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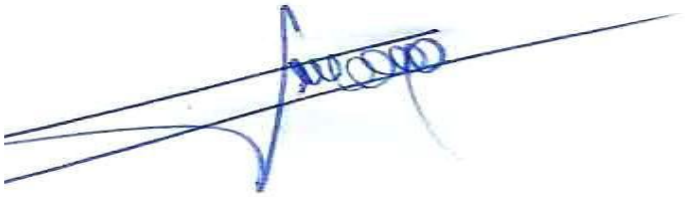
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Affidavit

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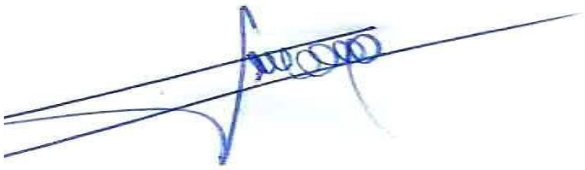
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Liste de publications et participation aux conférences

1) **<u>Liste des publications réalisées dans le cadre de ma thèse (premier auteur)</u>**:

1. **Pascal Dembélé**, Stéphane Ranque. "Factors influencing healthcare provider's adherence to national guidelines for the diagnosis and treatment of malaria cases in sub-saharan Africa". (**Revue, en cours de correction**).

2. **Dembélé, Pascal**, Mady Cissoko, Adama Zan Diarra, Lassana Doumbia, Aïssata Koné, Mahamadou H. Magassa, Maissane Mehadji, Mahamadou A. Thera, and Stéphane Ranque. 2024. "Evaluation of the Performance of Rapid Diagnostic Tests for Malaria Diagnosis and Mapping of Different *Plasmodium* Species in Mali".
International Journal of Environmental Research and Public Health 21, no. 2: 228. https://doi.org/10.3390/ijerph21020228

3. **Pascal Dembélé**, Adama Zan Diarra, Papa Mouhamadou Gaye,, Armel Agokeng, Li Bing, Lassina Doumbia, Mahamadou A Thera and Stéphane Ranque. "Quantitative polymerase chain reaction from malaria rapid diagnostic tests to detect *Borrelia crocidurae* in febrile patients in Mali". (**soumis à *Zoonoses and Public Health)***

4. **Pascal Dembélé**, Manthia Diallo, Papa Mouhamadou Gaye, Mady Cissoko, Mahamadou A Thera and Stéphane Ranque. " Molecular Epidemiology of *Mansonella perstans* in Mali. (**Soumis à Current Research in Parasitology & Vector- Borne Diseases**)

5. **Pascal Dembélé**, Seydou Fomba, Sébastien Cortaredona, Sekou Kouata, Lassina Doumbia, Sory Ibrahima Yoroté, Mady Cissoko, Mahamadou A Thera and Stéphane Ranque. "Adherence to malaria diagnosis and treatment guidelines among healthcare providers in Mali ". (**Soumis à Clinical Epidemiology and Global Health**)

2) Autres publications :

6. Mady Cissoko, Issaka Sagara, Jordi Landier, Abdoulaye Guindo, Vincent Sanogo, Oumou Yacouba Coulibaly, **Pascal Dembélé**, Sokhna Dieng, Cédric S. Bationo, Issa Diarra, Mahamadou H. Magassa, Ibrahima Berthé, Abdoulaye Katilé, Diahara Traoré, Nadine Dessay & Jean Gaudart Sub-national tailoring of seasonal malaria chemoprevention in Mali based on malaria surveillance and rainfall data. https://**doi.org** 10.1186/s13071-022-05379-4

7. Katile A, Sagara I, Cissoko M, Bationo CS, Dolo M, Thera I, Traore S, Kone M, **Dembele P**, Bocoum D, et al. Spatio-Temporal Variability of Malaria Incidence in the Health District of Kati, Mali, 2015–2019. *International Journal of Environmental Research and Public Health*. 2022; 19(21):14361. https://**doi.org**/10.3390/ijerph192114361

2) **Participation aux conférences au cours de la période de thèse** :

1. EPICLI 2023, 17ème conférence d'épidémiologie clinique, Poster, 10-12 mai 2023, Nancy, France
2. Journées InfectioPôle, Poster, 07 Juillet 2023, Marseille, France
3. Journées InfectioPôle, Poster, 5 Juillet 2024, Marseille, France

Résumé

Les maladies vectorielles constituent un problème de santé publique en Afrique en raison de leur morbi-mortalité et de leur impact socio-économique. Plusieurs facteurs, liés notamment à l'environnement, aux ressources économiques et aux comportements, contribuent à maintenir cette situation. Le paludisme est la première maladie parasitaire en Afrique subsaharienne et le Mali est l'un des pays les plus affectés, avec une morbidité de 37,7 % et une mortalité de 24,4 % (DHIS2 2023). Une bonne connaissance de son épidémiologie est nécessaire pour mettre en place des mesures de lutte efficaces. Les espèces de *Plasmodium* responsables de la maladie présentent des différences qu'il est nécessaire de prendre en compte pour mettre en place des mesures de lutte efficaces. *P. falciparum* présente fréquemment des résistances aux antipaludiques utilisés pour son traitement, allant de la chloroquine aux dérivés de l'artémisinine. Le traitement de *P. vivax* nécessite l'utilisation de médicaments actifs sur les hypnozoïtes afin d'empêcher les rechutes. Bien que le paludisme soit la première maladie parasitaire en Afrique, les autres maladies vectorielles ne doivent pas être négligées pour mettre en place des moyens de diagnostic et éviter un traitement inadéquat qui pourrait favoriser l'apparition de résistances aux antimicrobiens.

Nos travaux de recherche nous ont conduits à étudier l'épidémiologie de trois maladies vectorielles (paludisme, borréliose et mansonellose). Après avoir décrit l'état des connaissances sur l'adhésion aux recommandations sur les méthodes de diagnostic et de traitement du paludisme en Afrique subsaharienne, ce travail a essentiellement porté sur l'évaluation des méthodes de diagnostic des maladies fébriles, notamment le paludisme, la borréliose et la mansonellose au Mali.

Dans un second travail, nous avons évalué la performance des tests de diagnostic rapide (TDR) pour le diagnostic du paludisme et cartographié les différentes espèces de *Plasmodium* au Mali. À partir de l'ADN extrait de ces TDR, nous

avons effectué des PCR en temps réel (qPCR) spécifiques des espèces plasmodiales. *P. falciparum* était ubiquitairement distribué au Mali, avec une prévalence des qPCR positives allant de 78,9 % à 100 %, selon les régions. Dans l'ensemble, les TDR présentaient des indices diagnostiques acceptables pour le diagnostic du paludisme à *P. falciparum* ; cependant, leurs performances étaient moindres dans les régions du Nord, où la prévalence de *P. vivax* culminait à 21,1 %.

Dans un troisième travail, nous avons utilisé les mêmes échantillons d'ADN extraits des TDR du paludisme pour détecter les infections zoonotiques par les techniques de biologie moléculaire. L'ADN de *B. crocidurae* a été détecté dans 5 % (9/180) des échantillons collectés dans le district sanitaire de Diéma, situé dans la région de Kayes. Les autres régions ont donné des résultats négatifs.

Dans un quatrième travail, toujours à partir des mêmes échantillons d'ADN extrait des TDR du paludisme, nous avons utilisé des qPCR pour étudier la prévalence des filarioses sanguicoles. L'ADN de *Mansonella perstans* a été détecté dans 1,1 % (2/180) des échantillons dans la région de Koulikoro. La recherche a été négative dans les autres régions du Mali.

Enfin, la cinquième et dernière partie de ce travail consistait à étudier les connaissances, attitudes et pratiques influant sur le respect des directives nationales pour le diagnostic et le traitement du paludisme chez les prestataires de soins de santé et les patients du district sanitaire de Kita. Divers facteurs identifiés, en particulier la disponibilité d'un laboratoire fonctionnel, ont été positivement associés avec l'adhésion aux directives nationales de diagnostic et de traitement du paludisme parmi les prestataires (OR = 6,90 ; intervalle de confiance à 95 % [1,05-47,65]). Concernant les patients, la pratique de l'automédication était significativement plus élevée dans les zones rurales que dans les zones urbaines.

Ces travaux de thèse fournissent des informations précieuses sur l'épidémiologie du paludisme et la dynamique de la transmission au Mali. En outre, les résultats confirment la présence d'agents de la borréliose à fièvre

récurrente et de la mansonellose chez les patients fébriles. Notre étude montre qu'une approche intégrée pourrait être utilisée pour détecter le paludisme et d'autres maladies transmissibles, comme la borréliose, à partir de TDR négatifs pour le paludisme.

Mots clés : Espèces plasmodiales, Test de Diagnostic Rapide, Prévalence, Mansonellose, Borréliose, PCR ; Mali.

Abstract

Vector-borne diseases are a public health problem in Africa because of their high mortality and socio-economic impact. A number of factors, particularly those related to the environment, economic resources and behaviour, contribute to maintaining this situation. Malaria is the most important parasitic disease in sub-Saharan Africa, and Mali is one of the most affected countries, with a morbidity rate of 37.7% and a mortality rate of 24.4% (DHIS2 2023). A good understanding of its epidemiology is essential if effective control measures are to be implemented. The *Plasmodium* species responsible for the disease differ in ways that need to be taken into account if effective control measures are to be implemented. *P. falciparum* often develops resistance to the antimalarial drugs used to treat it, ranging from chloroquine to artemisinin derivatives. Treatment of *P. vivax* requires the use of drugs active against hypnozoites to prevent relapses. Although malaria is the leading parasitic disease in Africa, other vector-borne diseases must not be neglected in order to establish diagnostic methods and avoid inadequate treatment, which could encourage the emergence of antimicrobial resistance.

Our research has focused on the epidemiology of three vector-borne diseases (malaria, borreliosis and mansonellosis). After describing the state of knowledge on adherence to recommendations for malaria diagnosis and treatment in sub-Saharan Africa, this work focused on evaluating methods for diagnosing febrile diseases, particularly malaria, borreliosis and mansonellosis in Mali.

In a second study, we evaluated the performance of rapid diagnostic tests (RDTs) for malaria diagnosis and mapped the different *Plasmodium* species in Mali. Using DNA extracted from these RDTs, we performed species-specific real-time PCR (qPCR). *P. falciparum* was ubiquitous in Mali, with prevalence of positive qPCRs ranging from 78.9% to 100%, depending on the region. Overall, the RDTs provided acceptable diagnostic indices for the diagnosis of *P. falciparum* malaria;

however, their performance was poorer in the northern regions, where the prevalence of *P. vivax* peaked at 21.1%.

In a third study, we used the same DNA samples extracted from malaria RDTs to detect zoonotic infections using molecular biology techniques. *B. crocidurae* DNA was detected in 5% (9/180) of the samples collected in the Diéma health district, in the Kayes region. Negative results were obtained in the other regions.

In a fourth study, still using the same DNA samples extracted from malaria RDTs, we used qPCR to investigate the prevalence of blood-borne filariasis. *Mansonella perstans* DNA was detected in 1.1% (2/180) of the samples in the Koulikoro region. The test was negative in the other regions of Mali.

Finally, the fifth and final part of this work was to assess the knowledge, attitudes and practices that influence compliance with national guidelines for diagnosis and treatment of malaria among health care providers and patients in the Kita health district. Several identified factors, in particular the availability of a functional laboratory, were positively associated with adherence to national guidelines for malaria diagnosis and treatment among providers (OR = 6.90; 95% confidence interval [1.05-47.65]). Among patients, self-medication was significantly higher in rural than in urban areas.

Our research provided valuable information on the epidemiology of malaria and its regional specificity in Mali. The presence of relapsing fever borreliosis and mansonellosis was also confirmed by our results. Our results suggest that an integrated approach could be used to screen for malaria and other transmissible diseases, such as borreliosis, using malaria-negative RDTs.

Keywords: *Plasmodium* species, rapid diagnostic test, prevalence, Mansonellosis, Borreliosis, PCR; Mali.

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INTRODUCTION

De nombreuses maladies à transmission vectorisée sont provoquées par des arthropodes hématophages qui assurent une transmission des agents pathogènes. Les arthropodes sont le groupe d'animaux le plus diversifié [1], ils transmettent des maladies parasitaires , bactériennes ou virales [2]. Avec une incidence près de 247 millions de cas recensés dans le monde en 2022, associés à 619 000 décès environ, le paludisme est une parasitose tropicale associée à une morbidité et une mortalité importante [3]. L'Organisation mondiale de la santé (OMS) le classe parmi les maladies prioritaires nécessitant, avec la tuberculose et le VIH/SIDA, une intervention très rapide pour sauver des vies humaines. L'agent pathogène est un hématozoaire Apicomplexa du genre *Plasmodium* qui se transmet par la piqûre de certains moustiques anophèles femelles infectées [3]. Actuellement, cinq espèces de parasites de paludisme sont identifiés comme pathogènes pour l'homme. *Plasmodium falciparum* et *P. vivax* sont les plus dangereux [3].

De nombreux efforts ont été déployés, notamment en Afrique, dans la lutte contre le paludisme avec des progrès importants au cours des dernières années, ce malgré la pandémie du Covid 19 [4]. L'Afrique subsaharienne supporte une charge disproportionnée de morbidité et de mortalité liée au paludisme, représentant 94 % de tous les cas et 95 % de tous les décès dus au paludisme dans le monde [3]. Les symptômes comme la fièvre, les maux de tête, les troubles gastro-intestinaux, les douleurs articulaires et musculaires, les frissons causés par *P. falciparum* sont aspécifiques, ce qui fait qu'il possible de confondre le paludisme avec d'autres maladies fébriles non palustres lorsqu'une confirmation par un examen biologique n'est pas effectué. L'infection peut être simple, ou évoluer très rapidement en paludisme forme grave lorsque la prise en charge est tardive et/ou inefficace.

Le paludisme simple se manifeste cliniquement le plus souvent chez l'homme par une fièvre (température axillaire ≥ à 37,5°C) ou antécédent de fièvre

avec les plus souvent des maux de tête, une faiblesse musculaire, des troubles digestifs, les frissons et des courbatures, confirmé par un examen biologique TDR ou GE/FM) [5]. Le paludisme grave se caractérise par une confirmation biologique (TDR ou GE/FM) avec la présence de *P. falciparum* associé à une ou plusieurs des manifestations cliniques et /ou biologiques comme les troubles de la conscience, une dysfonction rénale, ou encore l'anémie grave [5].

Le Mali est l'un des pays où le nombre de cas et de décès est le plus élevé. Le taux d'incidence du paludisme sur l'ensemble de la population par exemple est passé de 125‰ en 2020 à 172‰ en 2021 [6]. Toutefois, le paludisme demeure une préoccupation majeure de santé publique car elle représente l'un des premiers motifs de consultation dans les formations sanitaires publique avec un taux de morbidité (37,7%) et de mortalité (24,4%) selon le DHIS2 2023 [6]. Les enfants de moins de 5 ans et les femmes enceintes sont les plus touchés [3].

Le paludisme est endémique avec une forte transmission au cours de la saison des pluvieuse dont la durée est variable en fonction des zones éco-climatiques montrant (a) une zone soudano guinéenne à transmission saisonnière longue de 4 à 6 mois ; (b) une zone de transmission saisonnière courte de 3 à 4 mois ;(c) une zone de transmission sporadique voire épidémique couvrant les régions du Nord et certaines des régions du sud comme Koulikoro et Kayes ;(d) des zones de transmission bi ou plurimodale comprenant le delta intérieur du fleuve Niger et les zones de barrage(e) des zones peu propices à l'impaludation particulièrement les milieux urbains comme Bamako et Mopti où le paludisme est hypoendémique [7]. Les principales espèces rencontrées sont *P. falciparum* (plus de 85%), *P. malariae* (10-15%) et *P. ovale* (1%). *P. falciparum* est à l'origine de formes graves et compliquées de paludisme et présente un taux de létalité élevé [7]. Des cas de *P. vivax* ont été documentés au Mali [8,9]. Les principales espèces vectrices sont *Anophèles gambiae* sensu stricto et *Anopheles funestus, Anophèles coluzzii* et *Anophèles arabiensis* [10].

En 2018, la prévalence du paludisme chez les enfants âgés de 6 à 59 mois était de 19 % selon les résultats des TDR. Les prévalences les plus élevées ont été observées dans les régions de Sikasso, Ségou, Mopti, et Koulikoro (30%, 26%, 25% et 22%, respectivement). La prévalence était faible, entre 1 à 10% dans les régions de Kidal, Taoudéni et Tombouctou et 1% dans le district de Bamako. Dans la région du Nord, la prévalence du paludisme à Gao et Ménaka était >10% [7]. En 2021, la prévalence nationale était stable de 19,4 % chez les enfants âgés de 6 à 59 mois [8]. Le poids sanitaire a été un levier important qui a suscité l'engagement des plusieurs décisions politiques telles que la gratuité des TDR pour tous les patients suspectés de paludisme et le traitement gratuit réservé pour chez les enfants de moins de 5ans et les femmes enceintes [5].

Sous le leadership du Programme National de Lutte contre le Paludisme (PNLP), le Mali, avec l'appui de ses partenaires, a entrepris la mise à échelle des stratégies de contrôle du paludisme basées sur les recommandations de l'OMS, notamment : le diagnostic précoce et le traitement précoce et approprié des cas confirmés ; l'utilisation de moustiquaires imprégnées d'insecticide à longue durée (MILD) ; le Traitement préventif intermittent (TPI) à la Sulfadoxine-Pyriméthamine (SP) chez la femme enceinte ; la Pulvérisation Intra Domiciliaire à effet rémanent (PID) ; et la Chimio Prévention du paludisme Saisonnier (CPS) chez les enfants de 3 – 59 mois [5]. Toutes ces mesures ont déjà prouvé leur efficacité sur la diminution de la mortalité et de la morbidité dans les pays endémiques [3]. Il parait indéniable que la prise en charge des cas à travers un diagnostic précis et un traitement efficace est une stratégie essentielle de lutte contre du paludisme.

Deux méthodes de diagnostic biologique des cas suspects sont utilisées au Mali, à savoir l'examen microscopique sanguin (goutte épaisse et frottis minces) et les TDR. La microscopie, bien qu'elle soit fiable, est souvent limitée par sa complexité, la nécessité d'un personnel qualifié et l'accessibilité restreinte dans

les zones reculées. Les tests de diagnostic rapide (TDR), basés sur l'immunochromatographie à flux latéral [11], sont apparus comme un outil précieux pour le diagnostic du paludisme en raison de leur facilité d'utilisation, de la rapidité du résultat, et de leur applicabilité dans des pays à faibles ressources. Plusieurs études ont démontré qu'ils offrent des performances comparables à celle de la microscopie par des experts [11–14].

Suite à l'émergence de la chloroquinorésistance chez *P. falciparum*, constatée dans beaucoup de pays endémiques, la chloroquine a été remplacée en 2005 par des combinaisons thérapeutiques à base d'artémisinine (ACT). Ensuite les TDR ont été introduits pour le diagnostic du paludisme en 2010 selon les recommandations de l'OMS [15,16]. Malgré la publication des directives nationales pour le traitement du paludisme, les pratiques de prise en charge du paludisme révèlent des insuffisances concernant le respect des directives nationales de diagnostic et de traitement dans les établissements de santé publique et privés. Les recherches indiquent que divers facteurs systémiques et comportementaux contribuent à ce problème, ce qui a un impact sur l'efficacité de la prise en charge du paludisme [17,18]. Notre travail est scindé en quatre parties distinctes :

Dans la première partie de ce travail, nous avons d'abord réalisé un état des lieux des connaissances actuelles sur les facteurs qui influencent l'adhésion aux directives nationales de diagnostic et de traitement du paludisme parmi les prestataires de soins en Afrique subsaharienne (**Article 1, partie I)**. Par la suite, nous avons réalisé une étude similaire dans le district sanitaire de Kita, région de Kita. L'objectif de ce travail était d'identifier les facteurs qui influencent l'adhésion aux directives nationales dans le district sanitaire de Kita. En outre, vu l'émergence d'espèces de *Plasmodium* autres que *P. falciparum* dans certaines régions du Nord et au centre du Mali ainsi que la mauvaise perception sur la fiabilité des résultats des TDR chez certains prestataires de soins, nous avons

entrepris une étude sur l'évaluation de la performance des TDR pour le diagnostic du paludisme, combinée à une cartographie des espèces. L'objectif visait à fournir des informations précieuses sur la distribution des différentes espèces de *Plasmodium* au Mali et les performances des tests de diagnostic rapide par rapport à la qPCR (**Article 2, Article 3 partie II).**

Dans la troisième partie du travail, nous avons constaté que l'un des défis majeurs pour les pays africains où le paludisme est hautement endémique est l'absence d'une méthode diagnostique dotée d'un pouvoir discriminant suffisant pour identifier les causes de fièvres d'origine palustres et non palustres. Cela nous a amené à utiliser la PCR en temps-réel à partir des TDR du paludisme, pour détecter l'ADN *Borrelia* spp. chez des patients fébriles au Mali **(Article 4, partie III).**

Enfin, la cinquième et dernière partie du travail, visait à l'utiliser le diagnostic moléculaire à partir des TDR du paludisme pour la détection de l'ADN de *Mansonella perstans* chez les patients fébriles au Mali **(Article 5, partie IV)**

PARTIE I : REVUE DE LA LITTERATURE

A study of the factors influencing healthcare provider's adherence to national guidelines for the diagnosis and treatment of malaria cases in sub-saharan Africa.

ARTICLE 1

Factors influencing healthcare provider's adherence to national guidelines for the diagnosis and treatment of malaria cases in sub-saharan Africa

Pascal Dembélé, Mahamadou Ali Thera and Stéphane Ranque

Factors influencing healthcare provider's adherence to national guidelines for the diagnosis and treatment of malaria cases in sub-saharan Africa

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RESUME

L'Organisation mondiale de la santé recommande de confirmer le paludisme par microscopie ou par un test de diagnostic rapide (TDR) avant le traitement. Le non-respect des directives nationales conduirait à un surdiagnostic du paludisme, et donc une prescription inappropriée des médicaments antipaludiques, exposerait les patients à des coûts de traitement inutiles et mais également aux effets secondaires des médicaments Cette revue narrative vise à d'évaluer le niveau d'adhésion aux directives nationales pour la prise en charge des cas de paludisme et identifier les facteurs affectant leur respect parmi les prestataires de soins de santé en Afrique subsaharienne publiés dans la littérature afin d'optimiser la prise en charge des cas du paludisme.

Une recherche complète portant sur les facteurs affectant l'adhésion des prestataires de soins aux directives nationales du diagnostic et du traitement des cas de paludisme en ASS a été menée à l'aide des bases de données PubMed, ScienceDirect, Google Scholar et Web of Science en utilisant des mots et expressions clés. Seuls les articles publiés entre 2014 et 2024 ont été sélectionnées sur la base de leur pertinence.

Sur un total de 535 articles initialement identifiés après lecture du titre et du résumé, seuls 32 articles pertinents répondants aux critères d'inclusion ont été inclus dans l'analyse quantitative. Les études observationnelles (24) ont examiné les facteurs prédictifs d'observance/non-observance et leurs conséquences. Divers facteurs identifiés au niveau des agents de santé, des patients et des établissements de santé influencent le respect des directives notamment la formation des agents de santé 20/32 (62.5%), la disponibilité des médicaments antipaludiques 15/32 (46.9%), la pression des patients et les attentes du traitement malgré les résultats des tests négatifs 13/32 (40.6%), les perceptions des prescripteurs envers les tests parasitologiques (méfiance dans les résultats des TDR et la microscopie dans

12/32 (37.5%), les symptômes cliniques 10/32 (31.3%), la connaissance des agents de santé 9/32 (28.1%).

Malgré les efforts visant à améliorer la prise en charge des cas de paludisme grâce à la disponibilité de meilleurs outils de diagnostic et de traitement du paludisme, l'Afrique subsaharienne reste confronter à divers défis en matière de respect des directives. Les facteurs potentiels qui compromettent l'adhésion comprennent des insuffisances en matière de formation, la mauvaise perception des prescripteurs envers les tests parasitologiques (méfiance dans les résultats des TDR et de la microscopie), l'approvisionnement irrégulier en produits antipaludiques et diagnostiques des établissements de santé, et l'insuffisance de ressources humaines, la pression des patients.

Pour améliorer le respect des directives nationales et parvenir à l'élimination du paludisme, il est nécessaire de renforcer la surveillance des pratiques des agents de santé par des interventions ciblées telles que la formation pour les prestataires de soins, la supervision, la disponibilité constante des services de diagnostic et de médicaments antipaludiques à moindre coût, la sensibilisation accrue des prestataires de soins de santé et des patients.

Mots clés : adhésion ; directives nationales ; conformité, diagnostic et traitement; paludisme ; Afrique subsaharienne

INTRODUCTION

On estime que le paludisme touche 3,4 milliards de personnes, soit environ la moitié de la population mondiale, dans 85 pays et territoires [1]. Sa prévalence estimée à 249 millions de cas en 2022 a été associée à près de 619 000 décès [1]. Il n'est pas surprenant que la répartition géographique de ces pays se situe principalement dans la région de l'Afrique subsaharienne (ASS), le Nigeria représentant la part la plus importante (29 %), suivi par les 12 % de la République démocratique du Congo, les 5 % de l'Ouganda, les 4 % du Mozambique et les 3,4 % de l'Angola, entre autres [2]. Les enfants de moins de 5 enfants en raison de leur immunité sous-développée et les femmes enceintes restent les couches les plus vulnérables touchées par le paludisme [1]. Les dépenses nationales dans la région africaine de l'OMS consacrées à la prise en charge des cas de paludisme ont connu d'une augmentation substantielle en 2022, avec des investissements de plus de 1,5 milliard de dollars [1]. Cette charge élevée pourrait s'expliquer en partie par le recours tardif des patients à la recherche des soins de santé lié le plus souvent à des obstacles financiers pour faire face aux coûts élevé de certains médicaments et d'autre part, il peut s'agir des aussi erreurs de diagnostic, dont le dysfonctionnement de l'établissement de santé serait à l'origine des difficultés de distinction entre le paludisme et d'autres maladies infectieuses notamment indisponibilité des services des laboratoire, la rupture fréquente des CTA ou des TDR, ou à une mauvaise observance des lignes directrices de traitement recommandé engendrant à une prescription excessive et abusive des médicaments antipaludiques [3].

La chimiorésistance du *Plasmodium falciparum* a été l'une des obstacles observés au traitement des cas de paludisme par suite d'une utilisation inappropriée des antipaludiques par la population et le non-respect des directives « tester et traiter » par les agents de santé [4,5]. Ainsi la chloroquine a été vite remplacée en 2005 en faveur des combinaisons thérapeutiques à base

d'artémisinine (ACT). Afin de relever le défi posé par cette maladie, des directives pour la prise en charge du paludisme ont été formulées à l'échelle nationale et mondiale. La dernière version des directives de traitement du paludisme de l'Organisation mondiale de la santé (OMS) a été publiée en 2015 [6]. Cet ensemble de directives comprend des recommandations pour le diagnostic et le traitement du paludisme simples et graves. Par conséquent, la gestion rapide et efficace des cas de paludisme est donc primordiale pour parvenir à de meilleurs résultats de contrôle et d'élimination du paludisme [6]. Ainsi, il serait bien de rappeler que les prestataires de soins doivent toujours privilégier le diagnostic biologique du paludisme après un examen clinique du patient bien conduit du patient avant toute prescription d'un médicament antipaludique. Cette politique de l'Organisation mondiale de la santé (OMS) visant à passer du traitement présomptif au diagnostic biologique de tout cas suspect de paludisme est en vigueur. En terme clair dorénavant la prescription des ACT est limitée uniquement aux patients ayant un test de paludisme positif et refusée à ceux ayant un résultat négatif [6].

Le respect des lignes directrices de diagnostic et de traitement du paludisme améliore la qualité des soins reçus par les patients et réduit qu'ils soient exposés inutilement aux risques des effets indésirables des médicaments antipaludiques et les échecs thérapeutiques [7]. Cela nécessite une confirmation parasitologique de tous les patients suspects de paludisme soit microscopie ou les tests de diagnostic rapide (TDR). Le test de diagnostic rapide (TDR) a été introduit dans le diagnostic du paludisme en 2010. Il a contribué largement à améliorer le diagnostic biologique du paludisme dans les pays d'Afrique subsaharienne où les ressources sont limitées pour la réalisation d'une microscopie optique de qualité dans les d'établissements de santé [8,9]. Cette approche diagnostique utilise la chromatographie et l'interaction antigène-anticorps pour identifier les antigènes parasitaires, tels que la protéine 2 riche en histidine (HRP-2) ou la lactate

déshydrogénase (pLDH) [10,11]. Les tests de diagnostic rapide (TDR) éliminent le besoin d'électricité ou d'appareils spécialisés et fournissent des résultats en (15 à 20 minutes) [10,11]. La confirmation biologique des parasites permet donc l'utilisation efficace des médicaments antipaludiques et permet d'identifier rapidement d'autres causes de fièvre et de les prendre en charge de manière appropriée [12]. La mise en œuvre d'une thérapie combinée à base d'artémisinine (CTA) et de tests de diagnostic rapide (TDR) a permis d'améliorer considérablement la prise en charge des cas de paludisme.

Néanmoins, l'émergence et la diffusion de la résistance à l'artémisinine dans certains pays de l'Asie comme la sous- région du Grand-Mékong [13], suscitent une surveillance accrue des pratiques pour éviter le recul des progrès récents réalisés dans le contrôle le paludisme dans les pays d'Afrique subsaharienne où le plus grand nombre de cas de paludisme et de décès sont enregistrés à moins que les prestataires de soins de santé ne respectent strictement les directives de diagnostic et de traitement établies [14].

Au-delà de la coordination des activités se rapportant à la lutte contre le paludisme, le (PNLP) dans les pays d'endémie palustre, procède régulièrement à la mise à jour des directives nationales de diagnostic et de traitement du paludisme selon le contexte local puis veille ensuite à leurs stricts respects par les prestataires de soins à tous les niveaux de la pyramide sanitaire.

Toutefois, plusieurs auteurs ont rapporté que dans la plupart des pays d'endémie d'Afrique subsaharienne nombreux facteurs militent contre le respect des directives nationales de traitement du paludisme par les prestataires de soins de santé [15–17]. Cependant, la rupture fréquente des médicaments dans les établissements de santé, la disponibilité des services de laboratoires, des TDR, l'accès aux directives nationales, le coût élevé des CTA et des écarts entre la connaissance et la pratiques des agents de santé ont été des préoccupations majeures rendant difficiles l'exécution correcte des directives [18,19].

Une meilleure compréhension des facteurs liés aux prestataires de soins, aux patients et au système de santé s'avère donc nécessaire pour dresser des interventions concrètes pour une mise en œuvre efficace de la politique de traitement et de diagnostic de la gestion des cas de paludisme. C'est pourquoi il nous a paru pertinent de faire cette revue narrative afin d'explorer l'état des lieux des données disponibles sur l'observance des prestataires de soins en Afrique subsaharienne et étudier les facteurs affectant le respect des directives nationales du diagnostic et du traitement du paludisme. Nous espérons que cette étude apportera donc une valeur ajoutée à l'ensemble des connaissances existantes et fournira des recommandations plus enrichies aux responsables de la mise en œuvre des politiques afin d'améliorer l'adhésion.

METHODES

Recherche et filtrage dans la base de données :

Nous avons effectué des recherches dans PubMed, Google Scholar et Web of Science sur l'adhésion des prestataires de soins aux directives nationales du diagnostic et du traitement des cas de paludisme publiés entre 2014 et 2024 sur la base d'articles pertinents et disponibles en Afrique subsaharienne. En utilisant d'autres moteurs de recherche comme Google scholar, web of science pour élargir la recherche de données, en effet, ces dernières chevauchaient beaucoup avec notre source principale Pubmed.

Une combinaison de mots-clés et de rubriques médicales (termes MeSH) a été utilisée pour identifier les études évaluant les facteurs affectant le diagnostic et le traitement du paludisme.

Les références bibliographiques des études obtenues ont été largement analysées afin de découvrir d'autres recherches pertinentes. Après la suppression des doublons, le titre et le résumé des articles ont été examinés selon les critères d'inclusion et d'exclusion.

Une deuxième sélection a été réalisée sur les articles en texte intégral. Zotero a été utilisé pour gérer, dédupliquer les références et déterminer leur éligibilité. Cette recherche a utilisé les mots clés suivants: (((((((healthcare workers' adherence) OR (healthcare workers compliance)) OR (attitude of health personnel)) AND (malaria*)) AND (guideline treatment OR diagnosis)) AND (sub-saharan africa)).

Les critères d'inclusion pour cette revue comprenaient : les études qui ont été menées uniquement en Afrique subsaharienne. L'Afrique subsaharienne était le lieu d'intérêt en raison du fardeau bien documenté de *P. falciparum* [1]. L'adhésion aux directives faisait référence au respect des directives de traitement basées sur le fait que le prestataire de soins ou l'agent de santé a effectué un frottis sanguin ou un TDR du paludisme avant la prescription [20] ; si le test est positif, il reçoit uniquement un antipaludique recommandé ; et s'il est négatif, il ne reçoit aucune prescription d'antipaludique. Cette étude a défini le traitement approprié comme la prescription d'antipaludiques aux patients TDR positifs et la non-prescription d'antipaludiques aux patients TDR négatifs ; un traitement inapproprié était défini comme la prescription d'antipaludiques à des patients TDR négatifs. Le non-respect des directives signifiait qu'un patient suspecté de paludisme qu'un test de confirmation biologique du paludisme n'avait pas été effectué, le test sanguin était négatif mais on lui a tout de même prescrit un antipaludique ou bien le test sanguin était positif mais aucun antipaludique correct comme la CTA n'a été prescrit [21]. Enfin notre recherche s'est limitée aux études publiées en langues anglaise et française entre 2014 et 2024. Les bases de données ont été consultées pour la dernière fois le 30 juin 2024.

Les critères d'exclusion étaient les suivants : études en dehors de l'Afrique subsaharienne, les revues et les thèses, les articles incomplets ou non détaillés, les études en dehors de la plage de dates et les articles qui n'abordent pas les facteurs affectant l'adhésion aux directives nationales du diagnostic et du traitement des

cas du paludisme. Les titres et les résumés ont d'abord été examinés, et sélectionné les articles complets pertinents en texte intégral.

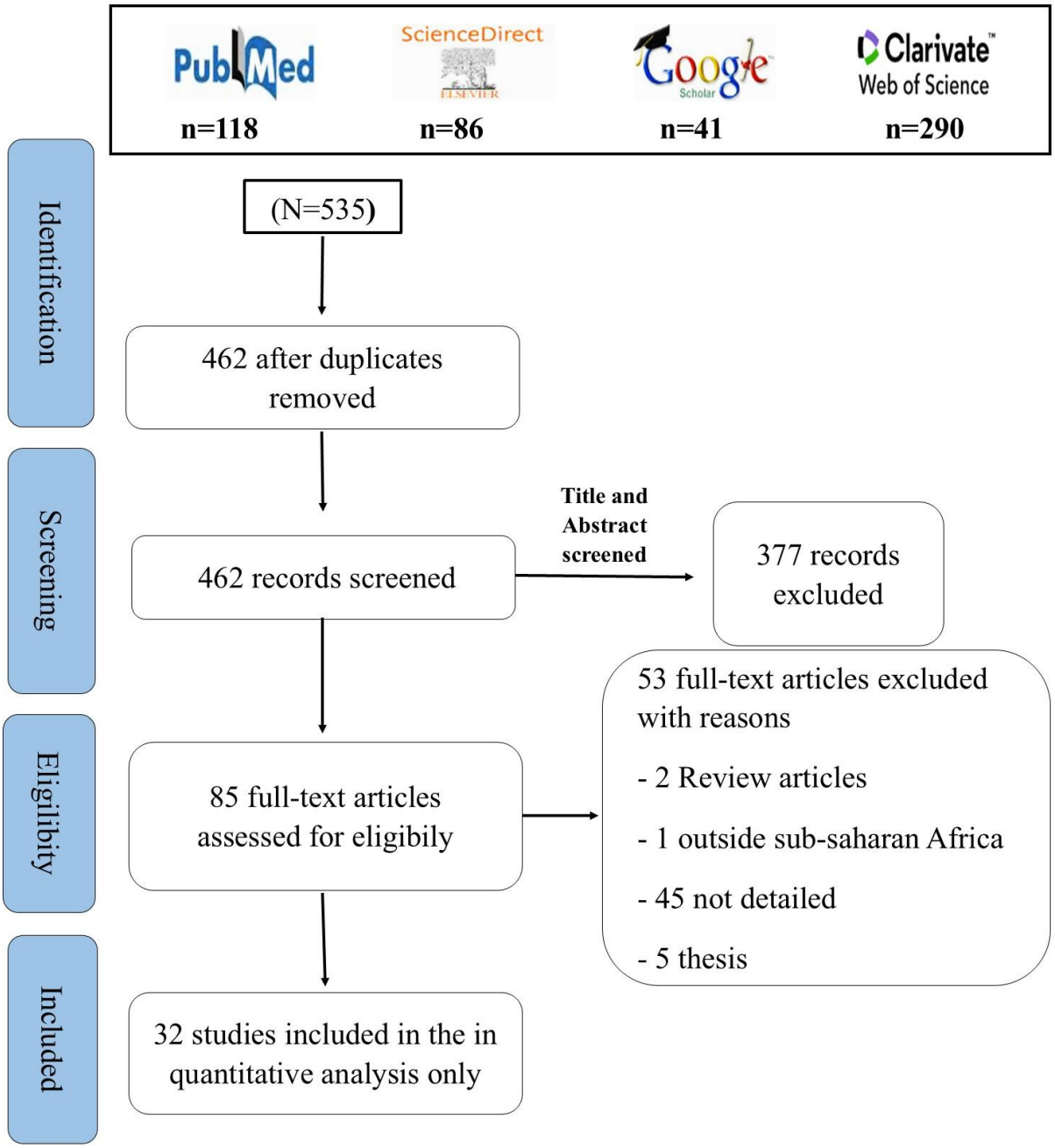
A l'aide de la méthodologie de synthèse narrative, qui utilise une approche textuelle pour résumer les résultats de la revue les différents facteurs contribuant au respect des directives nationales de la prise en charge des cas de paludisme ont été rapportées [22].

Résultats et Discussion

Sélection des études

Les résultats de la recherche nous ont permis d'identifier 535 articles au total, dont 73 étaient en doublons. Sur les 462 titres restants après la suppression des articles en double, 377 titres et résumés ont été exclus. Les articles en texte intégral (85) ont été considérés pour l'éligibilité ; À l'issu du processus de sélection, 32 études ont été finalement incluses dans cette analyse quantitative [14, 16, 17, 19,23–49]. Un diagramme de flux du processus de sélection décrit ci-dessus est présenté dans la **figure** 1.

