Original Research Article

Molecular Epidemiologyof *Plasmodium falciparum* Infections in Jos, Nigeria usingCross-Sectional PCR-based assays

ABSTRACT

Aim: To validate the prevalence of *Plasmodium falciparum* isolates in the Jos North area of Plateau State, Nigeria.
Study Design: Cross-sectional molecular epidemiological study
Place and Duration of Study: This study was conducted in Jos, Plateau State, Nigeria, where the samples were originally collected between October 2019 and January 2021.
Methodology: After receiving ethical permission, 136 microscopically screened 2 plus (++) and above positive malarial whole blood samples were obtained in EDTA bottles from two hospitals in Jos, Plateau State, Nigeria. The DNA extraction was performed according to the manufacturer's instructions using Zymo Research extraction kits. *Plasmodium genus* and *Plasmodium falciparum* were detected in the samples using the PCR method and gel electrophoresis.
Results: In the results, 47.8% (65/136) of the total malaria-positive samples collected were confirmed for the presence of the *Plasmodium genus* using PCR techniques. Out of these 65 positive samples, 63 were found to be *Plasmodium falciparum*.
Conclusion: This study clearly demonstrates that *Plasmodium falciparum* remains the predominant malaria species in Jos, Plateau State, comprising approximately 96.9% (63/65) of the malarial cases. This indicates that only about 3% of malaria cases affecting the residents of Jos, Plateau State might be caused by the other four species of malaria parasites (*Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi*).

Keywords: Epidemiology,PCR, Plasmodium falciparum,Malaria

1. INTRODUCTION

Malaria, a widespread and life-threatening disease, is caused by various Plasmodium parasites, including *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi*[1]. Out of these, *Plasmodium falciparum* is the most widespread and lethal in Africa [2,3]. Nigeria, the Democratic Republic of the Congo, the United Republic of Tanzania, and Niger account for more than half of all global malaria deaths, underscoring the urgency of implementing comprehensive control measures [3].

Although, between 2005 and 2017, there was a noteworthy reduction in the burden of *P. falciparum* malaria which signifies progress in control efforts [4], however, the battle against malaria is far from being won, as Nigeria still grapples with cases of asymptomatic malaria, hindering effective control measures. These asymptomatic infections contribute to ongoing parasite transmission and pose a significant challenge to malaria control efforts in the country [5].

It is important to know that, one of the complexities in malaria-endemic regions is the genetic diversity of *Plasmodium falciparum* strains, which can lead to the emergence of more virulent or drug-resistant forms of the disease [6]. A study identifying major subpopulations of *P. falciparum* in sub-Saharan Africa, including western, central, and eastern ancestries, as well as a highly divergent Ethiopian population, emphasizes the significance of understanding this diversity [7]. Notably, *P. falciparum* is the deadliest malaria parasite, responsible for over 90% of global malaria-related deaths, highlighting its importance in malaria research and control [8].

Since *Plasmodium falciparum* is the parasite responsible for severe and potentially fatal infections in humans [9], the infections caused by *P. falciparum* are highly likely to progress to severe forms of cerebral malaria which are characterized by symptoms such as seizures, confusion, and coma [10]. Besides cerebral malaria, *P. falciparum* infections can lead to other serious complications, including acute renal failure, where the kidneys cease functioning properly, and severe anaemia, a condition characterized by a lack of sufficient red blood cells to carry oxygen to the body's tissues [10]. Therefore, cerebral malaria, with its severe neurological complications, is one of the most concerning outcomes of *P. falciparum* infection [11].

The *Plasmodium falciparum*'s impact is not confined to its pathogenicity alone but actively evades the host's immune responses by producing and utilizing various parasite-derived molecules throughout its complex life cycle [12]. As a result, malaria's symptoms range from fever and flu-like illness to more severe manifestations such as anaemia, jaundice, and gastrointestinal problems are often felt. If malaria is Left untreated, it could culminate in kidney failure, seizures, mental confusion, coma, and even fatality [9]. The *P. falciparum*'s dominant role in severe and fatal malaria cases underscores the urgency of early diagnosis and timely treatment, particularly for travellers returning from malaria-endemic areas [8].

