Supplementary

Appendix

Multiple Regression Models assessing the contributors to breakthrough parasitaemia

The MLR equation is written as the generalized linear model for the $logit$ function as follows:

$$logit(p) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \ldots\ldots + \beta_m x_m$$

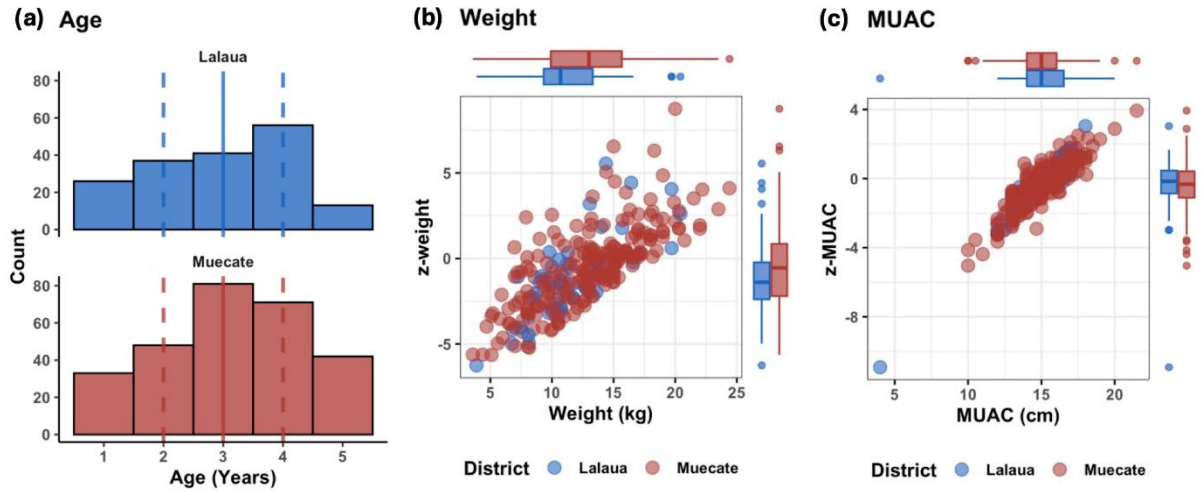
where, p is the probability of having positive parasitaemia on D28, β_0 is the intercept, and β_i is the regression coefficient indicating the relative effect of variable x_i. The values of model parameters were determined by maximizing the log likelihood function across n observations of outcomes

$$l(p) = \sum_{i=1}^{n} y_i log(p) + (1 - y_i) log(1 - p)$$

where, $y_i = 1$ when D28 parasitaemia was positive and $y_i = 0$ when D28 parasitaemia was negative. The MLR analysis was performed by constructing models using nine possible predictors; age, weight, z-score of weight, MUAC, z-score of MUAC, D7 capillary blood levels of sulphadoxine, pyrimethamine, and desethylamodiaquine, and the AUC from D7 to D28 of desethylamodiaquine levels. A total of 511 models were constructed using all possible combinations of the nine variables. The models were fitted with the same dataset from 162 patients.

Supplementary Figures

Figure S1: Demographic characteristics of participants. (a) Age distribution by district. Solid lines indicate medians and dashed lines represent interquartile ranges (IQRs). (b) Body weights and corresponding z-scores. (c) Mid-upper arm circumferences (MUAC) and corresponding z-scores.



