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1 **Significant efficacy of single low dose primaquine compared to stand alone artemisinin**
2 **combination therapy in reducing gametocyte carriage in Cambodian patients with**
3 **uncomplicated multidrug resistant *Plasmodium falciparum* malaria**

4
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15
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18
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24 **Abstract (250 words)**

25 Since 2012, single low dose of primaquine (SLDPQ, 0.25mg/kg) has been recommended with
26 artemisinin-based combination therapies, as first-line treatment of acute uncomplicated
27 *Plasmodium falciparum* malaria, to interrupt its transmission, especially in low transmission
28 settings of multidrug, including artemisinin, resistance. Policy makers in Cambodia have been
29 reluctant to implement this recommendation due to primaquine safety concerns and lack of data
30 on its efficacy.

31 In this randomized controlled trial, 109 Cambodians with acute uncomplicated *P. falciparum*
32 malaria received dihydroartemisinin-piperazine (DP) alone or combined with SLDPQ on the
33 first treatment day. Transmission-blocking efficacy of SLDPQ was evaluated on Days 0, 1, 2, 3,
34 7, 14, 21, 28 and recrudescence by reverse transcriptase polymerase chain reaction (RT-PCR)
35 (gametocyte prevalence) and membrane-feeding assays with *Anopheles minimus* mosquitoes
36 (gametocyte infectivity). Without the influence of recrudescence infections, DP+SLDPQ reduced
37 gametocyte carriage 3 fold compared to DP. Of 48 patients tested on Day 0, only three patients
38 were infectious to mosquitoes (~6%). Post-treatment, three patients were infectious: on D14
39 (3.5%, 1/29), and on the first and seventh day of recrudescence (8.3%, 1/12 for each); this overall
40 low infectivity precluded our ability to assess its transmission blocking efficacy.

41 Our study confirms effective gametocyte clearance of SLDPQ when combined with DP in
42 multidrug resistant *P. falciparum* and the negative impact of recrudescence infections due to poor
43 DP efficacy. Artesunate-mefloquine (ASMQ) has replaced DP and ASMQ-SLDPQ has been
44 deployed to treat all *P. falciparum* symptomatic patients to further support the elimination of
45 multidrug resistant *P. falciparum* in Cambodia.

46 **Key words:** direct-membrane feeding assays, transmission-blocking, malaria, primaquine

47 **Introduction**

48 Malaria remains a major public health challenge with an estimated 228 million cases recorded in
49 2018 (1). While considerable progress has been made since 2010 when the estimated case burden
50 was 251 million, the recent emergence and spread of both artemisinin and piperaquine resistant
51 *Plasmodium falciparum* lineage (KEL1/PLA1) in the Eastern Greater Mekong subRegion
52 (GMS), threaten this remarkable global achievement (2-7). Eliminating rapidly multidrug
53 resistant *P. falciparum* (MDRPf) is, therefore, the top priority for countries of the Greater
54 Mekong Subregion (GMS). Implementation of novel tools and strategies or repurposing existing
55 tools like primaquine that specifically aim at interrupting malaria transmission are essential to
56 reach this goal.

57 Although artemisinin derivatives are the more potent compounds in artemisinin-based
58 combination therapies (ACTs) and are active against early gametocyte stages (8-10) and reduce
59 transmission (11, 12), the only currently available drug effective against the transmissible mature
60 stage V gametocytes is primaquine. In 2012, the WHO recommended the addition of single low
61 dose of primaquine (SLDPQ, target dose 0.25mg/kg) to ACTs to interrupt malaria transmission,
62 primarily in low transmission areas of MDRPf like the GMS (13).

63
64 To date, several clinical studies conducted in Africa, Colombia and Cambodia, have assessed the
65 safety and efficacy of SLDPQ (14-24), mainly by measuring gametocyte carriage [microscopy or
66 Taqman reverse transcriptase polymerase chain reaction (RT-PCR)] as a surrogate marker of its
67 transmission blocking efficacy. This is due to the challenging logistical requirements of carrying
68 out mosquito infectivity studies. These studies showed that SLDPQ, dosed from 0.20 to 0.75
69 mg/kg, clearly reduced gametocyte carriage and increased their clearance in a dose dependent

70 manner (15-17, 19, 21-25). A small number of studies have investigated human-to-mosquito
71 transmission, based on infectivity measures, with variable results due to low pre- and/or post-
72 SLDPQ infectivity (16, 17, 19, 22, 24). Only two studies from Mali demonstrated conclusively
73 the transmission-blocking efficacy of SLDPQ which produced a 92.6% to 100% within-person
74 reduction in infectivity at Day 2 vs. baseline following 0.25 mg/kg of SLDPQ (16, 19).

