

Additional Figures and Table

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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Additional Figures

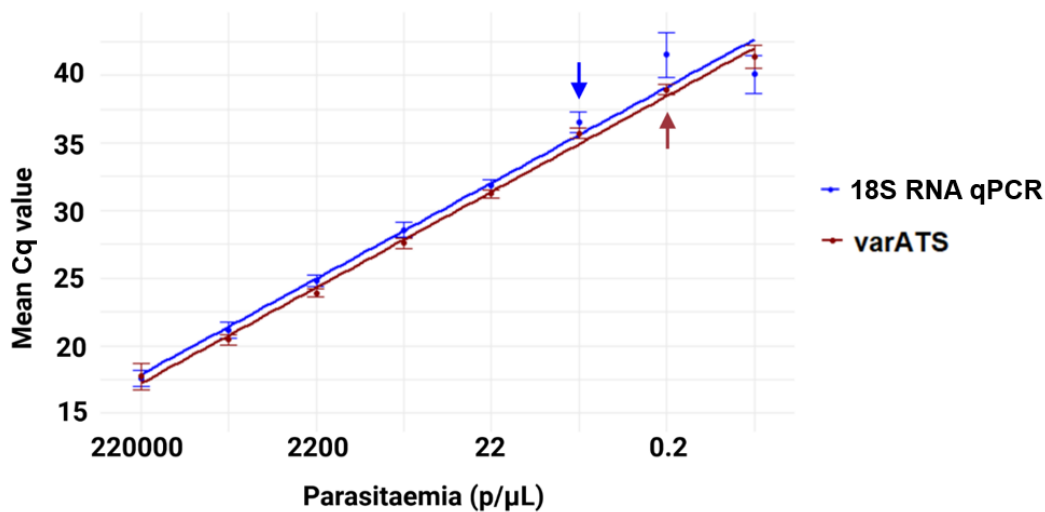


Figure S1. varATS and 18S RNA standard curves using 3D7 gDNA. The scatter plot represents the mean Cq value of the amplification of 8 replicates of the 10-fold serial dilution of 220000 parasites/ μ L of ring stage lab adapted *P. falciparum* 3D7 strain. The arrows indicate the detection limits of the two tests (2.2 parasites/ μ L for 18S RNA qPCR and 0.2 for varATS). Data were generated with the following method: Ring stage laboratory-adapted *P. falciparum* strain 3D7 was used to determine analytical sensitivity and parasitemia by 10-fold dilution of 220000 parasites/ μ L of parasitemia in 50% haematocrit from an uninfected blood donor [24]. Dilutions were prepared as a DBS and genomic DNA was extracted as described above. Each DNA sample was tested in duplicate (field isolates and 3D7 strain) and the quantification curve was made from the 3D7 standard dilution. Cut-off values for positivity for 18S rRNA qPCR and varATS were dilutions 6 (parasitaemia of 2.2 parasites/ μ L) and 7 (parasitaemia of 0.22 parasites/ μ L), respectively. Samples were defined as *P. falciparum* positive if the Ct value was less than or equal to the Ct of the cut-off. Samples that exhibited amplification for both duplicates and had a visually clear amplification curve were considered positive if their CQ value exceeded dilution 7 but remained below the upper limit of the 95% confidence interval for this parasitemia. Parasite densities were determined using standard curves generated from 3D7 genomic DNA amplifications of all dilutions of each plate with known parasite density.

