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Factors associated with the development of chronic *P. falciparum* infection in the Sahel

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Eric P. TWOMEY, MD
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Location of the practicum:
Université Aix Marseille
Institute SESSTIM
Faculté des Sciences Médicales
27 boulevard Jean Moulin
13005 Marseille

Professional advisor:
Jordi Landier, PhD
Institute SESSTIM

Academic advisor:
Pr. Aurore Gely-Pernot, PhD
EHESP Rennes

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Abbreviations

ACT ..... *artemisinin-based combination therapies*
BDHSS ..... *Bandafassi Demographic Health Surveillance System*
CFR ..... *case-fatality-ratio*
CHW ..... *community healthcare workers*
DBS ..... *dried blood spots*
DP ..... *dihydroartemisinin-piperaquine*
HCP ..... *Healthcare Professionals*
hsRDT ..... *high sensitivity rapid diagnostic tests*
IRS ..... *indoor residual sprays*
LLIN ..... *long lasting insecticidal nets*
MARS ..... Malaria Asymptomatic Reservoir in the Sahel
MDA ..... *mass drug administration*
NAAT ..... *Nucleic acid-based amplification techniques*
P ..... *Plasmodium*
PCR ..... *polymerase chain reaction*
PF ..... *Plasmodium falciparum*
PfEMP1 ..... *P. falciparum erythrocyte membrane protein 1*
PfHRP2 ..... *Plasmodium falciparum histidine rich protein 2*
PNLP ..... *Programme National de Lutte contre le Paludisme*
qPCR ..... *real time quantitative polymerase chain reaction*
RDT ..... *rapid diagnostic tests*
SDG ..... *Sustainable Development Goals*
SMC ..... *seasonal malaria chemoprevention*
SP ..... *sulfadoxine–pyrimethamine*
spp ..... *subspecies*
SSA ..... *Sub-Saharan Africa*
WHO ..... *World Health Organisation*

Abstract

Plasmodium infections range from severe disease to asymptomatic cases, with untreated infections potentially persisting for months or years. Asymptomatic individuals, forming a "reservoir," contribute substantially to malaria transmission. This study focuses on the factors influencing the persistence of *Plasmodium falciparum* infections in the Sahel region, considering sociodemographic, clinical, and environmental variables.

Data from the Malaria Asymptomatic Reservoir in the Sahel (MARS) Study, an open cohort study in eight villages across Senegal and Mali, were used. Four surveys were conducted from April 2021 to April 2022. Households were randomly selected, and participants' data were collected through questionnaires and biological samples. Real-time quantitative PCR was used to detect *P. falciparum*. The primary outcome was "dry season persistent plasmodium carriage," defined as qPCR positivity at subsequent surveys. Multilevel logistic regression in a generalized additive models (GAM) was employed to analyse the data, considering parasite density, age, sex, village, and period as variables.

Overall, 1,474 participants from 168 households were included, with 1,038 remaining after exclusions. During the late dry season (T0-T1), 54.84% of participants were persistent, compared to 15.38% in the early dry season (T2-T3). Higher parasite densities and ages 20-40 years were significant predictors of persistence. In univariable analysis, parasite density significantly increased the odds of persistence (OR = 2.01, $p<0.001$), and age also had a significant effect (OR = 1.65, $p<0.001$). Multivariable models showed a 20x increased odds of P. falciparum persistence during the late dry season when compared to the early dry season. Differences in persistence rates between Senegal and Mali were observed, emphasizing the role of robust healthcare systems.

This study highlights the importance of targeted interventions to reduce the malaria reservoir, particularly during the late dry season when persistence is highest. The identification of "superhosts" suggests the need for further research into genetic and immunological profiles. Localized public health strategies are essential to address transmission hotspots. Seasonal malaria chemoprevention and other interventions have shown efficacy and should be expanded. Continuous monitoring and treatment of asymptomatic carriers are crucial to disrupt the transmission cycle and prevent malaria reintroduction. Future efforts should focus on enhancing localized control measures, improving healthcare accessibility, and maintaining rigorous surveillance to achieve sustainable malaria eradication in the Sahel.

1 Introduction

1.1 History of Malaria

Malaria, a vector-borne disease, has been known since ancient times, with references dating back to 2700 BCE by the Chinese. The term "malaria" originates from the medieval Italian words "mala aria," meaning "bad air," reflecting the ancient belief that the disease was caused by the foul gases. In 1880, the French surgeon Charles Laveran discovered the parasite *Plasmodium*. This discovery was further expanded by the Italian malariologists Giovanni Grassi and who identified the *Anopheles* mosquitoes as the vector for malaria transmission. Public health interventions targeting malaria transmission have evolved significantly over the years. Initial efforts focused on vector control through environmental management to control mosquito populations. Protective measures recommended include the use of long-sleeved clothing, insect repellent, and bed nets. The first effective antimalarial drug, quinine, was isolated from the bark of the cinchona tree in the early 17th century by the Spanish. In the 20th century that a range of antimalarial drugs, including chloroquine and artemisinin-based combination therapies (ACTs), were developed, significantly improving malaria treatment outcomes. Malaria prevalence resurged in tropical countries from the 1970s to the 1990s because of a combination of relaxation of control efforts, increasing antimalarial drug resistance, and insecticide resistance in the mosquito vectors. Some countries achieved elimination in the past 20 years. Others, where local transmission no longer occurs, await World Health Organization (WHO) certification (1).

1.2 Malaria Disease

The malaria disease is caused by the *plasmodium* species. The parasites of the *Plasmodia species* are transmitted through vector from the *Anopheles* family, e.g. *Anopheles Gambiae.* Longevity, density, efficiency and biting habits of the mosquito vectors are the principal determinants of transmission intensity. In Africa, *Anopheles gambiae* commonly show the highest effectiveness as they often bree, occur in high densities, mostly in tropical regions and are yet somewhat resistant to environmental changes and relatively long-lived. This species is also known for their preference for feeding on humans (1). The amount of infectious bites per year and person, called entomological inoculation rate, is highly variable and ranges between 1 up to 1000 per year in certain areas (1–3).

1.2.1 Parasitical cycle of *Plasmodium spp.*

Plasmodia are protozoic parasites belonging to the *Hemosporoidae* (sprouting in the blood). The life cycles of most human pathogenic *Plasmodia* subspecies are parasitical ones, obligatory requiring humans and anopheles species hosts for sexual reproduction (4) (Figure 1). In general, female *Anopheles* mosquitos rely for the production of their eggs on proteins from human blood and therefore feed on humans once or twice per day (1).

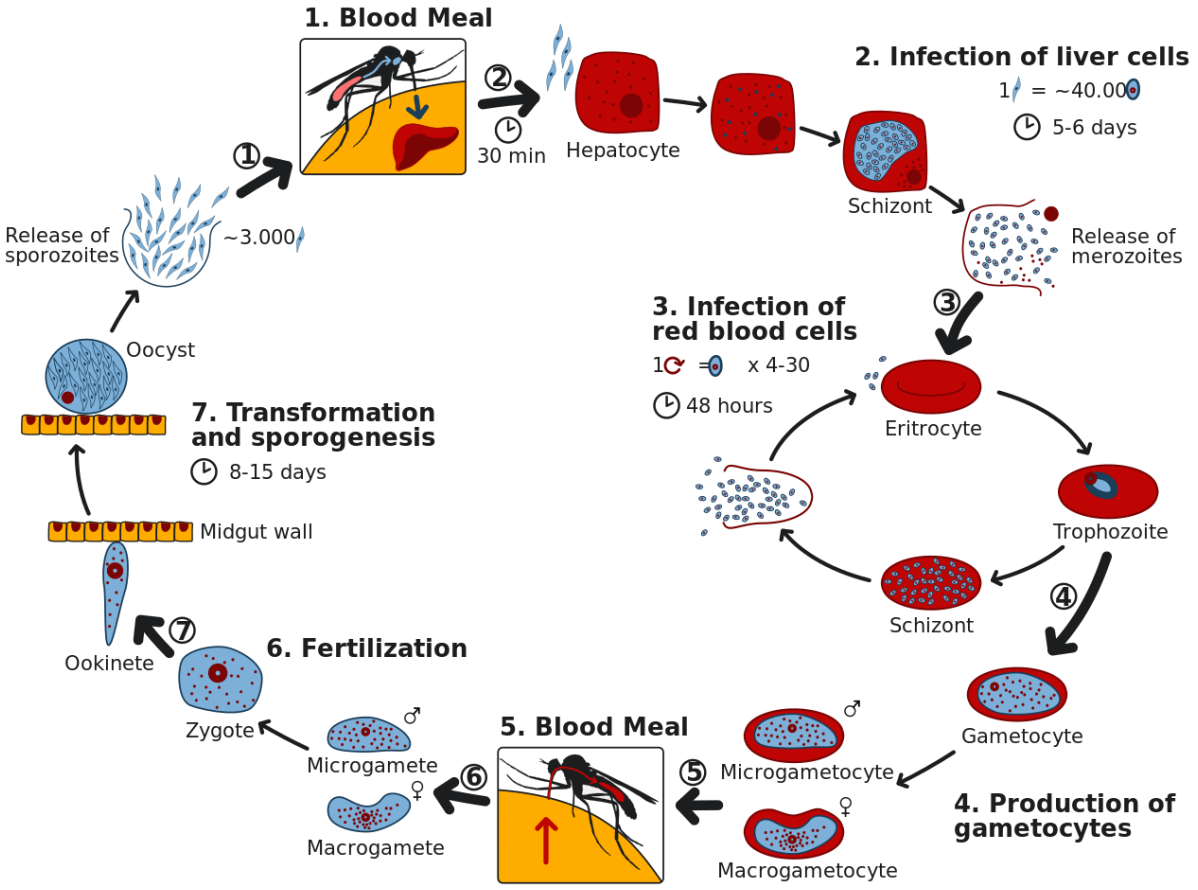


Figure 1: Parasitical Cycle of Plasmodium spp. The life cycle of Plasmodium spp. involves both humans and Anopheles mosquitoes. Female mosquitoes ingest Plasmodium gametocytes during a blood meal. These develop into sporozoites in the mosquito, which are transmitted to humans in a subsequent bite. In humans, sporozoites infect liver cells, forming schizonts that release merozoites. Merozoites infect red blood cells, undergoing asexual replication and forming trophozoites and schizonts. Some differentiate into gametocytes, which are ingested by mosquitoes, continuing the cycle. Human infections result in periodic fever spikes due to the synchronized erythrocytic cycle. Source: Wikimedia Commons, the free media repository

As the female *Anopheles* mosquitoes take a blood meal, they ingest ripe *Plamodia* of both sexes, which enter the sporogonic cycle within the mosquito. Sporozoitess enter the human organism on a different blood meal. There, they enter the exo-erythocytic cycle and mature within the human liver cells to schizozonts which later become meroyoits. 6–8 days after emerging from the liver, when parasite densities have reached roughly 50/µL of blood (roughly 100 million parasites in the blood of an adult). The incubation period is therefore usually 12–14 days from the infecting bite (1). During physiological splenic passage, asexual stages of *P.*

falciparum and *P. vivax* are retained in the spleen, initiating an endosplenic lifecycle in both species (5). There, cytoadherence is mediated by *P. falciparum* erythrocyte membrane protein 1 (*PfEMP1*), a clonally variant set of proteins exported to the infected erythrocyte surface and encoded by the *var* gene family (6,7). Schistozonts enter erythrozytes where they live in the cytoplasma (which is why they are called *Plasmodia*), develop into mero- and trophozoites. Eventually, trophozoits will develop into bisexual gametocytes, which again infect mosquitos, closing the parasitical cycle. These gametocytes are the name-giving form of the different *Plasmodia spp.*, detected under the microscope as a scythe-formed *P. falciparum* (*PF*, lat. *falx* = scythe), round-formed *P. malariae* and *vivax* or oval-formed for *P. ovale*.

1.2.2 Clinical presentation of the Malaria Disease

Fever is the most observed symptom, present in up to 90% of cases, followed by chills and sweats and headaches. Generalized seizures are associated specifically with *P. falciparum* malaria and might be followed by coma (cerebral malaria). Uncomplicated infections have few abnormal physical findings other than fever, mild anaemia, and, after several days, a palpable spleen (1). Age can influence the clinical manifestations of malaria. The liver can become enlarged, especially in young children, whereas mild jaundice is more likely in adults. Children under 5 years are particularly vulnerable to severe complications like respiratory distress, severe anaemia, and cerebral malaria (8). Recurrent *falciparum* and *vivax* malaria have pronounced adverse effects in young children, and interfere with growth, development, and schooling (1).