Les facteurs associés à l'adhésion ont été extraits dans un **tableau 1**. Les données extraites comprenaient le nom de l'auteur, l'année de publication, la population d'étude, le lieu d'étude, le type d'étude, les facteurs identifiés



221

222 **Figure 1.** Study selection flow

223 **Tableau 1** caractéristiques des études incluses

Etude en ligne First authors [Ref]	**Année**	**Pop d'étude**	**Pays**	**Type d'étude**	**Facteurs identifiés**
Mohamoud [16]	2022	Agents de santé et patients	Somalia	Etude transversale	La méconnaissance des directives nationales révisées sur le paludisme, la formation insuffisante des agents de santé, l'approvisionnement insuffisant de tests diagnostiques (RDT et la microscopie) et manque d'accès aux directives, les algorithmes cliniques et de tableaux muraux sur les doses de médicaments dans les établissements de santé, l'absence de supervision et de suivi régulier des agents de santé.
Owolabi Emmanuel,Sokefun[23]	2022	Agents de santé	Nigeria	Etude transversale	Les agents de santé du secteur public ont affiché un taux d'observance plus élevé que les professionnels du secteur privé principalement en raison d'un meilleur accès aux TDR dans les établissements publics Des facteurs tels que le manque de médicaments nécessaires en stock et le manque d'équipement influencent les pratiques de la gestion des cas de paludisme par les professionnels de la santé, le manque de fiabilité perçu des TDR par les praticiens privés a contribué à une faible adhésion
Agbemafle [24]	2023	Prescripteurs et les patients	Ghana	Enquête transversale	L'âge du patient, le nombre de scientifiques de laboratoire qualifiés dans un établissement, la surveillance du PNLP, l'âge et le sexe du prescripteur, la catégorie professionnelle, le nombre d'années de service et la formation
Amboko [17]	2022	Agents de santé et patients	Kenya	Enquêtes nationales transversales	L'endémicité du paludisme, la perception d'une zone à haut risque était également un prédicteur fort augmentant la probabilité de dépistage, Les patients fébriles des établissements appartement à des organisations confessionnelles /ONG, avaient plus de chance d'être testés. Les prescripteurs formés. L'âge du patient, les signes et symptômes cliniques des patients, (la température ≥ 37,5 °C, la plainte de fièvre), la formation continue, la connaissance correcte de la politique de traitement ciblée, les établissements de santé avec 3 scientifiques de laboratoires qualifiés.

Bamiselu [14]	2016	Agents de santé	Nigeria	Etude transversale comparative (établissements de santé public et privé)	La disponibilité des médicaments antipaludiques, la promotion des médicaments par les fabricants chez les prestataires privés, les agents de santé utilisant la méthode de diagnostic, la préférence des patients, le besoin de faire du profit et la demande des patients
Bawate [19]	2016	Agents de santé patients et établissements de santé	Uganda	Etude descriptive et transversale	le niveau de l'établissement, le cadre de l'agent de santé, la présence d'aides-travail dans les salles de consultation, la participation récente à une séance de formation médicale continue sur le paludisme et la présence d'un laboratoire fonctionnel
Bulafu [25]	2023	Agents de santé des établissements privés dans les bidonvilles	Uganda	Etude transversale	Le niveau d'étude (diplômes de l'enseignement supérieur), d'être une infirmière/sage-femme inscrite et une connaissance approfondie des directives de traitement du paludisme, le sexe et l'âge de l'agent de santé, la demande des patients et l'influence marketing des fabricants, La non-référence vers des établissements de santé de niveau supérieur, des facteurs liés aux patients tels que des niveaux élevés de pauvreté et de faibles niveaux de connaissances au niveau des ménages
Gindola[26]	2022	Dossiers médicaux des patients et entretiens avec agents de santé	Ethiopia	Conception de méthode mixte	le faible niveau de connaissance des lignes directrices, insuffisance de techniciens de laboratoire, la disponibilité des médicaments recommandés, disponibilité des fournitures de laboratoire de qualité
Kaula[27]	2018	Prestataires de soins, patients et établissement de santé	Uganda	Etude transversale	suivi d'une formation récente sur les lignes directrices
Khalid Mohamed[28]	2024	Agents de santé	Soudan	Conception de recherche qualitative avec discussion de focus groupe	les ruptures de stock d'antipaludique s et de TDR ; le manque de personnel ; la faible connaissance des protocoles de prise en charge des cas par les agents de santé (manque de formation et une supervision insuffisante) de la méfiance envers la microscopie du paludisme et les TDR ; et la pression des patients pour un diagnostic et un traitement.
Kolekang[29]	2022	Prescripteurs des établissements de santé privés, publics et confessionnels	Ghana	Etude exploratoire	Lacunes en matière de formation et de connaissance (formation inadéquate sur les directives), l'approvisionnement irrégulier des établissements de santé en kits de TDR, réticence des prescripteurs à l'égard des résultats de laboratoires
Moffitt[50]	2023	Patients sortis de l'hôpital régional de référence	Uganda	Etude rétrospective	Rupture des stocks de TDR, les heures de laboratoire de l'hôpital limitées, insuffisance de ressources humaines, pénurie de sang disponible
Arthur Mpimbaza [31]	2022	Agents de santé, patients fébriles et établissements de santé publics et privés	Uganda	Etude transversale	L'augmentation de l'âge du patient, la documentation de la température et le poids du patient, les établissements qui supervisés au cours des 3 derniers mois; disponibilité des services de dépistage du paludisme, les lignes directrices PCIME, les lignes directrices cliniques de l'Ouganda étaient plus

					susceptibles d'assurer prise en charge appropriée des cas de paludisme. Les travailleurs de la santé supervisés pour la gestion des cas de paludisme, le manque de tests et l'absence d'AL dans les établissements publics
Richard Kabaka,Mumali[32]	2023	Agents de santé et patients	Uganda	Etude transversale	Formation continue interrompue en raison de la de la pandémie du COVID-19, des ruptures de stock occasionnelles de médicaments antipaludiques, le bon niveau de connaissance et les compétences du personnel du laboratoire par rapport aux autres cadres.
Aliyu Mamman,Na'uzo[33]	2020	Agents de santé	Nigeria	Etude transversale	Les professionnels de santé ayant reçu une formation préalable à la gestion des cas de paludisme et la confiance des agents de santé dans les directives nationales du traitement, les agents de santé titulaires d'un certificat d'agent de vulgarisation communautaire junior, l'attentes des patients de recevoir un antipaludique, la présence de la fièvre chez un patients (symptômes cliniques) ont influencé significativement l'observance du traitement par les travailleurs de la santé.
Ruth J .Namuyinga [34]	2017	Agents de santé et patients	Malawi	Etude transversale	Plainte de fièvre au clinicien, le nombre de patients vus par le personnel de santé, nombre d'années de formation formelle reçue, nombre d'années d'expérience clinique, dernière formation sur la gestion des cas de paludisme et accès aux dernières directives de traitement du paludisme , le nombre de visites de supervision au cours des 6 mois, disponibilité des thermomètres, nombre de patients à l'établissement de santé et nombre de travailleurs du secteur de santé à l'établissement de santé, les ruptures de stock de TDR, et la possession d'une copie des directives nationales de traitement du paludisme de 2013, l'âge du matériel médical.
Obi[35]	2019	Agents de santé	Nigeria	Etude transversale	La mauvaise perception du prescripteur du TDR et influait sur la prescription d'ACT pour les patients dont les résultats de TDR étaient négatifs et le type d'établissement de santé (publics, en particulier dans des établissements tertiaires)
Odhiambo[36]	2019	Cliniciens, microscopistes et personnel de la pharmacie	Kenya	Etude transversale	Formation de recyclage sur la gestion des cas de paludisme pour les cliniciens et la sensibilisation des cliniciens au fait que le laboratoire de l'hôpital participe au programme national d'assurance qualité, la précision de diagnostic et les pratiques de traitement inappropriés dans les hôpitaux publics
Odhiambo [37]	2023	Personnel de points de vente de médicaments privés autonomes	Kenya	Etude transversale	Disponibilité d'un thermomètre fonctionnel, ceux qui ont reçu une visite de supervision ou d'inspection au cours des 3 mois précédents, ceux qui avaient été inspectés par la pharmacie et Poisons Board (PPB), le personnel de sexe masculin par rapport

					au personnel féminin, les titulaires d'un baccalauréat, personnels formés à l'utilisation des TDR,
Caroline B,Osoro[38]	2023	Prestataires de soins et des points de ventes de médicaments	Kenya	Etude qualitative	Lacune en matière de connaissances et de formation, dynamique des prestataires et des patients (préoccupation concernant la faible sensibilité des TDR entrainant l'hésitation quant à leur utilisation, la préférence des patients), la rupture des stocks d'antipaludiques essentiels en particulier la quinine et le coût des médicaments
Randriatsarafara [39]	2019	Prestataires de soins	Madagascar	Approche évaluative rétrospective et transversale	Les doutes sur les résultats du TDR et la non-participation aux revues périodiques du district sanitaire
Laura C Steinhardt[40]	2014	Agents de santé, patients et établissements de santé	Malawi	Enquête nationale transversale	Facteurs liés au traitement correct : les symptômes au niveau du patient (la fièvre et frisons, patients avec une température très élevée (≥ 37,5 °C), le type d'agent de santé était significativement associé à un traitement correct du paludisme. Les assistants médicaux, un cadre ayant deux ans de formation médicale, étaient 54 % plus susceptibles de traiter correctement le paludisme confirmé par rapport aux médecins ou aux cliniciens Facteurs liés au surtraitement des patients non atteints de paludisme. Patients sans paludisme confirmé qui se plaignaient de fièvre, vomissement ou de frissons étaient significativement plus susceptibles d'être surtraités alors que ceux qui se plaignaient de fatigue étaient moins susceptibles d'être surtraités pour le paludisme Les patients vus par assistants médicaux ou des infirmières étaient trois fois plus susceptibles d'être surtraités que ceux des médecins ou des cliniciens. Facteurs liés au diagnostic du paludisme par les agents de santé : les symptômes cliniques rapportés par les patients étaient les plus importants (fièvre, vomissement ou frissons), la toux et la fatigue moins de chance d'être diagnostiqué avec le paludisme. Les cadres inférieurs d'agents de santé étaient environ deux fois plus susceptibles que les médecins ou les cliniciens de diagnostiquer des patients atteints de paludisme. Les patients fréquentant les établissements où AL était en stock toute la journée étaient 15 % moins susceptibles d'être diagnostiqués avec le paludisme (p = 0,014), et ceux fréquentant les établissements CHAM étaient 25 % plus susceptibles d'être diagnostiqués avec le paludisme

BG Damien[41]	2018	Enfants fébriles	Bénin	Enquête transversale basée sur un échantillonnage en grappes et à plusieurs degrés	Les soignants informés de l'ACT et la fièvre confirmée par thermomètre, étaient significativement associées à l'utilisation des ACT
Christine Manyando[42]	2014	Enfants de moins de cinq ans ayant des antécédents de fièvre	Zambie	Etude prospective observationnelle	Les enfants dont le résultat du TDR était positif étaient plus susceptibles de se voir prescrire des antipaludiques que les enfants dont le résultat était négatif. Parallèlement, les enfants qui présentaient de la fièvre à l'admission étaient moins susceptibles de se voir prescrire des antipaludiques que les enfants qui n'avaient pas de fièvre.
Akinfemi Oyewumi, Akinyode[43]	2018	Agents de santé.	Nigéria	Etude transversale	Le manque de formation des agents de santé sur l'utilisation des TDR et la mauvaise connaissance des causes de la fièvre ont été associés à la prescription d'antipaludique s aux patients TDR négatifs
Ugwu I.Omale[44]	2024	Agents de santé.	Nigeria	Etude transversale analytique	Les facteurs associés et les prédicteurs de l'utilisation du TDR pour le diagnostic du paludisme étaient le fait de travailler dans un établissement de santé soutenu par l'USAID PMI était un prédicteur de l'utilisation habituelle (routine) du TDR pour le diagnostic du paludisme et de l'utilisation du TDR pour le diagnostic du paludisme pour tous ou la plupart des patients suspectés d'avoir eu le paludisme au cours du mois précédent. Les facteurs prédictifs d'une bonne pratique de prescription de médicaments antipaludiques étaient les suivants : avoir une bonne opinion de l'utilisation du TDR pour le diagnostic du paludisme ; avoir une bonne connaissance du diagnostic du paludisme et du TDR
Charles C Ezenduka[45]	2014	Dossiers des patients traités pour paludisme simple	Nigeria	Audit rétrospectif	La formation, la disponibilité d'outils de diagnostic et les connaissances des agents de santé, des ressources insuffisantes, telles que les ruptures de stock de tests de diagnostic rapide (TDR), le manque de fiabilité des résultats de laboratoire en raison de réactifs de laboratoire médiocres, l'insensibilité au TDR, le non-respect des résultats des tests par manque de confiance, le coût élevé des médicament
Yue Ge[49]	2023	Enfants de moins de cinq ans fébriles	19 pays d'Afrique subsaharienne Bénin, Burkina Faso, Burundi, Cameroun, Ghana, Guinée,	Etude transversale utilisant les EDS et MIS	Niveau d'études le plus élevé de la mère et du partenaire, le niveau d'éducation des parents, et le quintile de richesse ont montré une association positive avec la probabilité de subir des analyses sanguines et le quintile de richesse

			Libéria, Madagascar, Malawi, Mali, Mozambique, Nigéria, Rwanda, Sénégal, Sierra Leone, Tanzanie, Togo, Ouganda et Zambie)		
Jalal A. Bilal [46]	2015	Enfants fébriles de moins de cinq ans	Soudan	Etude transversale analytique	Manque de formation et de sensibilisation, pénurie de ressources (ruptures de stock de médicaments essentiels et de tests diagnostiques rapides (TDR), pressions comportementales (attentes des patients)
Cristolde A. Salomão[47]	2015	Dossiers médicaux des patients	Mozambique	Audit rétrospectif des dossiers médicaux	Les ruptures de stock de médicaments, une formation inadéquate et le manque de confiance dans les méthodes diagnostiques, la pression des patients et le manque de ressources
Singlovic Jan[48]	2016	Enfants fébriles	Burkina Faso, Nigeria et Uganda		Le lieu/la profession de l'ASC, résider dans un village autre que celui de l'agent de santé communautaire et la pression parentale

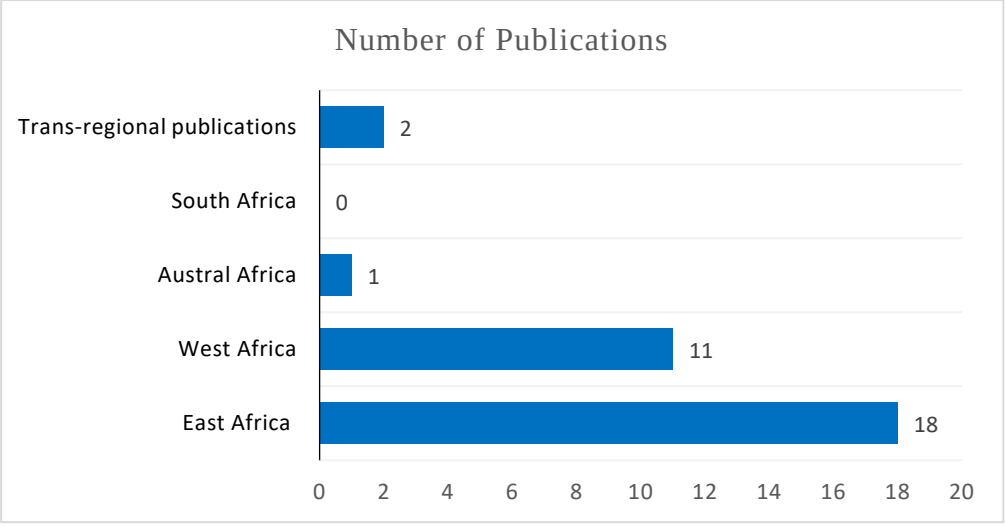
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Les principaux facteurs affectant l'adhésion des prestataires de soins aux directives nationales de diagnostic et du traitement du paludisme étaient, le manque de formation continue des agents de santé , la perception négative des prescripteurs dans les méthodes diagnostiques, approvisionnement insuffisants en médicaments antipaludiques et en produit de diagnostic dans les établissements de santé, la pression comportementale des patients et le manque de ressources, une méconnaissance des directrices sur la paludisme, les symptômes cliniques rapportés par les patients, le type de test de diagnostic disponible dans un établissement de santé et la précision diagnostique.

Les études incluses dans cette revue narrative comprenaient des études quantitatives observationnelles (**24**) menées auprès des prestataires de soins de santé, patients et des établissements de santé à travers l'Afrique subsaharienne. Des études qualitatives (**4**) et à méthodes mixtes (**2**) explorant les points de vue des prestataires et des patients sur les obstacles liés au respect des directives de prise en charge du paludisme dans les établissements de santé ont également été étudiées.

La **figure 2** met en évidence la localisation des différentes publications dans les régions de l'Afrique subsaharienne.

Figure 2 Location of included studies



Adhésion aux directives nationales de diagnostic et de traitement du paludisme

L'observation générale qui ressort de cette revue, est que les études réalisées dans les pays d'Afrique subsaharienne (ASS), montrent des différents niveaux d'adhésion à leurs directives nationales. En Ouganda, l'adhésion aux directives de traitement du paludisme grave était de 3% [30], en Somalie dans une étude conduite par Mahamoud et al seulement 33 (22 %) des agents ont déclaré respecter les directives, tandis que 117 (78,0 %) ont déclaré ne pas les respecter [16]. Au Nigéria, une différence significative a été trouvée entre les professionnels de santé publics et privés (60.6% et 27.3%) respectivement [14], au Soudan, la quinine était le médicament antipaludique le plus fréquemment prescrit en perfusion intraveineuse dans 65 cas (72.2%), Paradoxalement, 42/65 (64,6 %) traités par quinine ne présentaient aucun signe de paludisme grave [46]. Dans l'une des régions les plus endémiques de l'Ethiopie comme Gambela 107 cas (99,1%) de paludisme simple ont été gérés contrairement aux directives [26]. D'autre part, un faible taux de dépistage des cas de paludisme a été signalé au Malawi (34%), et au Soudan (43.5%) [46]. Toutes ces preuves constituent de véritables inquiétudes qui pourraient augmenter la mortalité et la mortalité liées au paludisme en Afrique subsaharienne.

On s'aperçoit que les résultats concernant l'adhésion peuvent varier d'un établissement de santé à un autre et évoluer aussi avec le temps. Ces variations sont surtout visibles entre les différents niveaux professionnels à cause des attitudes individuelles. Des conclusions similaires ont été tirées par exemple par Bawate et al en Ouganda où les médecins étaient susceptibles d'adhérer aux lignes directrices que les assistants médicaux et les infirmières prescripteurs [19]. De même qu'au Ghana, Agbemafle et al. ont aussi montré que l'adhésion était faible chez les assistants médicaux par rapport aux médecins militaires (AOR 0,004, IC à 95 % 0,004-0,02, $p < 0,001$). Des interventions beaucoup plus ciblées pour cette

catégorie de prestataires sont parfois nécessaires pour combler les déficits de leurs formations de base.

Au-delà de la connaissance, le comportement individuel du prescripteur peut l'amener à adhérer ou non aux directives thérapeutiques. Inversement, en Tanzani, Majige Selemani et al les cadres inférieurs étaient plus susceptibles de prescrire correctement les ACT (RCA 3,1 ; IC à 95 % 1,3-7,1 ; p = 0,009) que les médecins [51].

Les explications possibles pourraient être que les travailleurs de niveau académique supérieur, tels que les médecins et les cliniciens ont tendance à faire confiance à leur bagage intellectuel dans ces cas de figures, ils vont se fier uniquement des signes cliniques du patient, le discernement médical au mépris des directives nationales.

Une autre étude au Ghana, a indiqué que la formation à la prise en charge des cas de paludisme n'avait pas d'impact significatif sur le respect par les prescripteurs des directives établies en matière de traitement antipaludique [52]. Les prescripteurs ayant reçu une formation sur la prise en charge des cas de paludisme au cours de l'année précédente étaient quatre fois plus susceptibles de prescrire des ACT de manière inappropriée que ceux qui n'avaient pas reçu de formation (AOR : 4,1 ; IC à 95 % (1,5–11,6) ; p < 0,01). Les patients vus par des prescripteurs ayant été supervisés étaient huit fois plus susceptibles de se voir prescrire des ACT de manière inappropriée [52].

Plusieurs facteurs identifiés dans cette revue peuvent contribuer à une adhésion moins qu'optimale des prestataires de santé. Ces facteurs sont liés à l'attitude des prestataires de santé à l'égard des directives, aux facteurs institutionnels tels que la disponibilité des ressources nécessaires à la mise en œuvre efficace des directives et aux facteurs liés aux comportements des patients.

Facteurs liés aux agents de santé

La formation des agents de santé a été avérée dans cette revue comme un des facteurs majeurs d'adhésion aux directives nationales dans 62,5% (20/32) des publications [14,16,17,24,26–29,31,32,37–39,43,45–47]. Au Kenya, les chances des prestataires de soins titulaire d'un baccalauréat d'adhérer aux directives étaient 6 fois plus élevées que celles des autres (p = 0,0036) [37], de même que les personnes ayant reçu une formation sur l'utilisation des TDR avaient 4,4 fois plus de chances d'adhérer aux directives que celles n'ayant pas reçu de formation (p = 0,0132) [37]. Au Ghana, Agbemafle et al, ont montré les prescripteurs formés à la stratégie T3 (test, treatment, tract) avaient une meilleure adhésion (AOR : 99,33 IC à 95 % : 19,53–505,13, p<0,000) [24]. Le manque de connaissance sur l'utilisation des TDR du paludisme et la mauvaise connaissance des causes de la fièvre conduisaient la majorité des agents de santé à la prescription de médicaments antipaludiques aux patients négatifs aux tests au Nigéria [43]. Ces pratiques constituent la source de nombreuses erreurs diagnostiques et entrainent la surconsommation et le risque de résistance parasitaire aux antipaludiques.

La perception négative des prestataires de santé à l'égard du TDR du paludisme (manque de confiance dans les résultats des tests TDR, l'insensibilité au TDR et le manque de fiabilité des résultats de laboratoire en raison de réactifs de laboratoire médiocres) a été relevée par diverses études comme un prédicteur de la prescription d'ACT aux patients ayant des résultats de TDR négatifs [23,29,33,35,39,45,47]. Au Nigeria, les prestataires de soins qui avaient la mauvaise perception étaient susceptibles de traiter les patients suspects de paludisme de manière présomptive ou même de prescrire des ACT aux patients dont les résultats du TDR étaient négatifs [35].

Les prescripteurs ayant une mauvaise perception du TDR avaient 5,6 fois plus de chances de prescrire des ACT aux patients ayant des résultats de TDR négatifs par rapport à ceux ayant une bonne perception du TDR (AOR = 5,60, IC

à 95 % = 3,16–9,91) [35]. De plus, les prescripteurs des établissements publics avaient 2,8 fois plus de chances de prescrire des ACT aux patients ayant des résultats de TDR négatifs par rapport à ceux des établissements privés (AOR = 2,81, IC à 95 % = 1,44–5,46) [35].

En outre, les agents de santé des établissements de santé publics (OR = 4,3, IC à 95 % = 1,7–10,9) et privés (OR = 7,0, IC à 95 % = 3,3–14,7) utilisant la méthode de diagnostic du paludisme avaient des chances significativement plus élevées de respecter les directives [35]. Le sous-effectif des agents de santé affecte le respect des directives nationales surtout lorsqu'il ya un nombre élevé de patient. L'insuffisance de personnel de santé qualifié constitue un facteur qui compromet la qualité de la prise en charge du paludisme en particulier et les autres maladies en général dans les pays africains.

Pour améliorer le niveau d'adhésion aux directives nationales, les systèmes de santé des pays africains évaluent régulièrement leurs prestataires de soins afin de trouver des solutions locaux à certains obstacles notamment la motivation du personnel, la gestion équitable des ressources humaines le plan de carrière, renforcer en permanence les capacités des agents du publics et du secteur privé pour uniformiser la prise en charge du paludisme dans tous les établissements de santé si l'on veut progresser pour atteindre les objectifs de l'élimination de paludisme.

Facteurs liés aux patients

En ce qui concerne les facteurs liés aux patient associés à l'adhésion, nous avons observé que la pression des parents sur les prestataires de soins de santé ou l'attente du patient à recevoir un antipaludique était une observation constante dans plusieurs études [14,25,33,46,47]. Au Nigéria, Bamiselu et al ont monté que les préférences des patients, le besoin de faire des bénéfices et les demandes des patients ont été mentionnés par 18,5 %, 10,2 % et 5,6 % respectivement dans les

établissements de santé privés, pour influencer le traitement fourni, contre 3,7 %, 0,5 % et 0,9 % parmi ceux des établissements de santé publics, respectivement ($p < 0,05$) [14]. Très facilement le comportement du patient ou la pression du parent amène l'agent de santé à se plier aux caprices de son patient en mettant de côté les lignes directrices du traitement du paludisme. On observe en outre, que la présence de la fièvre chez un patient comme (symptômes cliniques) pouvait influencer significativement l'observance du traitement par les travailleurs de la santé [40]. Au bénin, Damien et al ont trouvé que les soignants informés sur l'utilisation des CTA et la fièvre confirmée par thermomètre, étaient significativement associées à l'utilisation des CTA [41]. Cela démontre la nécessité d'une bonne communication et la disponibilité des outils pour que le patient soit rassuré et qu'il utilise les médicaments prescrits avec plus de sérénité.

Yue Ge et al au cours d'une étude réalisée dans 19 pays d'Afrique subsaharienne ont aussi montré que le niveau d'éducation des parents et le quintile de richesse avaient une association positive avec la probabilité de subir des analyses sanguines dans l'étude de dans laquelle [49]. Il est important de renforcer la sensibilisation des parents ou des patients sur le respect des directives nationales du diagnostic et du traitement du paludisme.

Facteurs liés au système de santé

Des facteurs systémiques tels que la rupture fréquente des médicaments recommandés et les tests de diagnostic rapides influencent les pratiques la prescription du traitement des cas de paludisme par les professionnels de la santé [23, 34, 38, 46,47]. A cause de la disponibilité régulière les patients fébriles des établissements appartement à des organisations confessionnelles /ONG, avaient plus de chance d'être testés [17] En Outre, il a été prouvé en Ouganda, l'adhésion au directives nationales était plus élevée lorsque le laboratoire était fonctionnel [PRR = 0,47 (IC à 95 % 0,35 0,63)] [19].

L'absence de TDR dans les établissements de santé privés au Nigéria justifiait une faible adhésion parmi les professionnels de santé du secteur privé aux directives par rapport à ceux du public [14]. Pour réduire l'influence du marketing des fabricants au niveau d'établissements privés [14,25], Cette observation a été signalé dans une étude au Nigéria, le non-respect de la prescription des médicaments antipaludiques recommandés et de la confirmation en laboratoire a été signalé chez 33,9 % des agents de santé privés contre 11,3 % des agents de santé publics ($p < 0,001$) [14]. Il est important de mettre en place un système robuste capable d'approvisionner régulièrement en quantité et en qualité les établissements de santé en TDR de forte sensibilité analytique permettant de détecter une faible parasitémie. Les zones géographiques d'accès difficiles doivent avoir un stock de sécurité en quantité suffisante en médicaments et en TDR.

Parfois des préoccupations concernant la faible sensibilité des TDR ont été signalées et entrainaient l'hésitation quant à leur utilisation ainsi que la disponibilité des réactifs de laboratoire de bonne qualité [26, 29, 30,32]. Bien que le paludisme soit une maladie très mortelle mais il est évitable si la disponibilité des meilleurs outils de prise en charge du paludisme reste une garantie à des coûts abordables et accessibles pour les pays africains durement touchés par cette maladie.

CONCLUSION

Cette revue visait à évaluer les facteurs qui influent sur l'adhésion aux directives nationales de prise en charge des cas de paludisme dans les pays d'Afrique subsaharienne. Elle apporte des informations précieuses sur les facteurs liés aux prestataires de soins, aux patients et au système de santé.

Ces facteurs incluent la formation sous-optimale du personnel, la mauvaise perception de certains prescripteurs ou cliniciens à l'égard des tests de diagnostic (TDR ou microscopie), le comportement des patients, la pénurie de ressources

humaines et approvisionnement insuffisant en produits et les tests de diagnostic rapide dans les établissements de santé, le type d'établissement de santé et le coût élevé des médicaments efficaces.

D'ailleurs il semble que tous les aspects fondamentaux du système de santé aient été influencés de manières différentes selon le contexte de chaque pays. Il est donc impératif de mettre en œuvre des actions concrètes en vue de leurs améliorations, si on aspire à l'atteinte des objectifs de l'élimination du paludisme à l'horizon 2030.

La formation continue des prestataires de soins, la supervision régulière des activités et le renforcement de la communication pour le changement de comportement pourraient aider les pays d'Afrique subsaharienne à améliorer leur niveau d'adhésion aux directives nationales pour l'atteinte des objectifs nationaux et internationaux de la lutte contre le paludisme d'ici à l'horizon 2030.

Contribution des auteurs

Cette étude a été conçue par PD, MAT et SR.

Tous les auteurs ont lu et approuvé le manuscrit final.

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Les auteurs déclarent qu'ils n'ont aucun intérêt concurrent.

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PARTIE II

Directives Nationales de la prise en charges des cas de paludisme et cartographie des espèces plasmodiales

Préambule

Au Mali, le paludisme constitue un problème de santé publique majeur, nécessitant une gestion efficace pour réduire la morbidité et la mortalité qui lui sont associées. Les directives nationales et internationales recommandent un diagnostic parasitologique, par microscopie ou par un test de diagnostic rapide (TDR), avant toute prescription d'un traitement antipaludique avec les molécules recommandées, afin d'assurer une prise en charge appropriée. Cependant, la conformité des prestataires de soins à ces directives est essentielle pour optimiser les résultats du traitement et limiter les risques de résistance aux antipaludiques. Néanmoins, il est établi que plusieurs facteurs s'opposent au respect des directives nationales de diagnostic et de traitement des cas de paludisme par les prestataires de soins dans les pays africains hautement endémiques du paludisme.

A cause de l'émergence de la chloroquinorésistance de *P. falciparum* constatée dans de nombreux pays endémiques comme Mali, la chloroquine a été remplacée en 2005 par des combinaisons thérapeutiques à base d'artémisinine (CTA). L'artéméther-luméfantrine (AL) est la CTA de première intention pour le traitement du paludisme simple à *P. falciparum*. D'autres dérivés à base d'artémisinine, reconnus pour leur efficacité, sont utilisés comme la dihydroartémisinine / pipéraquine (DHA-PPQ), la pyronaridine / artésunate. En revanche, les injectables comme l'artésunate, l'artéméther ou la quinine sont recommandées pour le traitement des cas graves, selon la disponibilité, avec un relai par une dose complète de CTA dès que le patient est capable de prendre des médicaments par voie orale en toute sécurité. Le TDR a été introduit pour le diagnostic du paludisme en 2010. Il est l'outil le plus utilisé pour le diagnostic du paludisme au Mali dans les formations sanitaires et au niveau communautaire. Cette politique de traitement et de diagnostic du paludisme a fortement contribué à diminuer la morbidité et la mortalité liées au paludisme.

Au niveau national, des directives sont élaborées et mises à jour régulièrement avant d'être disséminées à tous les niveaux de la pyramide sanitaire. Elles s'appliquent à tous les prestataires de soins impliqués dans la prise des cas de paludisme. Une prise en charge correcte a été définie comme, en cas de test positif, la prescription d'un traitement antipaludique recommandé (CTA) au patient ; en cas de test négatif celui-ci ne devrait pas recevoir d'antipaludique. Le non-respect des directives nationales conduirait à un surdiagnostic du paludisme, et donc une prescription inappropriée des médicaments antipaludique, une exposition des patients aux effets indésirables, à un risque d'émergence, au fil du temps, d'une résistance parasitaire aux CTA et aussi à une augmentation des dépenses de santé.

Une surveillance des connaissances, des attitudes et des pratiques s'avère donc nécessaire pour garantir une mise en œuvre efficace des directives nationales de diagnostic et de traitement du paludisme dans les établissements sanitaires publics et privées. La présente visait d'identifier les différents facteurs associés à l'adhésion aux directives nationales dans le district sanitaire de Kita au Mali. Une étude descriptive et transversale a été réalisée afin de mesurer quantitativement la prévalence de ces facteurs associés à l'adhésion aux directives nationales. À l'aide d'un modèle de régression logistique, des rapports de risque de prévalence ajustés et leurs intervalles de confiance à 95 % ont été déterminés en fonction de l'adhésion aux directives nationales du diagnostic et du traitement du paludisme.

Au total, 27 agents de santé, 168 patients traités pour le paludisme et neuf gérants de pharmacie ont été interrogés. Les prestataires de soins qui ont déclaré avoir lu les directives nationales de diagnostic et de traitement du paludisme étaient (55,6%) 15/27. L'adhésion globale des agents de santé aux directives nationales du diagnostic et du traitement du paludisme était de (90.4%). Les prestataires de soins qui utilisaient les TDR étaient beaucoup plus susceptibles de respecter les directives (p =0,001). La disponibilité d'un laboratoire était positivement associée avec l'adhésion aux directives. En outre, la pratique de

l'automédication était très significative chez les individus vivants les localités rurales comme Kobiri et Kokofata par rapport à ceux du milieu urbain. L'âge des prestataires de soins, la formation, le niveau d'éducation n'ont pas été statistiquement significativement associés au respect des directives nationales.

Bien que des efforts importants aient été faits pour aligner les pratiques de diagnostic et de traitement du paludisme avec les directives établies, des améliorations restent nécessaires pour garantir une adhésion uniforme à ces recommandations dans les établissements de santé publics et privés. Il est crucial d'améliorer la formation continue des prestataires, la supervision, le suivi régulier et la sensibilisation des prescripteurs, d'assurer la disponibilité des ressources nécessaires, et d'adapter les infrastructures de santé pour soutenir une mise en œuvre plus cohérente des directives de traitement du paludisme.

Par ailleurs, bien que la microscopie soit une méthode de référence (gold standard) dans le diagnostic du paludisme force de constater que sa réalisation est le plus souvent très limitée dans les zones reculées. Elle nécessite de l'électricité, un personnel qualifié pour la lecture des lames et le temps d'exécution plus long (1 heure au maximum). Les tests de diagnostic rapide (TDR) sont apparus comme un outil précieux pour le diagnostic du paludisme depuis leur introduction en 2010, en raison de leur facilité d'utilisation, de la rapidité des résultats et de leur applicabilité dans des environnements à faibles ressources. L'évaluation de la performance (sensibilité et spécificité) des TDR dans le contexte très hétérogène du paysage épidémiologique du paludisme au Mali, informera les prestataires de soins de santé sur la fiabilité de ces tests dans les conditions locales. Nous avons entrepris cette étude qui avait pour objectif d'évaluer la performance diagnostique des différents TDR disponibles au Mali et à cartographier la distribution des espèces de *Plasmodium* dans le pays.

Ainsi des TDR négatifs et positifs pour le paludisme ont été collectés dans 41 sites répartis dans 9 régions du Mali. A partir de l'ADN extrait de la bande de nitrocellulose du TDR, nous avons utilisé des méthodes de biologie moléculaire

(qPCR) pour l'identification des espèces *P. falciparum, P. malariae, P. vivax* ou *P. ovale*.

Sur les 1 496 TDR testés, 258 (18,6 %) étaient positifs pour *Plasmodium* spp. dont 96,9 % pour *P. falciparum* et confirme la circulation de *P. vivax* dans la partie Nord du pays (Kidal et Ménaka) avec une prévalence variant de 1,9% à 21,1 %. Les TDR présentaient des indices diagnostiques acceptables ; les limites inférieures de l'IC95% des indices de Youden étaient toutes ≥ 0,50, sauf dans le nord (indice de Youden 0,66 (IC95% [0,44-0,82]) et 0,63 (IC 95 % [0,33-0,83]). Dans l'ensemble, les indices diagnostiques des TDR sont adéquats pour le diagnostic biologique du paludisme au Mali.

Cette étude contribuera à l'optimisation des pratiques de diagnostic, à l'amélioration de la surveillance du paludisme et à mieux comprendre l'épidémiologie du paludisme. En fin de compte, elle contribuera à la réduction de la morbidité et de la mortalité dues au paludisme dans la région. Pour cela, nous recommandons l'utilisation de TDR détectant les antigènes spécifiques de *P. vivax* dans le nord du pays

Article 2

Adherence to malaria diagnosis and treatment guidelines among healthcare providers in Mali

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Résumé

L'adhésion des agents de santé aux normes nationales de soins a un impact positif sur la qualité des soins qu'ils fournissent. Cette étude visait à identifier les facteurs influençant l'adhésion aux directives de prise en charge des cas de paludisme parmi les prestataires de soins de santé dans le district sanitaire de Kita au Mali.

Nous avons mené une étude transversale descriptive pour collecter des informations sur les prestataires de soins de santé, les établissements de santé et les patients atteints de paludisme dans le district sanitaire de Kita. Une régression logistique multivariable, ajustée en fonction de l'âge et du sexe, a été utilisée pour identifier les facteurs associés à l'adhésion aux directives nationales de diagnostic et de traitement du paludisme

Au total, 27 prestataires de soins de santé, 168 patients présentant de la fièvre et neuf gérants de pharmacies ont été interrogés. Quinze (55,6 %) des 27 prestataires ont déclaré avoir lu les directives de diagnostic et de traitement du paludisme. L'adhésion aux directives de traitement du paludisme était de 90,4% pour les patients dont le test était positif et de 82,3% pour les patients dont le test était négatif ($p < 0,0001$). L'utilisation de TDR était associée à une adhésion de 100 % contre 90,9 % ($p < 0,001$) pour ceux qui n'utilisaient pas de TDR. Après ajustement pour l'âge et le sexe, la disponibilité d'un laboratoire était positivement associée à l'adhésion des agents de santé (OR= 6,92, 95%CI [1,05 - 45,65]). Le risque d'automédication était 4 à13 fois plus élevé pour les patients vivant dans les zones rurales telles que Kobiri et Kokofata que pour ceux vivant dans les zones urbaines. L'adhésion à la directive nationale n'était pas associée à l'âge, à la formation ou au niveau d'éducation des agents de santé.

Conclusion. L'adhésion aux directives nationales pour la prise en charge des cas de paludisme était satisfaisante, mais l'introduction de stratégies efficaces de surveillance et de sensibilisation pourrait faciliter une plus grande adhésion.

MOTS CLÉS : Paludisme ; Adhésion ; prestataire de soins ; Directives pour la prise en charge des cas ; Mali.

Adherence to malaria diagnosis and treatment guidelines among healthcare providers in Mali

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Abstract

Context. Health workers' adherence to national standards of care has a positive impact on the quality of care they provide. This study aimed to identify factors influencing adherence to malaria case management guidelines among health care providers in the Kita health district of Mali.

