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MAJOR ARTICLE

Circulation of Non-*falciparum* Species in Niger: Implications for Malaria Diagnosis

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Background. Niger's National Malaria Control Programme and its partners use histidine-rich protein 2–based RDTs, which are specific to *Plasmodium falciparum* diagnosis. This study aimed to screen for the circulation of non-*falciparum* species in Zinder, a region of Niger, West Africa.

Methods. A cross-sectional study was carried out from July to December 2022 at the district hospital of the Zinder region of Niger. *P falciparum* histidine-rich protein 2–based rapid diagnostic tests were performed, and dried blood spot samples were collected for further laboratory multiplexed photo-induced electron transfer–polymerase chain reaction (PET-PCR) analysis on positive light microscopy from all patients with fever who attended the Zinder district hospital during the study period.

Results. In total, 340 dried blood spots were collected and analyzed by PET-PCR. Overall, 73.2% (95% CI, 68.2%–77.9%; 249/340) were positive for *Plasmodium* genus and species and represented the study population. *Plasmodium* species proportions were 89.5% (95% CI, 85.1%–93.1%; 223/249) for *P falciparum*, 38.5% (95% CI, 32.5%–44.9%; 96/249) for *P malariae*, 10.8% (95% CI, 7.3%–15.4%; 27/249) for *P vivax*, and 1.6% (95% CI, .4%–4.1%; 4/249) for *P ovale*. Single infection with *Plasmodium* species counted for 61.8% (95% CI, 55.5%–67.9%; 154/249), and the mixed infections rate, with at least 2 *Plasmodium* species, was 38.1% (95% CI, 32.1%–44.5%; 95/249). Single non-*falciparum* infections represented a rate of 10.0% (95% CI, 6.6%–14.5%; 25/249).

Conclusion. This study confirms the first evidence of *Plasmodium vivax* by PET-PCR in Niger in addition to the other 3 *Plasmodium* species. These findings underline the need to adapt malaria diagnostic tools and therapeutic management, as well as the training of microscopists, for recognition of non-*falciparum* plasmodial species circulating in the country. This will better inform the strategies toward malaria control and elimination, as well as the decision making of the health authorities of Niger.

Keywords. non-*falciparum*; PET-PCR; diagnosis; Republic of Niger; Zinder.

In Niger, malaria is a major public health problem. In 2020, 4 332 909 malaria cases were confirmed, causing 6098 deaths in the country's health facilities [1]. Malaria is the leading cause of morbidity and mortality, accounting for >28% of all illnesses and 50% of all deaths recorded in the country. Niger is among the 6 African countries that together record >50% of malaria cases and deaths globally and 1 of the 11 countries to bear 70% of the global malaria burden. The country accounts for 3.3% of global malaria cases, 2.8% of global malaria deaths, and 6.7% of malaria cases in West Africa [2, 3]. Although malaria is endemic throughout Niger, the national health statistics reports show that the burden is disproportionately higher in the southern part of the country [1, 4].

Four *Plasmodium* species—*P falciparum*, *P malariae*, *P vivax*, and *P ovale*—are generally responsible for malaria in Africa, and *P falciparum* is, by far, the most widespread parasite in the World Health Organization (WHO) African Region, including Niger [5, 6]. Therefore, almost all malaria control and elimination efforts target *P falciparum*. Indeed, malaria diagnosis in peripheral health facilities in Niger is based on the detection of *P falciparum* via *P falciparum* histidine-rich protein 2 (PfHRP2)–based rapid diagnosis tests (RDTs), which are specific to *P falciparum* diagnosis [5]. Light microscopy, which is the gold standard for malaria diagnosis, is available in some health centers and hospitals. It relies on the experience of the technicians, and because of the preponderance of *P falciparum*, microscopists will be less accustomed to the detection of non-*falciparum* species. Therefore, several non-*falciparum* species could escape RDTs and light microscopy [7, 8].

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Several studies carried out in neighboring countries, such as Mali and Burkina Faso (to the west) and Nigeria and Benin (to the south), have confirmed the presence of non-*falciparum* species such as *P malariae*, *P ovale*, and *P vivax* in their territory [9–13]. A study carried out between 2016 and 2018 in Magaria in the Zinder region of Niger reported proportions of parasitemia with mixed infections by light microscopy with *P falciparum* and *P ovale* (2.6%), *P falciparum* and *P malariae* (0.5%), as well as monoinfections by *P ovale* (1.3%) and *P malariae* (0.2%), confirming the presence of these non-*falciparum* plasmodial species in Niger territory except for *P vivax*, which was not yet reported [14].