1.2.3 Malaria Diagnostics

Until today, a thick blood film or smears remain the diagnostic gold standard, confirming clinical suspicion of malaria cases with a microscope (9). The detection threshold for microscopy is generally around 50-100 plasmodia/µL, making it relatively sensitive but less so than some modern diagnostic methods (10). However, rapid diagnostic tests (RDT) are used as an alternative or adjunct to microscopy. In some regions, they now predominate as the first-line investigation with a wide range of devices available, which are as good as is routine microscopy in the diagnosis of *falciparum* malaria (1,11). Standard RDTs have a detection threshold that varies but can be as low as 100-200 plasmodia/µL. High-sensitivity RDTs (hs-RDTs) have been developed to offer even greater sensitivity, with detection thresholds as low as approximately 0.2-2 plasmodia/µL. Nonetheless, RDTs that detect *Plasmodium falciparum histidine rich protein 2* (*PfHRP2*), an antigen exclusively produced by *P. falciparum*, are usually sensitive for diagnosis of this species, but cannot be used to evaluate treatment response because *HRP2* can persist in once-infected, parasite-free erythrocytes after splenic pitting (12). *HRP2*-based tests therefore might remain positive for weeks after acute infection, which

limits usefulness in high-transmission areas (13,14). Nucleic acid-based amplification techniques (NAAT), such as polymerase chain reactions (PCR) represents the pinnacle of malaria diagnostic sensitivity and specificity, capable of detecting as few as 1-5 plasmodia/µL. PCR-based methods offer the highest sensitivity and can distinguish between different *Plasmodia spp*., making them invaluable for research purposes and for confirming diagnosis in challenging cases (15).

1.2.4 Malaria Treatments

The treatments of malaria are stratified based on the severity of the disease and the specific *Plasmodia spp*. involved, with particular attention to *P. falciparum* due to its potential to cause severe illness. The treatment for uncomplicated *P. falciparum* malaria relies primarily on artemisinin-based combination therapies (ACTs), which are recommended by the WHO (16). Severe malaria is a medical emergency requiring immediate treatmentwith parenteral artesunate as soon as possible (17). Additionally, management of severe malaria includes symptomatic treatment and supportive care in an intensive care unit (16). Untreated, uncomplicated malaria cases have a case-fatality-ratio (CFR) of approximately 0.1% to 0.3%. The CFR for severe malaria cases can exceed 10-20% in the absence of proper treatment. However, with prompt and effective treatment, the CFR for uncomplicated malaria can be significantly reduced, and the CFR for severe malaria can be lowered to 1-5% (18). Malaria treatments are not always curative. Treatment failure usually presents as a recurrence of symptoms with detectable parasitaemia 2-6 weeks after an apparently successful treatment and is not always due to drug resistance (19–22).

1.3 Epidemiology of Malaria

Globally, the number of malaria cases in 2022 was significantly higher than in 2019, before the start of the COVID-19 pandemic. From 2000 to 2019, the global malaria incidence fell from 243 million to 233 million. There were an additional 11 million cases in 2020, no change in 2021 and an increase of 5 million cases in 2022, to reach a total of about 249 million cases (23). Endemic in 84 countries, malaria causes an estimated 619 000 deaths annually. Of an estimated 249 million malaria cases annually, 235 million (95%) occur in malaria-endemic countries in Africa, where 99% of infections are caused by *P. falciparum*. The age distribution of clinical malaria and malaria-associated mortality is highly determined by the intensity of malaria transmission (24). In many parts of sub-Saharan Africa, individuals receive more than ten infectious mosquito bites per year (25). Unfortunately, global malaria incidence has increased in the past 5 years and new challenges, such as spreading artemisinin resistance,

social upheaval from natural disasters, civil unrest, epidemics and the COVID-19-related disruption to health systems threaten malaria control (26–29).

1.3.1 Geographical Distribution of Malaria

Malaria remains endemic on the American continents as well as in Sub-Saharan Africa (SSA), Asia and Oceania, where it continues to be a significant public health challenge. Globally, most cases are concentrated in humid tropical and subtropical areas with continuous rainfall throughout the year, where conditions favour the breeding of *Anopheles* mosquitoes.

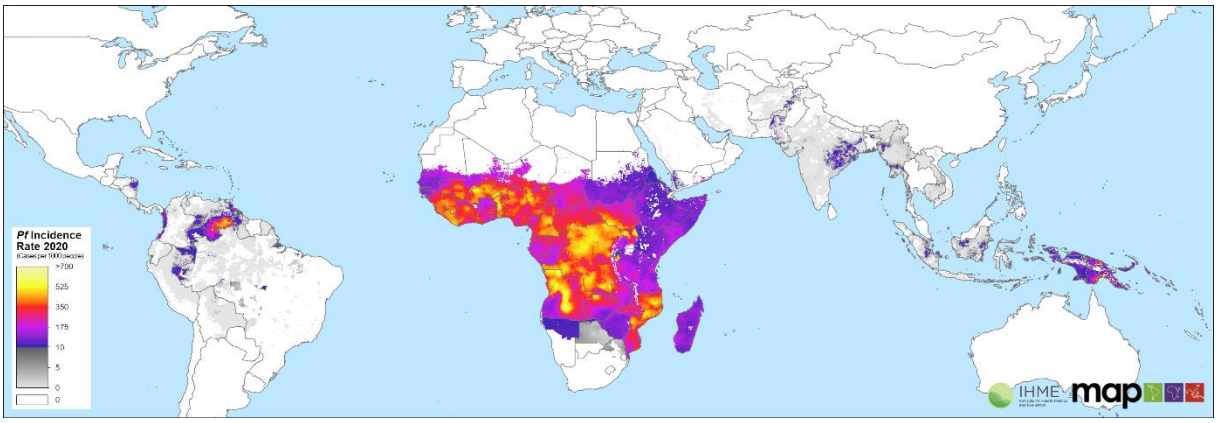


Figure 2: Plasmodium falciparum incidence rate in all age groups for 2020. Malaria is endemic in the Americas, SSA, Asia, and Oceania, with the highest incidence in SSA due to favourable conditions for Anopheles mosquitoes. The map shows malaria incidence rates in 2020, with most P. falciparum cases occurring in SSA. Source: www.malariaatlas.org

In semi-arid areas, such as the Sahel zone, malaria exhibits seasonality, peaking during the rainy seasons and diminishing during dryer periods (26). This pattern reflects the breeding requirements of *Anopheles* mosquitoes, which thrive in stagnant water. The great majority of *P. falciparum* malaria occurs in SSA (approx. 190 million cases) where transmission remains intense in many locations, although there is considerable variation in incidence within and between countries (30,31) (Figure2). The semi-arid Sahel alone makes up for 20% of global cases. Predictions as to the effect of climate change on global malaria distribution in the future vary, but have suggested the population at risk of malaria will increase, in particular in tropical highland areas (32). Within endemic countries, the incidence and prevalence of malaria can vary significantly from 1 to >1000 cases per 100,000 inhabitants annually. The disease incidence depends on environmental suitability for local vectors in terms of altitude, climate, vegetation, and implementation of control measures, and hence is inextricably linked to poverty, natural disasters, and war (33).

1.3.2 Historical, Current and New Strategies against Malaria

The evolution of malaria incidence and prevalence over the last 100 years has been marked by significant fluctuations, shaped by factors such as population growth, the spread of drug resistance, and environmental changes. Launched in 1955, the Global Malaria Eradication Program ended without success 14 years later (34). Since then until recently there has been no further time-limited commitment to global malaria eradication and its feasibility continues to be debated (1). From the 1970s to 1990s, global malaria cases, despite numerous public health initiatives aimed at controlling the disease, malaria cases in many areas, particularly in SSA, remained high or even increased and ended with an estimated 450 million cases annually, with a notable burden in SSA. This was due to factors such as resistance to chloroquine, the primary antimalarial drug of the era, the spread of mosquito vectors, and challenges in public health infrastructure. Today, malaria elimination - defined by WHO as “the interruption of local transmission of a specific malaria parasite species in a defined geographical area due to deliberate activities” - is being pursued in multiple countries with unstable malaria transmission. The Sustainable Development Goals (SDG) adopted by WHO in 2016 call for a reduction in malaria incidence and mortality of at least 90% and elimination of malaria in at least 35 countries by 2030 (35,36). Therefore, the E-2025 initiative, launched by WHO in 2021, aims to eliminate malaria in 25 countries primarily across Asia and Latin America by 2025 marks a new effort of, at least regionally, eliminating malaria (23). WHO has therefore developed a global strategic framework for integrated vector management, which advocates an evidence-based, integrated approach for vector control and offers guidance for successful operationalisation of the different approaches (37,38)

Malaria prevention measures generally fall into two categories: those aimed at reducing transmission and those focused on preventing morbidity and mortality, i.e. severe malaria. Some interventions affect both objectives. Vector control, consisting of the use of long-lasting insecticidal nets (LLINs), indoor residual sprays (IRS) and water engineering affect both, transmission and morbidity. LLINs are regarded most effective in high-transmission areas where vectors rest indoors at night (39). Pyrethroid-insecticide-treated mosquito nets reduced all-cause mortality by roughly 20% in children younger than 5 years (40). In addition to protecting the user, LLIN protect the community by killing anopheline mosquitoes, the so-called mass effect (41,42). IRS with insecticides that persist and kill mosquitoes is an important component of malaria control (43–45). Bite-prevention strategies including topical repellents, however, have not been shown to have an effect on malaria incidence (46,47). Achievements made through these LLIN and IRS, particularly in moderate-to-high transmission settings are now threatened by ongoing evolution of mosquito resistance to pyrethroid insecticides (48). Engineering solutions for vector-control, adapted to local topography and climate, aim to reduce stagnant waters as mosquito breeding sites and have substantially reduced malaria

morbidity (49,50). Access to healthcare, treatment with ACTs and seasonal malaria chemoprevention (SMC) reduce morbidity and mortality of already infected individuals. ACTs for the treatment of uncomplicated malaria and intravenous artesunate for severe malaria and improved parasitological diagnosis contributed to a substantial decline in global malaria mortality (51,52). Intermittent preventive treatment is applied in pregnant women receiving sulfadoxine–pyrimethamine (SP) at all antenatal care visits from second trimester (minimum dose interval of 1 month). Infants in moderate transmission areas receive SP with routine immunisations. SMC is directed to children aged 3–59 months in areas of seasonal transmission in the Sahel (intermittent SP plus amodiaquine at treatment doses, maximum four courses) (53). Mass drug administration (MDA) and vaccination prevent severe disease but also reduce transmission if widespread. The former can also deplete *Plasmodium* reservoirs. In African phase 3 clinical trials, three doses of the vaccine administered 1 month apart followed by a booster dose 18 months later reduced clinical malaria episodes over 3–4 years. It reduced it by 36% in young children first vaccinated between 5 months and 17 months of age and by 26% in infants first vaccinated between 6 months and 12 weeks of age (54). MDA was effective in smaller, well-defined populations in some settings but not in others, thus this approach has been little used in recent years (55–57). Pilot studies of MDA of ACT with single-dose primaquine to accelerate elimination of drug-resistant malaria in Southeast Asia, however, have taken place and early reports suggest it is effective and safe (58,59). Other approaches under consideration are mass treatment with ivermectin and dihydroartemisinin-piperaquine (DP), which shortens mosquito survival, and transgenic mosquitoes (55,60–62). The progress achieved in the fight against malaria since 2000 is estimated to be largely attributable to the use of LLINs and for a substantial minority by ACTs, however, it is geographically very uneven. In many SSA countries, where transmission is highest, eliminating malaria has proved more difficult and there are signs that progress in this direction has stalled (31,63). There is evidence that access to high quality ACTs is still much too low (<25%) in some areas (64). *Anopheles stephensi* has recently spread from Asia to the Horn of Africa (65). This highly efficient vector of both *P. falciparum* and *P. vivax* prefers breeding in human made containers and urban environmentsm and thus poses a substantial risk of increased receptivity to local transmission in urban Africa.

1.4 Burden of Malaria Disease

The global burden of malaria remains a significant public health challenge. In West Africa, where malaria transmission is intensely high, individuals can contract the disease multiple times per year, leading to an average of several sick days annually, significantly impacting economic productivity and educational attainment. The economic impact of malaria extends

beyond the immediate cost of treatment to include lost productivity, wages, education and innovation, straining both household finances and broader economic systems (1). In SSA, the economic burden of malaria has been profound, with estimates suggesting costs ranging from 2 to 7% of some countries' GDP (66).

