

# Persistence of *P. Falciparum* Ring-Stage Parasites 42 Days After Artemisinin and Non-Artemisinin Combination Therapy in Naturally Infected Malians

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## Research

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# Abstract

**Background:** Malaria control in sub-Saharan Africa relies upon prompt case management with artemisinin-based combination therapy (ACT). Ring-stage parasites, measured by *sbp1* quantitative reverse-transcriptase PCR (qRT-PCR), were previously reported to persist after ACT treatment and hypothesized to reflect temporary arrest of the growth of ring-stage parasites (dormancy) following exposure to artemisinins. Here, we examined the persistence of ring-stage parasitemia following ACT and non-ACT treatment.

**Methods:** We used samples from naturally infected Malian gametocyte carriers who received dihydroartemisinin-piperaquine (DP) or sulfadoxine-pyrimethamine (SP-AQ) with or without gametocytocidal drugs. Gametocytes and ring-stage parasites were quantified by qRT-PCR during 42 days of follow-up.

**Results:** At baseline, 89% (64/73) of participants had measurable ring-stage parasites. Following treatment, the proportion of ring-stage parasite-positive individuals and ring-stage parasite densities declined for all four treatment groups. Participants who received DP had 81% lower post-treatment ring-stage parasite density compared to participants who received SP-AQ ( $p < 0.001$ ). Gametocytocidal drugs did not influence ring-stage parasite persistence. Ring-stage parasite densities on days 14 and 28 after initiation of treatment were higher among individuals who subsequently experienced recurrent parasitemia compared to those who remained free of parasites until day 42 after initiation of treatment ( $p_{\text{day 14}} = 0.011$  and  $p_{\text{day 28}} = 0.068$ ). We observed no association of ring-stage persistence with gametocyte carriage.

**Conclusions:** Our findings of lower ring-stage persistence after ACT treatment without an effect of gametocytocidal partner drugs affirms the use of *sbp1* as ring-stage marker and argues against preferential persistence of low densities of rings following ACT treatment. The implications of our findings for monitoring drug sensitivity require further study.

## Background

Malaria is a leading cause of global morbidity and mortality. In 2018, an estimated 228 million cases and 405,000 deaths were reported worldwide (1). In sub-Saharan Africa, prompt diagnosis and treatment with artemisinin-based combination therapy (ACT) remains a key strategy for the treatment of uncomplicated *Plasmodium falciparum* malaria. ACT comprises of an artemisinin derivative that rapidly reduces parasite burden and a partner drug with a longer half-life that clears remaining parasitemia and provides prophylactic activity for weeks post-treatment. At present, artemisinin derivatives retain excellent efficacy throughout Africa despite reports of decreased sensitivity to some of its partner drugs (2). Whilst recent antimalarial efficacy trials in Africa have shown overwhelmingly high treatment success after ACT ( $\geq 95\%$ ) (2), parasites may persist shortly after initiation of treatment (3). Though this parasite persistence may not necessarily reflect drug resistance, which also depends on initial parasite density, host immunity,

and drug absorption (4, 5), it is important to better understand what parasite populations persist and whether parasite persistence has consequences for later recrudescence (5).

Post-treatment detection of parasite DNA may reflect both (remnants of) asexual parasites and gametocytes (6, 7), the latter commonly persisting after ACT treatment (8). A study in travelers in Sweden (9) indicated that residual parasite DNA can be detected by qPCR for up to 42 days after successful treatment without evidence of viable asexual parasites or gametocytes. Recently, mRNA transcripts specific to ring-stage parasites (skeleton binding protein; *sbp1*) were reported following ACT treatment (6, 10). This persistence of low-level asexual parasitemia after ACTs may be explained by the “dormancy theory” (11) which postulates that under artemisinin pressure, a subpopulation of young ring-stage parasites undergo developmental arrest where they remain metabolically inactive. It has been suggested that these low-density ring-stage parasites may represent ‘sleeping beauties’ (i.e. dormant parasites that tolerate artemisinin treatment, but are sensitive to other antimalarials) (12, 13) and this mechanism may explain why certain individuals experience recrudescence in the absence of actual artemisinin resistance.