75
76 In Cambodia, glucose-6-phosphate dehydrogenase deficiency (G6PDd) is a common X-linked
77 disorder of the red blood cells with frequencies ranging from 10.8% to 29.6% in males (26) and a
78 prevalence rate in malaria patients seen at health centers estimated at 13.9% (27). This is the
79 main reason why Cambodian policy makers have been reluctant to deploy SLDPQ without local
80 data on the safety and the efficacy of SLDPQ. Therefore, we conducted a trial assessing the
81 tolerability and the safety of SLDPQ (0.25mg/kg) in Cambodia in 2015-2016 (28), which
82 followed on from a safety trial of 0.75 mg/kg/week of PQ in vivax patients (29). Here, we present
83 our investigations on the transmission-blocking efficacy of this treatment through the evaluation
84 of gametocyte prevalence dynamics as well as infectivity measures on a subset of patients using
85 membrane-feeding assays with *Anopheles minimus* mosquitoes, one of the main malaria vectors
86 in Southeast Asia.

87

88 **Methods**

89 Study design

90 This open-label randomized control trial assessing the tolerability and the safety of SLDPQ was
91 carried out in Banlung, Rattanakiri province in northeastern Cambodia in 2015-2016

92 (NCT02434952), as described in Dysoley *et al.* (28). Briefly, 109 non pregnant, non breast
93 feeding patients aged ≥ 1 year with acute uncomplicated falciparum malaria (≥ 1 asexual
94 parasite/500 white blood cells equivalent to 16 asexual parasites/ μl) and a hemoglobin
95 concentration $\geq 6\text{g/dL}$ were recruited to receive either dihydroartemisinin piperazine (DP, Duo-
96 Cotecxin®, D40 mg and P320 mg, Zhejiang Holley Nanhu Pharmaceutical Co. Ltd, Jiaxing,
97 Zhejiang, China) alone or combined with SLDPQ (0.25mg/kg given with the first dose of DP,
98 Thai Government Pharmaceutical Organization, 15mg of primaquine base). Clinical and
99 laboratory assessments were performed on Days (D) 0, 1, 2, 3, 7, 14, 21 and 28. *Anopheles*
100 *minimus* mosquitoes were fed on blood samples collected from a subset of 55 patients and on the
101 days of recurrent (Drec) falciparum parasitaemia (defined by the WHO as late treatment failures)
102 depending on mosquito availability using direct-membrane feeding assays (DMFA).

103 The sample size calculation was based on the primary outcome of a reduction in the mean D7
104 hemoglobin (Hb) concentration of 1 g/dL in the G6PDd DP+SLDPQ arm vs. G6PDn DP+SLDPQ
105 arm. Assuming a mean and standard deviation of 11.27 (1.74) g/dL, calculated from a SE Asian
106 database of ~6,800 ACT-treated, falciparum-infected patients of all ages (WRJT, unpublished), a
107 two-sided alpha of 0.05, power of 80%, the sample size was 48/arm, rounded up to 50. G6PD
108 status in the field was diagnosed initially using the fluorescent spot test to allow for the allocation
109 of SLDPQ. The qualitative Carestart® (AcessBio, Solersset, NJ) rapid diagnostic test was assessed
110 in parallel.

111

112 Mosquito infection

113 DMFAs were carried out to assess individual malaria infectivity as described previously (30).
114 Briefly, 3-to-5-day-old *An. minimus* females were fed through membranes on patient's blood.

115 Mosquitoes were starved for 24h before being provided a blood meal. Venous blood samples
116 were collected in heparinized tubes and 400µl of blood were made available in membrane feeders
117 maintained at 37°C. Females were fed only once on freshly drawn blood. Post-feeding, unfed
118 females were discarded and fed females were kept in cages (20x20x20cm) with constant access to
119 a 10% sucrose solution. Patient infectivity was determined by assessing infection prevalence (i.e.
120 proportion of infected females) and infection intensity (i.e. number of *Plasmodium sp.* oocysts in
121 infected females). Midguts were dissected in a 1% Mercurochrome[®] stain and the presence and
122 the number of oocysts were determined under a microscope (20x magnification). Dissections
123 were performed 7-day post-blood meal. The mean number of dissected females was 49 (range:
124 15-85, median: 50).

