

GAMETOCYTEMIA AND INFECTIVITY TO MOSQUITOES OF PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA ATTACKS TREATED WITH CHLOROQUINE OR SULFADOXINE PLUS PYRIMETHAMINE

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Abstract. *Plasmodium falciparum* gametocytemia and its related infectivity for mosquitoes was studied in 115 patients (median age = 18 years, range = 4–45) with simple malaria attacks who lived in the hypoendemic area of Dakar, Senegal. Patients were included in a 28-day *in vivo* sensitivity test after treatment with chloroquine (CQ, $n = 82$) or sulfadoxine plus pyrimethamine (SP, $n = 33$). The prevalence of resistant infections was 58.5% in those treated with CQ and 0% in those treated with SP. The gametocytemia peaked at day 7 after treatment. The maximal gametocyte prevalence was 38.2% in the CQ-sensitive infection group, 89.6% in the CQ-resistant group, and 97.0% in those treated with SP. The maximal geometric mean gametocytemia was 2.19/ μ l in the CQ-sensitive infection group, 29.12/ μ l in the CQ-resistant group and 85.55/ μ l in those treated with SP. The period between appearance of the first clinical symptom and treatment was positively related to gametocyte prevalence at days 0 and 2. Experimental infection of wild *Anopheles arabiensis* using membrane feeders was performed at days 0 and 7, and mosquito infectivity was measured by oocyst detection on the midgut. At day 0, 14.1% of the patients had infected at least 1 mosquito, and at day 7, this value was 38.5%. The mean percentage of infected mosquitoes was 3.2% at day 0 and 12.6% at day 7. At day 7 after treatment with CQ, the relative risk for patients with resistant infections of infecting anophelines was 4.07 higher than in those with sensitive infections. No difference was observed in infectivity for mosquitoes between RI-type resistance and the RII + RIII-type resistance. A sporonticidal effect of SP was observed at day 7 after treatment. These data show that *P. falciparum* gametocytes and their infectivity for mosquitoes were differentiated according to the drug used, its efficacy, and the duration of symptoms before treatment; they were not dependent on the density of asexual stages. Prompt treatment of malaria cases performed at the beginning of symptoms could limit the spread of resistant parasites.

The factors that trigger and regulate the switch from asexual to sexual development of the malaria parasite remain largely mysterious,^{1,2} but may involve both genetic mechanisms³ and environmental mechanisms,⁴ especially when conditions deteriorate. Completion of *Plasmodium falciparum* gametocytogenesis (from merozoite to morphologically mature gametocyte) takes 10–12 days *in vivo*,^{5,6} an estimate that is consistent with the 10 days observed *in vitro*.⁷ In the peripheral blood stream, the mature gametocyte has a half-life of 2.4 days and 1 gametocyte generation may persist for up to 3 weeks.⁸ In continuous culture, the progeny of a single schizont is either only asexual parasites or only gametocytes, indicating a commitment to 1 or the other path of development prior to the merozoite stage.⁹

The passage of the malaria parasite from humans to the mosquito vector is characterized by one word: variability. This intriguing phenomenon has been observed by many investigators. To identify and evaluate each factor controlling these processes is a challenge that could lead to a better understanding of this complex biologic event. These factors have been reviewed recently^{10–13} and have updated some older reviews.^{14–16} Clearly, one of these factors is antimalarial drugs.

Antimalarial drugs often have an effect (positive or negative) on various phases of gametocytogenesis, on gametocyte infectivity, and/or on parasite development in the anophelines; this heterogeneous situation is also complicated by different effects of the same drug on different plasmodial species. The 8-aminoquinoline class of antimalarials, such as primaquine and WR-238605, has the unusual property of activity against mature gametocytes. Within other antimalarial classes, chloroquine (CQ), sulfadoxine (S), and pyrimethamine (P) are schizonticides with no effect on mature

