Plasmodium falciparum carriage in a population under long-term, intensive malaria control in Kedougou region, Senegal: a 1-year cohort study

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Summary

Background In Sahelian Africa, successful interventions against malaria include vector control, improved access to care, and seasonal malaria chemoprevention (SMC) in children. However, malaria incidence has increased in the past 5 years. Up-to-date evidence is necessary to design additional interventions and restore progress towards elimination. In this study, we aimed to describe subclinical *Plasmodium falciparum* infections in the general population and understand the changes in prevalence, parasite densities, and clinical incidence across age groups and seasons, and to identify factors associated with *P falciparum* carriage in Kedougou, Senegal's most affected region.

Methods We included all individuals older than 6 months from randomly selected households of four villages in a 1-year open cohort. During four surveys spanning the dry and wet seasons, we collected sociodemographic and behavioural data, and detected *P falciparum* using quantitative PCR on capillary dried blood samples. We analysed risk factors associated with *P falciparum* carriage using multilevel logistic regression.

Findings We included 763 participants from 69 households, and they were followed up from April 13, 2021, to March 30, 2022. *P falciparum* prevalence was lowest in SMC-eligible children (aged <10 years) and remained below 10% across wet and dry seasons. Older age groups had similar dry season prevalence at baseline (10–15%). During the wet season, prevalence increased in individuals aged 15–24 years (32·1%) and 35–49 years (24·7%). The highest clinical burden was in participants aged 10–14 years (527 cases per 1000 person-years) and 15–19 years (631 cases per 1000 person-years), over five-fold higher than children aged 6 months to 4 years (93 cases per 1000 person-years). Outdoor night-time activity was associated with *P falciparum* infection.

Interpretation In this setting, ongoing intensive control reduced malaria in SMC-eligible children. Older individuals bear an important clinical burden and harbour high prevalence during the wet season. Elimination-oriented interventions must tackle the parasite reservoir, involving whole communities and specifically young adults.

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For the French translation of the Summary see **Online** for appendix 1

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Introduction

In the Sahel region of west Africa, malaria incidence decreased from 359 cases to 290 cases per 1000 person-years between 2011 and 2018, but progress is stalling.[1] *Plasmodium falciparum* remains responsible for the heaviest burden.[2] While vector control remains central to malaria control strategies, improved access to health-care and chemoprevention in population groups at risk of severe malaria have become essential.

In Senegal, the malaria control strategy relies on vector control campaigns of long-lasting insecticidal net distribution and indoor residual spraying (IRS); free, community-based access to rapid diagnostic tests and treatment; and intermittent preventive treatment in pregnant women and seasonal malaria chemoprevention (SMC) in children younger than 10 years.[2,3] SMC involves monthly curative and prophylactic sulfadoxine–pyrimethamine and amodiaquine treatments, administered door-to-door under directly observed treatment supervision (appendix 2 p 3).[4]

The gradual implementation and strengthening of these strategies in Senegal reduced malaria burden between 2013 and 2017, but progress has stalled since 2018.[3] Three regions (Kedougou, Kolda, and Tambacounda) accounted for 11·3% of the country's population but 78·5% of cases in 2021, and Kedougou had the highest burden of malaria in the country (536·5 cases per 1000 person-years).[2,3]

Insecticide resistance and changes in vector bionomics make vector control increasingly challenging.[3,5] Despite considerable improvement in access to diagnosis and treatment, malaria case management only addresses symptomatic infections, overlooking infected carriers. Still, low-density, asymptomatic infections play an important role in malaria persistence in seasonal settings such as Kedougou.[6–11]

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See **Online** for appendix 2

Research in context

Evidence before this study

In 2023, 18 African countries administered seasonal malaria chemoprevention (SMC) in regions of strong seasonal transmission, covering 58 million children. The beneficial impact of SMC on morbidity and mortality in targeted age groups has been described extensively. However, few epidemiological studies have described the effect of SMC on the prevalence and distribution of plasmodium infections in the general population, especially in the long term. We searched for articles published in English or French from Jan 1, 2012 to Oct 31, 2024 documenting the reservoir of plasmodium infections using molecular methods or feeding assays in west African countries implementing SMC. We identified 265 articles using the search phrase "(malaria OR *Plasmodium falciparum*) AND (PCR or qPCR or QT-NASBA or RT-qPCR or feeding) AND (reservoir OR asymptomatic OR subclinical) AND (Burkina Faso OR Mali OR Senegal OR Niger OR Chad OR Cameroon OR Benin OR Nigeria OR Ghana OR Gambia OR Guinea OR Togo OR (Seasonal Malaria Chemoprevention) NOT Papua) AND ("2012 "[Date - Publication]: "3000 "[Date - Publication])". Of these articles, after excluding studies reporting a single age group, reporting immunological or parasite genetics outcomes only, and taking place in regions not implementing SMC (eg, central Cameroon or southern Ghana), we considered 23 studies reporting prevalence across diverse age groups.

20 studies (83%) took place before SMC implementation and reported the historical epidemiology: children had cumulative exposure to malaria since birth and acquired immunity to clinical disease proportionally to the local transmission intensity (often before age 10 years). Of these studies, only two characterised the asymptomatic, infectious reservoir, and highlighted a relationship between high asexual parasite densities, high gametocytaemia, and high infectivity to mosquitoes. In the absence of SMC, children younger than 15 years were estimated to contribute 50–80% of the infectious reservoir.

Only three studies reported prevalence in a context of SMC. One of these studies documented prevalence in Senegal, where SMC is administered up to age 10 years. It was conducted in a high prevalence village of less than 200 inhabitants, where SMC-eligible children had the lowest prevalence. The other two studies originated from The Gambia and Burkina Faso, where SMC is administered up to age 5 years only. In all studies, the general population was presented in three age categories (<5 years, 5–9 years or 5–14 years, and ≥10 years or ≥15 years). SMC-eligible children presented the lowest prevalence, irrespective of the overall prevalence level.

Added value of this study

Presenting disaggregated age data, we showed that SMC alters the historical paradigm of cumulative acquisition of immunity against plasmodium infection. Indeed, while children remain eligible for SMC, they are largely protected from clinical malaria and have low plasmodium carriage only. Full exposure to bloodstream infections only begins from age 10 years onwards, when children no longer receive chemopreventive treatments. As a result, individuals aged 10–24 years become central to malaria epidemiology during the wet season in Kedougou: first bearing the clinical burden (age 10–19 years), then harbouring high prevalence of asymptomatic infections (age 15–24 years). Specifically, individuals aged 15–19 years display the highest parasite densities, suggesting an important contribution to the infectious reservoir.

