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

M. Villa, M. Buysse, A. Berthomieu, Ana R Rivero

Ms. Ref. No.: IJPara20_351R1

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]

[1]MIVEGEC (CNRS – IRD – Université de Montpellier)

[2]CREES (Centre d'Écologie et Évolution de la Santé), Montpellier

911 avenue Agropolis, 34394 Montpellier, France

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

Declaration of interests: none

Abstract

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

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

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

2. Material and Methods

2.1. Mosquito and parasite protocols

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

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

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

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

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

2.2.1. Sulfadoxine-pyrimethamine experiment

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

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

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

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

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

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

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

2.4. Statistical analyses

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

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

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

2.5. Ethics statement

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

The authors declare no conflict of interests.

3. Results

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

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

3.1.1. Sulfadoxine-Pyrimethamine (SP) experiment

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

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

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

3.1.2. Artesunate (AS) experiment –

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

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

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

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

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

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

3.2.1. Sulfadoxine-Pyrimethamine experiment

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

Treatment had a significant effect on the prevalence of sporozoites within the mosquitoes (model 20, $\chi^2 = 10.394$, $p < 0.01$, Figure 2b). On average, parasites developing in mosquitoes having fed on an SP-treated host had a significantly lower probability of reaching the sporozoite stage than their control counterparts (55% vs 82%, respectively). Sporozoite burden was also significantly lower in mosquitoes having fed on an SP-treated host, irrespective of the dissection date (model 21, *treatment*: $\chi^2 = 9.8898$, $p < 0.01$; *date*: $\chi^2 = 3.1579$, $p = 0.2062$; Figure 2d). As above, these results are consistent across all the birds used in the experiment (Supplementary Materials, Figures S5 and S6).

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

3.2.2. Artesunate experiment

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

5. Acknowledgements

We would like to thank Tanguy Lagache for his help with the experiments and Bethsabée Sheid, the Vectopole coordinator. The Vectopole is one of the platforms of the Vectopole Sud network and is funded through the ANR "Investissements d'avenir" program (ANR-10-LABX-04-01). The experiments were funded through the ANR-16-CE35-0001-01 ('EVODRUG') to AR.

6. References

Aikawa, M., Beaudoin, R.L., 1968. Studies on nuclear division of a malarial parasite under pyrimethamine treatment. J. Cell Biol. 39, 749–754. doi:10.1083/jcb.39.3.749

Aleshnick, M., Ganusov, V. V., Nasir, G., Yenokyan, G., Sinnis, P., 2019. Experimental determination of the force of malaria infection reveals a non-linear relationship to mosquito sporozoite loads. bioRxiv 1–40. doi:10.1101/830299

Azevedo, R., Markovic, M., Machado, M., Franke-Fayard, B., Mendes, A.M., Prudêncio, M., 2017. Bioluminescence method for in vitro screening of *Plasmodium* transmission-blocking compounds. Antimicrob Agents Chemother 61, e02699-16.

Beavogui, A.H., Djimde, A.A., Gregson, A., Toure, A.M., Dao, A., Coulibaly, B., Ouologuem, D., Fofana, B., Sacko, A., Tekete, M., Kone, A., Niare, O., Wele, M., Plowe, C. V., Picot, S., Doumbo, O.K., 2010. Low infectivity of *Plasmodium falciparum* gametocytes to *Anopheles gambiae* following treatment with sulfadoxine-pyrimethamine in Mali. Int. J. Parasitol. 40, 1213–1220. doi:10.1016/j.ijpara.2010.04.010

Blagborough, A.M., Churcher, T.S., Upton, L.M., Ghani, A.C., Gething, P.W., Sinden, R.E., 2013. Transmission-blocking interventions eliminate malaria from laboratory populations. Nat. Commun. 4, 1–7. doi:10.1038/ncomms2840

Bolker, B.M., 2008. Ecological models and data in R, Ecological Models and Data in R. doi:10.1111/j.1442-9993.2010.02210.x

Bomblies, A., 2014. Agent-based modeling of malaria vectors: the importance of spatial simulation. Parasites and Vectors 7, 308. doi:10.1186/1756-3305-7-308

Bray, R.S., Burgess, R.W., Fox, R.M., Miller, M.J., 1959. Effect of pyrimethamine upon sporogony and pre-erythrocytic schizogony of *Laverania falciparum*. Bull. World Health Organ. 21, 233–238.

Burgess, R.W., Young, M.D., 1959. The development of pyrimethamine resistance by *Plasmodium*

falciparum. Bull. World Health Organ. 20, 37–46.

Burri, C., Ferrari, G., Ntuku, H.M., Kitoto, A.T., Duparc, S., Hugo, P., Mitembo, D.K., Lengeler, C., 2014. Short report: Delayed anemia after treatment with injectable artesunate in the Democratic Republic of the Congo: a manageable issue. Am. J. Trop. Med. Hyg. 91, 821–823. doi:10.4269/ajtmh.14-0149

Butcher, G., 1997. Antimalarial drugs and the mosquito transmission of *Plasmodium*. Int. J. Parasitol. 27, 975–987.