gametocytes. Nevertheless, CQ and SP are active against young gametocytes before their appearance in the peripheral circulation.^{17,18} The once-common view that gametocytogenesis induction is not observed after treatment with CQ or SP¹⁹ has faded considerably following the demonstration that CQ increases the gametocytogenesis of *P. chabaudi* *in vivo* and *P. falciparum* *in vitro*.^{20,21} Pyrimethamine is clearly sporontocidal; it inhibits dihydrofolate reductase in sensitive strains, damaging ookinetes and reducing oocyst numbers. Sulfadoxine might increase gametocytogenesis in drug-resistant isolates of *P. gallinaceum*.¹⁸ Sulfadoxine is sporonticidal against *P. berghei* but not against *P. falciparum*.^{22,23} Hogg and others observed that CQ enhances *P. falciparum* infectivity to mosquitoes, while SP reduces it.²⁴ Recent publications have emphasized that antimalarials must be considered together for their impact on gametocytes and infectivity for vector mosquitoes.^{18,24–30} Consequently, our study focuses on the effect of antimalarials on the gametocyte stage in humans and its infectivity for anopheline vectors. It involved volunteers with uncomplicated malaria attack who were treated with 1 of the 2 antimalarials most commonly used in Africa, CQ and SP. The study was carried out in an area where resistance to CQ is frequent, although CQ remains the first-line treatment recommended by public health national authorities, a situation highly prevalent in Africa.

MATERIALS AND METHODS

Study area. The study was carried out in Dakar, Senegal. In this area of hypoendemic malaria, transmission occurs mainly from September to November with an annual entomologic inoculation rate <1 ,^{31,32} a very low level of malaria transmission that permits consideration of all treatment fail-



ures as resistance and not as new infections. In children, at the end of the season of transmission, the plasmodic index ranges from 1.3% to 7.5%, depending on the district.^{33,34} Chloroquine resistance was reported in this area for the first time in 1988 and presently reaches approximately 50%.³⁵

Patients and gametocytemia. Patients were recruited from September to December in 1996 and 1997 when presenting at dispensaries located in the 3 districts of Gibraltar, Derklé, and Pikine Ancien. Patients were eligible to join the study if they were currently having a simple *P. falciparum* malaria attack (asexual parasitemia $>2,000/\mu\text{l}$, a temperature $\geq 38^\circ\text{C}$ or recent pyrexial antecedents, and absence of symptoms relevant to other pathologies), were living in the Dakar area, denied use of any specific antimalarial drug for the current period of illness, and if they (or their parents) gave informed verbal consent to participate in the study. The protocol was approved by the Ministry of Health of Senegal. A coinfection by a plasmodial species other than *P. falciparum* was an exclusion criterion. The disease history was recorded by asking patients or their parents when the present symptomatic period had started.

The treatments given were CQ (CQ phosphate; Société Industrielle Pharmaceutique de l'Ouest Africain, Dakar, Senegal), 25 mg/kg of body weight given over a 3-day period: 10 mg/kg on days 0 and 1 and 5 mg/kg on day 2 or SP (Fansidar®; F Hoffmann-LaRoche, Basel, Switzerland), 25 mg/kg of sulfadoxine plus 1.25 mg/kg of pyrimethamine given in a single dose. Treatment with CQ was given from September 1996 to November 1997, and treatment with SP was given from November 1997 to December 1997. If necessary, patients were provided with antipyretics (paracetamol tablets, 30 mg/kg/day) at days 0 and 1. All drug intake was controlled by a nurse.

The follow-up of patients was carried out using intravenous blood collected in heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) on days 0 and 7 for experimental infection of mosquitoes, and thick blood smears were prepared on days 0, 2, 4, 7, 14, 21, and 28. Thick smears were stained with Giemsa and 200 microscopic oil-immersion fields were systematically examined. For each thick smear, the mean number of leukocytes per field was evaluated for 5 fields. Gametocyte and asexual parasite densities were calculated assuming an average number of 8,000 leukocytes/ μl of blood. A second-line treatment with halofantrine (Halfan®; SmithKline Beecham, Worthing, United Kingdom) was administered if severe malaria and/or clinical failures occurred during the follow-up period; these patients were followed-up for 14 additional days. At the end of the study, all patients who had asexual *P. falciparum* parasitemia received a second-line treatment.

