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Balotin Fogang, Lionel Lellouche, Sukai Ceesay, Sainabou Drammeh, Fatou Jaiteh, et al.. Asymptomatic *Plasmodium falciparum* carriage at the end of the dry season is associated with subsequent infection and clinical malaria in Eastern Gambia. *Malaria Journal*, 2024, 23 (1), pp.22. 10.1186/s12936-024-04836-y . hal-04795643

HAL Id: hal-04795643

<https://hal.science/hal-04795643v1>

Submitted on 21 Nov 2024

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RESEARCH

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Asymptomatic *Plasmodium falciparum* carriage at the end of the dry season is associated with subsequent infection and clinical malaria in Eastern Gambia

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Abstract

Background Chronic carriage of asymptomatic low-density *Plasmodium falciparum* parasitaemia in the dry season may support maintenance of acquired immunity that protects against clinical malaria. However, the relationship between chronic low-density infections and subsequent risk of clinical malaria episodes remains unclear.

Methods In a 2-years study (December 2014 to December 2016) in eastern Gambia, nine cross-sectional surveys using molecular parasite detection were performed in the dry and wet season. During the 2016 malaria transmission season, passive case detection identified episodes of clinical malaria.

Results Among the 5256 samples collected, 444 (8.4%) were positive for *P. falciparum*. A multivariate model identified village of residence, male sex, age ≥ 5 years old, anaemia, and fever as independent factors associated with *P. falciparum* parasite carriage. Infections did not cluster over time within the same households or recurred among neighbouring households. Asymptomatic parasite carriage at the end of dry season was associated with a higher risk of infection (Hazard Ratio, HR = 3.0, $p < 0.0001$) and clinical malaria (HR = 1.561, $p = 0.057$) during the following transmission season. Age and village of residence were additional predictors of infection and clinical malaria during the transmission season.

Conclusion Chronic parasite carriage during the dry season is associated with an increased risk of malaria infection and clinical malaria. It is unclear whether this is due to environmental exposure or to other factors.

Keywords *Plasmodium falciparum*, Asymptomatic, Clinical malaria, Seasonal transmission

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Background

Malaria continues to be a significant public health challenge in several parts of the world, where it is responsible for a high burden of morbidity and mortality [1, 2]. Despite the impact of standard control interventions, asymptomatic *Plasmodium falciparum* infections continue to pose a challenge to control efforts as they maintain residual transmission [3]. Where malaria transmission is seasonal, most cases of clinical malaria occur between July and December, a period covering the seasonal rains and the 2 months immediately following them, with a peak in cases in November, while the dry season (January–June) is a period of low transmission, with mainly asymptomatic chronic infections acquired during the previous transmission season [4, 5].

As malaria transmission has declined considerably in The Gambia [1], thanks to wide scale-up of vector control interventions, the prevalence of infection has become increasingly heterogeneous [6–8]. To sustain the decline in malaria transmission and move towards elimination, there is a need to evaluate and improve existing community-based interventions through ongoing epidemiological surveillance and identification of associated factors.

A large proportion of asymptomatic individuals at the end of the transmission season remains infected for several months, from January to June [9]. However, the relationship between these chronic infections and the risk of infection and clinical malaria during the following transmission season remains unclear, with conflicting information; some studies report a protective effect [10–13] while others a higher risk of clinical malaria [14–17] or no consistent association [6, 18]. Such differences may be influenced by the age of the participants [17, 18], the intensity of malaria transmission [6, 18] and the number of circulating *P. falciparum* clones [13, 14, 19]. Importantly, variation in parasite exposure may also play a role.

Here, to characterize the dynamics of malaria transmission and identify associated risk factors, a total of 9 cross-sectional surveys were conducted during the dry and wet seasons, starting in December 2014. From July 2016, a longitudinal follow-up of treated asymptomatic and non-infected participants with passive *P. falciparum* detection was carried out to assess the impact of asymptomatic parasitaemia on subsequent infection and clinical malaria during the transmission season.

Methods

Ethical considerations

The study protocol was reviewed and approved by the Gambia Government/MRC Joint Ethics Committee (SCC 1476, SCC 1318, L2015.50) and by the London School of Hygiene & Tropical Medicine ethics committee (Ref. 10982). The field studies were also approved by local

administrative representatives, the village chiefs. Written informed consent was obtained from participants over 18 years old and from parents/guardians for participants under 18 years. Written assent was obtained from all individuals aged 12–17 years.

Study design and population

The study was carried out in four villages (Madina Samako: K, Njayel: J, Sendebu: P, and Karandaba: N) in Upper River Region (URR), eastern Gambia (Fig. 1A) [9, 20]. *Plasmodium falciparum* in the area is heterogeneous, ranging from 21 to 49% by PCR in November 2012 [20]. Seasonal Malaria Chemoprevention (SMC) is offered to all children aged 3 to 59 months each September, October and November. Proportion of individuals in rural villages sleeping under bed net amongst clinic attendees reaches 84.8% [6].