Implications of all the available evidence

Our findings suggest that after 10 years of SMC in Senegal, *Plasmodium falciparum* circulation thrives in individuals immediately above the SMC age threshold and up to 15 years older. Most SMC-implementing countries currently provide SMC to children up to age 5 years, but 2024 WHO recommendations lifted age thresholds; countries can now consider implementing it up to 10 years or in school-aged children. Expanding the SMC age range will have a beneficial effect on malaria-related severe morbidity and mortality, but the effect on transmission will probably only be transient. Individuals who no longer receive SMC will likely contribute much more to transmission than individuals exposed to *P falciparum* since birth. Gradually increasing the population under chemoprevention is probably less effective at reducing transmission compared with addressing the entire parasite reservoir.

Subclinical infections remain poorly documented in SMC settings. Malaria indicator or demographic health surveys focus on children younger than 5 years, who are protected by SMC, and underestimate general population prevalence.[12] Molecular methods (eg, PCR) are required to detect low-density infections accurately. The most recent state of the art studies (including molecular gametocytes detection and infectivity assays) were done pre-SMC (2007–08) in Burkina Faso and estimated that individuals younger than 15 years were responsible for 50–80% of the infectious reservoir.[13,14] SMC reduces clinical incidence in children by 80%,[4] and is likely to decrease their contribution to transmission, thus changing the historical patterns of highest burden in youngest children.

Of the three studies we identified that describe the distribution of plasmodium infections in the general population in an SMC context using PCR, none provides sufficient information to understand the effect of SMC in the general population: adolescents and adults are pooled as a single category (>10 years or >15 years), and only one study reported parasite densities.[15–17]

Addressing *P falciparum* carriage could enable further transmission reduction;[3,5] to design effective strategies

against this carriage, it is necessary to update current epidemiological evidence on malaria, assessing both clinical burden and subclinical *P falciparum* infections.

In this Article, we describe subclinical *P falciparum* infections in the general population over a year in Kedougou, a seasonal transmission setting of the Sahel receiving a high coverage intervention package.[3] We aimed to understand the changes in prevalence, parasite densities, and clinical incidence across age groups and seasons, and to identify factors associated with *P falciparum* carriage.

Methods

Study design

The Malaria Asymptomatic Reservoir in the Sahel (MARS) open cohort study took place in Kedougou region, Senegal. Malaria transmission is seasonal, with a dry, low-transmission season from January to May, and a wet, high-transmission season from June to December.[2] During this study, the Kedougou health district routinely implemented community-based early diagnostic and treatment, and 4-monthly rounds of SMC (round 1 start: June 24, 2021). IRS campaigns were conducted in early June of 2020 and 2021 (appendix 2 p 3).

We selected four villages—Baraboye, Hamady Herry, Ibel, and Thiabedji—representing Fulani and Malinke ethnic groups, with different levels of accessibility to roads and to health-care facilities, but reachable in rainy season. Each village had a trained and motivated community health worker (CHW; as assessed by feedback from health post nurse supervisors and availability and quality of the CHW's patient records; appendix 2 p 5).

The National Ethics Committee for Health Research of Senegal approved the protocol of the MARS cohort study (No.0000052/MSAS/DPRS/CNERS).

Participants

In April, 2021, we randomly selected households from the population census of the Bandafassi Demographic and Health Surveillance System (DHSS).[18] Target sample size was 150 participants per village (see appendix 2 p 6 for detailed calculation). We included households for which there was consent obtained from the head of household, confirmation that members would remain in the study area during the 1-year follow-up, and agreement to participate from over 50% of inhabitants aged 15 years or older.

In consenting households, we included individuals aged 6 months or older and staying for at least 1 night in the household. New participants matching these criteria were included during follow-up surveys. Participants could withdraw at any time. If more than 50% of inhabitants aged 15 years or older living in a household withdrew, neighbouring households of similar size were approached by the CHW, and the first to consent was recruited to maintain the sample size. All participants (or their parent or legal guardian for participants <18 years) provided written informed consent before their participation in any research activity; a literate individual witnessed consent of illiterate participants.

Procedures

We conducted four surveys in April, 2021 (baseline; T0), at the end of June, 2021 (T1; immediately before the first SMC round), at the end of November, 2021 (T2), and in March, 2022 (T3; appendix 2 p 7).

During surveys, two teams visited households within the same 8–10-day period. A team of trained research assistants fluent in local languages conducted face-to-face data collection; questions addressed sociodemographic characteristics, malaria-related health behaviours, and daily lifestyle factors. A second team consisting of trained nurses visited households, tested *P falciparum* infections by rapid diagnostic test (RDT; SD Bioline Malaria Ag Pf, Abbott Diagnostics Korea, Gyeonggi-do, South Korea), and collected capillary dried blood samples (two to four spots) on Whatman 3MM filter paper. Nurses measured participants' temperature by infrared forehead thermometer, and recorded history of fever in the last 48 h. The CHW was informed about RDT-positive participants, conducted a home visit, and proposed antimalarials unless the participant had already taken antimalarials for 2 weeks or less.

Between April, 2021 and March, 2022, CHWs conducted weekly household visits to register absences and mobility of cohort participants. In addition, CHWs and staff of the nearest primary health-care facility (health post) passively recorded RDT-confirmed clinical malaria cases occurring in all inhabitants of the four villages, cohort members included.

All participant data were recorded on paper. A trained operator entered responses into REDCap (surveys) and Excel (mobility and clinical cases).[19] Quality control measures were implemented throughout the study (appendix 2 pp 7–9).

We analysed dry blood spots by real-time quantitative PCR (qPCR) assays detecting *P falciparum* multicopy nuclear *varATS* gene and *CytB* gene (PgMt19 with *P falciparum* positives distinguished from melting temperature).[20,21] Detailed molecular methods are provided in appendix 2 (pp 9–11).

Statistical analysis

Database construction and analyses were conducted using R 4.0 and mgcv, FactoMineR, factoextra, and survey packages (appendix 2 p 9).

We used passively recorded RDT-confirmed clinical cases for all inhabitants across the four villages to estimate weekly incidence over the 1-year cohort study, reported per person-week. We calculated yearly incidence, reported per person-year, for all inhabitants, cohort members, and cohort members within specific age groups (appendix 2 p 12).