Butcher, P., 1989. Mechanisms of immunity to malaria and the possibilities of a blood-stage vaccine: a critical appraisal. Parasitology 98, 315–327. doi:10.1017/S0031182000062247

Capan, M., Mombo-Ngoma, G., Makristathis, A., Ramharter, M., 2010. Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative *in vitro* study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin. Malar. J. 9, 303. doi:10.1186/1475-2875-9-303

Chotivanich, K., Sattabongkot, J., Udomsangpetch, R., Looareesuwan, S., Day, N.P.J., Coleman, R.E., White, N.J., 2006. Transmission-blocking activities of quinine, primaquine, and artesunate. Antimicrob. Agents Chemother. 50, 1927–1930. doi:10.1128/AAC.01472-05

Churcher, T.S., Bousema, T., Walker, M., Drakeley, C., Schneider, P., Ouédraogo, A.L., Basáñez, M.G., 2013. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and estimating the reservoir of infection. Elife e00626. doi:10.7554/eLife.00626

Chutmongkonkul, M., Maier, W.A., Seitz, H.M., 1992. *Plasmodium falciparum*: effect of chloroquine, halofantrine and pyrimethamine on the infectivity of gametocytes for *Anopheles stephensi* mosquitoes. Ann. Trop. Med. Parasitol. 86, 103–110. doi:10.1080/00034983.1992.11812639

Coatney, G.R., Cooper, W.C., Eddy, N.B., Greenberg, J., 1953. Survey of antimalarial agents: chemotherapy of *Plasmodium gallinaceum* infections; toxicity; correlation of structure and action. Public Heal. Serv. Publ.

Cohuet, A., Osta, M.A., Morlais, I., Awono-Ambene, P.H., Michel, K., Simard, F., Christophides, G.K., Fontenille, D., Kafatos, F.C., 2006. *Anopheles* and *Plasmodium*: from laboratory models to natural systems in the field. EMBO Rep. 7, 1285–1289. doi:10.1038/sj.embor.7400831

Cornet, S., Nicot, A., Rivero, A., Cator, L., 2019. Avian malaria alters blood feeding of *Culex pipiens* mosquitoes. Malar. J. 18, 82.

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., Gamo,
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., Gomorrai, 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.,

Ruecker, A., Marques, S., Sinden, R.E., Herreros, E., 2015. Imaging-based high-throughput screening assay to identify new molecules with transmission-blocking potential against *Plasmodium falciparum* female gamete formation. Antimicrob. Agents Chemother. 59, 3298–3305. doi:10.1128/AAC.04684-14

Nunes, J.K., Woods, C., Carter, T., Raphael, T., Morin, M.J., Diallo, D., Leboulleux, D., Jain, S., Loucq, C., Kaslow, D.C., Birkett, A.J., 2014. Development of a transmission-blocking malaria vaccine: progress, challenges, and the path forward. Vaccine. doi:10.1016/j.vaccine.2014.07.030

Omar, M.S., Collins, W.E., Contacos, P.G., 1973. Gametocytocidal and sporontocidal effects of antimalarial drugs on malaria parasites. Exp. Parasitol. 34, 229–241. doi:10.1016/0014-4894(73)90082-9

Paaijmans, K., Fernàndez-Busquets, X., 2014. Antimalarial drug delivery to the mosquito: an option worth exploring? Futur. Microbiol 9, 579–582. doi:10.2217/FMB.14.30

Paton, D.G., Childs, L.M., Itoe, M.A., Holmdahl, I.E., Buckee, C.O., Catteruccia, F., 2019. Exposing *Anopheles* mosquitoes to antimalarials blocks *Plasmodium* parasite transmission. Nature. doi:10.1038/s41586-019-0973-1

Peatey, C.L., Leroy, D., Gardiner, D.L., Trenholme, K.R., 2012. Anti-malarial drugs: how effective are they against *Plasmodium falciparum* gametocytes? Malar. J. 11, 34. doi:10.1186/1475-2875-11-34

Peterson, D.S., Walliker, D., Wellems, T.E., 1988. Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in *falciparum* malaria, Proc. Nati. Acad. Sci. USA.

Pigeault, R., 2015. Ecologie évolutive des interaction Hôte / Moustique / Plasmodium : sources d'hétérogénéité de l'infection des vecteurs. Université de Montpellier.

Pigeault, R., Vézilier, J., Cornet, S., Zélé, F., Nicot, A., Perret, P., Gandon, S., Rivero, A., 2015. Avian malaria: a new lease of life for an old experimental model to study the evolutionary ecology of *Plasmodium*. Philos. Trans. R. Soc. B Biol. Sci. 370, 20140300. doi:10.1098/rstb.2014.0300

Plouffe, D.M., Wree, M., Du, A.Y., Meister, S., Li, F., Patra, K., Lubar, A., Okitsu, S.L., Flannery, E.L., Kato, N., Tanaseichuk, O., Comer, E., Zhou, B., Kuhen, K., Zhou, Y., Leroy, D., Schreiber, S.L., Scherer, C.A., Vinetz, J., Winzeler, E.A., 2016. High-throughput assay and discovery of small molecules that interrupt malaria transmission. Cell Host Microbe 19, 114–

126. doi:10.1016/j.chom.2015.12.001

Ramakrishnan, S.P., Basu, P.C., Harwant, S., Wattal, B.L., 1963. A study on the joint action of diamino-diphenyl-sulphone (DDS) and pyrimethamine in the sporogony cycle of *Plasmodium gallinaceum*: potentiation of the sporontocidal activity of pyrimethamine by DDS. Indian J. Malariol. 17, 141–148.