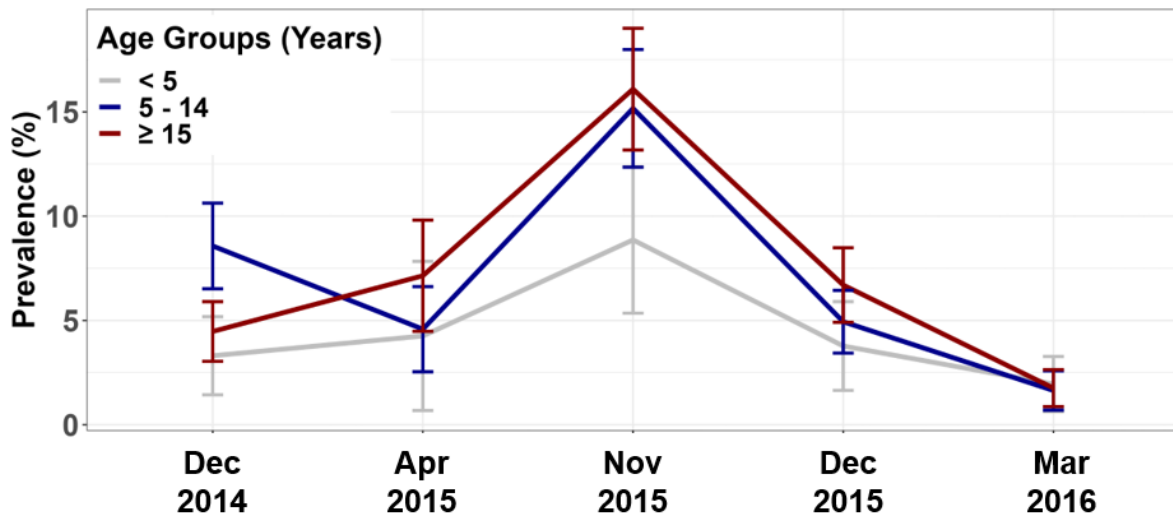


Figure S2: Trend analysis for *P. falciparum* infection prevalence by age groups in the first cohort, by nested PCR. Error bars represent 95% confidence intervals for prevalence.