The battle against malaria continues to be a global health concern, as recent statistics from 2021 revealed approximately 247 million cases of malaria, leading to 619,000 deaths [3]. Although, *Plasmodium falciparum* and Plasmodium vivax are identified as the primary threats in this ongoing battle, but *P. falciparum* is responsible for the majority of malaria-related deaths and is particularly prevalent in Africa, while P. vivax dominates malaria cases in most countries outside of sub-Saharan Africa.

2. MATERIAL AND METHODS

2.1 Sample Collection and Storage

Before DNA extraction, 136 clinically screened malarial samples that tested positive for 2 plus (++) and above were obtained from two hospitals in Jos, Nigeria and placed in EDTA containers.

2.2 DNA Extraction

Prior to DNA extraction, beta-mercaptoethanol was added to the Genomic Lysis Buffer at a 0.5% (v/v) dilution. For each blood sample, 100 µl of whole blood was combined with 400 µl of the prepared Genomic Lysis Buffer in a 4:1 ratio and vortexed for 2 minutes, then incubated at room temperature for 5 minutes. The lysate was transferred to a Zymo-SpinTM IICR column in a collection tube and centrifuged at 10,000 x g for 1 minute. The flow-through and collection tubes were discarded. The column was transferred to a new collection tube, and 200 µl of DNA Pre-Wash Buffer was added and centrifuged at 10,000 x g for 1 minute. The flow-through was discarded again. Next, 500 µl of g-DNA Wash Buffer was added to the column, centrifuged at 10,000 x g for 1 minute, and the flow through discarded. The column was placed in a clean microcentrifuge tube, 70 µl of DNA Elution Buffer was added, incubated at room temperature for 5 minutes, and then centrifuged at 14,000 x g for 30 seconds. Of the eluted DNA, 30 µl was stored at 4°C for immediate use while the remaining 40 µl was stored at -20°C as a backup. This 6-step extraction process was repeated for 36 blood samples.

2.3 Detection of *Plasmodium genus*

To amplify the 18S rRNA gene specific to the *Plasmodium genus*, forward (rPLU5 - 5′-CCTGTTGTTGCCTTAAACT TC-3′) and reverse (rPLU6 - 5′-TTAAAATTGTTGCAGTTAAAACG-3′) primers were utilized. The 25 µL PCR reaction mixture contained 12.5µl of One Tag Quick-load 2X Master Mix, 1µl each of 10µM forward and reverse primers, 5.5 µl of nuclease-free water, and 5 µl of DNA template. The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 2 minutes, and extension at 72°C for 2 minutes; final extension at 72°C for 10 minutes; and hold at 4°C indefinitely. The 1100bp amplified products were visualized by electrophoresis on a 1.5% agarose gel [22].

2.4 Detection of *Plasmodium falciparum*

For the amplification of *Plasmodium falciparum*, 5 µl (95µl) of the Plasmodium species' amplicons were used as template with the forward primer rFAL-1 (5′TTAAACTGGTTTGGGAAAACCAAATATATT-3′) and reverse primer rFAL-2 (5′-ACACAATGAACTCAATCATGACTACCCGTC-3’) [21]. The 25µL PCR reaction mixture contained 12.5µl of One Tag Quick-load 2X Master Mix, 1µl each of 10µM forward and reverse primers, 6.5 µl of nuclease-free water, and 4µl of amplicon template. Cycling conditions were as follows: initial denaturation at 95°C for 10 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes, and extension at 72°C for 2 minutes; final extension at 72°C for 10 minutes; and hold at 4°C indefinitely. The 205-base pair (bp) amplified products were visualized by electrophoresis on a 1.5% agarose gel [22].

3. RESULTS AND DISCUSSION

3.1 Gel Result for *Plasmodium falciparum* Gene

This study provides crucial insights into the prevalence of *Plasmodium falciparum* within in Jos, Plateau State as shown in Figures 1, 2, 3 and 4. Out of the 136 samples shown to be positive for malaria using the microscopic approach, only 47.8% (65/136) were confirmed positive using the PCR technique as shown in Figure 1. This raises questions about the accuracy of diagnostic methods in the study area, highlighting the necessity for improved techniques like PCR, which offer higher sensitivity and specificity in malaria detection.