Rivero, A., Gandon, S., 2018. Evolutionary ecology of avian malaria: past to present. Trends Parasitol. 34. doi:10.1016/j.pt.2018.06.002

Robert, V., Awono-Ambene, H.P., Le Hesran, J.-Y., Trape, J.-F., 2000. Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. Am. J. Trop. Med. Hyg. 62, 210–216. doi:10.4269/ajtmh.2000.62.210

Ruecker, A., Mathias, D.K., Straschil, U., Churcher, T.S., Dinglasan, R.R., Leroy, D., Sinden, R.E., Delves, M.J., 2014. A male and female gametocyte functional viability assay to identify biologically relevant malaria transmission-blocking drugs. Antimicrob. Agents Chemother. 58, 7292–7304. doi:10.1128/AAC.03666-14

Sanders, N.G., Sullivan, D.J., Mlambo, G., Dimopoulos, G., Tripathi, A.K., 2014. Gametocytocidal screen identifies novel chemical classes with *Plasmodium falciparum* transmission blocking activity. PLoS One 9, e105817. doi:10.1371/journal.pone.0105817

Saraiva, R.G., Kang, S., Simões, M.L., Angleró-Rodríguez, Y.I., Dimopoulos, G., 2016. Mosquito gut antiparasitic and antiviral immunity. Dev. Comp. Immunol. 64, 53–64. doi:10.1016/j.dci.2016.01.015

Schneider, P., Greischar, M.A., Birget, P.L.G., Repton, C., Mideo, N., Reece, S.E., 2018. Adaptive plasticity in the gametocyte conversion rate of malaria parasites. PLoS Pathog. 14, 1–21. doi:10.1371/journal.ppat.1007371

Sekar, V., Rivero, A., Pigeault, R., Gandon, S., Drews, A., Ahren, D., Hellgren, O., 2020. Gene regulation of the avian malaria parasite *Plasmodium relictum*, during the different stages within the mosquito vector. bioRxiv 2020.07.16.204198. doi:10.1101/2020.07.16.204198

Shinondo, C.J., Norbert Lanners, H., Lowrie, R.C.J., Wiser, M.F., 1994. Effect of pyrimethamine resistance on sporogony in a *Plasmodium berghei*/*Anophele stephensi* model. Exp. Parasitol. 78, 194–202.

Shute, P.G., Maryion, M., 1954. The effect of pyrimethamine (Daraprim) on the gametocytes and oocysts of *Plasmodium falciparum* and *Plasmodium vivax*. Trans. R. Soc. Trop. Med. an Hyg.

48, 50–63.

Sinden, R.E., 2017. Developing transmission-blocking strategies for malaria control. PLoS Pathog. 13, e1006336. doi:10.1371/journal.ppat.1006336

Sinden, R.E., 2010. A biologist's perspective on malaria vaccine development. Hum. Vaccin. doi:10.4161/hv.6.1.9604

Sinden, R.E., Carter, R., Drakeley, C., Leroy, D., 2012. The biology of sexual development of *Plasmodium*: the design and implementation of transmission-blocking strategies. Malar. J. 11, 1–11. doi:10.1186/1475-2875-11-70

Smith, D.L., McKenzie, F.E., 2004. Statics and dynamics of malaria infection in *Anopheles* mosquitoes. Malar. J. 3. doi:10.1186/1475-2875-3-13

Sowunmi, A., Akano, K., Ntadom, G., Ayede, A., Oguche, S., Agomo, C., Okafor, H., Watila, I., Meremikwu, M., Ogala, W., Agomo, P., Adowoye, E., Fatunmbi, B., Aderoyeje, T., Happi, C., Gbotosho, G., Folarin, O., 2017. Anaemia following artemisinin-based combination treatments of uncomplicated *Plasmodium falciparum* malaria in children: temporal patterns of haematocrit and the use of uncomplicated hyperparasitaemia as a model for evaluating late-appearing anaemia. Chemotherapy 62, 231–238. doi:10.1159/000449366

Teklehaimanot, A., Nguyen-Dinh, P., Collins, W.E., Barber, A.M., Campbell, C.C., 1985. Evaluation of sporontocidal compounds using *Plasmodium falciparum* gametocytes produced in vitro. Am. J. Trop. Med. Hyg. 34, 429–434. doi:10.4269/ajtmh.1985.34.429

Terzakis, J.A., 1971. *Plasmodium gallinaceum*: drug-induced ultrastructural changes in oocysts. Exp. Parasitol. 30, 260–266.

Terzian, L.A., 1970. A note on the effects of antimalarial drugs on the sporogonous cycle of *Plasmodium cynomolgi* in *Anopheles stephensi*. Parasitology 61, 191–194.

Terzian, L.A., Stahler, N., Daw, A.T., 1968. The sporogonous cycle of *Plasmodium vivax* in *Anopheles* mosquitoes as a system for evaluating the prophylactic and curative capabilities of potential antimalarial compounds. Exp. Parasitol. 23, 56–66. doi:10.1016/0014-4894(68)90042-8

Valkiunas, G., 2004. Life cycle and morphology, in: Avian Malaria Parasites and Other Haemosporidia. Boca Raton : CRC Press, Washington DC, pp. 17–45. doi:10.3390/v8030082

Van Eijk, A.M., Hill, J., Alegana, V.A., Kirui, V., Gething, P.W., ter Kuile, F.O., Snow, R.W., 2011. Coverage of malaria protection in pregnant women in sub-Saharan Africa: a synthesis and analysis of national survey data. Lancet Infect. Dis. 11, 190–207. doi:10.1016/S1473-

3099(10)70295-4

Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2015. *Plasmodium* infection brings forward mosquito oviposition. Biol. Lett. 11, 2–6. doi:10.1098/rsbl.2014.0840

Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2012. *Plasmodium* infection decreases fecundity and increases survival of mosquitoes. Proc. Biol. Sci. 279, 4033–41. doi:10.1098/rspb.2012.1394

Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2010. Insecticide resistance and malaria transmission: infection rate and oocyst burden in *Culex pipiens* mosquitoes infected with *Plasmodium relictum*. Malar. J. 9. doi:10.1186/1475-2875-9-379

Vincke, I.H., 1970. The effects of pyrimethamine and sulphormethoxine on the pre-erythrocytic and sporogonous cycle of *Plasmodium berghei berghei*. Ann. Soc. Belg. Med. Trop. (1920). 50, 339–358.

