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The transmission-blocking effects of antimalarial drugs revisited: fitness costs and sporontocidal effects of artesunate and sulfadoxine-pyrimethamine

Villa M^{1*}, Buysse M^{1,2}, Berthomieu A^{1,2}, Rivero A^{1,2}

¹MIVEGEC (CNRS – IRD – Université de Montpellier)

²CREES (Centre d'Écologie et Évolution de la Santé), Montpellier
911 avenue Agropolis, 34394 Montpellier, France

*Corresponding author (manon.villa@ird.fr)

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25 **Abstract**

26 Assays used to evaluate the transmission-blocking activity of antimalarial drugs are largely
27 focused on their potential to inhibit or reduce the infectivity of gametocytes, the blood stages of the
28 parasite that are responsible for the onward transmission to the mosquito vector. For this purpose, the
29 drug is administered concomitantly with the gametocyte-infected blood, and the results are evaluated
30 as the % reduction in the number of oocysts in the mosquito midgut.

31 We report the results of a series of experiments that explore the transmission-blocking potential
32 of two key antimalarial drugs, artesunate (AS) and sulfadoxine-pyrimethamine (SP), when
33 administered to mosquitoes already infected from a previous blood meal. For this purpose, uninfected
34 mosquitoes and mosquitoes carrying a 6-day old *Plasmodium relictum* infection (early oocyst stages)
35 are allowed to feed either on a drug-treated or an untreated host in a fully factorial experiment. This
36 protocol allows us to bypass the gametocyte stages and establish whether the drugs have a
37 sporontocidal effect, i.e. whether they are able to arrest the ongoing development of oocysts and
38 sporozoites, as would be the case when a mosquito takes a post-infection treated blood meal. In a
39 separate experiment, we also explore whether a drug-treated blood meal impacts key life history traits
40 of the mosquito relevant for transmission, and if this depends on their infection status.

41 Our results show that feeding on an AS- or SP-treated host has no epidemiologically relevant
42 effects on the fitness of infected or uninfected mosquitoes. In contrast, when infected mosquitoes feed
43 on an SP-treated host, we observe both a significant increase in the number of oocysts in the midgut,
44 and a drastic decrease in both sporozoite prevalence (-30%) and burden (-80%) compared to the
45 untreated controls. We discuss the potential mechanisms underlying these seemingly contradictory
46 results and contend that, provided the results are translatable to human malaria, the potential
47 epidemiological and evolutionary consequences of the current preventive use of SP in malaria-endemic
48 countries could be substantial.

49 **Keywords:** transmission-blocking interventions, vaccines, antimalarial drugs, avian malaria

50 **1. Introduction**

51 Synthetic antimalarial drugs are the mainstay for the prevention and treatment of malaria
52 throughout the world. Although the prime purpose for these antimalarials is to prevent or cure the
53 infection of the patients, it has become rapidly obvious that they can also be used to reduce the
54 prevalence of the disease in the population by reducing the onward transmission of the parasite by the
55 vector (Sinden et al., 2012; Wadi et al., 2019). The transmission-blocking effect of antimalarial drugs
56 can take place in three different, albeit non-exclusive, ways. Firstly, drugs may be able to kill, arrest
57 the maturation, alter the sex ratio or reduce the infectivity of gametocytes, the sexual stages of the
58 parasite that are present in the blood and are responsible for the transmission to the mosquito.
59 Secondly, drugs may have a sporontocidal effect, i.e. they may be able to hinder the sporogonic cycle
60 of the parasite within the mosquito. *Plasmodium* development inside the mosquito is complex and
61 involves the fusion of male and female gametocytes to form a zygote, the passage of the mobile zygote
62 through the midgut wall to form an 'oocyst' that grows, undergoing successive mitosis, ruptures and
63 releases thousands of 'sporozoites' that migrate to the salivary glands. Antimalarial drugs, or their
64 metabolites, can find their way to the mosquito midgut where they can block the parasite either
65 directly, by being toxic to any of the above stages of the parasite, or indirectly, by disturbing the fine-
66 tuned mosquito physiological pathways that are essential for parasite development (Sinden et al.,
67 2012). Finally, just as some antimalarials have unwanted secondary effects in the host, so they may be
68 able to adversely affect key life history traits of the mosquito essential for parasite transmission such as
69 its longevity or host seeking behaviour.

70 To date, the majority of experimental studies have focused on the gametocytocidal effects of
71 antimalarial drugs (Delves et al., 2018; Ruecker et al., 2014). Gametocytes are an attractive target for
72 transmission-blocking interventions because they constitute an important bottleneck in the life cycle of
73 the parasite and can be directly targeted due to their presence in the bloodstream of the host. While
74 some of the available compounds may be able to achieve a 100% gametocyte inhibition, and thus
75 completely block the transmission cycle, many of the compounds being tested result in a partial, if at

76 times substantial, gametocyte reductions (Sanders et al., 2014). Partial reductions may, however, fail to
77 accurately reflect the degree to which the mosquitoes become infected (Churcher et al., 2013; Sinden,
78 2017), and may even end up enhancing transmission due to higher mosquito survival rates associated
79 with lower oocyst burdens (Sinden, 2010). It has therefore been argued that interventions that aim to
80 target gametocytes should be combined with others that target the later stages of parasite development
81 within the mosquito (Blagborough et al., 2013; Paaijmans and Fernández-Busquets, 2014; Sinden,
82 2010). Whether antimalarial drugs have an effect on oocyst or sporozoite development, however, is
83 still largely unknown, as most protocols provide the drug with the infected blood meal thereby
84 conflating the effects on the gametocytes with the effects on the later stages (Delves et al., 2018; Wadi
85 et al., 2018).

86 Current WHO advice for the treatment of malaria in endemic countries relies heavily on the use
87 of two synthetic antimalarial drugs: artesunate and sulfadoxine-pyrimethamine (WHO, 2019).
88 Artesunate (henceforth AS), a potent and fast-acting artemisinin derivative, is used as a treatment for
89 severe/complicated malaria, or in combination with longer-acting antimalarial drugs such as
90 sulfadoxine-pyrimethamine, amodiaquine or mefloquine for the treatment of children and adults with
91 uncomplicated malaria (WHO, 2019). Sulfadoxine-pyrimethamine (henceforth SP), on the other hand,
92 is recommended in areas with moderate to high malaria transmission for the intermittent preventive
93 treatment (ITP) of pregnant women and infants (0-1 years) and, in areas of high seasonal transmission,
94 for the seasonal malaria chemoprevention (SMC) of young children (<5 years of age, WHO, 2019).
95 Each year, millions of people throughout the world get treated by one of these drugs (WHO, 2019).
96 Sulfadoxine and pyrimethamine act synergistically to inhibit the activity of dihydropteroate synthase
97 (DHPS) and dihydrofolate reductase (DHFR), respectively, thus inhibiting the folic acid metabolism of
98 the parasite (Peterson et al., 1988). They are both long-lasting drugs, with plasma concentrations being
99 found up to 42 days post treatment (Karunajeewa et al., 2009).

100 The gametocytocidal potential of both drugs has been the subject of numerous studies (review
101 in Butcher, 1997; Wadi et al., 2019). AS has a demonstrated cytotoxic activity against mature and

102 immature gametocytes *in vitro* (Chotivanich et al., 2006; Peatey et al., 2012). Evidence of the
103 gametocytocidal effects of SP, on the other hand, is contradictory. Certain studies have found that SP
104 has no gametocidal activity (Miguel-Blanco et al., 2015; Plouffe et al., 2016), others that SP inhibits
105 male gametocyte formation (Delves et al., 2012, 2013) and yet others that pyrimethamine
106 administration results in an increased gametocyte production, possibly as an adaptive response of the
107 parasite to stressful conditions (Schneider et al., 2018).

108 Here, we report the results from a series of experiments that aim to investigate the
109 transmission-blocking potential of AS and SP downstream from their putative cytotoxic effects on the
110 gametocytes. For this purpose, we performed a series of factorial experiments feeding infected and
111 uninfected mosquitoes either on drug-treated or control hosts. Experiments are carried out using the
112 avian malaria parasite, *Plasmodium relictum* and its natural vector, the mosquito *Culex*
113 *quinquefasciatus*, one of the few available systems in which these experiments are both technically and
114 ethically possible. In order to bypass the gametocytocidal effects of the drug, the 'infected' mosquitoes
115 were exposed to the drug while carrying a 6-day old infection from a previous blood meal
116 (corresponding the early oocyst stages, Pigeault, 2015). Under our standard laboratory conditions, 5-6
117 days is the average length of the *Cx quinquefasciatus* gonotrophic cycle.

118 Avian malaria has played a key historical role in the study of human malaria, being a stimulus
119 for the development of medical parasitology (Rivero and Gandon, 2018). It has played a particularly
120 pivotal role in the screening and clinical testing of the first synthetic antimalarials (Coatney et al.,
121 1953; Hewitt, 1940; Rivero and Gandon, 2018) and in the study of their potential use as transmission-
122 blocking compounds (Gerberg, 1971; Ramakrishnan et al., 1963; Terzakis, 1971). Compared with
123 rodent malaria, the avian malaria system has the added advantage of using the parasite's natural vector
124 in the wild, the mosquito *Culex pipiens*, thereby sidestepping the issues associated with mosquito-
125 parasite combinations without a common evolutionary history (Cohuet et al., 2006; Dong et al., 2006).

126 Our aims were to establish: 1) whether drugs administered to infected mosquitoes arrest the
127 ongoing development of oocysts and/or sporozoites, and 2) whether the drugs have an effect on the

128 fitness of infected and uninfected mosquitoes. Our results provide insights into the multiplicity of
129 effects that a given drug may have in the different stages of the parasite's sporogonic cycle. We discuss
130 the potential epidemiological and evolutionary consequences of using AS and SP to reduce
131 transmission of *Plasmodium* in the field.

132

133 **2. Material and Methods**

134

135 **2.1. Mosquito and parasite protocols**

136 All experiments were carried out using a laboratory strain of *Culex pipiens quinquefasciatus*
137 (SLAB strain). *Culex* mosquitoes are the most important natural vector of avian malaria in Europe and
138 the Americas (Vézilier et al., 2010). The larvae in all the experiments were reared at a constant density
139 per tray (n=300 larvae) following previously published laboratory protocols (Vézilier et al., 2010).
140 Larval trays (n=22) were placed individually inside an “emergence cage” (40 cm x 28 cm x 31 cm) and
141 emerged adults were allowed to feed *ad libitum* on a 10% glucose water solution. Rearing and
142 experiments took place at our standard insectary conditions (24-26 °C, 60-80% RH, and 12:12 L:D
143 photoperiod).

144 *Plasmodium relictum* (lineage SGS1) is the aetiological agent of the most prevalent form of
145 avian malaria in Europe. The parasite lineage was isolated from blue tits (*Parus caeruleous*) collected
146 in the Montpellier area in October 2016 and subsequently passaged to naïve canaries (*Serinus canaria*)
147 by intraperitoneal injection. Since then, it has been maintained by carrying out regular passages
148 between our stock canaries through intraperitoneal injections with the occasional passage through the
149 mosquito.

150

151 **2.2. Impact of antimalarials on Plasmodium-infected and uninfected mosquito traits**

152 The purpose of these experiments was to establish whether feeding from a sulfadoxine-

153 pyrimethamine (SP) or an artesunate (AS) treated host can negatively influence mosquito traits such as
154 longevity and fecundity. For this purpose, two separate experiments were set up (see Figure 1a).

155 2.2.1. Sulfadoxine-pyrimethamine experiment

156 To obtain infected and uninfected mosquitoes to use in the experiment, 200 female mosquitoes
157 were placed in a cage containing either an infected (parasitemia: 1.21%, 2.01%, 4.43% and 4.57%) or
158 an uninfected bird (n=4 and n=3 cages of infected and uninfected birds respectively). Infected birds
159 were obtained by injecting them with 100 μ L of blood from our *P. relictum*-infected canary stock.
160 Mosquito blood feeding took place 10 days after the injection, to coincide with the acute phase of the
161 *Plasmodium* infection in the blood (Cornet et al., 2014; Pigeault et al., 2015). After the blood meal,
162 which took place overnight, the bird was taken out of the cage, unfed mosquitoes were discarded and
163 engorged mosquitoes were provided with a 10% sugar solution.

164 Three days later, a tray with water was placed inside the cage to allow egg laying (and hence
165 the completion of the mosquito's gonotrophic cycle). Seven days post blood meal (pbm) 20
166 mosquitoes were haphazardly chosen from each of the 4 cages having contained an infected bird, and
167 were dissected under a binocular microscope to verify the existence of *Plasmodium* oocysts in their
168 midgut using mercurochrome staining. These dissections confirmed that the large majority of the
169 mosquitoes (91 %) had become infected. The average number of oocysts in mosquitoes sampled from
170 each of the cages were 214.76 ± 46.76 , 76.88 ± 23.29 , 40.05 ± 9.61 and 12.07 ± 2.75 .

171 To explore the impact of SP on the fecundity and longevity of mosquitoes, infected and
172 uninfected mosquitoes were allowed to take a second blood meal on either an SP-treated or a control
173 bird (Figure 1). For this purpose, four days prior to the blood meal, 3 birds (henceforth SP-treated
174 birds) had a daily subcutaneous injection of 30 μ l of a sulfadoxine-pyrimethamine solution (Sigma
175 S7821 and 46706, 320 mg/kg sulfadoxine, 16 mg/kg pyrimethamine solubilized in DMSO) while 3
176 additional (control) birds were injected with 30 μ l of DMSO. The concentrations given to the birds
177 were higher than the standard human dose (c.a. 30 mg/kg sulfadoxine, 1.5mg/kg pyrimethamine) to

178 account for differences in metabolism (birds have a higher basal metabolic rate than humans) and
179 mode of administration (oral in humans, peritoneal injection in birds). The red blood cell count of birds
180 (number of red blood cells per ml of blood) was quantified immediately before the blood meal using
181 flow cytometry (Beckman Coulter Counter, Series Z1). One hour after the last injection, 100 infected
182 and 80 uninfected mosquitoes were placed in a cage containing either an SP-treated or a control bird.
183 To allow the identification of the infected and uninfected mosquitoes, they were previously marked
184 using a small amount (2.5 µg/female) of coloured fluorescent powder (RadGlo® JST) as a dust storm.
185 Preliminary trials have shown that, at this concentration, the dust has no effect on mosquito traits
186 (Vézilier et al., 2012). On day 1 pbm, the number of blood-fed mosquitoes in each of the cages was
187 counted and unfed females discarded. Cages containing infected and uninfected mosquitoes were
188 alternated in the shelves and regularly rotated to ensure uniform environmental conditions.