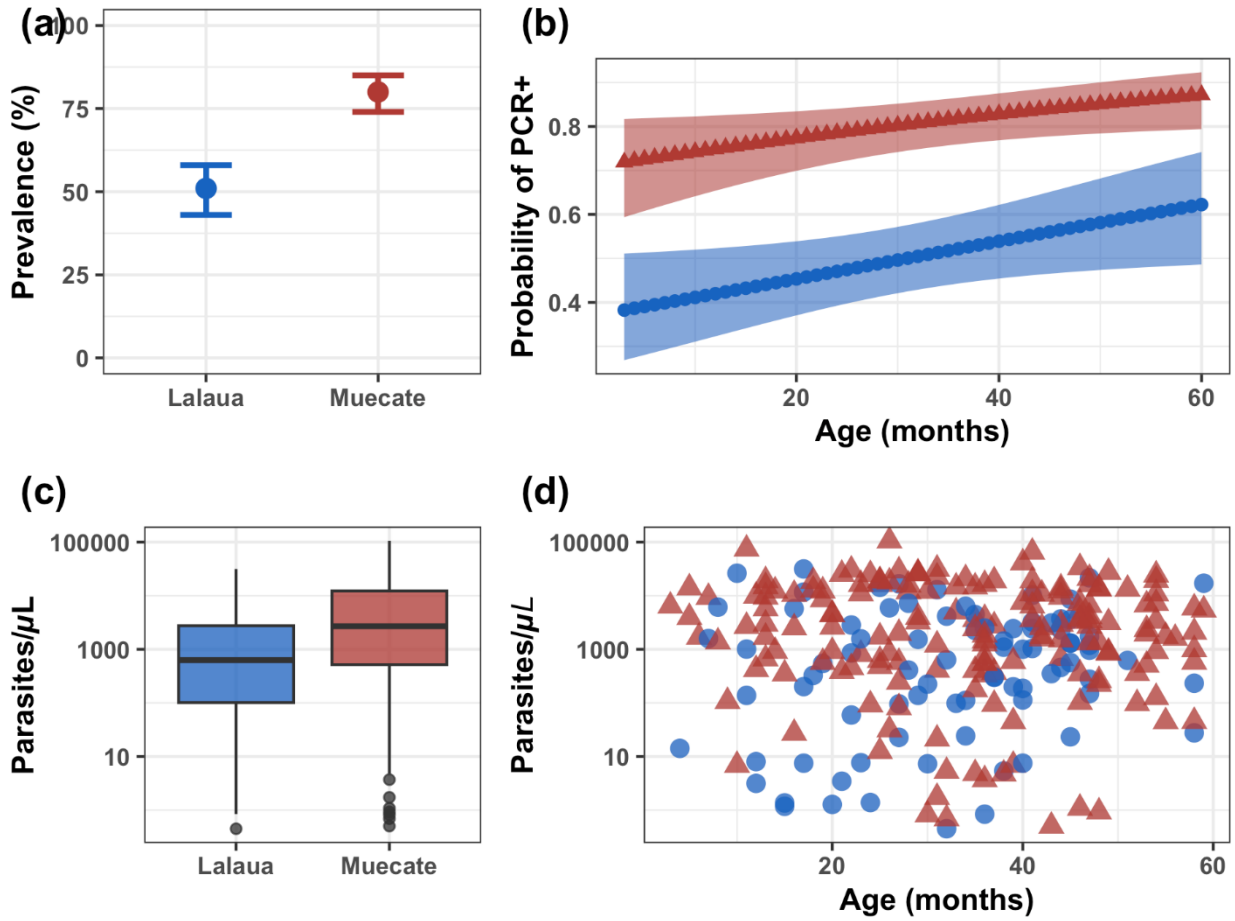


Figure S2: Baseline malaria characteristics. **(a)** Malaria prevalence by district, with 95% confidence intervals (CIs). **(b)** Estimated probability of testing PCR-positive on Day 0. **(c)** Geometric mean (95%CI) baseline parasite densities by site. **(d)** Baseline parasite densities stratified by age (blue circles-Lalaua, red triangles Muecate).

Figure S3: Correlation between microscopy and qPCR estimates of parasite densities

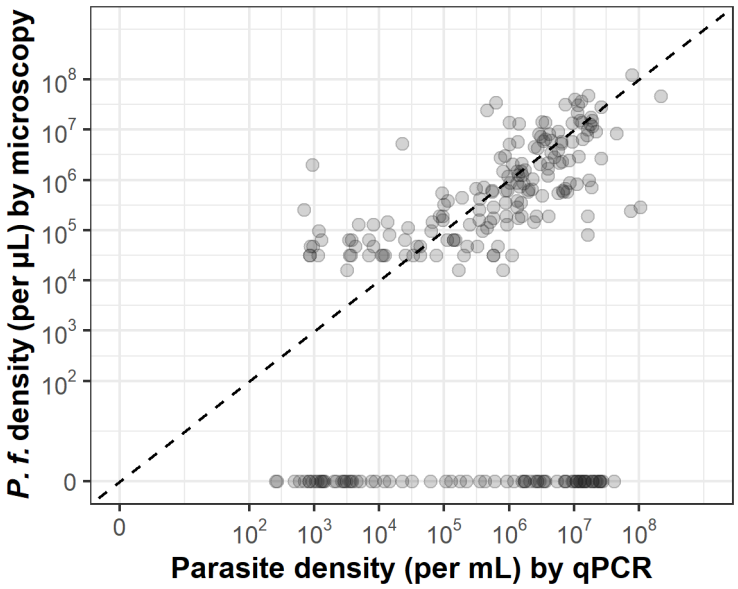


Figure S4: Capillary whole blood drug levels by site. Sulphadoxine and pyrimethamine are co-formulated in the same tablet. Desethylamodiaquine is the bioactive desethyl metabolite of amodiaquine.