Wadi, I., Anvikar, A.R., Nath, M., Pillai, C.R., Sinha, A., Valecha, N., 2018. Critical examination of approaches exploited to assess the effectiveness of transmission-blocking drugs for malaria. Future Med. Chem. 10, 2619–2639. doi:10.4155/fmc-2018-0169

Wadi, I., Nath, M., Anvikar, A.R., Singh, P., Sinha, A., 2019. Recent advances in transmission-blocking drugs for malaria elimination. Future Med. Chem. 11, 3047–3088. doi:10.4155/fmc-2019-0225

WHO, 2019. World Malaria Report 2019. Geneva.

Zélé, F., Nicot, A., Berthomieu, A., Weill, M., Duron, O., Rivero, A., 2014. *Wolbachia* increases susceptibility to *Plasmodium* infection in a natural system. Proc. R. Soc. B 281. doi:10.1098/rspb.2013.2837

Zhou, G., Kohlhepp, P., Geiser, D., Frasquillo, M. del C., Vazquez-Moreno, L., Winzerling, J.J., 2007. Fate of blood meal iron in mosquitoes. J. Insect Physiol. 53, 1169–1178. doi:10.1016/j.jinsphys.2007.06.009

[dataset] Villa, Manon (2020), “Villa & al 2020_IJP”, Mendeley Data, V1, doi: 10.17632/phzy3tmbzm.1