Since RDTs and light microscopy could miss low-density infections and those due to non-*falciparum* species could be missed by light microscopy, these infections could go undiagnosed and therefore untreated and may lead to severe cases and continued transmission.

Photo-induced electron transfer–polymerase chain reaction (PET-PCR) is a low-cost single PCR technique and an efficient tool for laboratory screening methods for malaria species. It has also demonstrated its ultrasensitivity as compared with RDTs and light microscopy for the rapid detection of *P falciparum* and non-*falciparum* species in the laboratory [15, 16]. Here, PET-PCR was used to determine the frequency of the main *Plasmodium* species (*P falciparum*, *P malariae*, *P ovale*, and *P vivax*) among febrile cases received in the health facilities in Niger.

MATERIALS AND METHODS

Study Region

The Zinder region is in the southeast of Niger, West Africa. It is bordered by the Agadez region to the north, the Diffa region to the east, the Maradi region to the west, and the Federal Republic of Nigeria to the south. It covers an area of 155 778 km^2 and extends in latitude between the parallels 12° 48′ and 17° 30′ north and in longitude between 7° 20′ and 12° 0′ east (Figure 1). The average temperature of the coldest month (January) is 22.3 °C (72.2 °F), and that of the warmest month (May) is 33.9 °C (92.9 °F). Precipitation amounts to 450 mm (17.7 in) per year. It ranges from 0 mm (0 in) in the driest months (January, February, November, December) to 185 mm (7.3 in) in the wettest one (August) [17]. The population of Zinder was estimated in 2016 at 4 132 321 inhabitants, mainly composed of the following ethnic groups: Hausa, Kanuri, Tuareg, Fulani, Toubou, and Arab. The average growth rate is around 3.2%. Agriculture occupies >80% of the population, who utilize nearly 40% of the developed land, which totals 2 937 616 ha. The irrigable potential represents some 18 000 ha [18, 19].

Study Type, Population, and Sample Collection

A cross-sectional study was carried out from July to December 2022 at the district hospital of the city of Zinder. It covered the rainy season (July–September) of high malaria transmission and the post–rainy season (October–December) of low transmission at the study site. Patients with fever visiting the Zinder district hospital were recruited for the study. Patients

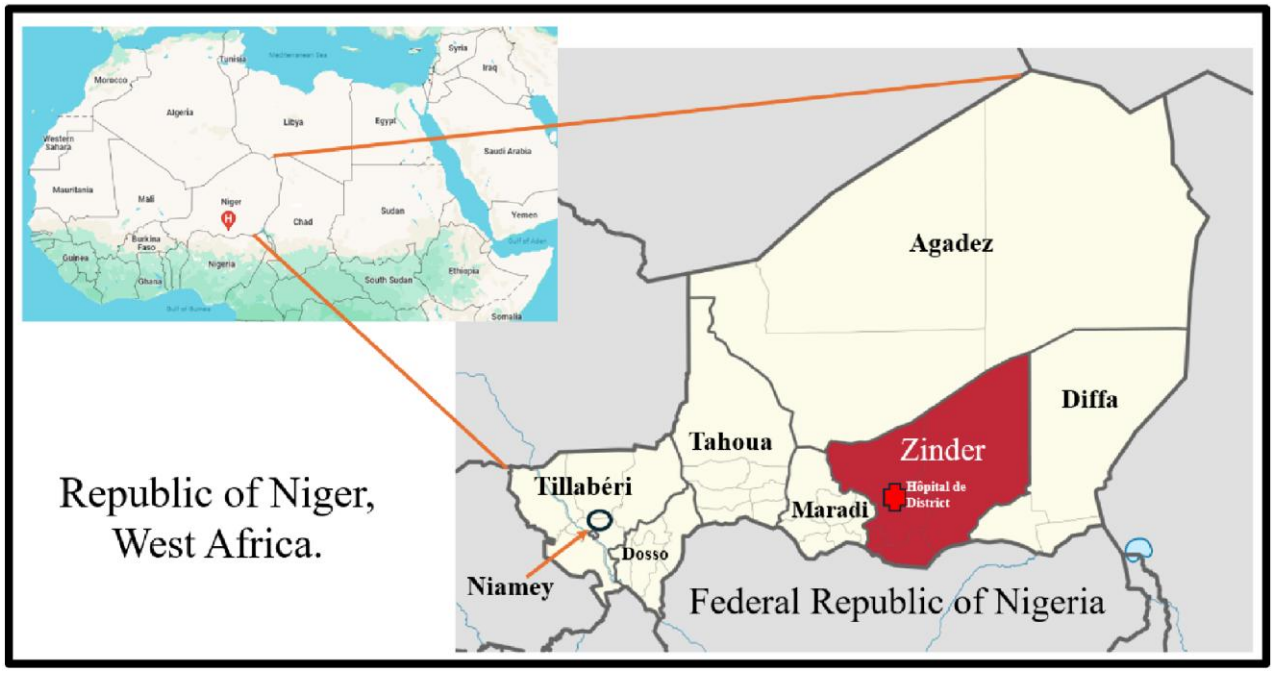

Figure 1. Zinder region, Republic of Niger, West Africa (2024).

