Reported sleeping under an insecticide-treated net the night before the illness. |
| Season | Dry/hot season March–June; rainy season July–November; winter season December–February (according to the transmission calendar described in [1]). |

# 2. Review of literature

## 2.1 Species distribution in Sudan

A national-scale analysis of routine surveillance data from 461 facilities (2017–2019) confirmed *P. falciparum* as the dominant species but estimated that *P. vivax* now accounts for about 10% of cases, higher in eastern and central states and present across the country [1]. *P. falciparum* peaks after the rains (July–November), whereas *P. vivax* peaks between March and May; Khartoum State showed the most stable and highest *P. vivax* transmission in every month [1]. The authors link rising urban *P. vivax* to the arrival of *Anopheles stephensi*, detected in Khartoum, Red Sea, Kassala and Gedarif states [1].

A cross-sectional survey of 1,226 suspected malaria cases at 21 sites in 10 states reported a *P. vivax* prevalence of 26.6% among malaria cases, differing significantly between states and by residence status, and warned that *P. vivax* is rising and spreading to new areas [2]. At Ed-Babiker Health Center in Khartoum State, 33 of 100 suspected cases were positive by microscopy: 24 *P. falciparum* and 9 *P. vivax* (27% of positives), with the 6–15-year group most affected [3]. At El-Mak Nimr Hospital, Shendi, 30% of 300 suspected cases were positive, with *P. falciparum* in 25% and *P. vivax* in 5% (about 17% of positives) [4]. Together these studies show a wide range of *P. vivax* proportions (roughly 5–27%) depending on location, setting, method and period, which is the variation this study aims to describe for Northern State.

## 2.2 Evidence from neighboring and comparable settings

A systematic review of 18 studies from southern Ethiopia, a country bordering Sudan, found a pooled malaria prevalence of 19.2% (95% CI 14.7–23.7) with *P. falciparum* in 56.5%, *P. vivax* in 40.6% and mixed infections in 3.0% of positives, with extreme between-study heterogeneity ($I^2 \approx 99.99\%$) attributed to season, diagnostic method and location; most primary studies used microscopy [9]. In Pakistan, a four-province survey of 801 febrile patients found that, of 707 positive samples, 76% were *P. vivax*, 18% *P. falciparum* and 6% mixed infections, with marked regional variation and the importance of misdiagnosis for chloroquine-based treatment [7]. In Punjab, a study of 16,075 suspected patients found that mixed infections rose from 9.6% by microscopy to 12.7% by PCR, with peaks in summer and post-monsoon and higher infection in males and in the 1–20-year group [8]. In India, a study of 1,412 patients with acute undifferentiated fever found 19% malaria prevalence, with *P. falciparum* in 46%, *P. vivax* in 38%, mixed infections in 11% and *P. malariae* in 5% of speciated cases [6].

## 2.3 Diagnostic accuracy: light microscopy and RDT

Light microscopy of Giemsa-stained thick and thin films is the routine standard for malaria diagnosis and species identification in Sudanese health facilities. It is inexpensive, shows parasite species, stages and density, and needs no specialized equipment, but its accuracy depends on staff skill, stain quality and parasite density. Studies using molecular methods have shown where microscopy is weakest: in a nationwide study of 5,260 symptomatic patients in Ghana, about one third of falciparum infections were submicroscopic, and most *P. malariae* and *P. ovale* infections were missed by microscopy [5]; an earlier Ghanaian community

study found a high rate of misidentification of non-falciparum parasites [10]. In India, microscopy and RDT had sensitivities of 29% and 24%, respectively, against PCR [6]; in Pakistan, mixed infections were often reported as single-species infections [7]. Sudan also faces pfhrp2/3 gene deletions and the absence of a non-HRP2 test that distinguishes *P. falciparum* from *P. vivax* in routine use [1].

These findings do not argue against microscopy; they show that its results depend on reader competence and procedure. This study therefore uses expert, standardised light microscopy as its identification method, with safeguards drawn from earlier protocols [3,5]: two independent, blinded, WHO-trained readers, a third reader to resolve discordance, a defined number of fields before a film is called negative, and re-reading of 10% of slides. An RDT is run on the same sample for comparison, and the study's limitations for low-density and mixed infections are stated openly (section 3.11).

## 2.4 Demographic, behavioural and seasonal factors

Across settings, school-age children and young people and males are often the most affected, explained by outdoor exposure and activity patterns [3,5,8]. Ghanaian data showed *P. falciparum* and *P. ovale* concentrated in children aged 5–15 years and *P. malariae* in those aged 10–20 [5]. In Sudan, *P. vivax* proportions are higher in urban residents, children, males and lower wealth quintiles, and are influenced by population movement, refugees and proximity to endemic neighbouring countries [1,2]. Seasonal timing also affects species mix, which is why sampling across months is advisable [7,9].