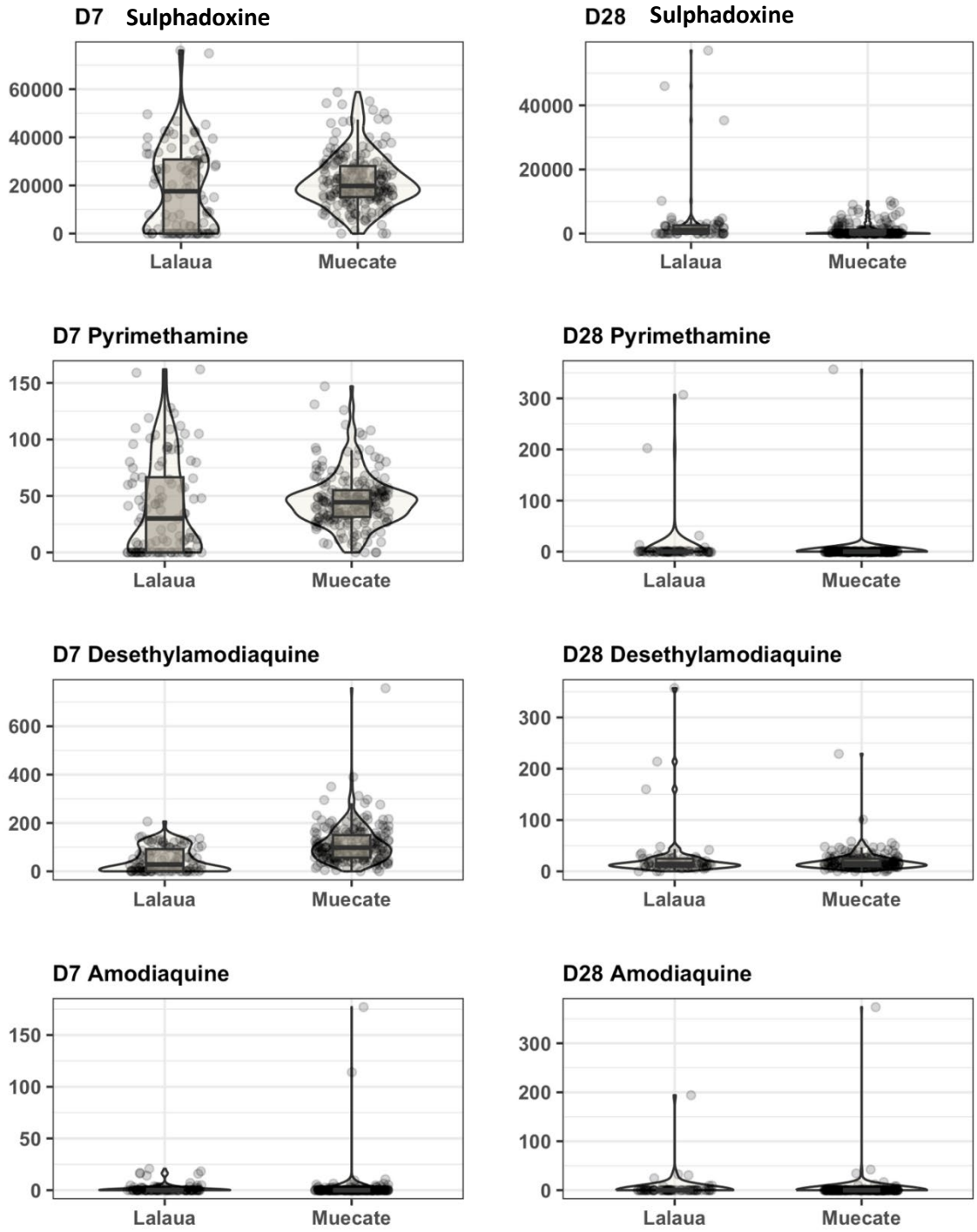


Figure S5a: Predicted probability of testing malaria-positive at D28 as a function of (right) D28 desethylamodiaquine concentration and (left) desethylamodiaquine exposure between day 7 and day 28. (Top) Predicted probabilities were estimated using a logistic regression model that adjusts for age (modeled with a smooth term), geographical location, and calendar time. The predictions assume a median child age of 33 months, in Muecate district, on 2022-02-25. Shaded areas represent the 95% confidence interval (CI). **(Bottom)** Distribution of D28 desethylamodiaquine concentrations and desethylamodiaquine exposures. Histogram counts (pale grey) are shown on the right y-axis, while the cumulative count is displayed on the left y-axis (thick line).