presenting with severe malaria were not included in the study. For all patients, PfHRP2 RDTs (SD-BIOLINE Malaria *P falciparum* Ag Test/HRP-2; Standard Diagnostics, Inc) and thick and thin smears for light microscopy diagnosis were performed, and capillary blood was spotted on Whatman filter paper and dried at room temperature (dried blood spots) for further laboratory analysis [20–22].

Malaria Diagnosis

All patients (adults and children) were diagnosed at the Zinder health facility with the PfHRP2 RDTs and light microscopy for confirmation and determination of parasite density for positive samples. The limits of detection are 100 to 200 parasites/μL for the PfHRP2 RDTs, 20 to 100 parasites/μL for the light microscopy, and <1 parasite/μL for the PCR. Furthermore, all the samples were analyzed by PET-PCR, which consists of using fluorogenic primers for the detection of the genus *Plasmodium* sp and plasmodial species by real-time PCR. Briefly, the multiplex PET-PCR assay consists of the amplification of the *Plasmodium* genus (forward primer: GGCCTAACATGGCTATGACG; reverse primer: FAM-aggcgcatagcgcctggCTGCCTTCCTTAGATGTGGTAGCT) or *P falciparum* (forward primer: ACCCCTCGCCTGGTGTTTTT; reverse primer: HEX-aggcgcatagcgcctggTCGGGCCCCAAAAATAGGAA), which is performed in a 20-μL reaction containing 2× TaqMan Environmental buffer 2.0 (Applied BioSystems), 125 nM each of forward and reverse primers, except for the *P falciparum* HEX-labeled primer, which was used at a 62.5 nM. For each sample, duplicate PET-PCR reactions were run with 2 μL of DNA template used in the PCR reaction with the following cycling parameters: initial hot start at 95 °C for 10 minutes, followed by 45 cycles of denaturation at 95 °C for 10 seconds and annealing at 60 °C for 40 seconds. The correct fluorescence channel was selected for each fluorescently labeled primer set, and the cycle threshold values were recorded at the end of annealing step [15, 16]. In this study, multiplex amplification of DNA from *P falciparum* and the genus *Plasmodium* was carried out with a reaction volume of 20 μL containing 5 μL of DNA template [15, 16]. The analysis was carried