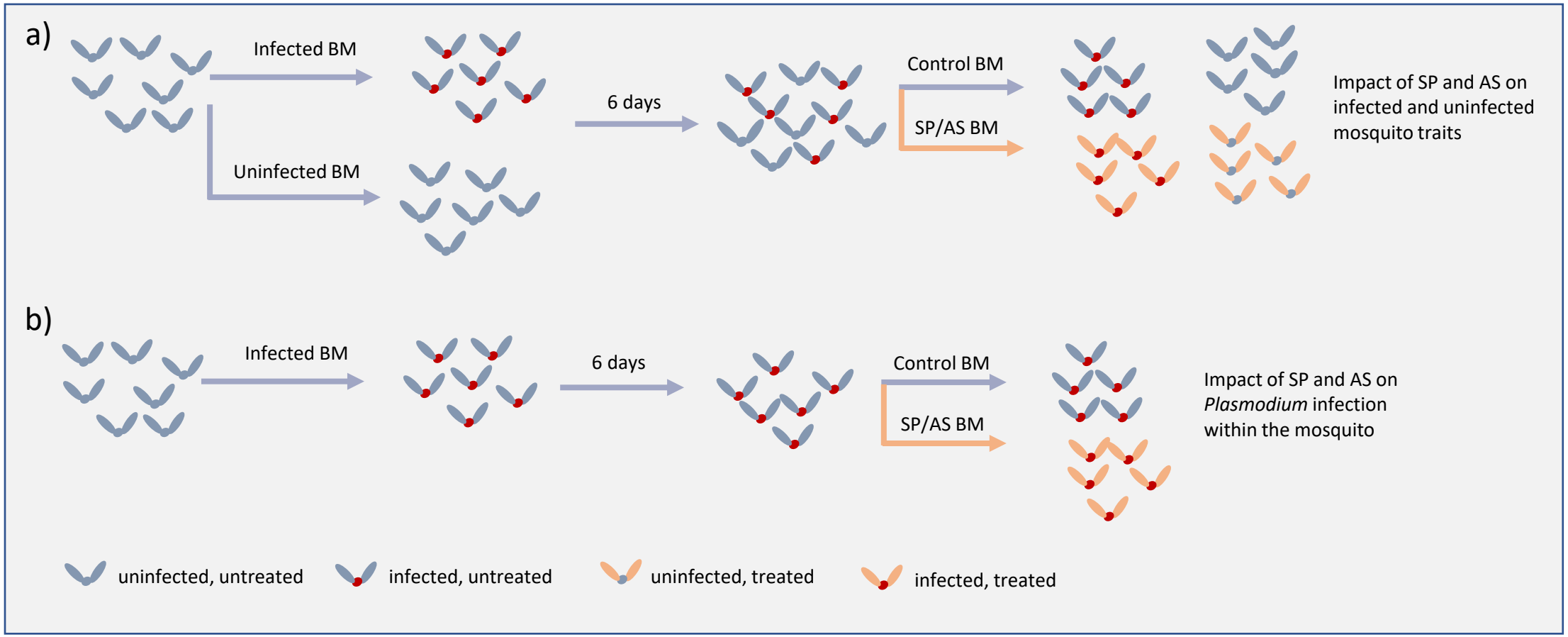
Figure Legends

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

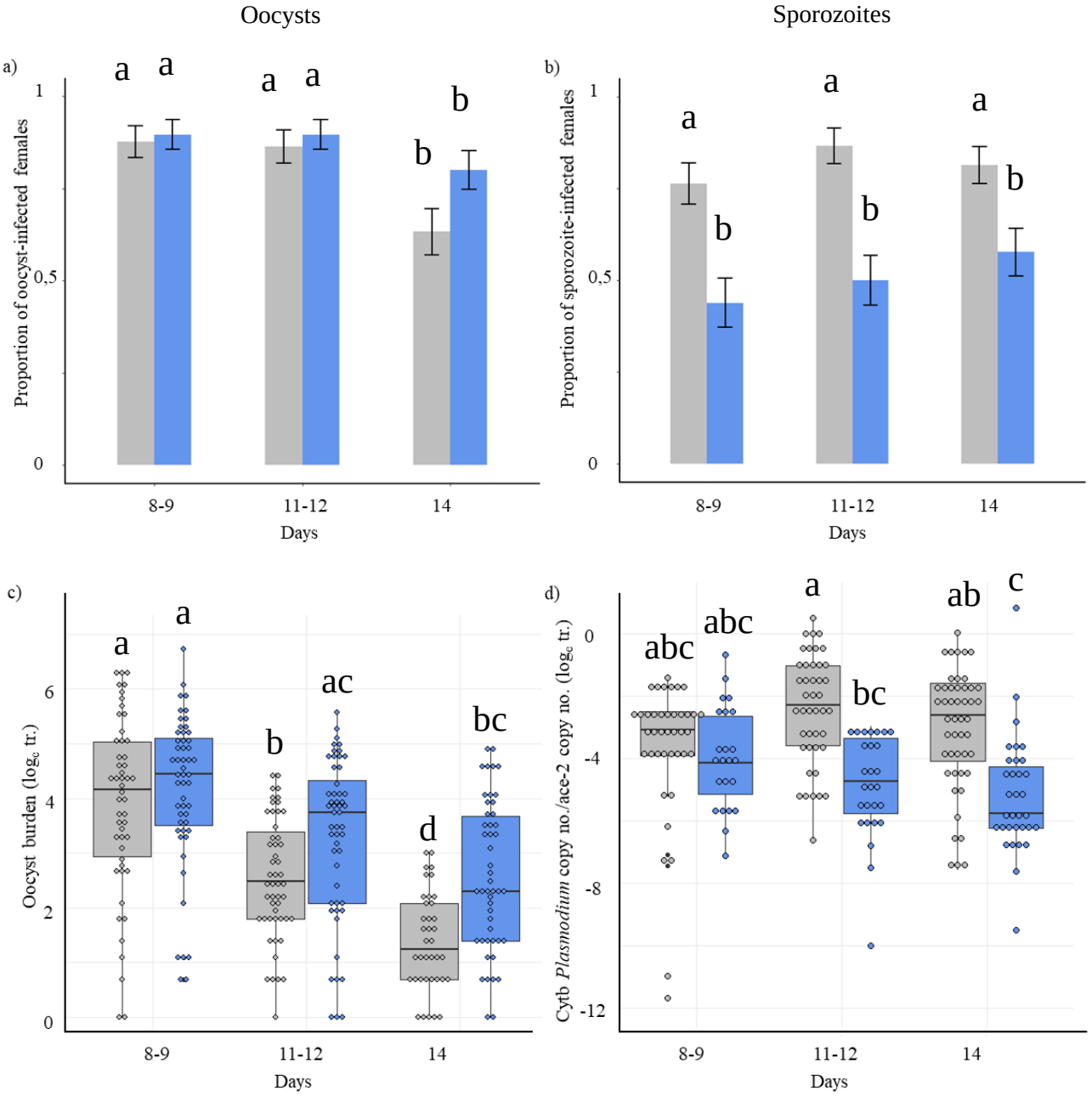
Figure 2. Prevalence and burden of oocysts and sporozoites in mosquitoes fed on a control- (grey) or SP-treated (blue) host for each sampling day (number of days post infection). a), b): oocyst and sporozoite prevalence, respectively. c), d): oocyst and sporozoite burden, respectively. Prevalence is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