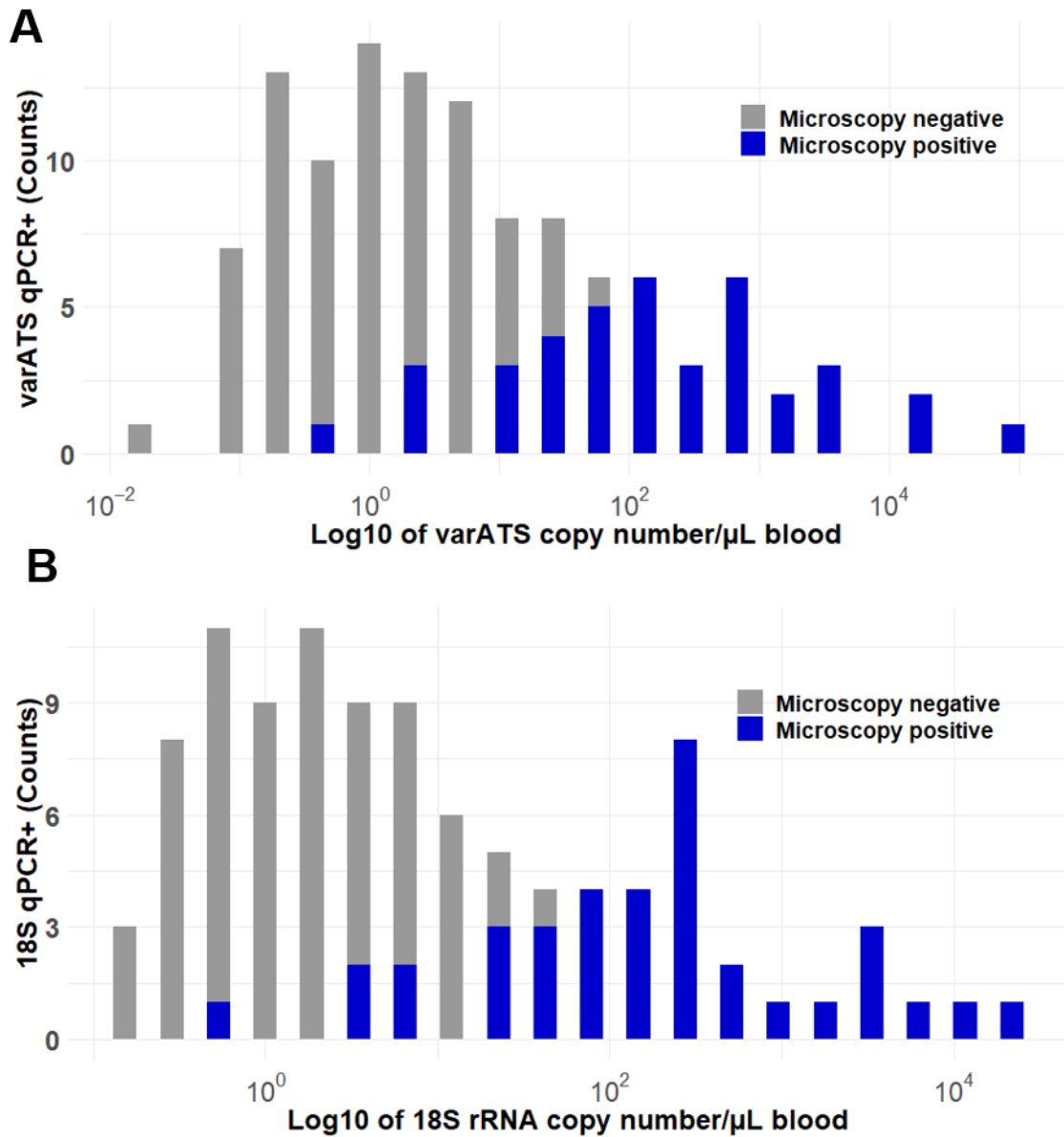


Figure S3. Parasites prevalence by microscopy according to parasite densities measured by varATS (A) and 18S rRNA qPCR (B) molecular tools. Parasite densities quantified by varATS and 18S rRNA qPCR are presented (log10 scale with a bin size of 20) for both microscopy negative and microscopy positive.