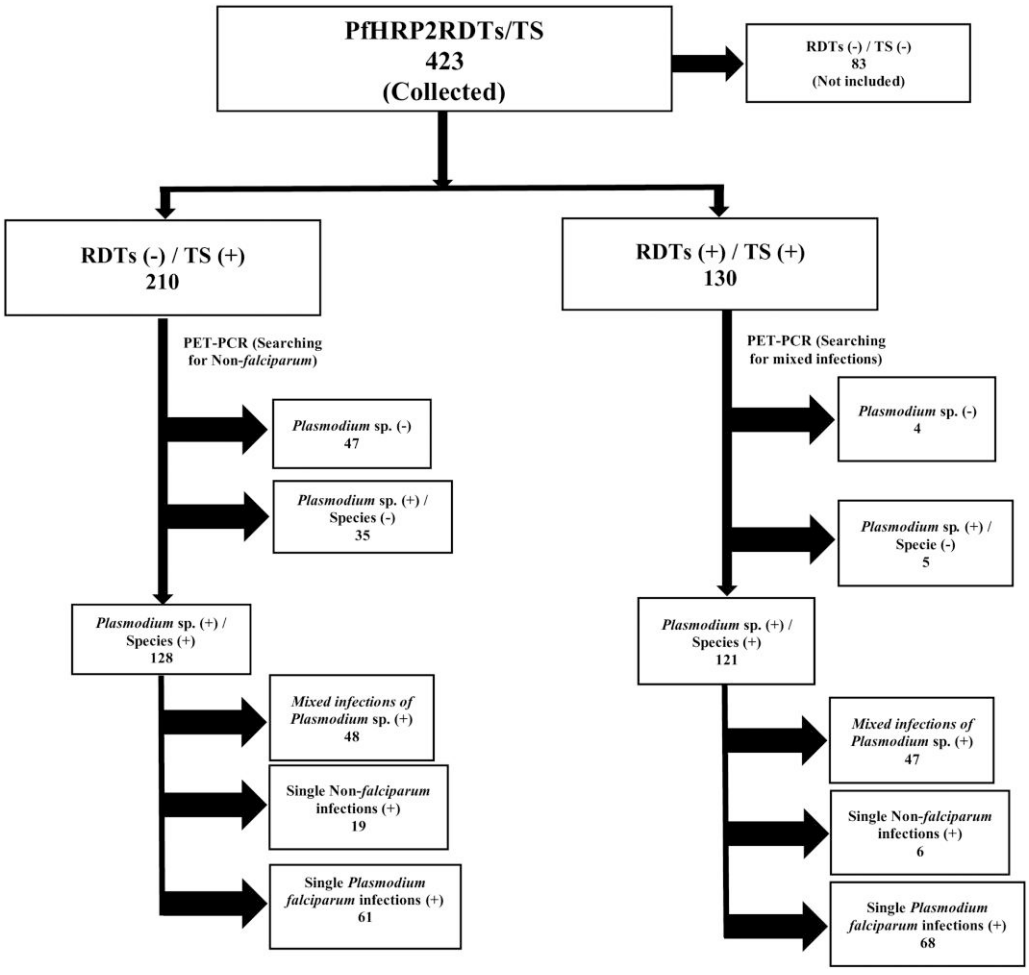

Figure 2. Flowchart of sample processing and malaria infection detection. +, presence; –, absence; PET-PCR, photo-induced electron transfer–polymerase chain reaction; PfHRP2, *P falciparum* histidine-rich protein 2; RDT, rapid diagnosis tests TS: thick/thin smear.

out with QuantStudio 5. Samples with a cycle threshold ≤40 were scored as positive. Distilled water was used as negative template control.

RESULTS

A total of 340 samples were included among the 423 patients with fever (adults and children) received at the Zinder district hospital during the recruitment period; 83 patients presenting a negative light microscopy result were not included in the study, because the study was designed to assess PfHRP2 deletion. Among those included, 61.7% (95% CI, 56.4%–67.0%; 210/340) of samples were light microscopy positive and RDT negative, and 38.2% (95% CI, 33.0%–43.6%; 130/340) were light microscopy positive and RDT positive (Figure 2). The PfHRP2 RDTs and the light microcopy diagnosed only *P falciparum* in this study.

Photo-Induced Electron Transfer–Polymerase Chain Reaction

Among the 340 dried blood spots analyzed with PET-PCR, 73.2% (95% CI, 68.2%–77.9%; 249/340) were positive for *Plasmodium* genus and species and represented the study population. The remaining 26.7% (95% CI, 22.1%–31.8%; 91/340) of samples were light microscopy false positives confirmed by a level 1 WHO microscopist. Among the samples that were RDT negative and thick/thin smear positive, 47 were negative with PET-PCR (35 were PET-PCR positive but failed to amplify for species identification). Among samples positive for RDT and thick/thin smear, 4 were negative with PET-PCR, and 5 were positive for *Plasmodium* genus and failed to identify species (Figure 2).

The overall proportion of *Plasmodium* species was 89.5% (95% CI, 85.1%–93.1%; 223/249) for *P falciparum*, 38.5% (95% CI, 32.5%–44.9%; 96/249) for *P malariae*, 10.8% (95% CI, 7.3%–15.4%; 27/249) for *P vivax*, and 1.6% (95% CI, .4%–4.1%; 4/249) for *P ovale* (Table 1). Single infections for *Plasmodium* species counted for 61.8% (95% CI, 55.5%–67.9%; 154/249), and the mixed infections rate, with at least 2 *Plasmodium* species, was 38.1% (95% CI, 32.1%–44.5%; 95/249; Table 2). Among these single infections, *P falciparum* represented 83.7% (129/154), *P malariae* 11.6% (18/154), *P vivax* 3.8% (6/154), and *P ovale* 0.6% (1/154). The overall single non-*falciparum* infections represented a rate of 10.0% (95% CI, 6.6%–14.5%; 25/249). Of the 95 mixed infections, *Pf/Pm* represented 74.7% (71/95), *Pf/Pv* 15.7% (15/95), *Pf/Pm/Pv* 5.2% (5/95), *Pf/Po* 2.1% (2/95), *Pm/Pv* 1.0% (1/95), and *Pf/Pm/Po* 1.0% (1/95).