Methods. We conducted a descriptive cross-sectional study to collect information on health care providers, health facilities and malaria patients in the Kita health district. Multivariable logistic regression, adjusted for age and sex, was used to identify factors associated with adherence to national malaria diagnostic and treatment guidelines.

Results. A total of 27 health care providers, 168 patients who present with fever, and 9 drug store managers were surveyed. Fifteen (55.6%) of the 27 providers reported having read the malaria diagnosis and treatment guidelines. Adherence to malaria treatment guidelines was 90.4% for patients testing positive and 82.3% for patients testing negative ($p < 0.0001$). The use of RDTs was associated with 100.0% adherence compared to 90.9% ($p < 0.001$) for those who did not use RDTs. After adjustment for age and sex, the availability of a laboratory was positively associated with adherence among health workers (OR= 6.92, 95%CI [1.05 - 45.65]). The risk of self-medication was 4 to 13 times higher for patients living in rural areas such as Kobiri and Kokofata than for those living in urban areas. Adherence to the national guideline was not associated with the age, training or educational level of the health workers.

Conclusion. Adherence to national guidelines for malaria case management was satisfactory, but the introduction of effective surveillance and awareness strategies could facilitate increased compliance.

KEYWORDS: Malaria; Healthcare provider adherence; cases management guidelines; Mali.

Introduction

Malaria is a complex disease whose epidemiology and impact on public health vary from one region of the world to another. In 2022, it was estimated that nearly 247 million new cases of malaria caused around 619,000 deaths (1). Sub-Saharan Africa bears a disproportionate burden of malaria-related morbidity and mortality, accounting for 94% of all cases and 95% of all deaths attributable to malaria worldwide. Mali is one of the countries with the highest number of cases and associated deaths. The incidence rate of malaria in the general population rose from 125‰ in 2020 to 172‰ in 2022. Malaria case fatality was estimated at 1.25‰ or 1498 deaths out of 1 197 864 confirmed severe cases. However, malaria remains a major public health concern as it represents one of the main reasons for medical visit in public health facilities with a morbidity rate (34%) and mortality rate (22%) (2). In 2018, the prevalence of malaria among children under 59 months was 19% according to the results of the Demographic and Health Survey (EDS-VI-M, 2018) (3). Despite the country's security challenges and the major global health crisis marked by COVID 19, Mali has made major progress in the fight against malaria over the last ten years. The prevalence fell drastically between the Demographic and Health Survey (DHSM-V) carried out in 2012-2013 and the Malaria Indicator Survey (MIS) in 2021, from 47% to 19% (4,5).

In Mali, malaria is endemic, with high transmission during the rainy season. The duration of which varies according to eco-climatic zone, showing (a) a Sudano-Guinean zone with long seasonal transmission lasting 4 to 6 months;(b) a zone with short seasonal transmission lasting 3 to 4 months; (c) a zone of sporadic or even epidemic transmission covering the northern regions and some of the southern regions such as Koulikoro and Kayes;(d) zones of bi- or multi-modal transmission including the delta of the River Niger and the dam zones;(e) zones that are not very conducive to malaria, particularly urban areas such as Bamako and Mopti where malaria is hypo-endemic (6). To reduce malaria morbidity and

mortality rates, Mali updated the national malaria control strategic documents following its participation to the Amsterdam ministerial conference (7) and joined the Rock Back Malaria (RBM) initiative in 1999 (8).

Following the emergence of chloroquine resistance in *Plasmodium falciparum* in many endemic countries, including Mali, chloroquine was replaced in 2005 by artemisinin-based combination therapy (ACT), and the Rapid diagnosis test (RDT) was introduced for malaria diagnosis in 2010 (9,10). This policy in line with WHO guidelines is used to develop and update malaria cases management guidelines before being disseminated to all levels of the health system (11).

If healthcare providers adhere to the guidelines, they will not only be able to achieve better control of malaria in the country, but will also help to eliminate it by 2016-2030(12). The Malian government has developed policies such as providing all malaria treatment free of charge for children under 5 and pregnant women (13). This decision has made a significant impact on malaria morbidity and mortality rates among the population.

National guidelines state that all suspected cases of malaria should be tested before any anti-malarial medication is prescribed. Artemether-lumefantrine (AL) is the first-line ACT for the treatment of uncomplicated *falciparum malaria*. Other artemisin-based derivatives known for their efficacy are used such as Dihydroartemesinine/Piperaquine (DHA-PPQ), Pyronaridine/artesunate, while injectables such as artesunate, artemether or quinine are recommended for severe malaria cases. with a ACT in relay once oral medication is possible (14–16).

Monitoring of knowledge, attitudes and practices is necessary to ensure effective implementation of the guidelines and to promote good practice in malaria cases management (17,18). Previous studies carried out in several endemic countries such as Mali (19,20), indicate that adherence to parasitological

testing and malaria treatment policies by health workers was inadequate in health facilities. In response to this need, this study was designed to assess diagnostic and treatment practices for febrile patients attending public, private and faith-based health facilities and to identify factors influencing adherence to national guidelines.

METHODS

Definitions

The definitions used in the study are taken from the National Malaria Management Guidelines of the National Malaria Control Program (NMCP), revised in October 2020 (11).

- The guidelines recommend parasitological confirmation of malaria by one of two methods (microscopy or RDT) before any treatment for patients with fever or a history of fever in all age groups (21).
- Compliance with the guidelines means that a patient suspected of having malaria: has malaria microscopy or RDT; if positive, receives only a recommended antimalarial; if negative, receives no antimalarial.
- Non-compliance with guidelines means that a patient suspected of having malaria did not have a malaria diagnostic test; or an antimalarial was prescribed despite a negative blood test; or the blood test was positive but the antimalarial prescribed was not in accordance with the national guideline.

Study site and design

With a surface area of 35,250 km2, the health district of Kita (Kita region) is located 187 km south-west of the capital Bamako, at latitude 13° 2' 5 North and longitude -9° 29' 22 West. The climate is of the Sudano-Sahelian type, with a Sahelian zone in the north where the dry season is the longest (8 months), making

it impossible to carry out agricultural activities throughout the year. In the south, there is a Sudanian zone with abundant rainfall of up to 1,000 to 1,200 mm in rainy season. Its population is estimated at 502,818 according to the District Health Information Software 2 (22).

The Kita health district includes a reference health center (Centre de Santé de Référence), 40 community health centers (CSCom) functional and 71 community health workers (CHWs) established at village level.

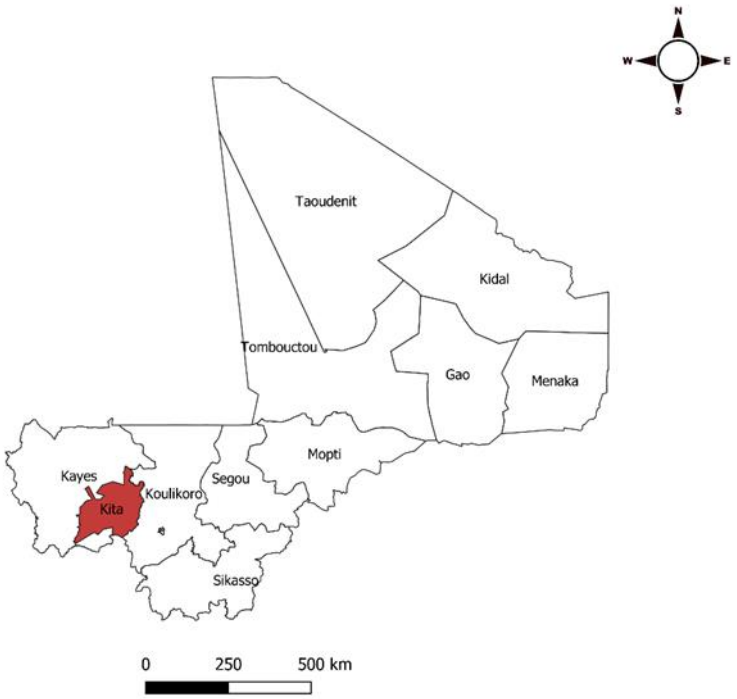


Figure 1. Health district of Kita Mali

This descriptive cross-sectional study was conducted from 03 February to 03 March 2023. Information was collected from 27 health professionals involved in the management of malaria cases, 168 patients with fever and 9 managers of drug stores. The research participants were from nine public, private and religious health facilities and participation was voluntarily. A questionnaire was in French and pre-tested in 2 facilities. The survey data were collected electronically using the Open Data Kit (ODK) software installed on tablets. Each device was able to capture GPS coordinates to provide the location of facilities. ODK data were

uploaded to a central server based in Bamako, where they were checked for completeness by a data manager. Data collector were trained in data collection for this study During data collection, the questionnaire was submitted in French to healthcare providers but translated into the local language for patients

Inclusion and non- inclusion criteria

All healthcare staff working in the selected facility who were directly or indirectly involved in the malaria cases management and who voluntarily agreed to answer the questionnaire were included. All staff who had not the above inclusion criteria were not included. Patients with fever and for whom informed consent had been obtained were considered. Other patients suffering from conditions other than malaria, and those who refused to answer the questionnaire, were excluded from the study.

Sample

Kita was chosen because of its epidemiological profile, high rainfall, and the presence of rivers along the year. Kita is located in an area where malaria transmission is moderate. The prevalence of malaria in the Kayes region was 12.2% according to the malaria indicator survey (MIS 2021) carried out in 2021. Information was collected based on the characteristics of healthcare providers, the level of knowledge of national malaria cases management guidelines, the availability of RDTs and ACTs, the level of adherence to and use of RDTs and ACTs in urban, rural and private health facilities, and factors influencing adherence to malaria cases management guidelines. Given the likely differences in perception between rural and urban areas, all urban and rural health areas were randomly sampled from the list provided by the DHIS2. This random selection was made using Excel 2013 after each center had been given a number. Private facilities accredited by the health authorities were chosen at random. If several healthcare providers worked in the center to be surveyed, those who were solely involved in malaria management were selected.

Variables

The variables included provider-level factors (address, age, sex, job, structure, level of education, professional experience), training/supervision, knowledge, attitudes and practices for diagnosing and anti-malaria drug prescribing for simple and severe malaria cases in line with national guidelines, then patient-level factors: address, age, sex, job, structure, level of education, professional experience, etc.) instruction, knowledge and means of prevention to protect themselves against, attitudes in case of fever, opinion on malaria diagnosis and treatment, medicines already taken at home. Finally, factors at the health facility level were based on information on the availability of microscopy services, RDTs, and antimalarial drugs.

Statistical analysis

Categorical variables were presented as the frequency and percentage; quantitative variables were presented as mean and standard deviation. Fisher's exact and Wilcoxon's tests were used to compare respondents' characteristics according to care providers' adherence to national guidelines. Multivariable logistic regression, adjusted on age and sex, were also performed. A two-sided α value of less than 0.05 was considered as statistically significant. Analyses were carried out using the R software.

ETHICAL APPROVAL AND CONSENT

The study protocol was approved by the Ethics Committee of the Faculty of Medicine and Odontostomatology **(N° 2023/ 29/CE/USTTB/, January 23, 2023)** for review of ethical aspects prior to the start of the work. Respondents' anonymity and confidentiality were guaranteed throughout the study. The free and informed consent of each participant (healthcare provider and patient) was obtained in writing prior to any study procedure. and we also obtained the written consent from the parent/guardian of each participant under 18 years of age.

RESULTS

Health workers' characteristics

The socio-demographic characteristics of the health care providers are summarised in Table 1. In total, there were 27 providers from the nine health facilities in the Kita health district, including 6 doctors, 9 nurses, 4 midwives, 5 auxiliary nurses and 3 community health workers. We found that 15/27 (55.5%) of the providers had secondary education, 11/27 (40.7%) had tertiary education and 1/27 (3.8%) had primary education. The majority of care providers were men: 14/27 (52%). The mean age of providers was 37.6 (±9.7) years. The sex ratio was 1.1 in favour of males. Sixty-three percent (17/27) of the health workers had received no specific training in malaria case management other than their basic training; this proportion was 100% (2/2) for health workers working in private facilities and 26% (7/27) in the faith-based centre. Health workers' age, training and level of education were not associated with compliance with national guidelines.

Patients' characteristics

The 168 malaria patients included in the study are described in Table 2. The mean age of the patients was 25.1 (±15.5) years. 56.5% 95/168 were female. Those not attending school accounted for 46.4%, with primary, secondary and tertiary education levels of 35.1%, 16.1% and 2.4% respectively. Of the 168 patients who responded to the questions, 102 (60.7%) indicated that the health centre was their first preference in the event of fever, compared with 14 (8.3%) who first sought care from traditional health providers. After adjusting for age and sex, we also found that the practice of self-medication was significantly higher among patients living in rural areas such as Kobiri (OR = 4.43, 95% CI [0.88 - 22.28]) and Kokofata (OR = 13.32, 95%CI [2.36 - 75.07]) compared to those living in urban areas.

Multivariate analysis confirmed a independent association between self-medication, locality and care-seeking ($p < 0.001$).The risk of self-medication was 10 times higher for patients who sought care late between 2 and 5 days (OR 95% CI 10.69 1.78 - 64.35) or 4 times higher for those who sought care according to clinical severity (OR = 4.37, 95%CI[1.44 - 13.26]) than for those who came early.

Table 1: Socio-demographic characteristics of the healthcare providers according to their adherence to national guidelines (n=27).

Variable		Adherence to guidelines		p-value*	OR 95%CI**	p-value	Total (n=27)
		No (n=10) n (%)	Yes (n=17) n (%)				%
Age	Mean (std)	38.1 (11.2)	37.3 (9.0)	0,862	0.96 [0.87-1.06]	0.434	37.6 (9.7)
	<25	0,0	100,0				44,4
	25-34	41,7	58,3				33,3
	35-44	22,2	77,8	0,625			11,1
	45-54	66,7	33,3				7,4
	55-58	50,0	50,0				3,7
Sex	Female	30,8	69,2	0,695	1,0		48,0
	Male	42,9	57,1		0.82 [0.14-4.71]	0.827	52,0
Residence	Rural	55,6	44,4				33,3
	Urban	18,8	81,2	0,018	NA		59,3
Availability of the laboratory	No	63,6	36,4	0,040	1,0		40,7
	Yes	18,8	81,2		**6.92 [1.05-45.65]**	**0.045**	59,3
RDT done	No	90,9	9,1	<0.001	NA		40,7
	Yes	0,0	100,0				**59,3**
Results of doubtful RDT	No	22,7	77,3	0,003	NA		40,7
	Yes	100,0	0,0				**59,3**
Malaria management training	No	41,2	58,8	0,692			63,0
	Yes	30,0	70,0				37,0
Monitoring	No	53,9	46,1	0,120			40,7
	Yes	21,4	78,6				59,3

:** Fisher's exact test /Wilcoxon Two-Sample test for age; *: Odds ratios with 95% confidence interval. Multivariable logistic regression (with Firth-correction) adjusted on age and sex; NA: not applicable for the multivariable analysis (zero counts)

773 **Table 2:** Socio-demographic characteristics of the malaria patients according to
774 their self-medication practices (n=168)

Variable		Self-medication		p-value*	OR 95%CI**	p-value	Total (n=168)
		No (n=32) n (%)	Yes (n=136) n (%)				N (%)
Age	Mean (std)	25.7 (19.8)	25.0 (14.3)	0,630	0.99 [0.96-1.01]	0,347	25.1 (15.5)
	0 - 5	5 (23.8)	16 (76.2)	0,605			21(12.5)
	6 -14	5 (23.8)	16 (76.2)				21(12.5)
	15 - 24	6 (20.7)	23 (79.3)				29(17.3)
	25 – 34	8 (13.6)	51 (86.4)				59(35.1)
	35 – 44	3 (14.3)	18 (85.7)				21(12.5)
	>44	5 (29.4)	12 (70.6)				17(10.1)
Sex	Female	19 (20.0)	76 (80.0)	0,843	1.0		95(56.6)
	Male	13 (17.8)	60 (82.2)		0.77 [0.31 - 1.93]	0,573	73(43.4)
Education	No education	13 (16.7)	65 (83.3)	0,631			78(46.4)
	Primary	11 (18.6)	48 (81.4)				59(35.1)
	Secondary	7 (25.9)	20 (74.1)				27(16,1)
	Higher	1 (25.0)	3 (75.0)				4(2.4)
Residence	Rural	12 (12.9)	81 (87.1)	0,030			93(55.4)
	Urban	20 (26.7)	55 (73.3)				75(44.6)
Distance to health center	< 1 km	25 (21.6)	91 (78.4)	0,299			116(69.0)
	> 5 km	7 (13.5)	45 (86.5)				52(31.0)
Distance to pharmacy	< 1 km	26 (22.0)	92 (78.0)	0,196			118(70.2)
	> 5 km	6 (12.0)	44 (88.0)				50(29.8)
Villages	Saint-Félix	8 (47.1)	9 (52.9)	<0.001	1.0		17(10.1)
	Darsalam	0 (0.0)	29 (100.0)		58.64 [1.78 - +∞]*	0,008	29(17.3)
	Kokofata	2 (7.4)	25 (92.6)		**13.32 [2.36 - 75.07]**	**0,003**	27(16.0)
	Makandianbougou	12 (41.4)	17 (58.6)		1.09 [0.26 - 4.54]	0,904	29(17.3)
	Tambaga	7 (29.2)	17 (70.8)		1.72 [0.38 - 7.90]	0,484	24(14.3)
	Kobiri	3 (7.1)	39 (92.9)		**4.43 [0.88 - 22.28]**	**0,071**	42(25.0)
Use of medical care	24 hours	25 (30.9)	56 (69.1)	<0.001	1.0		81(48.2)
	According to gravity	6 (14.0)	37 (86.0)		**4.37 [1.44 - 13.26]**	**0,009**	43(25.6)
	2 to 5 days	1 (2.3)	43 (97.7)		10.69 [1.78 - 64.35]	0,010	44(26.2)

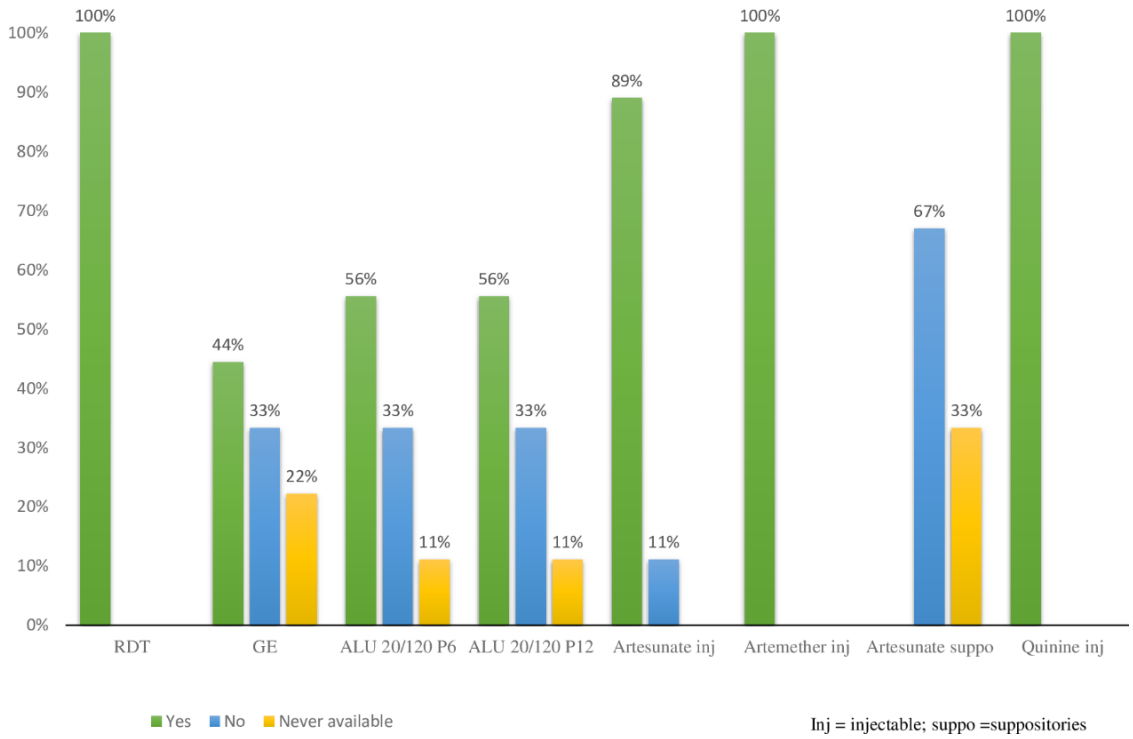
775 *: Fisher's exact test /Wilcoxon Two-Sample test for age; **: Odds ratios with 95% confidence
776 interval. Multivariable logistic regression (with Firth-correction) adjusted on age and sex.

NA: not applicable for multivariable regression (zero counts).

Characteristics of health facilities

Of the 9 health facilities surveyed, 6 (66.6%) were managed by the Ministry of Health and 3 were private. National guidelines for the management of malaria cases were available at 6 of the health facilities visited. Posters on the diagnosis and treatment of malaria cases were also available in 62.9% of the health centres visited. Hence, the results revealed that the health facilities had stocks of RDTs 100%, and 25% had microscopy services. After adjustment on age and sex, healthcare providers in facilities with laboratory unit were 7 times more likely to adhere to national guidelines than those without (OR 95%CI 6.90 1.05 - 45.65, p = 0.045).

The availability of antimalarial drugs in health facilities was relatively satisfactory in all the health facilities visited as illustrated in **S2. Figure 2**.



S2. Figure 2. Availability of inputs for treatment in the health care facilities on the day of the survey.

In addition, some health facilities had experienced stock-outs for a few days in the last three months prior to our visit, particularly malaria microscopy reagents, ALU blister packs of 6 and 12, injectable or suppository artesunate, and the keeping of stock sheets for good traceability of each molecule.

Artesunate suppositories 100% (8/8), reserved for treatment of febrile children prior to transfer from Community Health Worker (CHW) sites to Community Health Centre (CSCOM), were not available in any of the facilities visited. Artemether/Lumefrantrine 20/120mg (blister packs of 6 and 12) was out of stock for 7 days in one urban health facility. Injectable antimalarials reserved for treatment of severe cases (artesunate, artemether and quinine) were available in 100% of health facilities. The observed stock-outs were due to either poor needs assessment at CSCOM level or temporary stock-outs at district level.

Knowledge of health care providers

Of the 27 health care providers surveyed, 15 (55.6%) had read the national case management guidelines revised in October 2020. The clinical diagnosis of fever as the main sign of malaria was mentioned by 100% of providers for whom parasitological testing is recommended by national guidelines. Of these, 17 (63.0%) knew that malaria diagnostic tools were available in their health facilities. Fourteen (51.9%) of the health care providers surveyed said that ACTs were the first-line treatment for malaria. Quinine tablets were mentioned by five paramedics, one nurse, one auxiliary nurse, two midwives and one private practitioner.

Attitudes and practices towards national guidelines among healthcare providers

Our study found that 5 (18.5%) of the 27 healthcare providers had a negative perception of RDTs due to the unreliability of the RDT result, which they described as doubtful. Faced with this diagnostic dilemma, 33% of providers

performed both the RDT and blood smear microscopy on the same patient during a consultation. Confirmation of suspected cases was not systematic for all healthcare providers (14.8%) 4/27.

Of the 27 health providers, 23 (85.2%) reported that they systematically use RDTs for the biological diagnosis of malaria. They find them useful and practical for peripheral health facilities that lack laboratory services. As shown in Table 1, providers who used RDTs were much more likely to follow the guidelines (100.0% vs. 90.9%, $p < 0.001$).

Health care providers' adherence to national guidelines for malaria treatment and diagnosis

Overall, 90.4% of health care providers adhered to national guidelines for malaria diagnosis and treatment. Of the 168 patients, 162 (96.4%) were tested for malaria, of which 99 (58.9%) were positive and 68 (40.5%) were negative. Malaria treatment was appropriate in 91 (91.9%) of the 99 patients who tested positive, compared to 56 (82.3%) of the 68 patients who tested negative ($p<0.0001$). Although the majority of test-positive patients were treated appropriately, 9.1% of them did not receive antimalarial drugs, while 17.6% of test-negative patients were prescribed antimalarial drugs. Lack of provider confidence in RDT results, lack of a functioning laboratory, not using national guidelines, locality, self-medication practices and health care utilisation are some of the factors responsible for not following national guidelines. After adjustment for age and sex, the availability of a laboratory was positively associated with adherence to guidelines among health workers (OR = 6.92, 95%CI [1.05 - 45.65]). Health worker age, training and education level were not associated with adherence to national guidelines.

Patients' adherence to anti-malarial treatment

Of the 168 patients, 121 (72.0%) preferred injections to oral medication. The main reasons given were the bad taste of tablets (35%), forgotten tablets

(26%) and the large number of tablets to be taken at once (12%). However, the cost of malaria treatment at health centres was considered too high by 44.6% (75/168) of patients. Among patients, the practice of self-medication was significantly higher among those living in rural areas such as Kobiri (OR = 4.43, 95% CI [0.88 - 22.28]) and Kokofata (OR = 13.32, 95% CI [2.36 - 75.07]) compared to those living in urban areas. Neither cost of treatment, sex nor patients' level of education were significantly associated with the practice of self-medication.

DISCUSSION

This study aimed to identify provider-, patient- and facility-related factors affecting adherence to national guidelines for malaria diagnosis and treatment in Mali. Although the 90.4% compliance rate with national guidelines was satisfactory, we still found variations in malaria case management. Despite the availability of guidelines (either in paper or electronic form) in 66.7% of health facilities, only 55.6% of them had read them. The NMCP, with the technical and financial support of its partners, is making great efforts to improve the training of health care providers involved in the diagnosis and treatment of malaria, in particular by organising regular training sessions specific to malaria case management, cascade supervision, and by providing posters, algorithms and national and international normative documents to ensure appropriate practices in the field.

Nevertheless, adherence to recommended guidelines remains a concern in many malaria-endemic countries, including Mali, for reasons related to the behaviour of health care providers, patients or sometimes the health facility itself, such as difficulties in obtaining regular supplies of RDTs and microscopy reagents in certain insecure areas of the country and the unavailability of certain commodities at the health facility level. Factors associated with compliance were lack of provider confidence in the results of RDTs, use of RDTs, lack of a

functional laboratory, location, self-medication practices and late initiation of therapy.

Knowledge and attitudes

It's important to be aware of the recommended national guidelines for the management of malaria cases, but it's even more important to follow them in practice. WHO has stressed the importance of adherence to guidelines for diagnosis and treatment of malaria at different stages of malaria control. Based on these guidelines, the Malian NMCP has developed guidelines that are regularly updated and distributed to all levels of the health system.

Our results showed that 96.4% of healthcare providers were aware of the value of performing a malaria biological diagnostic test (RDT or microscopy) on patients with fever before prescribing antimalarial drugs to confirmed positive cases. However, these results fall short of the National Strategic Plan 2018-2022 target of 100% of suspected malaria cases seen in public, parapublic, faith-based, community and private health facilities to be biologically confirmed (22). Of patients with negative test results, 17.6% received antimalarial drugs. A study conducted in Nigeria found very low adherence, with more than 50% of patients with a negative test receiving an antimalarial drug (23). Doubts about the reliability of RDTs (18.5%) were the main reason for this striking discrepancy. This hypothesis is supported by the study by Fidiniaina Mamy Randriatsarafara and others in Madagascar (18) and Oluyomi F Bamiselu and others in Nigeria (24), which found that 55.6% and 40.9% of providers respectively had doubts about the results of RDTs in practice. Health workers who used either method of laboratory diagnosis of malaria (RDT or microscopy) were significantly more likely to adhere to national treatment guidelines in health facilities. These results are similar to those of Marycelina et al. in Tanzania, where overall health worker adherence to national treatment guidelines was 90.5% (25).

Despite our relatively small sample size, knowledge of healthcare providers' perceptions of the usefulness of malaria parasite diagnostics and its impact on their treatment practices could provide useful information to promote appropriate management of malaria cases (24). However, direct observation of their practices in managing a febrile case allows us to assess and ensure that management is in line with current national guidelines. Doubts about test sensitivity remain a concern for achieving malaria elimination targets, as false-negative results in the case of PfHRP2 deficiency can lead to inappropriate treatment of a potentially fatal disease (26,26). Therefore, RDTs should be adapted to the local malaria epidemiology (27). This study did not find an association between provider training in case management and adherence to guidelines. This is consistent with other studies showing that training or supervision does not necessarily translate into adherence to guidelines (28).

In our study, 43.75% of nurses followed the guidelines for diagnosis and treatment of malaria. One doctor, based on his experience, doubted the results of RDTs before prescribing, and the same observation has been made in several cross-sectional studies in Tanzania and Khartoum (29-32). The same observation was made by Selemani et al. in Tanzania, who reported correct prescribing of ACTs by nurses or junior staff rather than by doctors (29). The fact that some health care providers continue to prescribe quinine tablets or injectable quinine for the treatment of simple malaria attacks is an inappropriate practice that contradicts national guidelines and shows a lack of knowledge that should be addressed.

Malaria education for healthcare providers

As part of the monitoring and evaluation process, the National Malaria Control Programme plans and carries out various training activities for health care providers. In our study, 51.9% of health workers surveyed had received training in malaria case management. Our findings are similar to those in Ogun State,

Nigeria, where 51.2% of health providers had received training in malaria case management (22).

Adherence to guidelines

National guidelines state that ACTs should only be appropriately prescribed to patients with a positive malaria test, but 37% of staff were of the opinion that biological diagnosis of suspected cases is not always necessary. They prescribe antimalarial drugs, usually ACTs, based on their professional experience or the patient's clinical symptoms. The majority of test positive patients were treated appropriately, but 9% of them did not receive antimalarials, while 17.6% of test negative patients received antimalarials. The fact that some providers ignore test results before prescribing ACTs is based on their clinical experience. In addition, the unreliability of RDTs has sometimes led to over-prescription and waste of limited financial resources for RDT-negative patients (33,34). Adequate training of health care providers and equipping health facilities with RDTs that are adapted to the local malaria epidemiology (27), as well as management of patients with positive and negative RDT results, ensures that health care workers are able to identify and treat non-malarial causes of fever, an important element of adherence (35). In the study conducted in Mali by Fomba et al, 44.2% of 215 children who tested negative received an ACT-type antimalarial drug (20)

Overprescribing of parenteral artemisinine or quinine injections was observed in 72% of uncomplicated malaria cases. This finding is lower than that of Ofori-Adjei et al, who reported an 85% preference for injections. The reasons given were mainly socio-cultural and included patient demand and attitudes (23). Injections remain costly for both the NMCP and the patient and are associated with adverse events such as abscesses at the injection site, paralysis of a lower limb, or severe pain from the needle. However, when used appropriately, injections can be beneficial to a patient with severe malaria, when prescribed and administered correctly to a patient who is unable to eat orally, or in the case of

vomiting. The misuse of injections has a strong psychological impact on many patients and health workers, who also give in to patient demands, and many believe that injections are quicker and more effective than oral medicines, which are difficult to monitor. Other studies in some countries have reported similar beliefs (36,37). Lack of awareness among the population, lack of confidence among health workers, insufficient supervision and capacity building in the private sector, and patient demand are the causes of injecting drug abuse. A study in Kenya showed that health care providers' confidence in the guidelines affected patients' confidence and adherence to treatment. Patients who tested negative (49%) but received ACTs were less likely to adhere to treatment than those who tested positive (39,40). The availability of RDTs and laboratory facilities in health facilities significantly improves malaria case management. Our results show that health care providers in facilities with laboratory facilities were seven times more likely to adhere to national guidelines than those without (OR: 6.9; 95% CI (1.05-45.65) p=0.004). In addition to this notion of availability, geographical accessibility of a health centre and rurality were factors that significantly influenced patient adherence to malaria treatment. To reduce the practice of self-medication among people living in rural areas, it is necessary to strengthen communication about the dangers of self-medication, inform the public about free patient care, raise awareness and provide appropriate advice to mothers, caregivers and drug sellers for the appropriate management of febrile illnesses at the nearest health centre, as other studies in Cameroon and Nigeria have confirmed that the practice was common but that malaria resistance was low (41,42).

Notably, 45% of patients surveyed reported that the cost of antimalarial drugs in public and private health centres was very high (between €18.29 and €22.86). Despite the national policy of free malaria treatment for children aged 3 to 59 months in Mali in accordance with NMCP guidelines, a study conducted in the community of Kalaban Coro in Mali showed that the average overall cost of

malaria treatment was 24,207 CFA francs (€69), with a standard deviation of 7,921 CFA francs (€12.08). It ranged from 5,115 CFA francs (€7.80) to 45,265 CFA francs (€36.90) (38). Finally, availability, correct completion of stock records and average monthly consumption remain challenges that need to be improved.

CONCLUSION

Although the majority of healthcare providers had a high level of knowledge of the basic concepts of malaria, some gaps were observed in practice. We observed that their non-compliance with national guidelines could probably not be attributed to structural factors, such as the availability of RDTs, or to the knowledge of healthcare providers, but rather to perceptions. The NMCP in Mali needs to develop a communication plan for healthcare providers and patients to encourage behaviour change.

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AUTHORS' CONTRIBUTIONS

This study was designed by PD, SF, MAT, SR. Data were collected by PD, SK, LD, SIY with data analysis and interpretation by PD, SF, SC, MD and PD, SF wrote the first draft of the manuscript. All authors have read and approved the final manuscript

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AVAILABILITY OF DATA AND MATERIALS

Data can be made available by the corresponding author upon reasonable request.

CONSENT TO PUBLICATION

All participants in the study gave their consent for the publication of the research results.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Article 3

Evaluation of the Performance of Rapid Diagnostic Tests for Malaria Diagnosis and Mapping of Different Plasmodium Species in Mali

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Résumé

Le paludisme constitue un problème majeur de santé publique au Mali. Le diagnostic biologique du paludisme au Mali est principalement basé sur l'utilisation des tests de diagnostic rapide (TDR), qui détectent dans sang l'antigène *Histidin Rich Protein*2, spécifique de *P. falciparum.* Toutefois des études réalisées dans le nord et le centre du Mali ont documenté une prévalence variante entre 1 à 20% des autres espèces de *Plasmodium* comme *P. malariae, P. vivax* et *P.* ovale. L'évaluation de la performance des TDR (sensibilité et spécificité) dans le contexte du paysage épidémiologique hétérogène du Mali informera les prestataires de soins de santé sur la fiabilité de ces tests dans les conditions locales. Notre étude, basée sur un diagnostic de référence par Polymerase Chain Reaction (PCR), visait à évaluer la performance diagnostique des différents TDR disponibles au Mali et à cartographier la distribution des espèces de *Plasmodium* dans le pays.

Ainsi nous avons sélectionné au hasard 150 TDR négatifs pour le paludisme et 30 TDR positifs pour le paludisme dans 41 sites répartis dans 9 régions du Mali. L'ADN extrait de la bande de nitrocellulose du TDR a été analysé à l'aide d'une qPCR pan-*Plasmodium*. Les échantillons positifs ont ensuite été analysés avec des qPCR spécifiques à *P. falciparum*, *P. malariae, P. vivax* ou *P. ovale*.

Nos résultats montrent, sur les 1 496 TDR testés, que 258 (18,6 %) étaient positifs pour *Plasmodium* spp., dont 96,9 % pour *P. falciparum*. La prévalence de *P. vivax* atteignait 21,1 % dans le nord. Les TDR présentaient des indices diagnostiques globalement acceptables ; les limites inférieures de l'IC95% des indices de Youden étaient toutes ≥ 0,50, sauf dans le nord (indice de Youden 0,66 (IC95% [0,44-0,82]) et 0,63 (IC 95 % [0,33-0,83]). Dans l'ensemble, les indices diagnostiques des TDR se sont montrés adéquats pour le diagnostic biologique du paludisme au Mali.

Cette étude contribuera à l'optimisation des pratiques de diagnostic, à l'amélioration de la surveillance du paludisme et de comprendre l'épidémiologie du paludisme et, en fin de compte, contribuera à la réduction de la morbidité et de la mortalité dues au paludisme dans la région. Au plan pratique, nous avons recommandé l'utilisation de TDR détectant les antigènes spécifiques de *P. vivax* dans le nord du pays.

Mots clés : Espèces plasmodiales ; Tests de diagnostic rapide ; Sensibilité ; Spécificité ; PCR ; Mali

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Article

Evaluation of the Performance of Rapid Diagnostic Tests for Malaria Diagnosis and Mapping of Different *Plasmodium* Species in Mali

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Abstract: Background: The first-line diagnosis of malaria in Mali is based on the use of rapid diagnostic tests (RDT) that detect the Histidin Rich Protein 2 (HRP2) antigen specific to *Plasmodium falciparum*. Our study, based on a real-time polymerase chain reaction (qPCR) gold standard, aimed to describe the distribution of the *Plasmodium* species in each administrative region of Mali and to assess the performance of RDTs. Methods: We randomly selected 150 malaria-negative and up to 30 malaria-positive RDTs in 41 sites distributed in 9 regions of Mali. DNA extracted from the RDT nitrocellulose strip was assayed with a pan-*Plasmodium* qPCR. Positive samples were then analyzed with *P. falciparum*-, *P. malariae*-, *P. vivax*-, or *P. ovale*-specific qPCRs. Results: Of the 1496 RDTs, 258 (18.6%) were positive for *Plasmodium* spp., of which 96.9% were *P. falciparum*. The *P. vivax* prevalence reached 21.1% in the north. RDT displayed acceptable diagnostic indices; the lower CI95% bounds of Youden indices were all $\geq$0.50, except in the north (Youden index 0.66 (95% CI [0.44–0.82]) and 0.63 (95% CI [0.33–0.83]. Conclusions: Overall, RDT diagnostic indices are adequate for the biological diagnosis of malaria in Mali. We recommend the use of RDTs detecting *P. vivax*-specific antigens in the north.

Keywords: *Plasmodium* species; Malaria; rapid diagnostic tests; sensitivity; specificity; PCR; Mali

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1. Introduction

Despite numerous efforts, malaria remains a major public health problem around the world [1]. According to the latest estimates of the World Health Organization published in December 2022, there were 247 million cases of malaria in 2021 versus 245 million in 2020 [2]. About 619,000 deaths were attributed to this disease in 2021 versus 625,000 deaths in 2020 [1].

The WHO African region alone will account for over 95% of all malaria cases and 96% of all deaths attributable to malaria in 2021. Four African countries account for almost half of all malaria cases worldwide: Nigeria (26.6%), the Democratic Republic of Congo (12.3%), Uganda (5.1%) and Mozambique (4.1%) [1]. Children under five years of age are the most vulnerable targets affected by malaria, constituting 80% of all malaria cases and 96% of all malaria deaths in 2021 [1]. Mali is one of the countries with the highest number of cases and associated deaths. The malaria case fatality was estimated to be 1.25‰ or 1498 deaths

out of 1,197,864 confirmed severe cases. However, malaria remains a major public health concern as it represents one of the main reasons for medical visit in public health facilities with a morbidity rate of 34% and mortality rate of 22% [3].