In terms of mortality, the WHO reported an estimated 627,000 deaths globally in 2020. Again, the majority of these deaths occur in SSA, affecting predominantly children under the age of five and pregnant women, leaving a lasting impact on families and communities (23). Despite these efforts, today, malaria still kills roughly 2000 people per day, most of whom are young children in Africa. In terms of mortality and morbidity, it is the most important parasitic disease of human beings (1). Therefore, WHO declared that a continuous investment in malaria control and elimination is crucial for reducing the disease's burden and achieving the global malaria targets set by the (67).

1.5 Plasmodium Reservoirs

Plasmodium infections can present a wide range of clinical manifestations, from severe malaria disease to subclinical or asymptomatic cases. Untreated, the symptomatic phase of most *plasmodium* infections typically resolves within approximately 10 to 15 days. However, completely clearing the parasites from the body, and thus curing the infection, may take up to 4 weeks when untreated, In low-transmission settings, infections can persist for many months or years (or decades in *P. malariae* infections) (1,68–70). The sum of these individuals with an asymptomatic parasitaemia is described as “reservoir” and is suspected to provide an ongoing source of infection of mosquitos, being a major contributor to malaria transmission (71). In highly endemic settings, severe malaria in adults is unusual, but a substantial proportion of the population will have asymptomatic, submicroscopic parasitaemia (25,72). It is assumed that cure latency or tendency to reservoirs has to do with individual susceptibility to malaria (73,74). The individual immune system appears to have the highest influence on the occurrence of malaria disease and clearance of the plasmodium parasites (75). In addition, endogegeous factors such as the genetic profile, age, sex and secondary diseases on the one hand and environmental factors such as healthcare access, nutritional status and frequency of previous malaria infections are assumed to be possible factors of inter- and intra-individual variability in malaria clearance (76,77). Maternal anaemia is exacerbated, but most mothers remain asymptomatic despite intense accumulation of infected erythrocytes in the placental microcirculation (1,78). There are indications that immune protection against *Plasmodium*, called premonition, i.e. protection against high parasitaemia and illness without eliminating the infection, increases with age and the number of previous infections, skewing the burden of severe malaria to children younger than 5 years (25).

In this exploration of *plasmodium* reservoirs, an intentional differentiation is made between asymptomatic and symptomatic parasitaemia. "Malaria Disease" is used exclusively to describe symptomatic parasitaemia, where individuals exhibit clear symptoms prompting medical consultation. The notion of truly asymptomatic parasitaemia is contentious, with evidence suggesting that individuals with parasitaemia may experience minor symptoms, like mild fatigue, that do not necessarily lead to seeking medical care (74). To address this, we will refer to "asymptomatic parasitaemia" as to describe infections which leads to individuals affected not pursuing any medical attention. These infections, whether causing mild symptoms or no symptoms, are likely to remain untreated, evolve towards chronicity and thus, contribute to malaria transmission. The definition of “asymptomatic infection" recognises the gradient of symptom severity and its impact on public health responses. It serves as a pragmatic classification, helping to clarify the role of asymptomatic carriers in malaria dynamics and informing targeted control strategies. This distinction is crucial for refining surveillance and interventions aimed at curbing malaria transmission within populations.

1.5.1 Malaria Susceptibility, Immunogenicity, and Latency

Understanding the persistence of infections during the dry season and identifying carriers is essential for targeted interventions to reduce the malaria reservoir and prevent reintroduction during the wet season. Asymptomatic, genetically complex infections are common in high-transmission areas (79). The host-specific response to malaria plays a significant role in the establishment of chronic infections. Protection against severe malaria develops relatively quickly (80,81), but the ability to control parasitaemia at subclinical levels emerges more slowly with repeated exposure (82). In Mali, individuals infected during the dry season had higher *P. falciparum*-specific antibodies than uninfected individuals, suggesting that exposure and immunity are necessary for asymptomatic infections (83,84). Older children were more likely to be infected at the end of the dry season compared to younger children, indicating that immunity plays a role in asymptomatic infection (85). In Uganda, older children carried infections longer than children under five and adults, suggesting that immune responses associated with persistent asymptomatic infection differ from those that clear infections (76,86). It is unclear whether parasite-specific antibody responses provide protection or merely indicate previous exposure (87,88). Host factors such as hemoglobinopathies and anaemia, known to influence malaria risk and severity, may also affect the duration of asymptomatic carriage (89–91).

Parasites themselves may be pre-programmed or adapt to persist during the dry, non-transmission season. Establishing clinically silent infections that do not trigger immune responses could be an evolutionary strategy to survive the dry season and ensure transmission

continuity (77). In Mali, parasite populations sampled during the wet and dry seasons were similar, but those from the dry season appeared to adapt their phenotype, resulting in longer peripheral circulation and increased splenic clearance, potentially maintaining low parasitaemia without eliciting a strong immune response (83).

Heterogeneity in host transmission potential is a common feature of many infectious diseases. Identifying individuals who disproportionately contribute to transmission is essential. For vector-borne diseases like malaria, this requires estimating host infectivity and assessing the effective contact rate with the vector population. Up to 17% of mosquito infections are caused by submicroscopic parasite carriage in humans (92). Although children are often more infectious, adults receive more mosquito bites, amplifying their contribution to mosquito infections (77). In high-transmission settings like Burkina Faso, *P. falciparum* infection rates are higher compared to moderate and low transmission areas. Only a small proportion of individuals are infectious to mosquitoes at any given time, with this proportion decreasing as transmission intensity decreases. Additionally, 14.5% of mosquito blood meals contained DNA from multiple human sources, indicating repeated feeding, which increases malaria transmission models (85). In low-transmission areas, erratic, or focal (often termed “unstable transmission”), full protective immunity from malaria is not acquired, and symptomatic disease can occur at all ages. In such areas, changes in environmental, economic, or social conditions—eg, heavy rains after drought, large population movements—together with a breakdown in malaria control and prevention services (often because of armed conflicts) can result in epidemics, with substantial mortality in all age groups (14). However, Immunity is lost steadily after an individual leaves an endemic area, or in a population with falling transmission (33).

1.5.2 Malaria Seasonality

In contrast to subtropical and tropical regions, where malaria is continuously found throughout the year, many regions word wide, but especially in the Sahel from SSA, show a pattern of increasing and decreasing malaria transmissions according to the seasons (1). During dry season, most *Anopheles spp*. die, while their eggs prevail until the next season. Due to this lack of female *Anopheles* mosquitos, malaria transmission is halted during dry season. However, most malaria-related illness and death in Africa occurs in children aged 3–59 months in the Sahel sub-region during 4 months of the rainy season (1). Therefore, WHO has recommended the aforementioned implementations of SMC campaigns to all children aged 3–59 months in this region from the start of the yearly transmission season (93).

As described in the chapter “Malaria Reservoirs”, most individuals being infected during the wet season, clear their infection within some weeks of the dry season. However, some

individuals were described to stay with an ongoing, often mild or totally symptom free malaria, not seeking any medical attention and potentially contributing to transmission at the onset of the next wet season (33,92,94,95). Therefore, these individuals are thought to be the causal temporal link between transmission seasons, reinfecting *Anopheles* populations with the beginning of the next rain season and thus maintaining an active, trans-seasonal parasitical cycle in any given region. This unique feature of semi-arid regions such as the African Sahel creates a situation where, theoretically, all members of a community could be malaria-free at least once throughout the year. Based on these observations, public health interventions aimed at identifying and eradicating the human malaria reservoir during the rainy season are a promising strategy to reduce the malaria burden. To investigate the characteristics of this human reservoir, this study focuses on two representative countries within the African Sahel strip.

1.6 Malaria in Senegal

The West African country Senegal with a population of 18 million inhabitants has ever since been a hotspot for malaria. Mostly the semi-arid Sahel bordering with The Gambia, Guinee and Guinea Bissau in the south and with Mali in the southeast, provide a seasonal habitat for *Anopheles* mosquitos and therefore conditions for malaria transmission. Near the big rivers Senegal in the north and Gambia in the south and their affluent streams, and next to other bodies of water, however, malaria transmission is independent of seasonality as mosquitos can breed all year round (96). In all other regions, *Anopheles* mosquitos are dependent on the seasonal rainfall between June and November. Almost no transmission is seen in the dry season between November and April. As of 2021, Senegal had 537,000 confirmed cases of malaria over all age groups, which translates into an incidence of 31.2 per 1,000 inhabitants. This shows a stark incline from 355,000 confirmed cases in 2019. However, 78.5% of these cases were concentrated in only 3 regions in the southern and south-eastern parts of Senegal showing a highly heterogeneous pattern of geographical distribution. In 2021, the highest incidence was observed in Kédougou region, with 536.5 per 1,000 inhabitants (97). Despite a growing population of approximately 2,9% per year (98), malaria cases and malaria-relates deaths have consistently decreased since 2010 until 2019. This can be, at least partially, counted as a consequence and therefore success of the national program in fight against malaria (Programme National de Lutte contre le Paludisme - PNLP), which was established by the Senegalese Ministry of Health and Social Action. Within the last 17 years, this program introduced many different public health interventions, such as the introduction of free malaria diagnostics and treatment in the whole country. Since 2014, the SMC is available for all children from year 3 to 119 months. Until the year 2023, this meant 4 doses of the drug SP

during the rainy season in intervals of 4 weeks. Since 2023, a fifth dose was being introduced in Kédougou region, after evidence suggested, that the intervals were too long and the rounds too few (99). In 2021, 75,3% of households possessed ITNs, remarking a 6-year's low (97).

1.7 Malaria in Mali

Mali, another West African sub-Saharan country, shares climatic similarities with Senegal, featuring a Sahara region in the north and a Sahel region in the south-west, where the majority of its roughly 22 million inhabitants reside and where most malaria cases are reported. The country experiences a rainy season from June to November and an arid season from December to May, while areas around the Niger River maintain constant humidity and have flooding seasons following the rainy period between August and December (100). This creates seasonal malaria transmission patterns similar to those in Senegal. Since 2015, malaria cases have significantly decreased from 4.5 million to 2.7 million in 2021, despite an average annual population growth of 3.0% (100,101). Malaria incidence varies greatly across regions; Kayes, Koulikoro, and Sikasso regions account for 65% of the incidence (101). In 2013, Mali introduced a comprehensive malaria strategy called "Programme National de Lutte contre le Paludisme" (PNLP) by the Ministry of Health and Social Development, aiming to reduce malaria cases by 60% by 2030 (101). Part of this strategy includes the free provision of diagnostics such a RDTs for all and free treatments like (ACTs) to children under 5 years old, pregnant women, and other vulnerable groups (101). Additionally, since 2016, Mali has implemented an SMC campaign for children under 5 years old, consisting of four doses of SP and amodiaquine at monthly intervals during the rainy season (101). MDA campaigns were introduced in 2020 during malaria outbreaks to target specific areas for eradication efforts (PNLP, 2021). Further public health measures include the distribution of LLINs, introduced in 2006, and IRS implemented in 2018 (101).

2 Objectives

As described in the introduction, approximately 20% of today's malaria cases occur in the semi-arid Sahel zone. Due to the alternating dry and rainy seasons along this geographical area and the significant reduction of the *Anopheles* mosquito population during the dry season, the persistence of *Plasmodium* parasites relies on the existence of human reservoirs during the dry season. This presents an intriguing theoretical target for a tailored public health intervention. One feasible approach is targeted testing and treatment at the end of the dry season and before the onset of the next rainy season for individuals who exhibit a typical profile for trans-seasonal *Plasmodium* carriage. However, for economic and ethical reasons, it is impractical to test and treat the entire population. Therefore, precise knowledge of the human *P. falciparum* reservoir is necessary, especially of its sociodemographic, clinical and geographic characteristics. Based on this, we derive the following objectives for this master's thesis:

2.1 General Objective:

Characterize *P. falciparum* carriage reservoir and describe its evolution during the dry season in the general population.

2.2 Specific Objectives:

- Identify and quantify risk factors contributing to the persistent carriage of *P. falciparum* during the dry season.
- Analyse the spatial distribution of *P. falciparum* infection during the dry, low transmission season

3 Methods

3.1 Study Design

3.1.1 Setting & design

To answer these questions, data from the Malaria Asymptomatic Reservoir in the Sahel (MARS)-Study was used. This open cohort study comprised eight villages, four in Senegal and four in Mali, with various ethnic groups and different grades of accessibility, yet to be reached during rainy season. Throughout one year, four main surveys were conducted beginning right before the start of the rain season with a baseline survey in April and Mai 2021, denominated T0. The second survey (T1) was performed in June and July directly before the first SMC rounds, another one (T2) in November/December 2021 and a fourth and concluding survey (T3) in March and April 2022.