125
126 Biological investigations
127 Parasite RNA was extracted from the Trizol[®] (Life Technologies Holdings Pte Ltd, Singapore)
128 conserved whole blood samples using QIAamp RNA Blood Mini Kit[®] (Qiagen, Germany),
129 following the protocol recommended by the manufacturer. A two-step semi-quantitative real time
130 PCR was performed to detect malaria parasites, as previously described (31). Following PCR
131 amplification, falciparum positive samples were analyzed for the presence of gametocytes by a
132 Taqman RT-PCR, using primers spanning an exon-exon junction and targeting the “*Plasmodium*
133 *falciparum* meiotic recombination protein DMC1-like protein” gene (AF356553), as described
134 previously (32). Gametocyte dilution series based on *in-vitro* cultured local strain were used to
135 estimate gametocyte blood concentrations.

136
137 Ethical statement

138 Ethical approvals were obtained from the Cambodian National Ethics Committee for Health
139 Research (0370NECHR) and the trial registered at the clinicaltrials.gov (NCT02434952). The
140 protocols conformed to the Helsinki Declaration on ethical principles for medical research
141 involving human subjects (version 2002) and informed written consent was obtained for all
142 volunteers.

143
144 Statistical analyses
145 Initially, univariate Generalized Linear Mixed Models (GLMMs) with a binomial distribution
146 were fitted to model the PCR measured, gametocyte prevalence rates. In these models, follow-up
147 days (D1, 2, 3, 7, 14, 21 and 28), treatment (DP vs. DP+SLDPQ), presence of gametocytes prior
148 to treatment on D0, recrudescence status (recrudescent vs. cured patient) and hemoglobin
149 concentration prior to treatment on D0 were included in the model as fixed factors. Patient ID
150 was coded as a random factor to account for repeated measures on the same individual. The
151 univariate analyses were followed by a multivariable GLMM to model the dynamics of
152 gametocyte prevalence post-treatment including all the significant factors from the univariate
153 analysis.

154 Gametocyte clearance times were not compared in the study for three principal reasons: (i) of the
155 21 patients with microscopically detected D0 gametocytes, only 7 had complete follow up
156 microscopy data, (ii) Taqman RT-PCR measured gametocyte densities were highly variable
157 during the 28 days follow-up, and, (iii) by D28, 10/34 patients with gametocytes on D0 by
158 Taqman RT-PCR were still gametocytemic (n=1, DP+SLDPQ arm). *P*-values <0.05 were deemed
159 significant. All analyses were performed in R v.3.5.1 (33).

160

161 **Results**

162 One hundred nine patients with uncomplicated falciparum malaria were enrolled and treated with
163 the standard 3-day DP alone (48.6%, 53/109) or DP+SLDPQ (51.4%, 56/109). Seven patients
164 were lost to follow up (6.4%). Of the remaining 102 patients, 28 (27.4%) had PCR-proven *P.*
165 *falciparum* recrudescences; 24 occurred within the 28-day follow up on Days 12 (1), 14 (1), 17
166 (2), 18 (1), 21 (2), 22 (2), 23 (1), 24 (2), 25 (1), 26 (2), 27 (1), 28 (8), and four on D44, 46, 52,
167 and 124. Of the 28 recrudescence patients, 10 were retreated with the standard 3-day DP and 18
168 with quinine or quinine plus tetracycline; six of the 10 patients retreated with DP experienced a
169 second recrudescence.

170 At D0, gametocytes were detected in 47/109 patients (43.1%) by Taqman RT-PCR (Table 1; see
171 30 for further details), with no significant difference between arms ($P=0.3057$).

172

173 *Gametocyte dynamics*

174 Examining the dynamics of Taqman RT-PCR gametocyte carriage to D28, drug arm, baseline
175 gametocytemia, recrudescence status, baseline hemoglobin and Day of follow up were all
176 significant factors for gametocytemia in the univariate analysis (Table 2).

177 The DP recipients were more likely to be gametocytemic than DP+SLDPQ recipients during the
178 follow-up, seen more clearly on D7 and D14 (Table 2; Figure 1) as were patients with baseline
179 gametocytemia (Table 2; Figure 2) and recrudescence vs. cured patients (Table 2).