Throughout the study period, 61.4% (95% CI, 55.1%–67.5%; 153/249) of the infections were recorded during the month of September 2022 (Figure 3).

Table 1. Overall Proportion of *Plasmodium* Species in the Study Population (n = 249)

Plasmodium sp	% (No.)
P falciparum	89.5 (223)
P malariae	38.5 (96)
P vivax	10.8 (27)
P ovale	1.6 (4)

DISCUSSION

To facilitate appropriate diagnosis of malaria cases, it is important to know the prevalence of circulating malaria species in the country. The objective of this study was to screen for the circulation of non-*falciparum* species among patients with febrile malaria seen in health facilities in Niger. In this study, caried out in the Zinder region of Niger, West Africa, *P falciparum* was the main species with 89.5% (223/249) of malaria infection. In addition to *P falciparum*, *P malariae*, and *P ovale*, which were reported by a recent study carried out in Zinder [14], a substantial proportion of *P vivax* (10.8%, 27/249) was identified for the first time.

All 4 species have already been identified in Mali and Burkina Faso, which are bordering countries to the west, and Nigeria and Benin, which are bordering countries to the south [9–13, 23–27]. The circulation of *P vivax* is now well documented in sub-Saharan Africa among individuals who are Duffy positive [9, 28, 29]. The use of RDTs based on HRP2 detection, which detect only *P falciparum*, and light microscopy, which depends on the operator and requires considerable experience, may explain not only the high proportion of light microscopy false positives revealed by the PET-PCR but also why these species are not

Table 2. *Plasmodium* Species Infections in the Study Population: Overall, Single, and Mixed

Plasmodium sp Infections	% (No.)
Overall	
Single	61.8 (154)
Mixed	38.1 (95)
Total	100 (249)
Single	
P falciparum	83.7 (129)
P malariae	11.6 (18)
P vivax	3.8 (6)
P ovale	0.6 (1)
Total	100 (154)
Mixed	
Pf/Pm	74.7 (71)
Pf/Pv	15.7 (15)
Pf/Po	2.1 (2)
Pf/Pm/Pv	5.2 (5)
Pf/Pm/Po	1.0 (1)
Pm/Pv	1.0 (1)
Pf/Pv/Po	0
Pm/Po	0
Pv/Po	0
Total	100 (95)

Abbreviations: *Pf, P falciparum; Pm, P malariae; Po, P ovale; Pv, P vivax.*

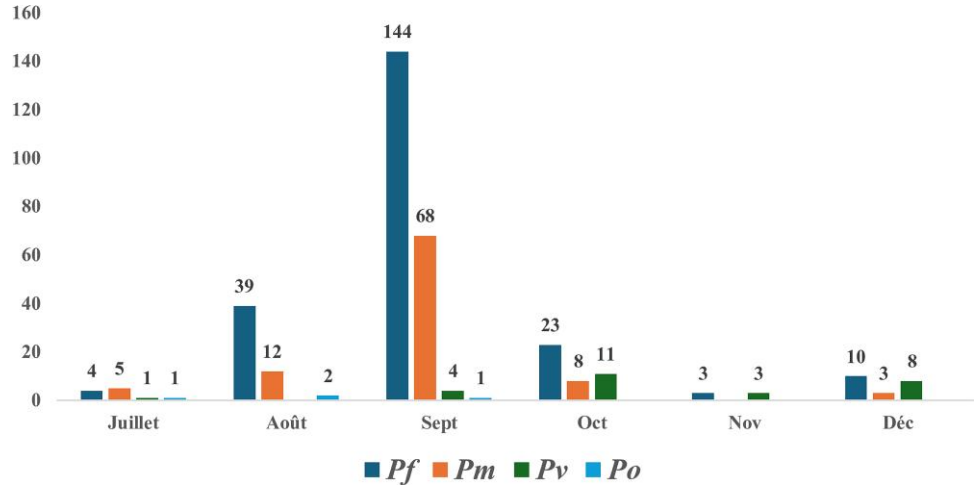

Figure 3. Seasonal distribution of *Plasmodium* species infections by photo-induced electron transfer–polymerase chain reaction. *Pf, P falciparum; Pm, P malariae; Po, P ovale; Pv, P vivax.*

detected or are detected at low frequency in endemic areas of sub-Saharan Africa. In addition, *P falciparum* has higher parasite densities than the other species, which could mask them when identified by light microscopy.