189 To quantify haematin (a proxy for blood meal size) and fecundity, 80 females from each cage
190 (40 infected and 40 uninfected) were haphazardly chosen and placed individually in numbered 30 ml
191 *Drosophila* tubes, covered with a mesh ('haematin tubes'). Food was provided in the form of a paper
192 strip soaked in a 10% glucose solution. Three days later (day 4 pbm), all mosquitoes were transferred
193 to a new tube containing 7 mL of mineral water to allow the females to lay their eggs ('fecundity
194 tube'). The amount of haematin excreted at the bottom of each tube was quantified as an estimate of
195 the blood meal size following previously published protocols (Vézilier et al. 2010). The fecundity
196 tubes were provided with a paper strip soaked with 10% sugar solution. The fecundity tubes were
197 checked daily for 4 consecutive days for the presence of eggs. The egg laying date was recorded and
198 egg rafts were photographed using a binocular microscope equipped with a numeric camera. Eggs
199 counted using the Mesurim Pro freeware (Academie d'Amiens, France).

200 To quantify longevity, the rest of the infected and uninfected mosquitoes were kept in the cages
201 and provided with *ad libitum* drinking water, as well as a tray of water for egg laying for the first 6
202 days. Survival of these mosquitoes was assessed daily by counting dead individuals lying at the bottom
203 of each cage until all females died.

204 2.2.2. *Artesunate experiment*

205 The protocol used was identical to the one used in the SP experiment with only a few minor
206 modifications. The parasitaemias of the birds used to infect the mosquitoes for this experiment were
207 5.62%, 6.5%, 7.81% and 9.99%. Here, four days prior to the blood meal, 3 birds (henceforth AS-
208 treated birds) had a subcutaneous injection of 50 µl of an artesunate solution (16 mg/kg artesunate,
209 Sigma A3731, in a 50mg/kg bicarbonate solution) twice daily (9am and 6pm) while 3 additional
210 (control) birds were injected with 50 µl of the bicarbonate solution. As in the previous experiment,
211 mosquito dissections confirmed that the large majority of the *Plasmodium* mosquitoes (95 %) were
212 indeed infected. The average number of oocysts in mosquitoes sampled from each of the cages were
213 (513.3 ± 98.28 , 590 ± 191.47 , 649.78 ± 133.73 and 38.11 ± 20.49).

214

215 2.3. *Impact of antimalarials on Plasmodium infection within the mosquito*

216 The purpose of these experiments was to establish whether antimalarial drugs can have an
217 effect on the development of *Plasmodium* within the mosquito. For this purpose we infected
218 mosquitoes following an identical protocol as above. The parasitaemias of the birds used to infect the
219 mosquitoes were 1.58%, 1.79%, 2.27%, 3.12%, 3.25% and 4.72%. We then allowed these previously-
220 infected mosquitoes to feed on either SP-treated, AS-treated or control birds (n=3 birds each, 80
221 mosquitoes per cage, see Figure 1b). At the time of feeding, mosquitoes had been infected for 6 days
222 from a previous blood meal. Protocols used to infect mosquitoes and treat the birds were identical to
223 those used in the two previous experiments (Figure 1).

224 To assess the impact of the drugs in the blood meal on the *Plasmodium* parasites developing
225 within the mosquitoes, 15-20 mosquitoes were haphazardly chosen from each cage at three different
226 intervals: 8-9 days, 11-12 days and 14 days post-infection (corresponding to 2-3 days, 5-6 days and
227 8 days after the treated blood meal). Based on previous results (Pigeault, 2015) these intervals
228 correspond to the expected peak oocyst numbers, start of sporozoite production and peak sporozoite
229 production, respectively. At each of these time points, each mosquito was dissected to count the

230 number of oocysts in the midgut under the microscope, and its head-thorax was preserved
231 individually at -20°C for the quantification of the sporozoites. Midguts were dissected under a
232 binocular microscope in 100 µl of 0.01 M phosphate-buffered saline (PBS). The dissected midguts
233 were transferred with a pin to a slide containing a drop of PBS with 5% mercurochrome. The slide
234 was observed under a phase contrast microscope equipped with a 40x oil immersion objective.
235 Sporozoites were quantified using real-time quantitative PCR as the ratio of the parasite's *cytb* gene
236 relative to the mosquito's *ace-2* gene (Zélé et al. 2014). As in the other experiments a large majority
237 of the mosquitoes were infected (82%-87%).

238

239 **2.4. Statistical analyses**

240 Analyses were carried out using the R statistical package (v3.4.4). The different statistical
241 models used are described in the Supplementary Materials (Tables S1 & S2). The general procedure to
242 build models was as follows: treatment (AS, SP, control), and infection status (infected/uninfected)
243 were fitted as fixed explanatory variables. Birds were fitted as a random effect. Where appropriate,
244 haematin and dissection day were introduced into the model as an additional fixed variable. Since we
245 observed differences between the different plates used for the colorimetric quantification of the
246 haematin (Vézilier et al., 2010) the models were fitted with the haematin residuals of a model
247 containing haematin as a response variable and plate as a fixed explanatory variable. Maximal models,
248 including all higher order interactions, were simplified by sequentially eliminating non-significant
249 terms and interactions to establish a minimal model. The significance of the explanatory variables was
250 established using a likelihood ratio test (LRT) which is approximately distributed as a chi-square
251 distribution (Bolker, 2008) and using $p = 0.05$ as a cut-off p-value.

252 Survival data were analyzed using Cox proportional hazards mixed effect models (coxme).
253 Proportion data (blood-fed females, egg laying females, oocyst and sporozoite burden) were analyzed
254 using mixed linear models and a binomial distribution. Response variables that were highly
255 overdispersed (number of eggs per raft, oocyst burden) were analyzed using mixed negative binomial

256 models (glmmTMB). *A posteriori* contrasts were carried out by aggregating factor levels together and
257 by testing the fit of the simplified model using a LRT (Crawley, 2007) or using the 'emmeans' package
258 in R (<https://cran.r-project.org/web/packages/emmeans/vignettes/comparisons.html>). Because of the
259 small number of replications, differences in red blood cell counts between the birds in the different
260 treatments were tested using Kruskal-Wallis non-parametric tests. Raw data have been deposited in
261 the MendeleyData open repository ([doi: 10.17632/phzy3tmbzm.1](https://doi.org/10.17632/phzy3tmbzm.1)).

262

263 **2.5. Ethics statement**

264 Bird manipulations were carried out in strict accordance with the “National Charter on the
265 Ethics of Animal Experimentation” of the French Government. Experiments were approved by the
266 Ethical Committee for Animal Experimentation established by the authors’ institution (CNRS) under
267 the auspices of the French Ministry of Education and Research (permit number CEEA- LR-1051).

268 The authors declare no conflict of interests.

269

270 **3. Results**

271

272 **3.1. Impact of antimalarials on Plasmodium-infected and uninfected mosquito traits**

273 Mosquitoes were provided either with a *Plasmodium*-infected or an uninfected meal. Six days
274 later (corresponding to the early oocyst stages (Sekar et al., 2020, submitted) they were provided with
275 a second (uninfected) blood meal which was either treated with SP, AS, or their untreated controls
276 (Figure 1a). The aim of the experiments was to establish whether the drugs have an impact on the life
277 history traits of the mosquitoes (i.e. fecundity, longevity) and if this depends on whether the mosquito
278 is infected or not.

279 **3.1.1. Sulfadoxine-Pyrimethamine (SP) experiment**

280 The vast majority of mosquitoes (97-100%) blood fed, independently of whether they were
281 provided with an SP-treated or a control bird (model 1, $\chi^2 = 0.1306$, $p = 0.7178$, Figure S3a) and of
282 their infection status (model 1, $\chi^2 = 3.1086$, $p = 0.0779$). The amount of blood ingested (quantified as
283 the amount of haematine excreted) was also similar across experimental conditions (model 2,
284 *treatment*: $\chi^2 = 0.6545$, $p = 0.4185$; *infection*: $\chi^2 = 0.0001$, $p = 0.9802$, Figure S3b). There was no
285 difference in the haematocrit of SP-treated and untreated birds (model 3, $\chi^2 = 0.4286$, $p = 0.5127$).

286 The probability of laying an egg raft was overall very high (85-95%) except for infected
287 mosquitoes feeding on control birds (65%, model 4, *treatment*infection*: $\chi^2 = 11.372$, $p = 0.001$, see
288 Supplementary Materials, Figure S3d). Overall, females having fed in SP treated birds laid eggs earlier
289 than those fed on control birds (model 5, LR.stat = 10.243, $p = 0.001$). Egg laying date also depended
290 on the interaction between blood meal size and the infection status of the mosquito (model 5, LR.stat =
291 7.2853, $p = 0.007$, Figure S3c). While for infected females blood meal size had no impact on
292 oviposition day, uninfected females who took larger blood meals laid eggs earlier than those who took
293 a smaller blood meals (LR.stat = 6.7296, $p = 0.0094$). Mosquito fecundity (number of eggs per raft)
294 decreased with egg laying date (model 6, $\chi^2 = 15.808$, $p < 0.001$) but was independent of both
295 treatment (model 6, $\chi^2 = 0.2478$, $p = 0.6186$) and infection status (model 6, $\chi^2 = 0.1048$, $p = 0.7462$,
296 Figure S3e),

297 Uninfected mosquitoes lived longer than their infected counterparts (model 7, $\chi^2 = 7.4768$, $p =$
298 0.006). Although statistically significant, this effect was biologically marginal (HR = 0.79, i.e.
299 uninfecteds have 21% higher chance of survival than their uninfected counterparts, CI: 0.1 - 34%).
300 Treatment with SP had no effect on survival (model 7, $\chi^2 = 0.0557$, $p = 0.8134$, Figure S3f). The
301 results were identical when analyzing survival to day 14, the time at which sporozoite production
302 peaks (model 8).

303 3.1.2. Artesunate (AS) experiment –

304 As above, the vast majority of mosquitoes (95-98%) blood fed, independently of whether they
305 were provided with an AS-treated or a control bird (model 9, $\chi^2 = 2.4543$, $p = 0.1172$, Figure S4a) and

306 of their infection status (model 9, $\chi^2 = 0.1085$, $p = 0.7418$). The amount of blood ingested was also
307 similar across experimental conditions (model 10, *treatment*: $\chi^2 = 0.0009$, $p = 0.4185$; *infection*: $\chi^2 =$
308 0.787 , $p = 0.375$, Figure S4b). There was no difference in the haematocrit of AS-treated and untreated
309 birds (model 11, $\chi^2 = 1.4727$, $p = 0.2888$).

310 The probability of laying an egg raft was overall very high (81-88%). As in the SP experiment,
311 infected mosquitoes had a slightly lower chance of laying eggs than their uninfected counterparts,
312 though here this effect was dependent of whether they had fed on a treated or an untreated bird (model
313 12, $\chi^2 = 4.3911$, $p = 0.0361$, Figure S4d). For mosquitoes feeding on AS-treated birds, the probability
314 of laying an egg raft depended heavily on the amount of blood ingested: treated females that took a
315 small blood meal saw their probability of laying eggs significantly reduced (mean \pm s.e probability of
316 egg laying for treated females in the lowest blood meal quartile: 55.4 ± 6.7 %, in the highest blood
317 meal quartile: 92.6 ± 3.0 %). No such difference was found in mosquitoes that fed on untreated birds
318 (lowest quartile: 78.2 ± 5.6 %, highest quartile 85.5 ± 4.8 % ; model 12, *treatment*haematine*: $\chi^2 =$
319 8.0323 , $p = 0.0046$, see Supplementary Materials, Figure S4d). The egg laying date was independent
320 of the treatment (model 13, LR.stat = 0.203, $p = 0.6523$) but was negatively correlated with the size of
321 the blood meal: females that took smaller blood meals laid eggs later (model 13, LR.stat = 12.498, $p <$
322 0.001 , Figure S4c). Mosquito fecundity (number of eggs per raft) increased with blood meal size
323 (model 14, $\chi^2 = 36.875$, $p < 0.001$, Figure S4e) but was independent of both treatment (model 14, $\chi^2 =$
324 0.2784 , $p = 0.5978$) and infection status ($\chi^2 = 0.9796$, $p = 0.3223$).

325 Neither the artesunate treatment (model 15, $\chi^2 = 0.0577$, $p = 0.8102$) nor the mosquito infection
326 status (model 15, $\chi^2 = 0.3266$, p -value = 0.5677) had an impact on overall mosquito survival (Figure
327 S4f). The results were identical when analyzing survival to day 14, the time at which sporozoite
328 production peaks (model 16).

329

330 **3.2. Impact of antimalarials on Plasmodium infection within the mosquito**

331 Mosquitoes were provided with a *Plasmodium*-infected meal. Six days later, when the infection
332 was in the early oocyst stages, they were allowed to feed on uninfected birds that had been previously
333 treated with either SP, AS, or given a solvent injection (DMSO as a control for SP, bicarbonate as a
334 control for AS, Figure 1b). The aim of the experiments was to establish whether the drugs have an
335 impact on the development of the parasite within the mosquito. For this purpose the mosquitoes were
336 dissected at different time intervals and the number of oocysts in the midgut, and of sporozoites in the
337 head-thorax fraction were quantified.