Experimental infection of mosquitoes. To obtain the best gorging rate of mosquitoes and their maximal survival 1-week post-bloodmeal, preliminary studies in our insectary have shown that wild *Anopheles arabiensis* must be 3–4 days old after emergence and feed through a baudruche membrane (a natural film extracted from the cecum of a cow or lamb).^{36,37} *Anopheles arabiensis* were collected at larval stages in market-garden wells in urban Dakar and kept at the insectary at 27–29°C and a relative humidity of 70–90%. Larvae were fed daily with Tetra Baby Fish Food L® (TetraWerke, Melle, Germany). Pupae were placed in cages

in which emergence occurred and adults had permanent access to a 3% sucrose solution. Sixty females (3–4 days old) belonging to the F_0 generation were placed without males into a 200-ml paper cup covered with a mosquito net and were starved for 5–7 hr. The human heparinized bloodmeal (lithium heparinate) was given using a baudruche membrane feeder kept at 37°C with a surface area of 15.9 cm².³⁸ Mosquitoes were allowed to feed for 15 min in the dark, and any that were not fully engorged were removed. Fed mosquitoes were maintained with permanent access to sucrose solution and without any further bloodmeals. After 8 days, surviving mosquitoes were dissected for midgut examination by light microscopy and any oocysts were counted using mercurochrome stain.

Data analysis. Variables considered in the analysis were related to 1) the densities of *P. falciparum* gametocytes and trophozoites, 2) the patient at day 0: age, sex, duration of the symptoms before treatment, 3) prevalence of infected mosquitoes, and 4) mean number of oocysts per infected mosquito. Geometric mean densities were expressed using the geometric mean of Williams [= exponential (arithmetic mean $(\ln(x_i + 1))) - 1$]. Discrete data were compared between groups using either the chi-square test or Fisher's exact test. Differences in group means were analyzed using either the Student's *t*-test or the nonparametric Mann-Whitney U test. Various gametocyte prevalences depending on the duration of symptoms before treatment were compared using the tendency chi-square test.

RESULTS

A total of 127 patients with simple malaria attacks were included in the study; 89 were treated with CQ and 38 with SP. Twelve patients (10.4%) were lost to follow-up or excluded: 8 traveled, 2 refused the blood tests, and 2 received uncontrolled additional antimalarials. Overall results are for 115 patients (82 CQ and 33 SP) between 4 years and 45 years of age (mean \pm SD = 19.8 ± 8.4 years, median = 18). The patients in these 2 groups did not differ in mean age (19.3 ± 8.8 years versus 20.8 ± 7.2 , respectively; $P = 0.30$, by Mann-Whitney U test). At day 0 these 2 groups did not differ in the duration of symptoms before treatment (4.6 ± 4.7 days versus 5.2 ± 5.3 ; $P = 0.70$), trophozoite density ($53,895 \pm 52,975/\mu\text{l}$ of blood versus $40,881 \pm 44,117$; $P = 0.17$), gametocyte prevalence (31.7% versus 51.5%; $P = 0.13$, by Fisher's exact test), and gametocyte density ($17.2 \pm 61.8/\mu\text{l}$ versus 28.2 ± 74.7 ; $P = 0.07$, by Mann-Whitney U test), or in the proportion of patients that were male (52% versus 64%; $P = 0.38$ by Fisher's exact test).

The overall prevalence of *in vivo* resistant infection was 58.5% and 0%, respectively, after treatment with CQ and SP (Table 1). The efficiency of treatment with CQ was not significantly linked to the age of the patients (20.5 ± 8.3 years in the sensitive group versus 18.5 ± 9.2 in the resistant group; $P = 0.20$, by Mann-Whitney U test) or with the duration of symptoms before treatment (3.8 ± 1.5 days in the sensitive group versus 5.3 ± 6.0 in the resistant group; $P = 0.54$, by Mann-Whitney U test). Seventy percent (39 of 61) patients probably infected by an infected vector within the Dakar area harbored infections resistant to CQ compared with 43% (9 of 21) infected outside the Dakar area. Al-

TABLE 1
Number of *Plasmodium falciparum* infections, according to treatment*

Treatment	No.	Sensitive	Number of infections							Total R	% R
			R I day 7	R I day 14	R I day 21	R I day 28	R II	R III			
CQ	82	34	3	15	12	5	11	2	48	58.5	
SP	33	33	0	0	0	0	0	0	0	0	

* R = resistant; CQ = chloroquine; SP = sulfadoxine plus pyrimethamine.

though this difference was not significant ($P = 0.12$, by Fisher's exact test), it is consistent with a well known higher resistance in African urban areas, especially in Dakar.³⁵

Gametocytemia. Post-therapeutic gametocytemia was higher in patients treated with SP than in those treated with CQ both for prevalence and density (Figure 1). Gametocyte prevalences peaked at day 7 at 62.2% and 97.0% for CQ and SP, respectively ($P < 10^{-4}$, by Fisher's exact test). Geometric mean densities of gametocytes peaked at day 7 at 10.1 and 85.6/ μ l of blood for CQ and SP, respectively ($P < 10^{-4}$, by Mann-Whitney U test).