Children under 5 and pregnant women are the most affected [3]. The main species encountered were *Plasmodium falciparum* (over 85%), *Plasmodium malariae* (10–15%) and *Plasmodium ovale* (1%) [4]. *P. falciparum* causes severe and complicated forms of malaria and has a high fatality rate [4]. Notably, cases of *Plasmodium vivax* have been documented in Mali [5,6].

In 2018, the prevalence of malaria in children aged 6–59 months was 19% according to RDT results. The highest prevalences were found in the regions of Sikasso, Segou, Mopti, and Koulikoro (30%, 26%, 25% and 22%, respectively). The prevalence was low, between 1 and 10% in the regions of Kidal, Taoudenit, and Timbuktu and <1% in the district of Bamako. In the north region, the malaria prevalence in Gao and Menaka was >10% [7]. The infant and child mortality was 101‰ [7]. In 2021, an RDT test-based Malaria Indicator Survey in Mali (MIS 2021) reported a stable 19.4% national prevalence among children aged 6–59 months [8]. Despite the great efforts made by the State and its partners, the 125‰ malaria incidence remains below the expected target of 81‰ in Mali following the DHIS2 2020 (District Health Information Software) [3].

The national malaria control policy in Mali is in line with several international initiatives [9] aiming to "Roll Back Malaria" [10]. Case management through early diagnosis and effective treatment remain the cornerstone of the fight against this disease.

To diagnose suspected cases of malaria, Mali uses microscopic examination of thick and thin blood smears and/or RDTs based on lateral flow immunochromatography [11]. The introduction of RDTs as a diagnostic tool for malaria between 2005 and 2009 has greatly improved the quality of malaria case management in Mali. The proportion of suspected cases who received a biological diagnosis by RDT was 87% and RDTs remain the most widely used tool in health facilities [12].

The RDT is based on the detection of the antigen Histidin Rich Protein 2 (HRP2) antigen in the blood, specific to *Plasmodium falciparum*, which is the most prevalent species in Mali [11]. It is most frequently used in primary healthcare facilities and at the community level by community health workers (CHWs) because of its easy handling [13,14]. It is advantageous in providing results within 15 min and discriminating malaria from non-malaria fevers through the detection of at least one specific antigen. The most used antibodies react to HRP2; other antigens, namely aldolase and plasmodial lactate dehydrogenase (pLDH), are less frequently used. Proper use of RDTs should optimize malaria diagnosis and avoid antimalarial drug resistance; however, the increase in false-negative RDT results poses a new challenge to malaria control [15,16]. Malaria RDT false-negative results can be explained by asymptomatic malaria with parasite densities below the RDTs' limit of detection [17,18]. Another explanation is the increasingly reported deletion of the *PfHRP2/3* genes encoding the HRP2 protein. Whatever the cause, false-negative RDT results in untreated patients who carry parasites, which maintain malaria transmission [19]. All of these issues are a serious threat to National Malaria Control Program (NMCP) [20]. PCR-based diagnostic tests display improved sensitivity, specificity for *Plasmodium* species detection and identification. PCRs detect mixed infections more accurately than traditional methods [21,22]. Although the PCR is not currently optimized for routine diagnosis, it is of major importance in epidemiological studies [23].

However, studies conducted in northern and central Mali have documented other species of *Plasmodium* such as *P. malariae*, *P. vivax*, and *P. ovale* with a prevalence ranging from 1 to 20% [5,6]. In the context of strengthened malaria control policies and the emergence of *Plasmodium* species other than *Plasmodium falciparum*, the present study aimed to provide useful information on (i) the distribution of different *Plasmodium* species in Mali and (ii) the performance of rapid diagnostic tests compared with qPCR.

2. Methods

2.1. Study Sites

Mali is a landlocked country in West Africa located between the 10- and 25-degree north latitudes and between the 4- and 12-degree west longitudes. It covers a 1,241,238 km^2 area. Malaria is generally endemic in most parts of Mali, with a recrudescence during the rainy season [24]. The level of malaria endemicity in Mali varies from one eco-climatic region to the other. Factors responsible for variations in endemicity include rainfall, altitude, temperature, hydro-agricultural development and urbanization [24]. There are 3 climatic zones in Mali that extend from south to north: the Sudano-Guinean zone, which covers 25% of the territory and has a rainfall of approximately 1300 to 1500 mm per year; the Sahelian zone, which covers 50% of the territory and receives rainfall of 200 to 800 mm per year; and the Saharan desert zone, which represents 25% of the territory. This zone is marked by irregular rainfall, often less than 200 mm per year. For this study, we collected RDT cassettes from 41 sites in the following regions of Mali: Kayes, Koulikoro, Sikasso, Ségou, Mopti, Timbuktu, Gao, Kidal and Menaka, from June to December 2021. No sample was collected in the Taoudeni region or the capital city Bamako.

2.2. Study Design

2.2.1. Rapid Diagnostic Test (RDT)

In Mali, the purchase and distribution of rapid diagnostic tests is an essential part of the master plan for the supply and distribution of essential medicines (SDADME). To control consumption needs and guarantee their availability and quality, they are managed similarly to other medicines, by using the same management tools. Decision No. 2011-774/MS-SG of 11 July 2011 made their application mandatory. Its objective is to ensure the correct supply of health products to the population by the Popular Pharmacy of Mali (PPM), which is the State's preferred tool for the supply, storage and distribution of health products through a State-PPM contract plan. This system is supplemented by the private sector through the Private Import and Wholesale Establishments (EPIWG) of approved private suppliers of pharmaceutical products. The RDTs used in our study sites were the SD Bioline Pf Ag (Standard Diagnostics, Inc, 05FK50), Adv Dx Malaria Pf Ag HRP2 (J. Mitra & Co. Pvt. Ltd, IR016025), which both detect the *P. falciparum* specific Histidin-rich Protein 2; and First Response Malaria Ag pLDH/HRP2 (Premier Medical Corporation Ltd, I16FRC25), which detects the protein Lactate Dehydrogenase (pLDH) of *P. falciparum* and screens for other *Plasmodium* species (Pan). RDTs were performed by health workers to reach a diagnosis of malaria in febrile outpatients presenting at health facilities (Figure 1). They were selected proportionally to the different collection sites in the region before including positive and negative RDTs.

2.2.2. Inclusion and Exclusion Criteria

Our study included RDT cassettes that were either negative for *P. falciparum*, with a single band visible in the control window "C" Test, or positive for *P. falciparum*, with a band both in the "C" control and in the "T" test windows. RDT cassettes with no band in the "C" control window were excluded.

2.2.3. DNA Extraction Technique

The selected RDT cassettes were opened under a Type 2 Microbiological Safety Station (MSS) using a dissecting needle (LANCEOLEE Models LT2304, Pakistan), dissecting forceps, and a pair of scissors. The nitrocellulose tape was removed from the plastic cassette and then cut into small 3 × 3 mm pieces using scissors for each sample. The scissors were decontaminated between each sample with 70° ethanol [25]. The cut nitrocellulose samples were then introduced into 1.5mL collection tubes for incubation at room temperature in 400 µL of Nuclisens EasyMag Lysis Buffer (bioMérieux, Craponne, France). The tubes were then centrifuged for 2 min at 13,000 rpm using Lyse&Spin basket tubes (Qiagen, Courtaboeuf, France) to retain the nitrocellulose pieces and carry out extraction

of the filtrate. DNA was extracted from 200 µL of the filtrate following the DNA Blood EZI Advanced XL protocol (QIAGEN Instruments Hombrechtikon, Switzerland), with a final elution volume of 50 µL. Random samples were assayed using a Qubit fluorometer (QUBIT2.0, Life Technologies Villebon sur Yvette, France) to control the DNA extraction quality. The DNA was then stored at −20 °C.

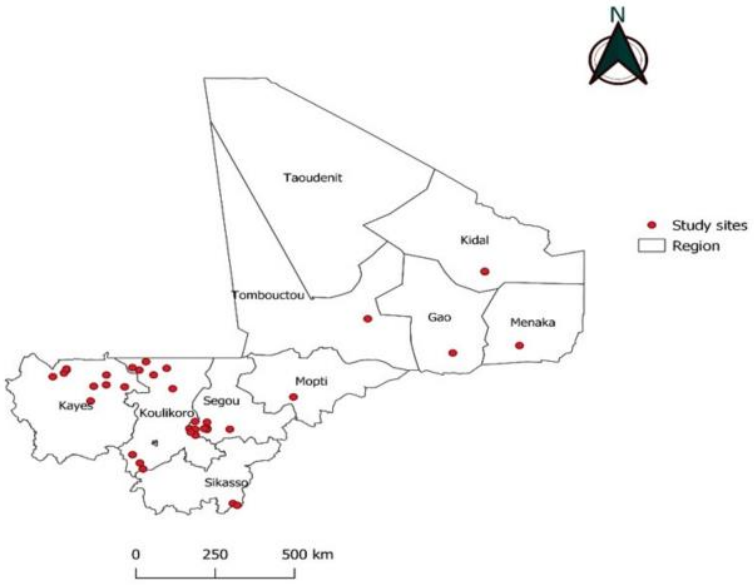


Figure 1. Location of the study sites in the nine administrative regions of Mali where malaria is endemic.

2.2.4. Plasmodium Species Detection by qPCR

DNA extracted from the nitrocellulose strip of malaria-positive and malaria-negative RDT cassettes were first subjected to pan-*Plasmodium* qPCR. In a second step, only the samples that were positive were tested with qPCR systems specific for *P. falciparum*, *P. malariae*, *P. vivax*, or *P. ovale* to identify the *Plasmodium* species [26]. For the amplification reactions, we used 100 µL of Roche Mix (Roche diagnostics GmbH, Mannheim, Germany), 18 µL of each primer (forward and reverse), 12 µL of probe (Var ATS Probe) and 2 µL of distilled water. Then, 20 µL of this mix plus 2 µL of added DNA were distributed in the plate. For the first (pan-*Plasmodium*) qPCR, the samples were deposited in duplicate with three negative controls and one positive control. The PCR conditions are summarized in (Table 1). Real-time PCRs were performed on a CFX96 (BIO-RAD, Marnes-la-Coquette, France) or LightCycler480 II (384-well) (Roche Diagnostic International Ltd, Rotkreuz, Switzerland) thermocycler. The amplification reaction program for the identification of the plasmodial species was as follows: 2 min at 50 °C, 5 min at 95 °C, 45 cycles (10 s at 95 °C, 30 s at 54 °C, 1 min at 60 °C), and 30 s at 40 °C [27]. Several negative controls and a positive control for each species were included in each PCR plate. Samples with an amplification threshold (Ct) value less than 39 were considered positive.

2.2.5. Case Definition

We considered the *Plasmodium*-specific PCR assay as the malaria diagnosis gold standard. Irrespective of the result, a malaria case had to display a positive *Plasmodium*-specific PCR result. True positive = (positive RDT/positive qPCR), false positive = (positive RDT/negative qPCR); false negative = (negative test/positive qPCR), true negative = (negative RDT/negative qPCR). Sensitivity was the proportion of positive RDT among the positive qPCR malaria cases. Specificity was the proportion of negative RDT among the negative qPCR non-malaria cases.

Table 1. List of the primers and probes, targeting the *Plasmodium* spp. 18s rRNA genes, used in our study.

Target Species	Primers and Probes	Sequences
Plasmodium spp.	VAR ATS-F	CCCATACACAACCAAYTGGA
	VAR ATS-R	TTCGCACATATCTCTATGTCTATCT
	Var ATS-Probe	FAM-TRTTCCATAAATGGT
Plasmodium falciparum	Pf-F	TAGCATATATTAAAATTGTTGCAG
	Pf-R	GTTATTCCATGCTGTAGTATTCA
	Pf-probe	6FAM-CGGGTAGTCATGATTGAGTTCATTC
Plasmodium malariae	Pm-F	TAGCATATATTAAAATTGTTGCAG
	Pm-R	GTTATTCCATGCTGTAGTATTCA
	Pm-probe	6FAM- TGCATGGGAATTTTGTTACTTTGAGT
Plasmodium ovale	Po-F	TAGCATATATTAAAATTGTTGCAG
	Po-F R	GTTATTCCATGCTGTAGTATTCA
	Po-probe	6VIC- TGCATTCCTTATGCAAAATGTGTTC
Plasmodium vivax	Pv-F	AGCATATATTAAAATTGTTGCAG
	Pv-R	GTTATTCCATGCTGTAGTATTCA
	Pv-probe	6VIC- CGACTTTGTGCGCATTTTGC

Hybridization temperature was 60 °C for each PCR, and we used Taqman™ hydrolysis probes [27].

2.3. Data Analyses

Data were processed in Excel (version 2013) and analyzed using JavaStat 2-way contingency table analysis [28]. The diagnostic performance of malaria RDTs was determined via sensitivity (Se), specificity (Sp), and Youden and Kappa indices, each with their 95% confidence intervals (CI95%). The agreement between the two diagnostic methods was estimated using Cohen's Kappa index [29]. The Kappa index indicates strong agreement when greater than 0.8 and poor agreement when less than 0.53. The Youden index ($Y = Se + Sp - 1$) combines both sensitivity and specificity to assess the validity of a diagnostic test.

2.4. Ethical Considerations

Our study was authorized by the National Malaria Control Program (number 042/MSDS-SG/NMCP, 4 February 2023), in accordance with Malian regulations on ethics and medical research.

3. Results

During the study period, 3338 malaria RDT cassettes were collected from 41 Health facilities in the 9 regions of Mali, of which 1019 (30.5%) were positive (Table 2). In Table 2, the number of RDTs and the prevalence of positive RDTs for malaria is detailed in each study site. Overall, 3338 RDT cassettes were collected, of which 69.5 tested malaria negative Table S1.

Table 2. Details of the total number collected and the proportions of positive and negative Malaria rapid diagnostic tests in each health center and administrative region.

Area	Health District	RDT Collection Site	Result		Total
			Positive N (%)	Negative N (%)	
Kayes	Diéma	Torodo	6 (2.2)	272 (97.8)	278
		Lakamané	3 (3.4)	89 (96.7)	92
		Lattakaf	0 (0.0)	47 (100)	47
		Débomassassi	0 (0.0)	48 (100)	48
		Koungo	3 (75.0)	1 (25.0)	4
		Lambidou	0 (0.0)	13 (100)	13
		Diancounté Camara	3 (6.4)	44 (93.6)	47
	Yélimané	Kodié	12 (19.0)	51 (81.0)	63
		Csréf	6 (4,1)	139 (95.9)	145
		Dogofry	0 (0.0)	23 (100.0)	23
		Bandiougoula	0 (0.0)	11 (100.0)	11
sub total			33 (4.3)	738 (95.7)	771
Koulikoro	Nara	Bagoini	0 (0.0)	50 (100.0)	50
		Mourdiah	5 (7.4)	68 (100.0)	73
		Kassakaré	4 (12.1)	29 (87.9)	33
		Alasso	7 (20.0)	28 (80.0)	35
		Tiapato	3 (14.3)	18 (85.7)	21
		Waourou	(0.0)	13 (100.0)	13
	Kangaba	Naréna	2 (1.6)	126 (98.4)	128
		Cscom Central	14 (28.0)	36 (72.0)	50
		Séléfougou	0 (0.0)	30 (100.0)	30
sub total			35 (8.1)	398 (91.9)	433
Sikasso	Kadiolo	Cscom central	110 (59.5)	75 (40.5)	185
		Kebeni	12 (16.4)	69 (85.2)	81
		Zégoua	28 (38.9)	44 (61.1)	72
sub total			150 (44.4)	188 (55.6)	338
Segou	Barouéli	Cscom Central	5 (41.7)	7 (58.3)	12
		Dioforogo	13 (65.0)	7 (35.0)	20
		Tamani	7 (35.0)	13 (65.0)	20
		NGara	16 (80.0)	4 (20.0)	20
		Tigui	20 (100.0)	0 (0.0)	20
		Bananido	7 (50.0)	7 (50.0)	14
		N'Gossola	5 (31.2)	11(68.8)	16
		Nianzana	20 (66.7)	10 (33.3)	30
		Yerebougou	16 (80.0)	4 (20.0)	20
		C.S. Réf.	8 (6.2)	122 (93.8)	130
		Ndjila	10 (50.0)	10 (50.0)	20
sub total			127 (39.4)	195 (60.6)	322
Mopti	Mopti	Soufouroulaye	79 (34.3)	151 (65.7)	230
		Fatoma	0 (0.0)	35 (100.0)	35
		Sévaré II	136 (91.9)	12 (8.1)	148
sub total			215 (52.1)	198 (47.9)	413
Tombouctou		Gourma Rharous	400 (66.9)	198 (33.1)	598
Gao	Gao	C.S. Réf. d'Ansongo	30 (13.4)	194 (86.6)	224
Menaka	Menaka	Menaka	21 (36.8)	36 (63.2)	57
Kidal	Kidal	Cscom d'Aliou	9 (14.3)	54 (85.7)	63
		C.S. Réf. de Kidal	20 (14.3)	120 (85.7)	140
sub total			29 (14.3)	174 (85.7)	203
Total			1019 (30.5)	2319 (69.5)	3338

We then randomly selected 30 positive and 150 negative RDTs in each administrative region for further qPCR analyses, except in two regions, namely Menaka and Kidal, where all positive tests (21 and 29 for Menaka and Kidal, respectively) and all negative tests (n = 36) in Menaka were included. Among the 1502 malaria RDT cassettes selected, 6 RDTs

were excluded as invalid, 260 were positive and 1236 negative for malaria. All 1496 RDTs were further analyzed using *Plasmodium* spp. qPCR. Among them, 258 had a positive *Plasmodium* spp. qPCR. Among these 258 *Plasmodium*-positive samples, 251 (96.9%) were positive for *P. falciparum*, 5 (1.9%) for *P. vivax* and 2 (0.8%) for *P. malariae* (Figure 2). We detected two cases of mixed infections, where *P. falciparum* was combined either with *P. vivax* or *P. malariae*, in Kidal or Koulikoro, respectively (Table 3).

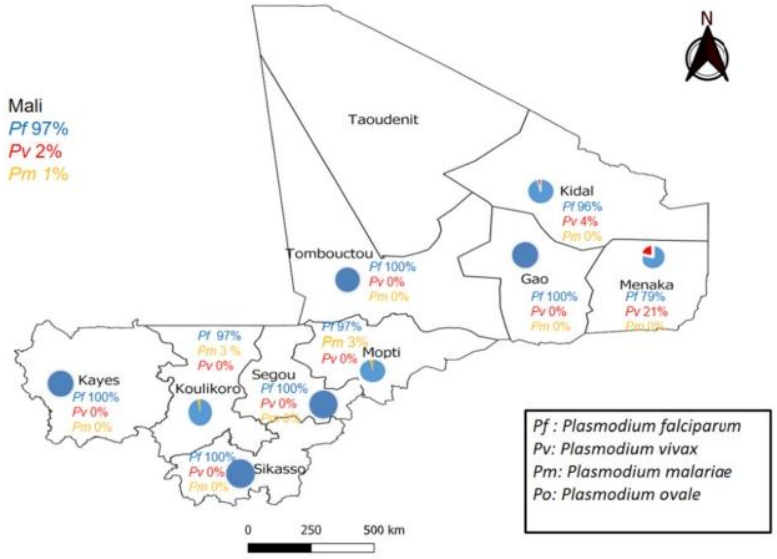


Figure 2. *Plasmodium* species distribution in the nine administrative regions of Mali where malaria is endemic.

Table 3. Distribution of plasmodial species as identified using qPCR according to research sites.

Regions	*P. falciparum*		*P. vivax*		*P. malariae*		*P. ovale*		*P. falciparum* + *P. malariae*		*P. falciparum* + *P. vivax*		Total
	n	%	*n*	%	*N*	%	*n*	%	*N*	%	*n*	%	
Kayes	33	100	0	0	0	0	0	0	0	0	0	0	33
Koulikoro	28	96.6	0	0	1	3.4	0	0	1	3.4	0	0	29
Sikasso	30	100	0	0	0	0	0	0	0	0	0	0	30
Ségou	42	100	0	0	0	0	0	0	0	0	0	0	42
Mopti	33	97.1	0	0	1	2.9	0	0	0	0	0	0	34
Tombouctou	30	100	0	0	0	0	0	0	0	0	0	0	30
Gao	18	100	0	0	0	0	0	0	0	0	0	0	18
Kidal	21	91.3	1	4.3	0	0	0	0	0	0	1	4.3	23
Ménaka	15	78.9	4	21.1	0	0	0	0	0	0	0	0	19
Total	251	96.9	5	1.9	2	0.8	0	0	1	0.4	1	0.4	258

The *P. falciparum* distribution was ubiquitous with a prevalence ranging from 78.9% to 100% of the positive PCRs, depending on the region. *P. vivax* was identified in 21.1% (4/19) and 4.3% (1/23) of the positive PCRs in Menaka and Kidal, respectively. *P. malariae* was identified in 2.9% (1/34) and 3.4% (1/29) of the positive PCRs in Mopti and Koulikoro, respectively. *P. ovale* was detected in none of our samples. Remarkably, we identified two cases of *P. vivax*, for example, in Menaka (4) (Figure 3) and Kidal (1), on negative RDTs, which only detected the HPR2 antigen specific to *P. falciparum*.

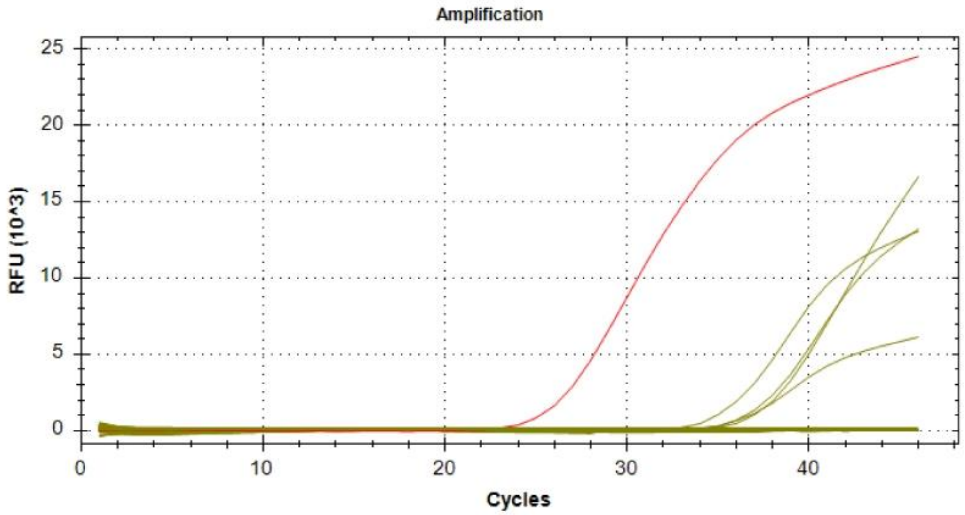


Figure 3. Amplification curves for real-time PCR targeting 18S rRNA of *Plasmodium vivax* species in Menaka (positive control in red and positive samples in green).

We observed 77 discordant RDT results, namely 14.3% (37/258) false-positive and 3.0% (40/1236) false-negative results.

We observed both the high sensitivity (Se) (96.6%) and high specificity (Sp) (98.7%) of the RDTs in the Koulikoro region. In contrast, Se and Sp were lowest in the Kidal (73.9% and 92.4%, respectively) and Menaka (78.9% and 84.2%, respectively) regions. The Youden and Cohen's Kappa indices of RDTs in each region are detailed in Table 4. Overall, RDTs displayed acceptable diagnostic indices. The lower bounds of the 95% confidence intervals (95%CI) of Cohen's Youden and Kappa indices were all$\geq$ 0.50, except in the Kidal and Menaka regions, where the Youden and Kappa indices were 0.66 (95% CI [0.44–0.82]) and 0.60 (95% CI [0.40–0.74], 0.63 (95% CI [0.33–0.83] and 0.62 (95% CI [0.32–0.80], respectively.

Table 4. Diagnostic indices (95% confidence intervals), when compared to the qPCR gold standard, of the rapid diagnostic tests (RDT), compared to the qPCR gold standard, used for the biological diagnosis of malaria in each administrative region of Mali.

Regions	Sensitivity	Specificity	Youden	Kappa
Kayes	87.9% [76.9–90.7]	99.3% [96.9–100]	0.87 [0.74–0.91]	0.90 [0.77–0.94]
Koulikoro	96.6% [84.9–99.8]	98.7% [95.7–98.6]	0.95 [0.81–0.99]	0.94 [0.80–0.98]
Sikasso	80.0% [65.3–90.0]	96.0% [93.1–97.9]	0.76 [0.58–0.87]	0.76 [0.58–0.88]
Segou	71.4% [62.7–71.4]	100% [97.3–100]	0.71 [0.60–0.71]	0.79 [0.67–0.79]
Mopti	88.2% [79–88.0]	100% [98–100]	0.88 [0.76–0.88]	0.92 [0.80–0.92]
Tombouctou	93.3% [80.6–98.7]	97.3% [94.8–98.4]	0.91 [0.75–0.97]	0.88 [0.73–0.95]
Gao	94.4% [73.4–99.7]	92.6% [90.3–93.2]	0.87 [0.64–0.93]	0.68 [0.50–0.73]
Kidal	73.9% [54.5–87.8]	92.3% [89.4–94.4]	0.66 [0.44–0.82]	0.60 [0.40–0.74]
Ménaka	78.9% [59.0–92.0]	84.2% [74.2–90.7]	0.63 [0.33–0.83]	0.62 [0.32–0.80]

4. Discussion

Our findings confirm that *P. falciparum* is the most prevalent species overall in Mali. Yet, this study's key finding is that non-*Plasmodium* species are also endemic, notably *P. vivax* in the northern region of Mali. Remarkably, we observed markedly lower diagnostic indices of malaria RDTs in these regions.

We report a *P. falciparum* prevalence ranging from 79% to 100% of the positive malaria RDTs according to the regions of Mali. Our results are consistent with those of Doumbo

et al., who reported that *P. falciparum* accounted for 98.2% of the positive *Plasmodium* spp. tests since 1988 [4]. In 2011, an increase in the prevalence of *P. falciparum* from 74.13% during the dry season to 63.72% during the cold season was observed in a study by Koita et al. [6]. The 97% *P. falciparum* prevalence in the Mopti region is in line with Konate et al., who reported a 98% prevalence in Badiangara in 2020 [30]. This high, meso- to hyper-endemic, prevalence could be explained by the highly seasonal malaria transmission as well as the climate, which is characterized by 400 to 700 mm of rainfall per year, a short rainy season, from June/July to August/September, and a longer dry season. The region is irrigated by the Niger river and a tributary, the Yamé, which provides numerous breeding sites for *Anopheles gambiae* and *Anopheles funestus* during the 5 months of transmission each year [31].

Remarkably, we found a 21% and 4% prevalence of *P. vivax* in the Menaka and Kidal regions, respectively. Our results are in line with those of Bernabeu et al., who reported a 30% prevalence of *P. vivax* in five cities (Goundam, Timbuktu, Gao, Bourem, and Kidal) located in northern Mali [5]. Briefly, the *P. vivax* prevalence has been increasing in the northern part of the country, especially in Menaka, where we observed a higher prevalence than that observed by Koïta et al. in 2011.

In 2016, a study conducted in four West African countries, Burkina Faso, The Gambia, Ghana and Mali, verified that the prevalence of non-*falciparum* infections was higher in Mali (3.81%, 95% CI [2.22–5.68]) than in The Gambia (0.17%) [32]. To support this assertion, *P. vivax* and *P. malariae* have been found in black African women living in the Bamako area [32]. We also observed *P. malariae* infections in two regions of Southern Mali, namely 3.4% in Koulikoro and 2.9% in Mopti. Our findings are similar to those of a study carried out in the same Koulikoro region, which reported a 2.7% prevalence [33]. *P. malariae infection* is involved in nephrotic syndrome and chronic glomerulopathy, which can progress to end-stage renal failure [34,35] and has been associated with a high burden of anemia [36,37] and death [38]. It is therefore critical for the NMCP to control all non-*P. falciparum* human infections to achieve the malaria elimination goal.

We found no *P. ovale* in our samples. However, this parasite species, despite being less dangerous than *P. falciparum*, remains a public health burden that should be included in malaria elimination programs [39–41]. *P. ovale* has been reported to have a prevalence of around 2% in Mali. [42]. Conventional PCR followed by DNA sequence analysis is commonly used to differentiate the two subspecies *P. ovale curtisi* and *P. ovale wallikeri*, which have been documented in Mali and other West African countries [40,43,44].

The rapid detection and identification of *Plasmodium* parasite species with improved diagnostic tools are key for operative NMCPs [31]. In our study, the sensitivity of RDTs was higher than 80% in each region, except in Kidal and Segou, where it was moderate. The relatively lower performance of RDTs observed in these regions could be explained by the enhanced analytical sensitivity of the qPCR compared to RDTs. The majority of false-negative RDTs had either a fine band or weak staining, which could be related to low *Plasmodium* density in the blood, as already assumed in previous studies [45,46]. This condition could explain a decrease in both the sensitivity and specificity of RDTs. Another explanation could be the emergence of HRP-2/3 gene deletion [16,47]. The problem of false positives has been previously addressed; it could be due to the persistence of the HRP-2 protein in the patient's blood up to several weeks after post-treatment parasite clearance [48]. In the Kidal and Menaka regions, the Youden and Kappa indices were lower than in the other regions. In Kidal, *P. vivax* was detected in a false-negative RDT that was designed to detect only the *P. falciparum*-specific HRP2 antigen. This relative emergence of non-*P. falciparum Plasmodium* species could be explained by the inability to detect other *Plasmodium* species of RDTs based solely on the detection of the *Pf HRP-2* antigen, which is specific to *P. falciparum*. Therefore, adapting the diagnostic tools to the regional epidemiology should strengthen malaria control in Mali. In particular, the use of RDTs that are capable of detecting pan-*Plasmodium* antigens, such as lactate dehydrogenase

(pLDH) or aldolase, should be advocated in regions where non-*P. falciparum Plasmodium* species are endemic [14,49].

The main strength of our study was its country-wide wide coverage, which allowed us to obtain a comprehensive picture of RDTs performance and *Plasmodium* species distribution in Mali. In contrast, our study also has some limitations, the main one being potential selection bias. Indeed, the samples were collected through a healthcare-facility-based epidemiological surveillance system, and only 40% of the population attends these healthcare facilities. Another limitation of our study was the deliberate selection of sites due to political insecurity constraints.

5. Conclusions

This study confirms that malaria rapid diagnostic tests (RDTs) contribute to significant improvement in the quality of malaria management in Mali. The results of this study suggest that the NMCP should recommend the use of RDTs with high analytical sensitivity that would be able to detect low parasitemia and set up a surveillance system for HRP2/3 deletion in patients, as it has already been documented in asymptomatic individuals in Mali. The NMCP should also recommend adapting RDTs' specificity to the local *Plasmodium* species epidemiology to enhance malaria diagnosis in Mali.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph21020228/s1, Table S1: Detail of the total number and the prevalence of positive and negative Malaria Rapid Diagnostic Tests in each health center.

Author Contributions: P.D., M.C., M.A.T. and S.R. designed the study and wrote the first version of the manuscript. P.D., M.C., A.Z.D., L.D. and M.M. performed the analysis and interpretation of the results. M.H.M. and A.K. contributed to the collection and transport of samples. M.C., M.A.T. and S.R. contributed to the critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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PARTIE III

Evaluation épidémiologique de la prévalence de la Fièvre récurrente à tiques au Mali

Article 4

Quantitative polymerase chain reaction from malaria rapid diagnostic tests to detect *Borrelia crocidurae* in febrile patients in Mali

Pascal Dembélé, Adama Zan Diarra, Papa Mouhamadou Gaye, Armel Agokeng, Li Bing, Mahamadou A Thera and Stéphane Ranque

Résumé

Dans cette partie de notre étude, nous nous sommes orientés à l'évaluation épidémiologique de la prévalence de la borréliose à tique au Mali. Après le paludisme, la fièvre récurrente à tique constitue l'une des premières causes de morbidité par maladie vectorielle. Les fièvres récurrentes transmises (TBRF) sont des infections fébriles négligées causées par les tiques molles du genre *Ornithodoros* spp. Ces tiques vivent dans les terriers des petits mammifères et piquent l'homme occasionnellement quand les terriers s'ouvrent surtout dans les habitations.

Actuellement, on sait très peu de chose sur la morbidité de cette maladie au Mali malgré l'existence des rapports antérieurs sur la présence de *Borrelia crocidurae* chez la tique vectrice et les petits mammifères rongeurs qui sont les réservoirs et la possibilité d'une co-infection humaine avec le paludisme est possible. Le manque des services de diagnostic appropriés, ou d'expertise technique capable de discriminer les patients suspects de paludisme des autres causes de maladie fébrile d'origine inconnue fait que la maladie transmise par les tiques reste toujours négligée et sous-diagnostiquée chez les patients fébriles au Mali. Cette maladie fébrile aiguë produit de multiples récidives de signes et symptômes non spécifiques, notamment de la fièvre, des maux de tête, une hépatomégalie, une splénomégalie, une anémie des myalgies et des arthralgies. Ces symptômes peuvent se confondre parfois à ceux du paludisme. L'objectif de cette étude visait à rechercher l'ADN de *B. crocidurae* chez des patients fébriles au Mali à partir des cassettes TDR du paludisme à l'aide de la biologie moléculaire.

Entre juin à décembre 2021, des TDR, négatifs et positifs pour le paludisme, ont été collectés dans 41 sites des neuf régions du Mali. Des outils moléculaires (qPCR et PCR standard) ont été utilisés pour détecter la présence de l'ADN de *B. crocidurae.*

L'ADN de *B. crocidurae* a été détecté dans (5% (9/180) des TDR au paludisme collectés seulement dans la région de Kayes.

Pour la première fois au Mali nous avons utilisé la qPCR pour évaluer la prévalence de la borréliose à tique à partir des cassettes TDR du paludisme chez les patients fébriles. Il est nécessité de renforcer le système de surveillance des maladies à transmission vectorielle comme la borréliose dans la communauté et nous plaidons pour la mise en place des outils de diagnostic de la TBRF sensibles et spécifiques à *B. crocidurae*, pour une meilleure prise en charge de cette infection zoonotique endémique. Un renforcement des compétences des microscopistes permettrait également d'améliorer le diagnostic de cette maladie.

Mots clés : *Borrelia crocidurae,* TDR, Paludisme, qPCR, Mali

Quantitative polymerase chain reaction from malaria rapid diagnostic tests to detect *Borrelia crocidurae* in febrile patients in Mali

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Abstract

Tick-borne relapsing fever (TBRF) is caused by *Borrelia* species transmitted to humans by soft ticks of the genus *Ornithodoros*. At present, very little is known about the morbidity of this disease in Mali, despite the fact that it exists with a risk of human co-infection with malaria. The lack of appropriate diagnostic services, or technical expertise to discriminate malaria suspects from other causes of febrile illness of unknown origin, means that the tick-borne disease remains neglected and underdiagnosed in febrile patients in Mali. Our study looked at finding the DNA of *Borrelia crocidurae* in febrile patients in Mali from malaria RDTs using molecular biology.

Methods: Between June and December 2021, negative and positive RDTs for malaria were collected at 41 sites in Mali's nine regions. Molecular tools (qPCR and standard PCR) were used to detect the presence of *B. crocidurae* DNA.

Results: *B. crocidurae* DNA was detected in 5% (9/180) of malaria RDTs collected only in the Kayes region.

Conclusion: The present study demonstrates the presence of agents of borreliosis with relapsing fever in febrile patients, although this disease is rarely diagnosed in Mali.

Key words: *Borrelia crocidurae*; RDT; Malaria; epidemiology; qPCR; Mali

Introduction

Transmitted recurrent fevers (TBRF) are neglected febrile infections caused by soft ticks of the genus *Ornithodoros* spp .[1,2]. Wild rodents and insectivores are common reservoir hosts. They are caused by several spirochetes of the genus *Borrelia* (*B. crocidurae, B. duttonii, B. recurrentis, and B. hispanica*), which are endemic in subtropical regions worldwide [3–5]. This acute febrile illness causes multiple recurrences of nonspecific signs and symptoms, including fever, headache, hepatomegaly, splenomegaly, anemia, myalgias, and arthralgias. These symptoms are similar to those of malaria [6,7]. The first clinical cases of (TBRF) have long been described as a major cause of morbidity and mortality in many parts of Africa [8,9].

In West Africa, *B. crocidurae* is recognized as the only vector responsible for (TBRF) disease, especially in many rural populations living in extremely precarious conditions [10,11]. Studies have shown that the incidence of TBRF in West Africa is high, accounting for 13% of febrile illnesses treated in rural health facilities [2]. In Tanzania, particular attention is currently being paid to TBRF, which is one of the ten leading causes of death in children under 5 year of age [12].

Microscopy is used to diagnose the disease. It is usually based on observation of the spirochete in thick blood smears during the acute febrile phase, a technique identical to that used to detect malaria haematozoa [13]. On the other hand, molecular methods (PCR or qPCR) remain the best diagnostic tools because of their high sensitivity and specificity [14,15]. The lack of diagnostic tools in Africa has been identified as a major gap in healthcare delivery on the continent [16]. In Mali, for example, studies have reported the presence of *B. crocidurae* in tick vectors and small mammal rodents, which are the reservoirs [17–19]. It has been shown that West African tick-borne relapsing fever (TBRF) is widespread, especially in the Saharan, Sahelian and Sudano-Sahelian regions [3], where average annual precipitation is between 50 and 250 mm, 250 and 500

mm and 500 and 750 mm respectively [3]. The persistence of drought in sub-Saharan countries potentially contributes to the proliferation of *Ornithodoros sonrai* responsible for *B. crocidurae* [20,21]. Most studies on TBRF in West Africa have been carried out in Senegal [14,22,23], where the TBRF vector ticks are geographically distributed over the northern two-thirds of the country, north of the 750 mm isohyet, and the southern limit of the vector tick corresponds approximately to latitude 13°40'N [3].

However, TBRF remains the second challenge for many malaria-endemic African countries, including Mali, where blood smears are tested only for Plasmodium in blood [24]. Because of the rarity of appropriate diagnostic services, such as molecular biology laboratories, it is clear that TBRF cannot be diagnosed quickly and reliably [23]. Indeed, when misdiagnosed, many cases of TBRF will be considered treatment-resistant malaria, as studies in Togo have shown, where febrile patients were often misdiagnosed as malaria [6]. Rapid diagnostic tests (RDTs) remain the most used tool in healthcare establishments, due to their ease of use. However, several studies have pointed to their usefulness as a potential source of DNA for molecular analysis[7,25–27]. Therefore, we used DNA extracted from RDTs to determine the prevalence of TBRF as a cause of fever in RDT-negative patients in Mali.