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

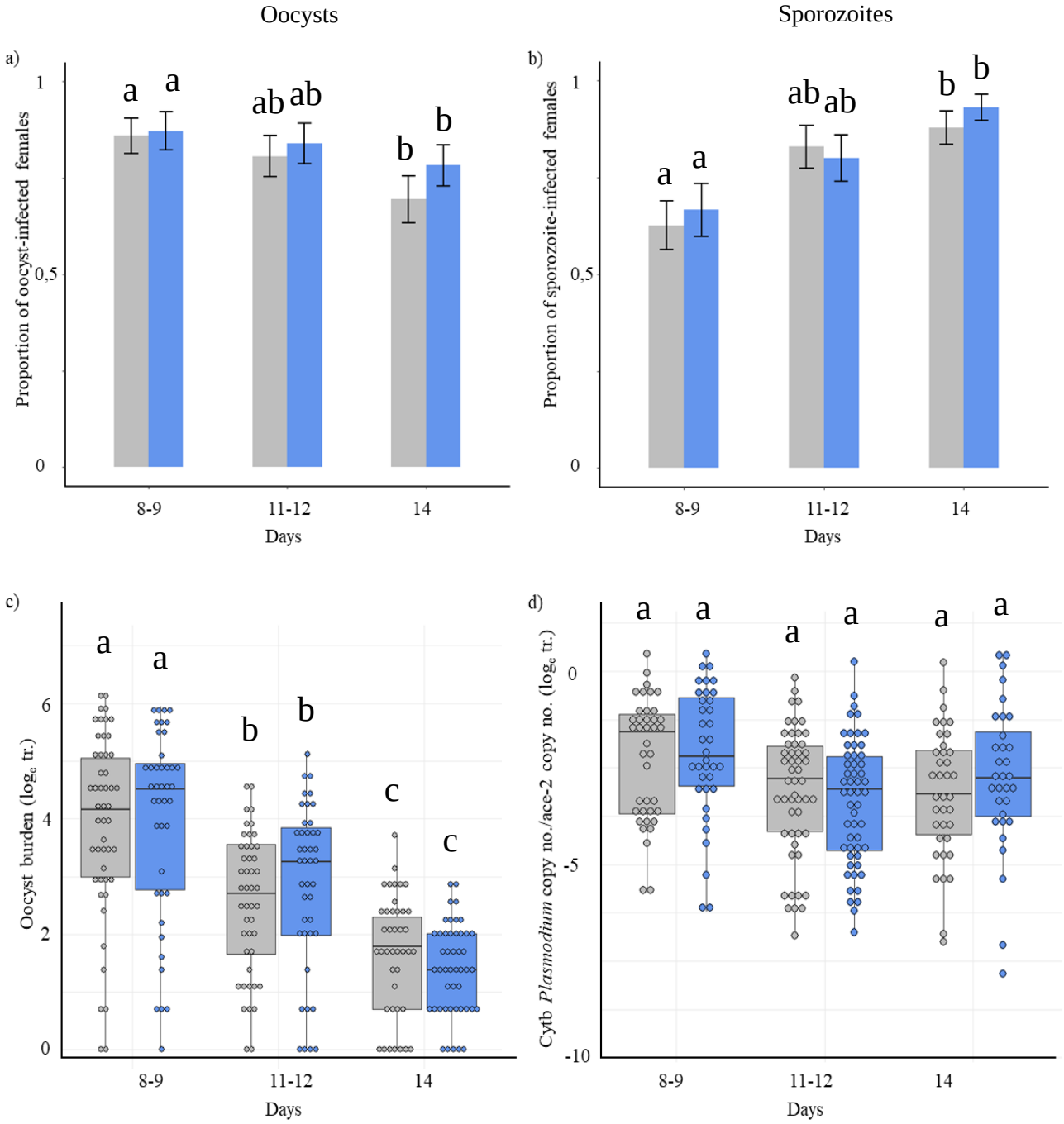
812 different. Post-hoc contrasts were carried out using the emmeans package in R (https://cran.r-
813 project.org/web/packages/emmeans/vignettes/comparisons.html).
814


a)
Infected BM
Uninfected BM
6 days
Control BM
SP/AS BM
Impact of SP and AS on infected and uninfected mosquito traits
b)
Infected BM
6 days
Control BM
SP/AS BM
Impact of SP and AS on *Plasmodium* infection within the mosquito
uninfected, untreated
infected, untreated
uninfected, treated
infected, treated

SP


Oocysts
Sporozoites
a)
b)
c)
d)
Proportion of oocyst-infected females
Proportion of sporozoite-infected females
Oocyst burden (log$_e$ tr.)
Cytb *Plasmodium* copy no./ace-2 copy no. (log$_e$ tr.)
Days
8-9
11-12
14
a
b
ac
bc
d
abc
ab
c

AS


Oocysts
Sporozoites
a)
b)
c)
d)
Proportion of oocyst-infected females
Proportion of sporozoite-infected females
Oocyst burden (log$_e$ tr.)
Cytb *Plasmodium* copy no./ace-2 copy no. (log$_e$ tr.)
Days
8-9
11-12
14
a
ab
b
c
0
0,5
1
2
4
6
-5
-10

Tables

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.

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

[*] 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.