Here, we expand upon our earlier observations by assessing ring-stage parasitemia among trial participants that were followed for 42 days after being randomized to ACTs or non-ACTs with and without gametocytocidal drugs. This study allowed us to examine whether persisting ring-stage parasitemia is specific to ACTs, and whether the persistence of ring-stage parasites is associated with parasite recrudescence and/or continued gametocyte production.

## Materials And Methods

### Study cohort and sample collection

This study used samples obtained from participants of a trial in Ouélessébougou, Mali who were randomized 1:1:1:1 to receive either sulfadoxine-pyrimethamine (SP-AQ), SP-AQ with single low-dose primaquine (SP-AQ + PQ), dihydroartemisinin-piperaquine (DP), or dihydroartemisinin-piperaquine with methylene blue (DP + MB) (14). Eligible participants were males with asymptomatic *Plasmodium falciparum* mono-infection, between 5 and 50 years of age, who were glucose-6-phosphate dehydrogenase (G6PD)-normal by CareStart G6PD rapid diagnostic test (Access Bio, Somerset, NJ, USA), had a hemoglobin concentration of  $\geq 10$  g/dL, and had a *P. falciparum* gametocyte density of  $\geq 2$  gametocytes/500 white blood cells by thick film microscopy. Participants were excluded if they had a serious or chronic illness (including signs of severe malaria), weighed 80 kg or more, reported antimalarial use within 7 days of screening, or reported allergies to study drugs.

Details on the study procedures are described in the original paper (14). In brief, participants were followed for 42 days and blood samples were obtained on days 0, 1, 2, 7, 14, 28 and 42 after initiation of treatment. Blood smear microscopy was conducted on blood samples taken by finger prick on day 0 and all days of follow-up to assess for asexual parasite and gametocyte density. For the measurement of ring-stage and gametocyte density by qRT-PCR, 100  $\mu$ L of blood was collected in EDTA tubes and

immediately transferred to RNAprotect (Qiagen) and stored at  $-80^{\circ}\text{C}$  until extraction by MagNAPure LC automated extractor (Total Nucleic Acid Isolation Kit-High Performance; Roche Applied Science, Indianapolis, IN, USA).

## Laboratory analysis

Ring-stage parasites were quantified by qRT-PCR targeting the *sbp1* mRNA transcript using previously described methods (6). Male and female gametocyte densities were quantified by qRT-PCR, targeting male *PfMGET* and female *Pfs25* mRNA transcripts as described elsewhere (14). For samples that were microscopy-positive for asexual *P. falciparum* parasites on days 7, 14, 28, and 42, PCR genotyping of *glurp* (*glurp2*), *msp2* (Fc27), *msp1* (K1, MAD20 and RO33) and *lc1* alleles were performed on samples obtained at enrollment and day of post-treatment failure. Pre- and post-treatment pairs were analyzed and classified as either recrudescence, re-infection, or indeterminate infections according to World Health Organization guidelines (7, 15).

## Statistical analysis

All analyses were performed using Stata 14.0 (StataCorp, College Station, TX, USA) and R (version 3.5.0; R Project for Statistical Computing; <http://www.r-project.org/>). Comparisons between proportions were conducted using Chi-squared or Fisher's exact test and Mann-Whitney tests were used to compare differences in parasite densities, unless otherwise specified. Correlations between ring-stage and gametocyte parasite densities were assessed by Spearman's rank correlation coefficient using the  $\log_{10}$  transformed versions of these variables. Multivariate linear regression was used to model  $\log_{10}$  ring-stage parasite density (parasites/ $\mu\text{L}$ ). Mixed-effects regression with random intercepts for participants and random slopes for each follow-up visit was used to estimate the average effect of treatment on ring-stage parasite density over time. These models specified a binary indicator of whether an individual received DP (0/1) and adjusted for follow-up visit, receipt of gametocides, and baseline  $\log_{10}$  SBP-1 parasite density. An interaction term between DP and follow-up visit was included to assess whether participants of the DP group cleared parasitemia at a more rapid rate than SP-AQ. An overall F-test was used to compute the p-value testing joint effect of the interaction terms. Log-binomial regression was used to assess what factors were associated with persistent parasitemia on day 7 post-treatment. All tests were two-sided with an alpha set to 0.05. P-values  $< 0.05$  were considered statistically significant.