Over a 2-year period, two cohorts of individuals at least 2 years old were recruited; one cohort was followed up between December 2014 and March 2016, and the other between July and December 2016 (Fig. 1B). All households were invited to participate in the study. The number of participants per household ranged from 1 to 79, with a median of 7 (Q1, Q3 4, 11). Five surveys covering the end of the transmission season (December 2014 and November and December 2015) and the following dry season (April 2015 and March 2016) were carried out in the first cohort in two-villages (J and K) (Fig. 1B). Surveys on the second cohort were carried out at the end of the 2016 dry season (July) and during the transmission season (October, November, and December) in the four study villages (Fig. 1B). During the surveys, structured questionnaires were used to record individual information (village, household, age, sex, bed net use, history of fever, temperature) from all participants. All sampled individuals who tested positive for *P. falciparum* by qPCR were treated with artemether-lumefantrine. Moreover, households in the villages J and K were geolocated using a global positioning system (GPS). During the 2016 transmission season, a resident nurse delivered complimentary basic healthcare to all participants. Suspected malaria cases (axillary temperature > 37.5 °C) were tested with a Rapid Diagnostic Test (CareStart) and, if positive, treated with artemether-lumefantrine.

Sample collection and malaria diagnostic

At each survey, approximately 200 µL of blood was collected by fingerprick into an EDTA-coated tube. A few drops were used for a thick blood film and dry blood spots on filter paper (Whatman 3 Corporation, Florham Park, NJ, USA). Haemoglobin (Hb) was measured by HaemoCue 301 machine. Blood samples from EDTA tubes were processed on the same day for leucodepletion

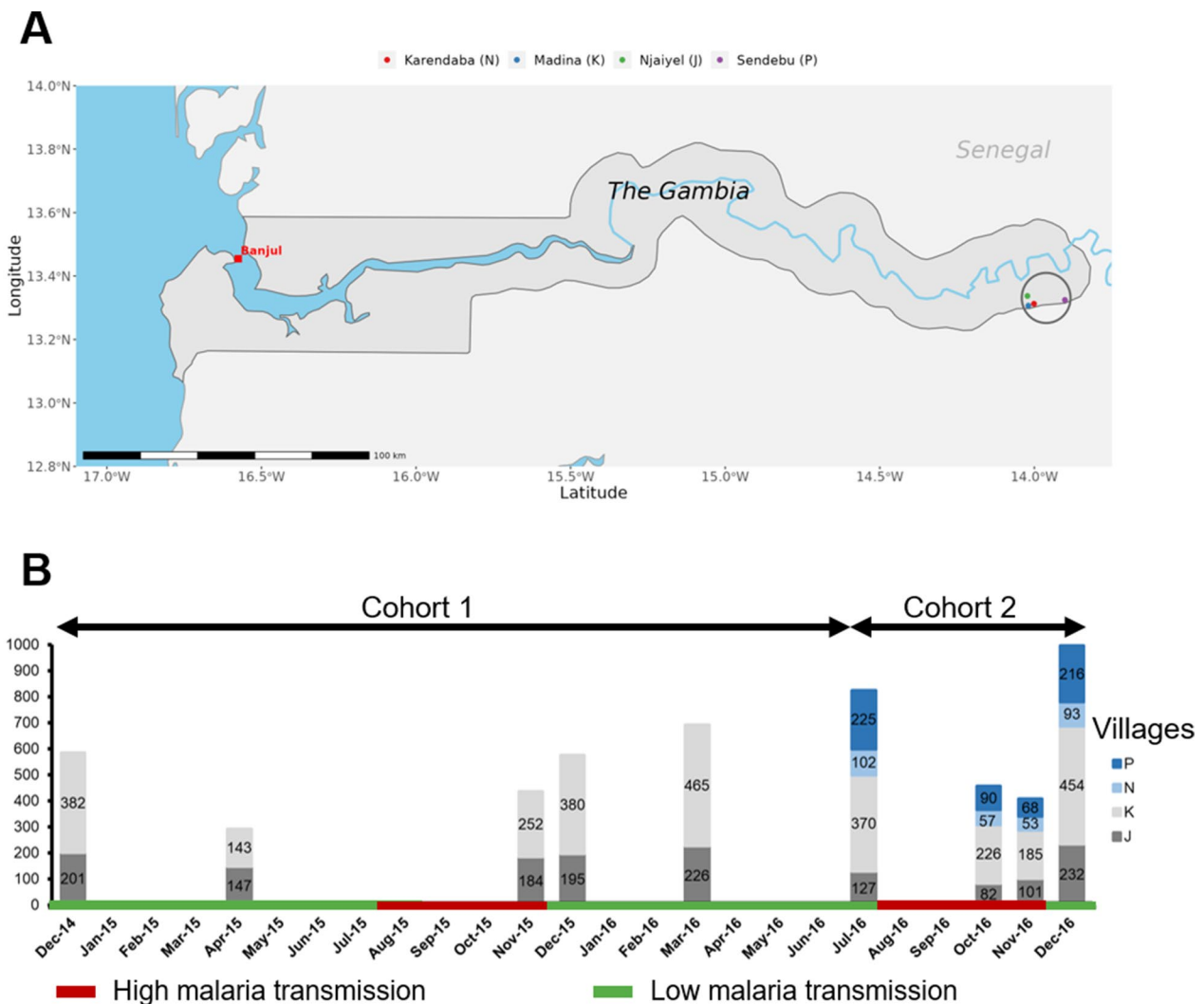


Fig. 1 Study site and design. **A** Map of The Gambia with the study site (4 villages) circled. Made with Natural Earth. **B** Presentation of the two cohorts and the 2-year sampling period in the four villages. The bar charts show the cumulative number of fingerprick samples per timepoint for each village. The green colour represents the low malaria transmission season and the red colour represents the high transmission malaria season in The Gambia. The colours in the bars indicate village of residence (P=blue, N=light blue, K=light grey, J=grey)

using a cellulose-based column (MN2100ff, similar to CF11) [21].

