

Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Malaria species in Sokoto, Northwestern Nigeria: The truth, the whole truth and nothing but the truth

Agom Daniel Dansy^{*1}, Matthias Gamde Solomon², Ogboi Sonny Johnbull³, Zainab M.³, Murtala Mohamed M.⁴, Jazuli Mohammed⁵, Ibrahim Abdullahi⁵, Muawaya, A.⁵, Sani Gatawa Yakubu⁶, Aliyu, G.B.⁷, Garba Z. G.⁷

Agom Daniel Dansy; Matthias Gamde Solomon; Ogboi Sonny Johnbull; Murtala Mohamed M.; Jazuli Mohammed; Ibrahim Abdullahi; Sani Gatawa Yakubu

- 1- Department of Hematology, School of Medical Laboratory Usmanu Danfodiyo University, Sokoto State, Nigeria.
- 2- Department of Histopathology, Faculty of Health Sciences and Technology, University of Jos, Plateau State, Nigeria.
- 3- Integrated Health Programme Sokoto, Nigeria.
- 4- Directorate of Case Management Sokoto and State Malaria Elimination Agency.
- 5- Ministry of Health Sokoto State, Nigeria.
- 6- Hospital Service Management Board Sokoto, Nigeria.
- 7- Gada Local Government Area, Sokoto State, Nigeria.

ARTICLE INFO

Article history:

Received 24 July 2022

Accepted 24 September 2022

Keywords:

Malaria
Plasmodium falciparum
Plasmodium ovale
Plasmodium malariae
Plasmodium vivax
 Sokoto

ABSTRACT

Background: Malaria is a serious public health problem in many parts of the world, especially in Sub Saharan Africa where Nigeria bears the bulk of the disease. In Nigeria, though *Anopheles gambiae* is the most dominant species across the country, *Anopheles arabiensis* is the most prevalent in the northern part of Nigeria like Sokoto, while the *Anopheles* males are predominant in mangrove coastal zones of the country. There are now known five different species of *Plasmodium* parasites that cause malaria havoc in the globe; *Plasmodium falciparum* (*P. falciparum*), *P. ovale*, *P. malariae*, *P. vivax* and *P. knowlesi* but, out of these species, *P. falciparum* is the most prevalent and virulent that accounts for about 95 % of all malaria infections in Nigeria. The significance of *P. malariae*, *P. ovale* and *P. vivax* are neglected as non- *P. falciparum* parasites, which are currently invisible to most public health authorities in Sokoto and Nigeria at large. **Aim:** Based on those challenges, this study aims to determine this existing *Plasmodium* species in Sokoto and establish the gold standard malaria diagnosis. In our knowledge, this is the first cross sectional laboratory based descriptive study of plasmodium species in Sokoto, North-Western Nigeria. **Result:** Our result showed that *P. falciparum*, *P. ovale*, *P. malariae*, and *P. vivax* are present in Sokoto. **Conclusion:** Based on our finding, we recommend malaria microscopy as the gold standard of malaria evidence based diagnosis.

Introduction

Malaria is a serious public health problem in many parts of the world, especially in Sub Saharan Africa where Nigeria bears the bulk of the disease [1]. Documented evidences abound that

Nigeria accounts for 25% of the malaria burden of the entire African continent [2]. The disease can be severe and can lead quickly to death if untreated [3]. Malaria is a vector borne parasitic disease caused by

Plasmodium species transmitted by infected female anopheles mosquito.

We found that most studies and routine diagnosis report mostly *Plasmodium falciparum* (*P. falciparum*), while other species are not reported. Based on our knowledge, this is the first cross sectional laboratory based descriptive study of *Plasmodium* species in Sokoto, North-Western Nigeria. The objective is to find the truth about the existence of species in Sokoto, North-Western Nigeria in order to strengthen malaria diagnosis and treatments in Sokoto.