	T0 (n=597)	T1 (n=621)	T2 (n=655)	T3 (n=637)
Sociodemographic characteristics				
Village				
Hamady Herry	155 (26·0%)	170 (27·4%)	173 (26·4%)	159 (25·0%)
Baraboye	143 (24·0%)	134 (21·6%)	160 (24·4%)	150 (23·5%)
Thiabedji	150 (25·0%)	156 (25·1%)	166 (25·3%)	156 (24·5%)
Ibel	149 (25·0%)	161 (25·9%)	156 (23·8%)	172 (27·0%)
Sex				
Male	283 (47·4%)	297 (47·8%)	316 (48·2%)	303 (47·6%)
Female	314 (52·6%)	324 (52·2%)	339 (51·8%)	334 (52·4%)
Age				
<5 years	96 (16·1%)	107 (17·2%)	110 (16·8%)	116 (18·2%)
5–9 years	115 (19·3%)	117 (18·8%)	122 (18·6%)	120 (18·8%)
10–14 years	88 (14·7%)	91 (14·7%)	98 (15·0%)	97 (15·2%)
15–19 years	67 (11·2%)	72 (11·6%)	66 (10·1%)	61 (9·6%)
20–24 years	36 (6·0%)	39 (6·3%)	47 (7·2%)	39 (6·1%)
25–34 years	54 (9·0%)	52 (8·4%)	63 (9·6%)	61 (9·6%)
35–49 years	81 (13·6%)	81 (13·0%)	85 (13·0%)	84 (13·2%)
≥50 years	60 (10·1%)	62 (10·0%)	64 (9·8%)	59 (9·3%)
***P falciparum* infection status and fever**				
P falciparum infections: positive RDT (T0–T3 surveys)				
Yes	12 (2·0%)	10 (1·6%)	49 (7·5%)	8 (1·3%)
No	571 (95·6%)	573 (92·3%)	595 (90·8%)	608 (95·4%)
Missing data	14 (2·3%)	38 (6·1%)	11 (1·7%)	21 (3·3%)
P falciparum infections: positive qPCR (T0–T3 surveys)				
Yes	65 (10·9%)	34 (5·5%)	111 (16·9%)	22 (3·5%)
No	517 (86·6%)	551 (88·7%)	532 (81·2%)	594 (93·2%)
Missing data	15 (2·5%)	36 (5·8%)	12 (1·8%)	21 (3·3%)
P falciparum infections: positive qPCR in dry season (T0 or T1 surveys)				
Yes	..	..	70 (10·7%)	..
No	..	..	498 (76·0%)	..
Missing data because new arrivals*	..	..	87 (13·3%)	..
Body temperature†				
<37·5°C	334 (55·9%)	546 (87·9%)	508 (77·6%)	451 (70·8%)
≥37·5°C	65 (10·9%)	8 (1·3%)	23 (3·5%)	155 (24·3%)
Missing data	198 (33·2%)	67 (10·8%)	124 (18·9%)	31 (4·9%)
History of fever over the previous 2 days†				
Yes	155 (26·0%)	54 (8·7%)	126 (19·2%)	120 (18·8%)
No	402 (67·3%)	522 (84·1%)	508 (77·6%)	490 (77·0%)
Missing data	40 (6·7%)	45 (7·2%)	21 (3·2%)	27 (4·2%)
Malaria health behaviours				
SMC during last rainy season				
Yes	207 (34·7%)	203 (32·7%)	241 (36·8%)	229 (35·9%)
No	11 (1·8%)	11 (1·8%)	11 (1·7%)	9 (1·4%)
Not eligible (age >10 years)	372 (62·3%)	390 (62·8%)	396 (60·5%)	382 (60·0%)
Missing data	7 (1·2%)	17 (2·7%)	7 (1·1%)	17 (2·7%)
Bednet use				
Every night	242 (40·5%)	524 (84·4%)	591 (90·2%)	265 (41·6%)
Not every night or never	344 (57·6%)	79 (12·7%)	33 (5%)	358 (56·2%)
Missing data	11 (1·8%)	18 (2·9%)	31 (4·7%)	14 (2·2%)

(Table 1 continues on next page)

P falciparum prevalence referred to the proportion of individuals carrying *P falciparum* infection at the time of the survey, regardless of clinical symptoms. Within the cohort, *P falciparum* prevalence was calculated by dividing the count of positive samples (as established by RDT or PCR) by the total number of samples tested. To extrapolate *P falciparum* prevalence to the general population, we estimated a weighted prevalence, accounting for a stratified (ie, village-level) and clustered (ie, household-level) sampling design and the population structure by village, age, and sex, based on census data (appendix 2 p 13).[22,23]

We considered sociodemographic variables (village, age, and sex), malaria preventive behaviours (reported SMC participation and bednet use), daily lifestyle behaviours (mobility frequency and outside night activity) and history of RDT-confirmed malaria episodes from the start of the trial period up to each timepoint or since the previous timepoint (appendix 2 p 14).

We monitored the type and the frequency of outdoor night-time activities at each survey and summarised this information in one variable constructed by a hierarchical ascendant clustering after principal component analysis on the combined four surveys (appendix 2 p 16).

The primary outcome, *P falciparum* carriage, was defined as qPCR-confirmed *P falciparum* individual infection.

In the prespecified analysis, we investigated the association between the primary outcome and age and sex across all surveys (model 1). Then, we focused on the high-transmission season visit (T2) to investigate the association with behavioural (model 2) or exposure-related factors (model 3).

First, we evaluated the association between *P falciparum* carriage and age as a continuous variable, sex, and visit, using a generalised additive multilevel logistic regression model with random intercepts at village, household, and individual levels. We used splines to estimate non-linear effects of age according to each visit (model 1).

Second, we studied the association between *P falciparum* carriage at T2 and malaria preventive behaviours (model 2). We only analysed behaviours at the end of wet season survey (T2), since *P falciparum* infections detected during T0, T1, and T3 would more likely result from persisting carriage than from an infective bite sustained in the dry, low-transmission season.

Third, we examined the association between *P falciparum* carriage at T2 and history of RDT-confirmed clinical malaria during the interval between T1 and T2, and history of *P falciparum* carriage during previous dry season visits (T0, T1, or both; model 3). Models 2 and 3 used multilevel logistic regression with random intercepts at village and household levels. Variables included in the models were chosen to minimise correlations between risk factors, as evaluated through pairwise comparisons using χ^2 or Kruskal–Wallis tests.

Pairwise comparisons using the Kruskal–Wallis test were conducted to compare median parasite density across seasons and age groups. Bonferroni correction was applied to adjust for multiple tests.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

At baseline (T0), we visited 79 households and enrolled 66 (83·5%; 597 participants) in the cohort, and they were followed up from April 13, 2021, to March 30, 2022. Reasons for non-enrolment were head of household refusal (n=3), household unable to participate in the follow-up (n=2), household relocated or scattered (n=4), or household located near a household already included (n=4). During T1, two head of households withdrew consent and one relocated permanently; all three were replaced with three new households at T2. Over one year, 597 participants were initially enrolled and 166 newcomer participants enrolled. 126 (16·5%) of the total 763 participants seen in at least one survey were lost to follow-up. Overall, the cohort included 763 participants in 69 households and 449 (58·8%) completed the four visits (appendix 2 p 17).