## Results

### Characteristics of sample population

Our study included 73/80 (91%) males from the original trial (14) (Table 1) who were randomized to receive SP-AQ ( $n = 18$ ), SP-AQ + PQ ( $n = 18$ ), DP ( $n = 18$ ); or DP + MB ( $n = 19$ ). In this population, recruited based on microscopy-detectable gametocyte carriage, 64 (89%) had a measurable density of ring-stage parasites by qRT-PCR. Baseline ring-stage parasites by qRT-PCR was positively correlated with microscopy-detected asexual parasite density (Spearman's  $\rho = 0.83$ ,  $p < 0.0001$ ). Younger participants

(between 5 to 14) had higher baseline ring-stage parasite densities compared to those 15 years and above ( $p < 0.02$ ). Baseline ring-stage parasite density did not significantly vary between randomized treatment groups ( $p = 0.61$ ).

| Baseline Characteristics   | All (n=73)      |
|--|-----------------|
| Age group in years, n (%)  |                 |
| 5 to <15   | 55 (75)         |
| 15 to <25  | 12 (16)         |
| 25+  | 6 (8)           |
| Weight in kg (mean $\pm$ SD)   | 33.4 $\pm$ 15.2 |
| Treatment, n (%)   |                 |
| SP-AQ  | 18 (25)         |
| SP-AQ +PQ  | 18 (25)         |
| DP   | 18 (25)         |
| DP +MB   | 19 (26)         |
| Asexual parasite prevalence by microscopy, n (%)                                 | 43 (59)         |
| Asexual parasite density by microscopy (per $\mu$ L), median [IQR]               | 107 [0, 400]    |
| Ring-stage parasite prevalence by qRT-PCR, n (%)                                 | 64 (89)         |
| Ring-stage parasite density by qRT-PCT (per $\mu$ L), median [IQR]               | 633 [7, 2028]   |
| Gametocyte prevalence by qRT-PCR, n (%)  | 72 (100)        |
| Gametocyte density by qRT-PCR (per $\mu$ L), median [IQR]                        | 57 [32, 173]    |
| Note: Values may not up to 100% due to missing values. IQR = interquartile range |                 |

Table 1

Characteristics of study population

## Parasite kinetics following treatment

Following antimalarial treatment, the prevalence and density of ring-stage parasites reduced across all four treatment arms (Fig. 1; Tables 2–3). DP was associated with a more rapid reduction in ring-stage parasitemia than SP-AQ ( $p_{DP \times time} < 0.0001$ ). Participants who received DP had, on average, 81% [95% CI: 63, 90] lower ring-stage parasite density compared to SP-AQ ( $p < 0.001$ ) (Fig. 1). Despite antimalarial treatment, ring-stage parasites were still detectable at a prevalence of  $\geq 11\%$  across all days of follow-up and across all treatment arms (Table 2). On day 7 post-treatment, 14 (19%) participants had detectable levels of ring-stage parasitemia and only one was microscopy-positive for asexual parasites, who was

classified as having a recrudescence infection. Multivariate log-binomial regression was used to determine what factors were associated with the presence of ring-stage parasites on day 7. Participants with persistent parasitemia were more likely to have higher ring-stage parasite densities at baseline compared to those without detectable levels of ring-stage parasites at day 7 (relative risk ratio = 1.31 [95 CI: 1.00, 1.73] increase in risk for every 1% increase in log<sub>10</sub>-transformed baseline SBP1 parasite density; p = 0.049). In this population, age (p = 0.84), weight (p = 0.92), and treatment type (p = 0.30) were not significantly associated with increased risk of persistent parasitemia.

| Visit  | Prevalence, n (%) |         |         | SP-AQ:DP          |                      |
|--|-------------------|---------|---------|-------------------|----------------------|
|  | All               | SP-AQ   | DP      | PR [95% CI]       | p-value <sup>1</sup> |
| Day 0  | 64 (89)           | 31 (89) | 33 (89) | 1.01 [0.86, 1.19] | 0.93                 |
| Day 1  | 54 (75)           | 29 (81) | 25 (69) | 0.86 [0.66, 1.13] | 0.28                 |
| Day 2  | 45 (63)           | 25 (71) | 20 (54) | 0.76 [0.53, 1.09] | 0.13                 |
| Day 7  | 19 (26)           | 9 (25)  | 10 (27) | 1.08 [0.50, 2.35] | 0.84                 |
| Day 14   | 14 (19)           | 7 (19)  | 7 (19)  | 0.98 [0.38, 2.50] | 0.95                 |
| Day 28   | 8 (11)            | 6 (17)  | 2 (5)   | 0.32 [0.07, 1.50] | 0.12                 |
| Day 42   | 24 (33)           | 18 (50) | 6 (17)  | 0.33 [0.15, 0.74] | 0.0027               |
| Abbreviations: PR=prevalence ratio; SP-AQ=sulfadoxine-pyrimethamine and amodiaquine; DP=dihydroartemisinin-piperaquine |                   |         |         |                   |                      |
| <sup>1</sup> p-values computed using Chi-squared test or Fisher's exact test   |                   |         |         |                   |                      |