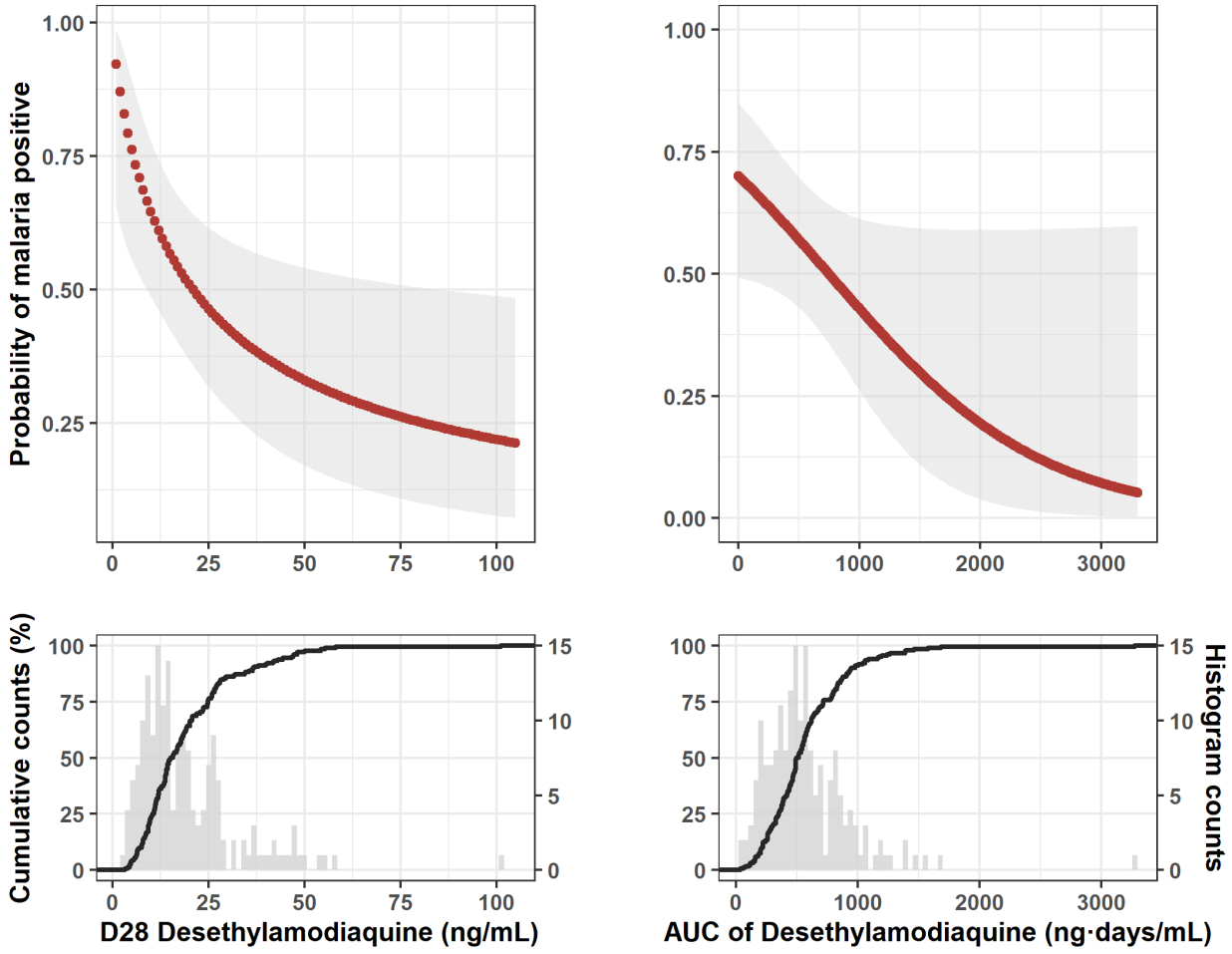


Figure S5b. The probability of an average child in the Mozambique study sites (aged 33 months old, weight 12.7 kg, Z-score = -0.81) being parasitaemic on D28 in relation to the $AUC_{7\text{-}28}$ of whole blood desethyl amodiaquine (DAQ) levels derived from the multiple regression model. Dashed lines represent 95%CI.

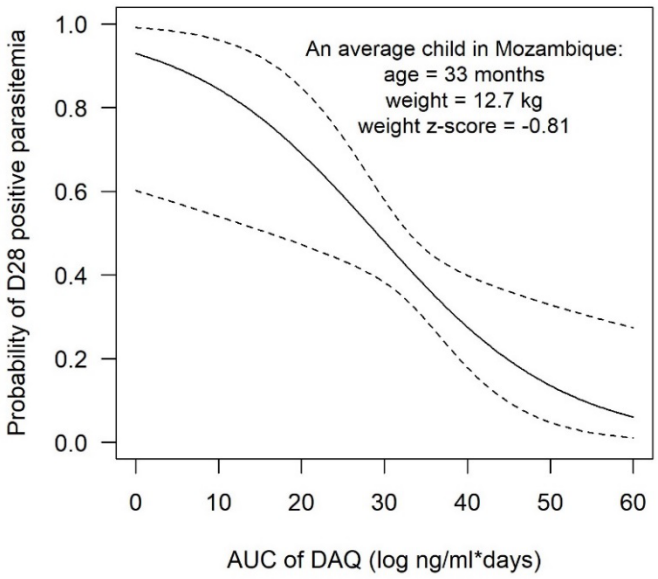


Figure S6. Day 7 whole capillary blood sulphadoxine concentrations in children whose blood samples were malaria parasite negative at D28 compared with those who were parasitaemic.