## 2.5 Gap in knowledge

Recent Sudanese hospital-based data on species distribution in Northern State are absent, and those from Khartoum State and Shendi are small, microscopy-only [3,4], or from surveys that may predate recent changes in vectors, floods and population movement [1,2]. No recent study combines a sufficiently large facility-based sample, blinded double-reader microscopy with arbitration, and analysis of associated factors in Dongola Locality. This proposal is designed to fill that gap.

# 3. Methodology

## 3.1 Study design

Descriptive, facility-based cross-sectional study.

## 3.2 Study area

public health facilities in Dongola Locality with functioning malaria microscopy services (Dongola Specialized Hospital ،Dongola Military Hospital ،Dongola Police Hospital) And private labs

## 3.3 Study population

**Source and target population:** all patients with confirmed malaria attending the selected facilities during the study period.

**Inclusion criteria**

- Patients of any age with clinically suspected malaria and a positive blood film at the facility.
- Resident in Dongola Locality or Northern State, or visiting but presenting at a study facility

**Exclusion criteria**

**-** Patient too ill to give a sample or provide history and without an available guardian/attendant.
- Refusal of consent or inadequate blood sample (unreadable film).

## 3.4 Sample size

the primary outcome is the proportion of malaria patients with P. vivax. Using the single-proportion formula $n = Z^2 \cdot P\ (1 - P)/d^2$, with an expected proportion P = 0.266 (reported by the multi-state Sudanese survey [2]), 95% confidence (Z = 1.96) and a precision (d) of 7%:
n = \frac {(1.96) ^2 \times 0.266 \times 0.734} {(0.07) ^2} \approx. 153
Because participants are clustered within three facilities, a design effect of 1.5 is applied (n = 230), and 10% is added for incomplete data or unreadable slides, giving 253, rounded to 250 patients. The precision was relaxed from 5% to 7% to suit a low-transmission setting with limited caseload, and the expected proportion (26.6%) comes from a multi-state survey [2]; the true proportion in Northern State may differ. If caseload is lower than expected, enrolment will continue within the study window and the achieved precision will be reported. Estimates for rarer species such as P. malariae and P. oval will be descriptive only

## 3.5 Sampling technique

A multistage approach will be used: (1) Dongola Locality facilities are grouped as urban Dongola or surrounding riverside villages; (2) three facilities are purposively selected on the basis of microscopy capacity, patient volume and access; (3) the 250-patient sample is allocated to facilities in proportion to their malaria caseload and spread across the data collection timetable (4) within each facility, all eligible confirmed patients are enrolled consecutively until the monthly quota is reached. Because facility selection is purposive, results will be generalized to similar facility attenders rather than to the whole population.

## 3.6 Variables

| Type | Variables |
|---|---|
| Dependent (outcome) | *Plasmodium* species by microscopy: *P. falciparum*, *P. vivax*, mixed, other; parasite density. |
| Independent | Age, sex, Blood group, residence (urban/rural), occupation, displacement status, travel history, previous malaria, bed-net use, month/season of presentation, facility |
| Diagnostic | Light microscopy result |

## 3.7 Data collection methods and instruments

### Questionnaire

An interviewer-administered, pre-tested structured questionnaire in Arabic (Appendix A) collects demographic data, blood group,residence, displacement status, travel history, bed-net use, previous malaria and symptoms. Data collectors will be laboratory staff

Laboratory procedures

- Sample collection: after consent for the thick and thin films .
- Slide preparation and staining
- Light microscopy (species identification): films read under a light microscope Species and stage are identified on the thin film, with the thick film used to detect low-density and mixed infections. Parasites are counted against 200 white blood cells for density.

Mixed infection rule: a film is reported as mixed only when two species are each clearly identified by the reader, on the thin film or in both films

**Laboratory procedures**

- **Sample collection:** after consent, a finger-prick (heel-prick for infants) capillary blood sample is taken by a trained laboratory worker for the thick and thin films .
- **Slide preparation and staining:** one thick and one thin film per patient on the same slide; thin film fixed with methanol; both stained with 10% Giemsa (pH 7.2) for 10 minutes, air-dried and coded.
- **Light microscopy (species identification):** films read under a light microscope with the 100× oil-immersion objective. At least 100 oil-immersion fields are examined on the thick film before it is called negative. Species and stage are identified on the thin film, with the thick film used to detect low-density and mixed infections. Parasites are counted against 200 white blood cells for density. Two independent WHO-trained microscopists read each slide blind to each other's result; a third reader resolves discordance in positivity or species (as in earlier Ghanaian and Sudanese protocols [3,5]). Slides are kept for re-reading.
- **Mixed infection rule:** a film is reported as mixed only when two species are each clearly identified by the reader, on the thin film or in both films.

## 3.8 Pilot study and pre-testing

Before fieldwork, the questionnaire and procedures will be pre-tested on 20 patients at a non-study facility, to check clarity, consent flow, slide preparation and staining, and the double-reader workflow. Problems will be corrected and the pilot data excluded from the final analysis.