Materials and methods

Study sites

The samples in this study came from the same areas as those in our previous study [28]**.** The RDTs were performed for the standard routine medical diagnosis at health care centres. They have been deidentified for respect of patient's confidentiality. According to the Malian regulations on ethics and medical research, regulatory approval has been granted by the competent authority of the National Malaria Control Program under number 064/MSDS-SG/NMCP, February 8, 2023.

Molecular analysis

DNA extraction technique used for *Borrelia* detection from malaria RDTs

DNA was extracted from RDT cassettes (30 positive and 150 negative) as previously described [29].

Real-time PCR detecting *Borrelia* spp.

DNA samples extracted from the malaria RDT cassette band were subjected to real-time polymerase chain reaction using primers and probes targeting *Borrelia* spp. (Bor_ITS4_F 5'-GGCTTCGGGTCTACCACATCTA-3'; Bor_ITS4_R 5'-CCGGGAGGGGA GTGAAATAG-3' and a probe Bor_ITS4_ P (6FAM-TGCAAAAGGCACGCCATCACC) TAMRA [18]. The samples tested positive for *Borrelia* spp. using the *ITS4* gene were subjected to a second qPCR using the *glpQ* gene specific for *B. crocidurae* using a primer (croci_glpQ_F5'-CCTTGGATACCCCAAATCATC-3';croci_glpQ_R5'-GGCAATGCATCA ATTCTAAAC-3'and a probe *B croci*_glpQ_P (6FAM-ATGGACAAATGACAGGTCTTAC –MGB [30], [31].

To ensure the reliability of our results, each PCR analysis was run with negative controls and a positive control derived from the DNA of *B. crocidurae* strains grown in the laboratory. Samples were considered positive only if the qPCR cycle threshold (Ct) was less than 36 [32].The mix composition and qPCR program were the same as those already described by Keita et al [33].

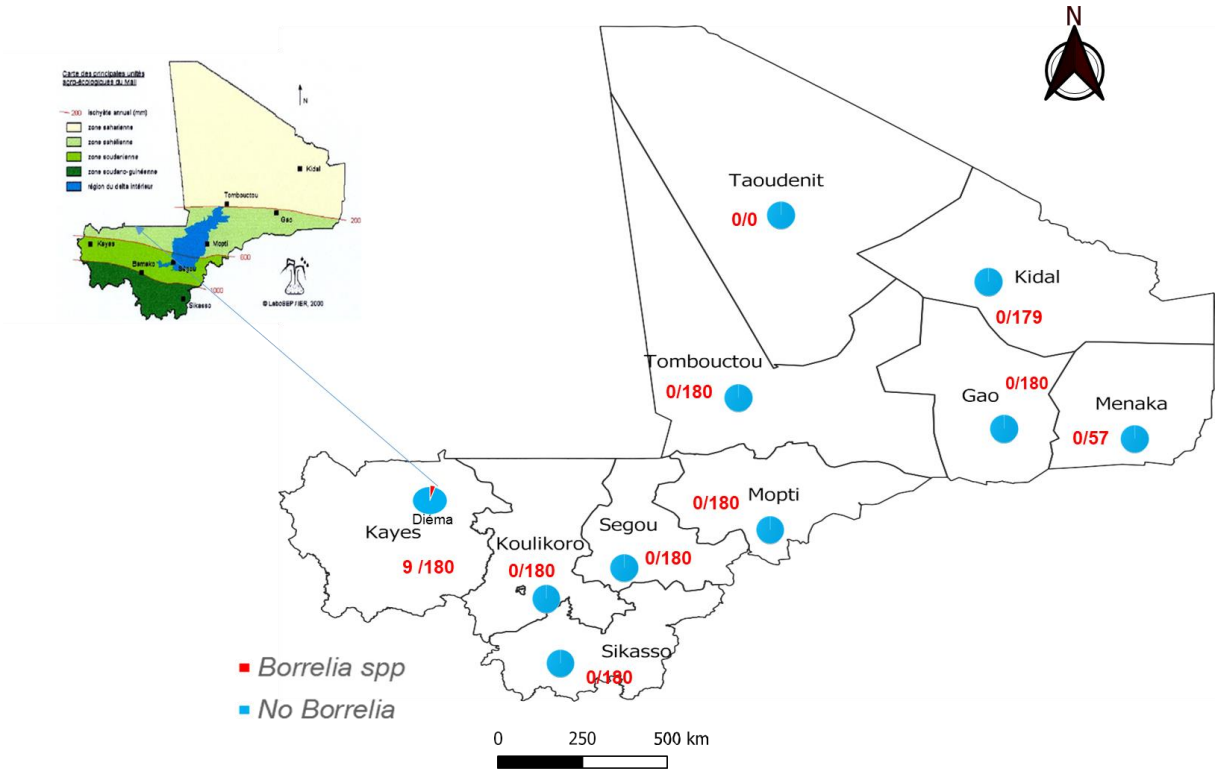
DNA based sequencing

All samples positive for the two genes in qPCR were subjected to standard PCR and sequencing. The standard PCR amplification targeting the (*flaB*) gene (B_F1 (5'-TAATACGTCAGCCATAAATGC-3' and B_R1 (5'-GCTCTTTGATCAGTTATCATTC-3') [14] was carried out using a thermocycler (Applied Biosystems, Foster City, CA, USA). The composition of the mix and the standard PCR program and sequence were the same as those already described by Rahal M and al. [34]. The amplified products were purified

using a Macherey Nagel plate (NucleoFast 96 PCR, Düren, Germany) and sequenced using the same primers (B_F1 and B_R1). Sequencing was performed using the same primers as for PCR with BigDye Terminator v1.1, v3.15× sequencing buffer (Applied Biosystems, Warrington, UK) and run on an ABI 3100 automated sequencer (Applied Biosystems).The sequences obtained were assembled and processed using ChromasPro (www.technelysium.com.au/ chromas.html) [35] and aligned with BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The corrected sequences were compared with existing sequences in GenBank (http://blast.ncbi.nlm. nih.gov/Blast.cgi) using basic local alignment search tool (BLAST) analysis. The sequences of samples positive for *borrelia crocidurae* by qPCR are presented in figure 2. Phylogenetic trees were constructed using maximum likelihood method with model selection determined by Molecular Evolutionary Genetics Analysis (MEGA) v7.0.26 software (Tamura K, 1993). The statistical support of the inner branches of the trees was assessed by bootstrapping with 1000 iterations.

Results

A total of 1496 malaria RDT samples (260 positive and 1236 negative) from febrile patients were tested for *Borrelia* spp. by real-time PCR. Of these, only (0.6%) 9/1496 RDTs were positive for both *ITS4* and *glpQ* genes (**figure1**). *Borrelia crocidurae* DNA was detected in 5% of RDTs tested (9 out of 180 randomly selected), collected in the Kayes region. Querying all the sequences obtained against the GenBank database shows that they have 99 to 100% coverage and 100% identity with the *B. crocidurae* sequence (JX119098) from Senegal. The phylogenetic tree shows that our sequences are close to those already found in Mali, Mauritania and Senegal (Figure 2).



1423 **Figure**: Map of the distribution of *Borrelia* prevalence in malaria RDTs in Mali

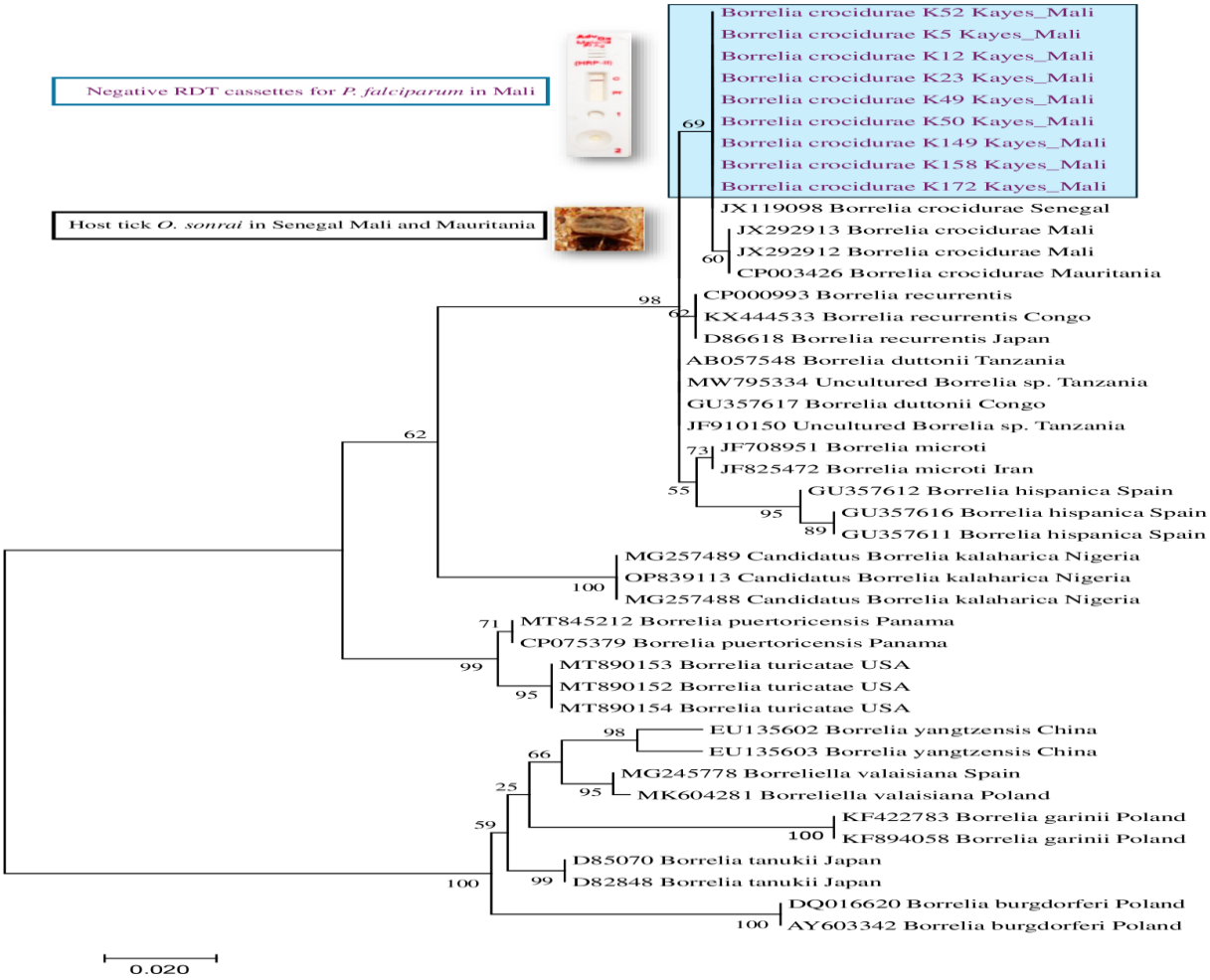


Figure 2: Phylogenetic tree (maximum likelihood method, bootstrap 1,000) of partial sequence data for the flagellin gene, (*FlaB*) of *B. crocidurae* identified from malaria-negative *P. falciparum* RDTs. Sequences of the *B. crocidurae FlaB* gene found in *O. sonrai* ticks and sequences from other *Borrelia* species were processed for comparison. The tree with the highest likelihood (-1092.19) is shown.

Discussion

Tick-borne relapsing fever (TBRF) is the most common vector-borne bacterial disease in humans in West Africa. Between December 2007 and October 2011, a study carried out in 20 villages in southern Mali reported the presence of the vector *O. sonrai* in small mammals and ticks collected from rodent burrows, and found that 17.3% of ticks were infected with spirochetes [17]. In this study,

we recorded a relatively high prevalence of 6% (9/150) *B. crocidurae* in negative malaria RDTs from febrile patients at a rural health center in the Diéma health district, located in the Kayes region bordering Senegal and Mauritania, two *Borrelia*-endemic countries [2,37].

Most studies on TBRF in West Africa have been carried out in Senegal. Our results are comparable to those of Mediannikov et al, carried out in Senegal between June 2010 and October 2011, according to which (7.3%) or 115 out of 1,566 blood samples from febrile patients were positive for *B. crocidurae*. The highest proportion of positive samples was found in Niakhar, at 19.1% (33/173) [38]. Patients aged 7 to 15 years were the most affected: 13.5% (33/173). The proportion of *Borrelia*-positive samples was significantly higher for samples collected during the dry season (16.9%, 40/237) than during the rainy season (6.9%, 30/432). Several previous studies have already demonstrated the influence of climate change on infectious diseases [21,39,40]. December 2016, on febrile patients examined respectively in one health center and three health posts in Niakhar, Ngayokheme, Toucar and Diohine, revealed that borreliosis cases accounted for 12% (94/800) of fever episodes, and all age groups were infected, with children and young people aged 8 to 14 and 22 to 28 the most infected by the disease (16% and 18.4%).

The increase in the number of cases is demonstrates that TBRF really is a public health problem in Senegal and other neighbouring countries such as Mali. Contrary to data from the previous study, the highest number of TBRF cases occurred in August [22], which also very often coincides with the period of high malaria transmission. To avoid over-diagnosis of malaria cases, health care providers must adhere to the following malaria case management guidelines [41], because the correct use of RDTs should optimize malaria diagnosis and prevent resistance to antimalarial drugs. They are also considered a better source of DNA for molecular analysis [25]. In this context, a similar study carried out by Ndiaye

EHI et al at the IHU Méditerranée Infection laboratory recorded a 7% prevalence of *B. crocidurae* in malaria *P. falciparum* RDTs collected in Senegal [7].

The *B. crocidurae* bacteria detected in our malaria-negative RDT DNA samples, confirmed by standard PCR, has already been identified in *O. sonrai* ticks in Senegal, Mauritania and Mali [1,42]. Phylogenetic analysis of the *flaB* gene showed that the *Borrelia* we obtained were similar to those already found in Senegal [17,42]. The results presented in this study confirm the unique circulation of the *B. crocidurae* pathogen causing TBRF in febrile patients in Mali.

Conclusion

There is a need to strengthen the surveillance system for vector-borne diseases such as borreliosis in the community, and to introduce diagnostic tools for TBRF that are sensitive and much more specific to *B. crocidurae*, for better management of this endemic zoonotic infection. Enhancing the skills of microscopists could improve diagnosis of the disease.

Author contributions

P.D., A.Z.D., M.A.T. and S.R. conceived the study and drafted the first version of the manuscrip**t.**

P.D., A.Z.D., P.M.G., L.B., and., A.A analyzed and interpreted the results.

A.Z.D., M.A.T. and S.R. contributed to the critical revision of the manuscript. All authors have read and approved the version

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Conflicts of interest

The authors declare no conflict of interest**.**

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Partie IV

Evaluation épidémiologique de la prévalence de la mansonellose à *M.perstans* au Mali

Article 5

Molecular Epidemiology of *Mansonella perstans* in Mali

Pascal Dembélé, Manthia Diallo, Papa Mouhamadou Gaye, Mady Cissoko, Mahamadou A Thera and Stéphane Ranque

Résumé

La mansonellose est l'une des maladies tropicales les plus négligées. Elle est causée par des nématodes filaires du genre *Mansonella*, dont les différentes espèces sont : *Mansonella perstans (M. perstans), Mansonella ozzardi* et *Mansonella streptocerca*.

M. perstans est l'espèce la plus fréquente au Mali. Malgré qu'elle soit considérée comme une filariose mineure, et peu, voire pas du tout, symptomatique, des études ont démontré que *M. perstans* est capable de provoquer des démangeaisons, des douleurs articulaires, une hypertrophie des ganglions lymphatiques, des graves troubles neurologiques et des œdèmes de Calabar. Le diagnostic est généralement effectué en détectant les microfilaires, dans le sang périphérique ou la peau, dépourvues de périodicité. Il existe très peu de données sur l'épidémiologie de cette maladie au Mali et notre étude visait à décrire la distribution géographique de *M. perstans* au Mali.

De juin à décembre 2021, nous avons sélectionné au hasard 30 tests de diagnostic rapide (TDR) positifs et 150 négatifs pour le paludisme dans chaque région administrative pour les analyser par qPCR. Dans deux régions, à savoir Ménaka et Kidal, tous les positifs (21 et 29, respectivement) et tous les négatifs (n=36) à Ménaka ont été inclus à partir des patients fébriles dans 41 sites répartis dans 9 régions du Mali. Nous avons extrait l'ADN sanguin des bandes de nitrocellulose des TDR. Tous les échantillons ont été analysés à l'aide d'une PCR en temps réel (qPCR) pan-filaires. Les échantillons positifs ont été testés avec une RT-PCR spécifique de *M. perstans* ciblant l'espace transcrit interne 1 (ITS1) de la séquence du gène de l'ARNr. L'ADN de *M. perstans* a été détecté dans 2 (1,1%) des 180 échantillons analysés de la région de Koulikoro. Les 1494 échantillons des 8 autres régions du Mali étaient tous négatifs.

Nos résultats ont montré une prévalence relativement faible de *M. perstans* au Mali en utilisant la qPCR sur les TDR du paludisme. D'autres études sont

nécessaires pour évaluer la prévalence de cette infection filarienne vectorielle négligée, qui peut être limité géographiquement.

Mots-clés : *Mansonella perstans* ; TDR paludisme ; Mansonellose ; qPCR ; épidémiologie ; Mali

Molecular Epidemiology of *Mansonella perstans* in Mali

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Abstract

Context: Mansonellosis is one of the most neglected tropical diseases. It is caused by filarial nematodes of the genus *Mansonella*, the different species of which are *Mansonella perstans, Mansonella ozzardi* and *Mansonella streptocerca*. *M. perstans* is the most abundant species in Mali. The aim of our study was to describe the geographical distribution of *Mansonella* sp. in Mali.

Methods: From June to December 2021, we randomly selected 30 positive and 150 negative RDTs in each administrative region for further qPCR analyses, except in two regions, namely Menaka and Kidal, where all positive tests (21 and 29 for Menaka and Kidal, respectively) and all negative tests (n = 36) in Menaka were included from patients with fever in 41 sites distributed in the nine regions of Mali and. We extracted blood DNA from the RDTs nitrocellulose strips. All samples were screened using a pan-filariae real-time PCR (qPCR). The positive samples were tested with a (*M. perstans* specific qPCR and we analysed the internal transcribed spacer 1 (ITS1) of the rRNA gene sequence.

Results*: M. perstans* DNA was detected in 2 (1.1%) of the 180 samples from the Koulikoro region. All 1494 samples from the 8 other regions of Mali were negative.

Conclusions: Our results showed a relatively low prevalence of *M. perstans* in Mali using qPCR on malaria RDTs. Further studies are needed to assess the prevalence of this neglected vector-borne filarial infection, which may be geographically restricted.

Keywords: *Mansonella perstans*; malaria RDT; Mansonellosis; qPCR; epidemiology; Mali

Introduction

Mansonella perstans infection is a little-known parasitic disease caused by a filarial nematode. It is transmitted to humans by the bites of tiny blood-sucking flies of the genus *Culicoides*, commonly known as midges, which feed on the blood of their hosts and enable the transmission of filarial parasites. *M. perstans* is widespread in several regions of sub-Saharan Africa, where it is an often-underestimated public health problem. It is also found in some parts of Central and South America, although its geographical distribution is more restricted [1].

Three main species are responsible for human mansonellosis: *M. perstans, M. streptocerca* and *M. ozzardi* [2,3]. *M. perstans* and *M. streptocerca* are mainly found in West, East and Central Africa, where they often coexist with other parasitic infections. These regions are characterised by environmental conditions that favour the proliferation of biting midges, thereby increasing the risk of transmission. *M. ozzardi* is mainly found in Latin America, with an uneven geographical distribution. It is endemic in certain regions of Central and South America, as well as on several Caribbean islands [3]. In addition to *M. perstans*, a potential new species of *Mansonell*a sp "DEUX" has recently been identified in febrile children in Gabon [4].

M. perstans is endemic to a large part of sub-Saharan Africa, extending from Senegal to Angola, through Central Africa, north to south-west Sudan, east to Uganda, Kenya, Tanzania and south to Zimbabwe [5]. In the most affected countries, *M. perstans* is prevalent in poor rural populations [5,6]. It is important to note that more than 100 million people are currently infected in Africa alone, and approximately 600 million people live in 33 countries at high risk of *M. perstans* infection [1]. The main data on the geographical distribution of *Mansonella spp*. are based on the morphological identification of microfilariae in thick and/or thin blood smears [1]. Adult worms appear to reside in various serous membranes (pleura, peritoneum, pericardium and mesentery) [7], while their

larval form, the microfilariae, circulate in the peripheral blood or skin without any circadian periodicity [8]. It is uncertain how long mature *M. perstans* adults survive in the human body, but microfilariae live for about 4 months [9]. The life cycles of the three human *Mansonella* species parasites responsible for mansonellosis have distinct biological, clinical and epidemiological characteristics [1,3,10]. The biological characteristics of these three filariases are summarised in Table S1 [1,10].

It is now generally accepted that mansonellosis is often asymptomatic or associated with relatively mild symptoms. Blood eosinophilia is a common feature [10]. However, in mansonellosis specific clinical manifestations may be associated with worm migration, including allergic reactions such as Calabar oedema, pruritus, fever, headache, pericarditis and pleural effusion [1]. The time lag between infection and the appearance of microfilariae in peripheral blood remains unknown [5]. Conventional diagnosis of *M. perstans* infection is based on the detection of microfilariae in blood smears [7,11]. In a study carried out in a rural area of Senegal, the overall prevalence of *M. perstans* was 14.5% in febrile and non-febrile patients. The prevalence increased with age and reached up to 40% to 50% in rural areas [12,13]. since 1912, it is known that *M. perstans* is the endemic species in Mali [14].

In a study conducted in a rural area of Senegal, the overall prevalence of *M. perstans* was 14.5% in febrile and non-febrile patients. The prevalence increased with age, reaching up to 40-50% in rural areas [12,13]. *M. perstans* has been known to be endemic in Mali since 1912 [14]. Data on mansonellosis in Mali are relatively scarce. Thiroux found a 46% prevalence of M. perstans carriage in soldiers from the former French Sudan (now Mali) [14]. In 1978, a 6.7% prevalence of M. perstans carriage was reported in schoolchildren aged 6-17 years in Kadiolo (Central District) [14]. In 1979, Rougemont et al. found a mansonellosis prevalence of 65% in Masantola, a village in Kolokani Circle. The

prevalence of mansonellosis in the village of Lofigué in Kadiolo was 28.45%. The prevalence of mansonellosis ranged from 5.5% to 78% in the population of 10 villages of Kolokani district (Central District) in April 1978 [15-17]. Since then, no data have been collected on this disease in Mali. The aim of this study was to assess the prevalence of this neglected filariasis in the different regions of Mali using qPCR.

Methods

Study sites

Mali is a landlocked country in West Africa between 10- and 25-degrees north latitude and 4 and 12 degrees west longitude. It covers an area of 1,241,238 km2. There are three climatic zones in Mali, from south to north: the Sudano-Guinean zone, which covers 25% of the country and has an annual rainfall of between 1,300 and 1,500 mm; the Sahelian zone, which covers 50% of the country and has an annual rainfall of between 200 and 800 mm; and the Saharan desert zone, which covers 25% of the country. This zone is characterised by irregular rainfall, often less than 200 mm per year. In this study, we collected malaria RDT cassettes from June to December 2021 from 41 sites in the following regions of Mali: Kayes, Koulikoro, Sikasso, Ségou, Mopti, Timbuktu, Gao, Kidal and Menaka. Notably, no samples were collected from the Taoudeni region or the capital, Bamako. Of the 3338 malaria RDT cassettes collected from 41 health facilities in the 9 regions of Mali, 1019 (30.5%) were positive and 2319 (69.5%) were negative. We then randomly selected 30 positive and 150 negative RDTs in each administrative region for further qPCR analysis, except in two regions, namely Menaka and Kidal, where all positive tests (21 and 29 for Menaka and Kidal, respectively) and all negative tests (n = 36) in Menaka were included.

Ethical Considerations

The RDTs were performed for the standard routine medical diagnosis at health care centres. They have been deidentified for respect of patient's confidentiality. According to the Malian regulations on ethics and medical research, regulatory approval has been granted by the competent authority of the National Malaria Control Program under number 064/MSDS-SG/NMCP.

DNA extraction from RDTs cassettes

DNA was extracted from the RDTs cassettes using the EZ1 DNA Blood Kit (Qiagen) on an EZ1 BioRobot extraction device (Qiagen, Hilden, Germany) according to the manufacturer's instructions and as described in [18]. Genomic DNA was extracted from a total of 1496 RDTs cassettes that were previously used for the detection of *Plasmodium falciparum* (*P. f.*) as described in [18]. In this study we used the same DNA template to detect filariae parasites, including *M. perstans*.

Pan-filarial and M. perstans specific real-time PCR

Real-time PCR (qPCR) was used to detect all filaria species or *M. perstans* in all DNA extracts from RDT cassettes (n= 1496). The qPCR reactions targeted the 28S region for all filaria species and another specific region for *M. perstans*. The primers and probes used are listed in Table 1. The pan-filariae qPCR conditions were identical to those described in [19]. All pan-filariae positive samples were further tested with *M. perstans* specific qPCR using the conditions described in [12]. All qPCR reactions were performed on a CFX96 thermal cycler (Bio-Rad, Marnes-la-Coquette, France) in a 20 µL reaction mixture containing 5 µL DNA template, 10 µL master mix, 3.5 µL sterile distilled water, 0.5 µL of each primer and 0.5 µL TaqMan probe (Applied Biosystems, Foster City, CA, USA). The amplification programme consisted of an initial 2-minute denaturation step at 50°C, followed by 3-minute denaturation at 95°C, then 40 cycles of 95°C for

30 seconds and 60°C for 1 minute before holding the sample at 4°C. For each qPCR run, a negative control (sterile distilled water) and a positive control, either *Dirofilaria immitis* for pan-filariae qPCR or a plasmid developed in our laboratory for *M. perstans* qPCR, were used.

Table 1. Primers used in the real-time PCR, Pan-filaria and *Mansonella perstans* parasite and *Mansonella* spp. sequencing protocols.

Primer names	Target region	Organism	Primer sequences (5'-3')	Reference
qFil_F	28S rRNA	Pan-filaria	TTGTTTGAGATTGCAGCCCA	[19]
qFil_R			GTTTCCATCTCAGCGGTTTC	
Probe (FAM 5'–3'TAMRA)			CAAGTACCGTGAGGGAAAGT	
Mp_Hub_F	ITS	*M. perstans*	AGGATCATTAACGAGCTTCC	[12]
Mp_Hub_R			CGA ATATCACCGTTAATTCAGT	
Probe (FAM 5'–3'TAMRA)			TTCACTTTTATTTAGCAACATGCA	
Standard PCR and sequencing				
ITS1_F	ITS1	*Mansonella* spp.	GGTGAACCTGCGGAAGGATC	[20]
ITS1_R			CTCAATGCGTCTGCAATTCGC	

DNA sequencing

Only qPCR positive samples for both genes were subjected to the sequencing of the rRNA Internal Transcribed Spacer 1 (ITS 1) region to identify *Mansonella* species. PCR reaction targeting a 240 bp region of ITS1 was performed in a thermal cycler (Applied Biosystems, 2720, Foster City, United States) with AmpliTaq Gold 360 PCR master mix (Applied Biosystems, Waltham, United States). The ITS region was amplified using the ITS1_F and ITS1_R primers described in [20] (**Table 1**). PCR was performed in a reaction mixture consisting of 5 µL of extracted DNA, 12.5 µL of AmpliTaq Gold 360 PCR master mix (Applied Biosystems, Waltham, MA, USA), 0.75 µL of each primer, and 6 µL of sterile distilled water, in a final reaction volume of 25 µL. The amplification program consisted in an initial denaturation at 95˚C for 15 min, followed by 40 cycles at 95˚C for 30 s, at 58˚C for 30 s, 72˚C for 1 min and a final step at 72˚C for 5 min.

PCR amplification products were purified using a Macherey Nagel plate (NucleoFast 96 PCR, Düren, Germany). Sanger sequencing was performed using the same primers as for PCR with BigDye Terminator v1.1, v3.15× sequencing buffer (Applied Biosystems, Warrington, UK) and run on an ABI 3100 automated sequencer (Applied Biosystems). The resulting sequences were assembled using ChromasPro version 1.7.7 software (Technelyium Pty. Ltd, Tewantin, Australia) and aligned using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) with ClustalW to perform multiple alignments of our sequences. The corrected sequences were compared with existing sequences in the National Centre for Biotechnology Information (NCBI) online database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using basic local alignment search tool (BLAST) analysis [21]. A maximum likelihood

(ML) phylogenetic tree (Figure 1) was constructed using substitution model selection in the Molecular Evolutionary Genetics Analysis (MEGA) v7.0.26 software (Tamura K, 1993). The substitution model selected for this analysis was the T92+I model (Tamura-3 parameter + invariant sites), which provides more robust and reliable inference for phylogenetic tree construction with our nucleotide sequence data [22,23]. Statistical support for the internal branches of the trees was assessed by bootstrapping with 1,000 iterations.

Results

qPCR detection of filarial species and *M. perstans*

Among the 1502 malaria RDT cassettes selected, 6 RDTs were excluded as invalid, 260 were positive and 1236 negative for malaria. All 1496 RDTs were further analysed using both pan-filaria 28S and *M. perstans* specific qPCR systems. Of these samples, only 2/1496 (0.1%) were positive for both qPCRs (Fig S1). Notably, *M. perstans* species were only detected in TDRs collected in the Koulikoro region, giving a local prevalence of 1.1% figure (1)

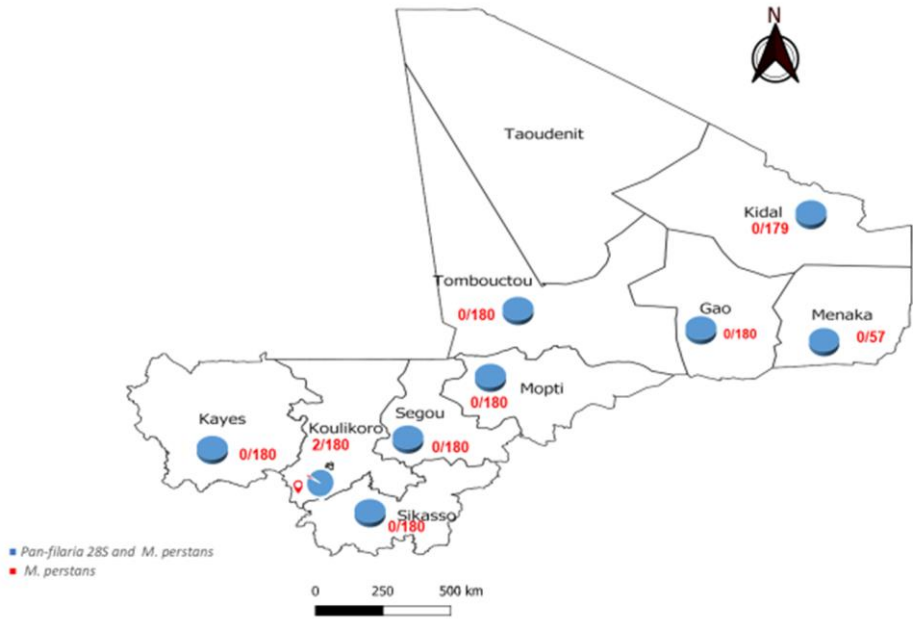


Figure1: Map of the distribution of *M. perstans* prevalence in malaria RDTs in Mali

DNA sequence-based identification of *Mansonella* parasites

ITS1 region of the rRNA sequence identification was carried out on the 2 *M. perstans* qPCR positive samples. The nucleotide sequences obtained were subjected to BLAST analysis against reference sequences from the GenBank (GB) database. BLAST analysis showed that our *M. perstans* sequences were 100.00% identical to their homologous sequences (GB accession numbers: PQ041650 and PQ041653) from Senegal. Our sequences were deposited in the GB database under accession numbers PQ282503 and PQ282504. The ITS phylogenetic tree based was constructed using our *M. perstan*s sequences in addition to other sequences downloaded from GenBank. The tree showed that our sequences are close to those already found in Senegal, Brazil and Gabon and that they form the same clade. The phylogenetic tree also showed interspecific discrimination (**Figure 2**).

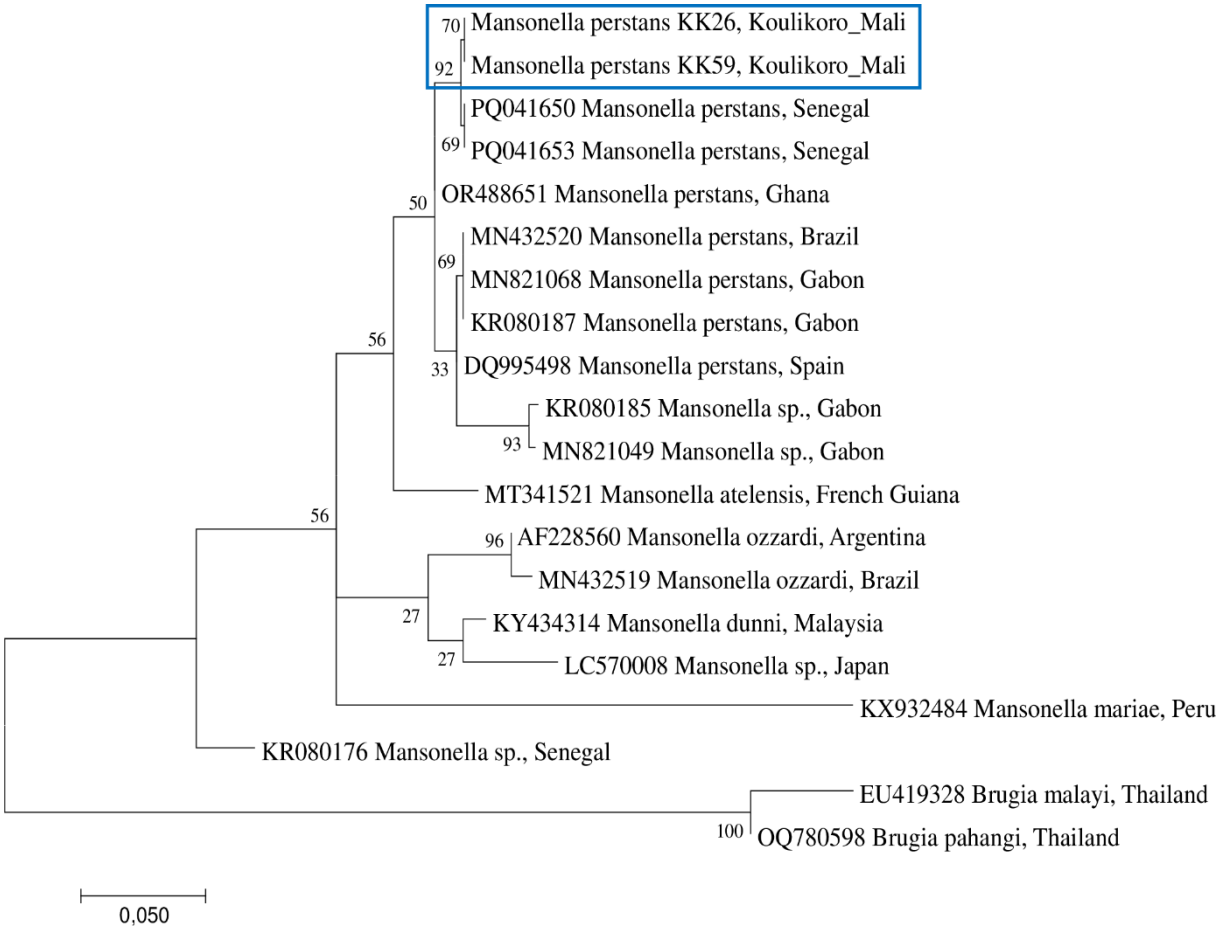


Figure 1. Maximum likelihood tree based on the nucleotide sequence of the ITS region, constructed using MEGA 7. Branch values are bootstrap support values based on 1000 replicates.

Discussion

Our results demonstrate the feasibility of *M. perstans* detection in Mali using DNA extracted from RDT cassettes. Among the 9 regions of Mali, we found positive *M. perstans* specific qPCR in patients attending health facilities in the Koulikoro region, located in the Sahel zone of Mali. We estimate the prevalence of mansonellosis in this region to be 1.1%.

Several studies have been carried out on mansonellosis caused by *M. perstans* in Mali, in particular in the south and north of the country; surveys carried out by the FMPOS Laboratory of Epidemiology of Parasitic Affections in the Kadiolo district (Central Arrondissement, in February 1978) showed that, out of 782 thick

diurnal drops in schoolchildren aged 6 to 17 years, the prevalence of *M. perstans* was about 6.7%, including 3.9% in children aged 7 to 10 and 10.9% in children aged 11 to 17 [14] perstans was about 6.7%, including 3.9% in children aged 7 to 10 and 10.9% in children aged 11 to 17. Similarly, in the village of Lofigué, the prevalence of *M. perstans* was 28.45% in 123 thick nocturnal droplets collected from adults, with M. *perstans* + *W. bancrofti* associations accounting for 8.13% [14]. The prevalence of *M. perstans* ranged from 5.5% to 78% in the 10 villages surveyed in the Kolokani district (Arrondissement Central, April 1978) [14]. In Gourma (April - May 1979), out of 608 thick daily drops taken from nomadic populations north of parallel 15º30'N on either side of the 300 mm isohyet, none showed *M. perstans* microfilariae [14]. Then, in 2003, Paul B Keiser et al. reported a prevalence of *M. perstans* of 75% in Mali [16]. All these results were obtained by microscopy. Although *M. perstans* infection is one of the known endemic human filariases in Mali, there are still very few studies on its epidemiology and its negative impact on the health of endemic populations.

The control and elimination of human filarial infections requires the implementation of prevention strategies, appropriate treatment and enhanced disease surveillance. Our approach demonstrates that an integrated approach can be used to detect bloodstream microfilariae, including *Mansonella* spp., using routinely performed malaria RDTs. We identified *M. perstans* by real-time PCR in two DNA samples extracted from negative and positive malaria RDTs from febrile patients, representing a prevalence of 0.1% (2/1496) in the country and 1.1% in the Koulikoro region. The low prevalence obtained in our results could be explained by the effects of repeated mass distribution campaigns of albendazole and ivermectin against helminthiasis, a strategy recommended by the

World Health Organization (WHO) to reduce the burden of neglected tropical diseases in the community [24]. For example, during the mass treatment campaign in 2018, 3 years before the collection of malaria RDT cassettes, the therapeutic coverage (ivermectin + albendazole) and single-dose ivermectin were 86.34% and 96.84%, respectively [24]. Ivermectin distributed during mass campaigns against lymphatic filariasis and onchocerciasis may have a long-term effect on the prevalence of *M. perstans* [25]. Another explanation may be that this neglected vector-borne filarial infection is geographically restricted. In contrast to our results, the overall prevalence of *M. perstans* in a rural area of Senegal was 14.5% (29/201) [12]. In a previous study comparing *M. perstans* detection techniques, the authors showed that qPCR could be successfully used to identify microfilariae from stained blood smears [12]. Given the interesting results obtained from this filaria-endemic region of Mali, it is important to note that conservation of RDT cassettes and lack of knowledge of patient demographics and clinical symptoms could be potential limitations of our approach.