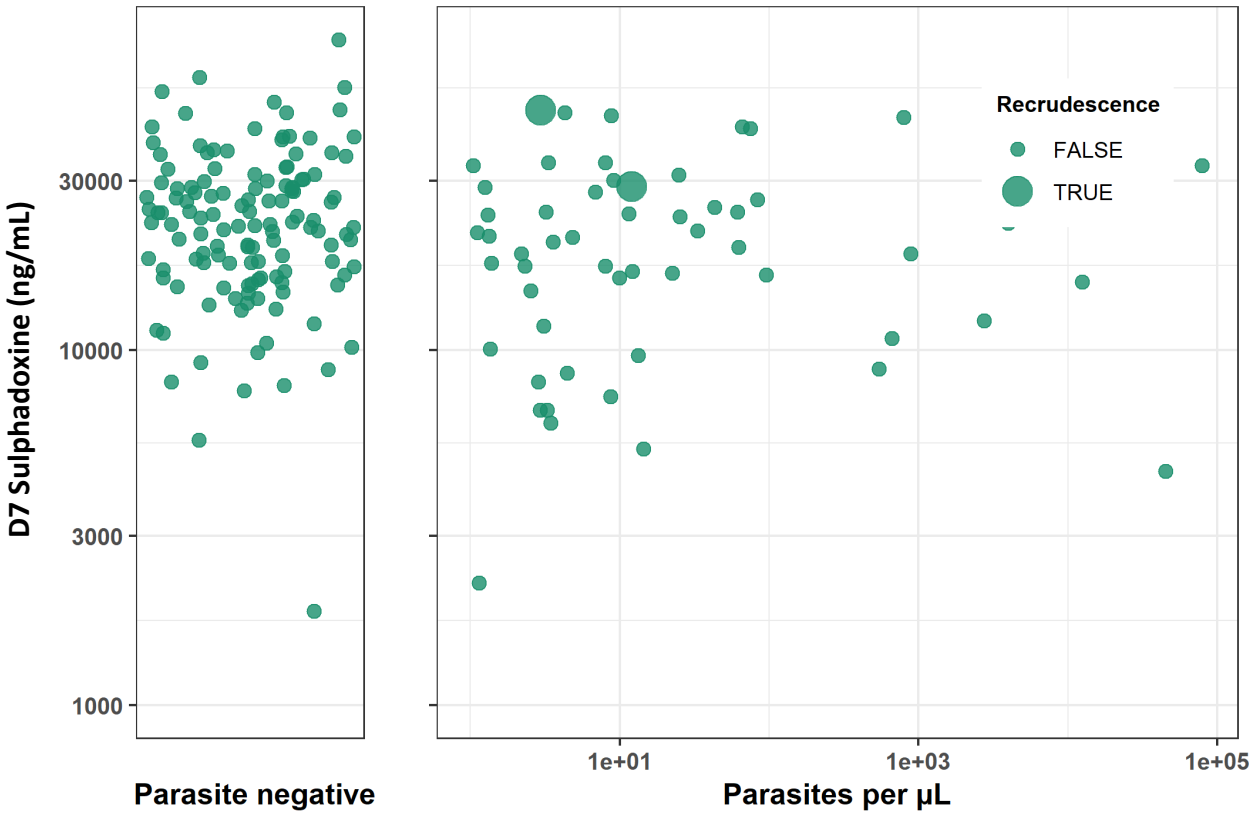


Figure S7. Day 7 capillary whole blood pyrimethamine concentrations in children whose blood samples were malaria parasite negative at D28 compared with those who were parasitaemic.

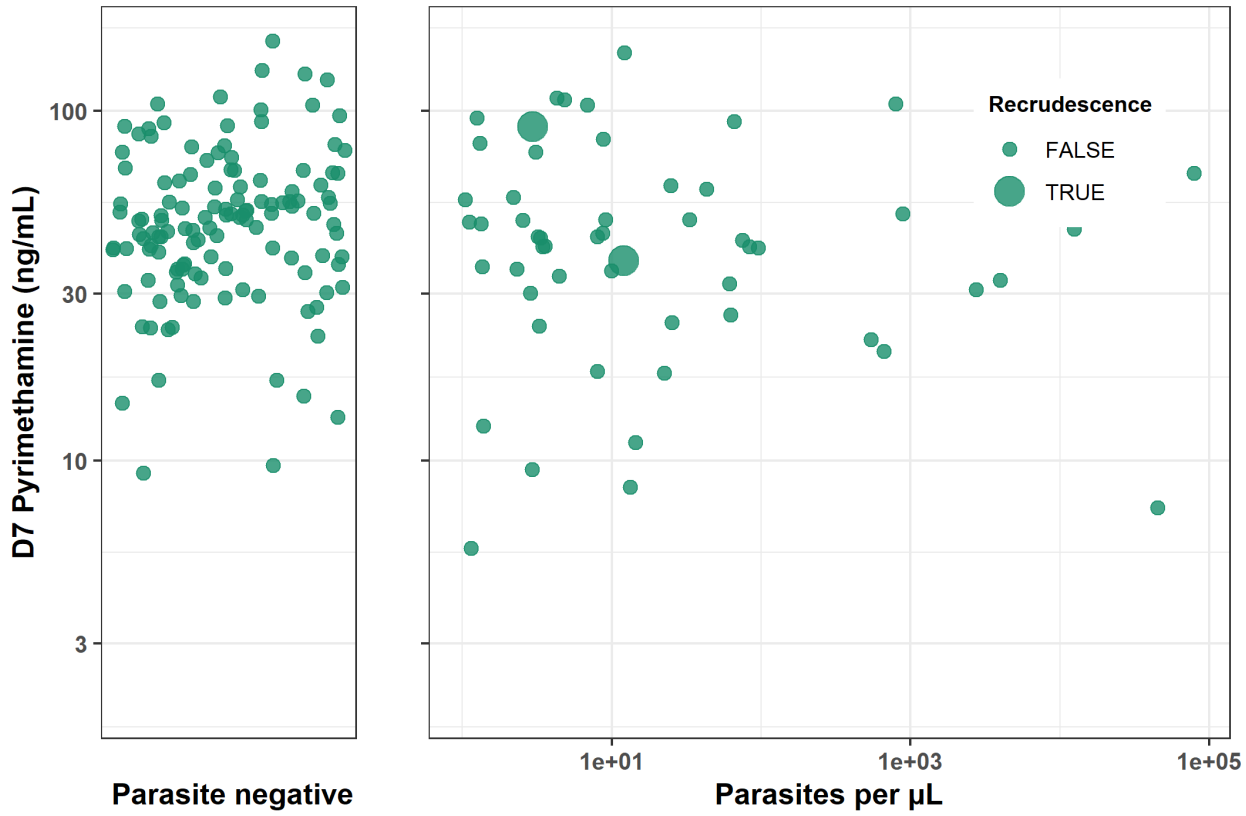


Figure S8. Proportions (95%CI) of children who were malaria parasitaemic on D28 in relation to the preceding day 7 sulphadoxine (left) and pyrimethamine (right) concentrations.