Since Sokoto State public health concern continued to echoes malaria threat to the wellbeing and socio-economic standard of the people living in the study population area, the study aimed to investigate the *Plasmodium* species in Sokoto, North-Western Nigeria

Material and Methods

Study area

Sokoto State is one of the most populous states in North-Western Nigeria. It is located at the extreme part of North-Western Nigeria between longitudes 3° and 7° east and between latitudes 10° and 14° North of the equator. It shares borders with Niger Republic to the North, Kebbi State to the South-West and Zamfara State to the East according to Systemic Screening for Behaviour Disorder [4]. The state covers a total land area of about 32,000 square kilometer and it has semi-arid climate and vegetation is largely Sahel Savannah with an annual rainfall of between 500 and 1300 mm and temperature range between 15 and over 40°C during warm days.

Materials and equipments used in the study

- EDTA treated Vacutainer 5 mL capacity (Lavender top), or
- EDTA treated tube of 5 mL capacity, one per donor
- Disposable syringes, 5 mL–10 mL capacity, one per donor
- Sterile syringe needles, 2L or 23 gauge, one per donor
- Latex protective gloves, minimum three per worker
- Sharps container
- Sterile lancets
- Methanol
- Wrapped clean micro-slides; numbers as required
- Cover-slips (sufficient size to cover thick and thin films)

- Mounting media
- Pasteur pipettes
- Micropipettes (Eppendorf type) 2µL–20µL capacity
- Pipette tips
- Templates for thick blood films
- Slide boxes to store 100 slides horizontally and protect from insects and dust
- Desiccators
- Hard plastic boxes with airtight lids to hold dried micro-slides
- Silica gel, active, for airtight boxes or desiccators
- Giemsa stain, in 25 mL–50 mL bottles, decanted from the stock solution
- Absorbent cotton wool
- De-ionized water buffered to pH 7.2
- Slide drying racks
- Timing clock, recording up to 60 minutes
- Binocular microscope fitted with x10 oculars, x40 and x100 objectives, a mechanical stage and an objective marker
- Mains or battery powered microscope lamp
- Immersion oil
- Tally counters, minimum 2
- Electronic calculator
- Record forms
- Pen and pencil
- Staining troughs to hold 20 slides placed back to back
- Measuring cylinder, capacity 100 mL–500 mL
- Measuring cylinder, capacity 10 mL–25 mL
- Flask or beaker—capacity will depend on the amount of stain to be made up [10].

Methodology

A Giemsa stained slides method was used in the study following the National guidelines and WHO SOPs [5].

Five-hundred twenty (52) blood samples from 26 secondary health facilities of 20 randomly selected blood film slides were carefully examined by WHO certified malaria micropists.

Collection of blood samples

A venous EDTA-blood sample was collected by venipuncture or alternative capillary puncture from each individual. After which thin and thick blood smears were prepared following WHO SOPs [6].

Venipuncture procedure

1. The venipuncture site was cleaned with a cotton pad dampened with 70% methanol and allowed to air dry.
2. Vacutainer, or a disposable syringe and 21–23 gauge needle was used to draw 3–5 mL of blood from an antecubital vein of participant.

3. The blood sample was transferred to an EDTA treated tube.
4. The tube was gently inverted a few times to mix blood and EDTA.

Capillary puncture procedure

After recording the patient's details on the form and register, protective latex gloves were worn

1. Holding patient's left hand, palm upward, selected the third finger from the thumb (the big toe was used for infants. The thumbs were not used for adults and children).
The finger was cleaned with piece of cotton wool lightly soaked in 70% alcohol, using film strokes to remove grease and dirt from the ball of the finger. Then the finger was clean with cotton cloth using film strokes to stimulate blood circulation.
2. The ball of the finger was punctured with sterile lancet, using a quick rolling action. Gentle pressure was applied on the finger to express the first drop of blood and then wiped it away with dry piece of cotton wool. It was ensured that the strands of cotton wool remain on the finger do not mix with blood sample collected.
3. Working quickly and handling clean slides only by the edges, collect the blood was followed [10].

Making thick films

One person delivered the measured amount of blood and another followed behind doing the spreading, both on their PPE (Personal Protective Equipment):

- (i) Using the micropipette and fitted tip, we worked quickly to avoid the blood drying, delivered 6 μ L of blood on to each of the indicated areas outlined in the templates. Using the corner of a clean slide, spread the blood evenly to cover all parts of the area of the template.
- (ii) Clots were ensured not to be formed by regular replacement of the pipette tip and spreader.
- (iii) Then, the slide was labeled properly and allowed to air-dry by keeping the slide on horizontal position. Precaution was taken during spreading and drying [6].