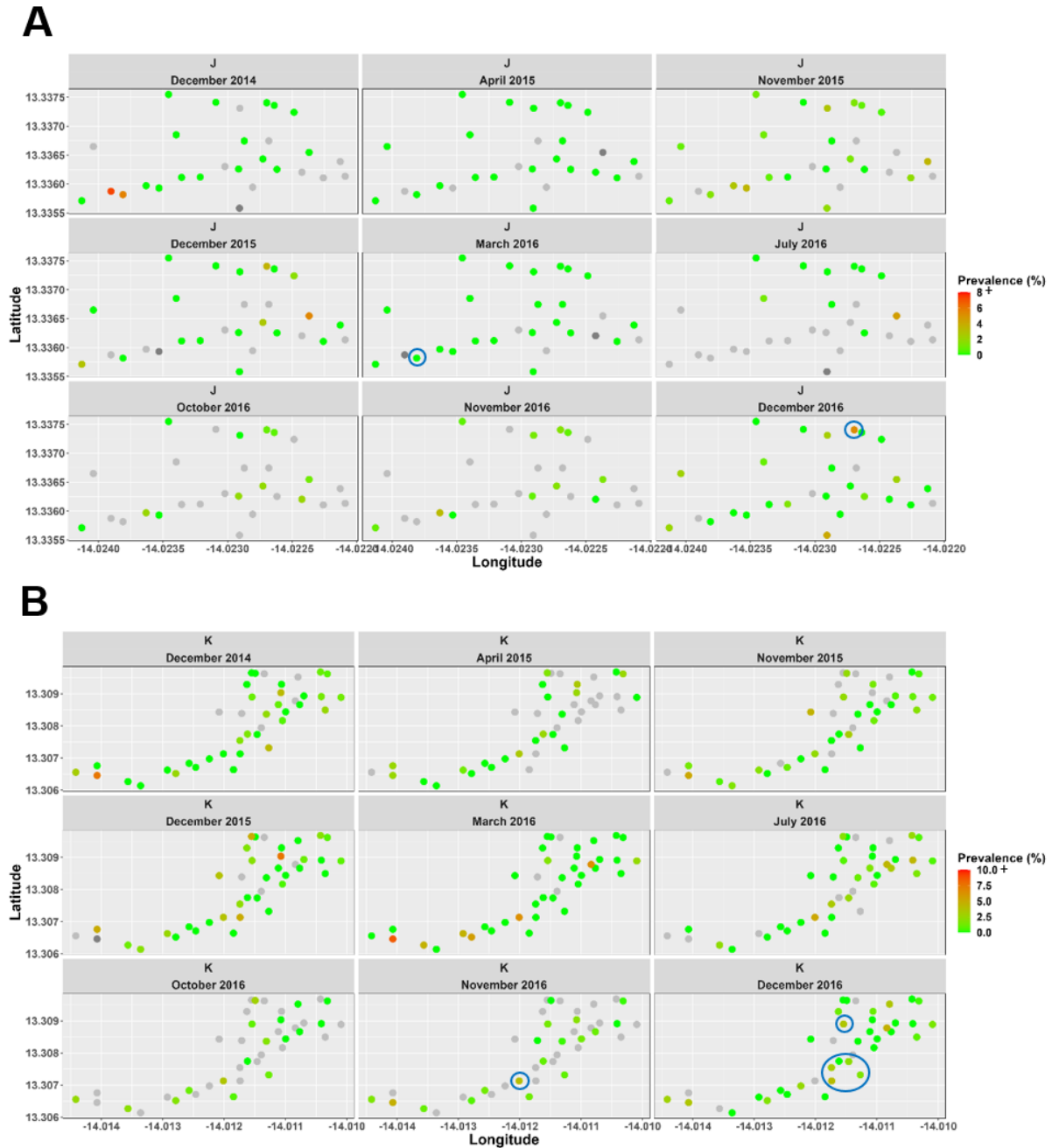


Figure S4. Spatio-temporal *P. falciparum* prevalence by household in villages J and K. *P. falciparum* prevalence in each household was normalized to the prevalence of infections per month in each village and only households with at least three individuals were considered. Grey colour represents the household with no data. The blue circles represent the significant hotspots ($p < 0.05$) determined using the Bernoulli spatial scan statistic in Satscan algorithm.

Additional Tables

Table S1. Hemoglobin levels (g/dl) and classification of anaemia (WHO, 2011)

Population	Anaemia status			
	No anaemia	Mild	Moderate	severe
Children 6 to 59 months	≥ 11.0	10.0-10.9	7.0-9.9	< 7
Children 6 to 11 years	≥ 11.5	11.0-11.4	8.0-10.9	< 8
Children 12 to 14 years	≥ 12.0	11.0-11.9	8.0-10.9	< 8
Non-pregnant women > 15 years	≥ 12.0	11.0-11.9	8.0-10.9	< 8
Pregnant women > 15 years	≥ 11.0	10.0-10.9	7.0-9.9	< 7
Men > 15 years	≥ 13.0	11.0-12.9	8.0-10.9	< 8

Table S2: Household-based assessment of *P. falciparum* infection clusters using Bernoulli spatial scan statistic

Villages	Sampling timepoint	Population	Infected	Prevalence of infections (%)	Identified Clusters	P-value
K	December 2014	373	30	8	K014	0.074
					K001, K003	0.126
					K037	0.638
					K032	0.999
	April 2015	141	14	9.9	K021, K022	0.53
					K001, K003, K002	0.85
					K009	0.95
	November 2015	247	36	14.6	K003	0.231
					K036	0.366
					K013, K014	0.954
	December 2015	375	25	6.2	K002, K003	0.107
					K021	0.478
					K020	0.794
					K038, K009, K010	0.863
	March 2016	460	9	2	K042, K005, K006, K041, K003	0.289
					K001, K003	0.255
	July 2016	366	28	7.7	K028, K030, K027, K031	0.444
					K010, K011, K036, K038, K009	0.622
K024					0.995	
K001, K003, K005, K041, K007, K009					0.173	
October 2016	226	16	7.1	K019	0.91	

				K015	0.997	
	November 2016	185	39	21.1	K009	0.031
					K003	0.065
					K017, K021	0.971
	December 2016	449	64	14.2	K017	0.0025
					K037, K036, K038, K010	0.0036
					K001, K003	0.334
					K023	0.712
					K030	0.776
					K026	0.985
					K008	0.999
	December 2014	201	3	1.5	J014, J016	0.382
	April 2015	147	1	0.7	NC	
	November 2015	184	26	14.1	J013, J017	0.342
					J003, J006, J004	0.664
					J023	0.938
	December 2015	12	6	50	NC	
	March 2016	226	3	1.3	J016	0.023
	July 2016	227	4	3.1	J005, J006, J011, J009, J012, J022, J004, J018	0.357
J	October 2016	82	10	12.2	J007, J009	0.66
					J005, J011	0.72
	November 2016	101	23	22.8	J017	0.265
					J005	0.963
					J027	0.974
	December 2016	232	19	8.2	J027	0.0054
					J022	0.841
					J003	0.996
					J028	0.996
					J015	0.999

P. falciparum infections clusters were identified using Bernoulli spatial scan analysis method in SaTScan version

10.1.2. NC: no cluster identified. In bold, the group with a significantly higher prevalence of *Plasmodium* infection ($p < 0.05$) compared to other households at a given time.