When only patients without gametocytes at day 0 ($n = 72$) were considered, the gametocyte prevalence of the 56 patients treated with CQ peaked at day 14 was 54%; in con-

trast, it peaked at 100% at days 4, 7, and 14 for the 16 patients treated with SP. The gametocyte density peaked at day 7 for both treatments and its geometric mean was 4.8/ μ l for patients treated with CQ and 58.0/ μ l for patients treated with SP ($P = 0.0002$, by Mann-Whitney U test).

Gametocytemia and parasitologic efficacy of treatment with CQ. Gametocyte prevalence was higher in resistant infections than in sensitive ones. This was systematically observed from day 0 to day 28. Prevalences were 17.7% and 41.7% at day 0 for sensitive and resistant infections, respectively; they peaked at day 4 at 38.2% for sensitive infections and at day 14 at 89.6% for resistant ones; at day 28, they were 11.8% and 66.7%, respectively. Significant differences were observed at days 0, 4, 7, 14, 21, and 28 ($0.04 > P > 10^{-5}$, by Fisher's exact test). This was not linked to the duration of symptoms before treatment.

Gametocyte density was higher in resistant infections than in sensitive ones. This was systematically observed from day 0 to day 28. Geometric means of densities were 0.49 and 2.72/ μ l of blood at day 0 for sensitive and resistant infections, respectively; they peaked at day 4 at 2.19/ μ l for sensitive infections and at day 7 at 29.12/ μ l for resistant ones, then decreased regularly to reach 0.21/ μ l and 3.47/ μ l, respectively, at day 28. Significant differences were observed at days 0, 4, 7, 14, 21, and 28 ($0.04 > P > 10^{-4}$, by Mann-Whitney U test).

Gametocyte prevalence and density did not differ between RI and RII + RIII. For instance, at day 7 prevalences were 87% for RI and 85% for RII + RIII ($P = 0.99$, by Fisher's exact test), and densities were 26.8/ μ l for RI and 34.5/ μ l for RII + RIII ($P = 0.53$ by Mann-Whitney U test).

When only patients without gametocytes at day 0 were considered, no differences were observed between sensitive and resistant infections at day 2 (Figure 2) ($P = 0.99$, by Fisher's exact test for gametocyte prevalence and $P = 0.92$, by Mann-Whitney U test for gametocyte density) or at day 4 ($P = 0.06$ and $P = 0.10$, respectively). However, at days 7, 14, 21, and 28 the gametocyte prevalence was higher in resistant infections than in sensitive ones ($P < 0.002$, by Fisher's exact test) but was not different between RI versus RII + RIII infections ($P > 0.34$, by Fisher exact test); the gametocyte density showed the same tendencies for the comparison of sensitive and resistant infections ($P < 0.0006$, but for the comparison of RI and RII + RIII infections, $P = 0.03$ at day 14 although P was always > 0.22 on other days). The relative risk of harboring gametocytes at day 4 for patients without gametocytes at day 0 was 1.83 higher in those with CQ-resistant infections than in those with CQ-sensitive ones (95% confidence interval = 0.93–3.77); at day 7 this risk was 3.50 (1.67–7.34).