Supplementary Materials

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

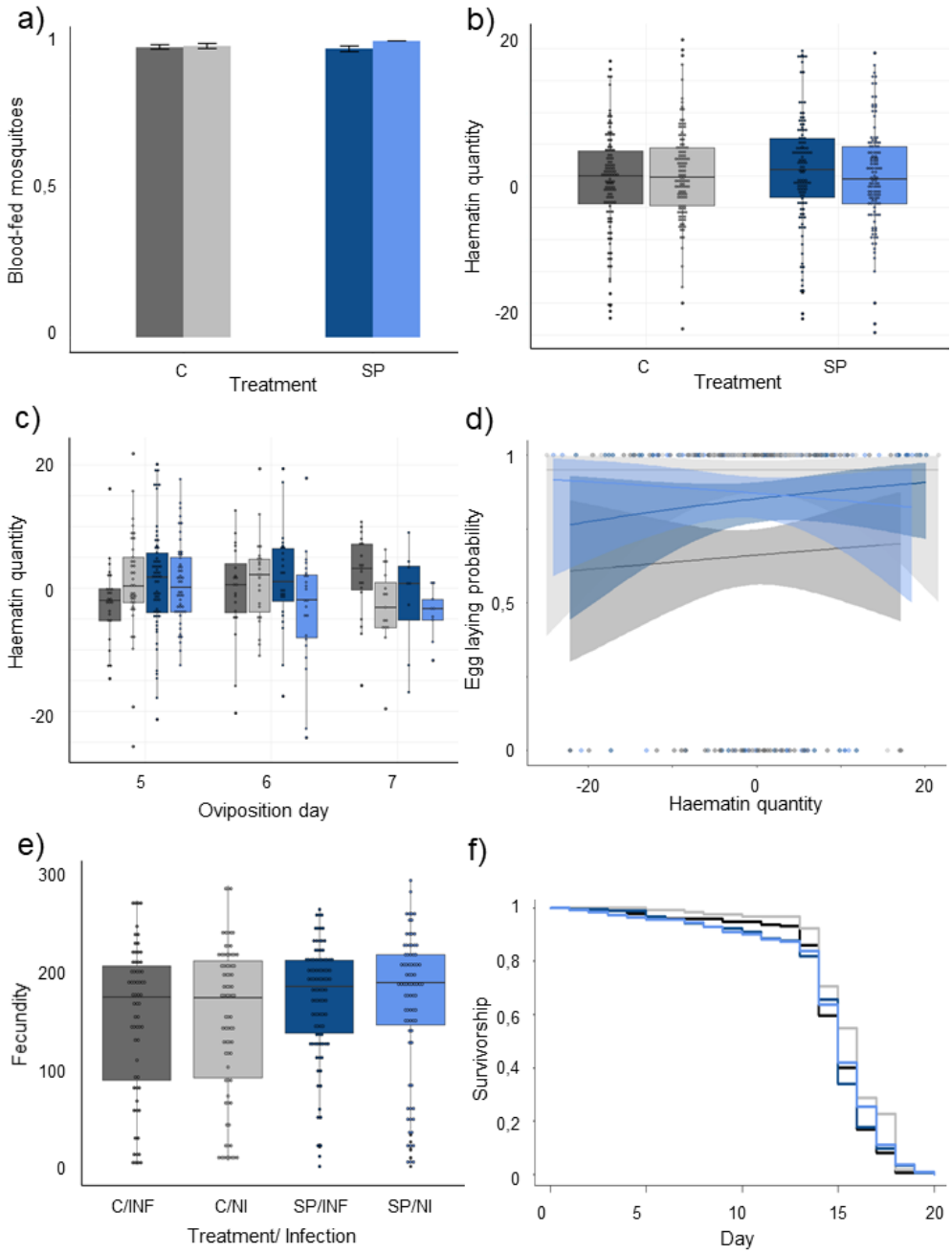
Table S1. Description of the statistical models used to analyze the impact of drugs on mosquito life history traits. Models with binomial error structure require a concatenated response variable binding together the number of successes and failures for a given outcome. N gives the number of mosquitoes included in each analysis. "Maximal model" represents the complete set of explanatory variables (and their interactions) included in the model. "Minimal model" represents the model containing only the significant variables and their interactions. N represents the number of replicates in each analysis. Round brackets indicate that the variable was fitted as a random factor. Square brackets indicate the error structure used (n: normal errors, b: binomial errors). date: sampling day, status: alive/dead on sampling day, fed/unfed: number of fed/unfed mosquitoes, hm: haematin excreted (proxi for blood meal size), plt: plate used for the colorimetric quantification haematin, hmr: residuals of hm by plate, eggs: number of eggs laid, inf: mosquito infection status (infected/uninfected), TR: mosquito fed on treated/untreated bird.

Variable of interest		Response variable	Model Nb.	N	Maximal model	Minimal model	R subroutine [err struct.]
Effect of AS on mosquito traits							
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
Effect of SP on mosquito traits							
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