Thick blood films were stained with a 10% Giemsa solution for 10 min and parasite density estimated by counting the number of parasites per at least 200 white blood cells (WBC) and assuming 8000 WBC/ μ L. Slides were considered negative if no parasites were detected by two microscopists after reading 500 WBC. A 20% error check was used to identify discrepancies between slide readers. All discordant results were read by a senior microscopist whose results were taken as final.

For samples collected between December 2014 and March 2016, DNA extracted from leukocyte-depleted red blood cells using the QIAgen MiniPrep kit was analysed

by nested PCR targeting *P. falciparum* 18S small subunit rRNA (ssRNA) genes [22].

For samples collected from July to December 2016, DNA was extracted from dry blood spot (DBS) by Chelex method [23] and analysed by 18S small subunit of ribosomal RNA (rRNA) qPCR and *var* gene acid terminal sequence (*varATS*) qPCR [24], as detailed in Additional file 1: Fig. S1.

Statistical analysis

Data were collected on paper-based case report forms (CRFs), double entered in Studytrax (© Sciencetrax LLC, USA), and checked for errors and discrepancies before statistical analysis. Statistical analyses were

performed using R studio version 4.2.3 [25]. Age was categorized into three groups: < 5 years, 5–14 years and ≥ 15 years. Anaemia was classified as mild, moderate or severe [26] (Additional file 1: Table S1). Comparisons of proportions between groups were performed using the chi-square χ^2 test; Spearman's correlation test was used to test the association between quantitative variables. 95% confidence intervals for prevalence were calculated using standard error based on Wilson Score Interval.

A multivariate mixed-effects logistic regression model, implemented with the R lme4 package, was used to determine factors independently associated with qPCR-detected *P. falciparum* infection. This modelling approach incorporates both fixed and random effects, making it suitable for analysing data with repeated or clustered observations. The mixed-effects logistic regression model assumes that observations within groups or clusters defined by random effects must be independent, and there should be no perfect separation in the data. In this model all fixed-effect variables were adjusted for clustering at different months of data collection (time of sampling), at the household level, and at the individual level, with the individual level nested within the household level. This adjustment helps mitigate potential biases stemming from temporal and household-level variability. The predictive factors (fixed-effects) included villages, age, sex, anaemia, history of fever in the past week and fever at inclusion (temperature > 37.5 °C). For this analysis, only individuals in the second cohort were included as complete epidemiological information for the first cohort was not available.

To assess the risk factors associated with subsequent infection or the first clinical malaria episode during the transmission season, both univariate and multivariate shared frailty mixed-effects survival models were applied. These models were implemented using the R survival and coxme packages. They integrate components of survival analysis and mixed-effects modelling, taking into consideration the inherent clustering or interdependence of observations within groups or clusters. The explained variables in separated models included the occurrence of infection or clinical malaria during the transmission season. In these models, individuals were clustered according to the timing of reinfection or the onset of clinical malaria, with each interval spanning 1 month. The random effect in the model pertained to households, and the covariates included the infection status at the end of dry season, villages, age, sex, anaemia status, history of fever in the past week, fever at inclusion (temperature > 37.5 °C). Kaplan–Meier survival curves were used to visualize the progression of malaria incidence or clinical cases during the transmission season.

Spatial analysis of clusters of *Plasmodium* infections (hotspot) was performed using the Bernoulli method in SaTScan version 10.1.2 [27]. In all analyses, P values < 0.05 were considered statistically significant. All the graphs were performed using R studio.

Results

Characteristics of the study cohorts

Over a period of 2 years, 5256 blood samples were collected from 1471 individuals aged 2 to 85 years (Fig. 1). The first cohort included 1003 participants and the second cohort 1284 participants, with 789 of them in both cohorts (Table 1). 46.3% (458) of cohort 1 were males and the median age was 11 years (Q1, Q3 5.5, 27). In the cohort 2, the median age was 12 years (Q1, Q3 6, 29), with more females than males. Anaemia prevalence was 42.8%, mostly mild (22.5%) and moderate (18.9%), with few cases of severe anaemia (1.4%) (Table 1). Fever at inclusion was rare (0.8%). Bed net use was high (76.4%).

Plasmodium falciparum prevalence in cohort 1

Between December 2014 and March 2016, 155 (6%) of 2575 samples collected were positive. Prevalence varied substantially over time, between 1.7 and 5.4% during the dry season and up to 14.4% during the transmission season (Additional file 1: Fig. S2). Children under 5 years of age were less likely to be infected (Additional file 1: Fig. S2). Parasite prevalence in older children and adults was similar and peaked towards the end of the transmission season (Additional file 1: Fig. S2).