## 3.9 Data handling and processing

Each participant receives a unique code; names are kept only on the consent form stored separately. Questionnaires are checked daily by the supervisor, coded in the field, and entered into EpiData (or SPSS) by the principal investigator using a data-entry screen with range and skip checks. A second person will re-enter a random 10% to check accuracy. Data cleaning and validation will be completed before analysis.

## 3.10 Data analysis plan

Analysis will be done in SPSS (version 26 or later) or Stata, structured by the specific objectives:

- **Objective 1:** frequencies and percentages with 95% confidence intervals for each species by final microscopy result.
- **Objective 2:** cross-tabulation of reader 1 against reader 2 (before arbitration); Cohen's kappa for inter-reader agreement on species; cross-tabulation of final microscopy against the RDT (*P. falciparum* versus non-falciparum) with kappa, and sensitivity, specificity, positive and negative predictive values of the RDT, with final microscopy as reference.
- **Objective 3:** chi-square or Fisher's exact test for associations between species category and age group, sex, residence, displacement, travel, season and facility.
- **Objective 4:** binary logistic regression (*P. vivax* vs *P. falciparum*) with variables significant at $p < 0.25$ in bivariate analysis; adjusted odds ratios with 95% CI. Model fit by Hosmer–Lemeshow test.
- **Objective 5:** median (IQR) parasite density by species compared with Mann–Whitney or Kruskal–Wallis tests.

Statistical significance will be set at $p < 0.05$ (two-sided). Continuous variables will be tested for normality before choosing tests. Appropriate charts (bar charts for categorical comparisons; box plots for parasite density) will accompany the tables.

## 3.11 Study period and timetable

| Activity | Dates | SEP | | | | OCT | | | | NOV | | | | DEC | | | | JAN | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 |
| 1. Submission of Research Proposals | 20–24 Sep 2026 | | | | | | | | | | | | | | | | | | | | |
| 2. Presentation of Research Proposals | 27 Sep – 01 Oct 2026 | | | | | | | | | | | | | | | | | | | | |
| 3. Data Collection | 04–22 Oct 2026 | | | | | | | | | | | | | | | | | | | | |
| 4. Data Analysis | 25 Oct – 05 Nov 2026 | | | | | | | | | | | | | | | | | | | | |
| 5. Results and Discussion Writing | 08–19 Nov 2026 | | | | | | | | | | | | | | | | | | | | |
| 6. Final Report Writing | 22 Nov – 03 Dec 2026 | | | | | | | | | | | | | | | | | | | | |
| 7. Electronic Submission of Projects | 06–10 Dec 2026 | | | | | | | | | | | | | | | | | | | | |
| 8. Project Evaluation (Judgement) | 13–24 Dec 2026 | | | | | | | | | | | | | | | | | | | | |
| 9. Project Modification (Revision) | 27–31 Dec 2026 | | | | | | | | | | | | | | | | | | | | |
| 10. Project Presentation (Viva) | 03–06 Jan 2027 | | | | | | | | | | | | | | | | | | | | |

## 3.12 Resources required and budget

Resources include trained laboratory staff and nurses at the facilities, light microscopes and laboratory space (provided by the facilities), Giemsa stain, slides and lancets, and a computer with statistical software. The budget below is an initial estimate in Sudanese pounds (SDG) and will be updated with current local prices before submission.

| Item | Cost (SDG) |
|---|---|
| Printing / Questionnaires | 19,000 |
| Blood tubes | 62,000 |
| Syringes, needles & gloves | 70,000 |
| Malaria microscopy consumables | 81,000 |
| CBC | 173,000 |
| Creatinine + Urea + Glucose | 156,000 |
| Transport / Sample storage | 92,000 |
| Transportation / Data collection | 138,000 |
| Data entry / SPSS | 57,000 |
| Report printing & binding | 35,000 |
| Internet / Communication | 25,000 |
| **Subtotal** | **908,000** |
| Contingency 10% | 92,000 |
| **Total** | **1,000,000** |

Funding: self-funded by the investigator, with seeking of institutional or National Malaria Control Programe support for laboratory consumables.

## 3.13 Ethical considerations

- **Approval:** the protocol will be submitted to the SMSB and the Northern State Ministry of Health research ethics committee before any data collection, with permission from each facility.
- **Informed consent:** written informed consent from adults and from parents/guardians of minors, with assent from children 12–17 years; consent form read aloud for those who cannot read (Appendix B). Participation is voluntary and refusal does not affect care.
- **Benefit and risk:** a finger-prick carries minimal risk. All patients receive standard diagnosis and treatment under the national protocol. If the final microscopy reading differs from the facility's initial report (for example a different species or a mixed infection), the result will be communicated to the treating clinician so that treatment can be adjusted.
- **Confidentiality:** coded identifiers; names stored separately under lock; only the research team has access; reports present aggregated data only.
- **Sample and data storage:** blood films used only for malaria species identification, kept in locked storage and destroyed after the study period; electronic data password-protected.

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