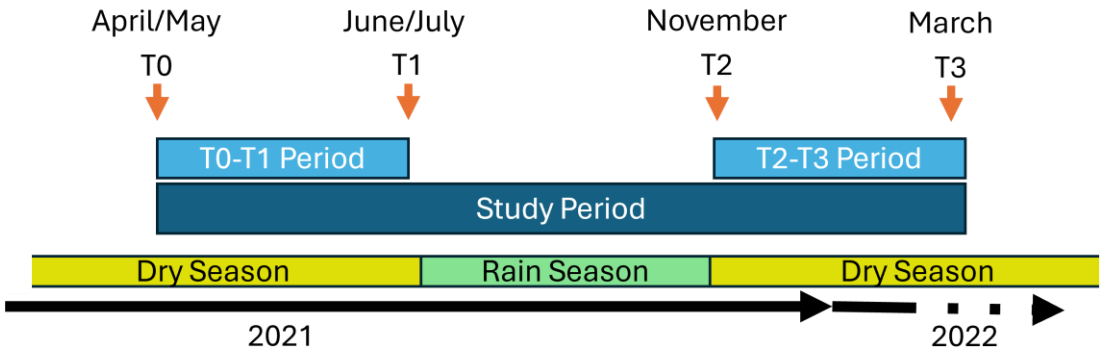


Figure 3. Study Design with T-visits and T-Periods. The MARS Study, as an open cohort study, comprised eight villages, four in Senegal and four in Mali, with various ethnic groups and different grades of accessibility. Throughout one year, four main surveys were conducted: a baseline survey in April and May 2021 (T0), a second survey in June and July before the onset of the rainy season and first SMC rounds (T1), another survey at the end of the rainy season and the beginning of the dry season in November/December 2021 (T2), and a concluding survey in March and April 2022 (T3).

3.1.2 Study population

Utilizing data of the last population census of the Bandafassi Demographic and Health Surveillance System (BDHSS) for the Kédougou district in Senegal and a dedicated census conducted in the four villages selected from Diré and Doneguebougou districts in Mali, households were randomly selected until reaching the targeted survey population of approximately 150 participants per village (102,103). Conditions for study inclusion were informed consent of the household's head, no intentions of moving outside the study area of most members of the household during the course of the study, and the informed consent of more than half of the members aged 15 years or older to take part in the study. Further inclusion criteria were age 6 months or older with the informed consent of one parent or

guardian until the age of 18 years and remaining at the household at least one night per week at the course of the entire study. As this was an open cohort study, all households fulfilling these criteria were eligible to replace households withdrawing throughout the year by either refusal to continue by the head of household or more than 50% of the members withdrawing individually. The exclusion criteria comprised the simultaneous participation in a different health study. The overall population was defined by all individuals and households that participated at least at one principal survey (102,103).

3.1.3 Surveys and Data Collection

Each survey consisted of two teams visiting the household in a period of maximum ten days. One team collected sociodemographic data such as age, sex education, information on malaria prevention such as SMC participation, bed net use, and behaviours such as outside night activity were collected through a face-to-face questionnaire on paper by a team of trained research assistants fluent in local languages. A second team consisting of trained nurses visited households to collect capillary dried blood spots (DBS) samples on Whatman 3M filter paper and perform RDTs onsite to diagnose present *P. falciparum* infections. In this setting, participants' temperature was measured using an infrared forehead thermometer while recording a possible history of fever within the last 48 hours. In Senegal, RDT-positive tested participants were referred to community healthcare workers (CHW) and/or health posts to receive a treatment by artemisinin combinations according to national guidelines (104). In Mali, RDT-positive participants presenting symptoms were referred for treatment by the nearest health post according to national guidelines. The REDCap digital data capture tools were used by trained operators to enter responses to an Excel sheet. (105,106). Complementary, weekly household visits by CHWs were conducted, registering absences and mobility of the participants throughout the whole year. Additionally, clinical suspicions of malaria were monitored and confirmed through RDTs by CHWs or Healthcare Professionals (HCPs) in the respective villages or affiliated health posts (Bandafassi and Thiabedji in Senegal and Diré and Doneguebougou in Mali) for not only study participants but all inhabitants of the study sites. Uncomplicated RDT-positive malaria cases were treated by CHW, while severe malaria cases and pregnant women with malaria were referred to the health post. All cases presenting at health post were treated. Data on absence, mobility and malaria cases were documented by trained operators.

3.1.4 Laboratory Analysis

Collected dry blood spots (DBS) were analysed by real time quantitative PCR (qPCR) assay to detect *P. falciparum* multi-copy *varATS* gene (107). In addition, *CytB* gene was detected enabling to identify *P. falciparum* distinguished by melting temperature gene (108,109). DBS

measuring 1cm in diameter were collected, standardizing the sampling process. These samples were then cut, dissolved in a solution of 475µL of Buffer G2 and 25µL of Proteinase K (using EZ1&2 DNA Investigator® kit from Qiagen®), then incubated at 56°C and 900 rpm for 2 hours. Investigator® Lyse&Spin Basket and EZ1&2 DNA Investigator® kits from Qiagen® were used to extract *Plasmodia* on EZ1.

3.1.5 Sample Size

Based on results from microscopy, a 10% prevalence of which half was detectable by PCR was hypothesized, leading to a total of 20% (77). Assuming a protection through SMC within children under 10 years, which correspond to 30-40% of the population, and including 150 participants per village, 60-80 cases of *P. falciparum* infections should be detected by the surveys in total. This should be able to detect a 14% increase of prevalence between low and high transmission periods (alpha=5%, power=80%, intraclass correlation coefficient=0.3, cluster design with 10 individuals per cluster, n=60 clusters) (Peerawaranun et al., 2019).

3.2 Ethical Approval

The protocol of the MARS cohort study was approved by the National Ethics Committee for Health Research of Senegal (N°0000052/MSAS/DPRS/CNERS) and the Ethics Committee of the Université des Sciences, Techniques et Techologies de Bamako (N°2020/297/CE/FMOS/FAPH). Written informed consents were obtained from all participants before any participation in research activities. Consents were witnessed by a literate individual. Parents or legal guardians gave written informed consent for participants under the age of 18 years.

3.3 Data Analysis

3.3.1 Outcome Definition and Prevalence Estimates

In order to analyse dry season persistence, we selected cohort participants who were qPCR positive during either T0 or T2. We defined the individual outcome “dry season persistent plasmodium carriage” as positive if participant was still qPCR positive at the next survey (T1, respectively T3), after excluding individuals with recorded antimalarial treatment during the interval, either due to a positive RDT during survey in Senegal (see above) or due to the diagnosis and treatment of a clinical malaria episode.

A *PF* positive RDT on T0 or a positive RDT on T2 led to the treatment of malaria and was therefore not considered to be an asymptomatic malaria reservoir and therefore excluded.

Also, individuals with arising clinical symptoms and treatment and possible subsequent reinfection between two surveys, were regarded as not “asymptomatic *plasmodium* reservoir” and therefore excluded from further analysis. Prevalence of asymptomatic infections referred to the proportion of individuals carrying a persistent PCR positive *P. falciparum* infection either between T0 and T1 or T2 and T3 in relation to those individuals without a persistent PCR positive *P. falciparum* infection during the corresponding period, i.e. positive at either T0 but not T1 or T2 but not T3.

3.3.2 Definition of exposures

In this study, we considered several exposure variables to investigate their association with dry season persistence of plasmodium carriage. The primary exposure variable was Parasite Density, which was measured in *P. falciparum* parasites per µl using qPCR and transformed using the log10 scale to normalize the data distribution. Sex was included as a categorical variable to account for potential differences between female and male participants. Age was treated as a continuous variable to capture the potential age-related effects on persistence. Village was incorporated as a categorical variable to represent different villages, accounting for potential geographical variability. The Period variable was used as a categorical variable to distinguish between the two study periods, T0T1 and T2T3. To capture participant-level variability between observations, we included the participants’ Record ID as a random effect, allowing for the assessment of individual differences beyond the fixed effects. Similarly, Household was included as a random effect to account for household-level clustering and variability in the data.

3.3.3 Statistical Analysis

To estimate the association between parasite density and asymptomatic *PF* persistence, we analysed the data using a multilevel logistic regression model with a binary outcome.

Given that all observations are nested within participants, who are in turn nested within households, villages, and regions, it is reasonable to assume that the observed values are not independent. External factors such as climate, flora and fauna, and healthcare systems, as well as internal factors such as genetic and immunological profiles, are likely to be more similar within individuals of a household, village, or region than between individuals of different regions. This structure of the data imposes a multilevel model that accounts for this by partitioning the residual variance across the levels.

As we did not want to assume a linear relationship between continuous variables and the outcome, we employed a generalized additive model (GAM) using splines. An increase of OR can therefore not be put into relation with a specified increase of a given continuous exposure unit, but has to be interpreted using the splines.

The GAM statement is declared as:

$$
\begin{aligned}
&Logit(Persistence) \\
&\quad = \beta_0(s(log10(Parasite\ Density))) + \beta_1(Sex) + \beta 2\ (s(Age)) \\
&\quad + \beta_3(Village) + \beta 4(Period) \\
&\quad + b1(i,j)(Participant\ within\ Household\ with\ Random\ Effect) \\
&\quad + b2(i)(Household\ with\ Random\ Effect) + \varepsilon i,j,k,t
\end{aligned}
$$

Uni- and multilevel multivariable models were performed using the {mgcv} package in R version 4.4.0.

In this study, SatScan was employed to perform cluster analysis using purely spatial analysis with Bernoulli discrete statistics, specifically targeting regions with at least two reported cases. An elliptic spatial window shape was utilised, which allowed for the detection of clusters of varying shapes and orientations. A medium non-compactness penalty was applied to balance between identifying compact clusters and allowing for more irregular cluster shapes. Standard p-value inference was used to determine the statistical significance of the identified clusters. Spatial analysis was performed to describe the spatial distribution using QGIS version 3.6.3 with OpenStreetMap and GoogleEarth Map and SatScan Version 10.1.3.