180 Baseline hemoglobin concentration and Day of follow-up were inversely associated with the
181 probability of being gametocytemic during the follow-up (Table 2).

182 When the aforementioned factors were tested in a multivariable model, all except drug arm
183 remained significant in the multivariate analysis (Table 3, model 1). However, when removing
184 recrudescence from the multivariable model, drug arm was a significant explanatory factor again
185 (Table 3, model 2).

186 Post-hoc calculation showed that there was a 99.1% power to detect a difference between the DP
187 and DP-PPQ treatment with the study sample sizes (n=49 and n=56 on D7 for DP and DP-PPQ
188 respectively).

189

190 *Mosquito infectivity*

191 A total of 55 patients were included in the DMFAs. Amongst the 292 feeding assays, only 113 of
192 14,444 (0.78%) dissected mosquitoes were infected (Table 4). Overall, 3 out of 48 ($6.3 \pm 6.8\%$)
193 individuals infected at least one mosquito at baseline, one individual was infectious on D14 (DP)
194 and two individuals with recrudescence infections (Tables 4 & 5) who were repeat DP treatment
195 failures: (i) at presentation on D124 (DP+SLDPQ), and (ii) D35 (7 days post DP started on D28).
196 The three individuals infectious on D0 were all gametocytemic by microscopy, received
197 DP+SLDPQ and were not infectious on D1 or D3 post-treatment (Table 5).

198

199 **Discussion**

200 In 2012, the WHO recommended adding 0.25 mg/kg SLDPQ to first-line treatments (ACTs) to
201 kill mature gametocytes and block human-to-mosquito transmission (13). As no data were
202 available at that time in Cambodia, we investigated the transmission-blocking efficacy of SLDPQ
203 when given with DP (the recommended first line standard ACT in 2015) by evaluating

204 gametocyte prevalence dynamics (Taqman RT-PCR) and *Anopheles minimus* infectivity by
205 DMFA.

206 Our results show that SLDPQ, given on the first day of the 3-day standard ACT treatment,
207 significantly decreased gametocyte carriage over time, starting on D3 and was least on D7
208 resulting in a 5.59 fold reduced risk, relative to DP alone, over 28 days in the univariate analysis.
209 This is consistent with recent reports on the efficacy of PQ in reducing gametocyte carriage (15-
210 17, 19, 21-25, 34) and earlier reports showing a lag between reduced gametocyte carriage and
211 rapid mosquito infectivity. The multivariable analysis revealed the significant effect of
212 recrudescence (resistant) infections on gametocyte carriage with a 3 fold reduction in gametocyte
213 carriage in the absence of resistance.

214
215 Our patients had a high rate of baseline gametocytemia (19% by microscopy, just under 45% by
216 Taqman RT-PCR), which is similar to that reported previously in 2010 in artemisinin resistant
217 western Cambodia (~19%) but is three fold higher than the 6% reported earlier in Rattanakiri
218 province (35). Given the high prevalence of *PfKelch13* mutant *P. falciparum* parasites (63%) in
219 our study and the very high recrudescence rate (~28%), our high gametocyte carriage rate is
220 consistent with the presence of ARPF, which has also led to piperaquine resistance (7). This is
221 consistent with another study in Cambodia which showed that DP treated patients from Oddar
222 Meanchey province (northern Cambodia) also had prolonged gametocyte carriage, irrespective of
223 the slow asexual parasite clearance time, due to ARPF, and in patients with recrudescence
224 infections (22, 35).

225

226 However, prolonged gametocyte carriage has also been seen in DP treated African patients
227 without ARPf who, nevertheless, had low human-to-mosquito transmission (16, 35, 36).
228 Moreover, several comparative studies have found that gametocyte carriage is higher in DP
229 compared to artemether lumefantrine (36-39), suggesting reduced gametocytocidal activity of
230 piperazine compared to lumefantrine.

231
232 Overall, we observed a biphasic pattern of gametocyte prevalence with a drop on D7, a rebound
233 on D14, followed by a decrease on D21 and D28. However, this effect was mainly driven by the
234 DP treated patients as their gametocyte carriage rate was similar over the 28-day follow-up
235 whereas it decreased markedly in the DP+SLDPQ treated patients (Figure 1).