VarATS qPCR is more sensitive than 18S qPCR and microscopy

To increase the sensitivity of the *P. falciparum* detection relative to microscopy, two quantitative PCR approaches targeting the 18S locus of the conserved small subunit of ribosomal RNA (18S rRNA, 5–8 copies/genomes) and the acidic terminal sequence of the *var* gene (*varATS*, ~60 copies/genomes) were evaluated using a set of samples from cohort 2. Using serial dilutions of the laboratory-adapted *P. falciparum* 3D7 strain to determine analytical sensitivity, *varATS* reached the limit of detection (LOD) of 0.22 parasites/ μ L of blood and was around 10 times more sensitive than standard 18S rRNA qPCR with the LOD of 2.2 parasites/ μ L (Additional file 1: Fig. S1). Out of 1202 fingerprick samples from this cohort screened by the 3 techniques, 11.7% (141), 10.1% (121) and 3.9% (47) samples were positive for *P. falciparum* using *varATS*, 18S rRNA qPCR and light microscopy, respectively (Fig. 2A). Of 166 *Plasmodium* infections detected by the three diagnostic tests combined, 18.9% (35) were only detected by *varATS*, while 10.2% (17) ($p=0.0066$) and 4.2% (7) ($p<0.0001$) were detected only by 18S rRNA

Table 1 Characteristics of the study population

Characteristics	Cohort 1: December 2014–March 2016, % (n/N)	Cohort 2: July 2016–December 2016, % (n/N)
Male sex	46.3 (458/989)	44.1 (565/1280)
Villages		
J	33.4 (335/1003)	20.6 (264/1284)
K	66.6 (668/1003)	48.4 (622/1284)
N	–	11.1 (142/1284)
P	–	19.9 (256/1284)
Age groups (years)		
< 5	22.1 (219/1003)	16.2 (207/1280)
5–14	33.9 (335/1003)	39.3 (503/1280)
> 15	44.0 (435/1003)	44.5 (570/1280)
Anaemia ^a		
Non-anaemic	–	57.2 (688/1203)
Mild	–	22.5 (271/1203)
Moderate	–	18.9 (227/1203)
Severe	–	1.4 (17/1203)
Slept under a LLIN last night	–	76.4 (916/1195)
History of fever in the past week	–	4.0 (48/1195)
Fever at inclusion	–	0.8 (10/860)

LLIN long-lasting insecticidal net; –: not determined

^a Anaemia is classified based on age and sex (WHO, 2011)

qPCR and microscopy, respectively (Fig. 2B and Additional file 1: Fig. S3). There was a high level of agreement between varATS and 18S rRNA qPCR, with 95.3% (1146 varATS+/18SRNA+ or varATS–/18SRNA–) samples with the same results for both methods (Additional file 1: Fig. S3). Parasite densities determined by varATS and 18S rRNA qPCR correlated strongly with parasitaemia quantified by microscopy ($r=0.76$, $P<0.0001$ and $r=0.86$, $P<0.0001$ for VarATS and 18S rRNA qPCR, respectively), as well as with each other ($r=0.94$, $P<0.0001$) (Fig. 2C). Because of the high concordance between the two molecular methods varATS and 18S rRNA qPCR, results from the two molecular *Plasmodium* detection tools were combined in a single analysis.

***Plasmodium falciparum* prevalence in cohort 2**

Plasmodium falciparum prevalence was 9.3% (77/824) in July, 9.7% (44/455) in October, 20.6% (84/407) in November, and 12.9% (128/995) in December. This trend was observed in all age groups (Fig. 3A). As shown in Fig. 3A, children under 5 years of age had the lowest prevalence while older children the highest prevalence (Fig. 3A). Bed net usage was high in all age groups (81% in <5, 74% in 5–14, 77% in ≥ 15 years). Median varATS parasitaemia was usually higher in the older children than adults and young children (5–14 vs <5 years, $p=0.1553$; 5–14 vs ≥ 15 years, $p<0.0001$), but not in all surveys (Fig. 3B).

Risk factors associated with *P. falciparum* infection

A multivariate mixed-effects logistic regression model shows that sex, age, anaemia, fever at inclusion, and village of residence were independent factors associated with *P. falciparum* infections (Table 2). Indeed, risk of infection was threefold higher (OR=3.134, $p<0.0001$) in older children and 2.5-fold higher (OR=2.616, $p<0.0001$) in adults than in young children, likely indicating the effect of SMC in this age group.

Residents of villages K and P were more at risk of infection than those of village N (OR=1.848, $p=0.0236$ and OR=3.499, $p<0.0001$ for villages K and P, respectively). Individuals with different degree of anaemia had higher odds of being infected by *P. falciparum* compared to non-anaemic individuals (Table 2). Fever at inclusion was strongly associated with infection (OR=4.374, $p=0.007$).

As malaria prevalence varied substantially between and within villages, the analysis investigated whether some households were hotspots of malaria transmission. Analyses focused on villages J and K, as data are available over a 2-year study period. By utilizing the Bernoulli spatial scan statistic in Satscan algorithm, two clusters in village J with a high prevalence of *P. falciparum* infections were identified (Additional file 1: Table S2). Similarly, in village K, three clusters of high *Plasmodium falciparum* infections at two different times (November and December 2016) were identified ($P<0.05$) (Additional file 1:

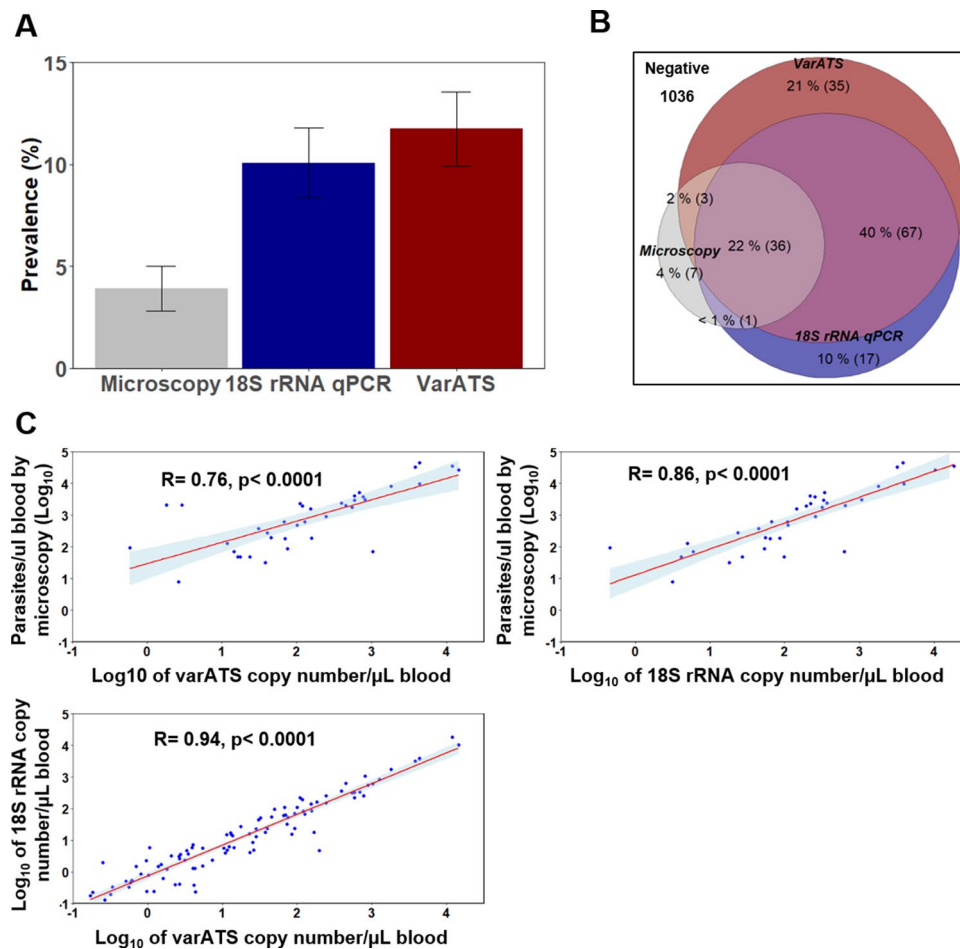


Fig. 2 *Plasmodium* detection by different diagnostic tools. **A** Proportion of *P. falciparum* detected with different diagnostic methods. **B** Venn diagram of *Plasmodium* positivity by varATS, 18S rRNA qPCR and/or microscopy. **C** Correlation in parasitaemia between diagnostic tools. Error bars in **A** correspond to 95% confidence interval for estimated prevalence. Spearman rank correlation (**C**) is presented as the best fit line and the coefficient (r), as well as the p -value (p), are shown for each correlation pair between parasitaemia. LM light microscopy, qPCR quantitative polymerase chain reaction, rRNA ribosomal ribonucleic acid, ATS acidic terminal segment

Table S2). However, when *P. falciparum* prevalence at the household level in the two villages was plotted over time, no evidence of clustering of infection over time within the same households or recurrent infections among neighbouring households was found (Additional file 1: Fig. S4). This suggests that transmission is stochastic, with no evidence of sustained malaria hotspots in these villages.

Asymptomatic parasitaemia at the end of dry season is associated with infection and clinical malaria during the following transmission season

The individual risk of being infected during the transmission season according to the infection status at the beginning of the transmission season was investigated (Fig. 4 and Table 3). In July 2016, 67 individuals were asymptomatic carriers and treated with artesunate-lumefantrine

anti-malarials; 68.7% (46) of them were re-infected during the transmission season. Among the 613 individuals non-infected in July 2016, 28.5% (175) of them became infected during the same period (HR=3.338, $p < 0.0001$) (Fig. 4 and Table 3). The association between infection status and risk of infection during the transmission season remains significant after adjustment for age and village (HR=3.0, $p < 0.0001$) (Table 3). Multivariate analysis shows that age and village of residence were independently associated with risk of infection (HR=2.767, $p < 0.0001$ for age 5–14 years, HR=3.063, $p < 0.0001$ for age ≥ 15 years and HR=2.375, $p = 0.0012$ for village P).

Individuals with a malaria parasite infection in July 2016 had a twofold higher risk of experiencing a clinical malaria episode than those without infection (HR=1.825, $p = 0.0086$); this effect persisted after adjusting for age, village and anaemia (HR=1.561, $p = 0.057$)

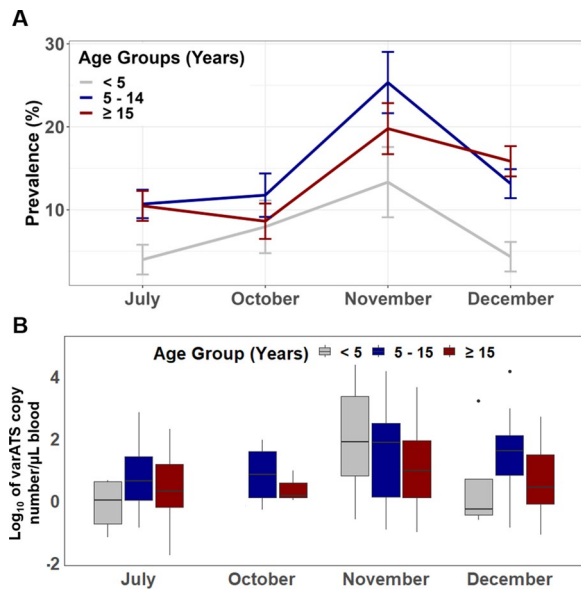


Fig. 3 Trend analysis for *P. falciparum* infection prevalence (A) and parasitaemia (B) by age groups in the second cohort (2016). In A, error bars represent 95% confidence intervals for prevalence. Box in B plots representing interquartile ranges (25th, median and 75th percentile, whisker percentile (1st and 99th)). B Parasite density by varATS was not available for October in the <5 year group