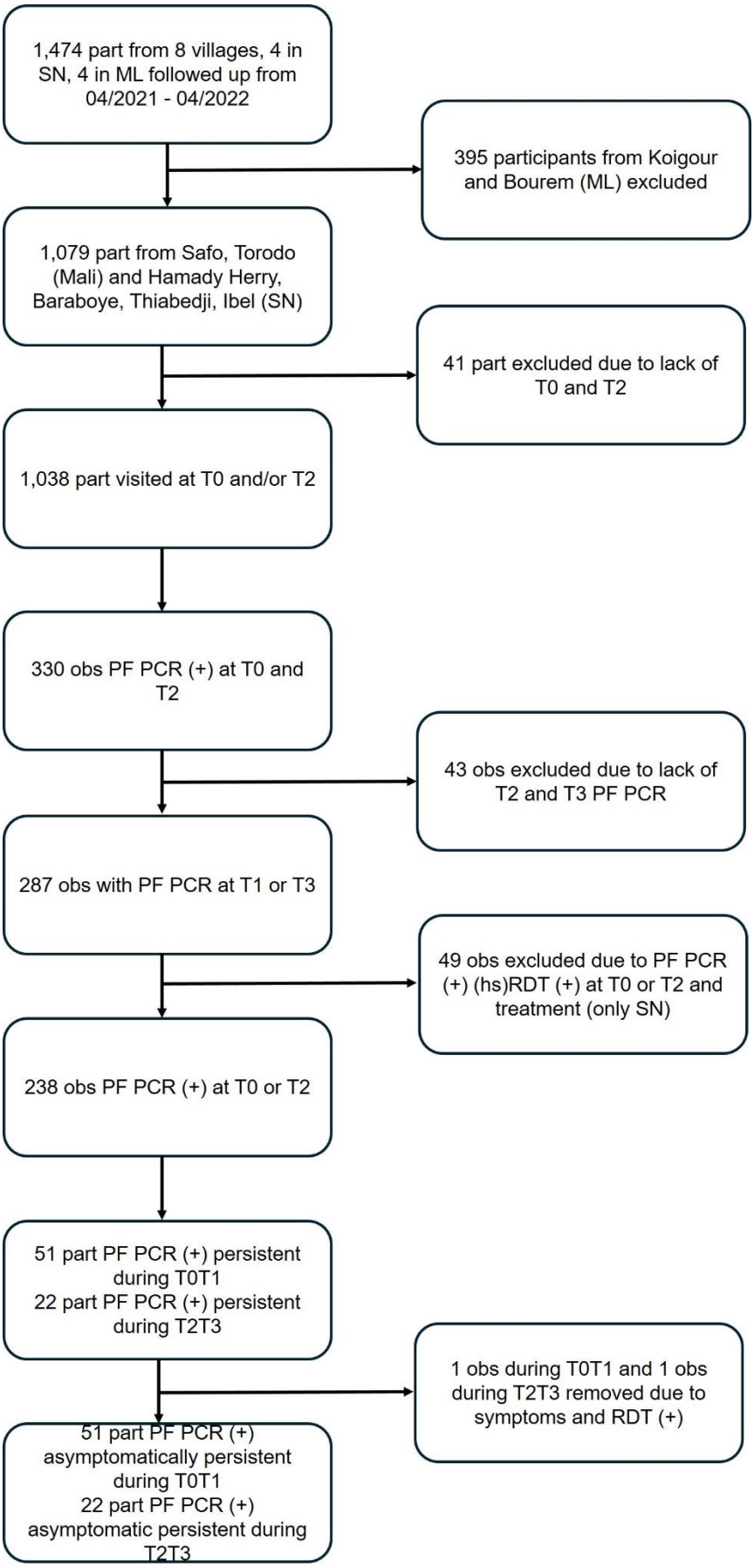


Figure 4: Flow diagram of participants' inclusion

4 Results

4.1 Study Structure and Participant Inclusion/Exclusion

Overall, 1.474 participants from 168 households from eight villages (01, Safo; 02, Torodo; 04, Koigour; 05, Bourem; 06, Hamady Herry; 07, Baraboye; 08, Thiabedji; 09, Ibel) were included in the follow-up over the course of the study. Including all types of follow-ups (surveys, weekly visits, and intercurrent visits for clinical malaria episodes), the database included 65,790 observations. However, two villages (04, Koigour and 05, Bourem, 395 participants) of the Diré district in Mali were excluded due to differences in geographical and meteorological conditions, including variations in the rainfall season, flooding, and proximity to the Niger River. From the remaining 1.079 participants, after excluding 41 individuals without sufficient information or negative at the start of the intervals where persistence was analysed, and those being RDT PF positive in Senegal or RDT PF positive and symptomatic in Mali (median parasite density of 0,175/µl, ICR 0,37 – 5,97), we retained 1.038 participants. Of these, 51 corresponded to the late dry season (T0-T1) and 22 to the early dry season (T2-T3) with a median parasite density of 0,66/µl (ICR 0,33 – 2,26) (see flowchart Figure 3).

4.2 Descriptive Profile of Participants

4.2.1 Distribution of Age

The age distribution of participants differs slightly between Mali and Senegal. In both countries, the largest group comprises individuals aged >10–25 years. In Mali, followed by the 0–10 years age group, followed by the group of >25 – 40 years and then > 40 years. In Senegal, however, in Mali, the second-largest group is those aged >40 years, followed by those aged 0 – 10 and ultimately >25 - 40. The categorisation of these age groups is based on the rationale that in Mali up to, SMC is provided free of charge for children up to 5 years old and in Senegal up to 10 years old, which is why they were put into the same age category.

4.2.2 Distribution of Parasite Density

The distribution of parasite densities was compared between the end of the dry season (T0 in blue) and the end of the rainy season, i.e. start of the dry season (T2 in red). The histogram of the log-transformed data reveals that participants during the T2T3 period exhibit higher counts of both high and low parasite densities, resulting in an overall higher mean parasite density. During the late dry season, the mean parasite density among persistent participants was 5,35/µl [Min 0.27, Max 128.40], which was higher than the mean parasite density of non-persistent participants at 2,18/µl [Min 0.27, Max 29,88]. Interestingly, in the early dry season,

the mean parasite density among persistent participants was 3,23/µl [Min 0.27, Max 24,62], substantially lower than that of non-persistent participants, which was 13,77/µl [Min 0.27, Max 588,91].

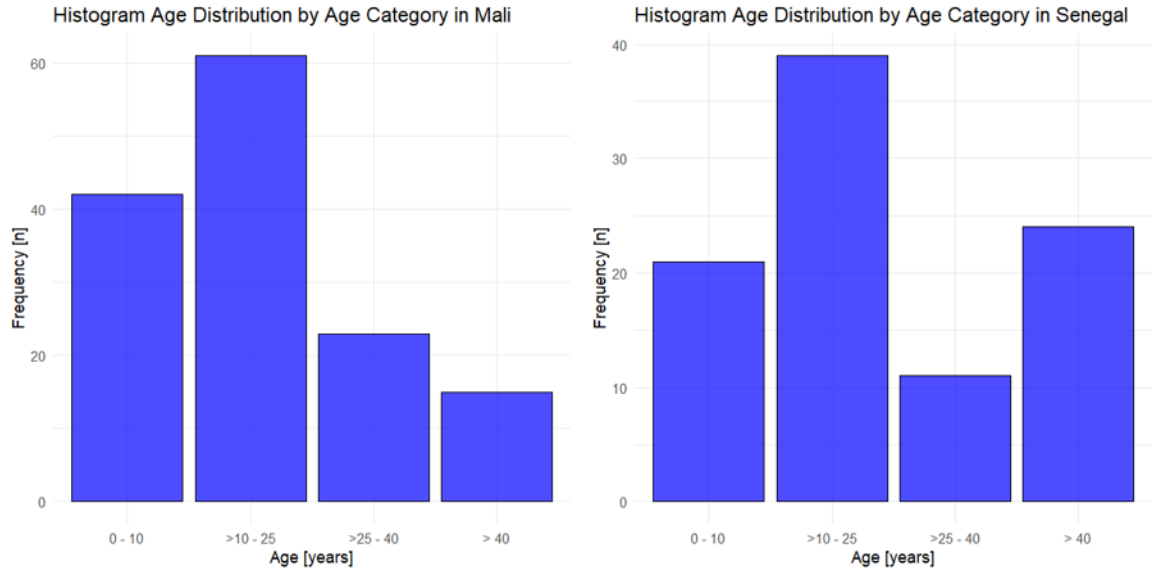


Figure 5 Age Distributions by Country. The age distribution of participants differs slightly between Mali and Senegal. In both countries, the largest group comprises individuals aged >10–25 years. In Mali, the next largest groups are 0–10 years, >25–40 years, and >40 years, respectively. In Senegal, the second-largest group is >40 years, followed by 0–10 years and >25–40 years. The categorization of these age groups reflects the availability of free SMC up to age 5 in Mali and up to age 10 in Senegal.

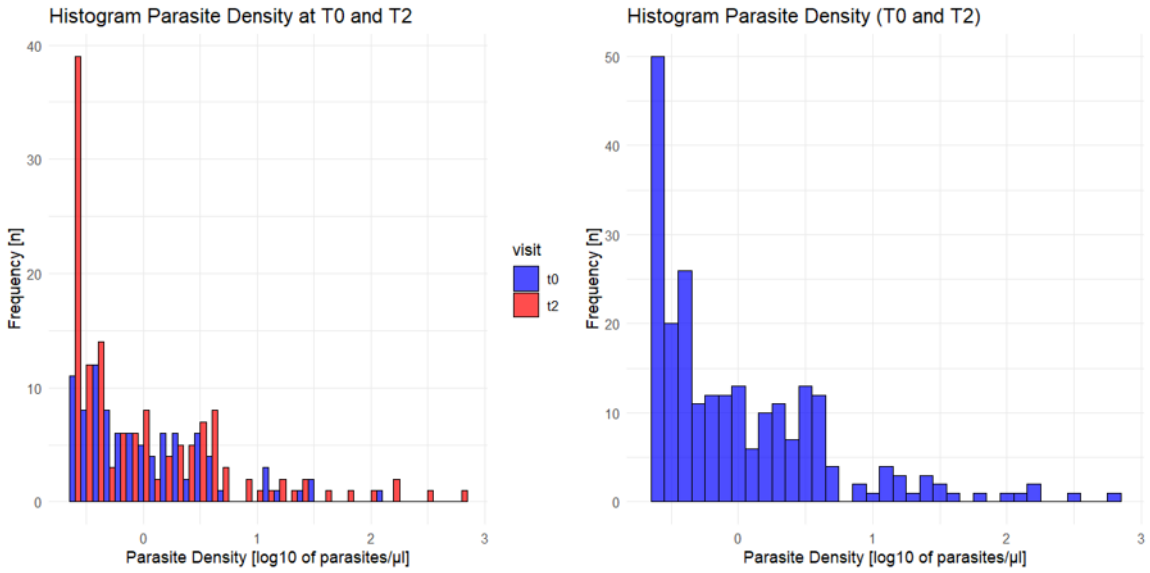


Figure 6 Distribution of Parasite Densities by Visit. The histogram compares parasite densities at the end of the dry season (T0, blue) and the start of the dry season (T2, red). Participants during T2 show higher counts of both high and low parasite densities, leading to a higher overall mean density. During the late dry season, persistent participants had a higher mean parasite density (5.35/µl) compared to non-persistent ones (2.18/µl). In the early dry season, persistent participants had a lower mean density (3.23/µl) than non-persistent participants (13.77/µl).

Table 1. Description of Sociodemographics, Geographic and Clinical Variables.

PF Persistence during T0T1 period			
Variable	**Overall**, N = 93[†]	**No**, N = 42[†]	**Yes**, N = 51[†]
Sex			
Male	56 (100%)	27 (48%)	29 (52%)
Female	37 (100%)	15 (41%)	22 (59%)
Age Category			
0 - 10	28 (100%)	14 (50%)	14 (50%)
>10 - 25	42 (100%)	17 (40%)	25 (60%)
>25 - 40	11 (100%)	3 (27%)	8 (73%)
> 40	12 (100%)	8 (67%)	4 (33%)
Village			
Safo	30 (100%)	10 (33%)	20 (67%)
Torodo	31 (100%)	9 (29%)	22 (71%)
Hamady Herry	10 (100%)	7 (70%)	3 (30%)
Baraboye	6 (100%)	5 (83%)	1 (17%)
Thiabedji	12 (100%)	8 (67%)	4 (33%)
Ibel	4 (100%)	3 (75%)	1 (25%)
Fever			
No	49 (100%)	27 (55%)	22 (45%)
Yes	16 (100%)	5 (31%)	11 (69%)
NA	28 (100%)	10 (36%)	18 (64%)

[†] n (%)

PF Persistence during T2T3 period			
Variable	**Overall**, N = 143[†]	**No**, N = 121[†]	**Yes**, N = 22[†]
Sex			
Male	65 (100%)	56 (86%)	9 (14%)
Female	78 (100%)	65 (83%)	13 (17%)
Age Category			
0 - 10	35 (100%)	33 (94%)	2 (5.7%)
>10 - 25	58 (100%)	46 (79%)	12 (21%)
>25 - 40	23 (100%)	17 (74%)	6 (26%)
> 40	27 (100%)	25 (93%)	2 (7.4%)
Village			
Safo	37 (100%)	31 (84%)	6 (16%)
Torodo	43 (100%)	37 (86%)	6 (14%)
Hamady Herry	17 (100%)	15 (88%)	2 (12%)
Baraboye	9 (100%)	7 (78%)	2 (22%)
Thiabedji	18 (100%)	15 (83%)	3 (17%)
Ibel	19 (100%)	16 (84%)	3 (16%)
Fever			
No	102 (100%)	89 (87%)	13 (13%)
Yes	9 (100%)	9 (100%)	0 (0%)
NA	32 (100%)	23 (72%)	9 (28%)
Previous persistence			
No	10 (100%)	10 (100%)	0 (0%)
Yes	26 (100%)	19 (73%)	7 (27%)
NA	107 (100%)	92 (86%)	15 (14%)

[†] n (%)

4.2.3 P. falciparum persistence varies through the dry season

The sample description is based on the sociodemographic profile (sex, age), place of residence (village), as well as clinical (fever, previous persistence) and laboratory data (*P. falciparum* load), stratified by observation period (late dry season – T0T1 vs. early dry season – T2T3) (Table 1). During the late dry season, of the 93 participants being *P. falciparum* (*PF*) PCR-positive on T0, 51 (54,84%) were still positive at the end of the period (T1), and thus considered persistent. In the early dry season (T2-T3), only 22 out of 143 (15,38%) showed persistence. The sex ratio is approximately equal in both periods, suggesting no difference in the distribution of persistence based on sex. The age distribution is discussed further below. During the T0T1 period, Villages in Mali (Safo and Torodo) showed almost consistently higher (67%, 71%) rates of persistence than villages in Senegal (Hamady Herry, Baraboye, Thiabedji, Ibel). During the T2T3 period, no substantial difference between villages or country could be observed. To investigate clinical symptoms as possible predictors of persistence, we

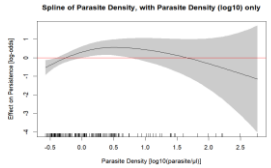
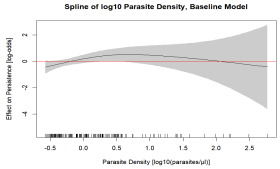
compared participants in regard of fever. At the beginning of the late dry season (T0), 11 out of 51 (21,57%) of the persistent participants had a fever, compared to 5 out of 42 (11,9%) of the non-persistent participants. At the beginning of the early dry season (T2), none of the persistent participants showed signs of fever while 9 out of the 121 (7,43%) non-persistent participants showed fever. It should be noted that fever in malaria was defined by WHO as a temperature of >37.5°C, which is quite low compared to clinical standards, making it a very sensitive but not specific predictor. Additionally, the dry season is also the hot season, with daily temperatures ranging from 42° to 45°C, which may externally contribute to elevated body temperatures. In the T2T3 period, which, per protocol, followed the T0T1 period, 7 out of 22 participants (31,81%) had previously shown persistence during the T0T1 period. This is contrasted by 19 out of 121 (15,7%) non-persistent (T2T3) participants which showed persistence in the previous T0T1 period This finding suggests an intraindividual propensity towards persisting PF carriage either across seasons or repeatedly.