338 3.2.1. Sulfadoxine-Pyrimethamine experiment

339 The prevalence of oocysts decreased with dissection time (model 17, $\chi^2 = 14.843$, $p < 0.01$), but
340 was independent of the antimalarial treatment (model 17, $\chi^2 = 2.7322$, $p = 0.0983$, Figure 2a). In
341 contrast, there was a very significant interaction between the SP-treatment and the time of dissection
342 on the number of oocysts developing inside the mosquitoes (model 18, $\chi^2 = 24.159$, $p < 0.01$, Figure
343 2c). Although the general trend was towards a decrease in the number of oocysts with time mosquitoes
344 having fed on a SP-treated bird had a consistently higher number of oocysts in their midgut than
345 mosquitoes having fed on their control counterparts. These results are consistent across all the birds
346 used in the experiment (Supplementary Materials, Figure S5 and S6). Fitting day as a continuous
347 (rather than discrete) variable in the model revealed that the rate of decline of oocysts with time was
348 significantly higher in control-fed mosquitoes (incidence rate ratio, IRR = 21%) than in SP-fed
349 mosquitoes (IRR = 9.4%).

350 Treatment had a significant effect on the prevalence of sporozoites within the mosquitoes
351 (model 20, $\chi^2 = 10.394$, $p < 0.01$, Figure 2b). On average, parasites developing in mosquitoes having
352 fed on an SP-treated host had a significantly lower probability of reaching the sporozoite stage than
353 their control counterparts (55% vs 82%, respectively). Sporozoite burden was also significantly lower
354 in mosquitoes having fed on an SP-treated host, irrespective of the dissection date (model 21,
355 *treatment*: $\chi^2 = 9.8898$, $p < 0.01$; *date*: $\chi^2 = 3.1579$, $p = 0.0762$; Figure 2d). As above, these results are
356 consistent across all the birds used in the experiment (Supplementary Materials, Figures S5 and S6).

357 Fitting day as a continuous (rather than discrete) variable in the model revealed that while in control-
358 fed mosquitoes the number of sporozoites stayed roughly constant with time (slope not significantly
359 different from 0, $t = 1.66$), in SP-treated mosquitoes, the number of sporozoites decreased significantly
360 with time ($t = 2.41$).

361 3.2.2. Artesunate experiment

362 Feeding on an AS-treated host had no impact on the prevalence of oocysts (model 23, $\chi^2 =$
363 0.854, p -value= 0.3554, Figure 3a). Oocyst burden, on the other hand, showed the same pattern of
364 decrease with time as in the previous experiment (model 24, $\chi^2 = 211.91$, $p < 0.01$, Figure 3c). There
365 was a significant effect of treatment in interaction with the date of dissection (model 24,
366 date*treatment: $\chi^2 = 7.3787$, $p = 0.025$). Post hoc analyses revealed the existence of a marginally
367 higher oocyst burden in treated hosts on days 11 and 12 ($\chi^2 = 3.8886$, $p = 0.05$) while no differences
368 were observed in day 8,9 ($\chi^2 = 0.0106$, $p = 0.9179$) and 14 ($\chi^2 = 3.5452$, $p = 0.0597$). Feeding on an
369 AS-treated host, however, had no effect on either sporozoite prevalence (model 25: $\chi^2 = 0.0106$,
370 $p = 0.9179$), or burden (model 26, $\chi^2 = 0.0002$, $p = 0.9885$, Figure 3d).

371

372 4. Discussion

373 Artesunate and sulfadoxine-pyrimethamine are the cornerstone of modern antimalarial
374 treatments in malaria-endemic areas. Millions of people across the world are treated every year with
375 these drugs. Both antimalarials are extremely efficient at clearing the parasite from the red blood cells
376 but, like most other drugs, they also come of a suite of adverse effects in humans (Medscape, 2020).
377 The aim of our study was to establish whether this double toxicity, for both *Plasmodium* and its host,
378 also takes place in the vector, thereby interfering on parasite transmission by mosquitoes. More
379 precisely we aimed to establish: 1) whether mosquitoes feeding on an AS or SP treated host suffer any
380 adverse fitness effects from the drugs, and 2) whether the drugs are toxic for the oocysts and
381 sporozoites developing inside a mosquito.

382 For this purpose, we carried out several factorial experiments feeding both uninfected
383 mosquitoes and mosquitoes with a 6-day old infection (corresponding to the early stages of oocyst
384 formation in *P. relictum*, Pigeault, 2015) on drug treated (AS or SP) and control hosts. We then
385 quantified the life history traits of the mosquito (fecundity, longevity) and the oocyst and sporozoite
386 stages of the parasites developing inside them.

387 Our results show what seem to be mostly minor effects of the drugs on the life history traits of
388 mosquitoes feeding from a treated host. Amongst the two life history traits quantified that are known
389 to be key for malaria transmission: mosquito longevity (Smith and McKenzie, 2004) and host feeding
390 probability (Cornet et al., 2019), neither were found to be affected by the drug treatments. Previous
391 work on the longevity effects of drugs has shown that *An. gambiae* mosquitoes membrane-fed on a
392 gametocyte culture containing high concentrations of SP (61 µg/ml sulfadoxine, 154 ng/ml
393 pyrimethamine) had significantly shorter lifespans (Kone et al., 2010). Whether this is due to
394 differences in the experimental system or, more likely, to key differences in experimental conditions
395 (Kone et al added a high SP dose to a gametocyte culture) is unclear. In our experiments, some
396 significant interactions were, however, found that may be worthy of further study. Females that fed on
397 an SP-treated bird laid eggs on average 8 hours earlier than those fed on control birds, a result that
398 agrees with previous studies showing that *Culex pipiens* mosquitoes are able to advance their
399 oviposition schedule when faced with adverse conditions (Vézilier et al., 2015). In addition,
400 mosquitoes taking small blood meals from AS-treated birds saw their probability of laying an egg raft
401 reduced by 37% as compared to their control counterparts. In humans, artesunate use is frequently
402 associated with haemolytic anaemia as evidenced by a decline in blood haemoglobin levels and an
403 increase in reticulocyte counts (Burri et al., 2014; Sowunmi et al., 2017). Had a similar phenomenon
404 taken place in our birds, mosquitoes taking a small blood meal from AS-treated hosts would not have
405 obtained enough haemoglobin to produce a batch of eggs (Ferguson et al., 2003; Vézilier et al., 2012;
406 Zhou et al., 2007). We found no difference in the total number of red blood cells between AS-treated

407 and untreated birds, but since our analysis did not allow us to distinguish between young (reticulocyte)
408 and mature red blood cells, we could not establish whether artesunate induces anaemia in this system.

409 In contrast to the effects observed on mosquito life history traits which are unlikely to bear
410 significant consequences for the epidemiology of the disease, the substantial reduction in both
411 sporozoite prevalence (- 30%) and burden (- 80%) in mosquitoes fed on an SP-treated blood meal, may
412 result in a drastic reduction in the transmission potential of the parasite. Sulfadoxine and
413 pyrimethamine act synergistically to inhibit the activity of dihydropteroate synthase (DHPS) and
414 dihydrofolate reductase (DHFR), respectively, thus inhibiting the folic acid metabolism of the parasite
415 (Hopkins Sibley et al., 2001). Folic acid is vital for the biosynthesis of purines and pyrimidines, which
416 are essential for DNA synthesis and cell multiplication (Kirk et al., 1976). The mitotic-blocking
417 properties of pyrimethamine were first gleaned through work done on *Plasmodium gallinaceum* where
418 birds treated with high concentrations of pyrimethamine showed arrested schizont division and fewer
419 merozoites were produced (Aikawa and Beaudoin, 1968). Since then, the schizontocidal effect of
420 pyrimethamine has been confirmed in several other systems (Delves et al., 2012; Vincke, 1970). In
421 contrast, work on the effect of pyrimethamine on *Plasmodium* sporogony in the mosquito has
422 produced contrasting results. The overwhelming majority of these studies tested the so-called
423 ‘prophylactic’ effect of pyrimethamine on the mosquito, that is, the effect of the drug when
424 administered prior to or concomitantly with the infected blood meal (Table 1). These studies found that
425 when administered with the infected blood meal, pyrimethamine averted the arrival of sporozoites to
426 the salivary gland. There was, however, no consensus on the mechanisms underlying this sporozoite-
427 inhibitory effect: pyrimethamine may have rendered gametocytes uninfected (Foy and Kondi, 1952),
428 prevented the ookinetes from traversing the midgut wall (Bray et al., 1959), or prevented the oocysts
429 from reaching maturity (Terzian, 1970; Terzian et al., 1968). More recent work seems to confirm that
430 pyrimethamine in combination with sulfadoxine, decreases the infectiousness of gametocytes
431 (Beavogui et al., 2010; Kone et al., 2010) and Delves et al. have reported that pyrimethamine and other
432 antifolates result in a strong (> 90%) inhibition of male gametocyte exflagellation, while having

433 virtually no effect on female gametocytes (Delves et al., 2012, 2013) thus effectively strongly skewing
434 the parasites' operational sex ratio. These studies collectively suggest that a prophylactic
435 administration of SP has transmission-blocking effect through the inhibition of the early (gametocyte)
436 stages within the mosquito.

437 Our experiments were carried out using a 'curative' protocol, i.e. the drug was administered to
438 mosquitoes carrying a 6-day old *Plasmodium* infection which, in this system, corresponds to the initial
439 stages of the oocyst invasion of the midgut. The drastic decrease obtained in both sporozoite
440 prevalence (Fig 2b) and burden (Fig 2d) demonstrate that SP has an additional effect on parasite
441 development, which is downstream from its toxicity to (male) gametes. Although the underlying
442 mechanism remains to be established, these results are consistent with the mitosis-blocking properties
443 of antifolates observed in the blood stages of parasites, which may here have prevented the multiple
444 rounds of cell division that take place inside the syncytial oocyst prior to the liberation of the
445 sporozoites (Gerald et al., 2011). Recent experiments using luciferase-expressing *Plasmodium berghei*
446 parasites cultured *in vitro* (Azevedo et al., 2017) have observed a significant reduction in the
447 luminescence of oocysts after adding 10 μ M pyrimethamine to the parasite culture. As the luciferase
448 was under the control of the parasite's circumsporozoite protein (*PbCSP*) promoter regions, a reduction
449 in the number of sporozoites produced inside the oocyst therefore seems like a plausible explanation
450 for the observed reduction in bioluminescence. Future studies should focus on the mechanisms
451 underlying the reduction in sporozoite numbers obtained in these experiments. One possible avenue of
452 research is to carry out a full transcriptomic analysis of oocysts, focusing on genes involved in the
453 folate pathway, which is the target of SP and is involved in the synthesis of DNA.

454 The strong reduction in sporozoite prevalence and burden in SP-fed mosquitoes is all the more
455 notable for being associated with a significant concomitant increase in the number of oocysts in the
456 midgut (Fig 2c). Ookinetes issued from the fussion of male and female gametes in the mosquito
457 midgut, start invading the midgut epithelium 24-48h after the blood meal (Valkiunas, 2004). They then
458 start growing in size as the sporozoites develop inside them. Previous work from our laboratory has

459 shown that the first oocysts are detectable through mercurochrome staining 4-6 days after the infected
460 blood meal, and mature oocysts reach their peak intensity on days 8-10. On 6 day pbm (day at which
461 the treated blood meal was ingested) on average, only between 30-50% of the mature oocysts found
462 during the peak oocyst intensity (which happens around day 8pbm) are present in the midgut. This
463 leaves a substantial window of opportunity (2-3 days) for the ingested drugs to have an effect on a
464 large fraction of the oocysts developing in the midgut. The mechanisms underlying the increased
465 oocyst intensity observed in mosquitoes having ingested a treated blood meal require further study, but
466 several potential avenues of research are possible. SP may have an effect on oocyst maturation
467 directly, through an effect on the pathways that contribute to oocyst maturation, or indirectly, through
468 alterations in the physiology of the mosquito that would affect the composition of mosquito-derived
469 molecules that are essential for *Plasmodium* development, or through a modification of the mosquito
470 microbiota as a result of SP's powerful antibiotic properties (Capan et al., 2010). Antibiotics have been
471 shown to enhance the susceptibility of mosquitoes to *Plasmodium* by disturbing their gut microbiota
472 (Gendrin et al., 2015). Microbiota, however, seem to exert their influence during the ookinete invasion
473 of the midgut (Dong et al., 2009); whether they may also have an effect during the growth and
474 maturation of the oocysts is not known. Further work is needed in order to establish the viability of
475 these supernumerary oocysts. In this system, however, standard human malaria methods to establish
476 oocyst viability are either not reliable (such as oocyst size, as this depends on the state of maturation)
477 or not yet available (as is the case for immune-fluorescence-antibodies). To our knowledge, this is the
478 first time that such an increase in oocysts following a drug-treated blood meal has been reported in any
479 study, which raises some interesting questions regarding the timing of SP administration with respect
480 to the maturation of oocysts in the midgut. In addition, we observed an interesting pattern whereby the
481 decline in oocyst numbers with time, a natural process that takes place as mature oocysts burst to
482 produce sporozoites, happens more rapidly in control-fed than in SP-fed mosquitoes, which may be
483 indicative of a delay in oocyst development in the latter. Other possibilities for the increased oocyst
484 intensity observed, such as an SP-induced immunosuppression or SP-induced facilitation of the of

485 oocyst development within the midgut would also merit further study. Irrespective of the underlying
486 mechanism, our results indicate that SP exerts two opposing effects on the parasite's sporogonic
487 development within the mosquito: one that facilitates the development of oocysts in the midgut,
488 followed by another that blocks the production of sporozoites within them.

489 In contrast to SP, AS treatment had no discernible effects on sporozoite burden and only a
490 minor effect on the number of oocysts. These results are in agreement with previous work showing
491 that artesunate and other artemisinin derivatives have a considerable gametocytocidal effect in
492 humans but no effect on the mosquito stages of the parasite (Butcher, 1997; Wadi et al., 2019). It is
493 worth noting that previous work using a curative dose of other drugs have obtained drastically
494 different results to the ones we have obtained with SP and AS. A curative dose of atovaquone
495 administered to *An. stephensi* mosquitoes carrying a 4-day old *P. berghei* infection resulted in a
496 decrease in oocyst numbers without a concomitant decrease in sporozoites (Fowler et al., 1995). Data
497 from *in vitro* experiments suggests that atovaquone inhibition of pyrimidine biosynthesis may prevent
498 meiotic DNA replication in the immature ookinete and its development into the oocyst stage (Fowler
499 et al., 1995). However, given that the treatment was given after the ookinetes had invaded the midgut
500 wall, atovaquone is likely to also negatively impact the process of oocyst maturation in the midgut
501 through, as yet, unknown mechanisms. In contrast, a curative dose of a primaquine-like
502 aminoquinoline, did not affect oocyst numbers but sporozoite production was entirely impaired,
503 possibly by generating reactive oxygen species and/or interfering with the parasite's electron transport
504 chain (Hamerly et al., 2019). These results highlight the need to understand the mechanisms
505 underlying the curative effect of drugs, as a first step towards their potential use as transmission-
506 blocking compounds in the field.