Furthermore, about 96.9% (63/65) of the *Plasmodium genus* detected in this study as presented in Figure 1 were confirmed as *Plasmodium falciparum* using PCR technique. This high percentage aligns with ongoing challenges faced by countries like Nigeria, where asymptomatic malaria cases persist, and impeding control efforts [5]. As a result, these cases might contribute significantly to continuous parasite transmission, thereby presenting a substantial obstacle to malaria control efforts. This significant presence of *Plasmodium falciparum* in Jos could have serious consequences. First of all, because *P. falciparum* is the deadliest malaria parasite and causes more than 90% of malaria-related fatalities worldwide [9, 8]. This emphasizes the importance of tailoring control measures to specifically target *P. falciparum* and implementing robust strategies to reduce its prevalence. Secondly, the prevalence of *P. falciparum* in the study area highlights the need for enhanced surveillance and early detection programs. This is because, early identification is imperative to prevent the progression of infections to severe and potentially fatal forms, such as cerebral malaria, acute renal failure, or severe anaemia [10].

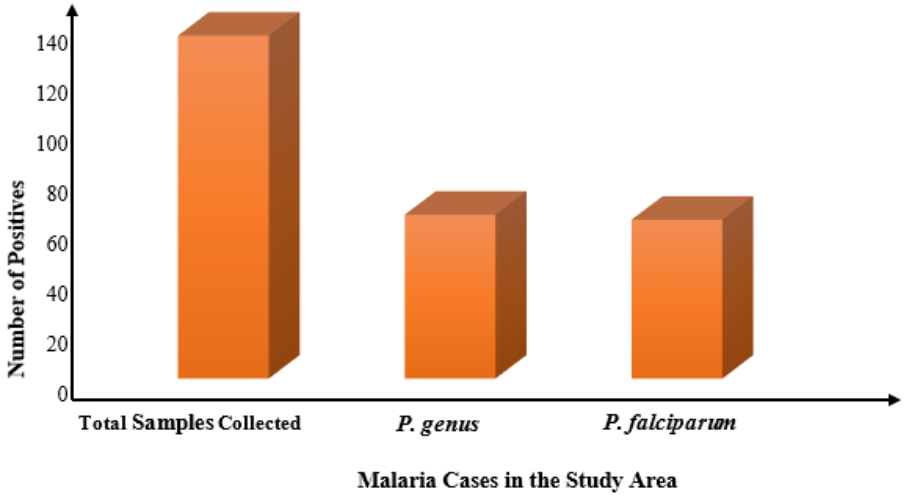

Figure 1 Graphical Distributions of *P. genus* and *P. falciparum* in Jos

Although earlier reports indicated that some successes in lowering the incidence of *P. falciparum* malaria in Nigeria between 2005 and 2017 [4,15], the recent high prevalence of this fatal Plasmodium species in Plateau State emphasizes the need for ongoing and intensive control efforts. This is crucial due to *P. falciparum*'s high prevalence in African countries like Nigeria, the Democratic Republic of the Congo, Tanzania, and Niger [3], necessitating continuous efforts to combat the illness, especially in these hotspot regions. Additionally, considering the variety of malaria complications such as cerebral malaria, acute renal failure, and severe anaemia that can arise from *P. falciparum* infections, early diagnosis and prompt treatment are of paramount importance, particularly for travelers returning from malaria-endemic areas [8]. Therefore, to implement individualized interventions and ensure successful treatments, an understanding of the genetic variety of *P. falciparum* strains found in sub-Saharan Africa has become essential [7]. The small sample size and lack of sequencing of the amplified products of *P. falciparum* for genetic variation analyses are the major limitations of this work.

Out of 136 microscopically positive samples, 65 were PCR positive for the *Plasmodium genus*, with 63 of those confirmed as *P. falciparum*.