On average, there were 10 participants per household (range 2–26). Half the participant population was younger than 15 years (380 [49·8%] of 763), and 394 (51·6%) were female and 369 (48·4%) were male (table 1, appendix 2 p 18). Over 90% of eligible children reported SMC participation in 2020 and 2021. Nightly bednet use varied from 242 (40·5%) of 597 participants during the dry season (T0) to 591 (90·2%) of 655 during the wet season (T2). Outdoor night-time activity patterns clustered in three profiles (table 1). For example, during the wet season, of the 655 respondents in the T2 survey, 375 (57·3%) often participated in eating, conversing, or cooking, 203 (31·0%) rarely engaged in outdoor night-time activities, and 18 (2·7%) often participated in cultural or religious ceremonies, work, or bush activities (activities or sleeping in bush or field; appendix 2 pp 19–21). During the T2 wet season survey, most participants (75·9%, n=497) reported no travels, 65 (9·9%) travelled less than once a month, and 18 (2·7%) travelled at least once a month.

Over the study period (April 13, 2021, to March 30, 2022), clinical malaria incidence was 309 cases per 1000 person-years in the cohort and 375 cases per 1000 person-years in the four villages' general population. Three villages showed a bimodal incidence pattern with peaks in August and October, whereas Baraboye had a distinct peak in July followed by decline until January (figure 1A). Malaria incidence was highest in young adults aged 15–19 years (631 cases per 1000 person-years) and in children aged 10–14 years (527 cases per 1000 person-years) and lowest among children younger than 5 years (93 cases per 1000 person-years; figure 1B).

	T0 (n=597)	T1 (n=621)	T2 (n=655)	T3 (n=637)
(Continued from previous page)				
Household participating in indoor residual spraying during last rainy season (n=66)				
Yes	61 (92·4%)	58 (87·9%)	58 (87·9%)	58 (87·9%)
No	5 (7·6%)	6 (9·1%)	6 (9·1%)	6 (9·1%)
Missing data	0 (0%)	2 (3·0%)	2 (3·0%)	2 (3·0%)
Daily life behaviours				
Outdoor night-time activity				
Often (work, bush, or ceremony)	55 (9·2%)	9 (1·4%)	18 (2·7%)	22 (3·5%)
Often (eat, converse, or cook)	306 (51·3%)	395 (63·6%)	375 (57·3%)	437 (68·6%)
Rarely	192 (32·2%)	171 (27·5%)	203 (31·0%)	145 (22·8%)	
Missing data	44 (7·4%)	46 (7·4%)	59 (9·0%)	33 (5·2%)
Mobility frequency since last survey				
None	..	528 (85·0%)	497 (75·9%)	535 (84·0%)
Less than once a month	..	65 (10·5%)	65 (9·9%)	46 (7·2%)
At least once a month	..	8 (1·3%)	18 (2·7%)	16 (2·5%)
Missing data	..	20 (3·2%)	75 (11·5%)	40 (6·3%)
Passive recording of RDT-confirmed symptomatic malaria cases				
Clinical malaria episode since last survey				
Yes	..	5 (0·8%)	146 (22·3%)	6 (0·9%)
No	..	557 (89·7%)	431 (65·8%)	608 (95·4%)
Missing data because new arrival	..	59 (9·5%)	78 (11·9%)	23 (3·6%)

Data are n (%). Data summarise the cohort during the four surveys (T0: April, 2021; T1: June, 2021; T2: November, 2021; T3: March, 2022). *P falciparum*=*Plasmodium falciparum*. PCR=quantitative PCR. RDT=rapid diagnostic test. SMC=seasonal malaria chemoprevention. *Or refusal of blood sampling at T0 and T1. †Body temperature could be influenced by high ambient temperature in T0 and T3 (around 40°C).

Table 1: **Cohort sociodemographic characteristics, clinical status and history, and relevant behaviours**

Of 2492 samples collected during the four surveys, 232 tested positive for *P falciparum* by qPCR (table 1, appendix 2 p 22). Individuals who were *P falciparum* positive were identified in 56 (81·2%) of 69 households across all surveys. Among 178 individuals who tested positive at least once, 136 (76·4%) had one positive sample, 32 (18·0%) had two, eight (4·5%) had three, and two (1·1%) had four.

Weighted *P falciparum* prevalence by qPCR was low (11·0% [95% CI 7·1–17·7]) during the dry season baseline survey (T0) and increased strongly (20·9% [14·5–27·9]) during the end of wet season survey (T2; figure 2A) with the exception of one village (Baraboye, 6·9%; figure 2B). 171 (73·7%) of 232 qPCR-detected *P falciparum* infections were undetectable by RDT. This proportion was highest at T0, with 53 (81·5%) of 65 infections undetected, and lowest at T2 with 28 (70·3%) of 111 infections undetected (appendix 2 p 23). Around 91% of samples had *P falciparum* parasitaemia below the threshold of 100 parasites per µL in the four surveys (T0 93·7%, T1 91·2%, T2 87·9%, and T3 100·0%; figure 2C).

Children younger than 10 years had the lowest *P falciparum* qPCR prevalence throughout the study period (figure 2D). Dry season prevalence was similar across all age groups older than 10 years: around

10–15% in T0, 5–10% in T1, and 5% in T3. During the wet season, individuals with the highest prevalence were those aged 15–19 years (22 [33·8%] of 65), 20–24 years (14 [29·8%] of 47), and 35–49 years (21 [24·7%] of 85).

Dry season parasitaemia was low (median <10 parasites per µL) across all groups (figure 2E; appendix 2 p 24). Wet season parasitaemia was more heterogeneous, and medians reached 15·8 parasites per µL in participants aged 15–19 years and 10·0 parasites per µL in those aged 50 years or older, but remained at 1·5 parasites per µL in children younger than 10 years. Median comparison across eight age groups was not significant, although this was likely due to small sample size.

Age was associated with *P falciparum* infection at T0 and T2 in univariate and multivariate analysis (table 2, figure 3; appendix 2 p 26); this association was non-linear. Using age 9 years as the reference, children younger had significantly lower odds in the dry season T0 (eg, odds ratio [OR] associated with age 5 years 0·49 [95% CI 0·31–0·78]), whereas adolescents and young adults (eg, 18 years OR 1·89 [1·08–3·31]) and adults (eg, age 50 years OR 2·29 [1·06–5·00]) had higher odds (figure 3A). This age pattern persisted in the wet season, and the magnitude of the effects increased (with reference to age 9 years: age 5 years OR 0·44 [0·30–0·64], age 18 years OR 2·77 [1·62–4·47], and age 42 years OR 2·49 [1·20–5·17]; figure 3C). Sex was not associated with parasite carriage (OR 0·78 [0·53–1·13], p=0·19; table 2).