507 We are acutely aware that the effects of curative administration of SP on oocyst and sporozoite
508 burden may not directly translatable to human malaria. SP is widely used as a preventive treatment for
509 uninfected children (SMC) and pregnant women (IPT), with millions of doses being provided every
510 year across the African continent (Van Eijk et al., 2011). The transmission-blocking effect of a

511 curative administration of SP demonstrated here would be relevant when infected mosquitoes bite
512 these SP-treated individuals (in the field, mosquitoes go through several gonotrophic, bloodmeal - egg
513 laying - bloodmeal, cycles, Bomblies, 2014). To confirm the curative effect of SP in human malaria
514 infections, experiments where infected mosquitoes are membrane-fed on treated uninfected blood
515 could be carried out, with the caveat that membrane feeding and direct feeding on human volunteers
516 may render different results (Beavogui et al., 2010; Butcher, 1989; Wadi et al., 2018). Provided the
517 results obtained here are repeatable in human malaria, the epidemiological and evolutionary
518 consequences of the preventive use of SP in malaria-endemic countries could be substantial. Fewer
519 sporozoite-carrying mosquitoes (-30%), and fewer sporozoites in the salivary gland (-80%) should
520 translate into lower transmission rates, even accounting for a non-linear correlation between sporozoite
521 load and transmission (Aleshnick et al., 2019). The evolutionary consequences may not be less
522 important. Current work largely assumes that the strongest selective pressures for drug resistance
523 operate in the treated host. As these results show, the strong bottleneck for sporozoites in the mosquito
524 may act as an additional selective pressure which may help maintain drug resistance in the field even
525 when the drug is not used to treat infected hosts, as is the case in the current mass administration of
526 pyrimethamine for ITP and SMC. More generally, these results also highlight the need for further
527 studies on the effects of the transmission-blocking compounds on each stage of the parasite's cycle
528 within the mosquito. The results of standard membrane feeding assays, considered to be the gold
529 standard for assessing the efficiency of transmission-blocking interventions, are reported as a percent
530 reduction in the number of oocysts compared to a control (Nunes et al., 2014; Paton et al., 2019), with
531 current efficacy thresholds set at around an 80% reduction. As shown here, drugs can have contrasting
532 effects on different stages of the parasite's sporogonic cycle highlighting the potential drawbacks of
533 assessing drug-based transmission-blocking interventions based on oocyst quantifications alone.

534

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536

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541

542 **6. References**

- 543 Aikawa, M., Beaudoin, R.L., 1968. Studies on nuclear division of a malarial parasite under
544 pyrimethamine treatment. *J. Cell Biol.* 39, 749–754. doi:10.1083/jcb.39.3.749
- 545 Aleshnick, M., Ganusov, V. V., Nasir, G., Yenokyan, G., Sinnis, P., 2019. Experimental
546 determination of the force of malaria infection reveals a non-linear relationship to mosquito
547 sporozoite loads. *bioRxiv* 1–40. doi:10.1101/830299
- 548 Azevedo, R., Markovic, M., Machado, M., Franke-Fayard, B., Mendes, A.M., Prudêncio, M., 2017.
549 Bioluminescence method for in vitro screening of *Plasmodium* transmission-blocking
550 compounds. *Antimicrob Agents Chemother* 61, e02699-16.
- 551 Beavogui, A.H., Djimde, A.A., Gregson, A., Toure, A.M., Dao, A., Coulibaly, B., Ouologuem, D.,
552 Fofana, B., Sacko, A., Tekete, M., Kone, A., Niare, O., Wele, M., Plowe, C. V., Picot, S.,
553 Doumbo, O.K., 2010. Low infectivity of *Plasmodium falciparum* gametocytes to *Anopheles*
554 *gambiae* following treatment with sulfadoxine-pyrimethamine in Mali. *Int. J. Parasitol.* 40,
555 1213–1220. doi:10.1016/j.ijpara.2010.04.010
- 556 Blagborough, A.M., Churcher, T.S., Upton, L.M., Ghani, A.C., Gething, P.W., Sinden, R.E., 2013.
557 Transmission-blocking interventions eliminate malaria from laboratory populations. *Nat.*
558 *Commun.* 4, 1–7. doi:10.1038/ncomms2840
- 559 Bolker, B.M., 2008. Ecological models and data in R, *Ecological Models and Data in R.*
560 doi:10.1111/j.1442-9993.2010.02210.x
- 561 Bomblies, A., 2014. Agent-based modeling of malaria vectors: the importance of spatial simulation.
562 *Parasites and Vectors* 7, 308. doi:10.1186/1756-3305-7-308
- 563 Bray, R.S., Burgess, R.W., Fox, R.M., Miller, M.J., 1959. Effect of pyrimethamine upon sporogony
564 and pre-erythrocytic schizogony of *Laverania falciparum*. *Bull. World Health Organ.* 21, 233–
565 238.
- 566 Burgess, R.W., Young, M.D., 1959. The development of pyrimethamine resistance by *Plasmodium*

- 567 *falciparum*. Bull. World Health Organ. 20, 37–46.
- 568 Burri, C., Ferrari, G., Ntuku, H.M., Kitoto, A.T., Duparc, S., Hugo, P., Mitembo, D.K., Lengeler,
569 C., 2014. Short report: Delayed anemia after treatment with injectable artesunate in the
570 Democratic Republic of the Congo: a manageable issue. Am. J. Trop. Med. Hyg. 91, 821–823.
571 doi:10.4269/ajtmh.14-0149
- 572 Butcher, G., 1997. Antimalarial drugs and the mosquito transmission of *Plasmodium*. Int. J.
573 Parasitol. 27, 975–987.
- 574 Butcher, P., 1989. Mechanisms of immunity to malaria and the possibilities of a blood-stage
575 vaccine: a critical appraisal. Parasitology 98, 315–327. doi:10.1017/S0031182000062247
- 576 Capan, M., Mombo-Ngoma, G., Makristathis, A., Ramharter, M., 2010. Anti-bacterial activity of
577 intermittent preventive treatment of malaria in pregnancy: comparative *in vitro* study of
578 sulphadoxine-pyrimethamine, mefloquine, and azithromycin. Malar. J. 9, 303.
579 doi:10.1186/1475-2875-9-303
- 580 Chotivanich, K., Sattabongkot, J., Udomsangpetch, R., Looareesuwan, S., Day, N.P.J., Coleman,
581 R.E., White, N.J., 2006. Transmission-blocking activities of quinine, primaquine, and
582 artesunate. Antimicrob. Agents Chemother. 50, 1927–1930. doi:10.1128/AAC.01472-05
- 583 Churcher, T.S., Bousema, T., Walker, M., Drakeley, C., Schneider, P., Ouédraogo, A.L., Basáñez,
584 M.G., 2013. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density
585 and estimating the reservoir of infection. Elife e00626. doi:10.7554/eLife.00626
- 586 Chutmongkonkul, M., Maier, W.A., Seitz, H.M., 1992. *Plasmodium falciparum*: effect of
587 chloroquine, halofantrine and pyrimethamine on the infectivity of gametocytes for *Anopheles*
588 *stephensi* mosquitoes. Ann. Trop. Med. Parasitol. 86, 103–110.
589 doi:10.1080/00034983.1992.11812639
- 590 Coatney, G.R., Cooper, W.C., Eddy, N.B., Greenberg, J., 1953. Survey of antimalarial agents:
591 chemotherapy of *Plasmodium gallinaceum* infections; toxicity; correlation of structure and
592 action. Public Heal. Serv. Publ.
- 593 Cohuet, A., Osta, M.A., Morlais, I., Awono-Ambene, P.H., Michel, K., Simard, F., Christophides,
594 G.K., Fontenille, D., Kafatos, F.C., 2006. *Anopheles* and *Plasmodium*: from laboratory models
595 to natural systems in the field. EMBO Rep. 7, 1285–1289. doi:10.1038/sj.embor.7400831
- 596 Cornet, S., Nicot, A., Rivero, A., Cator, L., 2019. Avian malaria alters blood feeding of *Culex*
597 *pipiens* mosquitoes. Malar. J. 18, 82.
- 598 Cornet, S., Nicot, A., Rivero, A., Gandon, S., 2014. Evolution of plastic transmission strategies in

- 599 avian malaria. PLoS Pathog. 10. doi:10.1371/journal.ppat.1004308
- 600 Crawley, M.J., 2007. The R book, Imperial C. ed. UK.
- 601 Delves, M., Plouffe, D., Scheurer, C., Meister, S., Wittlin, S., Winzeler, E.A., Sinden, R.E., Leroy,
602 D., 2012. The activities of current antimalarial drugs on the life cycle stages of *Plasmodium*: a
603 comparative study with human and rodent parasites. PLoS Med. 9.
604 doi:10.1371/journal.pmed.1001169
- 605 Delves, M.J., Miguel-Blanco, C., Matthews, H., Molina, I., Ruecker, A., Yahiya, S., Straschil, U.,
606 Abraham, M., León, M.L., Fischer, O.J., Rueda-Zubiaurre, A., Brandt, J.R., Cortés, Á.,
607 Barnard, A., Fuchter, M.J., Calderón, F., Winzeler, E.A., Sinden, R.E., Herreros, E., Gamu,
608 F.J., Baum, J., 2018. A high throughput screen for next-generation leads targeting malaria
609 parasite transmission. Nat. Commun. 9, 1–13. doi:10.1038/s41467-018-05777-2
- 610 Delves, M.J., Ruecker, A., Straschil, U., Lelièvre, J., Marques, S., López-Barragán, M.J., Herreros,
611 E., Sinden, R.E., 2013. Male and female *Plasmodium falciparum* mature gametocytes show
612 different responses to antimalarial drugs. Antimicrob. Agents Chemother. 57, 3268–3274.
613 doi:10.1128/AAC.00325-13
- 614 Dong, Y., Aguilar, R., Xi, Z., Warr, E., Mongin, E., Dimopoulos, G., 2006. *Anopheles gambiae*
615 immune responses to human and rodent *Plasmodium* parasite species. PLoS Pathog. 2, e52.
616 doi:10.1371/journal.ppat.0020052
- 617 Dong, Y., Manfredini, F., Dimopoulos, G., 2009. Implication of the mosquito midgut microbiota in
618 the defense against malaria parasites. PLoS Pathog. 5, e1000423.
619 doi:10.1371/journal.ppat.1000423
- 620 Ferguson, H.M., Rivero, A., Read, A.F., 2003. The influence of malaria parasite genetic diversity
621 and anaemia on mosquito feeding and fecundity. Parasitology 127, 9–19.
622 doi:10.1017/S0031182003003287
- 623 Fowler, R.E., Sinden, R.E., Pudney, M., 1995. Inhibitory activity of the anti-malarial Atovaquone
624 (566C80) against ookinetes, oocysts, and sporozoites of *Plasmodium berghei*. J. Parasitol. 81,
625 452. doi:10.2307/3283831
- 626 Foy, H., Kondi, A., 1952. Effect of daraprim on the gametocytes of *Plasmodium falciparum*. Trans.
627 R. Soc. Trop. Med. Hyg. doi:10.1016/0035-9203(52)90084-9
- 628 Gendrin, M., Rodgers, F.H., Yerbanga, R.S., Ouédraogo, J.B., Basáñez, M.G., Cohuet, A.,
629 Christophides, G.K., 2015. Antibiotics in ingested human blood affect the mosquito microbiota
630 and capacity to transmit malaria. Nat. Commun. 6, 1–7. doi:10.1038/ncomms6921

- 631 Gerald, N., Mahajan, B., Kumar, S., 2011. Mitosis in the human malaria parasite *Plasmodium*
632 *falciparum*. Eukaryot. Cell 10, 474–482. doi:10.1128/EC.00314-10
- 633 Gerberg, E.J., 1971. Evaluation of antimalarial compounds in mosquito test systems. Trans. R. Soc.
634 Trop. Med. Hyg. 65, 358–363.
- 635 Gunders, A.E., 1961. The effect of a single dose of pyrimethamine and primaquine in combination
636 upon gametocytes and sporogony of *Laverania falcipara* (= *Plasmodium falciparum*) in
637 Liberia. Bull. World Health Organ. 24, 650–653.
- 638 Hamerly, T., Tweedell, R.E., Hritzo, B., Nyasembe, V.O., Tekwani, B.L., Nanayakkara, N.P.D.,
639 Walker, L.A., Dinglasan, R.R., 2019. NPC1161B, an 8-aminoquinoline analog, is metabolized
640 in the mosquito and inhibits *Plasmodium falciparum* oocyst maturation. Front. Pharmacol. 10,
641 1265. doi:10.3389/fphar.2019.01265
- 642 Hewitt, R., 1940. Bird Malaria, The american journal of hygiene.
- 643 Hogh, B., Gamage-Mendis, A., Butcher, G.A., Thompson, R., Begtrup, K., Mendis, C., Enosse,
644 S.M., Dgedge, M., Barreto, J., Eling, W., Sinden, R.E., 1998. The differing impact of
645 chloroquine and pyrimethamine/sulfadoxine upon the infectivity of malaria species to the
646 mosquito vector. Am. J. Trop. Med. Hyg. 58, 176–182. doi:10.4269/ajtmh.1998.58.176
- 647 Hopkins Sibley, C., Hyde, J.E., Sims, P.F.G., Plowe, C. V., Kublin, J.G., Mberu, E.K., Cowman,
648 A.F., Winstanley, P.A., Watkins, W.M., Nzila, A.M., 2001. Pyrimethamine-sulfadoxine
649 resistance in *Plasmodium falciparum*: what's next? TRENDS Parasitol. 17, 582–588.
- 650 Karunajeewa, H.A., Salman, S., Mueller, I., Baiwog, F., Gomorra, S., Law, I., Page-Sharp, M.,
651 Rogerson, S., Siba, P., Ilett, K.F., Davis, T.M.E., 2009. Pharmacokinetic properties of
652 sulfadoxine-pyrimethamine in pregnant women. Antimicrob. Agents Chemother. 53, 4368–
653 4376. doi:10.1128/AAC.00335-09
- 654 Kirk, D., Mittwoch, U., Stone, A.B., Wilkie, D., 1976. Limited ability of thymidine to relieve
655 mitotic inhibition by pyrimethamine in human fibroblasts. Biochem. Pharmacol. 25, 681–685.
- 656 Kone, A., Vegte-Bolmer, M. van de, Siebelink-Stoter, R., Gemert, G.J. van, Dara, A., Niangaly, H.,
657 Luty, A., Doumbo, O.K., Sauerwein, R., Djimde, A.A., 2010. Sulfadoxine-pyrimethamine
658 impairs *Plasmodium falciparum* gametocyte infectivity and *Anopheles* mosquito survival. Int.
659 J. Parasitol. 40, 1221–1228. doi:10.1016/j.ijpara.2010.05.004
- 660 Medscape, 2020. Artesunate dosing, indications, interactions, adverse effects, and more [WWW
661 Document]. URL <https://reference.medscape.com/drug/artesunate-342684> (accessed 4.21.20).
- 662 Miguel-Blanco, C., Lelièvre, J., Delves, M.J., Bardera, A.I., Presa, J.L., López-Barragán, M.J.,