Gametocytemia and duration of symptoms before

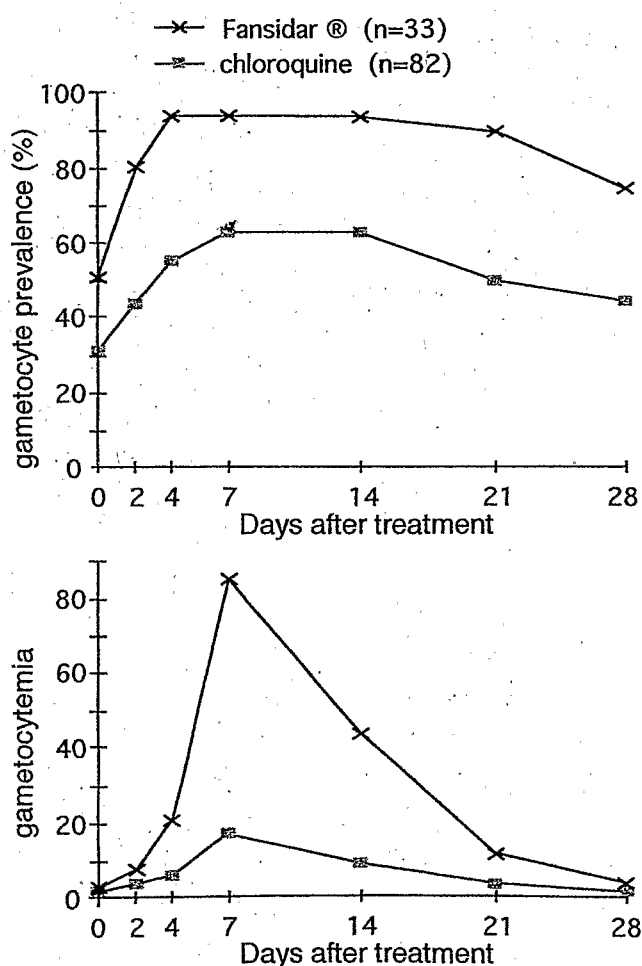


FIGURE 1. Evolution of *Plasmodium falciparum* gametocyte prevalence and geometric mean ($\ln(x + 1)$) gametocyte density per microliter of blood, according to treatment.

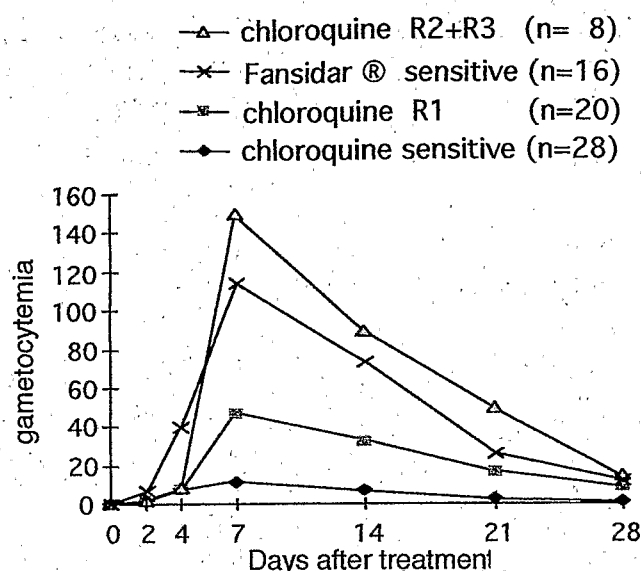


FIGURE 2. Dynamics of the arithmetic mean gametocytemia of *Plasmodium falciparum* per microliter of blood for patients without gametocytes at day 0, according to treatment and to parasitologic response.

treatment. The median duration of symptoms reported by children or their parents at the beginning of treatment (day 0) was 4 days (mean \pm SE = 4.82 ± 2.45 , range = 1–38 with 6 durations >10 days).

The mean \pm SE duration of symptoms was 6.30 ± 3.83 days among children with gametocytes at day 0 compared with 3.93 ± 1.49 days among children without gametocytes at day 0 ($P < 0.01$, by Mann-Whitney U test). Duration and gametocyte prevalence were also linked at days 0, 2, and 4, with a significant positive trend at day 2 (Table 2).

Gametocyte densities at day 2 were correlated with the duration of symptoms (after an \ln transformation of gametocyte densities and durations of symptoms, $r = 0.336$, $n = 112$; $P = 0.0003$). The geometric mean gametocyte density at day 2 increased from $2.1/\mu\text{l}$ when the duration of symptoms was 1 or 2 days to $7.1/\mu\text{l}$ when the duration of symptoms was ≥ 5 days.

Gametocytemia and asexual parasitemia. The link between asexual parasite density at day 0 and gametocyte prevalence at day 0 approached statistical significance: geometric mean asexual parasite density at day 0 = 33,442 for patients without gametocytes at day 0 versus 23,238 for patients pos-

itive for gametocytes ($P = 0.07$, by Mann-Whitney U test). Such a difference was not observed between asexual parasite density at day 0 and gametocyte prevalence at days 2, 4, and 7 ($0.87 > P > 0.11$ depending on the comparisons).

Although the sexual parasite density at day 0 was negatively correlated with gametocyte density at day 0 ($r = y - 0.216$, $n = 115$; $P = 0.02$), it was not correlated with gametocyte density at day 2 ($r = y - 0.059$, $P = 0.53$) or on any of the subsequent days ($P > 0.21$).