Individuals engaging regularly in outdoor night-time activities presented with a higher odds of infection at T2 compared with those participating rarely. The difference between types of activities (eating, conversing, and cooking compared with work, ceremonies, and bush activities) was not significant (table 2). Children younger than 10 years participated less often in outdoor night-time activities than older individuals (appendix 2 p 27).

Mobility frequency at T2 and bednet use were not associated with *P falciparum* carriage.

Participation in the SMC campaign was reported uniformly in eligible children (241 [96%] of 252), and therefore omitted from multivariable analysis (table 2).

Individuals infected with *P falciparum* during the previous dry season (T0 or T1) were at higher risk of a wet season *P falciparum* infection at T2 (OR 2·93 [95% CI 1·48–5·79]) compared with individuals without previous dry season infection (table 2). However, individuals with a recorded clinical malaria episode between T1 and T2 were not at higher risk of carriage at T2 (1·35 [0·77–2·36]). The interaction between the carriage of *P falciparum* infection at T0 or T1 and the occurrence of a clinical episode of malaria between T1 and T2 was not significant.

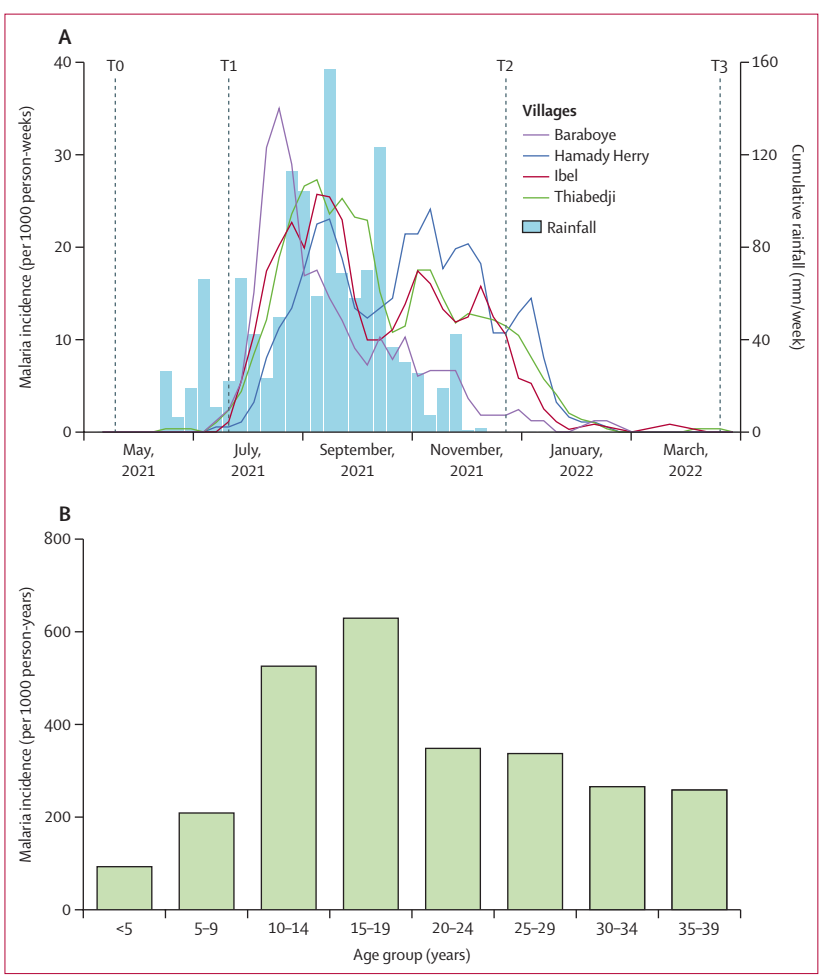
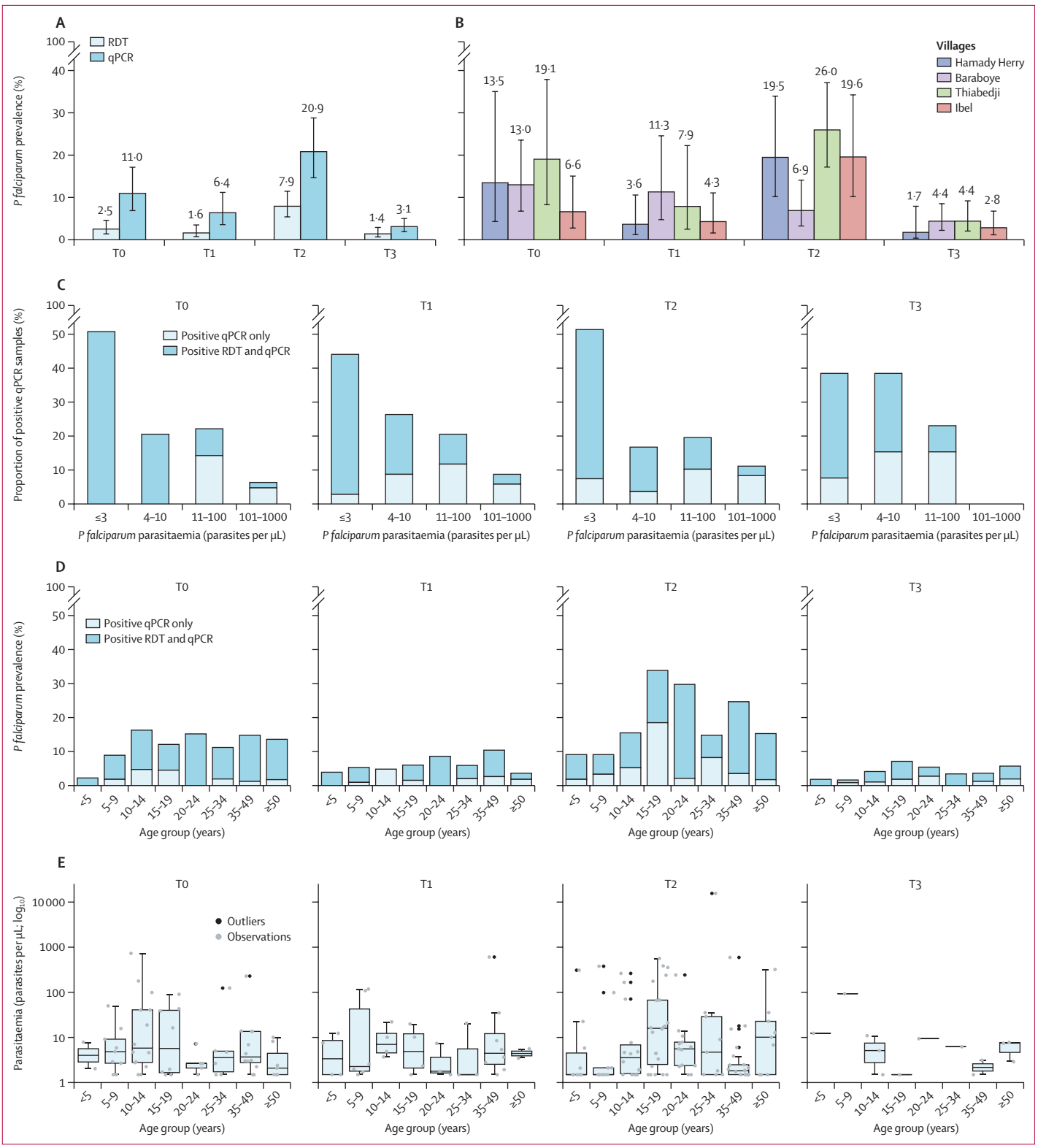