(Table 4). Additionally, age and villages were also independent predicting factors for clinical malaria during the transmission season (HR=3.404, p=0.0003, HR=3.951, p<0.0001 and HR=2.717, p=0.0054 for age 5–14 years, age ≥ 15 years and village P, respectively). Together, these data indicate that asymptomatic carriage of parasites during the dry season is associated with an increased risk of infection and clinical malaria during the following transmission season.

Discussion

In The Gambia, there is a need to evaluate and improve existing community-based interventions through ongoing epidemiological surveillance and the identification of risk factor for parasite carriage. The dynamics and the factors associated with *P. falciparum* infections, and the link between asymptomatic carriage before the transmission season and risk of infection and clinical malaria were investigated over 2 years.

Consistent with previous studies in The Gambia [6, 13, 20], the prevalence of infection was lower in children under 5 years of age than in older children and adults, possibly due to the seasonal malaria chemoprophylaxis programme (monthly antimalarial treatment

Table 2 Assessment of associated risk factors of *P. falciparum* infection

Factors	Description (n)	% infections (n)	OR [95% CI]	P value
Sex	F (1513)	11.4 (172)	Ref.	
	M (1162)	13.9 (162)	1.336 [1.031–1.731]	0.0280
Age groups (years)	< 5 (474)	6.33 (30)	Ref.	
	5–14 (1078)	13.8 (149)	3.134 [2.041–4.813]	<0.0001
	≥ 15 (1123)	13.7 (154)	2.616 [1.697–4.032]	<0.0001
Village of residence	J (542)	10.3 (56)	1.616 [0.895–2.916]	0.1110
	K (1235)	11.9 (147)	1.848 [1.085–3.146]	0.0236
	N (305)	7.9 (24)	Ref.	
	P (599)	17.7 (106)	3.499 [2.026–6.043]	<0.0001
History of fever in the past week	Yes (131)	14.5 (19)	1.021 [0.590–1.767]	0.9382
	No (2306)	12.49 (288)	Ref.	
Anaemia ^a	Non-anaemic (1390)	10.6 (147)	Ref.	
	Mild (550)	13.6 (75)	1.577 [1.155–2.155]	0.0042
	Moderate (493)	16.2 (80)	1.759 [1.294–2.392]	0.0003
	Severe (37)	29.7 (11)	3.942 [1.818–8.548]	0.0005
Fever at inclusion	Yes (19)	31.6 (6)	4.374 [1.496–12.78]	0.0070
	No (2446)	12.6 (307)	Ref.	

Only individuals from the second cohort (N=2681) were included in this multivariate mixed-effects logistic regression model because complete epidemiological information were only available for these individuals. In this model all fixed-effect variables were adjusted for clustering at different months of data collection (time of sampling), at the household level, and at the individual level, with the individual level nested within the household level

Significant estimates are bolded

OR odds ratio, CI confident interval, Ref. reference

^a Anaemia determined by age and sex according to the WHO 2011 report

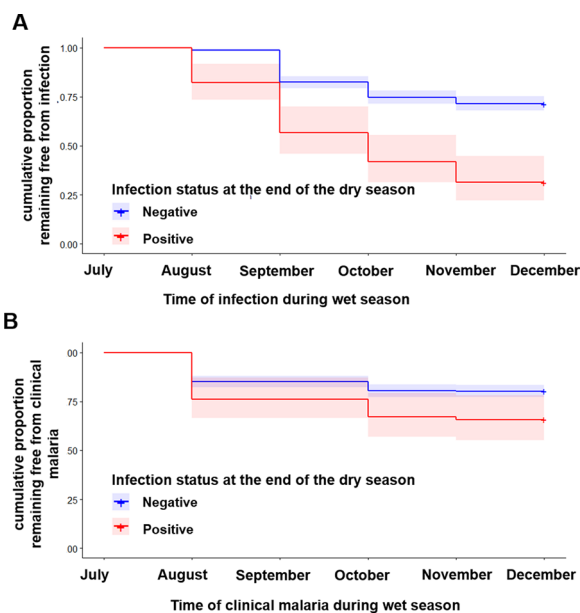


Fig. 4 Asymptomatic *P. falciparum* carriage at the end of dry season and during the subsequent transmission season. Being carrier in the dry season increases the risk of both *P. falciparum* infection (A) and malaria symptoms in the following transmission season (B). Time to infection and/or onset of clinical malaria were grouped into a 1-month interval

during the transmission season) [28, 29]. The higher prevalence of infection and high parasite densities in older children than in adults may be the result of acquired immunity that makes adults better protected and able to better control parasite growth when infected [30]. Furthermore, in line with acquired immunity with age, when infected during the transmission season, children under 5 years old had a higher parasite density compared to adults. Individuals with different degrees of anaemia were more likely to be infected with *P. falciparum* than non-anaemic individuals. This observation appears to be the consequence of *Plasmodium* infection rather than the cause of infection, given that malaria is known to be the main cause of anaemia in endemic areas [31, 32].