Preparation of thin film

After collection of one drop of blood on a clean grease free slide, thin film was made by spreading the blood using a smooth edged slide. Absolute methanol or ethanol was used to fix the thin film. Following steps were taken for fixing the thin film as described [6].

The slide was placed horizontally on a staining rack. A small drop of absolute methanol or ethanol was applied to the thin film. Then the slide was allowed to fix for 1-2 minutes.

Making thin blood films

(i) Working quickly by placing 2 μ L of blood in the centre of the slide on the mid-line of the slide then, spread the film using the edge of another clean slide spreader at an angle of 45° from the horizontal plane. A well-prepared thin blood film was judged by having a smooth tail end and free of vertical lines and holes. The slide was then labeled properly and allowed to evenly air dried and protected from dust and insects [6]. The slide was placed horizontally on a staining rack. A small drop of absolute methanol or ethanol was applied to the thin film. Then the slide was allowed to fix for about 1 minute.

(ii) The fixed slides were allowed overnight in the desiccator or warm cupboard to enhance staining by improving blood-film adherence and staining because, poor adherence affects EDTA treated blood.

Staining the blood films using the slow (3%) method of Giemsa staining

The slide was first placed on a staining rack. Then 10% Giemsa stain having a pH of 7.2 was poured gently on the fixed thin film and de-hemoglobinized thick film until the slide was totally covered. Then the slide was allowed to stain for 10 minutes out of the sunlight. Then the stain was washed with clean buffered water of 7.2 pH. Back of the slide was wiped and placed in a draining rack. The slide was then allowed for air dry [6].

Dried films were packed, face to back, and stored in a desiccator charged with activated silica gel.

Diagnosis by microscopy using thick and thin blood film staining procedure

The thick part of the blood film was examined using 100 x magnifications, and up to 100 fields were checked before considering the slide as negative. The thin film was examined for *Plasmodium* species identification. The slides were examined by two experienced microscopists as the international standard guideline [7].

Validation of blood films by microscopy

Each set of the examined slides was validated by a number of expert microscopists (the term "expert" implies that they have been prequalified and their competency confirmed) and also allowed to

monitoring of both inter-slide and inter-reader consistency.

Ethical considerations

Ethical clearance was obtained from the Ethics and Research Committee of Gada Local Government Council, Ministry of Health Sokoto State. Permission obtained from General Hospital Gada, Hospital services Management Board in Sokoto state, they were informed and consent from each participant was obtained prior to their participation in the study and their privacy issues intentionally were considered.

Ethical clearance code number:
Gad/MoH/PHC/002/Vol.1

Results

Among the 26% positive of the total 520 samples, *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax* species were discovered in Sokoto. They occurred in the following percentage distributions; *P. falciparum* 72%, *P. ovale* 17%, *P. malariae* 7% and *P. vivax* 3%.

Plate 1. *Plasmodium ovale* blood films.