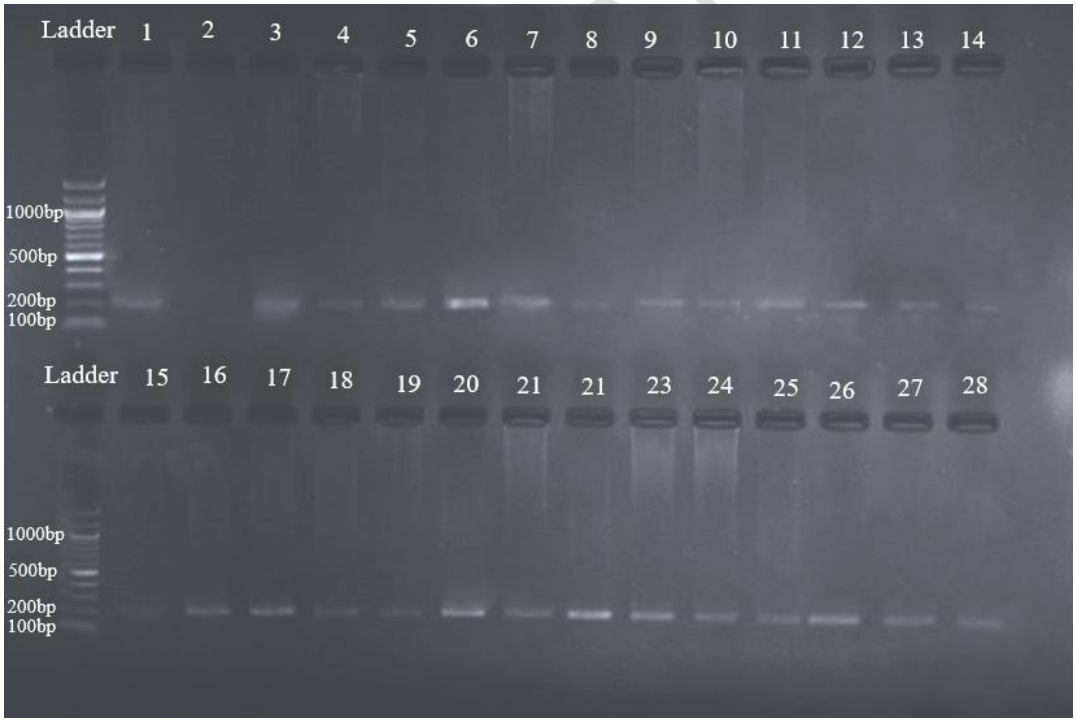

Figure 2 Gel Electropherogram of *P. falciparum* PCR Amplicons (Samples 1-28)
A 100bp ladder was used. Expected band size of 205 bp.