There was significant variability in malaria prevalence between villages. These findings demonstrate the heterogeneity of malaria transmission in the eastern Gambia, even among neighbouring villages. Similarly, in a previous study [20], significant variation in malaria prevalence in this region was observed, ranging from 21 to 49%. This discrepancy was attributed not only to the observed higher insecticide resistance [33, 34], but also to differences in vector composition. Indeed, *Anopheles gambiae*

sensu stricto (s.s.) is more common in villages away from The Gambia river, while *Anopheles arabiensis* and *Anopheles coluzzii* are more widespread near the river [34].

Due to the variation in malaria prevalence among neighbouring villages, there is a hypothesis regarding the presence of sustained malaria transmission hotspots within the villages, where certain households, based on the characteristics of the individuals and their environment, could sustain transmission in the area. Although some households exhibited significantly high malaria prevalence within villages, these households were not consistently the same, with no evidence of hotspot households spreading malaria, suggesting that the transmission pattern follows a stochastic pattern in the area [35]. Therefore, a targeted malaria prevention approach focusing households with highest prevalence is unlikely to yield a lasting effect in this area. However, treating all malaria cases identified in cohort 2 may have altered spatio-temporal patterns.

Asymptomatic carriage of *P. falciparum* at the end of the dry season was associated with both an increased risk of infection and clinical malaria during the following transmission season. The treatment given to detected asymptomatic individuals during the second cohort might have induced a local reduction of transmission, however this reduction would apply homogeneously across the population. Consistent with these results, studies in seasonal malaria transmission areas where the dry season lasts for ~6–8 months showed that asymptomatic *P. falciparum* infections at the end of dry season are associated with the increase risk of the infection [16, 36] or clinical malaria during the following transmission season [14–17, 37]. The main hypothesis is that although individuals were treated at the end of the dry season, they continued to reside in the same environment where they were initially infected, with potentially higher risk of being re-infected. Nevertheless, individuals testing negative at the end of the dry season and being able to control new infections without developing symptoms suggests a fairly effective immunity and the ability to suppress parasites below detection levels. However, this differs from other reports which show that asymptomatic *P. falciparum* infections at the end of the dry season were associated with a reduced risk of clinical malaria in the following transmission season [11–13, 36, 38]. Chronic infections and the maintenance of immunity could protect against high parasitaemia and clinical malaria [39, 40]. The discrepancy between asymptomatic infections being a protection or a risk for reinfection/clinical malaria

Table 3 Predicted hazard of *P. falciparum* infection during the malaria transmission season

Factors	Description (n)	% infections (n)	Univariate analysis		Multivariate analysis	
			HR [95% CI]	P value	HR [95% CI]	P value
Asymptomatic infection at the end of dry season	Yes (67)	68.7 (46)	3.388 [2.433–4.718]	< 0.0001	3.0 [2.149–4.190]	< 0.0001
	No (613)	28.5 (175)	Ref.		Ref.	
Sex	F (383)	31.3 (120)	1.155 [0.884–1.509]	0.29		
	M (295)	34.2 (101)	Ref.			
Age groups (years)	< 5 (129)	15.5 (20)	Ref.		Ref.	
	5–14 (296)	33.8 (100)	2.621 [1.617–4.245]	< 0.0001	2.767 [1.704–4.493]	< 0.0001
	≥ 15 (253)	39.9 (101)	2.917 [1.801–4.724]	< 0.0001	3.063 [1.888–4.970]	< 0.0001
Village of residence	J (107)	30.8 (33)	1.332 [0.748–2.373]	0.33	1.571 [0.870–2.837]	0.13
	K (304)	25.7 (78)	1.05 [0.626–1.760]	0.85	1.084 [0.637–1.845]	0.76
	N (73)	24.7 (18)	Ref.			
	P (196)	46.9 (92)	2.255 [1.355–3.751]	0.0017	2.375 [1.407–4.007]	0.0012
History of fever in the past week	Yes (21)	38.1 (8)	1.253 [0.608–2.582]	0.54		
	No (647)	31.7 (205)	Ref.			
Anaemia ^a	No (370)	32.7 (121)	Ref.			
	Mild (155)	29.0 (45)	0.861 [0.610–1.217]	0.40		
	Moderate (135)	33.3 (45)	1.051 [0.743–1.485]	0.78		
	Severe (10)	40.0 (4)	1.549 [0.567–4.235]	0.39		
Fever at inclusion	Yes (7)	14.3 (1)	0.320 [0.044–2.310]	0.326		
	No (663)	32.3 (214)	Ref.			

In this analysis only individuals with known infection status during the follow-up period were included (data on active or passive diagnosis of infection at least once during the follow-up period)

Significant estimates are bolded

HR hazard ratio, CI confident interval, Ref. reference

Only factors associated with infection in the univariate analysis ($p < 0.05$) were included in the multivariate analysis

^a Anaemia determined by age and sex according to the OMS 2011 report (OMS, 2011)

could be explained by the large range of malaria transmission intensity between the cited studies, as well as local environmental conditions. For example, a recent study conducted in The Gambia revealed that in the semi-urban villages dry season carriage was associated with an increased risk of clinical malaria during the following transmission season, whereas such risk was lower in rural villages [6]. Finally, a pooled analysis also demonstrated the importance of transmission intensity with risks of subsequent clinical malaria, as well as host age and polyclonality of infection [19], although this meta-analysis encompassed studies that evaluated clinical infections within the same asymptomatic infection.