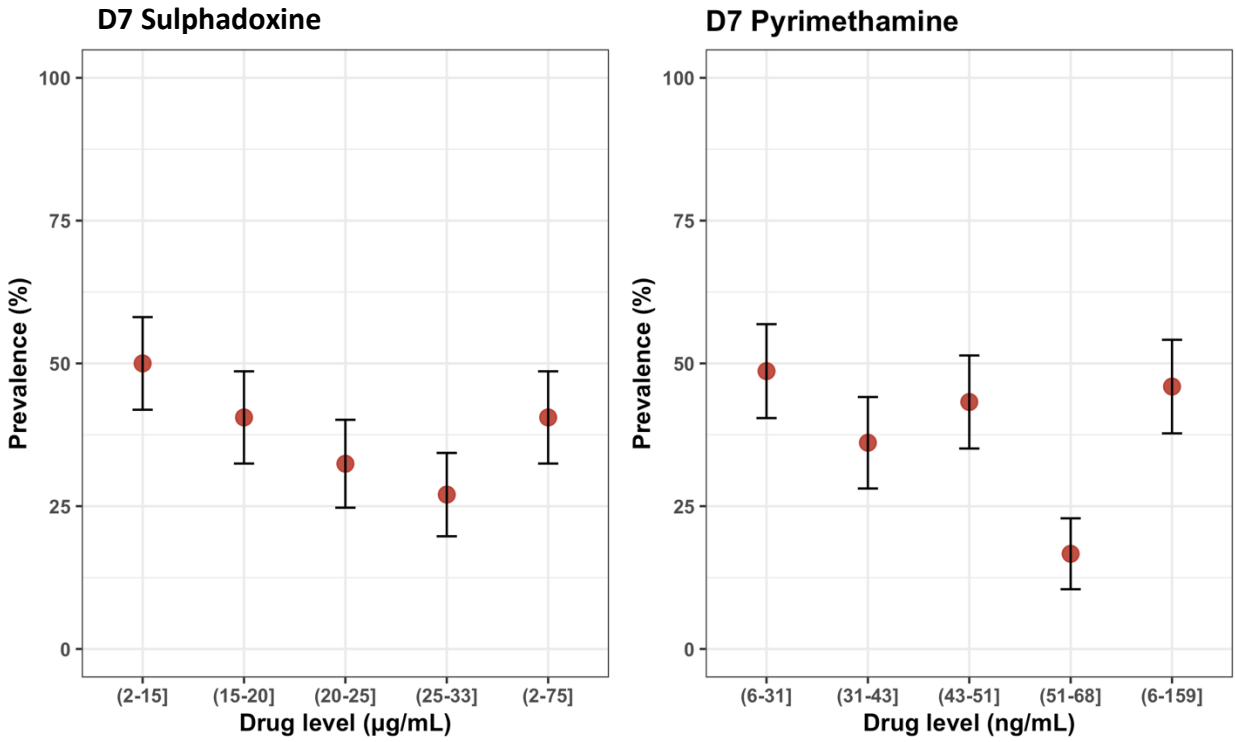


Figure S9: Relationship between malaria prevalence and parasite density across different weight bands. (a) malaria prevalence for each weight band, with error bars representing the 95% confidence intervals. (b) Parasite density in relation to weight, with data points colour-coded by age group

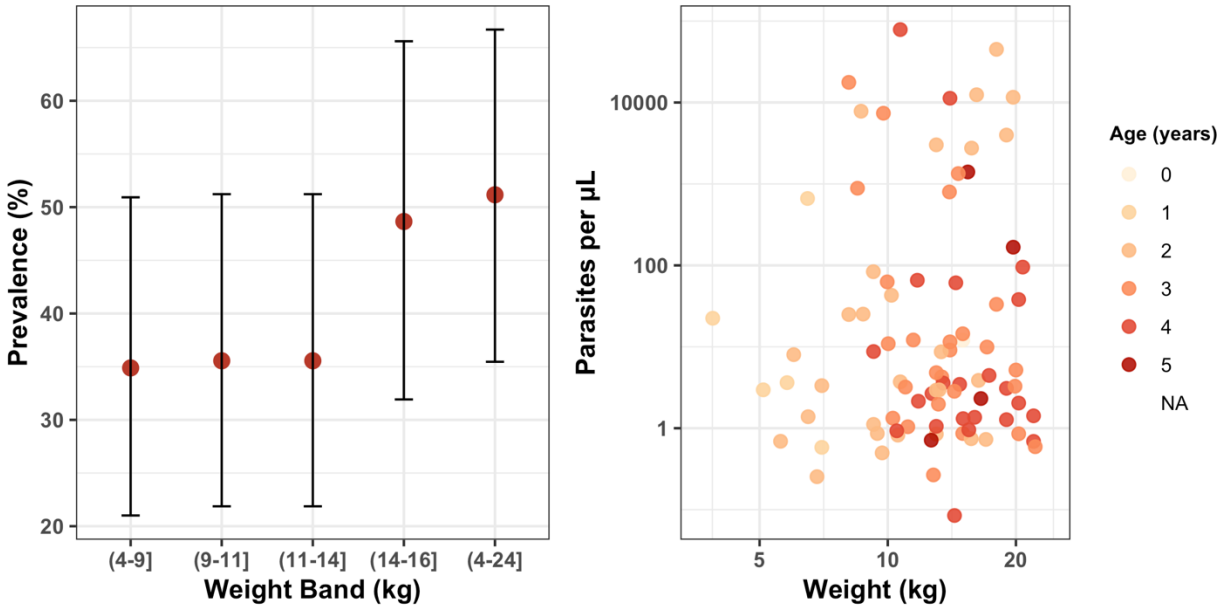


Figure S10. Predicted relationship between D28 desethylamodiaquine levels and breakthrough parasitaemia.