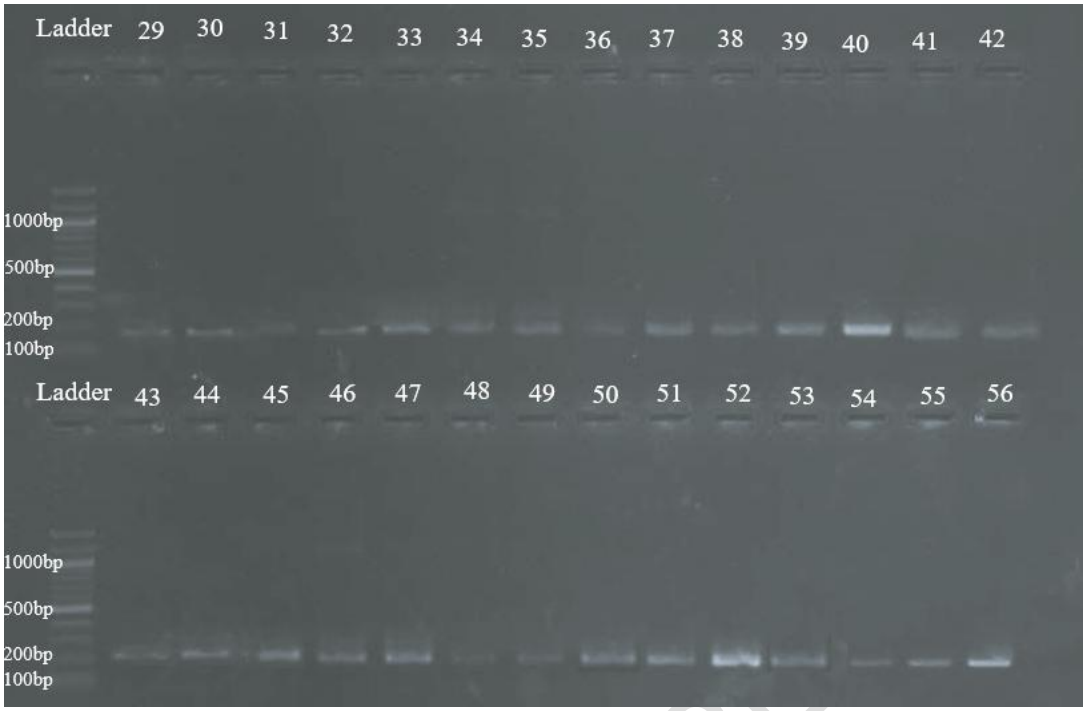

Figure 3 Gel Electropherogram of *P. falciparum* PCR Amplicons (Samples 29-56)
A 100bp ladder was used. The expected band size of 205 bp.

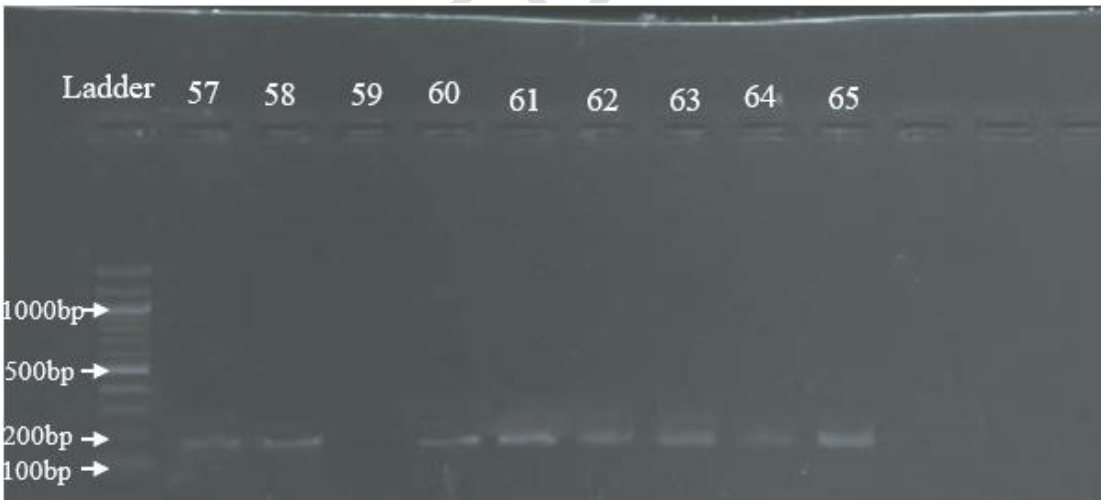

Figure 4 Gel Electropherogram of *P. falciparum* PCR Amplicons (Samples 57-65)
A 100bp ladder was used. The expected band size of 205 bp.

4. CONCLUSION

These findings serve as a reminder of the ongoing challenges of malaria disease caused by *Plasmodium falciparum* in Jos North, Plateau State. Also, the statistics from WHO in 2023 and these findings mutually emphasize the urgency of comprehensive control measures, tailored interventions, and

continued research efforts to mitigate the impact of this deadly disease, especially in regions heavily burdened by malaria. Therefore, the need to understand the prevalence, genetic diversity, and specific complications associated with *P. falciparum* infections remains fundamental in the global fight against malaria.

CONSENT

Informed consent was not required as this study did not involve direct contact with patients.

ETHICAL APPROVAL

This study obtained ethical approval from the Health Research Ethics Committee of the Hospitalsin Jos with numbers: NHREC/21/05/2005/00677 and NHREC/05/01/2010b. All experiments were performed in accordance with relevant guidelines and regulations for research involving human samples.

REFERENCES

1.Cohee, L. M., & Laufer, M. K. (2017). Malaria in Children. Pediatric Clinics of North America, 64(4), 851–866. https://doi.org/10.1016/j.pcl.2017.03.004

2.USAID. (2020). U.S. President's Malaria Initiative. Pmivectorlink.org. https://pmivectorlink.org/about/malaria-burden/#:~:text=Malaria%20is%20a%20preventable%20and,infections%20and%20deaths%20in%20Africa.