Table 2

Comparison of SBP-1 prevalence between SP-AQ and DP arms over time.

| Visit  | Median [IQR]        |                     | Difference in log10 SBP-1 Density |                                    | p-value |
|--|---------------------|---------------------|-----------------------------------|------------------------------------|---------|
|  | SP-AQ               | DP                  | SP-AQ: DP                         | SP-AQ: DP                          |         |
|  | SP-AQ               | DP                  | MD [95% CI] <sup>1</sup>          | % Difference [95% CI] <sup>2</sup> |         |
| Day 0  | 891.3 [8.2, 2065.4] | 330.4 [6.5, 1990.7] | 0.02 [-1.0, 1.0]                  | 3 [-91, 1019]                      | 0.98    |
| Day 1  | 21.2 [0.6, 212]     | 0.6 [0, 2.3]        | -1.5 [-2.0, -0.9]                 | -97 [-99, -89]                     | <0.001  |
| Day 2  | 0.6 [0, 11.2]       | 0.02 [0, 0.3]       | -1.2 [-1.8, -0.6]                 | -94 [-98, 76]                      | <0.001  |
| Day 7  | 0 [0, 0.003]        | 0 [0, 0.07]         | 0.09 [-0.4, 0.6]                  | 24 [-64, 320]                      | 0.73    |
| Day 14   | 0 [0, 0]            | 0 [0, 0]            | -0.3 [-0.8, 0.3]                  | -44 [-84, 96]                      | 0.36    |
| Day 28   | 0 [0, 0]            | 0 [0, 0]            | -0.4 [-0.8, 0.08]                 | -57 [-85, 19]                      | 0.10    |
| Day 42   | 0.03 [0, 7.1]       | 0 [0, 0]            | -1.2 [-2.1, -0.3]                 | -94 [-99, -46]                     | 0.012   |
| <p>Note: IQR = interquartile range</p> <p><sup>1</sup> Coefficient from linear regression modelling log10 SBP-1 parasite density adjusting for baseline SBP-1. A value of 0.001 was added to all values to account for zeroes.</p> <p><sup>2</sup> Calculated by 100*(10MD-1).</p> |                     |                     |                                   |                                    |         |

Table 3

Comparison of SBP-1 parasite density between DP and SP-AQ arms.

Spearman rank correlation tests were used to assess whether the persistence of ring-stage parasites was associated with later gametocyte density, which would be indicative of ongoing gametocyte production. Among those who had persistent ring-stage parasitemia on day 7 (n = 14), we found no significant correlation between their ring-stage parasite density on day 7 and gametocyte density on days 14 and 28 (Fig. 2).

## Factors associated with recurrent infections

Over the course of the 42-day follow-up, 10/73 (14%) participants experienced recurrent parasitemia detectable by microscopy. Conventional genotyping of polymorphic MSP-1, MSP-2 and GLURP genes (15) indicated that four of these were recrudescent infections, four were re-infections, and two were indeterminate. Seven of the recurrent infections were detected on day 42 (2 indeterminate, 2 recrudescent, and 3 reinfections) and the rest occurred on days 7 (1 recrudescent), 14 (1 recrudescent), and day 28 (1 reinfection) (Table 4). Recurrent infections were more common in the SP-AQ group (6/36 or 17%) than the

DP group (4/37 or 11%), though this finding did not reach statistical significance. Ring-stage parasite densities on days 14 and 28 after initiation of treatment were higher among individuals who subsequently experienced recurrent parasitemia compared to those who did not experience recurrent infection until day 42 after initiation of treatment (Mann Whitney test  $p_{\text{day 14}}=0.011$  and  $p_{\text{day 28}}=0.068$ ) (Table 4).