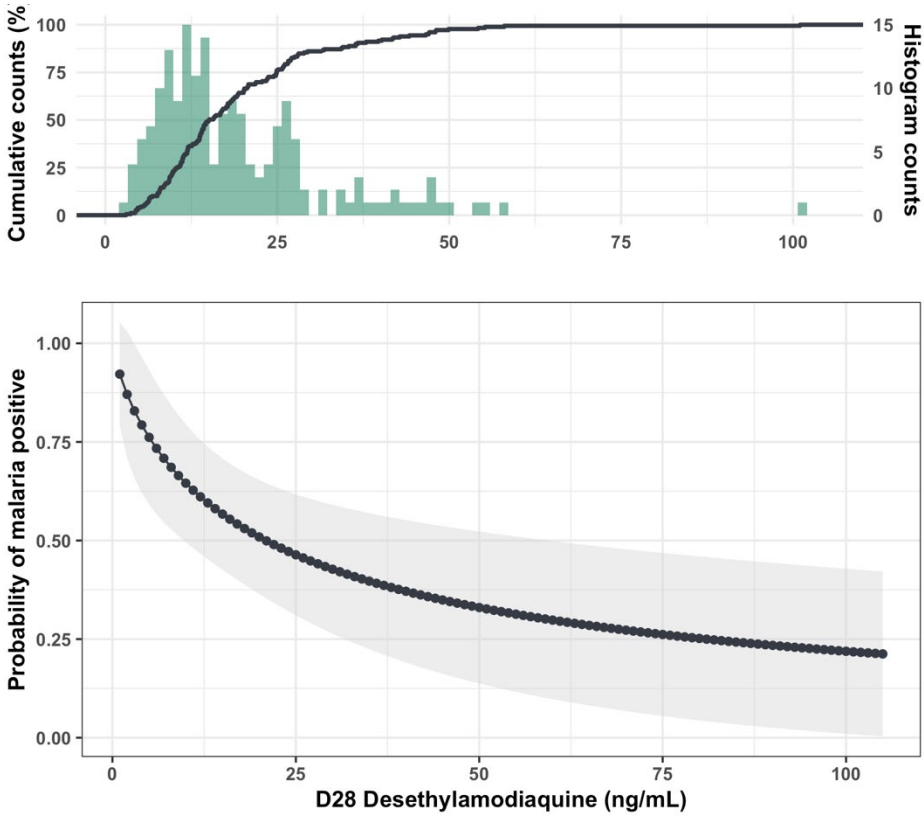


Figure S11: The relationship between the D7 whole blood desethyl amodiaquine concentrations and the area under the concentration-time curve ($AUC_{7\text{-}28}$) from D7 to D28

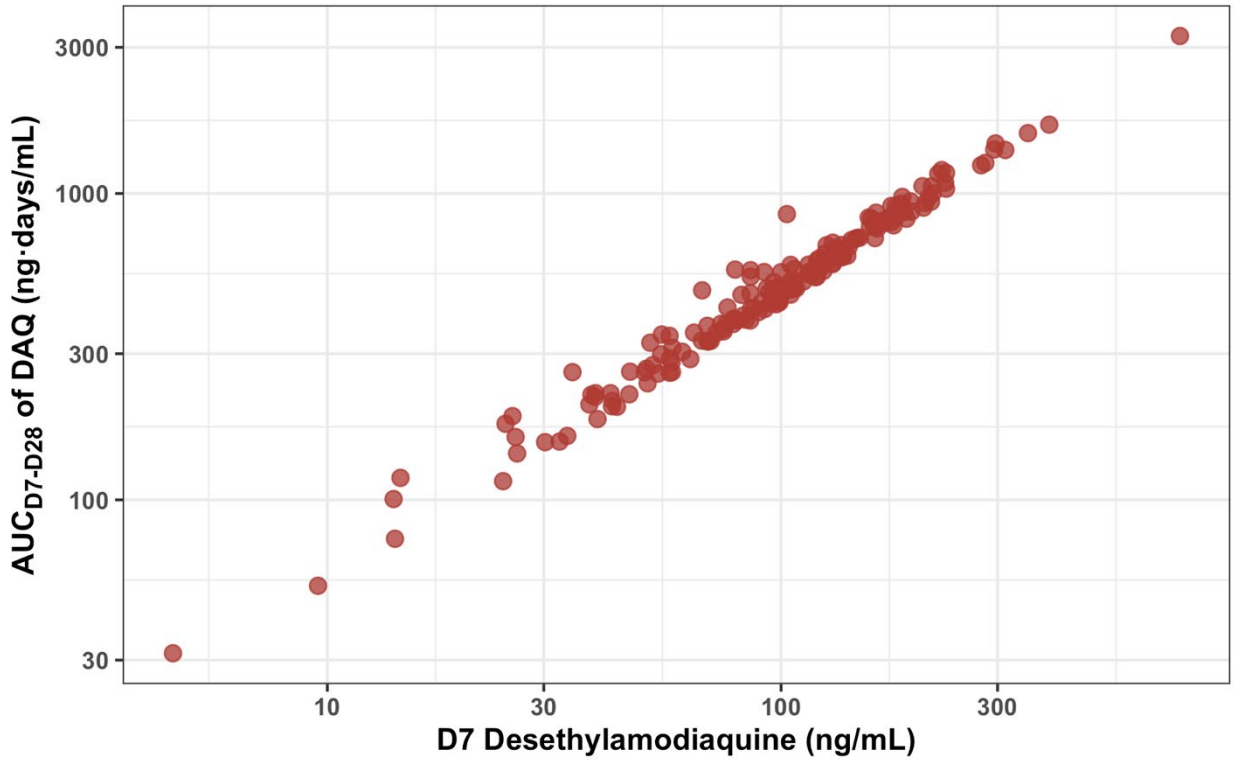
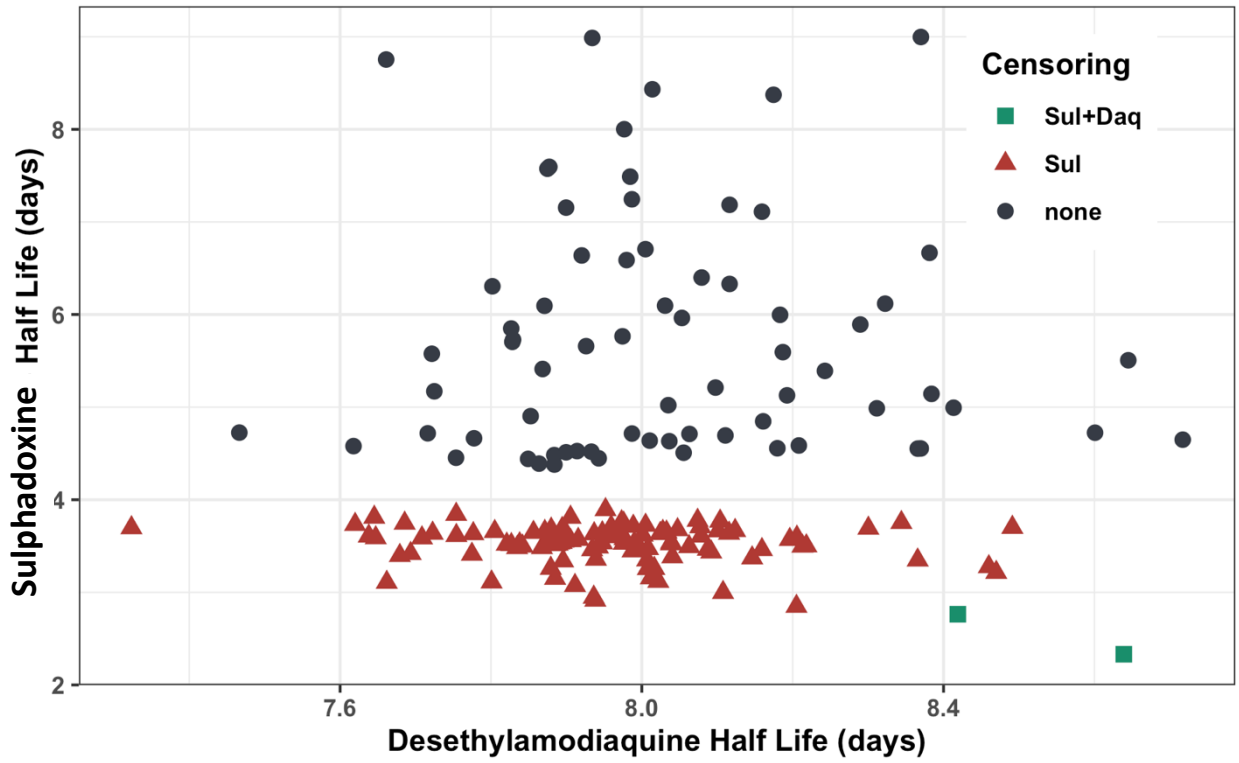