Conclusion

For the first time, the use of malaria RDT cassettes allowed qPCR-based screening of filarial species, including *M. perstans*, and revealed a low prevalence in central Mali and the Sahel zone. Further studies of this little-known human filariasis are needed to properly assess its public health significance. This will help to strengthen mansonellosis control strategies in Mali.

Author contributions: P.D., MD., M.A.T. and S.R. designed the study and wrote the first version of the manuscript.

P.D., M.D., P.M.G., and M.C., analysed and interpreted the results.

M.D., P.M.G., M.A.T. and S.R. Contributed to the critical revision of the manuscript. All authors read and approved the final version.

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and materials

The authors confirm that the data supporting the results of this study are available in the manuscript and its supplementary material. All nucleotide sequences described have been deposited in the NCBI GenBank nucleotide database and can be accessed by BLAST at http://blast.ncbi.nlm.nih.gov. Accession numbers detailed in the manuscript are as follows: PQ282503 and PQ282504.

Supporting information

S1 Table.

S1 Figure. Amplification curves for real-time PCR on CFX96 targeting the qFil 28S gene of *M. perstans* species in Koulikoro (T+: positive control and the two positive samples detected in the Kangaba health district (Koulikoro region).

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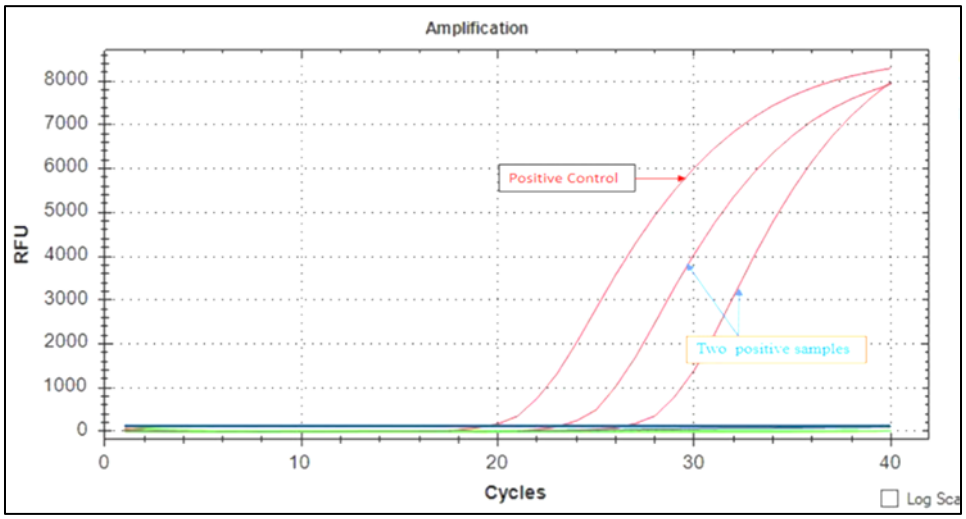
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Supplementary data

S1 Table. Main characteristics of human mansonellosis.

Characteristic	*Mansonella streptocerca*	*Mansonella ozzardi*	*Mansonella perstans*
Endemic areas	West and Central Africa	Central and South America, Caribbean	Sub-Saharan and Northern Africa, South America
Arthropod vectors	*Culicoïdes* spp.	*Culicoïdes* spp; S imulium amazonicum	*Culicoïdes* spp.
Location of adult worms	Dermis of the trunk and upper shoulder girdle	Lymphatic vessels, pericardium, pleura and peritoneum	Pericardium, pleura and peritoneum; mesentery and retroperitoneum
Location of microfilariae	Skin	Blood and skin	Blood



S1 Figure. Amplification curves for real-time PCR on CFX96 targeting the qFil 28S gene of *M. perstans* species in Koulikoro (T+: positive control and the two positive samples detected in the Kangaba health district (Koulikoro region).

ANNEXES

Publications en collaboration

Article 6

Spatio-Temporal Variability of Malaria Incidence in the Health District of Kati, Mali, 2015-2019

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Article

Spatio-Temporal Variability of Malaria Incidence in the Health District of Kati, Mali, 2015–2019

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Abstract: Introduction: Despite the implementation of control strategies at the national scale, the malaria burden remains high in Mali, with more than 2.8 million cases reported in 2019. In this context, a new approach is needed, which accounts for the spatio-temporal variability of malaria transmission at the local scale. This study aimed to describe the spatio-temporal variability of malaria incidence and the associated meteorological and environmental factors in the health district of Kati, Mali. Methods: Daily malaria cases were collected from the consultation records of the 35 health areas of Kati's health district, for the period 2015–2019. Data on rainfall, relative humidity, temperature, wind speed, the normalized difference vegetation index, air pressure, and land use–land cover were extracted from open-access remote sensing sources, while data on the Niger River's height and flow were obtained from the National Department of Hydraulics. To reduce the dimension and account for collinearity, strongly correlated meteorological and environmental variables were combined into synthetic indicators (SI), using a principal component analysis. A generalized additive model was built to determine the lag and the relationship between the main SIs and malaria incidence. The transmission periods were determined using a change-point analysis. High-risk clusters (hotspots) were detected using the SatScan method and were ranked according to risk level, using a classification and regression tree analysis. Results: The peak of the malaria incidence generally occurred in October. Peak incidence decreased from 60 cases per 1000 person–weeks in 2015, to 27 cases per 1000 person–weeks in 2019. The relationship between the first SI (river flow and height, relative humidity, and rainfall) and malaria incidence was positive and almost linear. A non-linear relationship was found between the second SI (air pressure and temperature) and malaria incidence. Two transmission periods were determined per year: a low transmission period from January to July—corresponding to a persisting transmission during the dry season—and a high transmission period from July to December. The spatial distribution of malaria hotspots varied according to the transmission period. Discussion: Our study confirmed the important variability of malaria incidence and found malaria transmission to be associated with several meteorological and environmental factors in the Kati district. The persistence of malaria during the dry season and the spatio-temporal variability of malaria hotspots reinforce the need for innovative and targeted strategies.

Keywords: malaria; environmental factors; hotspot; spatio-temporal dynamics; geoepidemiology

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1. Introduction

Malaria continues to be a serious threat to populations living in endemic or epidemic areas. According to the World Health Organization (WHO), the global number of malaria cases in 2019 was 229 million, with 94% of these occurring in sub-Saharan Africa [1]. Of the 409,000 malaria deaths reported in 2019, 67% occurred in children under five years and 95% were recorded in sub-Saharan Africa [1]. Unfortunately, the global decrease in malaria incidence over the 2015–2019 period was negligible, at 2% [1].

In Mali, 2.9 million cases of malaria were recorded in 2019, including 871,274 severe cases and 1454 deaths [2]. That same year, malaria was one of the leading causes of mortality (27%), morbidity (23%), and consultations (34%) [2,3]. Malaria transmission is highly heterogenous in Mali, showing (i) an endemic profile (hyperendemic or holoendemic) in the Sudano–Sahelian zone (center and southern zone), where 50 to 80% of children under five years are parasite carriers (i.e., plasmodium index); (ii) an epidemic profile in the northern Saharian zone, with a plasmodium index below 5%; (iii) a hyperendemic profile (bi- or pluri-modal) in irrigated/flood-prone areas (mainly bi-annual rice culture); and (iv) a hypoendemic profile in urban areas [4]. While the spatio-temporal variability of malaria transmission in Mali is largely related to environmental and meteorological factors [3], it is also increasingly linked to climate change, as in other regions of the world [3,5].

Over the last two decades, the National Malaria Control Program (NMCP) has been implementing malaria control strategies in Mali, based on WHO recommendations. These strategies consist of early diagnosis and case management, chemoprevention in pregnant women and children aged 3 to 59 months, and vector control using long-lasting insecticide nets (LLIN) and indoor residual spraying (IRS) [2].

In Mali, early diagnosis is currently performed using rapid diagnostic tests (RDTs) and early case management through the administration of artemisinin-based combination therapy (ACT). Both RDTs and ACT are free of charge in health facilities for children under five years. According to the Malaria Indicator Survey, only 14% of febrile children in Mali received an RDT and 51% failed to take ACT in 2015 [6]. In 2021, 23.3% of children received an RDT, 19.4% of whom were positive for malaria [7].

Chemoprevention in pregnant women is based on intermittent preventive treatment (IPT), which consists of the administration of three doses of sulfadoxine-pyrimethamine (SP) during pregnancy. Coverage has considerably improved since IPT was first introduced in Mali, in 2003 [8]. Thus, the rate of coverage increased from 35% in 2012 to 66% in 2015 for the first dose, and from 44% in 2015 to 56% in 2019, and to 57% in 2021 for the second dose [2,6]. Despite this significant increase, Mali has not reached the goal of universal coverage recommended by the WHO [3], nor has it even achieved the coverage target of 80% set in the 2018–2022 National Malaria Control Strategic Plan [6]. The low coverage rate is largely explained by poor adherence to treatment among pregnant women.

Since 2012, the WHO has recommended the administration of seasonal malaria chemoprevention (SMC) in children under five years to prevent malaria morbidity and mortality. A maximum of four doses of antimalarial treatment, a combination of SP and amodiaquine (AQ), should be administered at one-month intervals during the high transmission season [9]. According to the Malaria Indicator Survey, 36% of the targeted children in Mali received at least one dose of antimalarial treatment during the high transmission season, in 2015 [6]. Between 2016 and 2019, this rate increased significantly to 106% of the targeted children for the first and second doses, to 107% for the third dose, and to 105% for the fourth dose [2]. In spite of these significant improvements, SMC coverage is likely to have been hindered by the limited access to care among the Sahelian children and by low adherence to treatment after the first dose.

Vector control consists in preventing human contact with the mosquito vector and in limiting mosquito proliferation in the environment. This strategy specifically relies on the appropriate use of LLINs and IRS. In Mali, LLINs are distributed free of charge, both during prenatal consultations and during the distribution campaigns organized by the NMCP and its partners. According to the Malaria Indicator Survey, 71% of children under

five years, 78% of pregnant women, and 68% of the population who are at risk of malaria used LLINs in Mali, in 2015 [6]. While these figures are encouraging, Mali has yet to reach the goal of universal LLIN coverage, recommended by the WHO [10]. To date, IRS has been applied in only four health districts of Mali, protecting 90% of the population at risk in these areas [3].

In the health district of Kati, where malaria incidence was estimated at 68% in 2017 [11], the same control strategies are implemented as in the rest of Mali. However, these strategies have not been as successful as one would have hoped. While the RDT coverage rate in Kati was high, at 81%, in 2016, it had significantly fallen to 68% in 2017 [11]. The drop in antenatal visits from 37% in 2016, to 26% in 2017, and to 24% in 2018 suggests that the IPT coverage rate in pregnant women also decreased [9]. As for the LLIN coverage rate in pregnant women, it fell from 84.2% in 2017, to 51.68% in 2018 [11]. By contrast, SMC, which was introduced in the Kati district in 2016, has shown encouraging results, with a coverage rate of 109% of the targeted children in 2019 [11]. Note that no mass IRS campaign has been implemented in Kati, to date.

Despite the implementation of control strategies at the national scale, the malaria burden remains high in Mali. In this context, a new approach is needed, which accounts for the spatio-temporal variability of malaria transmission at the local scale. To help achieve this objective, the present study sought to answer the following question: "What meteorological and environmental factors influence the spatio-temporal variability of malaria transmission in the health district of Kati, in Mali?"

2. Methods

2.1. Study Site

The health district of Kati is located north-west of Bamako, in the Sudano–Sahelian zone of Mali. In 2019, the population of Kati was estimated at 695,921, with a density of 66 inhabitants/km^2, and a growth rate of 3.6%. The district has an area of 9636 km^2 and is subdivided into 23 communes and 35 health areas. It is crossed by various seasonal streams and by the Niger River, in the south-east. The vegetation consists mainly of grassy savannah, dotted with fruit trees and shrubs. The main economic activities are market gardening and cereal cultivation, both of which are facilitated by the presence of water reservoirs and seasonal streams, which constitute potential mosquito breeding sites. Livestock breeding is another important economic activity, with the commune of Kati hosting the country's largest weekly livestock fair.

Annual rainfall in the Kati health district is 1000 mm. The rainy season runs from June to October and peak rainfall occurs in August (304 mm). The temperature averages 20 °C during the cold season and 30 °C during the hot season.

Malaria transmission in Kati is moderate [12] and seasonal, with the transmission season running from June to December. In some areas, the entomological inoculation rate can range from 137 to 167 infecting bites per person during the transmission season [13].

2.2. Data Collection

This study retrospectively analyzed daily malaria cases recorded in the health district of Kati, over the period 2015–2019. Malaria data were collected from the consultation records of the district's 35 health areas. These records contained the following information on patients visiting community health centers (CSCOM): date of consultation, name, age, sex, place of residence, weight, clinical signs, diagnosis, and treatment prescribed. Only cases confirmed by thick blood smear or RDT were considered for analysis.

Meteorological data were collected from different sources. Data generated through remote sensing were extracted from the NASA Giovanni website [14], as follows: daily number of rainfall events and cumulative rainfall (mm); minimum and maximum day and night temperatures (°C); and minimum and maximum day and night relative humidity (%). Other data were extracted from the ERA5 database through Google Earth Engine [15]: mean air pressure (hPa); normalized difference vegetation index; mean wind speed (km/h).

The following hydrological data were obtained from the National Directorate of Hydraulics of Mali: Niger River height (cm) and flow (m^3).

The following land cover data were extracted from the Copernicus Global Land Cover [16–20]: bare soil, cropland, grass vegetation, shrubland, forest, seasonal inland water, and permanent inland water.

The geographic coordinates of the 35 health areas of Kati were extracted from the Geographic Location System during the field survey. The perimeter of each health area was delineated based on these geographic coordinates, using the Voronoi polygon method.

Malaria, meteorological, and environmental data were aggregated on a weekly basis.

2.3. Statistical Analysis

2.3.1. Temporal Analysis of Malaria Incidence and Associated Meteorological and Environmental Factors

An additive decomposition of the malaria incidence time series was performed to estimate trend, seasonality, and residual at the scale of the Kati health district.

Weekly malaria incidence was estimated and plotted. Rainfall, mean temperature, and Niger River height and flow were also plotted at health-district level.

To reduce dimension and, thus, account for collinearity, a principal component analysis (PCA) of meteorological and environmental factors was performed. Components were determined using the elbow method and the Kaiser criterion [21]. Each component represented a synthetic indicator (SI).

A univariate generalized additive model (GAM), using minimum generalized cross validation and maximum explained deviance, was performed to estimate the lags between the main SIs and malaria incidence [21].

A multivariate GAM, with negative binomial distribution, was built to account for overdispersion [22]. Spline function smoothing was performed to estimate non-linear relationships, and standardized incidence ratios were estimated by modeling the log-transformed population as the offset [23].

A change-point analysis of the malaria incidence time series was performed to determine transmission periods. The dates of significant changes in the mean and variance of malaria incidence were determined using the pruned exact linear time algorithm [24], with the AIC criterion [5,25,26].

2.3.2. Spatial Analysis of Malaria Incidence

The search for high-risk clusters or hotspots was carried out using Kulldorff's SatScan method, the precision of which increases with the size of the population at risk, the incidence rate, and the relative risk (RR) [27,28]. This method was selected because it circumvents the problem of test multiplicity by using the likelihood ratio test and the Monte Carlo approach [26]. Health areas with significantly high malaria incidence were grouped into high-risk clusters, in the form of a circular window of variable size [26], a shape that helped to avoid the poor performance associated with edge effects [29]. The characteristics of each hotspot were studied using supervised classification to identify the main associated factors and their combinations.

A map showing the spatio-temporal distribution of malaria incidence and hotspots was built for each year of the study.

2.3.3. Classification and Regression Tree Analysis

Health areas were ranked according to their level of malaria risk using classification and regression tree analysis (CART), a non-parametric classification method that analyzes historical data by means of a decision tree. The main variables associated with hotspot status were identified by constructing a tree whose leaves (i.e., the terminal nodes) were composed of health areas presenting the same level of malaria risk [30]. The CART method presents two main advantages: it can treat both numerical and categorical variables and it handles outliers more effectively than other statistical models (including PCA) [31].

2.3.4. Software

Malaria data were entered on tablets using REDCap software version 11.1.1 (Vandrbilt University, Nashville, TN, USA). Meteorological and environmental data extraction and statistical analyses were performed using R software, version 4.1.0 (18 May 2021, Copyright © 2022, R Foundation for Statistical Computing). SatScan, version 9.7 (Information management Services Inc, Calverton, MS, USA), was used to detect malaria hotspots. The incidence map with malaria hotspots was built with QGIS, version 3.16.7 (Open Source Geospatial Foundation Project, Beaverton, OR, USA). Figures and images were formatted and treated using Microsoft Paint.

3. Results

3.1. General Description of the Malaria Incidence Time Series

A total of 293,001 confirmed malaria cases, which were collected from 190 consultation records, were analyzed for the period 2015–2019.

The malaria incidence time series confirmed the seasonality of malaria transmission in Kati. The peak of malaria incidence generally occurred in October. The peak of incidence decreased over the study period, from 60 cases per 1000 person–weeks in 2015, to 27 cases per 1000 person–weeks in 2019 (Figure 1).

(**A**)

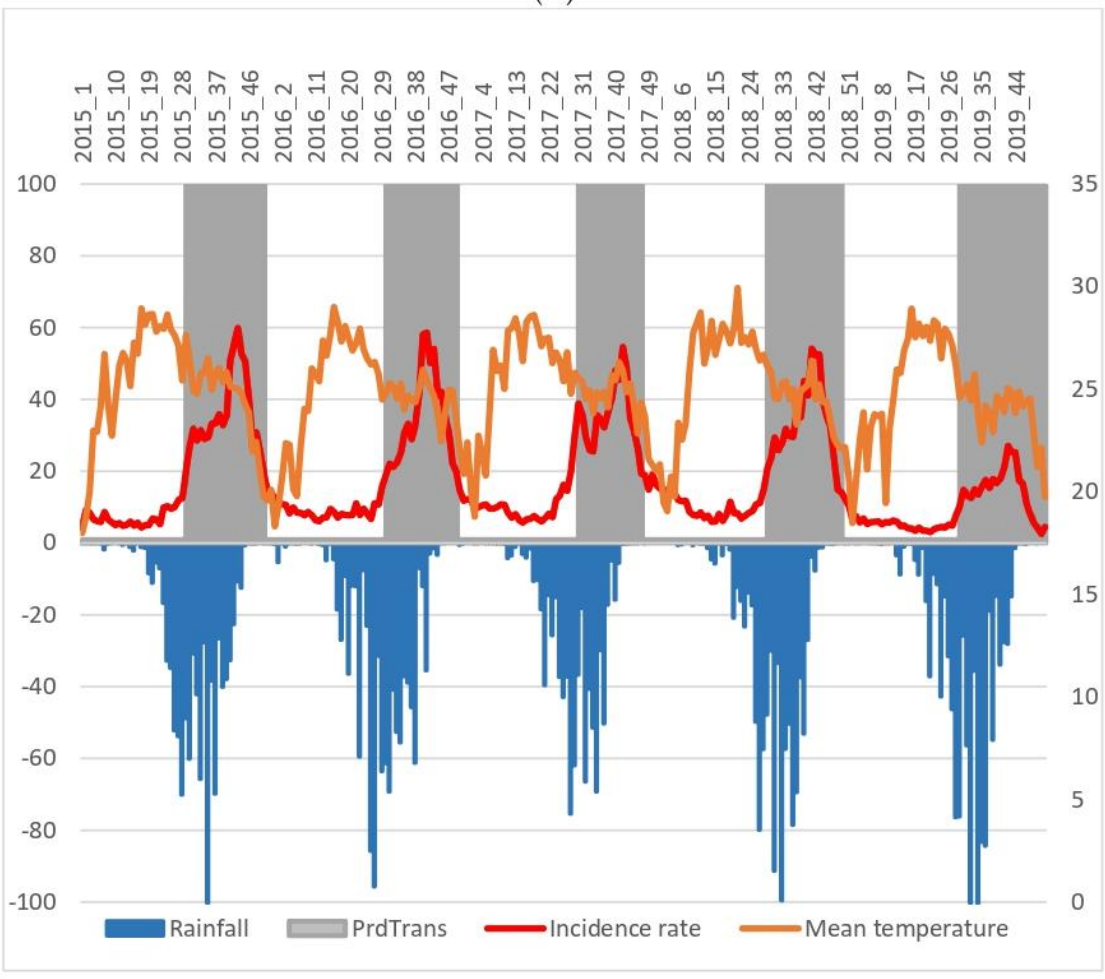


Figure 1. *Cont.*

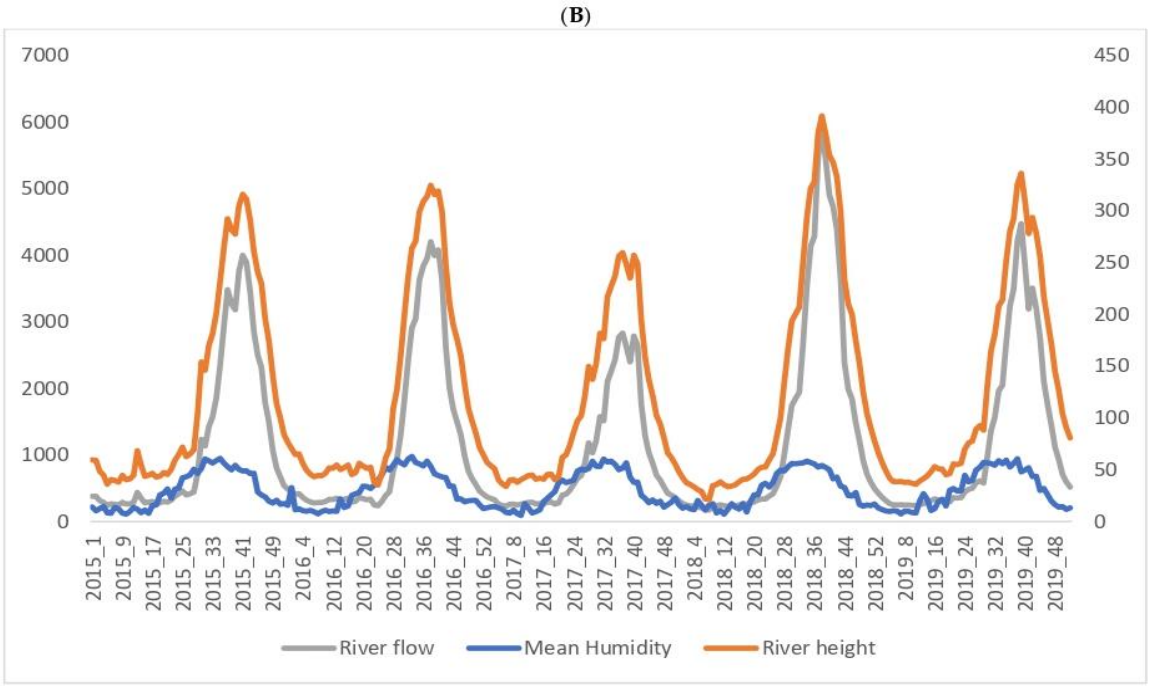


Figure 1. (**A**): Weekly malaria incidence (red curve) according to rainfall (blue), temperature (orange), and transmission period (PrdTrans: white band—low transmission period; grey band—high transmission period). (**B**): River height (orange curve), river flow (gray curve), and relative humidity (blue curve).

The peak rainfall was observed in August in 2015, 2018, and 2019, and in July in 2016 and 2018. This peak also decreased over the study period, from 146 mm in 2015, to 105 mm in 2019 (Figure 1).

The lag between the peak rainfall and the peak malaria incidence was 8 weeks in 2015, 2018, and 2019; 13 weeks in 2016; and 14 weeks in 2017.

Three SIs were determined using PCA, with the elbow method and the Keiser criterion. These SIs explained 88.5% of the total inertia (Figure 2). The first SI (SI#1), which explained 49.3% of the inertia, consisted of river flow and height, relative humidity, and rainfall. The second SI (SI#2), which explained 29.8% of the inertia, consisted of air pressure and temperature (Table 1).

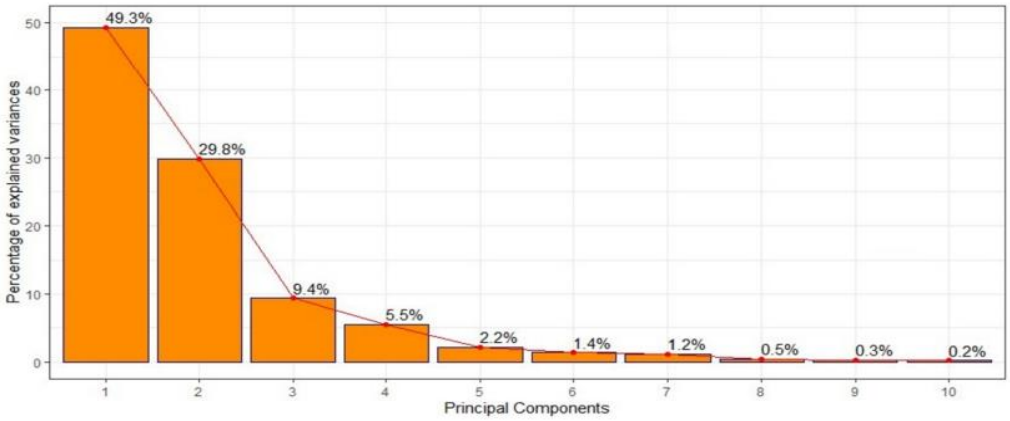


Figure 2. Percentage of inertia, explained by the synthetic indicators in principal component analysis.

Table 1. Correlation of the main synthetic indicators with malaria incidence.

Synthetic Indicators	Variables	Percentage of Inertia	Correlation Coefficient r	Percentage of Total Inertia
Synthetic Indicator #1	Mean river flow	7.05	0.87	49.3%
	Maximum river flow	7.12	0.88	
	Mean river height	7.49	0.90	
	Maximum river height	7.50	0.90	
	Minimum river height	7.44	0.90	
	Mean night relative humidity	7.92	0.93	
	Minimum night relative humidity	7.55	0.90	
	Mean day relative humidity	7.89	0.93	
	Maximum day relative humidity	7.71	0.91	
	Minimum day relative humidity	7.69	0.91	
	Cumulative Rainfall	4.47	0.70	
	Wind speed	3.87	−0.65	
Synthetic Indicator #2	Mean air pressure	7.61	0.71	29.8%
	Mean night temperature	14.52	0.98	
	Minimum night temperature	13.21	0.93	
	Maximum night temperature	14.24	0.97	
	Mean day temperature	14.52	0.98	
	Minimum day temperature	13.21	0.93	
	Maximum day temperature	14.24	0.97	

3.2. Temporal Analysis of Malaria Incidence and Associated Meteorological and Environmental Factors

The univariate GAM showed a lag of 4 weeks between SI#1 (river flow and height, relative humidity, and rainfall) and malaria incidence. A lag of 19 weeks was found between SI#2 (air pressure and temperature) and malaria incidence.

The multivariate GAM highlighted a significant relationship between the first two SIs (SI#1 and SI#2) and malaria incidence, with an explained deviance of 78.5%.

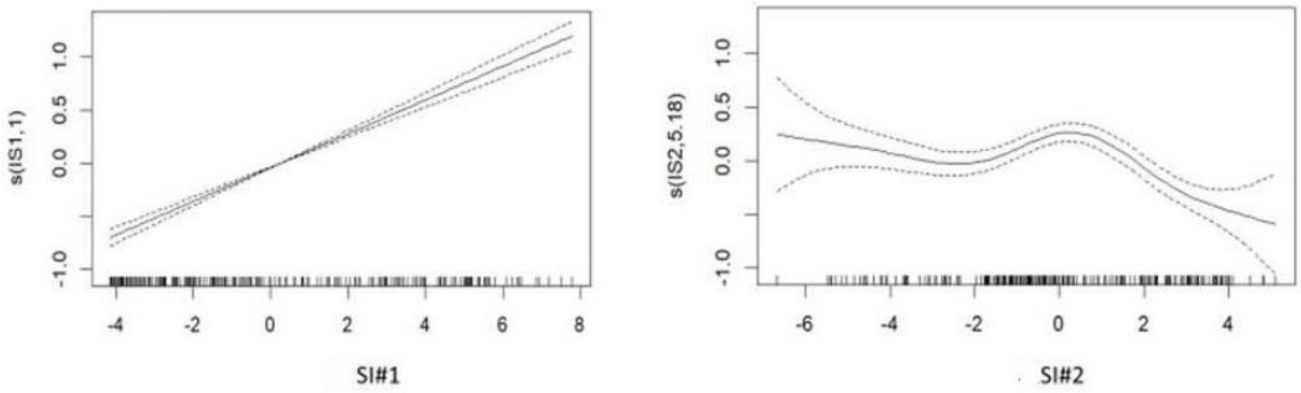
The relationship between SI#1 (river flow and height, relative humidity, and rainfall) and malaria incidence ($p < 0.001$) was positive and almost linear (Figure 3A). The SI#1 variables that most correlated with malaria incidence were river flow and height, and relative humidity (Table 1).

The relationship between SI#2 (air pressure and temperature) and malaria incidence was significantly non-linear ($p < 0.001$). The SI#2 variable that most correlated with malaria incidence was temperature (Table 1): the incidence of malaria increased significantly as the temperature increased, but decreased significantly at higher temperatures (Figure 3B).

Two transmission periods were determined using a change-point analysis, as follows: a low transmission period (LTP) and a high transmission period (HTP).

The mean duration of the LTP was 24 weeks. The LPT generally started in December and ended in July; however, it lasted from December to June in 2016–2017 and from November to July in 2017–2018.

The mean duration of the HTP was also 24 weeks. The HTP started in July and ended in December during every year of the study.



(A) (B)

Figure 3. Generalized additive model of synthetic indicators #1 (**A**) and #2 (**B**). The solid line represents the relationship between synthetic indicators #1 or #2 and malaria incidence, and the dotted line represents the 95% confidence interval.

3.3. Spatial Analysis of Malaria Incidence

According to the SatScan analysis, 25 out of 35 health areas were a malaria hotspot at least once during the study period. Specifically, three health areas were a malaria hotspot in the first year of the study, two health areas were a malaria hotspot in two consecutive years (2015–2016 or 2016–2017), 10 health areas were a malaria hotspot every year of the study, and 10 health areas were a hotspot without temporal regularity (Table 2).

Table 2. Distribution of health areas according to hotspot status.

	Never a Hotspot	Hotspot in the First Year of the Study	Hotspot in Two Consecutive Years	Hotspot Every Year of the Study	Hotspot without Temporal Regularity
Number of health areas	10	3	2	10	10

Malaria hotspots were detected during all LTPs.

A total of seven significant hotspots, covering nine health areas, were detected during the 2015–2016 LTP. The hotspot with the highest malaria RR (7.23; $p < 0.0001$) had a malaria incidence rate of 0.79 cases per 1000 person–weeks. This hotspot covered two health areas with a population of 10,026 inhabitants, within a radius of 11.15 km.

A total of five significant hotspots, covering 16 health areas, were detected during the 2016 HTP. The largest hotspot had an RR of 3.87 ($p < 0.0001$) and covered nine health areas with a population of 106,485, within a radius of 29.75 km.

A total of five significant hotspots, covering eight health areas, were detected during the 2017–2018 LTP. The hotspot with the highest RR (13.45; $p < 0.0001$) covered two health areas with a population of 8195 inhabitants, within a radius of 3.84 km.

Lastly, a total of seven significant hotspots, covering 13 health areas, were detected during the 2018 HTP. The largest hotspot had an RR of 3.17 ($p = 0.0001$) and covered six health areas with a population of 95,226 inhabitants, within a radius of 22.86 km. The hotspot with the highest RR (18.84; $p = 0.0001$) covered only one health area, with a population of 4423 inhabitants.

Table 3 describes the transmission periods according to the presence of hotspots and according to selected meteorological variables. Table 4 describes the health areas according to the number of times they were a hotspot and according to their land cover characteristics. Maps showing the spatio-temporal distribution of malaria incidence and hotspots in the Kati health district are presented in Figure 4.

Table 3. Transmission periods according to the presence of hotspots and selected meteorological variables.

Malaria Transmission Periods	Duration (Weeks)	Number of Health Areas That Were a Hotspot	Number of Hotspots	Incidence Rate (Cases per 1000 Person–Weeks)			Rainfall (mm)			Mean Temperature (°C)			Mean Wind (km/h)			Mean NDVI		
				Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.
Low transmission period, 2015 (W1–W28)	28	10	5	0.003	0.737	7.210	203	260	305	29	29	31	2.06	2.24	2.34	0.218	0.274	0.344
High transmission period, 2015 (W29–W50)	22	15	5	0.041	2.883	27.2	578	725	853	25	26	27	1.43	1.57	1.70	0.240	0.536	0.646
Low transmission period, 2015 (W51)–2016 (W29)	31	9	7	0.001	0.994	8.742	321	408	455	28	29	30	2.21	2.31	2.39	0.240	0.348	0.440
High transmission period, 2016 (W30–W49)	20	16	5	0.022	2.803	15.75	433	520	603	26	27	28	1.06	1.22	1.34	0.240	0.377	0.430
Low transmission period, 2016 (W50)–2017 (W26)	29	14	6	0.004	1.140	12.02	200	257	307	28	29	30	1.96	2.14	2.25	0.240	0.337	0.451
High transmission period, 2017 (W27–W47)	21	10	6	0.176	3.198	25.04	543	611	679	26	27	28	1.12	1.35	1.50	0.240	0.447	0.599
Low transmission period, 2017 (W48)–2018 (W27)	32	8	5	0.019	1.206	21.34	211	278	326	28	29	30	1.91	2.03	2.15	0.240	0.340	0.400
High transmission period, 2018 (W28–W49)	22	13	7	0.003	2.034	36.44	641	741	842	26	27	27	1.11	1.27	1.40	0.240	0.506	0.616
Low transmission period, 2018 (W50)–2019 (W27)	30	13	7	0.023	0.381	11.72	166	213	242	28	29	30	1.99	2.12	2.19	0.204	0.266	0.301
High transmission period, 2019 (W28–W46)	19	14	4	0.005	0.399	14.29	689	789	906	26	27	27	1.11	1.40	1.56	0.220	0.247	0.269

Table 4. Health areas according to the number of times they were a hotspot and their land cover characteristics.