| Infection Type    | Prevalence, n (%) | Ring-stage parasite density by qRT-PCR (per $\mu\text{L}$ ), median [IQR] |         |               |         |                |                      |
|-------------------|-------------------|---|---------|---------------|---------|----------------|----------------------|
|                   |                   | Day 7   | p-value | Day 14        | p-value | Day 28         | p-value <sup>1</sup> |
| Treatment success | 63 (86)           | 0 [0, 0.07]   | Ref 0   | [0, 0]        | Ref     | 0 [0, 0]       | Ref                  |
| Recurrent         | 10 (14)           | 0 [0, 0]  | 0.99    | 0.08 [0, 0.4] | 0.011   | 0 [0, 0.3]     | 0.068                |
| Recrudescent      | 4 (5)             | 0 [0, 6.5]  | 0.57    | 5.6 [0, 11.2] | 0.12    | 14.2 [0, 28.4] | 0.029                |
| Re-infection      | 4 (5)             | 0 [0, 0.4]  | 0.92    | 0.08 [0, 0.2] | 0.085   | 0 [0, 0]       | 0.61                 |
| Indeterminate     | 2 (3)             | 0 [0, 0]  | 0.40    | 0.3 [0, 0.7]  | 0.12    | 0.1 [0, 0.3]   | 0.041                |

Note: P-values were computed using the Mann-Whitney test. Comparisons are between recurrent infection categories to reference category (Treatment Success).

Table 4

Prevalence and density of ring-stage parasites by recurrent infection type. To assess whether prior ring-stage parasite densities were associated with increased risk of recurrent infections, parasite densities for each column of recurrent infections excludes those that were detected before or on that day of follow-up. For example, ring-stage parasite densities for recrudescent infections on day 14 excludes recrudescent infections that occurred on day 7 (n=1) and day 14 (n=1).

## Discussion

In this study of young Malian males with asymptomatic *P. falciparum* carriage, we found ring-stage parasites were detected up to 42 days after antimalarial treatment. Densities of post-treatment ring-stage parasites reduced at a more rapid rate following receipt of ACTs compared to non-ACTs, arguing against the hypothesis that this parasite population reflects dormant parasites that tolerate can artemisinin treatment. In our modestly sized population, few individuals experienced an episode of recurrent parasitemia (n = 10), but these individuals tended to harbor higher ring-stage parasite densities prior to recurrence than those who were successfully treated.

Whilst repeated assessments of parasite density shortly after initiation of treatment provide the most conclusive evidence on (changes in) parasite responsiveness (16), alternative metrics are used to compare the early effects of antimalarials. These include the proportion of individuals with residual parasitemia by microscopy (17) or PCR (3, 7) or the concentration of the histidine rich protein-2 parasite antigen (18). The current study, examining the kinetics of mRNA transcripts indicative of ring-stage parasitemia following treatment (10, 19), explicitly does not aim to present this measure as a proxy for parasite clearance half-lives or direct evidence of reduced susceptibility of parasites to treatment. A recent study from Mali that was specifically designed to assess parasite clearance half-lives following artesunate monotherapy observed indications for delayed clearance in one setting (3), highlighting the need for monitoring of (early) parasite clearance following ACTs. Here, we aimed to examine a previously reported phenomenon of persisting *sbp1* ring-stage transcripts following ACT treatment in more detail (6, 10). Previous studies reported weak (6, 10) or absent association with the concurrent presence of gametocytes (19). Along with *in vitro* experiments on synchronized parasite material (10), this make a strong case that this marker is indeed specific to the detection of ring-stage parasites. The current data further support this by reporting no measurable impact of gametocytocidal drugs on ring-stage mRNA persistence. We hypothesized that ring-stage parasites post ACT treatment were reflective of parasite dormancy, a phenomenon specific to artemisinin derivatives (13) where parasites are able to tolerate artemisinin treatment by entering a temporary growth-arrested state. Previous *in vitro* work indicated that parasites that became dormant after a single treatment with dihydroartemisinin were still receptive to other drugs (13). Our study, directly comparing ACT and non-ACT treatment, allowed us to test this hypothesis. We observed ring-stage parasites were present across all treatment arms and at higher prevalence and density following non-ACT treatment. This argues against the artemisinin-specific dormancy phenomenon as an explanation for our findings.