- 663 Ruecker, A., Marques, S., Sinden, R.E., Herreros, E., 2015. Imaging-based high-throughput
664 screening assay to identify new molecules with transmission-blocking potential against
665 *Plasmodium falciparum* female gamete formation. *Antimicrob. Agents Chemother.* 59, 3298–
666 3305. doi:10.1128/AAC.04684-14
- 667 Nunes, J.K., Woods, C., Carter, T., Raphael, T., Morin, M.J., Diallo, D., Leboulleux, D., Jain, S.,
668 Loucq, C., Kaslow, D.C., Birkett, A.J., 2014. Development of a transmission-blocking malaria
669 vaccine: progress, challenges, and the path forward. *Vaccine.*
670 doi:10.1016/j.vaccine.2014.07.030
- 671 Omar, M.S., Collins, W.E., Contacos, P.G., 1973. Gametocytocidal and sporontocidal effects of
672 antimalarial drugs on malaria parasites. *Exp. Parasitol.* 34, 229–241. doi:10.1016/0014-
673 4894(73)90082-9
- 674 Paaijmans, K., Fernández-Busquets, X., 2014. Antimalarial drug delivery to the mosquito: an option
675 worth exploring? *Futur. Microbiol* 9, 579–582. doi:10.2217/FMB.14.30
- 676 Paton, D.G., Childs, L.M., Itoe, M.A., Holmdahl, I.E., Buckee, C.O., Catteruccia, F., 2019.
677 Exposing *Anopheles* mosquitoes to antimalarials blocks *Plasmodium* parasite transmission.
678 *Nature.* doi:10.1038/s41586-019-0973-1
- 679 Peatey, C.L., Leroy, D., Gardiner, D.L., Trenholme, K.R., 2012. Anti-malarial drugs: how effective
680 are they against *Plasmodium falciparum* gametocytes? *Malar. J.* 11, 34. doi:10.1186/1475-
681 2875-11-34
- 682 Peterson, D.S., Walliker, D., Wellems, T.E., 1988. Evidence that a point mutation in dihydrofolate
683 reductase-thymidylate synthase confers resistance to pyrimethamine in *falciparum* malaria,
684 *Proc. Nati. Acad. Sci. USA.*
- 685 Pigeault, R., 2015. Ecologie évolutive des interaction Hôte / Moustique / Plasmodium : sources
686 d'hétérogénéité de l'infection des vecteurs. Université de Montpellier.
- 687 Pigeault, R., Vézilier, J., Cornet, S., Zélé, F., Nicot, A., Perret, P., Gandon, S., Rivero, A., 2015.
688 Avian malaria: a new lease of life for an old experimental model to study the evolutionary
689 ecology of *Plasmodium*. *Philos. Trans. R. Soc. B Biol. Sci.* 370, 20140300.
690 doi:10.1098/rstb.2014.0300
- 691 Plouffe, D.M., Wree, M., Du, A.Y., Meister, S., Li, F., Patra, K., Lubar, A., Okitsu, S.L., Flannery,
692 E.L., Kato, N., Tanaseichuk, O., Comer, E., Zhou, B., Kuhen, K., Zhou, Y., Leroy, D.,
693 Schreiber, S.L., Scherer, C.A., Vinetz, J., Winzeler, E.A., 2016. High-throughput assay and
694 discovery of small molecules that interrupt malaria transmission. *Cell Host Microbe* 19, 114–

- 695 126. doi:10.1016/j.chom.2015.12.001
- 696 Ramakrishnan, S.P., Basu, P.C., Harwant, S., Wattal, B.L., 1963. A study on the joint action of
697 diamino-diphenyl-sulphone (DDS) and pyrimethamine in the sporogony cycle of *Plasmodium*
698 *gallinaceum*: potentiation of the sporontocidal activity of pyrimethamine by DDS. Indian J.
699 Malariol. 17, 141–148.
- 700 Rivero, A., Gandon, S., 2018. Evolutionary ecology of avian malaria: past to present. Trends
701 Parasitol. 34. doi:10.1016/j.pt.2018.06.002
- 702 Robert, V., Awono-Ambene, H.P., Le Hesran, J.-Y., Trape, J.-F., 2000. Gametocytemia and
703 infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria
704 attacks treated with chloroquine or sulfadoxine plus pyrimethamine. Am. J. Trop. Med. Hyg.
705 62, 210–216. doi:10.4269/ajtmh.2000.62.210
- 706 Ruecker, A., Mathias, D.K., Straschil, U., Churcher, T.S., Dinglasan, R.R., Leroy, D., Sinden, R.E.,
707 Delves, M.J., 2014. A male and female gametocyte functional viability assay to identify
708 biologically relevant malaria transmission-blocking drugs. Antimicrob. Agents Chemother. 58,
709 7292–7304. doi:10.1128/AAC.03666-14
- 710 Sanders, N.G., Sullivan, D.J., Mlambo, G., Dimopoulos, G., Tripathi, A.K., 2014. Gametocytocidal
711 screen identifies novel chemical classes with *Plasmodium falciparum* transmission blocking
712 activity. PLoS One 9, e105817. doi:10.1371/journal.pone.0105817
- 713 Saraiva, R.G., Kang, S., Simões, M.L., Angleró-Rodríguez, Y.I., Dimopoulos, G., 2016. Mosquito
714 gut antiparasitic and antiviral immunity. Dev. Comp. Immunol. 64, 53–64.
715 doi:10.1016/j.dci.2016.01.015
- 716 Schneider, P., Greischar, M.A., Birget, P.L.G., Repton, C., Mideo, N., Reece, S.E., 2018. Adaptive
717 plasticity in the gametocyte conversion rate of malaria parasites. PLoS Pathog. 14, 1–21.
718 doi:10.1371/journal.ppat.1007371
- 719 Sekar, V., Rivero, A., Pigeault, R., Gandon, S., Drews, A., Ahren, D., Hellgren, O., 2020. Gene
720 regulation of the avian malaria parasite *Plasmodium relictum*, during the different stages
721 within the mosquito vector. bioRxiv 2020.07.16.204198. doi:10.1101/2020.07.16.204198
- 722 Shinondo, C.J., Norbert Lanners, H., Lowrie, R.C.J., Wisner, M.F., 1994. Effect of pyrimethamine
723 resistance on sporogony in a *Plasmodium berghei/Anophele stephensi* model. Exp. Parasitol.
724 78, 194–202.
- 725 Shute, P.G., Maryion, M., 1954. The effect of pyrimethamine (Daraprim) on the gametocytes and
726 oocysts of *Plasmodium falciparum* and *Plasmodium vivax*. Trans. R. Soc. Trop. Med. an Hyg.

- 727 48, 50–63.
- 728 Sinden, R.E., 2017. Developing transmission-blocking strategies for malaria control. *PLoS Pathog.*
729 13, e1006336. doi:10.1371/journal.ppat.1006336
- 730 Sinden, R.E., 2010. A biologist's perspective on malaria vaccine development. *Hum. Vaccin.*
731 doi:10.4161/hv.6.1.9604
- 732 Sinden, R.E., Carter, R., Drakeley, C., Leroy, D., 2012. The biology of sexual development of
733 *Plasmodium*: the design and implementation of transmission-blocking strategies. *Malar. J.* 11,
734 1–11. doi:10.1186/1475-2875-11-70
- 735 Smith, D.L., McKenzie, F.E., 2004. Statics and dynamics of malaria infection in *Anopheles*
736 mosquitoes. *Malar. J.* 3. doi:10.1186/1475-2875-3-13
- 737 Sowunmi, A., Akano, K., Ntadom, G., Ayede, A., Oguiche, S., Agomo, C., Okafor, H., Watila, I.,
738 Meremikwu, M., Ogala, W., Agomo, P., Adowoye, E., Fatunmbi, B., Aderoyeje, T., Happi, C.,
739 Gbotosho, G., Folarin, O., 2017. Anaemia following artemisinin-based combination treatments
740 of uncomplicated *Plasmodium falciparum* malaria in children: temporal patterns of
741 haematocrit and the use of uncomplicated hyperparasitaemia as a model for evaluating late-
742 appearing anaemia. *Chemotherapy* 62, 231–238. doi:10.1159/000449366
- 743 Teklehaimanot, A., Nguyen-Dinh, P., Collins, W.E., Barber, A.M., Campbell, C.C., 1985.
744 Evaluation of sporontocidal compounds using *Plasmodium falciparum* gametocytes produced
745 in vitro. *Am. J. Trop. Med. Hyg.* 34, 429–434. doi:10.4269/ajtmh.1985.34.429
- 746 Terzakis, J.A., 1971. *Plasmodium gallinaceum*: drug-induced ultrastructural changes in oocysts.
747 *Exp. Parasitol.* 30, 260–266.
- 748 Terzian, L.A., 1970. A note on the effects of antimalarial drugs on the sporogonous cycle of
749 *Plasmodium cynomolgi* in *Anopheles stephensi*. *Parasitology* 61, 191–194.
- 750 Terzian, L.A., Stahler, N., Daw, A.T., 1968. The sporogonous cycle of *Plasmodium vivax* in
751 *Anopheles* mosquitoes as a system for evaluating the prophylactic and curative capabilities of
752 potential antimalarial compounds. *Exp. Parasitol.* 23, 56–66. doi:10.1016/0014-
753 4894(68)90042-8
- 754 Valkiunas, G., 2004. Life cycle and morphology, in: *Avian Malaria Parasites and Other*
755 *Haemosporidia*. Boca Raton : CRC Press, Washington DC, pp. 17–45. doi:10.3390/v8030082
- 756 Van Eijk, A.M., Hill, J., Alegana, V.A., Kirui, V., Gething, P.W., ter Kuile, F.O., Snow, R.W.,
757 2011. Coverage of malaria protection in pregnant women in sub-Saharan Africa: a synthesis
758 and analysis of national survey data. *Lancet Infect. Dis.* 11, 190–207. doi:10.1016/S1473-

- 759 3099(10)70295-4
- 760 Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2015. *Plasmodium* infection brings forward
761 mosquito oviposition. *Biol. Lett.* 11, 2–6. doi:10.1098/rsbl.2014.0840
- 762 Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2012. *Plasmodium* infection decreases fecundity and
763 increases survival of mosquitoes. *Proc. Biol. Sci.* 279, 4033–41. doi:10.1098/rspb.2012.1394
- 764 Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2010. Insecticide resistance and malaria
765 transmission: infection rate and oocyst burden in *Culex pipiens* mosquitoes infected with
766 *Plasmodium relictum*. *Malar. J.* 9. doi:10.1186/1475-2875-9-379
- 767 Vincke, I.H., 1970. The effects of pyrimethamine and sulphormethoxine on the pre-erythrocytic and
768 sporogonous cycle of *Plasmodium berghei berghei*. *Ann. Soc. Belg. Med. Trop.* (1920). 50,
769 339–358.
- 770 Wadi, I., Anvikar, A.R., Nath, M., Pillai, C.R., Sinha, A., Valecha, N., 2018. Critical examination
771 of approaches exploited to assess the effectiveness of transmission-blocking drugs for malaria.
772 *Future Med. Chem.* 10, 2619–2639. doi:10.4155/fmc-2018-0169
- 773 Wadi, I., Nath, M., Anvikar, A.R., Singh, P., Sinha, A., 2019. Recent advances in transmission-
774 blocking drugs for malaria elimination. *Future Med. Chem.* 11, 3047–3088. doi:10.4155/fmc-
775 2019-0225
- 776 WHO, 2019. World Malaria Report 2019. Geneva.
- 777 Zélé, F., Nicot, A., Berthomieu, A., Weill, M., Duron, O., Rivero, A., 2014. *Wolbachia* increases
778 susceptibility to *Plasmodium* infection in a natural system. *Proc. R. Soc. B* 281.
779 doi:10.1098/rspb.2013.2837
- 780 Zhou, G., Kohlhepp, P., Geiser, D., Frasquillo, M. del C., Vazquez-Moreno, L., Winzerling, J.J.,
781 2007. Fate of blood meal iron in mosquitoes. *J. Insect Physiol.* 53, 1169–1178.
782 doi:10.1016/j.jinsphys.2007.06.009
- 783 [dataset] Villa, Manon (2020), “Villa & al 2020_IJP”, Mendeley Data, V1, doi:
784 10.17632/phzy3tmbzm.1
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Figure Legends

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789 **Figure 1. Experimental design.** a) Impact of SP and AS on mosquito life history traits. Mosquitoes
790 were either provided an infected (red 'body') or an uninfected (grey 'body') blood meal. Six days later
791 they were either given a control blood meal (grey 'wings') or a drug treated blood meal (orange
792 'wings'), b) Impact of SP and AS on Plasmodium development. Protocol was identical except for the
793 first blood meal, which was an infected blood meal for all the mosquitoes.