The circulation of *P vivax* has implications for treatment, as hypnozoites are not cleared by artemisinin-based combination therapy and are implicated in relapses, which are estimated to account for 50% to 96% of all posttreatment *P vivax* recurrences in Papua New Guinea, Thailand, and Ethiopia [26, 30–34]. The overall single non-*falciparum* rate doubled the 5% threshold recommended by the WHO, which is considered to be the point where the proportion of cases missed by PfHRP2 RDTs due to non-HRP2 expression may be greater than the proportion of cases that would be missed by the less sensitive *Plasmodium* lactate dehydrogenase (pLDH)-based RDTs [35].

This indicates the need to conduct similar studies in other regions of the country to determine the prevalence of all *Plasmodium* species to update diagnosis and treatment strategies to better control malaria in the country. The results of the seasonal distribution of *Plasmodium* sp have confirmed the seasonal transmission of malaria and its peak observed in September in Niger. This distribution is also observed in several neighboring West African countries [36–38]. Hence, this shows the need for the country's health authorities to adapt control strategies to target this period. A high proportion of infections due to non-*falciparum* species was identified by PET-PCR, showing a failure of light microscopy to detect these infections; therefore, more capacity building is needed for microscopist training, and/or less operator-dependent techniques should be utilized. The PfHRP2 RDTs used in Niger do not detect the non-*falciparum* species. Given the high proportion of these non-*falciparum* infections, the introduction of the combination of *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*'s lactate dehydrogenase (pan-LDH)–only RDTs and combined *P falciparum* pLDH and pan-LDH RDTs or the use of molecular techniques such as loop-mediated isothermal amplification should be considered. The latter is now approved for diagnosis by WHO and commercialized and does not need expertise on molecular biology to be performed [39, 40].

CONCLUSION

This study confirms the first presence of *Plasmodium vivax* by PET-PCR in addition to the other 3 malaria species (*P falciparum*, *P malariae*, and *P ovale*) in the Zinder region of Niger. The high rate of single non-*falciparum* infections slightly above the recommended threshold by the WHO indicates that the country should start planning the substitution of PfHRP2 RDTs with pan-LDH–only RDTs and combined *P falciparum* pLDH and pan-LDH RDTs. These findings underline the need to adapt malaria diagnostic tools and therapeutic management, as well as the training of microscopists, for recognition, detection, and identification of non-*falciparum* plasmodial species circulating in the country. This will better inform the strategies toward malaria control and elimination, as well as the decision making of the National Malaria Control Programme and the health authorities of the Republic of Niger.

Notes

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Author contributions. M. N. G., L. M. M., D. S., A. K., M. L. I., D. N., and A. S. B. designed the study and its setting, organized the sample collection, analyzed the generated data of the study. M. N. G., M. A. D., K. D., M. L. I., I. I., M. K. S., and L. M. M. designed the tables and figures. M. N. G., L. M. M., M. L. I., M. D., K. D., M. A. D., and A. S. B. contributed to the writing of the manuscript.

Consent for publication. All authors read and approved the final manuscript.

Consent to participate. No individual patient consent was obtained because remnant deidentified clinical specimens were used for secondary laboratory analysis.

Data availability. The data supporting the findings of this article are included within the article.

Ethics approval. FSS UASZ Niger No. 014/05/04/2022.

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Potential conflicts of interest. All authors: No reported conflicts.