From a public health perspective, it is important to examine whether persisting ring-stage parasites are predictors of recrudescence infections or the source of gametocyte production. Our study population was small and as the original study objective was to assess gametocyte clearance and infectivity (14), not all individuals harbored asexual parasites at the start of treatment. Only 10 episodes of recurrent parasitemia were observed and only four of these represented recrudescence infections by conventional parasite genotyping. Nevertheless, ring-stage parasitemia appeared higher prior to the occurrence of recurrent infection. In addition, our longer period of follow-up also allowed us to explore associations of ring-stage persistence with subsequent gametocyte carriage. Whilst data collection was not specifically designed for this, we were able to relate ring-stage densities with gametocyte densities 7 and 14 days later, roughly the period needed for gametocyte production (20), and observed no association. The investment of parasites that persist under drug-pressure in either asexual multiplication or gametocyte production reflects a delicate balance (21). A terminal investment in gametocyte production, sometimes hypothesized when increased gametocyte production is seen in partially resistant parasites (22, 23), was not observed here.

The study is subject to several limitations. First, we recognize that our sample size was small, which may have limited the statistical power of our study. Thus, future studies (e.g. pooled analyses) may be needed

to confirm findings. Second, our sample population consisted mostly of young males with high gametocyte densities at enrollment, which may limit the generalizability of our findings to other parasitized populations. Third, due to the uneven follow-up periods and longer tailed follow-up periods toward end of the study, we were unable to assess the exact time of recurrent infection and how long ring-stage parasitemia remains elevated prior to recurrence.

## Conclusion

In summary, we conclude that ring-stage parasites may persist at low concentrations following antimalarial treatment. Whilst this parasite population is unlikely to reflect dormant parasites following artemisinin treatment, the association of ring-stage persistence with subsequent detection of recurrent parasitemia by microscopy warrants further studies to examine whether they may reflect viable parasite populations that may recrudescence when the concentrations of antimalarials become permissive during follow-up.

## List Of Abbreviations

ACT artemisinin-based combination therapy

DP dihydroartemisinin-piperaquine

G6PD glucose-6-phosphate dehydrogenase

MB methylene blue

*P. falciparum* *Plasmodium falciparum*

PQ primaquine

qRT-PCR real-time reverse transcriptase polymerase chain reaction

SBP1 skeleton binding protein 1

SP-AQ sulfadoxine-pyrimethamine

## Declarations

## Ethics statement and consent to participate

Ethical approval for the study was granted by the Ethics Committee of the Faculty of Medicine, Pharmacy, and Dentistry of the University of Science, Techniques, and Technologies of Bamako (Bamako, Mali), the Committee on Human Research at the University of California San Francisco (UCSF; San Francisco, CA, USA), and the Research Ethics Committee of the London School of Hygiene & Tropical Medicine (London,

UK). All study participants or their parents/guardians provided written informed consent prior to enrolment.

## Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## Consent for publication

All authors approved submission of this manuscript for publication.

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## Author's contributions

TB conceived the study. RG, CD, AD, and TB secured funding for the trial used in this analysis. AM, HD, KS, KD, SMN, RG, IG, AD, TB, and MER were involved in the implementation of the trial. AM, TB, and MER contributed to data analysis and writing of the manuscript, with editorial input from KL, WG, HD, RG, IG, CD, and AD. KL and WG conducted laboratory analyses. All authors have reviewed and approved of the final version of the manuscript.