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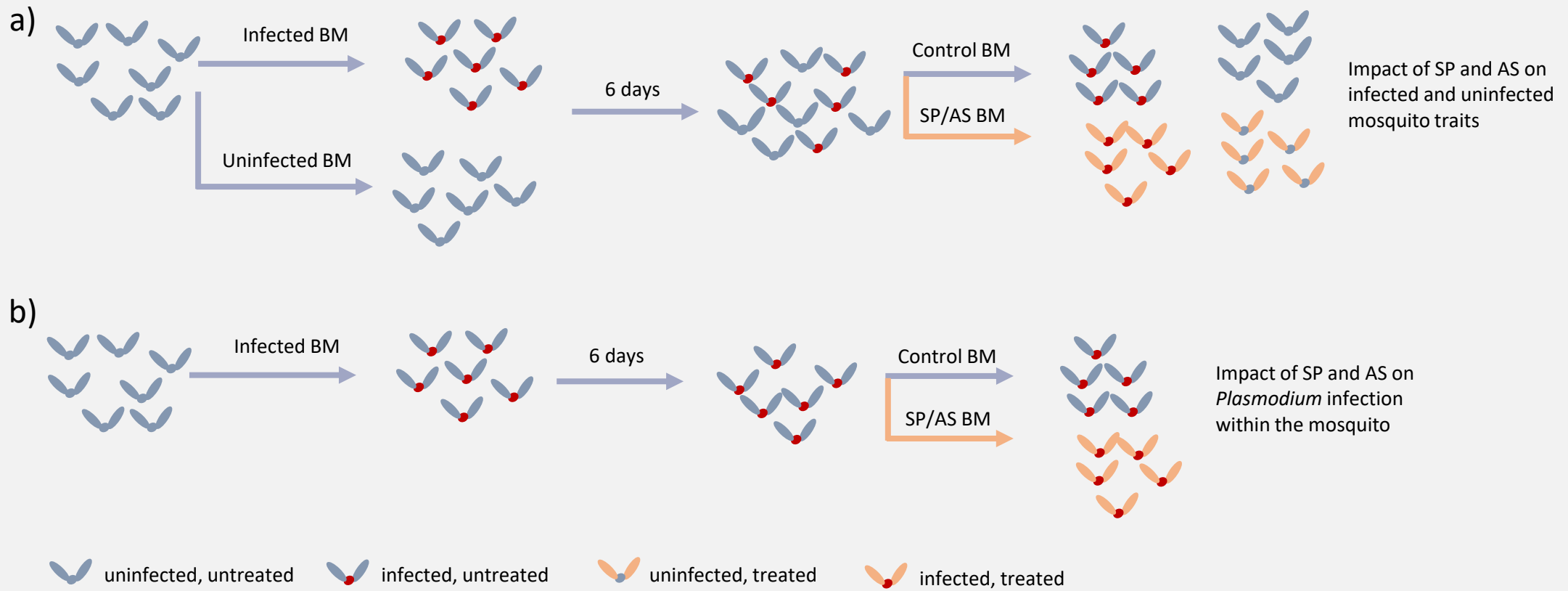
795 **Figure 2. Prevalence and burden of oocysts and sporozoites in mosquitoes fed on a control-**
796 **(grey) or SP-treated (blue) host** for each sampling day (number of days post infection). a), b): oocyst
797 and sporozoite prevalence, respectively. c), d): oocyst and sporozoite burden, respectively. Prevalence
798 is represented as the mean \pm standard error (calculated as $\sqrt{pq/n}$). Burden is represented as a
799 boxplot where with the median (horizontal lines), first and third quartiles (box above and below the
800 medians). Vertical lines delimit 1.5 times the inter-quartile range above which individual counts are
801 considered outliers and marked as circles. Bars not connected by the same letter are significantly
802 different. Post-hoc contrasts were carried out using the emmeans package in R ([https://cran.r-](https://cran.r-project.org/web/packages/emmeans/vignettes/comparisons.html)
803 [project.org/web/packages/emmeans/vignettes/comparisons.html](https://cran.r-project.org/web/packages/emmeans/vignettes/comparisons.html)).

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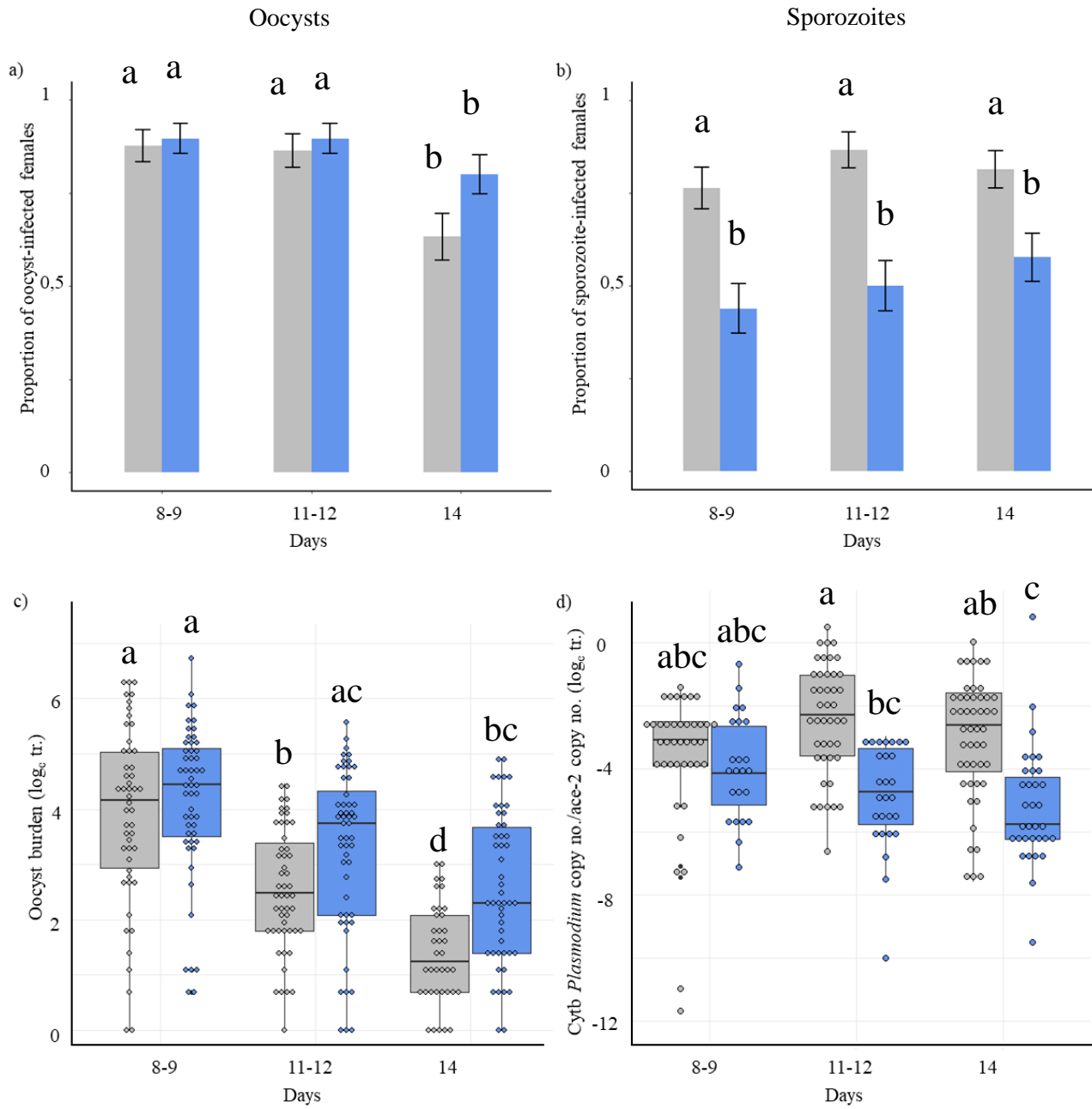
805 **Figure 3. Prevalence and burden of oocysts and sporozoites in mosquitoes fed on a control-**
806 **(grey) or AS-treated host (blue)** for each sampling day (number of day post infection). a), b): oocyst
807 and sporozoite prevalence, respectively. c), d): oocyst and sporozoite burden, respectively. Prevalence
808 is represented as the mean \pm standard error (calculated as $\sqrt{pq/n}$). Burden is represented as a
809 boxplot where with the median (horizontal lines), first and third quartiles (box above and below the
810 medians). Vertical lines delimit 1.5 times the inter-quartile range above which individual counts are
811 considered outliers and marked as circles. Bars not connected by the same letter are significantly

812 different. Post-hoc contrasts were carried out using the emmeans package in R ([https://cran.r-](https://cran.r-project.org/web/packages/emmeans/vignettes/comparisons.html)
813 [project.org/web/packages/emmeans/vignettes/comparisons.html](https://cran.r-project.org/web/packages/emmeans/vignettes/comparisons.html)).

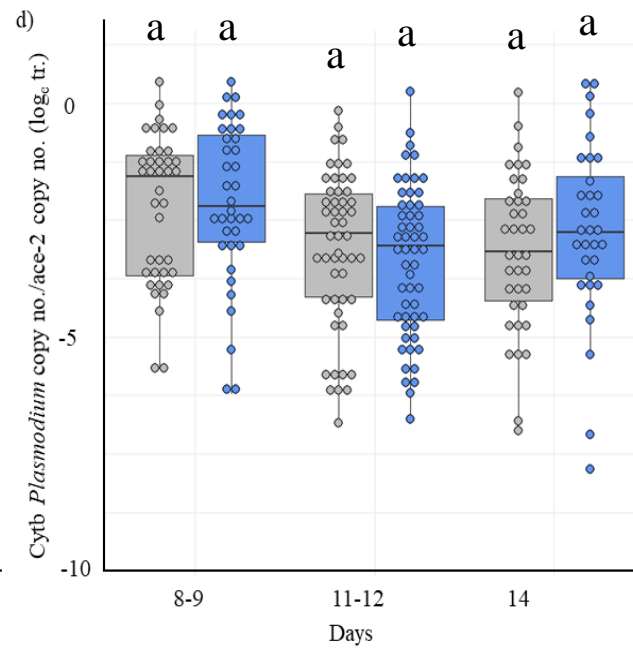
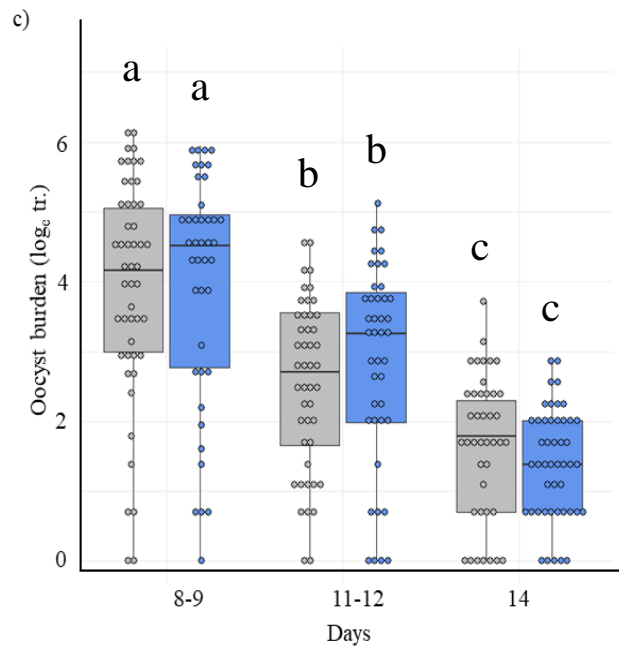
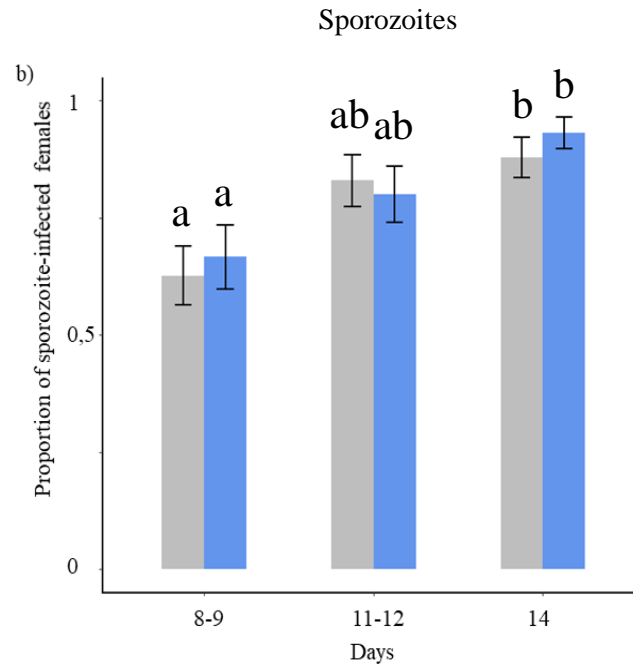
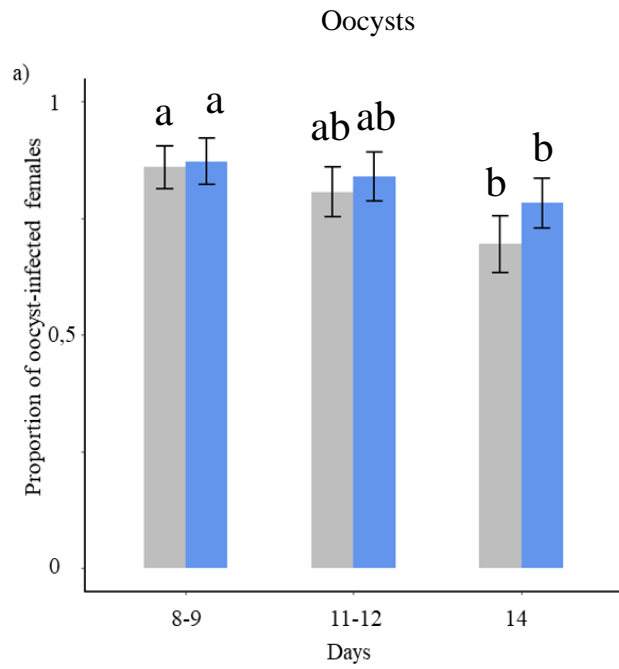
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Tables

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Table 1: Summary of the main studies investigating the inhibitory effect of pyrimethamine (PYR) alone or in combination with sulfadoxine (SFX) for oocyst and sporozoite formation. In prophylactic protocols the drug is administered before or concomitantly with the infected blood meal (mosquitoes ingest the drug at the same time as the infective gametocytes). In curative protocols, the mosquito is first infected and then provided with a second blood meal containing the drug.