Health Areas	Number of Times Health Area Was a Hotspot	Bare Soil			Cropland			Grass Vegetation			Shrubland			Forest			Seasonal Inland Water			Permanent Inland Water		
		Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.
BANCOUMANA	2	0	0.33	100	0	37.8	90	0	33.6	100	0	15.8	43	0	7.4	42	0	0	100	0	0	100
DABAN	4	0	0	23	0	29	87	0	40.6	81	0	20.8	47	0	7.6	54	0	0	81	0	0	0
DIAGO	4	0	0.67	30	0	31.6	87	0	39.4	76	0	17.8	44	0	7.4	39	0	0	6	0	0	0
DIALAKORODJI	0	0	0	34	0	15	78	0	39.4	74	0	16.4	43	0	6.4	38	0	0	0	0	0	0
DIO BA	4	0	0	20	0	15.4	85	0	42.2	79	0	24.8	47	0	9.8	42	0	0	5	0	0	0
DIO GARE	3	0	0.33	26	0	24.2	86	0	39.4	77	0	21.6	44	0	8.8	40	0	0	25	0	0	0
DJIGUIDALA	3	0	0	9	0	20.8	88	0	42	100	0	23.8	46	0	10.6	46	0	0	100	0	0	100
DJOLIBA	0	0	0	100	0	33.4	93	0	35	99	0	17.8	66	0	7.4	40	0	0	100	0	0	100
DOGODOUMA	2	0	0	24	0	6.8	39	0	43.8	73	0	24	40	0	15.6	48	0	0	0	0	0	0
DOMBILA	0	0	0	29	0	4.2	87	0	45.8	80	0	25.8	43	0	14	55	0	0	11	0	0	0
DOUBABOUGOU	0	0	0	16	0	10	82	0	44.2	78	0	25.4	43	0	11.8	46	0	0	2	0	0	0
FALADJE	4	0	0	26	0	21.6	86	0	42.8	84	0	23.8	45	0	8.8	53	0	0	70	0	0	0
FARABANA	5	0	0	69	0	6.4	82	0	41.2	99	0	24.4	46	0	13	62	0	0	100	0	0	100
FARADA	0	0	0.67	36	0	27.8	80	0	37.4	72	0	17.6	40	0	7.6	38	0	0	3	0	0	0
KABALABOUGOU	0	0	0	89	0	0	84	0	17.4	100	0	2.7	39	0	0	32	0	0	100	0	0	100
KALIFABOUGOU	5	0	0	22	0	23	86	0	42.2	79	0	23.2	46	0	8.8	45	0	0	7	0	0	0
KANADJIGUILA	0	0	0	28	0	3.8	79	0	45	75	0	25.4	41	0	13.2	43	0	0	0	0	0	0
KATICORO	3	0	0.33	16	0	18	79	0	40.4	74	0	15.2	36	0	5.8	33	0	0	0	0	0	0
KOKO	0	0	0	25	0	17.4	80	0	41.6	78	0	21.4	46	0	9.8	39	0	0	0	0	0	0
MALIBOUGOU	0	0	0.33	22	0	21.6	79	0	36.2	67	0	19	41	0	10	46	0	0	53	0	0	0
MORIBABOUGOU	4	0	0	94	0	4.4	72	0	42	100	0	22.2	45	0	8.6	41	0	0	100	0	0	63
NANA-KENIEBA	0	0	0	17	0	0	80	0	47.8	81	0	27.2	46	0	22.4	56	0	0	0	0	0	0
NEGUELA		0	0	30	0	5.4	87	0	42.2	80	0	27.2	50	0	11.8	64	0	0	17	0	0	0

Table 4. *Cont.*

Health Areas	Number of Times Health Area Was a Hotspot	Bare Soil			Cropland			Grass Vegetation			Shrubland			Forest			Seasonal Inland Water			Permanent Inland Water		
		Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.
NGABAKORO	3	0	0.33	59	0	23.2	85	0	39.8	100	0	18.4	66	0	7.6	43	0	0	100	0	0	100
NIAME	3	0	0	13	0	15.6	80	0	45.4	75	0	25.6	47	0	10.6	42	0	0	0	0	0	0
NIOUMA MAKANA	1	0	0	22	0	0.8	80	0	49.8	81	0	26.4	45	0	19	61	0	0	0	0	0	0
OUEZZINDOUGOU	4	0	0	63	0	19.8	83	0	31.8	87	0	19.8	42	0	9.2	55	0	0	100	0	0	100
SAFO	1	0	0	19	0	25.6	81	0	44.2	78	0	20.6	49	0	7	38	0	0	5	0	0	0
SANANFARA	0	0	0	37	0	10.2	43	0	43	77	0	22	42	0	8.4	49	0	0	0	0	0	0
SANDAMA	0	0	0	24	0	0	80	0	48.4	81	0	27.2	45	0	20.8	60	0	0	0	0	0	0
SANGAREBOUGOU	0	0	0	98	0	3.4	69	0	31	97	0	8.2	46	0	4	36	0	0	100	0	0	15.6
SIBY	0	0	0	28	0	4.4	85	0	43.6	79	0	23.8	46	0	13.2	53	0	0	75	0	0	0
SONIKEGNY	5	0	0	19	0	29.8	66	0	40.6	78	0	19	39	0	7.6	34	0	0	47	0	0	0
TORODO	4	0	0	32	0	27	86	0	41	75	0	22.2	47	0	8.2	43	0	0	26	0	0	0
YELEKEBOUGOU	5	0	0	28	0	25.6	85	0	43	79	0	22.4	46	0	8.2	43	0	0	55	0	0	0

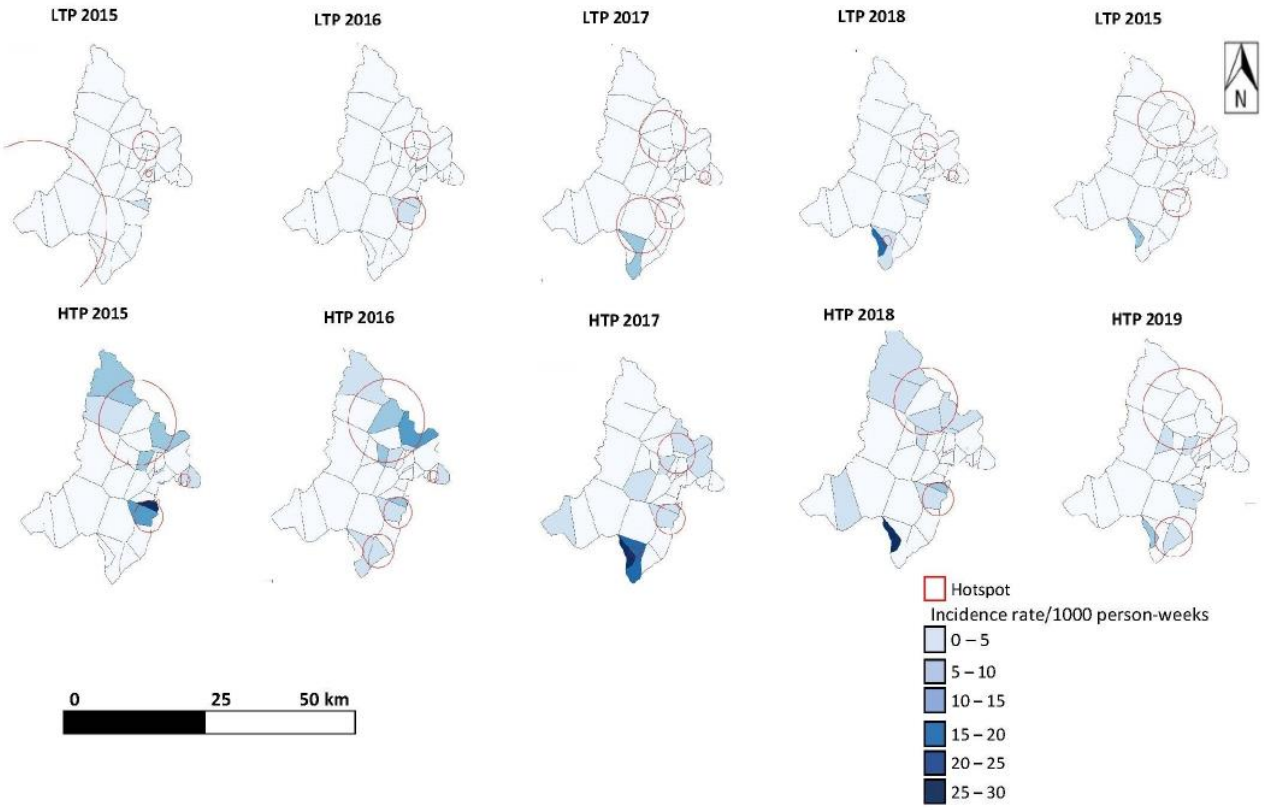


Figure 4. Maps showing the spatio-temporal distribution of malaria incidence and hotspots (red circles) in Kati health district. LTP—low transmission period; HTP—high transmission period.

3.4. Classification and Regression Tree Analysis

The CART method identified three classes of malaria risk: low, moderate, and high. Two variables were significantly associated with the different classes of risk: cropland cover and bare soil cover.

A total of 18 health areas were classified as having a low level of malaria risk (Class 1). These health areas had a mean cropland cover ≤ 23.25. The number of times these health areas were identified as a hotspot was less than one.

A high level of malaria risk (Class 2) was observed in nine health areas, which had a mean cropland cover > 23.25 and a mean bare soil cover ≤ 0.98. The number of times these health areas were identified as a hotspot was equal to four.

A total of eight health areas were classified as having a moderate level of malaria risk (Class 3). These health areas had a mean cropland cover > 23.25 and a mean bare soil cover > 0.98. The number of times these health areas were identified as a hotspot was equal to two.

The different classes of malaria risk are described in Table 5.

Table 5. Classes of malaria risk according to the number of health areas and to meteorological and environmental variables.

Class	1	2	3
Incidence (cases per 1000 persons–weeks)	288.62	425.89	262.80
Number of health areas	18	9	8

Table 5. *Cont.*

Class	1	2	3
Total Population	112,979	56,851	83,618
Cumulative Rainfall (mm)	992	902	950
Mean Temperature (°C)	27.78	28.24	27.95
Mean NDVI	0.37	0.35	0.33
Mean Cropland (SD)	17.68 (2.67)	29.83 (2.36)	29.88 (3.72)
Mean Shrubland (SD)	19.93 (2.13)	21.55 (0.81)	18.09 (1.40)
Mean Forest (SD)	12.82 (1.75)	10.07 (1.56)	9.22 (2.31)
Mean Permanent Inland Water (SD)	1.50 (9.67)	0.04 (1.77)	1.84 (9.56)
Mean Seasonal Inland Water (SD)	1.32 (8.82)	0.04 (1.14)	1.46 (7.84)
Mean Bare Soil (SD)	1.50 (2.45)	0.60 (0.43)	1.62 (1.35)
Mean Grass Vegetation (SD)	35.83 (3.57)	36.87 (0.89)	34.27 (2.31)

NDVI—normalized difference vegetation index; SD—standard deviation. Class 1 corresponds to a low level of malaria risk, Class 2 to a high level of malaria risk, and Class 3 to a moderate level of malaria risk.

Figure 5 shows the CART classification of the health areas, according to the level of malaria risk and to the cropland cover and bare soil cover. A map of the health areas, classified according to the level of malaria risk, is presented in Figure 6.

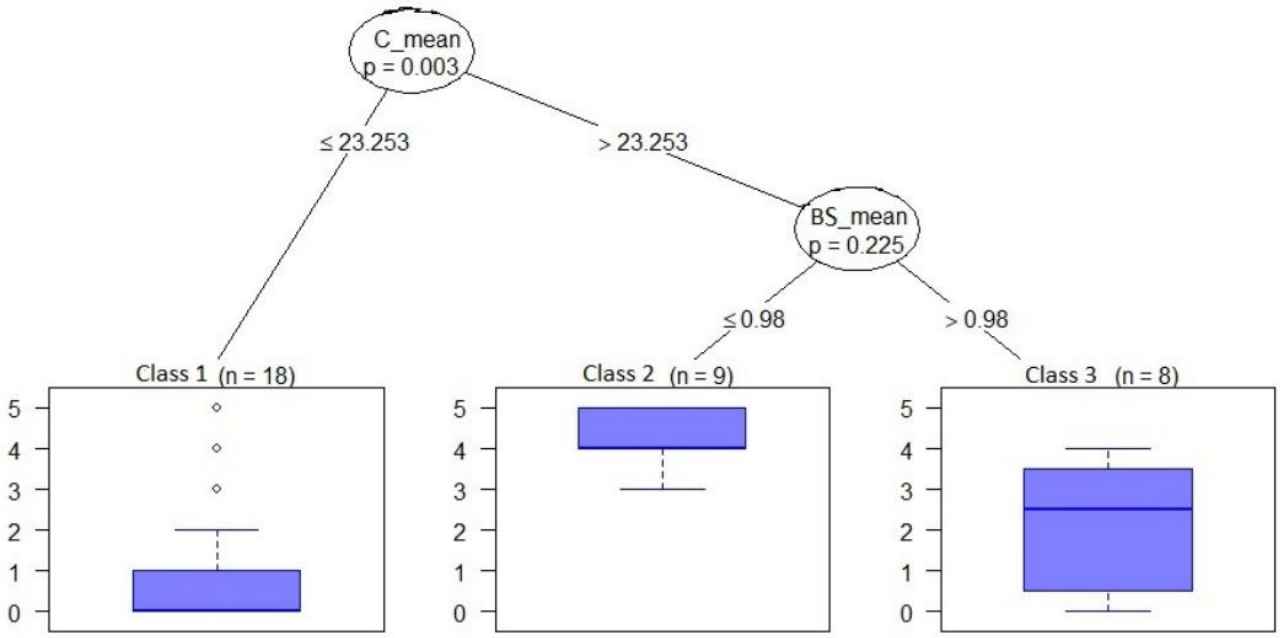


Figure 5. CART classification of health areas, according to the level of malaria risk and to cropland cover and bare soil cover. The blue box represents the number of times a health area was a hotspot. C_mean—mean cropland cover; BS_mean—mean bare soil cover.

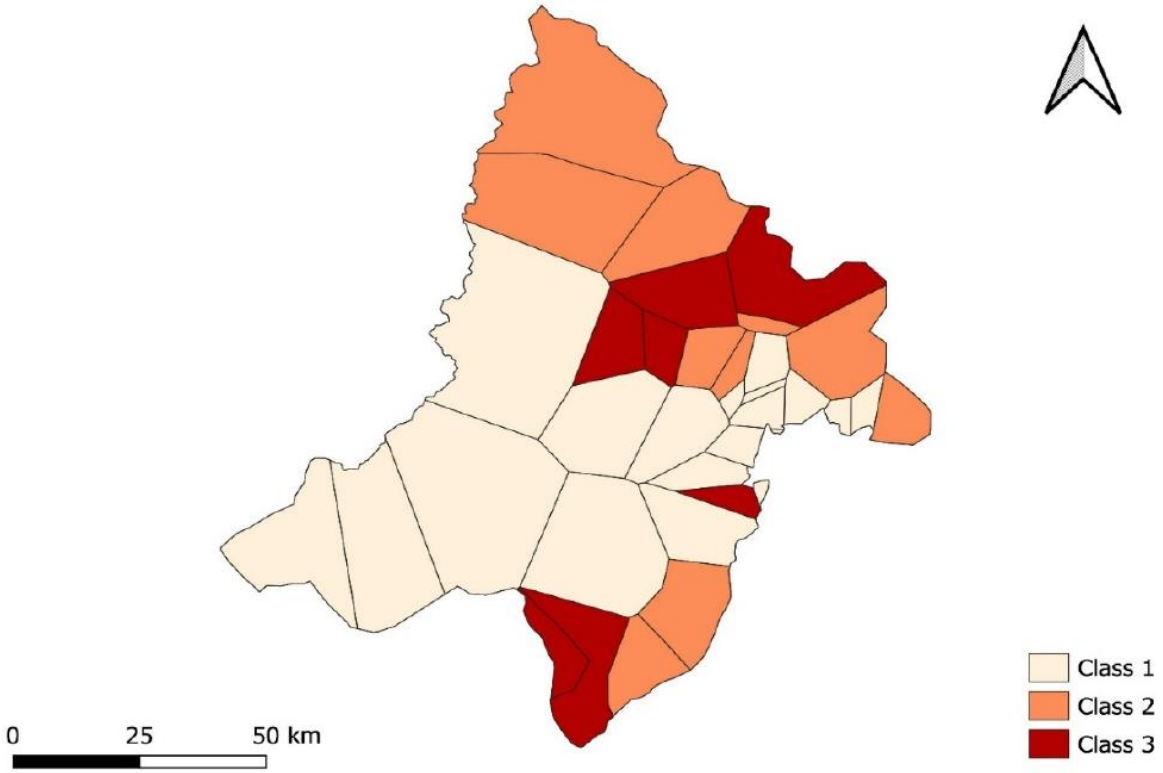


Figure 6. Map of health areas classified according to level of malaria risk. Class 1 corresponds to a low level of malaria risk, Class 2 to a high level of malaria risk, and Class 3 to a moderate level of malaria risk.

4. Discussion

This study aimed to describe the spatio-temporal variability of malaria incidence and the associated meteorological and environmental factors, in the health district of Kati, Mali, for the period 2015–2019. Our descriptive analysis of the incidence curve confirmed the seasonality of malaria transmission in the Kati district. The spatio-temporal variability of malaria incidence was high and was associated with several meteorological and environmental factors. Malaria hotspots were detected during all LTPs.

For most of the study period, the onset of the HTP occurred in July in the Kati health district—i.e., one month later than is usually observed in this, and other health districts of the Sudano–Sahelian zone. This finding was similar to that reported by Dieng et al. in Senegal [5], which can most likely be explained by the climatic similarities between the two study sites. However, it differs from that reported by Cissoko et al. in northern Mali (Diré health district) [21], who found that the HTP onset occurred in August.

Malaria incidence decreased gradually over the study period. This decrease may be due to the following factors: (1) the impact of SMC, which was introduced in the Kati district in 2016 [32], and that was likely to have helped to reinforce the malaria control strategies already in place; (2) the observed decrease in rainfall over the study period; or (3) a combination of the two. Note that a decrease in malaria incidence has been reported in several other regions of sub-Saharan Africa and in other parts of the world in recent years [15,25,26,33–35].

The lag between the peak rainfall and the peak malaria incidence in the Kati district was 8 weeks in 2015, 2018, and 2019; 13 weeks in 2016; and 14 weeks in 2017. Similarly, the studies by Ferrão et al. in Mozambique [36] and Diouf et al. in Senegal [37] reported a lag of 8 weeks between the peak rainfall and the peak of malaria incidence, while that by Teklehaimanot et al. in Ethiopia [38] found a lag of 12 weeks between the two events.

A lag of 4 weeks was observed between SI#1 (river flow and height, relative humidity, and rainfall) and malaria incidence. By contrast, Sissoko et al., in Mali [25], and Rouamba et al., in Burkina Faso [33], reported a lag of 8 and 9 weeks, respectively, between relative humidity and malaria incidence. Ouedraogo et al., in Burkina Faso [26], found a shorter lag of 2 weeks between these two variables.

The relationship between SI#1 (river flow and height, relative humidity, and rainfall) and malaria incidence was positive and almost linear. Interestingly, the relationship between relative humidity and malaria incidence was also positive and almost linear in the studies by Segun et al., in Nigeria [39], and by Ouedraogo et al., in Burkina Faso [26]. Moreover, a positive and almost linear relationship between river height and malaria incidence was found in the studies by Stefani et al., in French Guiana [34], and by Sissoko et al. [25] and Cissoko et al. in Mali [21]. In our study, the correlation between rainfall and malaria incidence was not as strong as that between the other SI#1 variables and malaria incidence. This suggests that the impact of rainfall on malaria incidence in the Kati district is always mediated by relative humidity and by river flow and height [5].

The relationship between SI#2 (air pressure and temperature) and malaria incidence was non-linear, with a lag of 19 weeks. The incidence of malaria increased gradually as the temperature increased but decreased significantly at higher temperatures—a phenomenon explained by the fact that *Anopheles gambiae* (the principal vector for this area) larvae and adults die at temperatures above 35 °C [40]. A non-linear relationship between temperature and malaria incidence was also observed in the studies by Sissoko et al., in Mali [25], and by Ouedraogo et al. [26] and Bationo et al., in Burkina Faso [15]. However, the lag between temperature and malaria incidence in these studies was 13, 2, and 16 weeks, respectively.

The LTP and HTP were determined using a change-point analysis, and followed the two climatic seasons of Mali: the dry season and the rainy season. Similar results were reported by Sissoko et al. [25] and Coulibaly et al. in Mali [41], by Dieng et al. in Senegal [5], and by Rouamba et al. [33] and Baragatti et al. in Burkina Faso [42].

The distribution of malaria hotspots varied significantly over the study period. Some health areas, most of them in urban areas, remained non-hotspots throughout the study. Others were hotspots in the first year of the study or in two consecutive years, and others were hotspots during every year of the study (Table 2). The non-negligible incidence observed during the LTPs reflected the persistence of malaria during the dry season. This phenomenon, which was also observed in Senegal by Dieng et al. [5], suggests that malaria control strategies need to be implemented earlier than is currently the case.

The CART analysis found a very low risk of malaria transmission in areas with little cropland. This risk was four times higher in areas with large cropland areas and little bare soil, and two times higher in areas with large cropland areas and large bare soil areas (Figure 5). This finding suggests that market gardening, which is widespread in the Kati district, increases the risk of malaria transmission—a phenomenon explained by the fact that the water reservoirs needed to sustain this activity constitute potential mosquito breeding sites. This finding is also in line with the studies conducted in other parts of the world. Thus, Cissoko et al. [21] and Bhattarai et al. [43] found malaria incidence to be associated with land use and the proximity of water sources in Mali and Nepal, respectively. For their part, Mitchell et al. [44] and Paul et al. [45] observed an association between greater cropland cover and increased malaria prevalence in Tanzania. Lastly, Nicholas et al. [46] found a link between agricultural practices and the proliferation of malaria vectors in Kenya.

The limitation of our study was the use of data from consultation records. Most of these records were not properly maintained. In addition, information was missing in some observations. As a corrective action, all incomplete observations were removed from the database. Another limitation of this study was the absence of the consideration of social and behavioral factors, which are important in the implementation of malaria control interventions.

5. Conclusions

Our study confirmed the important variability of malaria incidence in the health district of Kati and confirmed that malaria's variability was highly associated with a combination of meteorological and environmental factors, including land cover and land use. We found that malaria incidence was low in areas with little cropland areas, but much higher in areas with large cropland areas and little bare soil areas. The persistence of malaria cases during the dry season and the spatio-temporal variability of malaria hotspots reinforce the need for innovative and targeted strategies in Mali.

Author Contributions: A.K., J.G., M.S.S. and I.S. (Issaka Sagara) designed the study and wrote the first draft of the manuscript; A.K., M.C. and C.S.B. performed the statical analysis, under the supervision of J.G. and I.S. (Issaka Sagara); M.D., I.T., S.T. and M.K. conducted the field study, coordinated by A.K. and I.S. (Issaka Sagara); P.D., D.B., I.S. (Ibrahima Sidibe) and I.S. (Ismael Simaga) contributed to the data collection and description; J.L. contributed to the interpretation of the results and the critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

WHO	Word Health Organization
NMCP	National Malaria Control Program
LLIN	Long-lasting insecticide net
IRS	Indoor residual spraying
RDT	Rapid diagnostic test
ACT	Artemisinin-based combination therapy
IPT	Intermittent preventive treatment
SP	Sulfadoxine-pyrimethamine
SMC	Seasonal malaria chemoprevention
AQ	Amodiaquine
PCA	Principal component analysis
SI	Synthetic indicator
GAM	Generalized additive model
RR	Relative risk
CART	Classification and regression tree analysis
LTP	Low transmission period
HTP	High transmission period

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Article 7

Sub-national tailoring of seasonal malaria chemoprevention in Mali based on malaria surveillance and rainfall data

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Parasites & Vectors

RESEARCH Open Access

Sub-national tailoring of seasonal malaria chemoprevention in Mali based on malaria surveillance and rainfall data

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Abstract

Background: In malaria endemic countries, seasonal malaria chemoprevention (SMC) interventions are performed during the high malaria transmission in accordance with epidemiological surveillance data. In this study we propose a predictive approach for tailoring the timing and number of cycles of SMC in all health districts of Mali based on sub-national epidemiological surveillance and rainfall data. Our primary objective was to select the best of two approaches for predicting the onset of the high transmission season at the operational scale. Our secondary objective was to evaluate the number of malaria cases, hospitalisations and deaths in children under 5 years of age that would be prevented annually and the additional cost that would be incurred using the best approach.

Methods: For each of the 75 health districts of Mali over the study period (2014–2019), we determined (1) the onset of the rainy season period based on weekly rainfall data; (ii) the onset and duration of the high transmission season using change point analysis of weekly incidence data; and (iii) the lag between the onset of the rainy season and the onset of the high transmission. Two approaches for predicting the onset of the high transmission season in 2019 were evaluated.

Results: In the study period (2014–2019), the onset of the rainy season ranged from week (W) 17 (W17; April) to W34 (August). The onset of the high transmission season ranged from W25 (June) to W40 (September). The lag between these two events ranged from 5 to 12 weeks. The duration of the high transmission season ranged from 3 to 6 months. The best of the two approaches predicted the onset of the high transmission season in 2019 to be in June in two districts, in July in 46 districts, in August in 21 districts and in September in six districts. Using our proposed approach would prevent 43,819 cases, 1943 hospitalisations and 70 deaths in children under 5 years of age annually for a minimal additional cost. Our analysis shows that the number of cycles of SMC should be changed in 36 health districts.

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Conclusion: Adapting the timing of SMC interventions using our proposed approach could improve the prevention of malaria cases and decrease hospitalisations and deaths. Future studies should be conducted to validate this approach.

Keywords: Malaria, High transmission season, Rainfall, Sub-national, Tailoring

Background

In 2020, 482,790 deaths attributable to malaria were reported in children under 5 years of age worldwide, and 95% of the 242 million cases of malaria occurred in Africa [1]. In Mali, the Local Health Information System recorded 1454 hospital deaths attributable to malaria out of 2,884,837 confirmed cases in 2020. The 2018 Mali Demographic and Health Survey (DHS) reported a mortality of 101 per 1000 children [2], a figure considerably higher than the average mortality of 76 per 1000 children reported in 2019 by the World Bank for the Sub-Saharan Africa region [3].

The WHO technical guidelines for malaria control recommend the implementation of targeted interventions in malaria endemic countries [4]. Targeting consists of tailoring interventions to the local epidemiological context to make better use of available financial resources [5, 6]. Recommended interventions include community-based diagnostic and treatment, chemoprevention for children aged 3–59 months and pregnant women as well as vector control using long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and larviciding [4, 7]. In low-prevalence countries (i.e. malaria prevalence < 1%), mass drug administration is recommended to reduce asymptomatic carriage and to control epidemics [8]. Epidemiological surveillance allows the timing of outbreaks to be determined, epidemics to be detected and the development of parasite and mosquito resistance to be tracked, with the view to adapt the timing of interventions and to check the progress made [4]. Between 2010 and 2017, targeted control interventions helped to reduce the global incidence of malaria by > 40% [9].

In Mali, the multiplication of chloroquine resistance outbreaks prompted the National Malaria Control Program (NMCP) to revise its malaria management protocol in 2005 and then again in 2020 by integrating the new WHO recommendations. Since June 2006, uncomplicated malaria cases are treated with artemisinin-based combination therapy (ACT) and severe malaria cases with injectable artesunate, artemether or quinine (as a last resort) for a period of 7 days, with these injectables being replaced with ACT if the patient's condition improves rapidly. In places where the technical infrastructure does not allow the continuation of treatment for severe malaria, pre-referral treatment is recommended to reduce the risk of death. Diagnosis and treatment are provided in public and para-public health facilities: rapid diagnostic tests (RDTs) are free for all patients with suspected malaria, and treatment is free for pregnant women and children under 5 years of age [10].

In accordance with WHO recommendations, malaria prevention in Mali involves vector control using LLINs and IRS and chemoprevention in children and pregnant women [10]. A major control intervention is seasonal malaria chemoprevention (SMC) [11], which consists of the monthly administration of antimalarial drugs to children aged 3–59 months during the high malaria transmission season [12]. Since 2016, the NMCP has been implementing SMC interventions in all health districts on the basis of historical national data on malaria incidence or prevalence [13]. Together with financial partners, the NMCP carries out macro-planning in November to budget the costs of the following year's campaign based on available resources (purchase and transport of SMC drugs). Between May and June, the heads of health districts come together in regional micro-planning workshops to budget the costs of administering SMC at the operational scale (per diem, logistics, supervision, paperwork). Interventions are performed in four cycles of treatment during the high transmission season (from July to November) [13]. Antimalarial drugs (sulfadoxine-pyrimethamine and amodiaquine) are administered by community health workers to children aged 3–59 months either door-to-door or at fixed sites in town centres. Importantly, SMC interventions are increasingly cost-effective [14]: the average unit cost (per child and per cycle) went from 1.1 euro (€) (745 CFA franc) in 2012 [15] to 0.7€ (425 CFA franc) in 2014 [14]. (Note that this unit cost covers drugs, transport, per diems and logistics.)

The efficacy of SMC interventions in Mali has been demonstrated at the health district scale. An 80% reduction in uncomplicated and severe cases has been reported in the health district of Kati (in southern Mali) following the implementation of SMC [13]. One study reported a 61% difference in mortality between health districts with and without SMC interventions [14]. Another study found that deaths and hospitalisations attributable to malaria were 44% and 61% lower, respectively, in health districts where SMC was implemented compared to those where it was not [18]. However, no decline in malaria incidence was observed at the national scale in

children under 5 years of age between 2018 and 2020, even though the effectiveness of control interventions did not seem to be compromised by parasite resistance [9], suggesting a need for improving malaria control in Mali, including by tailoring the timing of SMC interventions to that of malaria transmission at the operational scale.

Malaria transmission depends on a combination of meteorological and climatic factors that determine the distribution and abundance of the disease vector *Anopheles* species. In Mali, the main vectors of malaria transmission are *Anopheles gambiae* sensu stricto, *An. funestus*, *An. coluzzii* and *An. arabiensis* [19]. These species share a similar life-cycle: after a period of aestivation [19, 20], they start laying eggs to increase their population as environmental conditions become favourable again (rainy season and irrigation). They then ingest, develop and transmit the plasmodium during their blood meal on asymptomatic human carriers [21]. In accordance with this life-cycle, the number of malaria cases increases after the onset of the rainy season, with a lag that corresponds to the delayed impact on transmission of the increase in mosquito activity [22, 23]. Across the country, the rainy season lasts from April to October, and the peak of malaria transmission occurs in September, with the lag between the onset of the rainy season and that of the high transmission season ranging from 5 to 18 weeks depending on the eco-climatic zone [22, 23]. However, in some of the northern regions, the rainy season lasts from July to October and is followed by a flooding season that lasts from November to February. A second peak usually occurs in December in these regions [24] as transmission can persist for several weeks in flooded areas (sometimes until February) due to the lasting presence of humid zones, aquatic vegetation and the malaria vector species *An. funestus* [24]. These data suggest important spatio-temporal heterogeneity of malaria transmission in Mali, a phenomenon that should be taken into consideration to improve SMC interventions in a context of limited resources. However, while this heterogeneity has been explored in specific regions of Mali [25–27], it has yet to be described at the scale of the entire country.

This study proposes a predictive approach for tailoring the timing and number of cycles of SMC in all health districts of Mali based on sub-national epidemiological surveillance and rainfall data. Our primary objective was to select the best of two approaches for predicting the onset of the high transmission season at the operational scale. Our secondary objective was to evaluate the number of malaria cases, hospitalisations and deaths in children under 5 years of age that would be prevented annually and the additional cost that would be incurred using the best approach.

Methods

Study site

Mali is a landlocked country with a surface area of 1,241,248 km^2. Its population was estimated at 22 million in 2021 (National Population Directorate (DNP) [28].

The country is split into four ecoclimatic zones: the Saharan zone in the north (annual rainfall: < 200 mm), the vast Sahelian zone in the centre (annual rainfall: ≤ 800 mm), the Sudanese-Sahelian zone in the southeast (annual rainfall: 1000 mm) and the Sudanese-Guinean zone in the extreme south (annual rainfall: > 1000 mm). There are two seasons in Mali: the dry season from November to March and the rainy season from April to October. The first rain events occur as intermittent thunderstorms around April or May.

Mali is divided into 75 health districts that correspond to the operational scale of SMC interventions (i.e. microplanning of activities). Each health district comprises one hospital and 10–20 health areas that provide primary care and conduct epidemiological surveillance.

Data and data source

Malaria data

In Mali, malaria cases are usually confirmed with RDTs in public, para-public and private health facilities at the community level or in hospital. As *Plasmodium falciparum* is responsible for 98% of malaria cases in the country, the RDTs currently in use are based on the detection of *P. falciparum* histidine-rich protein 2 (PfHRP-2) [29, 30]. Malaria cases are occasionally confirmed in hospital by microscopy examination of thick blood smears.

Data on malaria (suspected, tested and confirmed cases as well as deaths) are collected weekly at the health district level by health facility agents (community health workers, technical directors of community health centres, private institutions and hospital data managers) via the epidemiological surveillance system. The data are then reported to the NMCP and entered in the District Health Information Software version 2 (DHIS2) data system.

This study used weekly aggregated data on malaria cases, hospitalisations and deaths for the 75 health districts of Mali over the 2014–2019 period. No personal data were used.

Rainfall data

In Mali, 24 meteorological, climatological and agro-bioclimatological stations and 17 synoptic stations are currently in operation [31]. As these stations do not cover all 75 health districts, we used remote sensing rainfall data in our analyses. Weekly rainfall data by health district were extracted from the Climate Hazards Group Infra-Red Precipitation with Station data (CHIRPS) rainfall

dataset via the Google Earth engine interface [32, 33] for the 2014–2019 period.

Population data

Data on population by health district for the 2014–2019 period were obtained from the DNP. These data are updated yearly through multiplying population figures from the General Population and Housing Census of 2009 (GPHC 2009) by the annual population growth rate [34].

Intervention cost data

The average unit cost of SMC interventions (per child and per cycle) was obtained from the study by Diawara et al. [14]. This cost covers drugs, transport, per diems and logistics, but does not cover health infrastructure.

Data validation

Data on malaria are monitored weekly and monthly at the local level by a data manager. Feedback is provided to the health facility agents who entered th data into the DHIS2 data system.

With the support of the US President's Malaria Initiative (USAID and PMI Measure Malaria) [35], the NMCP organises bi-annual reviews of surveillance data in problem areas as part of quality improvement of malaria data (https://pnlp.ml). These reviews are accompanied by formative supervision for all health personnel involved in malaria control and management. Data are then processed and validated at the local, regional and national levels during a yearly workshop involving data management officers, funding partners and hospital and programme representatives [36].

Data analysis

In the first part of the analysis, we determined the median onset of the rainy season, the median onset of the high transmission season, the median lag between these two events and the median duration of the high transmission season in each health district for the 2014–2019 period.

We determined the median onset of the rainy season in each health district for the 2014–2019 period using weekly rainfall data. Adapting the definition proposed by Sivakumar et al. [37], we defined the onset of the rainy season as the week when rainfall reached 20 mm after 1 April.

The median onset and median duration of the high transmission season in each health district for the 2014–2019 period were determined by performing a change point analysis of weekly incidence data [38, 39]. For each health district, incidence per 1000 person-weeks was calculated by dividing the number of weekly cases by population × time.

The lag between the median onset of the rainy season and the median onset of the high transmission season reflected the delayed impact on malaria transmission of the increase in mosquito activity after aestivation. It was calculated as follows (Eq. 1):

$$\text{Median lag} = \text{median onset of high malaria transmission season} - \text{median onset of rainy season} \quad (1)$$

Two predictive approaches were evaluated. The first approach, App-A, predicted the onset of the high transmission season in 2019 using median rainfall data and median lag data for the 2014–2018 period. The second approach, App-B, predicted the onset of the high transmission season in 2019 using rainfall data for 2019 and median lag data for the 2014–2018 period.

The two approaches were evaluated by calculating the difference in weeks between the observed and predicted onset of the high transmission season in 2019. A difference between - 2 and + 2 weeks was considered acceptable. For each health district i:

$$\text{Error}_i = \text{observed onset}_i \text{of high transmission season} - \text{predicted onset}_i \text{of high transmission season} \quad (2)$$

The two approaches were also evaluated by calculating the standardised score (z) of the onset of the high transmission season for each health district [40]:

$$z = \frac{x - \mu}{\sigma}$$

where x is the predicted value of the onset of the high transmission season; μ is the mean value of the onset of the high transmission season in 2014–2019; and σ is the standard deviation of the onset of the high transmission season in 2014–2019.

The onset of the high transmission season was used to determine the best start date of SMC interventions in each health district. The duration of the high transmission season was used to determine the number of cycles of SMC needed in each health district.

Incidence data were presented in a box and whisker format. Health districts with irregular seasonality and/or low seasonal transmission were identified based on incidence figures and the results of the change point analysis.

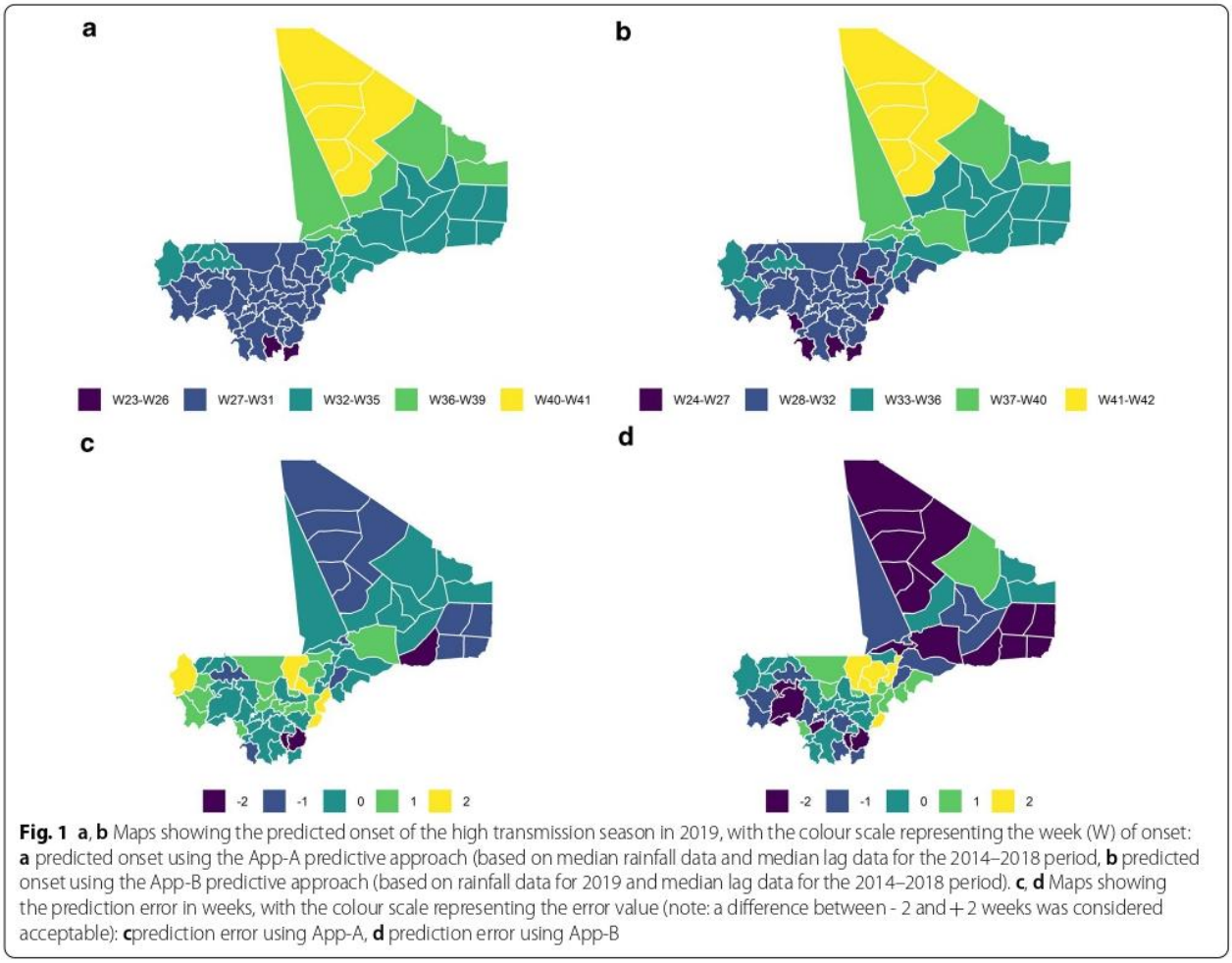


Fig. 1 **a**, **b** Maps showing the predicted onset of the high transmission season in 2019, with the colour scale representing the week (W) of onset: **a** predicted onset using the App-A predictive approach (based on median rainfall data and median lag data for the 2014–2018 period, **b** predicted onset using the App-B predictive approach (based on rainfall data for 2019 and median lag data for the 2014–2018 period). **c**, **d** Maps showing the prediction error in weeks, with the colour scale representing the error value (note: a difference between - 2 and + 2 weeks was considered acceptable): **c**prediction error using App-A, **d** prediction error using App-B

The second part of the analysis determined the number of malaria cases, hospitalisations and deaths in children under 5 years of age that would be prevented annually using the best of the two predictive approaches.

The additional cost of using the best of the two approaches was also estimated. This cost was the difference between the global cost of the current SMC intervention and the global cost that would be incurred using the best approach. Global costs were calculated through multiplying the average unit cost [14] by the number of children per cycle (as validated in the micro-plans).

Software and packages

All statistical analyses were performed using R software version 3.4 ® Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) packages {Changepoint}{ggplot}.

Results

Prediction of the onset of the high malaria transmission season

Over the 2014–2019 period, mean annual rainfall in Mali was 774 mm, varying significantly between health districts, from 60 mm in the north (Taoudenit region in the Saharan zone) to 1384 mm in the south (Sikasso region). The median onset of the rainy season ranged from week (W) 17 (W17; April) in the south (Sikasso region) to W34 (August) in the north (Taoudenit region).

Mean annual malaria incidence over the 2014–2019 period was 90 cases per 1000 person-years, ranging from one case per 1000 persons-years (Taoudenit region) to 285 cases per 1000 person-years (Sikasso and Koulikoro regions). The median onset of the high transmission season ranged from W25 (June) to W40 (October) depending on the health district.

The median lag between the onset of the rainy season and the onset of the high transmission season ranged from 5 to 12 weeks depending on the health district.

Table 1 Classification of health districts according to observed and predicted months of onset of the high malaria transmission season in 2019

Month of onset of the high transmission season	Number of health districts according to observed month of onset	Number of health districts according to predicted month of onset using App-A[a]	Number of health districts according to predicted month of onset using App-B[a]
May	4	2	1
June	36	40	43
July	21	21	15
August	14	12	10
September	0	0	6

[a] App-A and App-B are the predictive approaches used in the study. App-A predicted the onset of the high transmission season in 2019 using median rainfall data and median lag data for the 2014–2018 period. App-B predicted the onset of the high transmission season in 2019 using rainfall data for 2019 and median lag data for the 2014–2018 period

Using the first approach (App-A), the predicted onset of the high transmission season in 2019 ranged from W23 (June) to W40 (September). The predicted onset was in June in two health districts, in July in 46 health districts, in August in 21 health districts and in September in six health districts (Fig. 1a).