Figure 1: **Malaria incidence**
(A) Weekly malaria incidence per 1000 person-weeks (smoothing with a moving average of 3 weeks) and cumulative rainfall during the 1-year cohort period (April 13, 2021–March 30, 2022). Dotted blue lines indicate the occurrence of the four prevalence surveys (T0=mid-April, 2021; T1=mid-June, 2021; T2=end of November, 2021; T3=end of March, 2022).[24] Three villages displayed a bimodal pattern, with the highest peak occurring in August, followed by a decrease in September, and then a subsequent rise or plateau in October, before decreasing until January. Baraboye stood out with a sudden surge in incidence, peaking in July, followed by a gradual decline until January. (B) Malaria incidence (per 1000 person-years) of cohort participants during the 1-year study period across age groups.

Discussion

This 1-year cohort study showed a 57% relative increase in prevalence of *P falciparum* infection between the dry season baseline (T0) and the end of wet season survey (T2;

Figure 2: **Characteristics of *Plasmodium falciparum* carriage**
(A) Weighted *P falciparum* prevalence by RDT and qPCR during the four surveys in the general population. (B) Weighted *P falciparum* prevalence by qPCR across villages. (C) *P falciparum* parasitaemia during the four surveys (positive qPCR quantified at T0 =6·9% [n=63], T1=100% [n=34], T2=96·4% [n=107], T3=59·1% [n=13]). We considered only positive RDT confirmed by qPCR. One participant presenting with a malaria episode upon T2 survey with a parasitaemia of 15 592 parasites per µL is not represented for graph clarity and representation. (D) *P falciparum* infection prevalence detected by qPCR and RDT across age groups during the four prevalence surveys within the cohort. (E) *P falciparum* parasite density estimated by qPCR during the four surveys according to age groups. The central line represents the median number of parasites per µL, the boxes represent the IQR, and the bars above and below represent 1·5 × IQR above the third quartile and below the first quartile, respectively. qPCR=quantitative PCR. RDT=rapid diagnostic test.


A
RDT
qPCR
P falciparum prevalence (%)
2·5
11·0
1·6
6·4
7·9
20·9
1·4
3·1
T0
T1
T2
T3
B
Villages
Hamady Herry
Baraboye
Thiabedji
Ibel
13·5
13·0
19·1
6·6
3·6
11·3
7·9
4·3
19·5
6·9
26·0
19·6
1·7
4·4
4·4
2·8
C
Proportion of positive qPCR samples (%)
Positive qPCR only
Positive RDT and qPCR
≤3
4–10
11–100
101–1000
P falciparum parasitaemia (parasites per µL)
D
P falciparum prevalence (%)
<5
5–9
10–14
15–19
20–24
25–34
35–49
≥50
Age group (years)
E
Parasitaemia (parasites per µL; log10)
Outliers
Observations

	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Model 1: sociodemographic factors (T0–T3; n=2426 for multivariate analysis)*				
Survey				
T0	1 (ref)	..	1 (ref)	..
T1	0·48 (0·30–0·77)	0·0024	0·55 (0·33–0·92)	0·0218
T2	2·15 (1·47–3·14)	<0·0001	2·29 (1·50–3·51)	0·0001
T3	0·29(0·17–0·51)	<0·0001	0·34 (0·19–0·61)	0·0003
Sex				
Male	1 (ref)	..	1 (ref)	..
Female	0·78 (0·54–1·14)	0·21	0·78 (0·53–1·13)	0·19
Age–visit interaction				
Age–T0	Appendix p 26 (figure S9A)	0·0232	Figure 3A	0·0248
Age–T1	Appendix p 26 (figure S9B)	0·34	Figure 3B	0·33
Age–T2	Appendix p 26 (figure S9C)	0·0021	Figure 3C	0·0019
Age–T3	Appendix p 26 (figure S9D)	0·16	Figure 3D	0·15
Model 2: malaria prevention and exposure behaviours (T2; n=570 for multivariate analysis)†				
SMC during last rainy season (n=637)				
Yes	1 (ref)	..	NA	NA
No	1·69 (0·29–9·76)	0·56	NA	NA
Not eligible (age >10 years)	2·99 (1·75–5·12)	<0·0001	NA	NA
Bednet use (n=613)				
Every night	1 (ref)	..	1 (ref)	..
Not every night or never	1·36 (0·52–3·58)	0·53	1·23 (0·45–3·36)	0·69
Outdoor night-time activity (n=588)				
Rarely	1 (ref)	..	1 (ref)	..
Often (eat, converse, or cook)	2·34 (1·3–4·22)	0·0046	2·23 (1·23–4·04)	0·0082
Often (work, bush, or vigil)	7·24 (2·1–24·92)	0·0017	6·32 (1·8–22·12)	0·0039
Mobility frequency since last survey (n=569)				
None	1 (ref)	..	1 (ref)	..
Less than once a month	1·39 (0·59–3·26)	0·45	1·17 (0·47–2·91)	0·73
At least once a month	3·24 (0·91–11·54)	0·0697	1·82 (0·46–7·17)	0·39
Missing data	1·59 (0·67–3·76)	0·29	1·15 (0·47–2·82)	0·75
Model 3: previous *Plasmodium* infection history (T2; n=557 for multivariate analysis)‡				
Clinical malaria episode since last survey (n=643)				
No	1 (ref)	..	1 (ref)	..
Yes	1·47 (0·86–2·52)	0·16	1·35 (0·77–2·36)	0·29
Missing data because new arrivals	1·41 (0·6–3·29)	0·43	NA	NA
P falciparum infections: positive qPCR in dry season (T0 or T1 surveys; n=557)				
No	1 (ref)	..	1 (ref)	..
Yes	2·93 (1·48–5·79)	0·0019	2·93 (1·49–5·79)	0·0019

Model 1: Sociodemographic factors during the four surveys (n=2426). Model 2: behavioural factors linked to malaria prevention or exposure at the end of the wet season (T2 survey; n=570). Model 3: individual participant history of confirmed clinical or asymptomatic infection at the end of the wet season (T2 survey) (n=570). NA=not applicable. qPCR=quantitative PCR. *Multilevel model with random intercepts at village, household, and individual levels. Variables included are sex and age–visit interaction estimating visit-specific non-linear splines for age (continuous variable). †Multilevel model with random intercepts at village and household levels. Factors included are seasonal malaria chemoprevention during last rainy season, bednet use, outside night-time activity, and mobility frequency since last visit. ‡Multilevel model with random intercepts at village and household levels. Factors included are confirmed clinical malaria episode since last survey, and *P falciparum* PCR positive in dry season.