<i>Plasmodium</i> species	Mosquito species	Dose*	Oocyst inhibition	Sporozoite inhibition	Reference
Prophylactic administration					
<i>P. falciparum</i>	<i>An. gambiae</i>	20 mg PYR /ind	-	YES	(Foy and Kondi, 1952)
<i>P. falciparum</i>	<i>An. stephensi</i>	25 mg PYR /ind	YES ¹	NO ²	(Shute and Maryion, 1954)
<i>P. falciparum</i>	<i>An. gambiae</i> <i>An. melas</i>	25-50 mg PYR /ind	YES	YES	(Bray et al., 1959)
<i>P. falciparum</i>	<i>An. quadrimaculatus</i> <i>An. freeborni</i>	50-100 mg PYR /ind	YES ¹	YES	(Burgess and Young, 1959)
<i>P. falciparum</i>	<i>An. gambiae</i>	12-50 mg PYR/ind	YES ^{1,3}	YES ^{2,3}	(Gunders, 1961)
<i>P. falciparum</i>	<i>An. stephensi</i>	0.00001% PYR (ss)	-	YES ²	(Gerberg, 1971)
<i>P. falciparum</i>	<i>An. stephensi</i>	10 ⁻⁷ M PYR (mf)	YES ¹	-	(Chutmongkonkul et al., 1992)
<i>P. falciparum</i>	<i>An. gambiae</i>	75 mg PYR 1500 mg SFX	YES ¹	-	(Hogh et al., 1998)
<i>P. falciparum</i>	<i>An. gambiae</i>	1.25 mg/kg PYR 25 mg/kg SFX	YES	-	(Beavogui et al., 2010)
<i>P. falciparum</i>	<i>An. arabiensis</i>	25 mg/kg SFX 1.25 mg/kg PYR	YES ^{1,4}	-	(Robert et al., 2000)
<i>P. vivax</i>	<i>An. stephensi</i>	50 mg PYR /ind	YES ¹	NO ²	(Shute and Maryion, 1954)
<i>P. vivax</i>	<i>An. stephensi</i>	0.002 gr PYR /ml (ss)	YES ¹	YES	(Terzian et al., 1968)
<i>P. cynomolgui</i>	<i>An. stephensi</i>	0.001 gr PYR /ml (ss)	YES ¹	YES	(Terzian, 1970)
<i>P. cynomolgui</i>	<i>An. stephensi</i>	0.00001% PYR (ss)	-	YES ²	(Gerberg, 1971)
<i>P. cynomolgui</i>	<i>An. maculatus</i>	3 mg PYR /kg	YES	YES	(Omar et al., 1973)
<i>P. berghei</i>	<i>An. stephensi</i>	2.5 - 20mg PYR /kg	YES	YES	(Vincke, 1970)
<i>P. berghei</i>	<i>An. stephensi</i>	20mg PYR /kg	YES	YES	(Shinondo et al., 1994)
<i>P. berghei</i>	<i>An. stephensi</i>	Serum from PYR/SFX treated patients (mf)	YES ¹	-	(Hogh et al., 1998)
<i>P. gallinaceum</i>	<i>Ae. aegypti</i>	0.028 mg/kg PYR 210 mg/kg SFX	-	YES	(Ramakrishnan et al., 1963)
<i>P. gallinaceum</i>	<i>Ae. aegypti</i>	0.001% and 0.0001% PYR (ss)	YES ¹	YES ²	(Terzakis, 1971)
<i>P. gallinaceum</i>	<i>Ae. aegypti</i>	0.00001% PYR (ss)	-	YES ²	(Gerberg, 1971)
Curative administration					
<i>P. falciparum</i>	<i>An. gambiae, An. melas</i>	25-50 mg PYR 4 days post infection	YES ¹	NO ²	(Bray et al., 1959)
<i>P. falciparum</i>	<i>An. gambiae</i>	1µM PYR 2-4 days post-infection (mf)	YES ¹	NO ²	(Teklehaimanot et al., 1985)
<i>P. falciparum</i>	<i>An. stephensi</i>	10 ⁻⁷ M PYR 4 days post infection (mf)	NO	-	(Chutmongkonkul et al., 1992)

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* Drug administered directly to host unless otherwise stated: (ss): drug administered in sugar solution, (mf): drug added to blood in a membrane feeder. ¹ Inhibition was partial (some oocysts present); ² Sporozoites were observed but not quantified; ³ No untreated controls; ⁴ Chloroquine-treated patients used as a control.

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Supplementary Materials

The transmission-blocking effects of antimalarial drugs revisited:
fitness costs and sporontocidal effects of artesunate
and sulfadoxine-pyrimethamine (Villa et al)

837 **Table S1. Description of the statistical models used to analyze the impact of drugs on mosquito**
 838 **life history traits.** Models with binomial error structure require a concatenated response variable
 839 binding together the number of successes and failures for a given outcome. N gives the number of
 840 mosquitoes included in each analysis. "Maximal model" represents the complete set of explanatory
 841 variables (and their interactions) included in the model. "Minimal model" represents the model
 842 containing only the significant variables and their interactions. N represents the number of replicates in
 843 each analysis. Round brackets indicate that the variable was fitted as a random factor. Square brackets
 844 indicate the error structure used (n: normal errors, b: binomial errors). date: sampling day, status:
 845 alive/dead on sampling day, fed/unfed: number of fed/unfed mosquitoes, hm: haematin excreted (proxi
 846 for blood meal size), plt: plate used for the colorimetric quantification haematin, hmr: residuals of hm
 847 by plate, eggs: number of eggs laid, inf: mosquito infection status (infected/uninfected), TR: mosquito
 848 fed on treated/untreated bird.
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Variable of interest		Response variable	Model Nb.	N	Maximal model	Minimal model	R subroutine [err struct.]
<i>Effect of AS on mosquito traits</i>							
RBC	RBC/ml	RBC	11	5	TR	1	K-W
Survival	Overall survival	(date, status)	15	414	TR*inf + (1 bird)	(1 bird)	coxme
	% mosquitoes surviving until day 14	cbind (dead, alive)	16	10	TR*inf + (1 bird)	(1 bird)	lmer [b]
Blood meal size	Blood-fed	cbind (fed, unfed)	9	966	TR*inf + (1 bird)	(1 bird)	lmer [b]
	Blood meal size	hmr (lm (hm ~ plt)	10	414	TR*inf + (1 bird)	(1 bird)	lmer [n]
Fecundity	Egg laying probability	cbind (laid, not laid)	12	440	hmr*TR*inf + (1 bird)	TR*hmr + inf + (1 bird)	lmer [b]
	Oviposition day	day	13	337	hmr*TR*inf	hmr	clm
	Number of eggs per raft	eggs	14	337	hmr*TR + inf*day + (1 bird)	hmr + (1 bird)	glmmTMB
<i>Effect of SP on mosquito traits</i>							
RBC	RBC/ml	RBC	3	6	TR	1	K-W
Survival	overall survival	(date, statut)	7	564	TR*inf + (1 bird)	inf + (1 bird)	coxme
	% mosquitoes surviving until day 14	cbind(dead, alive)	8	12	TR*inf + (1 bird)	(1 bird)	lmer [b]
Blood meal	Blood-fed	cbind (fed, unfed)	1	1045	TR*inf + (1 bird)	(1 bird)	lmer [b]
	Blood meal size	hmres (lm (hm ~ Plq)	2	454	TR*inf + (1 bird)	(1 bird)	lmer [n]
Fecundity	Egg-laying probability	cbind (laid, not laid)	4	378	hmr*TR*inf + (1 bird)	TR*inf + (1 bird)	lmer [b]
	Oviposition day	day	5	312	hmr*TR*inf	hmr*inf + TR	clm
	Number of eggs per raft	eggs	6	312	hmr*TR + inf*day + (1 bird)	day + (1 bird)	glmmTMB

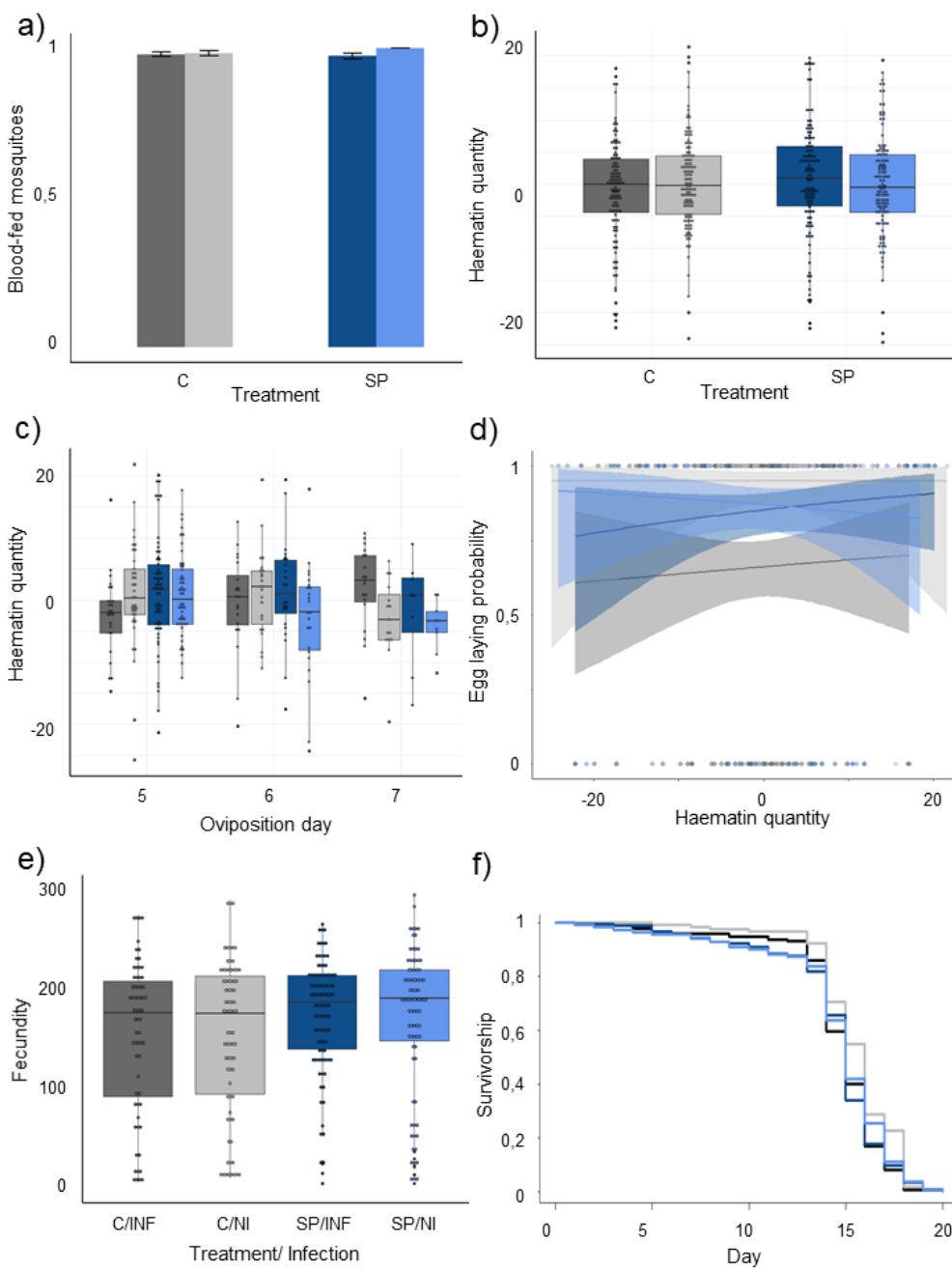
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853 **Table S2. Description of the statistical models used to analyze the impact of drugs on**
 854 ***Plasmodium* prevalence and burden.** Models with binomial error structure require a concatenated
 855 response variable binding together the number of successes and failures for a given outcome. N gives
 856 the number of mosquitoes included in each analysis. "Maximal model" represents the complete set of
 857 explanatory variables (and their interactions) included in the model. "Minimal model" represents the
 858 model containing only the significant variables and their interactions. N represents the number of
 859 replicates in each analysis. Round brackets indicate that the variable was fitted as a random factor.
 860 Square brackets indicate the error structure used (n: normal errors, b: binomial errors). date: mosquito
 861 dissection day (discrete variable), day: mosquito dissection day (continuous variable), inf: mosquito
 862 infection status (infected/uninfected), plt: plate used for the sporozoite quantification (qPCR), TR:
 863 mosquito fed on treated/untreated bird.
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 865