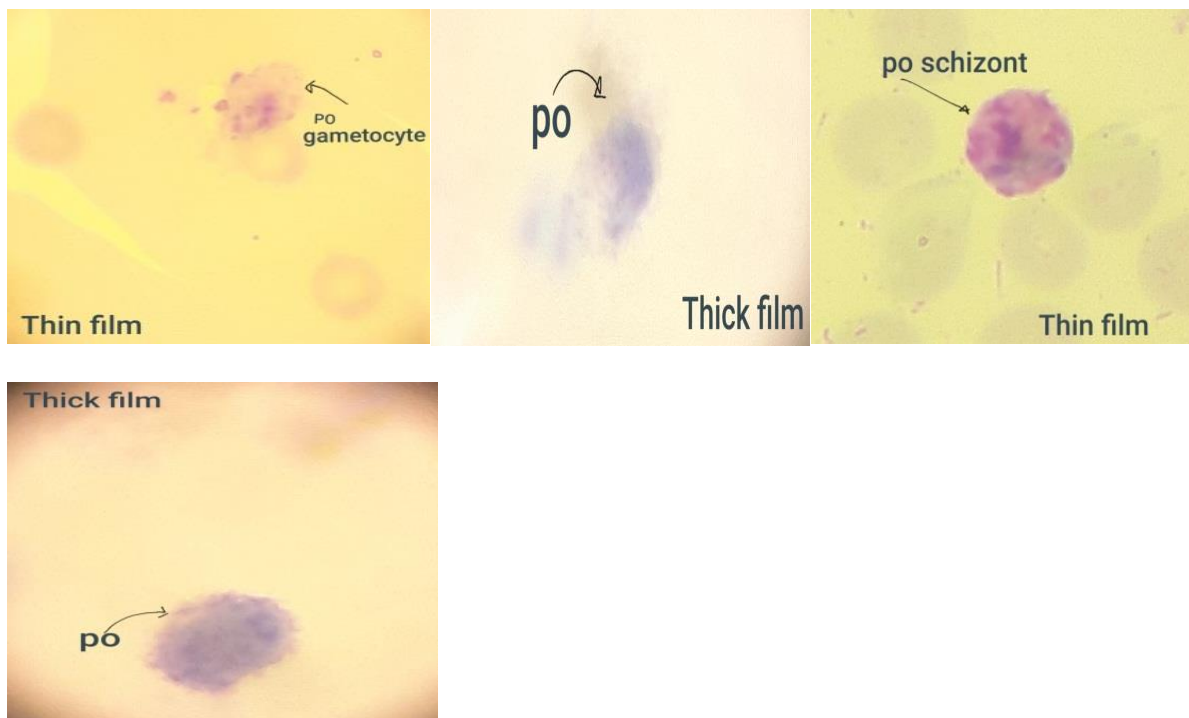
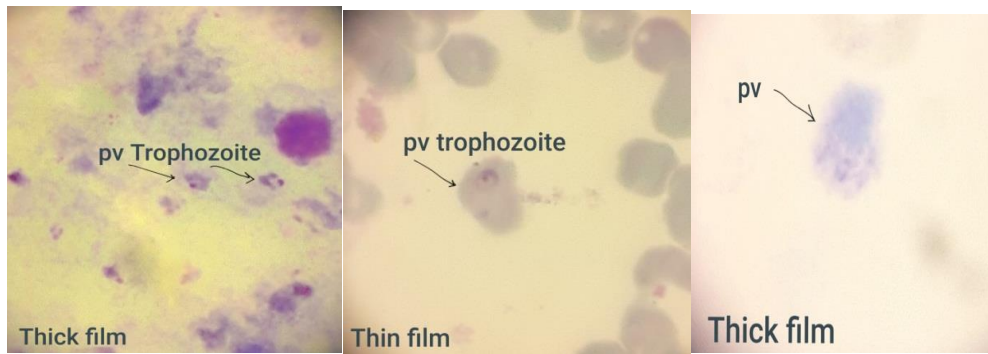
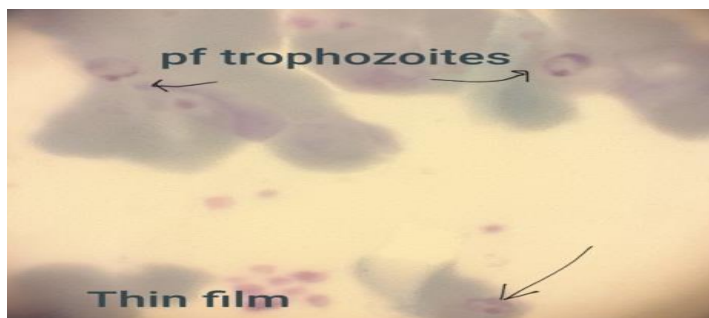


Plate 2. *Plasmodium malariae* blood films



Plate 3. *Plasmodium vivax* blood films**Plate 4.** *Plasmodium falciparum* blood film