References

1. US President's Malaria Initiative. Niger Malaria Operational Plan FY 2023. 2023. Available from: https://d1u4sg1s9ptc4z.cloudfront.net/uploads/2023/01/FY-2023.
2. Severe Malaria Observatory. Niger: Charge du paludisme. 2023. Available at: https://www.severemalaria.org/fr/countries/niger-0.
3. World Health Organization. OMS Niger-Rapport Activités 2020. 2023. Available at: https://www.afro.who.int/sites/default/files/2021-04/OMS%20Niger_Rapport%20activit%C3%A9s%202020.pdf.
4. US. Niger malaria operational plan FY 2022. 2023. Available at: https://d1u4sg1s9ptc4z.cloudfront.net/uploads/2022/01/FY-2022-Niger-MOP.pdf.
5. US President's Malaria Initiative. Efficacité des antipaludiques utilisés pour le traitement du paludisme simple à Plasmodium falciparum dans les sites sentinelles d'Agadez, Gaya et Tessaoua—Pmi_Impact_Malaria_Niger_TES_Rapport. 2019. 2023. Available at: https://assets.speakcdn.com/assets/2594/pmi_impact_malaria_niger_tes_rapport.pdf.
6. World Health Organization. World malaria report 2022. 2023. Available at: https://reliefweb.int/attachments/d5c71a5b-dcbc-4dba-b6af-e4abe7d95b9e/9789240064898-eng.pdf.
7. World Health Organization. Malaria rapid diagnostic test performance—results of WHO product testing of malaria RDTs: round 6 (2014–2015). 2023. Available at: https://iris.who.int/bitstream/handle/10665/204118/9789241510035_eng.pdf.
8. Diallo MA, Diongue K, Seck MC, et al. Quality control of malaria microscopy reveals misdiagnosed non-*falciparum* species and other microscopically detectable pathogens in Senegal. Ann Clin Microbiol Antimicrob **2018**; 17:8. doi:10.1186/s12941-018-0261-1.
9. Oboh MA, Badiane AS, Ntadom G, et al. Molecular identification of *Plasmodium* species responsible for malaria reveals *Plasmodium vivax* isolates in Duffy negative individuals from southwestern Nigeria. Malar J **2018**; 17:439. doi:10.1186/s12936-018-2588-7.
10. Herman C, Leonard CM, Uhomoibhi P, et al. Non-*falciparum* malaria infection and IgG seroprevalence among children under 15 years in Nigeria, 2018. Nat Commun **2023**; 14:1360. doi:10.1038/s41467-023-37010-0.
11. Dembele L, Aniweh Y, Diallo N, et al. *Plasmodium malariae* and *Plasmodium falciparum* comparative susceptibility to antimalarial drugs in Mali. J Antimicrob Chemother **2021**; 76:2079–87. doi:10.1093/jac/dkab133.
12. Oguike MC, Betson M, Burke M, et al. *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* circulate simultaneously in African communities. Int J Parasitol **2011**; 41:677–83. doi:10.1016/j.ijpara.2011.01.004.
13. Abdulraheem MA, Ernest M, Ugwuanyi I, et al. High prevalence of *Plasmodium malariae* and *Plasmodium ovale* in co-infections with *Plasmodium falciparum* in asymptomatic malaria parasite carriers in southwestern Nigeria. Int J Parasitol **2022**; 52:23–33. doi:10.1016/j.ijpara.2021.06.003.
14. Coldiron ME, Assao B, Guindo O, et al. Prevalence of malaria in an area receiving seasonal malaria chemoprevention in Niger. Malar J **2021**; 20:419. doi:10.1186/s12936-021-03953-2.
15. Lucchi NW, Narayanan J, Karell MA, et al. Molecular diagnosis of malaria by photo-induced electron transfer fluorogenic primers: pET-PCR. PLoS One **2013**; 8:e56677. doi:10.1371/journal.pone.0056677.
16. Badiane AS, Ndiaye T, Thiaw AB, et al. High prevalence of asymptomatic *Plasmodium* infection in Bandafassi, south-east Senegal. Malar J **2021**; 20:218. doi:10.1186/s12936-021-03746-7.
17. Climates to Travel. World climate guide—climate—Zinder (Niger). 2023. Available at: https://www.climatestotravel.com/climate/niger/zinder.
18. Gifex. Carte de la région de Zinder. 2023. Available at: https://gifex.com/fr/fichier/carte-de-la-region-de-zinder/.
19. Institut National de la Statistique. Niger data portal. 2024. Available at: https://niger.opendataforafrica.org/?lang=fr.
20. World Health Organization. Techniques de base pour le diagnostic microscopique du paludisme, 2e ed part 1. 2014. 2023. Available at: https://apps.who.int/iris/bitstream/handle/10665/164472/9789242547825_fre.pdf?sequence=1&isAllowed=y.
21. World Health Organization. Techniques de base pour le diagnostic microscopique du paludisme, 2e ed part 2. 2014. Available at: https://apps.who.int/iris/bitstream/handle/10665/164472/9789242547917_fre.pdf?sequence=2&isAllowed=y. **2023**.
22. World Health Organization. Consultation regionale sur la strategie technique mondiale 2016-2025 contre le paludisme. 2023. Available at: https://cdn.who.int/media/docs/default-source/malaria/gts/gts-consultation-african-region-brazzaville-march2014-eng.pdf?sfvrsn=1ab6d0cd_8&download=true.
23. Poirier P, Doderer-Lang C, Atchade PS, et al. The hide and seek of *Plasmodium vivax* in West Africa: report from a large-scale study in Beninese asymptomatic subjects. Malar J **2016**; 15:570. doi:10.1186/s12936-016-1620-z.
24. Baird JK. African *Plasmodium vivax* malaria improbably rare or benign. Trends Parasitol **2022**; 38:683–96. doi:10.1016/j.pt.2022.05.006.
25. Williams J, Njie F, Cairns M, et al. Non-*falciparum* malaria infections in pregnant women in West Africa. Malar J **2016**; 15:53. doi:10.1186/s12936-016-1092-1.
26. Boudin C, Robert V, Verhave JP, Carnevale P, Ambroise-Thomas P. *Plasmodium falciparum* and *P malariae* epidemiology in a West African village. Bull World Health Organ **1991**; 69:199–205. http://www.ncbi.nlm.nih.gov/pubmed/1677615
27. Dansy A, Solomon M, Johnbull O, et al. Malaria species in Sokoto, northwestern Nigeria: the truth, the whole truth and nothing but the truth. Microbes Infect Dis **2022**; 4:1059–64. doi:10.21608/mid.2022.152223.1357.
28. Oboh MA, Singh US, Ndiaye D, et al. Presence of additional *Plasmodium vivax* malaria in Duffy negative individuals from southwestern Nigeria. Malar J **2020**; 19:229. doi:10.1186/s12936-020-03301-w.
29. Oboh MA, Oyebola KM, Idowu ET, Badiane AS, Otubanjo OA, Ndiaye D. Rising report of *Plasmodium vivax* in sub-Saharan Africa: implications for malaria elimination agenda. Sci Afr **2020**; 10:e00596. doi:10.4269/ajtmh.23-0523.
30. Viljoen AM, Van Vuuren SF, Gwebu T, Demirci B, Başer KHC. The geographical variation and antimicrobial activity of African wormwood (*Artemisia afra* Jacq) essential oil. J Essent Oil Res **2006**; 18:19–25. doi:10.1080/10412905.2006.12067114.
31. Adekunle AI, Pinkevych M, McGready R, et al. Modeling the dynamics of *Plasmodium vivax* infection and hypnozoite reactivation in vivo. PLoS Negl Trop Dis **2015**; 9:e0003595. doi:10.1371/journal.pntd.0003595.
32. Robinson LJ, Wampfler R, Betuela I, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med **2015**; 12:e1001891. doi:10.1371/journal.pmed.1001891.
33. Abreha T, Hwang J, Thriemer K, et al. Comparison of artemether-lumefantrine and chloroquine with and without primaquine for the treatment of *Plasmodium vivax* infection in Ethiopia: a randomized controlled trial. PLoS Med **2017**; 14:e1002299. doi:10.1371/journal.pmed.1002299.
34. Aftab T, Khan MMA, Ferreira JFS. Effect of Mineral Nutrition, Growth Regulators and Environmental Stresses on Biomass Production and Artemisinin Concentration of Artemisia annua L. In: Aftab T Ferreira JFS Masroor M Khan A Naeem M, eds. Artemisia annua - Pharmacology and Biotechnology: Springer-Verlag, **2014**:157–72. Access date: 2024. doi:10.1007/978-3-642-41027-7_10.
35. World Health Organization. False-negative RDT results and implications of new reports of P falciparum histidine-rich protein 2/3 gene deletions. 2017. Available at: https://www.who.int/docs/default-source/documents/publications/gmp/false-