Variable of interest	Response variable	Model Nb.	N	Maximal model	Minimal model	R subroutine [err struct.]
<i>Effect of AS on Plasmodium</i>						
Oocyst prevalence	Number of mosquitos with at least 1 oocyst	cbind (inf, uninf)	23	330 TR *date + (1 bird)	date + (1 bird)	lmer [b]
Oocyst burden	Number of oocysts per infected mosquito	oocysts	24	266 TR*date + (1 bird)	date + (1 bird)	glmmTMB
Sporozoite prevalence	Number of mosquitos with sporozoites	cbind (inf, uninf)	25	287 TR *date + (1 plt)	date + (1 plt)	lmer [b]
Sporozoite burden	Ratio between mosquito and parasite DNA	log(ratio)	26	227 TR *date + (1 bird) + (1 plt)	date + (1 bird) + (1 plt)	lmer [n]
<i>Effect of SP on Plasmodium</i>						
Oocyst prevalence	Number of mosquitos with at least 1 oocyst	cbind (inf, uninf)	17	352 TR*date + (1 bird)	date + (1 bird)	lmer [b]
Oocyst burden	Number of oocysts per infected mosquito	oocysts	18	291 TR*date + (1 bird)	TR *date + (1 bird)	glmmTMB
Oocyst burden	Number of oocysts per infected mosquito	oocysts	19	291 TR*day + (1 bird)	TR *day + (1 bird)	glmmTMB
Sporozoite prevalence	Number of mosquitos with sporozoites	cbind (inf, uninf)	20	320 TR *date + (1 plt)	TR + (1 bird) + (1 plt)	lmer [b]
Sporozoite burden	Ratio between mosquito and parasite DNA	log (ratio)	21	225 TR *date + (1 bird) + (1 plt)	TR + (1 bird) + (1 plt)	lmer [n]
Sporozoite burden	Ratio between mosquito and parasite DNA	log (ratio)	22	225 TR *day + (1 bird) + (1 plt)	TR*day + (1 bird) + (1 plt)	lmer [n]

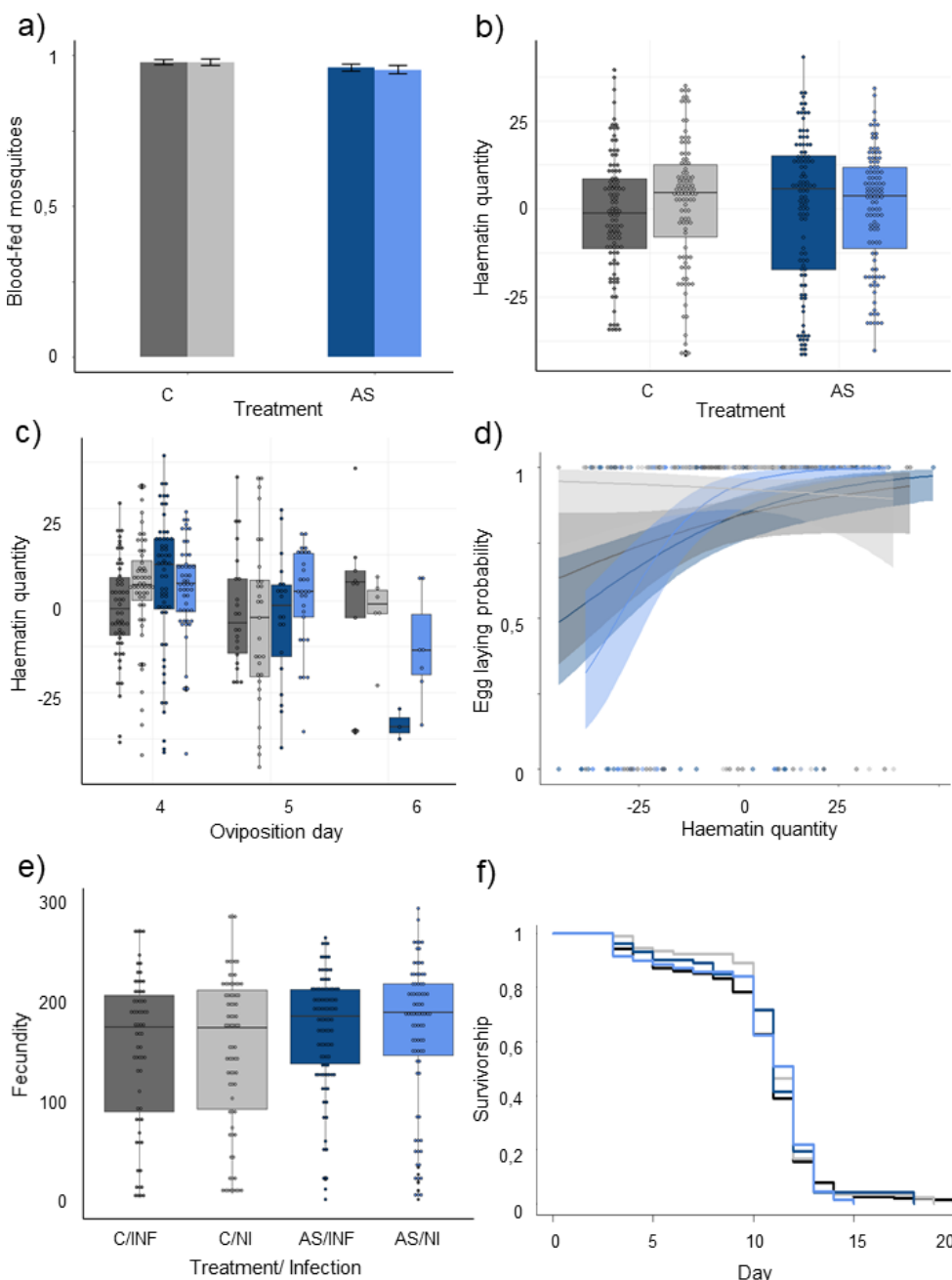
866

867 **Figure S3. Impact of SP on *Plasmodium*-infected and uninfected mosquito traits.** a) Proportion of
 868 mosquitoes that blood-fed, b) Blood meal size (represented by the residuals of a model containing
 869 'plate' as an explanatory variable, see materials and methods) as a function of treatment, c) Blood meal
 870 size as a function of oviposition day, d) Probability of laying an egg raft, e) Fecundity, f) Survival.
 871 Grey: mosquitoes fed on a control bird, blue: mosquitoes fed on an SP-treated bird. Dark grey, dark
 872 blue: infected mosquitoes; light grey, light blue: uninfected mosquitoes Bars (a) represent mean \pm
 873 standard error (calculated as $\sqrt{pq/n}$), boxplots (b, c, e) represent the median (horizontal lines), first
 874 and third quartiles (box above and below the medians), the 1.5 inter-quartile range (vertical lines) and
 875 the outliers (points above and below the iq range). Lines (d) are fitted using a logistic regression the
 876 colored areas are the 95% confident intervals.



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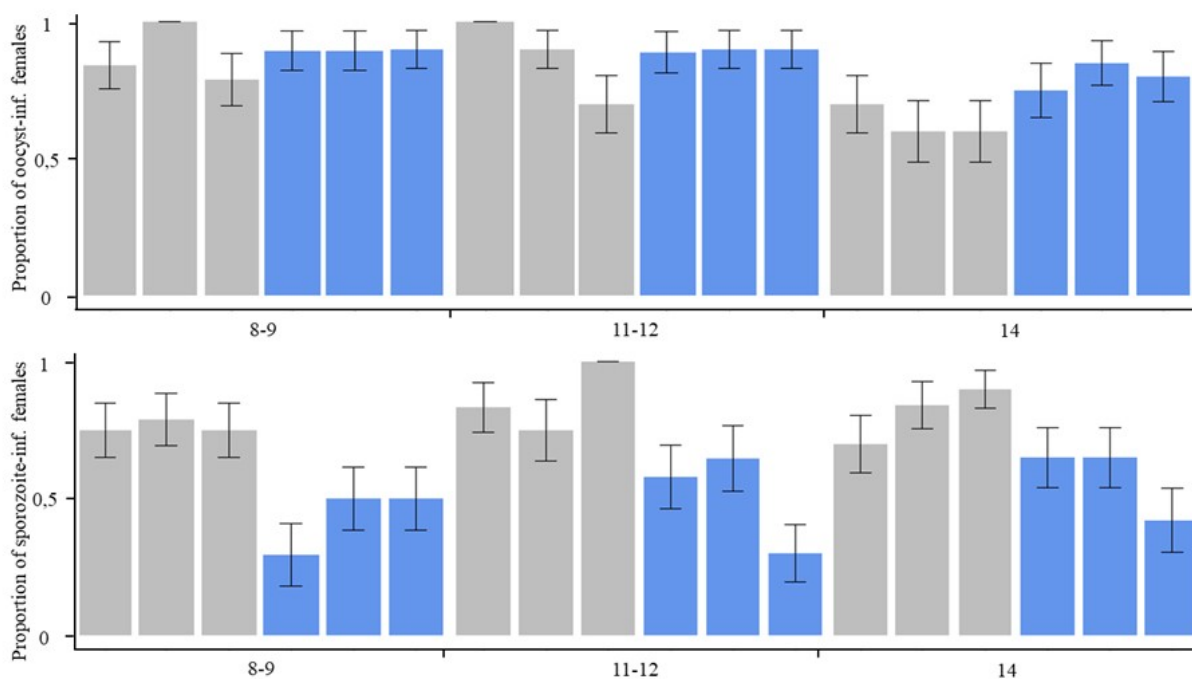
878 **Figure S4. Impact of AS on Plasmodium-infected and uninfected mosquito traits.** a) Proportion of
 879 mosquitoes that blood-fed, b) Blood meal size (represented by the residuals of a model containing
 880 'plate' as an explanatory variable, see materials and methods) as a function of treatment, c) Blood meal
 881 size as a function of oviposition day, d) Probability of laying an egg raft, e) Fecundity, f) Survival.
 882 Grey: mosquitoes fed on a control bird, blue: mosquitoes fed on an AS-treated bird. Dark grey, dark
 883 blue: infected mosquitoes; light grey, light blue: uninfected mosquitoes Bars (a) represent mean \pm
 884 standard error (calculated as $\sqrt{pq/n}$), boxplots (b, c, e) represent the median (horizontal lines), first
 885 and third quartiles (box above and below the medians), the 1.5 inter-quartile range (vertical lines) and
 886 the outliers (points above and below the iq range). Lines (d) are fitted using a logistic regression the
 887 colored areas are the 95% confident intervals.



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890 **Figure S5. Prevalence of oocysts (top) and sporozoites (bottom) in mosquitoes fed on each of the**
891 **3 control (grey) and SP-treated (blue) birds, for each of the 3 sampling days (8-9, 11-12 and 14).**
892 Between 15-20 mosquitoes were sampled per bird and per time point. Each mosquito was used to
893 estimate oocyst prevalence (abdomen dissection of midgut, top panel) and sporozoites (qPCR on head-
894 thorax, bottom panel, see Materials and Methods). Prevalence was established as presence/absence of
895 oocysts in the midgut (top panel) and the head-thorax (bottom panel). Prevalence is represented as the
896 mean \pm standard error (calculated as $\sqrt{pq/n}$).

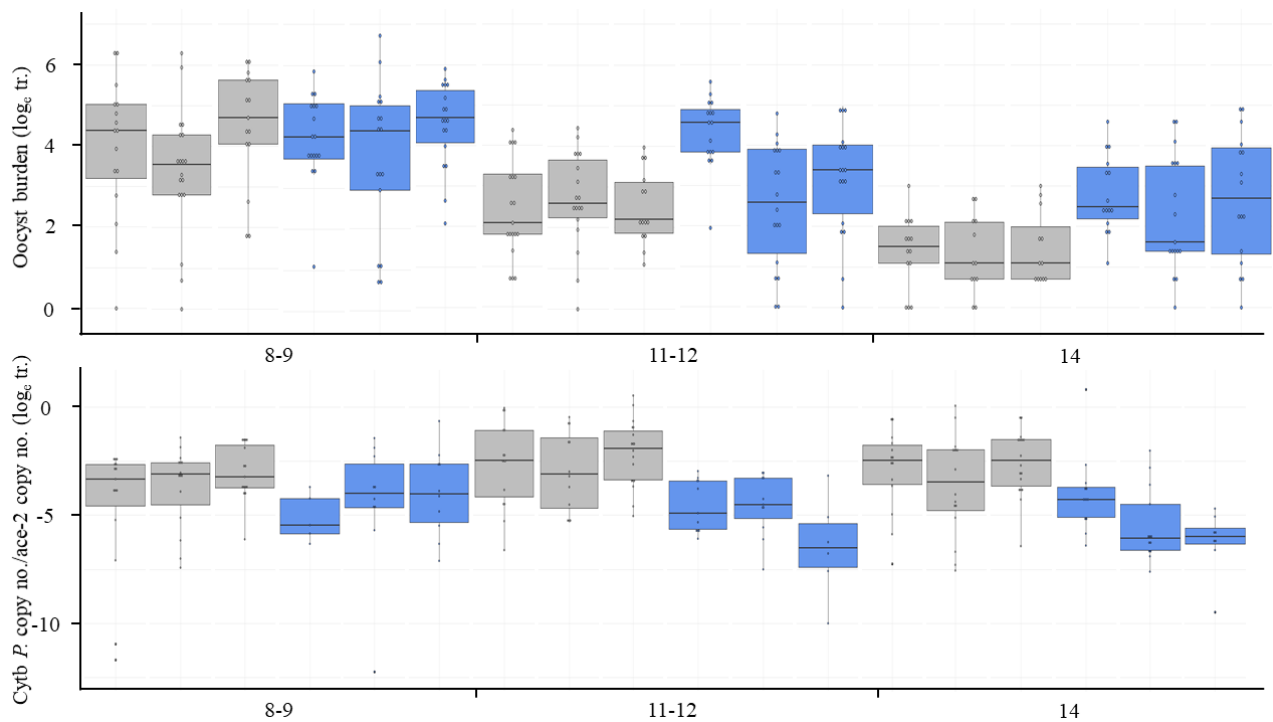
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900 **Figure S6. Oocyst (top) and sporozoite (bottom) burden in mosquitoes fed on each of the 3**
901 **control (grey) and SP-treated (blue) birds**, for each of the 3 sampling days (8-9, 11-12 and 14).
902 Between 15-20 mosquitoes were sampled per bird and per time point. Mosquitoes were dissected to
903 count the number of oocysts in the midgut (top panel) and the head-thorax fraction was used to
904 quantify the number of sporozoites (qPCR, bottom panel). Burden is represented as a boxplot where
905 with the median (horizontal lines), first and third quartiles (box above and below the medians).
906 Vertical lines delimit 1.5 times the inter-quartile range above which individual counts are considered
907 outliers and marked as circles.



908