Using the second approach (App-B), the predicted onset of the high transmission season in 2019 ranged from W24 (June) to W41 (September). The predicted onset was in June in one health district, in July in 43 health districts, in August in 15 health districts and in September in 16 health districts (Fig. 1b).

Both approaches predicted the onset of the high transmission season with an error of ± 2 weeks.

App-A had the best prediction results, with a median error of 0 weeks. No error was found in 34 health districts (45%) and an error of 2 weeks was observed in three health districts (Ansongo, Sikasso and Niena) (4%) (Table 1; Fig. 1c).

App-B showed a median error of 1 week. An error of 2 weeks was observed in the health districts with strong epidemic potential, most of which were located in the northern regions (Ménaka and Taoudenit regions) and in the District of Bamako (Table 1; Fig. 1d).

Both approaches showed high standardised scores. These ranged from a minimum of - 1 to a maximum of + 1 (Fig. 2).

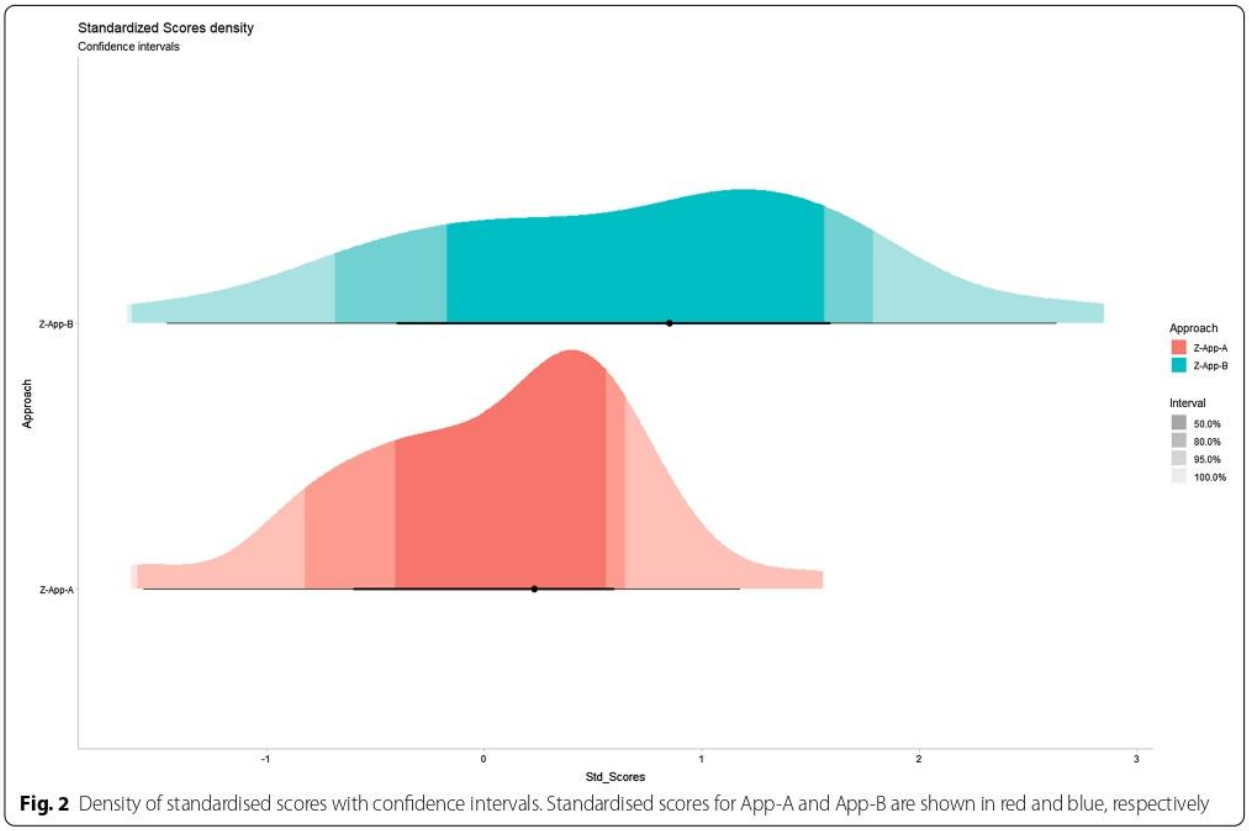


Fig. 2 Density of standardised scores with confidence intervals. Standardised scores for App-A and App-B are shown in red and blue, respectively

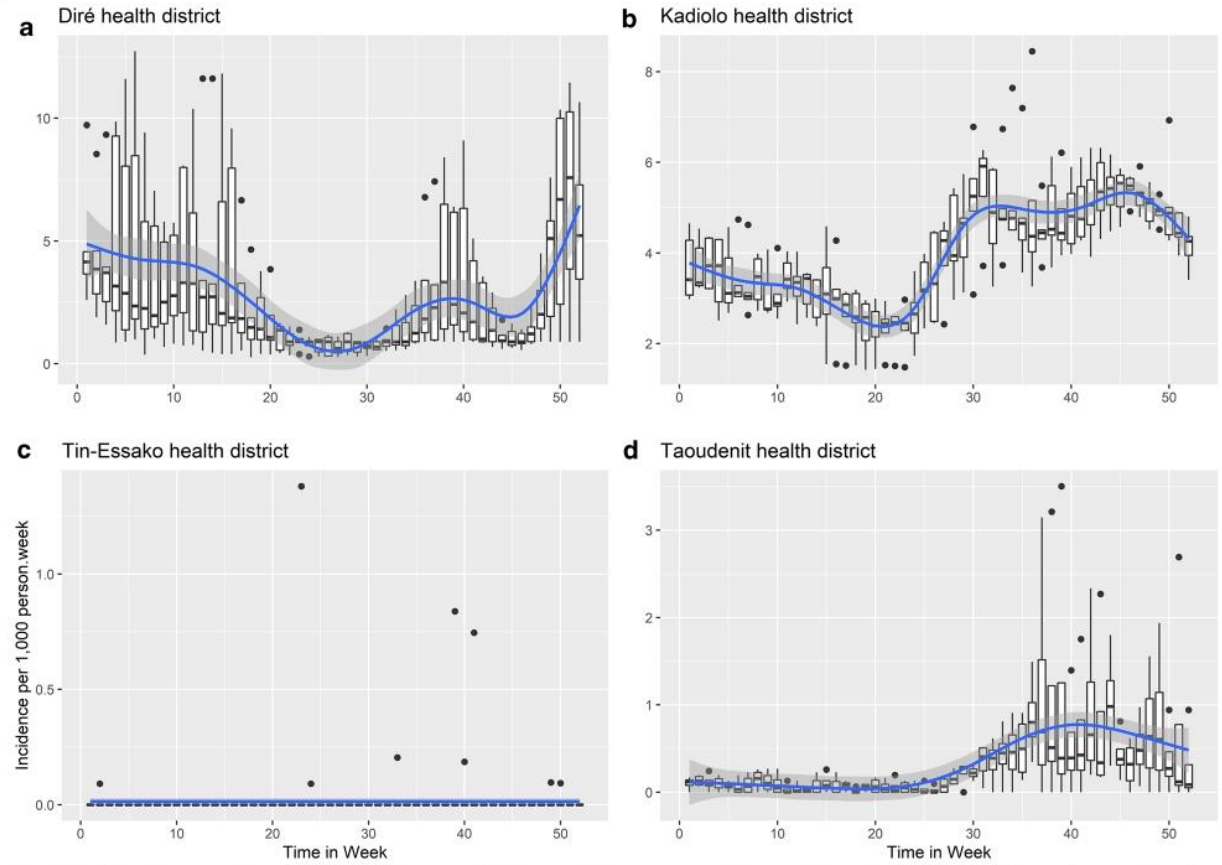


Fig. 3 The Blue line on a silver background represents the smoothing curve and the confidence intervals Duration and seasonality of malaria transmission in four representative health districts. **a** Bimodal seasonality, **b** usual seasonality (from July to December), **c** irregular seasonality, **d** low seasonal transmission

Duration and seasonality of malaria transmission

Over the 2014–2019 period, the mean duration of the high transmission season was 4 months, and the median duration ranged from 3 to 6 months (Fig. 3a). The high transmission season began between June and August and ended between October and February/March.

Usual seasonality of malaria transmission was observed in 73 health districts (97.33%). Seasonality was bimodal in eight health districts (Ansongo, Diré, Goundam, Gourma Rharous, Menaka, Niafunké, Timbuktu and Youwarou), all of which are located along the Niger river (Fig. 3b). Seasonality was irregular in the health districts of Tinessako, Tessalit and Tindermene (Fig. 3c). In the six health districts of Taoudenit region, seasonal transmission was very low, with a weekly incidence of < 1 case per 1000 person-weeks (Fig. 3d).

Our analysis showed that the current strategy of conducting four cycles of SMC between July and October is appropriate in 39 of the 75 health districts. In 36 of these health districts, the number of cycles of SMC should be adapted to the duration and seasonality of malaria transmission. Specifically, 20 health districts should receive five cycles due to bimodal transmission and/or a long high transmission season, seven should receive three cycles due to a short high transmission season and nine should be excluded from SMC interventions due to irregular seasonality and/or low seasonal transmission (Fig. 4).

Additional cost incurred and number of malaria cases, hospitalisations and deaths prevented annually using the best of the two predictive approaches

Based on an average unit cost of 0.7€, the overall cost of implementing SMC interventions in the 75 health districts using App-A would be 11,965,070€, compared to 11,404,705€ for the current approach. The additional cost incurred would be 560,365€, which represents less than 5% of the current cost.

Using the best of the two approaches (App-A) would prevent 43,819 cases, 1943 hospitalisations and 70 deaths in children under 5 years (Fig. 5) annually.

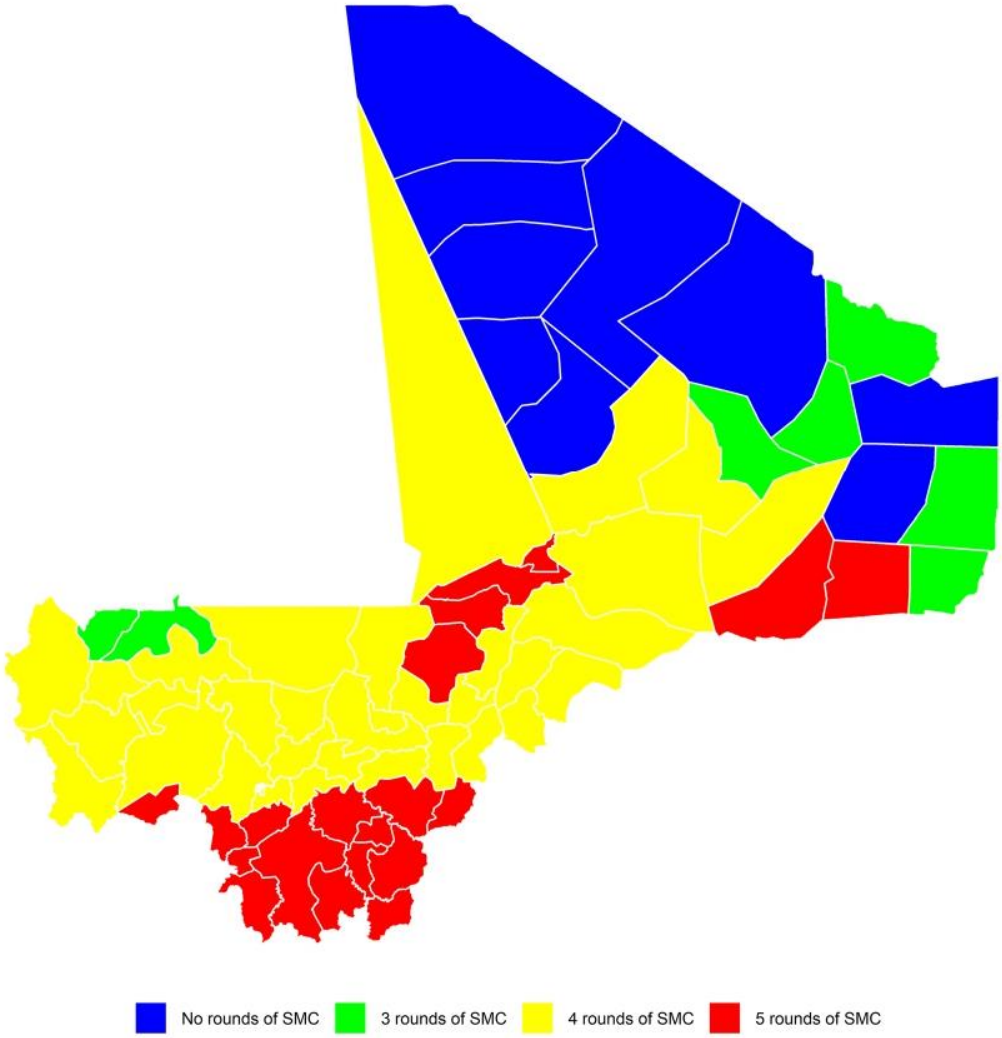


Fig. 4 Number of cycles of seasonal chemoprevention needed in each health district based on the duration and seasonality of malaria transmission. Colour scale represents the required number of cycles. SMC, Seasonal malaria chemoprevention

Discussion

Based on the results of this study, we propose a predictive approach for tailoring the timing and number of cycles of SMC in all health districts of Mali based on sub-national epidemiological surveillance and rainfall data. Our analysis shows that over the 2014–2019 study period, the onset of the rainy season varied between W17 (April) in the south and W34 (July) in the north along an environmental gradient. The onset of the high transmission season followed a similar pattern, with the high transmission season beginning in the Sahelian and Sudanese-Guinean zones one or two months earlier than in the Saharan zone. The median lag between the onset of the rainy season and the onset of the high transmission season ranged from 5 to 12 weeks, and the duration of the high transmission season varied between 3 and 6 months. Our findings suggest that the current malaria control strategy could be adapted in nearly half of Mali's health districts in order to yield additional morbidity and mortality reductions. Using our proposed approach (App-A) would prevent 43,819 cases, 1943 hospitalisations and 70 deaths in children under 5 years of age (Fig. 5) annually for a minimal additional cost (560,365€).

The current malaria control strategy of the NMCP consists in delivering four cycles of SMC each year during the high transmission season across the 75 health districts of Mali. The timing and number of cycles of SMC were selected, respectively, based on the average onset of the high transmission season (from July to November) and the average duration of the high transmission season

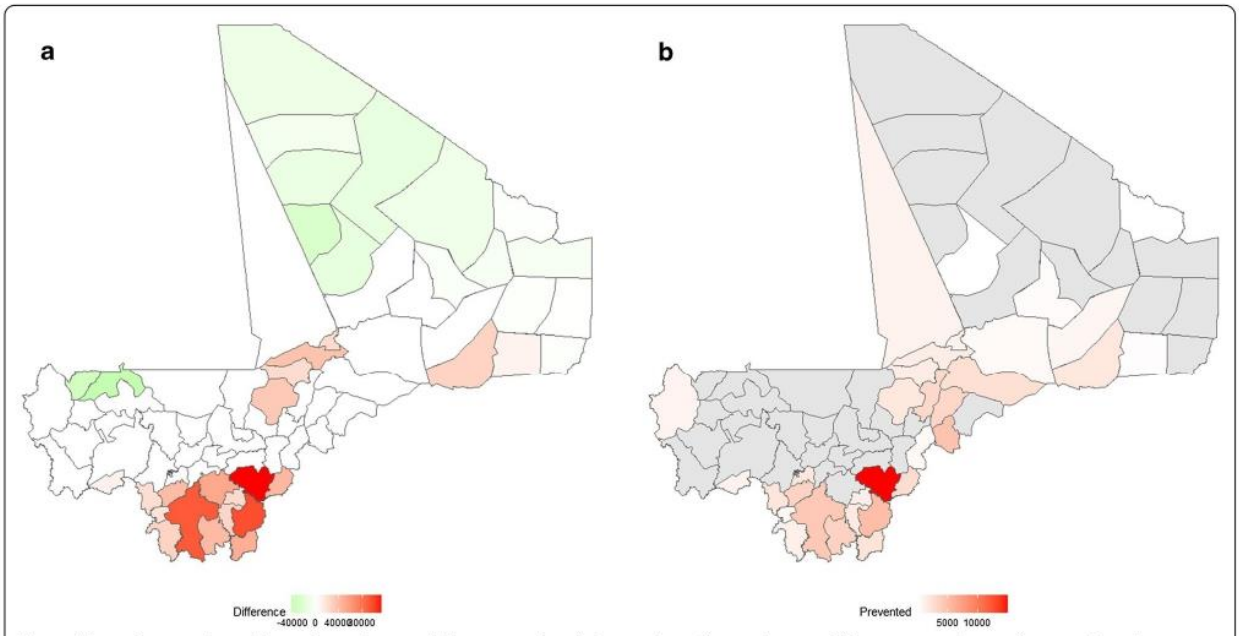


Fig. 5 Maps showing the additional cost that would be incurred and the number of cases that would be prevented annually using App-A. **a** Additional cost incurred per health district, with the colour scale representing the difference between the cost of using the current approach and the cost of using App-A. **b** Number of malaria cases prevented per health district, with the colour scale representing the estimated number of cases that would be prevented using App-A

(4 months) [13]. Because this strategy does not account for the spatio-temporal heterogeneity of malaria transmission in Mali, some health districts receive SMC long before or long after the onset of the high transmission season, which likely compromises the global efficacy of SMC.

Our findings confirm that an average of four cycles of SMC is needed across the country. However, they also indicate that the effectiveness of SMC interventions could be improved by adapting the timing and number of cycles in 36 of the 75 health districts. Specifically, the number of cycles should be increased to five in health districts with bimodal transmission (i.e. in flooded areas or areas next to dams) and/or a long high transmission season; this finding is in line with results reported by Tagbor et al. which showed that the malaria burden could be reduced by 50% in a region of Ghana with a long high transmission season if $\geq$ 5 cycles of SMC were distributed annually [41]. In contrast, those health districts with irregular seasonality and/or low seasonal transmission (i.e. without cases for several weeks) appear to benefit little from SMC interventions. These health districts, all of them located in the arid north, could be excluded from the SMC program. Interestingly, Noor et al. [49] reached a similar conclusion in a study using seasonality, an age-structured population, urbanisation and malaria endemicity to identify health districts suitable for SMC targeting in Sahelian countries.

In 2019, the District of Bamako was excluded from the SMC program, as the 2018 DHS reported a low prevalence of malaria in children aged 6–59 months [2]. However, this survey was carried out during the SMC intervention and thus at a time when children were protected from malaria infection, suggesting that the prevalence of malaria was underestimated. Our study observed a high incidence of malaria in the general population of the District of Bamako, indicating that the city may need to be reintegrated in the SMC program. Indeed, studies have shown that the persistence of malaria in the general population can lead to epidemic resurgence, with severe cases in children [43, 44]. Moreover, the absence of premunition in the population of health districts with low seasonal transmission is known to increase the risk of epidemics [45]. These concerns are supported by a recent study showing that the incidence of malaria in children aged 6–59 months nearly doubled in the District of Bamako between 2018 and 2021 [46]. At the very least, the peri-urban area of Bamako should be reintegrated in the SMC program, as indicated by a meta-analysis of urban malaria in sub-Saharan Africa, which found the entomological inoculation rate to be sixfold higher in peri-urban areas (45.8) than in city centres (7.1) [47].

Sub-national tailoring of SMC interventions is now recommended by the WHO as part of the High Burden High Impact Initiative. In Senegal, SMC interventions have been tailored to the onset and duration of the rainy season since 2015. Interventions are initiated 1 month after the onset of the rainy season, with three cycles delivered in the regions of Kolda, Sédhiou and Tambacounda and four cycles delivered in the region of Kédougou [48]. Considering that the lag between the onset of the rainy season and that of the high transmission season is highly variable, our approach for predicting the onset of the high transmission season based on rainfall data allows for an even more refined tailoring of SMC interventions.

The additional cost of using our proposed approach was estimated by multiplying the average unit cost of SMC [14] by the number of target children in all health districts, a method used by Noor et al. [49] for the population-based estimation of malaria drug needs in sub-Saharan Africa in 2015–2020 [50]. The estimated additional cost is relatively low (560,365€) because the exclusion of health districts with irregular seasonality and/or low seasonal transmission would compensate for the increase in the number of cycles in health districts with bimodal transmission and/or a long high transmission season. In practice, this cost would likely be even lower because the health districts to be excluded are all located in the north of the country, where a very low population density leads to greater transport needs (including vehicle rental) and therefore to a higher unit cost of SMC. In this regard, the unit cost of SMC has also been shown to vary greatly in other Sub-Saharan countries. In Senegal, where the average unit cost (0.5€) is similar to that in Mali (0.7€) and covers roughly the same expenses, the unit cost in small health posts with few target children was found to be almost fivefold higher (2.5€) than the average unit cost [51]. In Ghana, the unit cost of SMC (per child receiving at least the first dose of four courses) has been shown to vary greatly depending on the mode of delivery (from 4.2€ for delivery by veterinary health workers to 4.5€ for delivery by consultation nurses and to 5.1€ for delivery by Expanded Programme on Immunisation nurses) [52]. To further reduce the cost of our proposed approach, the number of distribution days per cycle could be reduced from 5 to 4 days. As it turns out, some of Mali's neighbouring countries (Senegal, Burkina Faso and Niger) have been distributing SMC over 3–4 days from the start [49].

In Mali, epidemiological surveillance data are mainly provided by the public and para-public sectors, as the rate of healthcare seeking in the private sector (including for specialised care) is very low at 3%. Indeed, private health care not developed to any great extent in the country, with 70% of private facilities located in the District of Bamako [53, 54]. Moreover, the vast majority of Malians prefer to seek care in the public and para-public sectors, where malaria testing and treatment for severe cases are free of charge. Currently, 26% of private healthcare facilities enter their malaria data directly in the DHIS2 sysem. Other private facilities send their data to community health centres and district hospitals, which in turn enter these data into the platform.

Monitoring activities in Mali have improved the quality of epidemiological surveillance data in recent years. In 2020, the data completeness rate was 98% (compared to 94.7% in 2016 and 76% in 2014) and the data promptness rate was 70.2% [33]. However, data quality has improved mainly in the south of the country. In the northern health districts, which suffer from political insecurity and low internet and telephone coverage, the completeness rate and the promptness rate were 60% and 42%, respectively, in 2020 [33]. These figures raise the possibility of underreporting in the Saharan zone. In the future, the quality of routine malaria data could be improved in this zone by adapting epidemiological surveillance to the local context. Should the low incidence of malaria transmission in the northern health districts be confirmed, malaria control could be strengthened by switching from weekly to daily epidemiological surveillance and by performing SMC interventions on a case-by-case basis. This approach has proven to be successful in the neighbouring Maghreb countries, which now have sporadic transmission and are considered to be malaria-free [55, 56].

The rainfall data used in our study were collected through remote sensing. Their quality could be compromised by poor satellite visibility, especially in the north where sand storms are common. However, several epidemiological studies have shown the relevance of using remote sensing rainfall data in the absence of observational data [57–59]. In particular, one study used remote sensing rainfall data to estimate malaria seasonality, populations at risk and areas to be targeted by SMC in Sahelian and sub-Sahelian countries [42]. In Mali, a recent study conducted in the northern health district of Diré found no difference between observational and remote sensing rainfall data [24], suggesting that the rainfall data used in our study are reliable. This reliability is supported by studies conducted in other malaria endemic countries, which reported lags between the onset of the rainfall season and the onset of the high transmission season that are consistent with our results [24, 60–62].

Given the variability of rainfall in the Sahelian zone [25, 63], some authors have suggested collecting rainfall data over several years for the planning of malaria control interventions in the area [64, 65]. This strategy has already shown its efficacy for agricultural production in the Gourma zone of Mali [66, 67]. It was also specifically

recommended for the epidemiological surveillance of malaria in a study conducted in the Sahelian zone of Niger (Zinder) [68]. The predictive approach proposed in our study accounts for the high variability of rainfall over time and can therefore contribute to improving malaria epidemiological surveillance in Mali.

The main limitation of our study is that we used routine malaria data, which do not perfectly reflect the epidemiological situation on the ground. As mentioned earlier, however, the implementation of periodic reviews by the NMCP has considerably improved the quality of malaria data in recent years, especially in the southern health districts. Moreover, to correct for underreporting in the northern health districts, we collected malaria data from local reports when these were missing from the DHIS2. Another limitation of our study is that the number of preventable malaria cases, hospitalisations and deaths was calculated for children under 5 years of age instead of children aged 3–59 months, the latter being the age range selected for SMC interventions in Mali. Yet, it is unlikely that the efficacy of our approach was overestimated given that malaria is rare in children aged under 3 months. Lastly, entomological data were not included in our analyses because these are only available for a few sites in Mali. We compensated for the absence of entomological data by using rainfall data, which have been shown to correlate with malaria vector distribution and abundance [69]. This allowed us to propose a simple and effective approach for predicting the onset of the high transmission season in Mali.

Our proposed approach for tailoring the timing and number of cycles of SMC interventions at the operational scale could improve malaria control and reduce the malaria burden in Mali for a minimal additional cost. Future studies using high-quality routine data could further refine our approach by providing detailed estimations of additional costs in selected locations and/or by determining the effects of temperature, vegetation [70], vector abundance and insecticide resistance on the dynamics of malaria transmission [71, 72]. Our approach could thus be extended to other malaria endemic African countries with similar characteristics.

Conclusion

The study proposes a simple and effective predictive approach for tailoring SMC interventions in Mali based on epidemiological surveillance and rainfall data. Future studies should be conducted to validate this approach with a view to extending it to other African countries with similar characteristics.

Abbreviations

ACT: Artemisinin-based combination therapy; CHIRPS: Climate Hazards Group InfraRed Precipitation with Station; DHS: Demographic and Health Survey; DHIS2: District Health Information Software version 2; DNP: National Population Directorate; GPHC: General Population and Housing Census; IRS: Indoor residual spraying; LLIN: Long-lasting insecticidal net; NMCP: National Malaria Control Program; RDT: Rapid diagnostic test; SMC: Seasonal malaria chemoprevention.

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Author contributions

OYM, PD and MM collected the primary data; MC and JG designed the study; MC, JL and CSB conducted the field study coordinated by MC; MC and JG developed the statistical analysis plan with the participation of JL, IS and MBC; MC carried out the statistical analysis under the supervision of JG; MC performed the mapping analysis with the participation of CSB and JL; MC, JG, AK, JL, VS, DT, SD, MM, ID, ND and CSB participated in the statistical analysis and validated the results; MC, IS, ND and JG drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data and background maps are available on request.

Declarations

Ethics approval and consent to participate

This study was authorised by the competent authority, the National Malaria Control Programme (number 042/MSDS-SG/PNLP, 14 January 2021), in accordance with Malian regulations on ethics and medical research.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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CONCLUSION GENERALE ET PERSPECTIVES

Nos travaux de thèse, dans leur ensemble nous ont permis d'approfondir nos connaissances sur l'épidémiologie du paludisme, de la borréliose et de la mansonellose au Mali. Nous avons scindé nos travaux en quatre parties liées aux aspects cités précédemment.

J'ai d'abord réalisé une revue de la littérature qui m'a permis d'identifier que les facteurs au sein des systèmes de santé d'Afrique subsaharienne, qui constituent un obstacle majeur à l'application effective des directives nationales de lutte contre le paludisme. Les facteurs identifiés, tels que la formation insuffisante du personnel, la mauvaise perception de certains prestataires de soins de santé envers les tests diagnostiques, les pressions des patients, les pénuries de ressources et les coûts élevés des médicaments, impactent de manière significative l'adhésion aux recommandations. Il apparaît clairement que les systèmes de santé dans leur ensemble sont affectés par ces problématiques, soulignant la nécessité d'une approche globale pour améliorer la prise en charge du paludisme.

Dans un deuxième travail, complémentaire au premier, nous avons étudié les connaissances, attitudes et pratiques de la prise en charge du paludisme, dans le district sanitaire de Kita. Cette étude a démontré que le niveau d'adhésion globale des prestataires de soins aux directives nationales de diagnostic et de traitement du paludisme était acceptable (90,4%). Nous avons observé que les prestataires de soins qui utilisaient les TDR étaient beaucoup plus susceptibles de respecter les directives nationales, et que la disponibilité d'un laboratoire était positivement associée avec l'adhésion aux directives (OR = 6.92, 95% CI [1.05 - 45.65]). En outre, la pratique de l'automédication était très significative chez les individus vivants les localités rurales comme Kobiri (OR = 4.43, 95%CI [0.88 - 22.28]) et Kokofata (OR = 13.32, 95%CI [2.36 - 75.07]) par rapport à ceux du milieu urbain.

Le respect des directives nationales de diagnostic et de traitement contribuera à réduire considérablement la morbidité et la mortalité liées au paludisme dans les pays d'endémie. Pour renforcer l'adhésion aux directives

nationales, il est impératif de mettre en œuvre des stratégies ciblées et durables. La formation continue des prestataires de soins, couplée à une supervision régulière des activités, constitue un premier pas essentiel dans cette direction. De plus, des campagnes de communication efficaces visant à modifier les comportements des professionnels de santé et des patients sont indispensables. En renforçant les systèmes de santé et en s'attaquant aux déterminants sociaux de la santé, les pays d'Afrique subsaharienne seront mieux équipés pour atteindre les objectifs de l'élimination du paludisme à l'horizon 2030.

Ensuite, compte tenu de l'importance du paludisme au Mali et de la diversité des espèces impliquées, nous avons trouvé qu'il était nécessaire de procéder à une évaluation des tests de diagnostic rapide pour le diagnostic du paludisme et la cartographie des différentes espèces plasmodiales. Les résultats de ces travaux ont fourni des informations précieuses sur l'épidémiologie du paludisme et la dynamique de la transmission. Pour aider les responsables du Programme National de Lutte contre le Paludisme à adapter plus efficacement leurs interventions en fonction de l'épidémiologie locale.

La troisième partie de cette thèse a porté sur la détection de *Borrelia crocidurae* chez des patients fébriles au Mali, à partir des TDR du paludisme en utilisant les méthodes de biologies moléculaires. L'ADN de *B. crocidurae* a été détecté dans 5% (9 sur 180) des TDR négatifs au paludisme collectés dans le district sanitaire de Diéma en zone sahélienne dans la région de Kayes frontalière avec le Sénégal et la Mauritanie. Les résultats issus de cette partie contribuent de manière significative à l'amélioration de nos connaissances sur la distribution géographique et la prévalence de la borréliose à tique au Mali ; une cause de fièvre non-palustre à considérer dans la région de Kayes.

La dernière partie de cette thèse consistait à utiliser la PCR en temps réel pour détecter *Mansonella perstans* par qPCR à partir des TDR du paludisme réalisés au Mali. L'ADN de *M. perstans* a été détecté seulement dans 1,1% des échantillons testés.

Ces résultats ouvrent de nouvelles perspectives de recherche et de santé publique visant à lutter contre le paludisme et autres maladies infectieuses à transmission vectorielle comme la borréliose et la mansonellose.

- La sensibilisation à la nécessité pour les prestataires de soins de se conformer aux directives nationales et d'informer les patients sur les dangers pour la santé de l'automédication.
- D'autres études plus larges restent à mener sur la fièvre récurrente transmise par la tique *Borrelia* dans le reste des districts sanitaires de la région de Kayes au Mali.

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Résumé

Les maladies vectorielles constituent un problème de santé publique en Afrique en raison de leur morbi-mortalité et de leur impact socio-économique. Plusieurs facteurs liés notamment à l'environnement, aux ressources économiques, aux comportements concourent à maintenir cette situation. Le paludisme constitue la première maladie parasitaire en Afrique sub-saharienne, le Mali est l'un des pays les plus affectés avec une morbidité de 37,7% et de mortalité de 24,4% (DHIS2 2023). Une bonne connaissance de son épidémiologie est nécessaire pour mettre en place des mesures de lutte efficaces. Les espèces de *Plasmodium* causant le paludisme ont des ressemblances mais aussi des différences qu'il est nécessaire de prendre en compte pour établir des mesures de lutte efficaces. *P. falciparum* présente fréquemment des résistances aux antipaludiques qui ont été utilisés pour son traitement allant de la chloroquine aux dérivés de l'artémisinine. Le traitement *P. vivax* nécessite l'utilisation de médicaments actifs sur les hypnozoïtes pour empêcher les rechutes. Bien que le paludisme soit la première maladie parasitaire en Afrique, les autres maladies vectorielles ne sont pas à négliger pour mettre en place des moyens diagnostics et éviter un traitement inadéquat qui peut pourrait être responsable de la propagation de la résistance aux antimicrobiens.

Notre thématique de recherche nous a conduit à étudier les aspects épidémiologie de trois maladies vectorielles (paludisme, borréliose et mansonellose). Après avoir fait l'état des lieux sur l'adhérence aux méthodes de diagnostic et de traitement du paludisme en Afrique sub-saharienne, ce travail a essentiellement porté sur l'évaluation des méthodes de diagnostic des maladies fébriles notamment le paludisme, la borréliose et la mansonellose au Mali.

Par la suite, dans un second travail, nous avons évalué la performance des tests de diagnostic rapide pour le diagnostic du paludisme et la cartographie des différentes espèces de *Plasmodium* au Mali. A partir de l'ADN extrait, des tests moléculaires ont été effectués. La distribution du *Plasmodium falciparum* était ubiquitaire avec une prévalence allant de 78,9 % à 100 % des PCR positives, selon les régions. Dans l'ensemble, les tests de diagnostic rapide (TDR) présentaient des indices diagnostiques acceptables à bien poser le diagnostic du paludisme dû à *P falciparum* cependant la prévalence de *P. vivax* a atteint 21,1 % dans le nord.

Dans un troisième travail, nous avons utilisé les échantillons d'ADN identiques extraits de tests de diagnostic rapide du paludisme à *P. falciparum*, positifs et négatifs, pour détecter les infections zoonotiques par les techniques de biologie moléculaire. L'ADN de *B. crocidurae* a été détecté dans 5 % (9/180) des TDR négatifs au paludisme tous collectés dans le district sanitaire de Diéma situé dans la région de Kayes.

Dans un quatrième travail, concerne l'utilisation de ce diagnostic moléculaire pour étudier la prévalence de la mansonellose chez les patients fébriles. L'ADN de *Mansonella perstans* a été détecté dans 1,1 % (2/180) des TDR négatifs au paludisme collectés dans la région de Koulikoro.

Enfin, la cinquième et dernière partie de ce travail, était l'étude des facteurs qui affectent le respect de des directives nationales de diagnostic et de traitement du paludisme chez les prestataires de soins de santé dans le district sanitaire de Kita. Divers facteurs identifiés, tels que la disponibilité d'un laboratoire fonctionnel, ont montré une association positive avec l'adhésion aux directives nationales de diagnostic et de traitement du paludisme parmi les prestataires (OR = 6,90, 95 % CI [1,05 - 47,65]). Concernant les patients, la pratique de l'automédication était significativement plus élevée dans les zones rurales que dans les zones urbaines.

Cette thèse fournit des informations précieuses sur l'épidémiologie du paludisme et la dynamique de la transmission au Mali. En outre, les résultats confirment la présence d'agents de la borréliose à fièvre récurrente et la mansonellose chez les patients fébriles. Notre étude montre qu'une approche intégrée pourrait être utilisée pour détecter le paludisme et la mansonellose ou la borréliose à l'aide de TDR.

Mots clés : Espèces plasmodiales, Test de Diagnostic Rapide, Prévalence, Mansonellose, Borréliose, PCR; Mali.

Abstract

Vector-borne diseases are a public health problem in Africa, due to their morbidity-mortality and socio-economic impact. Several factors, including the environment, economic resources and behavior, contribute to maintaining this situation. Malaria is the number one parasitic disease in sub-Saharan Africa, and Mali is one of the most affected countries, with a morbidity rate of 37.7% and a mortality rate of 24.4% (DHIS2 2023). A better understanding of the epidemiology of the disease is essential if effective control measures are to be put in place. The *Plasmodium* species that cause malaria have both similarities and differences, which need to be taken into account to establish effective control measures. *P. falciparum* frequently displays resistance to the antimalarial drugs used to treat it, ranging from chloroquine to artemisinin derivatives. Treatment of *P. vivax* requires the use of drugs active on hypnozoites to prevent relapses. Although malaria is the number one parasitic disease in Africa, other vector-borne diseases should not be overlooked in order to establish diagnostic means and avoid inadequate treatment, which could be responsible for the spread of antimicrobial resistance.

Our research theme led us to study the epidemiological aspects of three vector-borne diseases (malaria, borreliosis and mansonellosis). After taking stock of adherence to diagnostic and treatment methods for malaria in sub-Saharan Africa, this work focused on evaluating diagnostic methods for febrile diseases, notably malaria, borreliosis and mansonellosis, in Mali.

In a second study, we evaluated the performance of rapid diagnostic tests for malaria diagnosis and the mapping of different Plasmodium species in Mali. Molecular tests were carried out on extracted DNA. The distribution of *P. falciparum* was ubiquitous, with prevalence ranging from 78.9% to 100% of PCR positives, depending on the region. On the whole, rapid diagnostic tests (RDTs) showed acceptable diagnostic indices for the diagnosis of malaria due to *P. falciparum*, although the prevalence of *P. vivax* reached 21.1% in the north.

In a third study, we used identical DNA samples extracted from positive and negative *P. falciparum* malaria rapid diagnostic tests to detect zoonotic infections by molecular biology techniques. *B. crocidurae* DNA was detected in 5% (9/180) of RDTs tested in the Diéma health district in the Kayes region.

A fourth study concerns the use of this molecular diagnostic to study the prevalence of mansonellosis in febrile patients. *Mansonella perstans* DNA was detected in 1.1% (2/180) of malaria-negative RDTs collected in the Koulikoro region.

The last component of this study was an investigation into the factors influencing compliance with national guidelines for malaria diagnosis and treatment among healthcare providers in the Kita health district.

Various factors identified, such as the availability of a functional laboratory, showed a positive association with adherence to national malaria diagnosis and treatment guidelines among providers (OR = 6.90, 95% CI [1.05 - 47.65]). Among patients, the practice of self-medication was significantly higher in rural than in urban areas.

This thesis provides valuable insights into malaria epidemiology and transmission dynamics in Mali. The results also corroborate the presence of agents of relapsing fever, borreliosis, and mansonellosis in febrile patients. Our approach shows that an integrated approach could be used to detect malaria and Mansonella infection using RDTs.

Keywords: Plasmodium species, rapid diagnostic test, prevalence, Mansonellosis, Borreliosis, PCR, Mali.