negative-rdt-results.pdf?sfvrsn=ec917b72_2#:~:text=Athresholdof5%25was,-sensitivepLDH-basedRDTs. **2023**.
36. Delmont J. Paludisme et variations climatiques saisonnières en savane soudanienne d'Afrique de l'Ouest. Cah Etud Afr **1982**; 22:117–33. https://www.persee.fr/doc/cea_0008-0055_1982_num_22_85_2276. 2023.
37. Ba O. Thèse de Doctorat—Etude de la saisonnalité du paludisme à Plasmodium falciparum en milieu urbain de Bamako 2010. ADHL Mali Home Université des Sciences, des Techniques et des Technologies de Bamako Faculté de Médecine et d'Odontostomalogie. 2023. Available at: https://www.bibliosante.ml/handle/123456789/9254.
38. Direction de la Météorologie Nationale du Niger. Prévisions climatiques saisonnières. 2020. Available at: https://www.meteo-niger.org/content.php?page=131. **2024**.
39. Morris U, Aydin-Schmidt B. Performance and application of commercially available loop-mediated isothermal amplification (LAMP) kits in malaria endemic and non-endemic settings. Diagnostics (Basel) **2021**; 11:336. doi:10.3390/diagnostics11020336.
40. Garba MN, Dème AB, Diongue K, et al. Quality assessment of malaria microscopic diagnosis at the Aristide Le Dantec University Hospital of Dakar, Senegal, in 2020. BMC Res Notes **2024**; 17:68. doi:10.1186/s13104-023-06571-0.