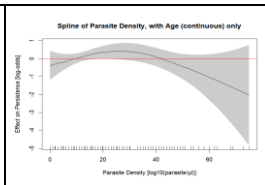
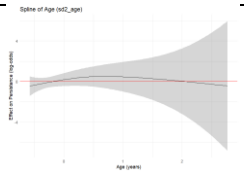
4.3 Inference Analysis with Uni- and Multivariable Models

4.3.1 *P. falciparum* Persistence depends on Parasite Density and Age

For the interpretation of the splines, the log-effect on persistence and Parasite Density log10 have to be transformed back by exponentiation with e. The multivariable model (Figure 6m Table 2), with n = 231 observations and 37,1% explained deviance (Dev), including all variables, shows a positive effect of parasite density between 0,64 and 1,8 parasites/µl, but it does not reach statistical significance at any point (p=0,176). Neither sex nor village showed a statistically significant effect on parasite persistence. However, comparing the periods upon *PF* persistence, in both, univariable and multivariable analysis, a statistically significant and substantial effect difference was observed. Comparing the late dry season (T0T1), the early dry season (T2T3) revealed an OR= 6,66 in the univariable and OR=20 in the multivariable analysis (p<0,001, n=231) with a substantial attribution do the explained deviance (26,2%), suggesting that late dry season is more strongly associated with *PF* persistence than early dry season.

Table 2 Uni- and Multivariable Analysis of Sociodemographic, Geographic and Clinical Factors on PF Persistence

	Univariable						Multivariable					
Variable	**OR**	**CI Low**	**CI High**	**p-value**	**n**	**Dev**	**OR**	**CI Low**	**CI High**	**p-value**	**n**	**Dev**
Parasite density log10				*0,001**	231	12,5%				0,176	231	37,1%

Age continuous				*0,001**	236	15,6%				0,182		
Sex	1,00	0,56	1,79	0,997	236	12,1%	1,26	0,60	2,64	0,541		
Torodo (ML)	0,88	0,37	2,09	0,780	236	12,0%	1,10	0,34	3,51	0,875		
Hamady Herry (SN)	0,38	0,11	1,30	0,122			0,70	0,14	3,46	0,666		
Baraboye (SN)	0,42	0,10	1,82	0,245			0,69	0,12	4,09	0,680		
Thiabedji (SN)	0,50	0,16	1,53	0,223			0,74	0,17	3,33	0,700		
Ibel (SN)	0,35	0,09	1,31	0,118			0,86	0,16	4,75	0,866		
Period (T2T3)	0,15	0,08	0,28	*0,001**	236	26,2%	0,11	0,05	0,24	*<0,001**		
Age >10 - 25	3,23	1,24	8,44	*0,017**	236	3,2%						
Age >25 - 40	3,85	1,27	11,64	*0,017**								
Age 0 - 10	1,87	0,66	5,29	0,237								

In comparison, in univariable analysis, parasite density was associated with a significantly higher odds of *PF* persistence. We found significant (p<0,001) higher odds (OR = 2,01) on *PF* persistence within a parasite density of 1,1 to 2,46/µl, (n=231, Dev=12,5%). Age also resulted to have significant (p<0,001) higher odds (OR=1,65) effect on parasite density between the age of 18 and 25 (n=236, Dev=15,6%).

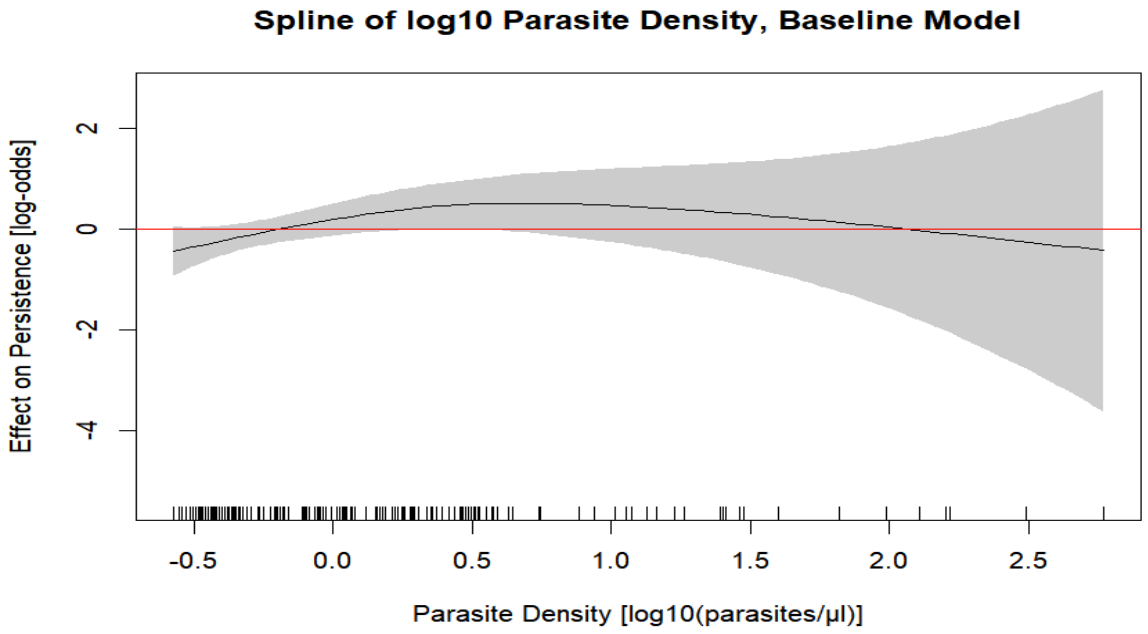


Figure 7 Spline Graph showing the Effect of Parasite Density on PF Persistence. Spline curves illustrate the relationship between variables by fitting smooth lines through the data. For interpretation, the log-effect on persistence and parasite density (log10) must be transformed back by exponentiation with e. The multivariable model (Figure 6, Table 2), with 231 observations and 37.1% explained deviance, indicates a positive effect of parasite density between 0.64 and 1.8 parasites/µl, though this effect is not statistically significant (p=0.176).

This result was mirrored by the univariable analysis of age categories with the categories of >10-25 years and >25-40 years being showing a statistically significant effect on parasite persistence (OR 3,23 – 3,85, p=0,017, n=236, Dev=3,2%). We also performed an analysis of a multivariable model with an interaction between age and sex, suggesting that an interaction was likely (p=0,067).

Additional sub models, containing only subsets of the dataset, i.e. per period (Annex, Table 5, Figure 10) or per country (Annex, Table 4, Figure 9) led to a borderline statistical difference at village level in Thiabedji (SN) and Baraboye (SN) could indicate a possible effect on village level.

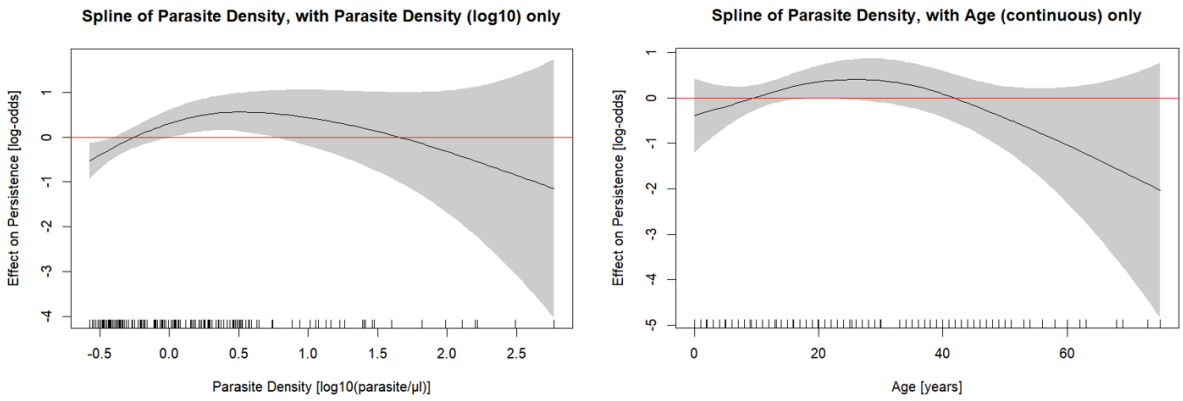


Figure 8. Spline Graphs comparing the Effect of Parasite Density or Age on PF Persistence. In univariable analysis, parasite density significantly increased the odds of PF persistence, with an OR of 2.01 for densities between 1.1 and 2.46 parasites/µl (p<0.001, n=231, Dev=12.5%). Age also significantly affected persistence, with an OR of 1.65 for ages 18 to 25 years (p<0.001, n=236, Dev=15.6%).

As the counties age distribution and health regulation on malaria diagnosis and treatment differ, we performed a subdivision by country, yielding more interesting results (Table 3, Figure 8). In the multivariable analysis of Senegal (n=93, Dev=24,9%), no explanatory variable reached statistical significance, however, in an univariable model of the participants age (n=95, Dev=10,7%), the effect on the odds on *PF* persistence was statistically significant higher amongst participants aging 20 to 40 years compared to those of 15 or 47 years (OR = 3,32, p<0,001), possibly making this group of participants in Senegal one of the driving factors for the model. In this subdivision, parasite density amongst participants in Senegal reaches no statistically significant difference. A similar observation can be made in the subdivision of Mali (n=138, Dev=17,09%). While a univariable analysis of the effect on *PF* persistence by participants age in Mali reaches a statistically significant difference (p=0,038, n=1,41, Dev=27,0%), an undulating spline with ORs pointing into different directions has to be interpreted with cautions as it is likely due to artefacts.

4.4 Exploratory Spatial Analysis of *P. falciparum* Prevalence

To describe *P. falciparum* prevalence at community level and compare the spatial distribution of *P. falciparum* persistence during the dry season, we conducted a cluster analysis of the six previously described villages. For this purpose, we used *P. falciparum* prevalence data from two time points, T0 (middle of the dry season) and T2 (end of the wet season/start of the dry season).

4.4.1 *P. falciparum* prevalence clusters remain at site but decrease over time

Figure 9 exemplifies a cluster analysis of *P. falciparum* prevalence clusters in the study village of Torodo, located in the Kati region of Mali. The yellow dots represent the surveyed households (n=22), while the ellipses indicate the clusters. Each ellipse encompasses areas with statistically significant higher *P. falciparum* prevalence compared to outside areas, thus clusters are to be interpreted relatively rather than absolutely.

In Torodo, at the end of the dry season (T0), three smaller clusters, with one, two and four households respectively, are identified. At the end of the subsequent rainy season and the beginning of the dry season (T2), a larger cluster comprising nine households is found in the northwest of the village. Four households remained within the high-risk group during both seasons. However, it must be mentioned that the shown households correspond to a sample of households. Unmeasured households in between could change the results.