236
237 We were not able to assess the transmission-blocking efficacy of SLDPQ due to the very low
238 infectivity rate observed in only three patients (~6%) at baseline and three infectious events
239 during follow up. This is a major limitation of DFMA, previously reported by Lin *et al.* (22) at
240 baseline using *Anopheles dirus* mosquitoes and on D7, without assessing baseline, by Okebe *et al*
241 (24). In another study in African children, despite a higher infectivity rate at enrollment after
242 microscopy-based selection of gametocyte positive asymptomatic children (38%), Gonçalves *et*
243 *al.* (17) were also unable to detect the added value of SLDPQ due to very low infectivity post-
244 treatments in all arms. As our clinical trial aimed primarily at assessing SLDPQ tolerability and
245 safety, we did not particularly select patent or high-density gametocyte carriers and thus would
246 have needed substantially more individuals to assess transmission-blocking efficacy. Only two
247 studies in Mali statistically confirmed high anti-infectivity efficacy (>90%) at 48h of SLDPQ,
248 when dosed at baseline, after selecting gametocyte positive participants by microscopy (16, 19).

249 However, we observed that the three SLDPQ treated infectious patients on D0 did not infect
250 mosquitoes on subsequent days despite the presence of gametocytes. Several reasons may explain
251 this finding. Following primaquine, an anti-infectivity effect is rapid ($\leq 24h$) and precedes the
252 decline in gametocytaemia (40). Male gametocytes seem to be more sensitive to primaquine than
253 female gametocytes (41) and *P. falciparum* sex ratio is biased towards females, potentially
254 resulting in delayed gametocyte clearance. The relationship between gametocytemia and
255 infectivity is nonlinear and is, thus, an indirect measure of infectivity (42-44).

256
257 To conclude, our study shows that the addition of SLDPQ to ACT treatment in symptomatic
258 falciparum malaria decreases substantially gametocyte carriage in patients with MDRPf.
259 Although unable to reconfirm the elegant studies of Dicko *et al.* (16, 19), SLDPQ, dosed on the
260 first day of treatment, is likely to further decrease the transmission potential of *P. falciparum*
261 patients in our setting when combined with an effective ACT.

262 Owing to the high failure rate of DP, Cambodia has switched to artesunate-mefloquine treatment
263 as first line treatment for uncomplicated *P. falciparum* and in January 2018 deployed SLDPQ in
264 the whole country, based partly on the results of these findings and the good tolerability of
265 SLDPQ in G6PDd patients (28). An age based dosing regimen of SLDPQ, designed for
266 Cambodia, will aid elimination efforts where weighing scales are unavailable (45).

267

268 **Conflicts of interest.** All authors declare no conflicts of interest.

269

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283

284

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- 468

469 **Tables**470 **Table 1.** Baseline characteristics of the two study groups of patients

	DP	DP + SLDPQ
No. enrolled	53	56
No. recrudescent	18	10
Male: Female	44:9	44:12
Mean age, yrs (SD)	25 (16)	26 (14)
G6PD deficient: G6PD normal	3:50	6:50
Number of gametocyte positive slide	14	7
Mean gametocytemia / μ l (SD, range)	64 (250, 0-1432)	40 (149, 0-787)
Number of positive gametocyte Taqman RT-PCR	26	21
Hemoglobin concentration (g/dL)	12.63	13.19
Anemia (Hb<11g/dL)	10/51	9/56

471

472 **Table 2.** Independent factors associated with change in gametocytemia over 28 days by
 473 univariate analysis. Odds ratio < 1 indicates a negative association.

Factor		OR	95% CI	P-value
Treatment	DP+SLDPQ			
	DP	5.59	1.71 , 18.27	0.0044
D0 status	D0 negative			
	D0 positive	15.83	4.83 , 51.91	<0.0001
Recrudescence status	Cured patient			
	Recrudescent patient	11.11	2.78 , 44.36	0.0006
D0 hemoglobin		0.46	0.34 , 0.63	<0.0001
Day of follow-up		0.94	0.91 , 0.96	<0.0001

474

475

476 **Table 3.** Multivariable models (with and without recrudescence) of the factors associated with
 477 changes in gametocytemia over 28 days.