When only patients without gametocytes at day 0 were considered, trophozoite density at day 0 was not correlated with the density of gametocytes on any days ($P > 0.42$). There was no correlation between trophozoite and gametocyte numbers in the CQ-resistant infection subgroup or when a log transformation was performed on the whole group.

The ratio of circulating sexual to asexual form densities at day 0 was not significantly correlated with host age ($r = y - 0.015$, $n = 115$; $P = 0.87$).

Gametocyte infectivity for mosquitoes. Results were obtained from 107 patients who had infected at least 1 mosquito or who had not infected any mosquitoes when at least 10 had been dissected. Depending on the availability of suitable mosquitoes to perform experimental bloodmeal, some patients were not included in this study, especially if they were not gametocyte carriers. In this context, at days 0 and 7, there were 78 and 91 patients, 1,810 and 1,732 dissected mosquitoes, 54 and 164 mosquitoes with at least 1 oocyst, and 127 and 1,442 oocysts, respectively.

Overall, 11 of 78 patients (14.1%) had infected at least 1 mosquito at day 0, and 35 of 91 (38.5%) did so at day 7 ($P = 0.0005$, by Fisher's exact test). The mean percentages of infected mosquitoes were 3.2% at day 0 and 12.6% at day 7 ($P = 0.0005$, by Mann-Whitney U test). The mean geometric means > 0 of oocyst number per mosquito was 0.28 at day 0 and 2.07 at day 7 ($P = 0.28$, by Mann-Whitney U test); when this calculation was performed on all values including the zeros it was 0.04 at day 0 and 0.80 at day 7.

When the groups treated with CQ and SP were compared, no significant differences were observed concerning 1) the proportion of patients infecting mosquitoes (13.3% versus 16.7%, respectively, at day 0; $P = 0.71$, by Fisher's exact test, and 35.4% versus 46.5% at day 7; $P = 0.35$), 2) the mean percentages of infected mosquitoes (2.8% versus 4.6%, respectively, at day 0; $P = 0.70$ by Mann-Whitney U test, and 12.4% versus 13.2% at day 7, $P = 0.76$), 3) the mean geometric means > 0 of oocyst number per mosquito

TABLE 2
Duration of symptoms before treatment and gametocyte prevalence (% \pm SD)

No.	Duration of symptoms (days)				P value of the chi-square test for linear trend
	1-2	3	4	≥ 5	
	24	26	25	37	
Gametocyte prevalence at					
Day 0	25.0 \pm 8.8	34.6 \pm 9.3	36.0 \pm 9.6	48.6 \pm 8.2	0.06
Day 2	37.5 \pm 10.0	53.8 \pm 9.7	56.0 \pm 9.9	67.6 \pm 7.7	0.03
Day 4	52.2 \pm 10.4	73.1 \pm 8.7	64.0 \pm 9.6	75.7 \pm 7.0	0.15
Day 7	75.0 \pm 8.8	61.5 \pm 9.5	60.0 \pm 9.8	83.8 \pm 6.1	0.32
Day 14	78.3 \pm 8.6	68.0 \pm 9.3	56.0 \pm 9.9	78.4 \pm 6.8	0.98

(0.29 versus 0.27, respectively, at day 0; $P = 0.83$ by Mann-Whitney U test, and 2.46 versus 1.33 at day 7; $P = 0.22$), and 4) this last mean calculated on all values including the zeros (0.039 versus 0.045, respectively, at day 0, and 0.87 versus 0.61 at day 7).

Gametocyte infectivity and efficacy of treatment with CQ. The patients with resistant infections infected more mosquitoes than those with sensitive ones. At day 0, 0 of 23 patients with infections sensitive to CQ infected at least 1 mosquito compared with 8 of 37 patients (21.6%) with resistant infections ($P = 0.02$, by Fisher's exact test). At day 7, these proportions were 4 (13.3%) of 30 and 19 (54.3%) of 35, respectively ($P = 0.0007$, by Fisher's exact test) and corresponded to a relative risk of 4.07 for 1 patient to be infectious for mosquitoes when harboring a resistant infection compared with a sensitive one (95% confidence interval = 1.56–10.65). At day 0, the mean percentages of infected mosquitoes was 0% in sensitive infections and 4.5% in resistant ones ($P = 0.02$, by Mann-Whitney U test). At day 7, these means were 5.3% and 18.4%, respectively ($P = 0.0008$); thus, 1 week after treatment with CQ, those with resistant infections infected 3.5 times more anophelines than those with sensitive ones.