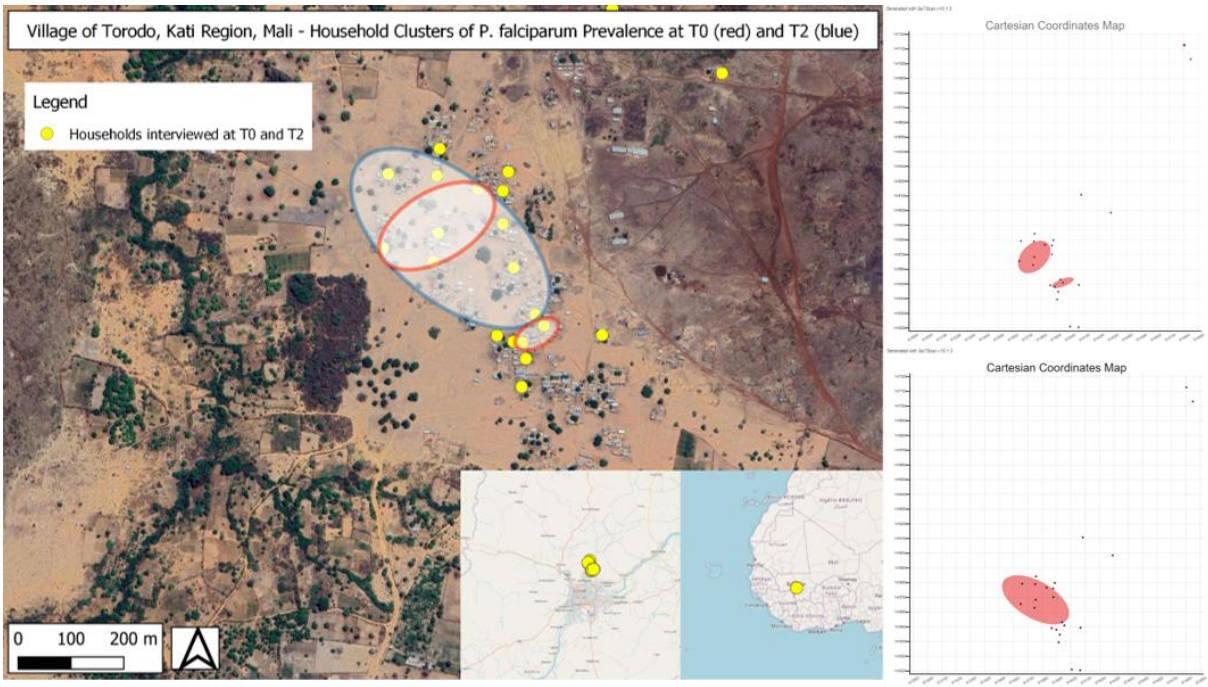


Figure 9. Cluster Analysis of PF Prevalance at T0 and T2 in Torodo. This figure illustrates a cluster analysis of *P. falciparum* prevalence in Torodo, Kati region, Mali. Yellow dots represent surveyed households (n=22), while ellipses indicate clusters of statistically significant higher prevalence. At T0, three smaller clusters are identified. By T2, a larger cluster of nine households emerges in the northwest, with four households consistently in the high-risk group across both seasons.

5 Discussion

The results of this study provide insights into the factors associated with the persistence of chronic *P. falciparum* infections in the Sahel region, particularly during the dry season. These findings are essential for understanding the dynamics of malaria transmission and developing targeted interventions.

In the multivariable analysis performed, only the time interval (T0-T1 i.e. late dry season 2021, vs T2-T3 i.e. early dry season 2022) resulted to have a statistically significant (p<0,001) effect on the *PF* persistence with a 20x higher odds of T0-T1 when compared to T2-T3. Parasite density and age as potential risk factors did not reach levels of statistical significance in the multivariable model (p = 0,182 and 0,176 respectively). However, an interaction between age and parasite density seems likely (p=0,067). When analysed independently in a univariable analysis both, parasite density (OR 2.01) and age (OR 1.65) result to show a statistically significant (p<0,001) effect on persistent *PF* infections, particularly during the late dry season. Specifically, parasite densities of approximately 1.1 – 2.5 parasites/µl appear to favour several months of *PF* persistence, aligning with our initial hypothesis. In light of the current literature, this seems plausible for several reasons. First, lower parasite densities are less likely to cause symptoms, making it unlikely for individuals to seek medical care, diagnostics, and treatment. Additionally, low *PF* densities fall below the detection thresholds of conventional RDTs and microscopy, making detection and treatment impossible (1,11,33) (10). This finding aligns with previous research indicating that higher parasite loads can contribute to the chronicity of P. falciparum infections (79). Ongoing genotyping of the same samples will also provide an insight on the previously reported longer persistence of multiple compared to single infections.

In the univariable analysis, we identified an age range of approximately 20 – 40 years that is associated with a higher likelihood of *PF* persistence. On the one hand, this association is plausible and supported by previous (25,72). On the other hand, we did not correct for exposure and health-seeking behaviour as potential confounders in this study. The age between 20 and 40 years represents the period of greatest economic activity, which in rural areas often involves outdoor work, leading to higher exposure and inoculation rates, and it was shown, that repeated exposition is required to develop an immune response able to control parasitaemia (82). This factor should be considered in future studies. Additionally, the observed age-related differences, with adults showing higher odds of persistence, are consistent with studies suggesting that immunity to malaria develops progressively with age and repeated exposure (76,85). This gradual acquisition of immunity likely influences the ability to maintain asymptomatic infections without progressing to clinical disease, thereby contributing to the reservoir of infection. A link between the use of SMC from 3 to 59 months

in Mali and 3 to 119 months in Senegal and postponed acquisition of protective immunity against *P. falciparum* can be assumed. A large sample size could have permitted to highlight country specific patterns in persistence.

Since the introduction of SMC in 2014, the level of exposure to *P. falciparum* infections has decreased among these children, compared to the rest of the population. They remain exposed to infective bites, but only undergo a fraction of bloodstream infections and clinical episodes compared to others. It is likely that children exiting the SMC eligibility age-range must acquire additional immunity from the age of 6 or 11 years onwards. This acquisition is also likely slower than for previous generations, due to the transmission reduction obtained through vector control.

Another factor strongly associated with *PF* persistence identified in our study is the timing within the dry season, i.e. beginning of the dry season (T2-T3) vs end of the dry season (T0-T1). Previous studies on persistence, which followed positive individuals over several weeks and months, have shown that the initial parasite load rapidly decreases in the form of an exponential curve/asymptote, and the infection either clears or persists at a low level (74). Considering this background, we explain the significantly higher odds of persistence during the late dry season by assuming that individuals who remain latent carriers and have an infection five months after the end of the last rainy season are likely to maintain this infection two months longer until the next rainy season. This interpretation seems to contradict the observed mean parasite loads of the early dry season (T2) of 3,23 parasites/µl compared to 5,35 parasites/µl in the late dry season (T0). A hypothesis to explain this difference could relate to the genetic profile of the *Plasmodia*. Andrade and colleagues postulated that there might be genetic variations and alleles within the *Plasmodia* that are activated either during the dry or rainy season (83). Some variations may trigger explosive replication with increased mosquito infection likelihood during the rainy season, while others favour a type of slow replication at low levels, possibly higher sequestration and employing immune-evasion mechanisms resulting in a "smouldering infection" without significantly activating the host's immune response. This subform could increase in number by the end of the dry season, tolerated by the immune system, thereby explaining the higher parasite loads. Describing the genetic profile of *PF* has already been done by Collins in The Gambia (74) and would be the next step in identifying *Plasmodia* with higher persistence capabilities in humans.

However, not only the genetic profile of the parasites plays a role. It is worth noting the relatively high proportion of nearly 32% of persistent carriers in the T2T3 period who had already shown persistence in the preceding dry season (T0T1). If this phenomenon can be replicated in future studies, it might indicate the existence of "superhosts," individuals whose immunological and/or genetic profiles, in combination with defensive *Plasmodia*, create optimal conditions for the formation of a human reservoir. The disproportionate contribution of specific individuals to

transmission was reported from a recent study assessing infectivity of asymptomatic carriers to mosquitos in Uganda: out of 104 individuals tested once or multiple times, 4 individuals only contributed >60% of all infected mosquitoes, and one of these remained infected over 18 months (68). Identifying and describing these individuals genetically and immunologically could lead to even more precise measures for the identification of and intervention on the human *Plasmodia* reservoir.

The spatial distribution analysis revealed clusters of *P. falciparum* prevalence within specific villages, emphasizing the importance of localized interventions. Most clusters remained at the same sites, nonetheless clusters at late dry season being smaller than clusters at early dry season, suggesting that while the overall prevalence decreases during the dry season, certain areas continue to harbour persistent infections. This observation aligns with a recent study suggesting a strong clustering on household level through different inoculation rates (110) and supports the notion that malaria control efforts need to be geographically targeted to address hotspots of transmission effectively (77).

The impact of environmental and sociodemographic factors was also evident in our study. Differences in *P. falciparum* persistence between villages and countries were noted, likely due to variations in malaria control measures, healthcare access, and diagnostic practices. For instance, in Senegal, where malaria diagnostics and treatment are more accessible and comprehensive, the persistence rates were lower compared to Mali, highlighting the importance of robust healthcare systems in managing malaria (97,101).

This study contributes to the growing body of evidence on the importance of targeted malaria interventions. By identifying and understanding the risk factors and spatial dynamics of *P. falciparum* persistence, public health strategies can be better tailored to address the unique challenges of malaria transmission in the Sahel. Future research should focus on the development and implementation of localized, evidence-based interventions that consider both the biological and environmental factors influencing malaria persistence (25,79).

6 Conclusion

This study elucidates the multifaceted factors influencing the persistence of chronic *P. falciparum* infections in the Sahel region, emphasizing on the one hand the interplay between parasite density, age and timing within the dry season, shown by the uni- and multivariable analysis, and on the other hand environmental conditions, and healthcare practices shown and discussed in the cluster analysis.

The timing within the dry season emerged as a crucial factor, with significantly and substantially higher odds of persistence observed in the late dry season. This aligns with the hypothesis that once a latent carrier sustains an infection several months after the rainy season, they are likely to maintain it further into the dry season. The possibility of genetic variations in *Plasmodium* that favour either an active replication or a passive conformation state could explain the persistence dynamics observed.

The identification of "superhosts," individuals who consistently harbour persistent infections across seasons, points to the need for further research into the genetic and immunological profiles that contribute to this phenomenon. Understanding these profiles could lead to more precise identification and intervention strategies targeting the human reservoir.

Our spatial analysis underscores the importance of localized approaches to malaria control, as persistent clusters of infection were identified within specific villages. This calls for geographically tailored public health strategies to effectively reduce transmission hotspots. The observed differences in persistence rates between Senegal and Mali highlight the critical role of robust healthcare systems in managing and preventing malaria.

SMC and other interventions during the rainy season have shown significant efficacy in reducing *P. falciparum* prevalence, supporting their continued implementation and potential expansion, possibly onto higher age groups. Continuous monitoring and treatment of asymptomatic carriers are essential to disrupt the transmission cycle and mitigate the risk of malaria reintroduction at the onset of the wet season. This could be achieved through the application of an MDA at the end of the dry season, aiming to eliminate a potential non-detected human reservoir before the onset of the next rainy season. Alternatively, understanding if RDT-detectable infections at the start of the dry season are more or less persistent could open perspectives for the design of seasonal screen and treat interventions against the reservoir, which are usually ineffective due to the low detectability of chronic infections.

In conclusion, this study provides valuable insights into the persistence of malaria infections in the Sahel, informing the development of precise, evidence-based interventions. Future efforts should focus on enhancing the effectiveness of localized malaria control measures, improving

healthcare accessibility, and maintaining rigorous surveillance to ultimately achieve sustainable malaria eradication in the region.