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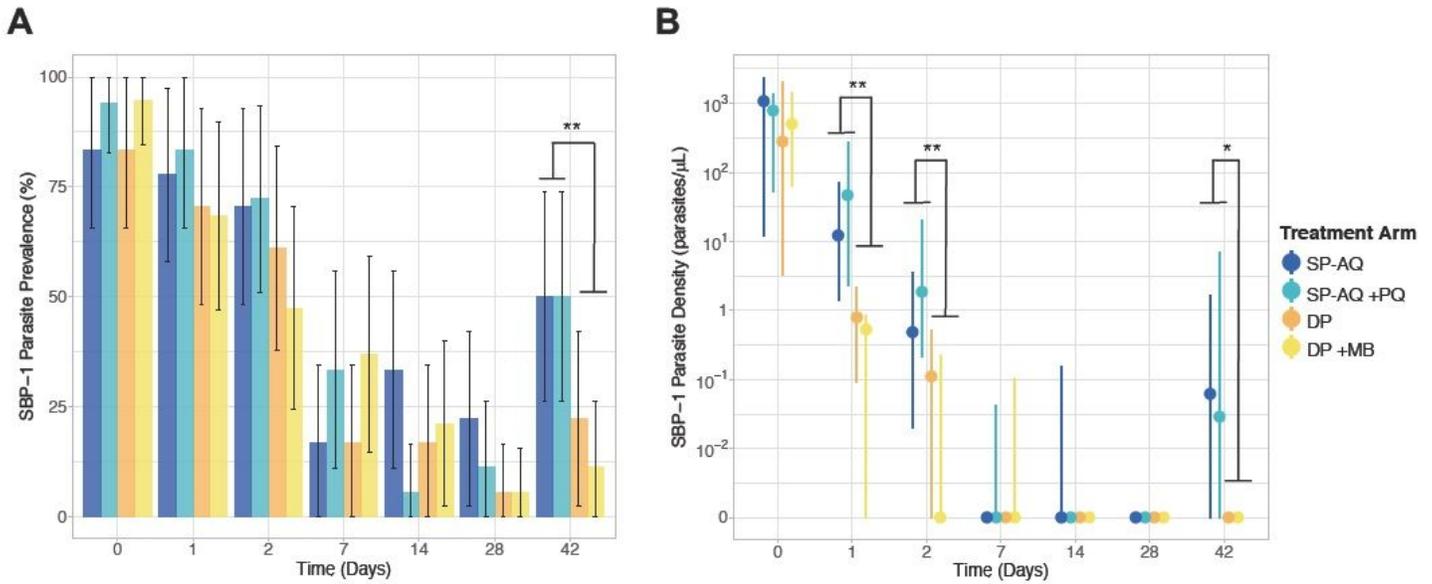
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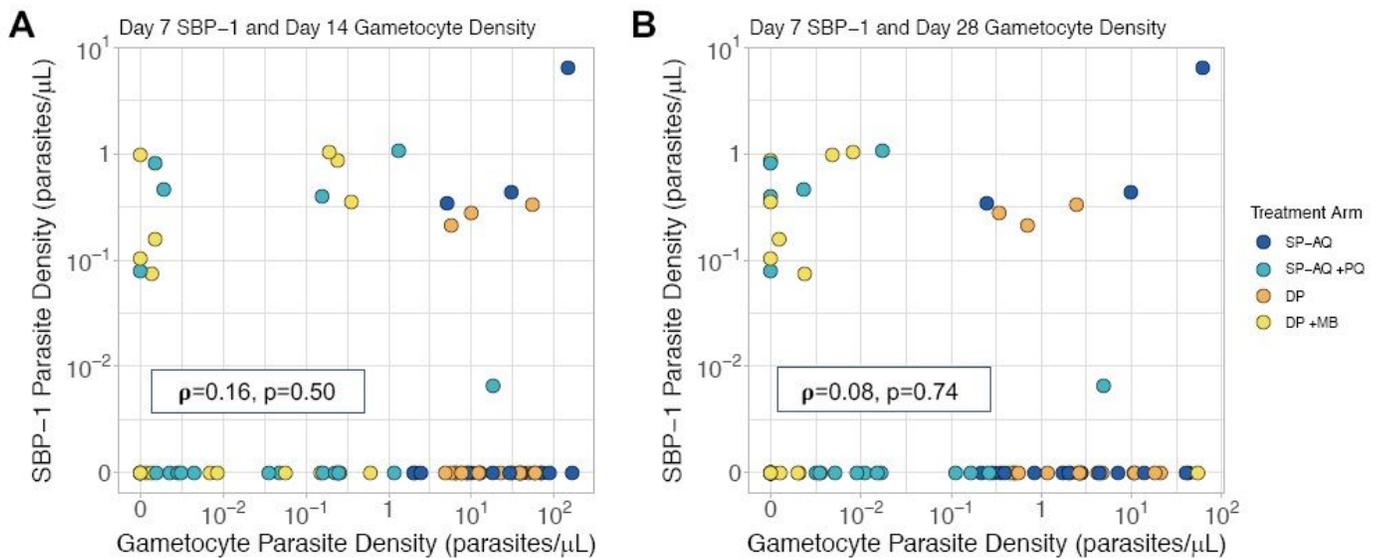
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## Figures