3.World Health Organization. (2023, March 29). Malaria. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/malaria

4.Weiss, D. J., Lucas, T., Nguyen, M., Nandi, A., Donal Bisanzio, Battle, K. E., Cameron, E., Twohig, K. A., Pfeffer, D., Rozier, J., Gibson, H. S., Rao, P. C., Casey, D., Bertozzi-Villa, A., Collins, E., Dalrymple, U., Gray, N., Harris, J., Howes, R. E., & Sun Yun Kang. (2019). Mapping the global prevalence, incidence, and mortality of *Plasmodium falciparum*, 2000–17: a spatial and temporal modelling study. The Lancet, 394(10195), 322–331. https://doi.org/10.1016/s0140-6736(19)31097-9

5.Abdulraheem, M. I., Weiss, D. J., et al. (2019). Noteworthy strides have been made in mitigating *P. falciparum* malaria between 2005 and 2017, indicative of advancements in control efforts, although persistent challenges remain, especially in regions like Nigeria where asymptomatic cases hinder comprehensive control strategies. PLoS One, 14(6), e0217980. doi:10.1371/journal.pone.0217980

6.Opute, A. O., Akinkunmi, J. A., Funsho, A. O., Obaniyi, A. K., &Anifowoshe, A. T. (2022). Genetic diversity of *Plasmodium falciparum* isolates in Nigeria. A review. Egyptian Journal of Medical Human Genetics, 23(1). https://doi.org/10.1186/s43042-022-00340-7

7.Amambua-Ngwa, A., Amenga-Etego, L., Kamau, E., Amato, R., Ghansah, A., Lemu Golassa, Randrianarivelojosia, M., Ishengoma, D. S., Apinjoh, T. O., Oumou Maïga-Ascofaré, Andagalu, B., Yavo, W., Marielle Karine Bouyou-Akotet, Oyebola, K. M., Mane, K., Archibald Worwui, Jeffries, D., Simpson, V., Umberto D'Alessandro, & Kwiatkowski, D. (2019). Major subpopulations of *Plasmodium falciparum* in sub-Saharan Africa. Science, 365(6455), 813–816. https://doi.org/10.1126/science.aav5427

8.Zekar, L., & Sharman, T. (2023, August 8). *Plasmodium falciparum* Malaria. Nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK555962/

9.CDC. (2023). Malaria. Centers for Disease Control and Prevention. https://www.cdc.gov/malaria/about/faqs.html#:~:text=Symptoms%20and%20Diagnosis,-What%20are%20the&text=Symptoms%20of%20malaria%20include%20fever,loss%20of%20red%20blood%20cells.

10.CDC. (2023). DPDx - Laboratory Identification of Parasites of Public Health Concern. Centers for Disease Control and Prevention. https://www.cdc.gov/dpdx/malaria/index.html#:~:text=Infections%20caused%20by%20P.,or%20acute%20respiratory%20distress%20syndrome.

11.Idro, R., Marsh, K., John, C. C., & Newton, C. R. (2010). Cerebral Malaria: Mechanisms of Brain Injury and Strategies for Improved Neurocognitive Outcome. Pediatric Research, 68(4), 267–274. https://doi.org/10.1203/pdr.0b013e3181eee738

12.Sakoguchi, A., & Arase, H. (2022). Mechanisms for Host Immune Evasion Mediated by *Plasmodium falciparum*-Infected Erythrocyte Surface Antigens. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.901864

13.Matrevi, S. A., Opoku-Agyeman, P., Quashie, N. B., et al. (2019). *Plasmodium falciparum*kelch propeller polymorphisms in clinical isolates from Ghana: 2007-2016. Antimicrob Agents Chemother. doi:10.1128/AAC.00802-19

14.Lloyd, Y. M., Esemu, L. F., Antallan, J., et al. (2018). PCR-based detection of *Plasmodium falciparum* in saliva using mitochondrial cox3 and varATS primers. Trop Med Health, 46(1), 1-6. doi:10.1186/s41182-018-0100-2

15.Abdulraheem, M. A., Ernest, M., Ugwuanyi, I., Abkallo, H. M., Nishikawa, S., Adeleke, M., Orimadegun, A. E., & Culleton, R. (2022). High prevalence of Plasmodium malariae and Plasmodium ovale in co-infections with *Plasmodium falciparum* in asymptomatic malaria parasite carriers in southwestern Nigeria. International Journal for Parasitology, 52(1), 23–33. https://doi.org/10.1016/j.ijpara.2021.06.003