Table 2: **Factors associated with *Plasmodium falciparum* infection detected by PCR**

from 11·0% to 20·9%). Most infections (74%) were undetected by RDT. Children younger than 10 years had the lowest prevalence and clinical incidence throughout the follow-up, confirming SMC protection. Participants aged 10–19 years had a heavy clinical burden during the high-transmission season. Participants aged 15–24 years and 35–49 years had a high prevalence of carriage at the end of the high-transmission season, 32·1% and 24·7%, respectively. Among these participants, those aged 15–19 years had the highest parasite densities when infected subclinically. In addition to age, outdoor night-time activities and previous dry season infection were associated with *P falciparum* infection.

The findings of this study are consistent with previous reports of lowest prevalence of *P falciparum* among children receiving SMC,[15,16,25] and parasite density lowest among participants aged 15 years or older.[16] In 2019, a study identified a higher prevalence (70% overall, 50% among SMC-eligible children) in a remote community of the same area.[15] Incidence among cohort participants is similar to a previous description in the Bandafassi DHSS (Kedougou district).[4] A 2011 SMC trial[26] provides comparison data for *P falciparum* incidence in children: in children younger than 5 years, incidence was 24 cases per 1000 person-months with SMC (*vs* 8 in our study) and 135 without SMC (not estimated in our study); and in children aged 5–9 years, incidence was 20 cases per 1000 person-months with SMC (18), and 122 without SMC (not estimated in our study; figure 2B). The lower SMC protection in children aged 5–9 years might relate to differential outdoors exposure, but further investigations should confirm if the current age-group-based dosing regimen provides a sufficient dose to all children in the same group.[4] Our results contrast with pre-SMC evidence which showed that younger children bore the heaviest burden of prevalence, parasitaemia, and gametocytaemia.[13,14]

Our study has several strengths. The study design as an open cohort of households enabled us to include 15–25% of village populations; this sample corresponded to the general population structure and was maintained throughout the study, but it decreased study power compared with individual sampling. The combination of RDT and qPCR enabled us to assess both patent and low-density infections (<10 parasites per µL). The repeated, detailed participant questionnaire on individual behaviours successfully captured seasonality of bednet use and epidemiological evidence, matching entomological results on outdoor mosquito biting in Kedougou.[27]

Limits in this study are also linked to its design, likely causing a cohort bias in visits separated by low-transmission periods. Lower *P falciparum* infection prevalence at T1 and T3 probably resulted from a natural clearance of parasites but also from treatment of RDT-positive individuals during T0 and T2 visits:

RDT-detected infections had higher parasitaemia and were more likely to persist.[25] We consider that cohort activities did not bias T2 infections and prevalence: cohort activities involved less than 25% of village populations and therefore are unlikely to have affected the village-level parasite reservoir. We conducted one engagement meeting with cohort households before each visit, which could have promoted more health-conscious attitudes among members. However, we did not observe a large difference in clinical malaria incidence: 375 cases per 1000 person-years at village level versus 309 cases per 1000 person-years in the cohort. We therefore assume that cohort participants and other villagers were exposed to similar transmission levels throughout the study.

Additionally, reliance on dry blood spot samples probably restricted the quantity of parasite material available and our ability to quantify densities lower than 2 parasites per µL. We did not include microscopy due to similar (or lower) performance than RDT in these settings, which implied only incremental sensitivity gains.[28,29]

In this study, we show that, after long-term deployment of control interventions, and specifically SMC, the historical understanding of the age distribution of parasites and of the infectious reservoir is no longer valid.[13,14] In Kedougou, SMC-eligible children younger than 10 years remain susceptible to severe malaria in case of infection, but they no longer bear the largest share of the clinical burden or parasite reservoir. Instead, *P falciparum* persists during the dry season at low prevalence across all age groups of 10 years and older; it expands during the wet season, particularly affecting individuals aged 10–24 years.

Individuals aged 10–19 years have higher parasite densities more frequently than individuals aged 20–24 years. Increasing malaria control after 2006 likely drives an age-specific history of exposure, which results in an increase over time of clinical burden and parasite density in older age groups (appendix 2 p 3).[30] Individuals aged 20 years or older were probably exposed to high levels of transmission during their childhood, with limited vector control and no SMC, which likely led them to build higher immunity levels earlier in life, resulting in lower burden. Individuals aged 15–19 years started benefiting from interventions in their childhood (bednets and partial SMC), and individuals aged 10–14 years grew up almost entirely under intensive interventions (bednets and SMC from age 3 months to 10 years);[31] their cumulative exposure is therefore likely lower than their older siblings at the same age, and they have a stronger clinical response.[30,31]