Model	Factors	adjOR	95% CI	P-value
Model 1	Treatment	2.39	0.81 , 7.08	0.1152
	D0 status	7.35	2.27 , 23.78	0.0009
	Recrudescence	6.02	1.70 , 21.26	0.0053
	D0 hemoglobin	0.57	0.42 , 0.77	0.0003
	Day of follow-up	0.93	0.90 , 0.96	<0.0001
Model 2	Treatment	3.15	1.09 , 9.11	0.0344
	D0 status	6.63	2.10 , 20.87	0.0012
	D0 hemoglobin	0.55	0.41 , 0.75	0.0001
	Day of follow-up	0.93	0.90 , 0.96	<0.0001

478

479

480 **Table 4.** Infectivity rates (No. of positive isolates/No. of tested isolates) defined by treatment
 481 assessed by direct-membrane feeding assays during the 28-day follow-up.

Follow-up day	DP	DP+SLDPQ	Total	Infectivity rate
D0	0/15	3/33	3/48	6.3 ± 6.8 %
D1	0/1	0/6	0/7	0
D2	0/12	0/26	0/38	0
D3	0/15	0/31	0/46	0
D7	0/11	0/24	0/35	0
D14	1/10	0/19	1/29	3.5 ± 6.6 %
D21	0/1	0/4	0/5	0
D28	0/6	0/22	0/28	0
rec1D0	0/4	1/8	1/12	8.3 ± 15.6 %
rec1D3	0/4	0/6	0/11	0
rec1D7	1/6	0/6	1/12	8.3 ± 15.6 %
rec1D14	0/4	0/1	0/4	0
rec1D28	NA	0/1	0/1	0
rec1D35	NA	0/1	0/1	0
rec2D0	0/4	0/1	0/5	0
rec2D3	0/4	0/1	0/5	0
rec2D7	0/4	0/1	0/5	0
Total DMFA	101	191	292	2 ± 1.6 %

482

483 DP – dihydroartemisinin piperazine
 25

484 SLDPQ – single low dose primaquine

485 Rec1 and Rec2 correspond to the first and second recrudescences, respectively.

486 Positive isolates are isolates which were able to infect at least one mosquito from direct-
487 membrane feeding assay (DFMA).

488

489 **Table 5.** Details of the five patients, infectious to *A. minimus* by direct-membrane feeding assays
 490 during the 28-day follow-up.

Day	Patient ID	Treatment Given	Sex	Age	Gametocytemia (gametocytes/ μ l)	Gametocytes detected by Taqman RT-PCR	Infection prevalence (proportion of infected females \pm 95%CI)	Infection intensity (mean number of oocysts \pm SE)
D0	D_002	DP +SLDPQ	F	29	562	Positive	41.7 \pm 14	4.7 \pm 0.97
D1					336	Positive	0	NA
D2					48	Positive	0	NA
D3					0	Positive	0	NA
D7					NA	Negative	0	NA
D14					NA	Negative	0	NA
D21					NA	Negative	0	NA
D28					0	Positive	0	NA
Rec1D0 (D124)	D_002	QN+TE			24	Positive	10 \pm 8.3	2.6 \pm 1.4
Rec1D3 (D127)					64	Positive	0	NA
Rec1D7 (D131)					168	Positive	0	NA
D0	N_015	DP+ SLDPQ	M	23	787	Positive	16 \pm 10	2.12 \pm 0.4

D1					2208	Positive	0	NA
D2					40	Positive	0	NA
D3					176	Positive	0	NA
D7					NA	Negative	0	NA
D14					NA	Negative	0	NA
D28					NA	Negative	0	NA
Rec1D0 (D28)	N_035	QN	F	8	1823	Positive	0	NA
Rec1D3 (D31)					11912	Positive	0	NA
Rec1D7 (D35)					7904	Positive	94.3 ± 7.7	82.6 ± 5.5
D0	N_046	DP	M	8	1432	Positive	0	NA
D2					2904	Positive	0	NA
D3					3952	Positive	0	NA
D7					1952	Positive	0	NA
D14					432	Positive	6 ± 6.5	1
D28					16	Positive	0	NA
D0	N_074	DP +SLDPQ	M	25	540	Positive	86.3 ± 9.4	11.3 ± 1.7
D3					8	Positive	0	NA