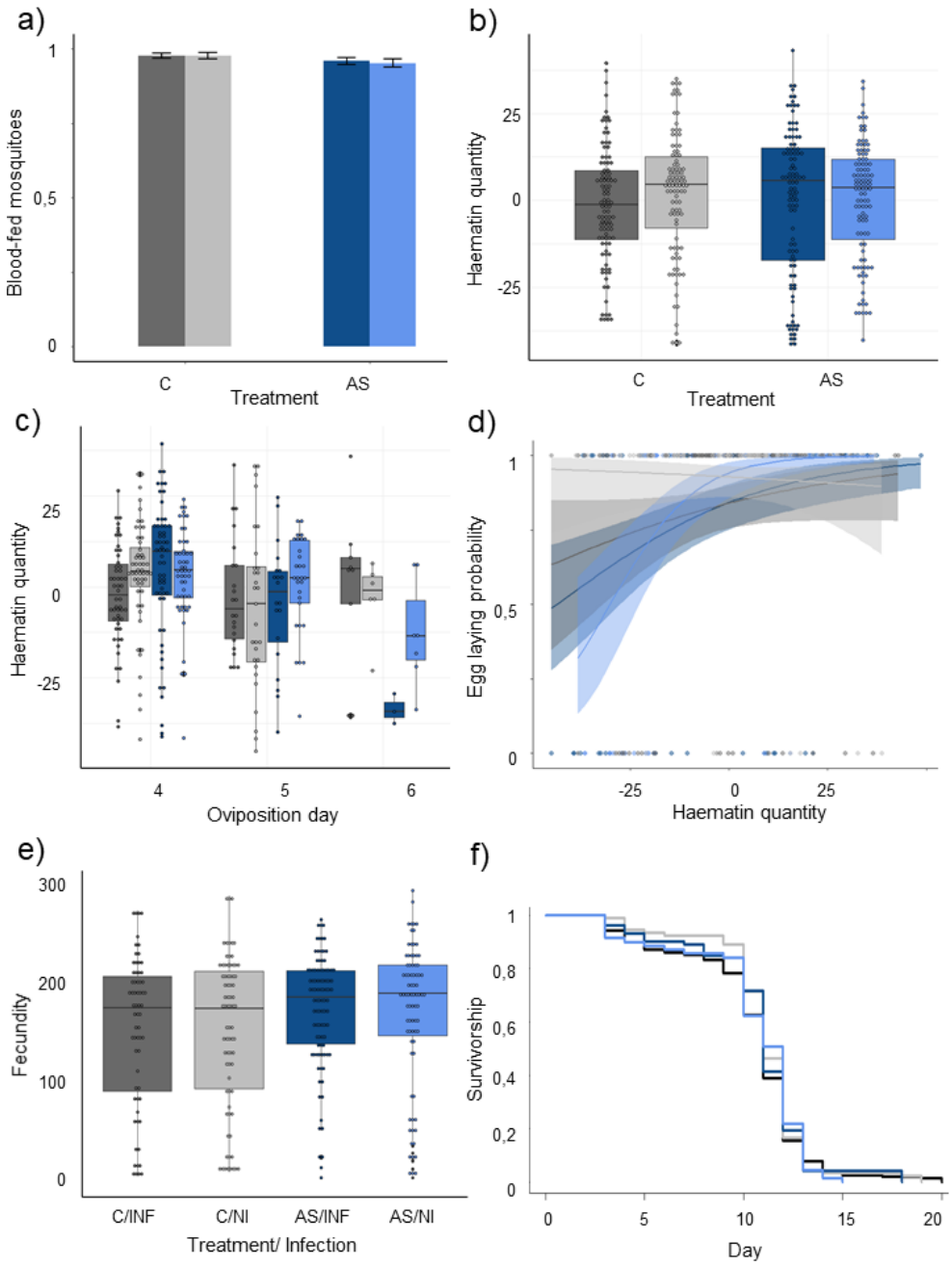
Table S2. **Description of the statistical models used to analyze the impact of drugs on *Plasmodium* prevalence and burden.** Models with binomial error structure require a concatenated response variable binding together the number of successes and failures for a given outcome. N gives the number of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

Variable of interest		Response variable	Model Nb.	N	Maximal model	Minimal model	R subroutine [err struct.]
Effect of AS on Plasmodium							
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]
Effect of SP on Plasmodium							
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|plq)	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]

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



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 ±
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.



888
889

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

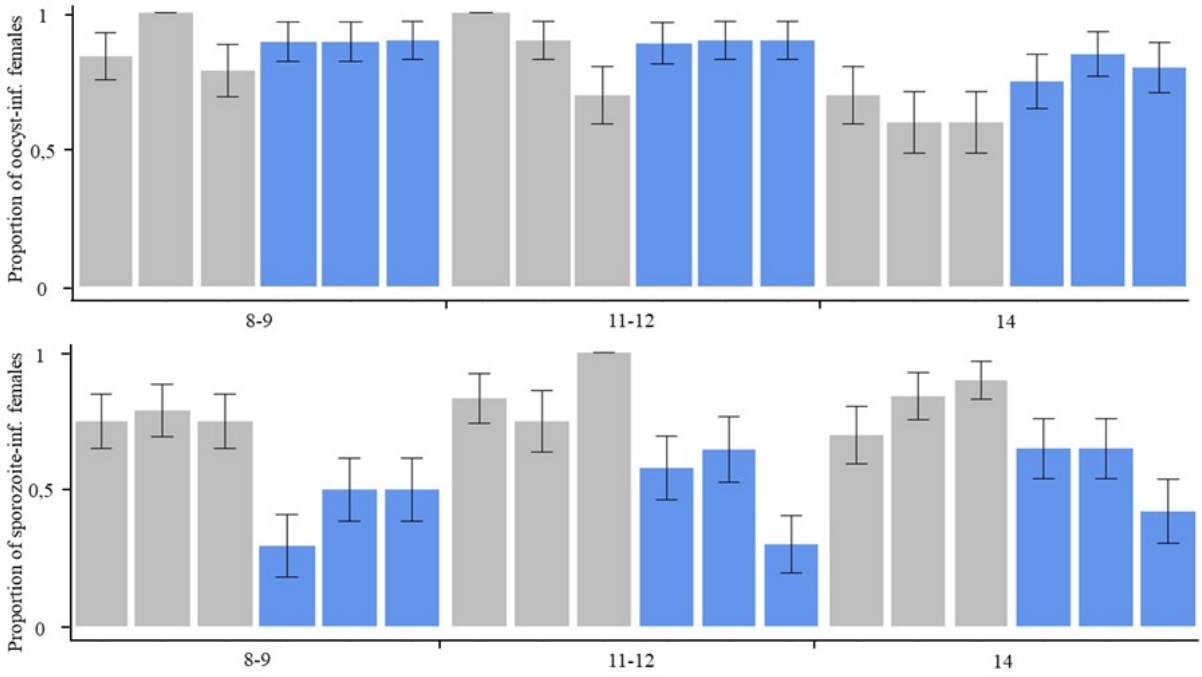


Figure S6. Oocyst (top) and sporozoite (bottom) burden in mosquitoes fed on each of the 3 control (grey) and SP-treated (blue) birds, for each of the 3 sampling days (8-9, 11-12 and 14). Between 15-20 mosquitoes were sampled per bird and per time point. Mosquitoes were dissected to count the number of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