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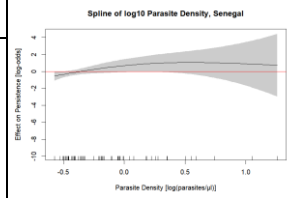
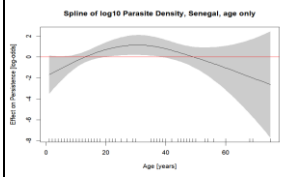
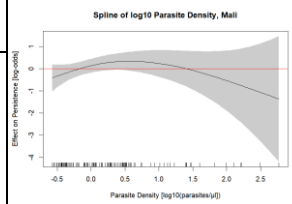
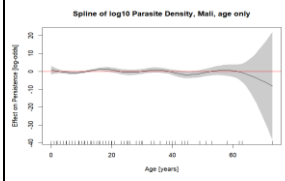
8 Appendices

Table 3. Uni- and Multivariable Analysis of PF Persistence by Country

	Univariable						Multivariable					
Variable	**OR**	**CI Low**	**CI High**	**p-value**	**n**	**Dev**	**OR**	**CI Low**	**CI High**	**p-value**	**n**	**Dev**
Senegal											93	24,9%
Parasite density log10										0,129		
Age continuous				*<0,001**	95	10,7%				0,075		
Record ID										0,913		
Household										0,979		
Sex							0,99	0,31	3,15	0,990		
Baraboye (SN)							0,69	0,10	4,63	0,701		
Thiabedji (SN)							0,86	0,17	4,29	0,858		
Ibel (SN)							0,56	0,10	3,25	0,515		
Mali											138	17,0%
Parasite density log10										0,251		
Age continuous				*0,038**	141	27,0%				0,551		
Record ID										0,934		
Household										0,075		
Sex							0,99	0,46	2,11	0,970		
Torodo (ML)							0,98	0,33	2,94	0,973		

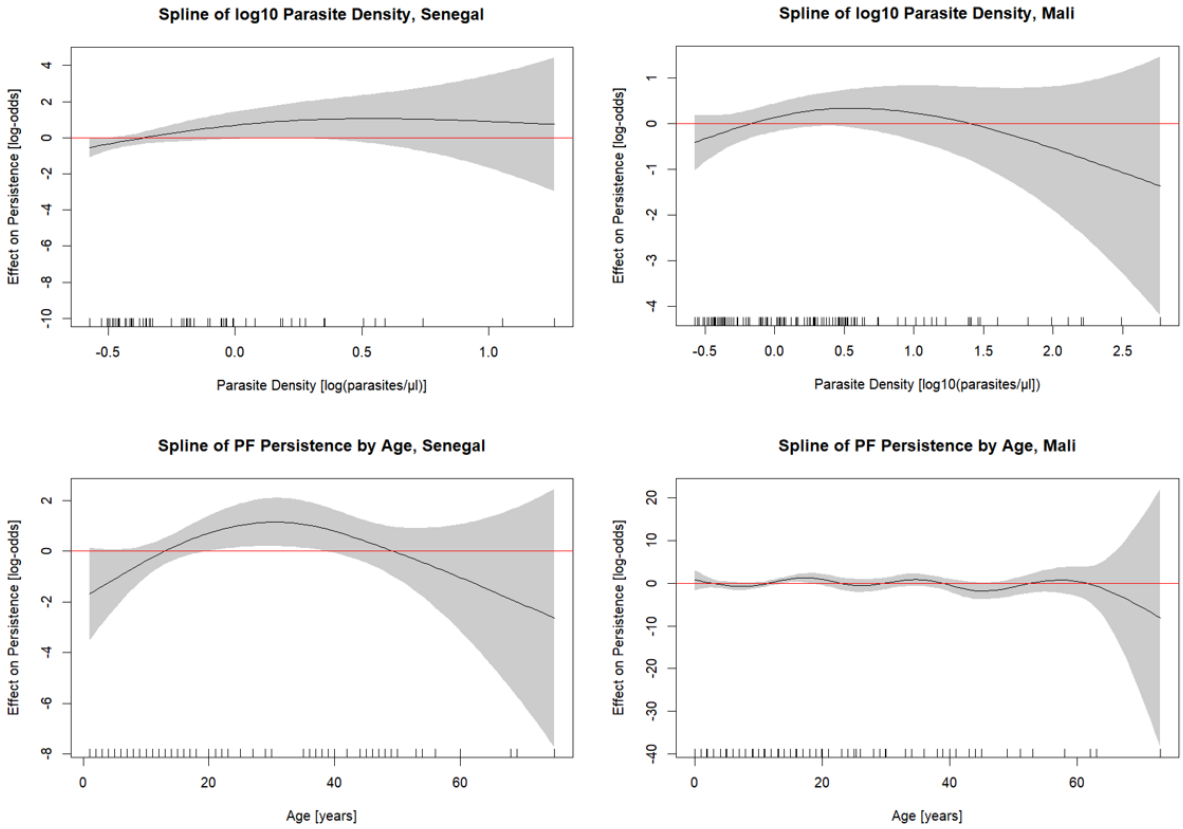
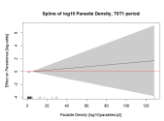
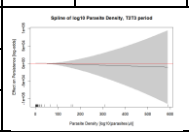


Figure 10. Comparison of Splines by Country, Age and Parasite Density. The splines illustrate the effects of age and parasite density on PF persistence in Senegal and Mali. In Senegal, the multivariable analysis (n=93, Dev=24.9%) showed no significant explanatory variables. However, a univariable model indicated significantly higher odds of PF persistence among participants aged 20 to 40 years (OR = 3.32, p<0.001). In Mali, a univariable analysis also showed a significant effect of age on PF persistence (p=0.038, OR=1.41, Dev=27.0%), though the undulating spline suggests potential artefacts.

Table 4. Multivariable Analysis of Factors influencing PF Persistence, by Period.

	Multivariable					
Variable	**OR**	**CI Low**	**CI High**	**p-value**	**n**	**Dev**
T0T1					93	13,9%
Sex	1,50	0,57	3,92	0,412		
Torodo (ML)	1,25	0,41	3,83	0,696		
Hamady Herry (SN)	0,24	0,05	1,20	0,083		
Baraboye (SN)	0,10	0,01	1,02	0,052		
Thiabedji (SN)	0,24	0,05	1,07	0,061		
Ibel (SN)	0,14	0,01	1,69	0,123		
Parasite density log10				0,539		
Age continuous				0,654		
Household				0,593		
T2T3					138	18,2%
Sex	1,41	0,47	4,27	0,544		
Torodo (ML)	2,17	0,39	11,96	0,374		
Hamady Herry (SN)	5,55	0,51	60,18	0,159		
Baraboye (SN)	5,13	0,50	52,94	0,170		

Thiabedji (SN)	6,04	0,72	50,47	0,097		
Ibel (SN)	4,96	0,60	40,83	0,137		
Parasite density log10				0,052		
Age continuous				0,265		
Household				0,455		

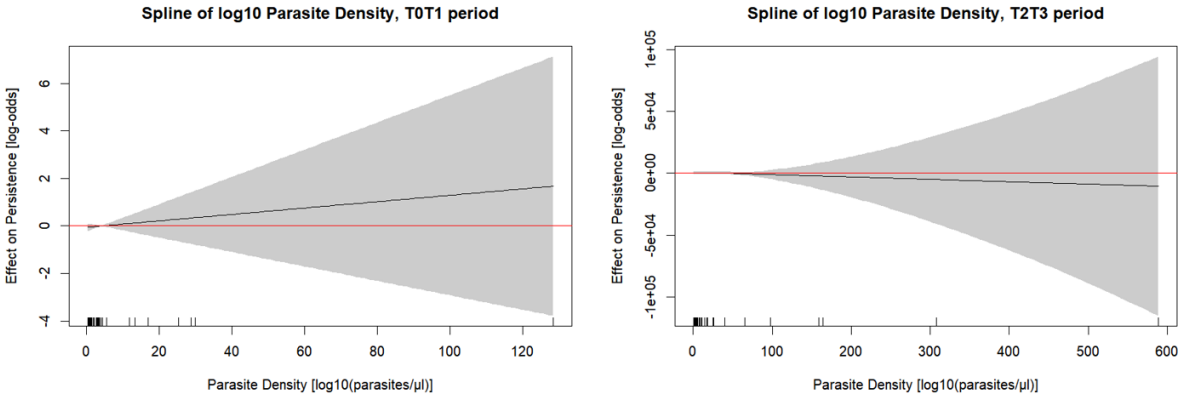


Figure 11. Splines of PF Persistence according to period.

9 Abstract in French

Les infections à *Plasmodium* varient de la maladie grave aux cas asymptomatiques, avec des infections non traitées pouvant persister pendant des mois ou des années. Les individus asymptomatiques, formant un « réservoir », contribuent substantiellement à la transmission du paludisme. Cette étude se concentre sur les facteurs influençant la persistance des infections à *Plasmodium falciparum* dans la région du Sahel, en considérant les variables sociodémographiques, cliniques et environnementales.

Les données de l'étude sur le réservoir asymptomatique de paludisme au Sahel (MARS), une étude de cohorte ouverte dans huit villages au Sénégal et au Mali, ont été utilisées. Quatre enquêtes ont été menées d'avril 2021 à avril 2022. Les ménages ont été sélectionnés au hasard et les données des participants ont été recueillies par des questionnaires et des échantillons biologiques. Une PCR quantitative en temps réel a été utilisée pour détecter *P. falciparum*. Le résultat principal était la « portage persistant du *Plasmodium* en saison sèche », défini par la positivité de la qPCR lors des enquêtes suivantes. Une régression logistique multiniveaux dans un modèle additif généralisé (GAM) a été employée pour analyser les données, en considérant la densité parasitaire, l'âge, le sexe, le village et la période comme variables.

Au total, 1.474 participants issus de 168 ménages ont été inclus, avec 1.038 restant après exclusions. Pendant la fin de la saison sèche (T0-T1), 54,84 % des participants étaient persistants, contre 15,38 % au début de la saison sèche (T2-T3). Des densités parasitaires plus élevées et des âges de 20 à 40 ans étaient des prédicteurs significatifs de la persistance. Dans l'analyse univariée, la densité parasitaire augmentait significativement les chances de persistance (OR = 2,01, p<0,001), et l'âge avait également un effet significatif (OR = 1,65, p<0,001). Les modèles multivariés montraient une augmentation de 20 fois des chances de persistance de *P. falciparum* pendant la fin de la saison sèche par rapport au début de la saison sèche. Des différences dans les taux de persistance entre le Sénégal et le Mali ont été observées, soulignant le rôle des systèmes de santé robustes.

Cette étude met en évidence l'importance des interventions ciblées pour réduire le réservoir de paludisme, en particulier pendant la fin de la saison sèche, lorsque la persistance est la plus élevée. L'identification des « superporteurs » suggère la nécessité de recherches supplémentaires sur les profils génétiques et immunologiques. Des stratégies de santé publique localisées sont essentielles pour aborder les points chauds de transmission. La chimioprévention saisonnière du paludisme et d'autres interventions ont montré leur efficacité et devraient être étendues. Une surveillance continue et le traitement des porteurs asymptomatiques sont cruciaux pour interrompre le cycle de transmission et prévenir la réintroduction du paludisme. Les efforts futurs devraient se concentrer sur l'amélioration des

mesures de contrôle localisées, l'accessibilité des soins de santé et le maintien d'une surveillance rigoureuse pour atteindre l'éradication durable du paludisme dans le Sahel.

10 Students Contribution

The MARS study was designed and supervised by Dr Jordi Landier, PhD for SESSTIM. All data collection was carried out by SESSTIM staff and partners in Senegal and Mali. Laboratory analyses were performed by the Department of Parasitology of the University of Aix-Marseille. The Master's student, Eric Twomey, MD, cleaned and restructured the data as part of his mandatory M2 internship at the SESSTIM of the University of Aix-Marseille and independently performed the descriptive part. Eric Twomey carried out the analysis with the multilevel multivariable model and the spatial analyses under the supervision of Jordi Landier. Eric Twomey carried out literature research, code writing with R, creation of the tables and figures, and writing of the master's thesis independently with subsequent content control by Jordi Landier.

During his internship, in April 2024, in addition to his main task of analysing the MARS data and preparing a manuscript for publication, Eric Phillip Twomey went on a mission to the Kédougou region of Senegal for and with the support of SESSTIM and IRD Dakar. There, together with a Senegalese colleague, Dr Harouna Thiam, MPH, he conducted a field study to explore the recent increase in severe malaria rates in the "District sanitaire de Kédougou", getting to know parts of the study setting of the MARS study. With the support of Dr Jordi Landier, PhD, Dr El-Hadji Ba, PhD, Pr. Dr Adama Faye, MPH, he designed, conceptualised and carried out a project during a one-month stay in Senegal. He designed and validated a qualitative interview guide, a quantitative questionnaire addressed to medical staff, and community health workers in 30 interviews and collected more than 400 medical records of severe malaria from local health centres and hospital.
The feasibility and financing of this side project had not yet been clarified at the start of the internship, which is why the content of this project was not included in the Master's thesis.