Conclusion

In conclusion, this 2-years study identified village of residence, anaemia, and fever at enrolment as independent factors associated with *P. falciparum* infections in the eastern Gambia. *P. falciparum* transmission pattern seems stochastic. The data further validate the notion that asymptomatic parasitaemia carriage at the end of dry season correlates with a higher risk of infection and clinical malaria during the following transmission season. Therefore, targeting asymptomatic infections during the dry season may reduce the risk of clinical malaria in the following transmission season.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12936-024-04836-y>.

Table 4 Predicted hazard of clinical malaria during the malaria transmission season

Factors	Description (n)	% symptomatic infections (n)	Univariate analysis		Multivariate analysis	
			HR [95% CI]	P value	HR [95% CI]	P value
Asymptomatic infection at the end of dry season	Yes (67)	34.3 (23)	1.826 [1.165–2.861]	0.0086	1.561 [0.988–2.467]	0.057
	No (241)	19.7 (121)	Ref.		Ref.	
Sex	F (383)	19.6 (73)	1.306 [0.941–1.815]	0.11		
	M (295)	24.1 (71)	Ref.			
Age groups (years)	< 5 (129)	7.7 (10)	Ref.		Ref.	
	5–14 (296)	23.6 (70)	3.404 [1.751–6.617]	0.0003	3.404 [1.746–6.638]	0.0003
	≥ 15 (253)	25.5 (64)	3.656 [1.751–7.131]	0.0001	3.951 [2.024–7.714]	< 0.0001
Village of residence	J (107)	23.4 (25)	2.024 [0.945–4.337]	0.07	2.115 [0.980–4.562]	0.056
	K (304)	16.4 (20)	1.347 [0.663–2.740]	0.41	1.299 [0.636–2.651]	0.47
	N (73)	12.3 (9)	Ref.		Ref.	
	P (196)	30.6 (60)	2.721 [1.350–5.484]	0.0051	1.218 [0.966–1.536]	0.095
History of fever in the past week	Yes (21)	19.0 (4)	0.889 [0.325–2.435]	0.82	2.717 [1.344–5.494]	0.0054
	No (647)	27.7 (134)	Ref.			
Anaemia ^a	No (370)	23.2 (86)	1.672 [1.044–2.677]	0.032	1.744 [1.187–2.797]	0.0231
	Mild (155)	14.2 (22)	Ref.		Ref.	
	Moderate (135)	22.2 (30)	1.619 [0.932–2.813]	0.087	1.620 [0.933–2.812]	0.087
	Severe (10)	20.0 (2)	1.459 [0.341–6.231]	0.61	1.335 [0.312–5.723]	0.07
Fever at inclusion	Yes (7)	14.3 (1)	0.535 [0.074–3.872]	0.676		
	No (663)	21.0 (139)	Ref.			

In this analysis only individuals with known infection status during the follow-up period were included (data on active or passive diagnosis of infection at least once during the follow-up period)

Significant estimates are bolded

HR hazard ratio, CI confident interval, Ref. reference

Only factors associated with symptomatic infections in the univariate analysis ($p < 0.05$) were included in the multivariate analysis

^a Anaemia determined by age and sex according to the OMS 2011 report (OMS, 2011)

Additional file 1: Figure S1. varATS and 18S RNA standard curves using 3D7 gDNA. **Figure S2.** Trend analysis for *P. falciparum* infection prevalence by age groups in the first cohort, by nested PCR. **Figure S3.** Parasites prevalence by microscopy according to parasite densities measured by varATS (A) and 18S rRNA qPCR (B) molecular tools. **Figure S4.** Spatio-temporal *P. falciparum* prevalence by household in villages J and K. **Table S1.** Hemoglobin levels (g/dL) and classification of anaemia (WHO, 2011). **Table S2.** Household-based assessment of *P. falciparum* infection clusters using Bernoulli spatial scan statistic.

Acknowledgements

Our thanks go to Julia Mwesigwa and all fieldworkers involved in the study. We would like to thank the inhabitants of the four villages (Madina Samako, Njayel, Sendebu, and Karanbada) in eastern part of The Gambia who took part in this study.

Author contributions

Conceptualization by AC and TB; field work and sample processing by SC, SD, FJ, CH, JF. Data analysis by BF, LL, MAG. Writing of original draft by BF. Reviewing and editing of manuscript by BF, MAG, CH, JL, DC, UDA, TB, AC. Funding acquisition by AC and TB.

Funding

This work was funded by grants from the Netherlands Organization for Scientific Research (Vidi fellowship NWO 016.158.306) and the Bill & Melinda Gates Foundation (INDIE OPP1173572), the joint MRC/LSHTM fellowship, CNRS, the French National Research Agency (18-CE15-0009-01). The "SPF-Post doc en

France" programme of the "Fondation pour la Recherche Médicale" funded the author "Balotin Fogang" (SPF202209015889).

Data availability

All anonymised data is available upon request.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Gambia Government/MRC Joint Ethics Committee (SCC 1476, SCC 1318, L2015.50) and by the London School of Hygiene & Tropical Medicine ethics committee (Ref 10982). The field studies were also approved by local administrative representatives, the village chiefs. Written informed consent was obtained from participants over 18 years old and from parents/guardians for participants under 18 years. Written assent was obtained from all individuals aged 12–17 years.

Competing interests

No conflicts of interest were reported by the authors.

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Received: 2 October 2023 Accepted: 3 January 2024

Published online: 17 January 2024

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