The RI and RII + RIII type resistances equally infected mosquitoes. At day 0, 7 (25.9%) of 27 patients with RI infections infected at least 1 mosquito compared with 1 (10.0%) of 10 with RII or RIII infections ($P = 0.40$, by Fisher's exact test). At day 7, these values were 13 (54.2%) of 24 and 6 (54.5%) of 11, respectively. At day 0, the mean percentages of infected mosquitoes were 5.4% in those with infections and 2.2% in those with RII or RIII infections ($P = 0.34$, by Mann-Whitney U test). At day 7, these means were 18.6% and 17.9%, respectively ($P = 0.93$).

Gametocyte infectivity and gametocyte density. A significant and positive correlation was observed between the gametocyte density and the percentage of infected mosquitoes (at day 0, $r = 0.314$, $n = 78$; $P = 0.05$, and at day 7, $r = 0.529$, $n = 91$; $P < 10^{-4}$).

A similar analysis was conducted taking into account the treatment (CQ or SP) and the parasitologic response of the infection after treatment with CQ (sensitive, RI, and RII + RIII). This showed a good adjustment of various points to a straight line (when forced to 0, $y = 0.1319x$ with $R^2 = 0.94$; calculation performed without SP at day 7) (Figure 3). Of interest is the poor infectivity power of gametocytes for mosquitoes at day 7 after treatment with SP. From the equation of the straight line, it was deduced that the infectivity of gametocytes 7 days after treatment with SP decreased 2.0 times.

Gametocyte infectivity and symptoms before treatment. At day 0, the proportion of patients who infected at least 1 mosquito was 5.7% (2 of 35) if their duration of symptoms was ≤ 3 days, and 19.0% (8 of 42) if their duration of symptoms was ≥ 4 days ($P = 0.10$, by Fisher's exact test). The mean percentages of infected mosquitoes were 1.41% in sensitive infections and 4.68% in resistant ones ($P = 0.06$, by Mann-Whitney U test). At day 7, these means were 6.1% and 16.7%, respectively ($P = 0.10$).

Gametocyte infectivity and density of trophozoites at day 0. The arithmetic mean trophozoite number was 36,371 for patients who infected at least 1 mosquito at day 0 and

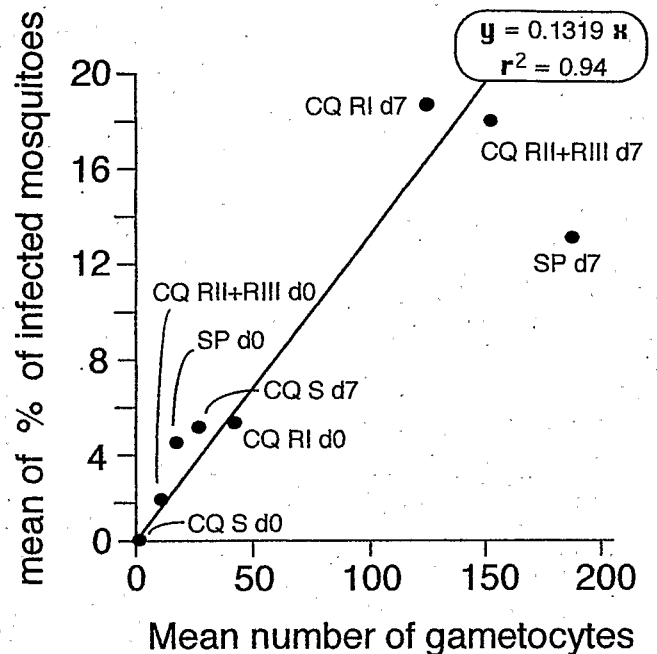


FIGURE 3. Relationship between gametocyte density and infectivity for mosquitoes. CQ = chloroquine; SP = sulfadoxine plus pyrimethamine; S = sensitive; R = resistant; d = day. The straight line equation was calculated without SPd7.

55,338 for those who did not ($P = 0.026$, by Mann-Whitney U test). The means for patients who infected at least 1 mosquito at day 7 were 42,176 and 57,408, respectively ($P = 0.030$, by Mann-Whitney U test). The correlation coefficient between trophozoite number at day 0 and the percentage of infected mosquitoes was $r = y - 0.198$ ($n = 78$; $P = 0.08$) at day 0 and $r = y - 0.045$ ($n = 91$; $P = 0.67$) at day 7.