Discussion

From our result, *P. malariae*, *P. ovale* and *P. vivax* are present in Sokoto, which clearly point finger at malaria drugs resistance recorded in Sokoto, which can possibly relate to the formerly unknown background existence of the different *Plasmodium* species in Sokoto, Nigeria. The present result is in tandem with the finding of **Abdulraheem et al.** [7] from Southwest Nigeria. This could be as a result of globalization of the populace and migrations across species endemic areas in and out Nigeria [8]. Malaria in Nigeria is principally due to *P. falciparum* and, to a lesser extent is thought that *P. malariae*, *P. ovale* and *P. vivax* to be absent in Nigeria and particularly in Sub-Saharan Africa and Africa in general. It may be due to the near fixation of the Dufy negative gene in the population. Nevertheless, there are frequent reports of *Plasmodium* Dufy negative individuals in the sub-region, including reports from two countries sharing border with Nigeria to the west (Republic of Benin) and east (Cameroon). The distinct pathological features of *P. vivax* parasite and other newly discovered species in Sokoto could be diversity of population movement [9].

Conflict of interest

The authors declare no conflict of interest.

Funding

The study was funded by the contributing authors.

Acknowledgement

I, Dansy D. A. sincerely appreciate Almighty God, all the authors, Sokoto State Ministry of Health, the patients and staff of all the health facilities that made this study successful and fruitful.

Author contributions

Dansy, D. A conceived this study, **Dansy D. A.** and **Gamde S. M** designed the study, **Jazuli, M** and **Ibrahim, A.** collected the samples, **Mutala, M. M** and **Muawaya, A.** performed the laboratory experiments, **Aliyu, G.B** did the data analyses, **Zainab, M, Sani, Y. G** and **Garba, Z. G** edited and proofread the write up. **Dansy, D. A.** drafted the first manuscript, sent to all the authors and they made their contributions and approved the final manuscript without any conflict of interest.

Abbreviations

BF: Blood film

EDTA: Ethylenediaminetetraacetic acid

RDT: Rapid Diagnostic Test

WHO: World Health Organization

References

- 1-**Aisha A, Gamde SM, Abdurrahman AM, Hauwa K, Khadijah AA, Anthony P, et al.** Histopathological and Biochemical Effects of Selected Mosquito Chemical Repellents on Liver of Adulth Wistar Rats. *EC Emergency Medicine and Critical Care* 2020; 4(4): 26 -36.
- 2-**Wondimagegnehu A, Mpazanje R, Ozor L, Abdullahi S.** Investigation of a Suspected Malaria Outbreak in Sokoto State, Nigeria, 2016. *Open Access Library Journal* 2017; 4: e4246.
- 3-**World Health Organization (WHO).** 2019. World malaria report 2019.
- 4-**Abdulraheem MA, Ernest M, Ugwuanyi I, Abkallo H M, Nishikawa S, Adeleke M, et al.** High prevalence of *Plasmodium malariae* and *Plasmodium ovale* in co-infections with *Plasmodium falciparum* in asymptomatic malaria parasite carriers in Southwest Nigeria. *International journal parasitol* 2021; 7519(21): 239-243.
- 5-**Cheesbrough M.** District Laboratory practice. In: *Tropical Countries, Part-1, Low price edition.* Bath press and bounded in at the University press, Cambridge. 1999: 239-258.
- 6-**Oluboyo BO, Enweani IB, Ekejindu IM, Oluboyo AO.** Prevalence of Malaria Parasitaemia among Children Resident in Orphanages in Anambra State, Nigeria. *African journal Biomedical* 2017; (20): 245 – 247.
- 7-**Samwal Haj Osman, Sababil Salih Ali, Alkhair Abd Almahmoud Idris, Romisa Mohamed Ahmed, Aya Mamoun Abdaltaif, Esra Khalid abdalla, et al.** Prevalence of *Plasmodium vivax* species in Elhajyosife area in Shergalnile locality in Khartoum State. *Microbes and Infectious Diseases* 2022; 3(2): 492-499.
- 8-**World Health Organization (WHO).** “Malaria Microscopy Quality Assurance Manual – Version 2. 1.Malaria - diagnosis. 2.Microscopy - standards. 3.Quality control I.” World Health Organization 2015. ISBN 978 92 4 154939 4.
- 9-**World Health Organization (WHO).** “Investing to overcome the global impact of neglected tropical diseases”: World Health Organization 2015. World Health Organization: 2015. Available at: <https://apps.who.int/iris/handle/10665/152781>.
- 10-**James R.** The blood micro sampling; Capillary Blood Collection: Advantages and Disadvantages 2021. Neoteryx