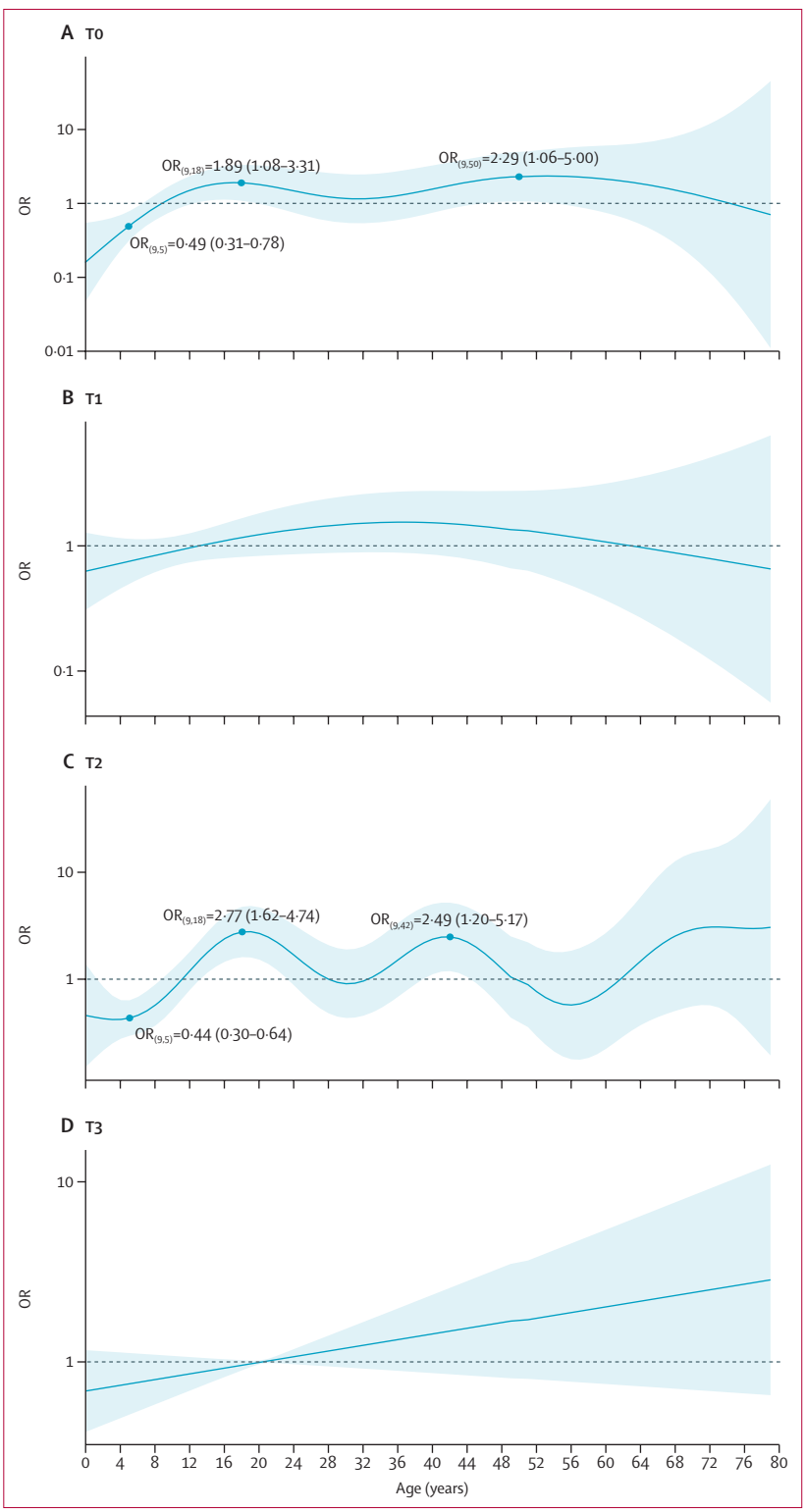


Figure 3: **Association between age and *Plasmodium falciparum* infection**
Non-linear association between age and *P falciparum* infection detected by PCR at T0 (A), T1 (B), T2 (C), and T3 (D); survey in multivariate analysis including sex, visit, and visit–age interaction, with a non-linear effect of age estimated separately for each visit (n=2426; table 2). Example ORs are provided for easier interpretation to see an effect of age, in which $OR_{(9,5)}$=0·49 indicates that, with reference to an age of 9 years, an age of 5 years is associated with a lower odds of *P falciparum* infection with an OR of 0·49. OR=odds ratio.

Our results also highlight that in a context of free, community-based access to diagnostics and treatment, adults aged 20 years or older present with frequent malaria episodes, suggesting that gratuity promotes the effective treatment of uncomplicated malaria in a large part of the population.[32]

There is a clear need for additional interventions addressing all plasmodium infections, not only in symptomatic individuals or those at high risk of severe outcomes. Any gain currently achieved on child morbidity and mortality depends on the annual repetition of SMC campaigns. These campaigns should not be reduced while active, intense parasite circulation persists in the rest of the population. In The Gambia, SMC uptake by eligible children was associated with a decrease in malaria risk among household members;[33] this study was, however, conducted in 2021, which was the first year when the Gambian National Malaria Control Programme extended SMC to children aged 5–9 years. Our results question whether this effect would persist when generations under SMC for 10 years grow older than the SMC age limit. Indeed, how children acquire immunity during and after SMC exposure and how they will contribute to the clinical burden as adults remains unknown. While artemisinin resistance emerges in Africa, the status quo is likely to yield a deteriorating situation. It is necessary to design transmission-reducing interventions that look beyond the reduction of the severity burden.

See **Online** for appendix 3

Bednet use was not associated with *P falciparum* infection in the wet season (T2). Yet, 90% of participants declared sleeping under a bednet during the wet season, and close to 50% during the dry, hot season. The intensity of indoors vector control in Kedougou, and the association of *P falciparum* carriage with outdoors night-time activities, suggest that outdoor transmission—already described in Kedougou 20 years ago—contributes substantially to malaria persistence.[34] While efficient outdoors vector control strategies remain under development, mass drug administration interventions are effective at short-term parasite reservoir depletion.[35] Short-term and long-term effect on transmission can already be evaluated.

In Sahelian countries, SMC targets more than 50 million children aged 5 years or younger, and extension up to age 10 years or school-age children is considered. In this study, we show that intensive control, including SMC, altered malaria epidemiology in a high burden region of Senegal. Yet, we also show persisting transmission in older, ineligible individuals. Involving all communities, with particular engagement of adolescents and young adults, will be crucial to address all *P falciparum* infections—irrespective of severity risk—and continue reducing transmission in the Sahel region of west Africa.

Contributors

JL, IS, JG, and E-HKCB designed the study, with contributions from CS and SR. JL, IS, E-HKCB, JG, MC, M-KB, AK, EL, and CL'O designed the methodological approach, study procedures, and tools. JL, E-HKCB, and EL supervised the operational project in Senegal and collected data with support from FD. CL'O, MM, and PS designed and conducted the PCR assays. JL and EL designed the statistical analysis plan and accessed and verified the data. EL conducted the analysis. JL and EL interpreted the findings. EL created the figures and wrote the first draft of the manuscript. All authors reviewed and revised the manuscript and approved this version of the Article for publication. All authors had access to the data and accept responsibility to submit for publication.

Equitable partnership declaration

The authors of this paper have submitted an equitable partnership declaration (appendix 3). This statement allows researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This statement is part of *The Lancet Global Health*'s broader goal to decolonise global health.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified individual participant data and corresponding data dictionaries are available with publication on reasonable request to the corresponding author via email and require approval from the principal investigators (E-HKCB, CS, and IS) via a data sharing agreement; a description of study objectives will be needed before requests are approved.

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