**Figure 1**

SBP-1 Parasite Prevalence (Panel A) and Density (Panel B) Between Treatment Arms Over Time. P-values were calculated using chi-squared or Fisher’s exact test to test differences in prevalence between ACT and non-ACT groups and Wilcoxon’s rank-sum test to test differences in parasite density. Note: \*\*p-value<0.0001, \*p<0.05



**Figure 2**

Correlation between Day 7 SBP-1 Parasite Density and Gametocyte Density on Days 14 (Panel A) and 28 (Panel B). Correlation ( $\rho$ ) and p-values were calculated only among those who had detectable levels of

ring-stage parasites on day 7 using Spearman's rank correlation. Different colors represent treatment arms.

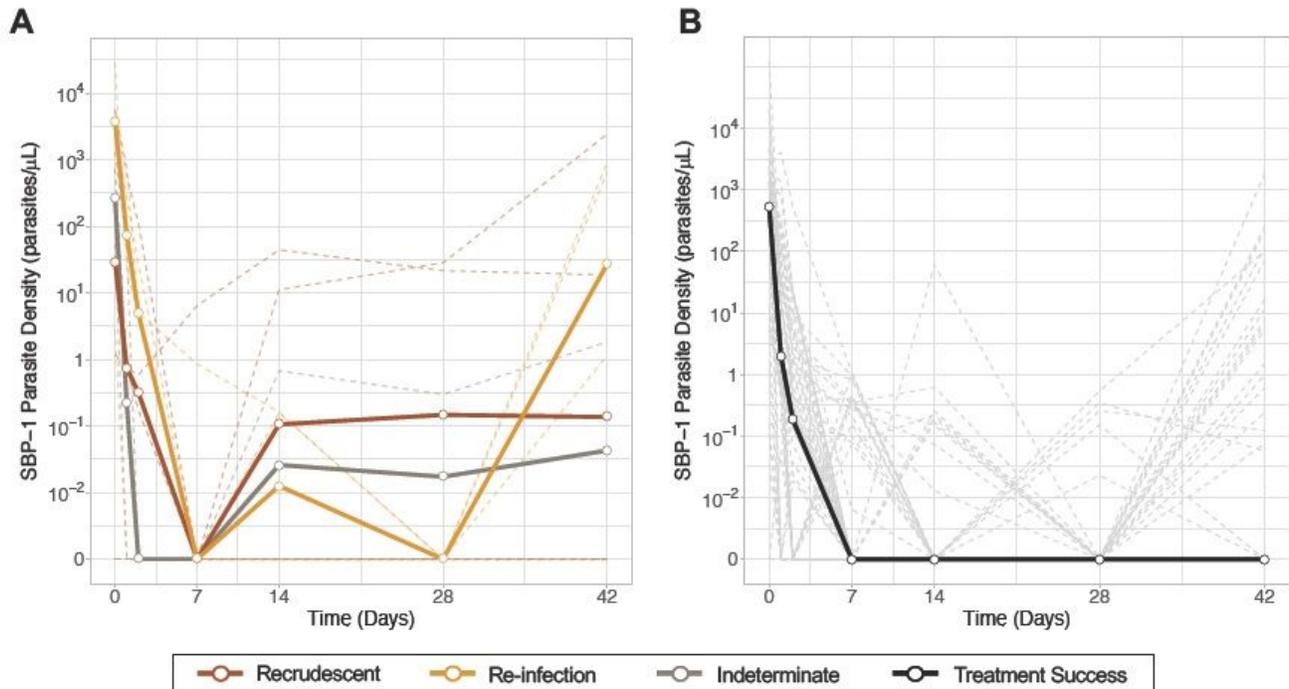


Figure 3

SBP-1 Parasite Density By Recurrent Infections and Days of Follow-up. Panel A includes participants with either recrudescent (dark red), re-infection (orange), or indeterminate (dark grey) infections. Panel B includes only participants who were successfully treated. Dark, bold lines indicate median values at each time point. Light, dashed lines indicate individual trajectories.

## Supplementary Files

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- [graphicalabstract.png](#)