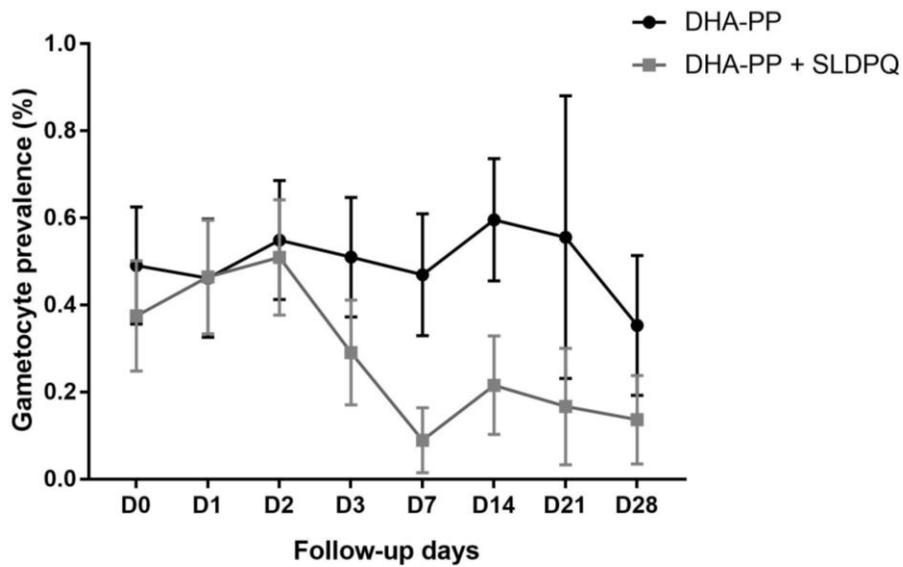
491

492 QN+TE: Quinine + tetracycline

493 Rec1 corresponds to the first recrudescence, Day post initial infection is indicated in brackets.

494 NA: Data not available

496 **Figures**

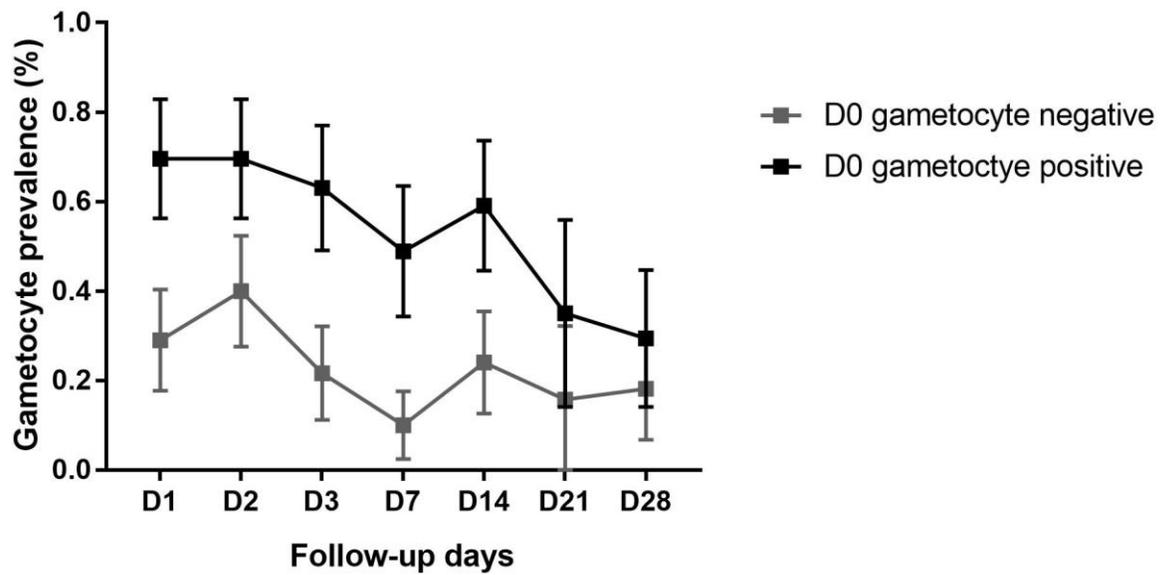


497

498 Figure 1.

499 Gametocyte prevalence by Taqman RT-PCR by follow-up day and treatment group. Data show
500 proportion \pm 95% confidence intervals.

501



502
 503 Figure 2.
 504 Gametocyte prevalence by Taqman RT-PCR by follow-up day and presence of gametocyte prior
 505 to treatment on D0 detected by Taqman RT-PCR. Data show proportion \pm 95% confidence
 506 intervals.
 507