Figure S12. The relationship between between desethylamodiaquine and sulphadoxine estimated elimination half-lives



Supplementary Tables

Table S1: The prevalence of malaria based on samples collected from children with data available for both dates.

	Before SMC	After SMC
Malaria positive (PCR)	139/194 (72%)	88/194 (45%)
P. falciparum	127/194 (66%)	83/194 (43%)
P. ovale	54/194 (28%)	2/194 (1%)
P. vivax	1/194 (0.5%)	0/194 (0%)
P. malariae	0/194 (0%)	0/194 (0%)
Undetermined*	4/194 (2%)	2/194 (4%)

Data are presented as n (%)

*If there was insufficient DNA then the malaria species could not be determined.

Table S2. Details of recrudescent *P. falciparum* infections.

	Age (months)	Parasite density (per mL) at D28	Desethylamodiaquine concentration (ng/mL) at D28
1	26	7,380,303	5.24
2	11	2,962	14.4
3	4	12,054	20.6

Table S3: Summary of detectable drug levels

Drug name	Detectable Samples (n)	Geometric mean (ng/mL)	Minimum (ng/mL)	Maximum (ng/mL)
Sulphadoxine				
D7	270/301	19402	1300	76100
D28	102/248	2349	843	57100
Pyrimethamine				
D7	266/301	42	4.67	162
D28*	7/248	45	4.53	356
Amodiaquine				
D7	54/302	4	1.88	177
D28*	19/251	14	2.05	374
Desethylamodiaquine				
D7	274/302	74	3.19	757
D28	243/251	8	3.01	357

* All detectable levels of sulphadoxine and amodiaquine were suspected to result from participants receiving their second round of SMC before, rather than after, the blood sample was taken.

127 Table S4: STROBE checklist

128 STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***
129

	Item No	Recommendation	Line No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-3 33-56
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	61-75
Objectives	3	State specific objectives, including any prespecified hypotheses	75-78
Methods			
Study design	4	Present key elements of study design early in the paper	77-78, 84-85
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	82-84 86-98 101-117
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	101-107
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	122-134
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	137-141
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	136-141
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions	136-141

		(c) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	154-238
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Fig 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	Tables 144-167
Outcome data	15*	Report numbers of outcome events or summary measures over time	169-265

130

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	169-265
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	supplement
Discussion			
Key results	18	Summarise key results with reference to study objectives	268-356
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3183-343
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	343-356
Generalisability	21	Discuss the generalisability (external validity) of the study results	353-356
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	380-385