Gametocyte infectivity and sex ratio of the patients. At day 0, 12.8% (5 of 39) of the females infected at least 1 mosquito compared with 15.4% (6 of 39) of the males ($P = 0.76$ by Fisher exact test). At day 7, these proportions were 42.9% (18 of 42) and 34.7% (17 of 49), respectively ($P = 0.52$).

Gametocyte infectivity and age of the patients. At day 0, the mean \pm SD age of patients who infected at least one mosquito was 24.5 ± 11.0 years compared with 18.4 ± 8.1 years in those who did not ($P = 0.07$, by Mann-Whitney U test). At day 7, these mean \pm SD ages were 19.0 ± 7.3 and 20.5 ± 8.7 years, respectively ($P = 0.62$).

DISCUSSION

Our results obtained with naturally infected patients and wild strains of the local vector clearly demonstrate that *P. falciparum* gametocyte responses and their infectivity for mosquitoes are dependent on at least 3 factors: the drug used, its efficacy, and the duration of symptoms before treatment.

Under CQ pressure, the main selective advantage of CQ-resistant parasites is their ability to achieve gametocytogenesis. High gametocytemias are observed with subsequently higher proportions of infected mosquitoes; this is to be ex-

pected considering that CQ enhances gametocytogenesis and does not demonstrate sporontocidal activity.^{18,20}

Under SP pressure, the SP-sensitive parasites achieved gametocytogenesis but a sporontocidal activity is observed prior to the stage of mature oocyst. By comparing what was observed with CQ, clearly different mechanisms are involved in the transmission of CQ-resistant or SP-resistant parasites. In our study, no SP-resistant parasites were observed; nevertheless, it seems reasonable to suppose that the sporontocidal activity of pyrimethamine on sensitive gametocytes¹⁸ would not exist in resistant ones, as has been the case with pyrimethamine-resistant *P. berghei* under drug pressure.³⁹

Although the gametocyte is a product of asexual schizogony, in our study the relationship between density of asexual stage at day 0 and subsequent gametocytemia was poor or absent, depending on the parameters considered. This implies a complex regulation of the sexual versus asexual strategies to optimize the global fitness of the parasite. Kitchen and Putnam observed an interval of approximately 10 days occurring between the first appearance of trophozoites and gametocytes and between their peak densities.⁴⁰ This time lag is in agreement with models proposed recently that predict that an optimizing pathogen should delay production of its transmission stages.^{41,42}

The higher gametocytemia before treatment in CQ-resistant infections in comparison with sensitive ones has already been observed in other studies in Senegal^{29,30} and the Solomon Islands.⁴³ This might be explained by a higher gametocytogenesis as observed *in vitro* in CQ-resistant parasites;²⁸ furthermore, Hogg and others observed that patients harboring CQ-resistant parasites were 4.4 times more likely to produce gametocytes as those harboring sensitive ones.²⁴ This mechanism might be that implicated in the increasing of the sporozoitic index in anopheline vectors after the appearance of CQ-resistant parasites in Tanzania.⁴⁴

At a time when the CQ resistance problem appears to have major consequences in terms of mortality,⁴⁵ any practical considerations that would limit the spread of resistance to antimalarials would be welcomed. In this regard, the present study leads to an important public health lesson: to limit the spread of resistance, the treatment of malaria cases has to be done as soon as possible. Although not statistically significant, a 3.3-fold increase was observed in the proportion of patients who became infectious for mosquitoes if treatment was performed after the first 3 days following the appearance of symptoms. Precocious treatment has to be performed not only to prevent complications, but also to limit the spread of resistance.

These results pose many questions, with two of them suggesting a new area of research. First, the appearance of a large amount of gametocytes in the peripheral blood after treatment is a strong indication of a CQ-resistant infection. At days 4 and 7 after treatment of patients without gametocytes at day 0 with CQ, the patients with resistant infections were much more likely to harbor mature gametocytes. It would be interesting to determine after treatment with CQ at which density of gametocytes an appropriate second-line treatment has to be taken to anticipate and prevent delayed clinical failure. Second, our data were obtained from patients who were symptomatic. It would be interesting to know if

our results concerning antimalarial-transmission relationships can be extrapolated to other categories of people, especially individuals who are infected but not symptomatic.

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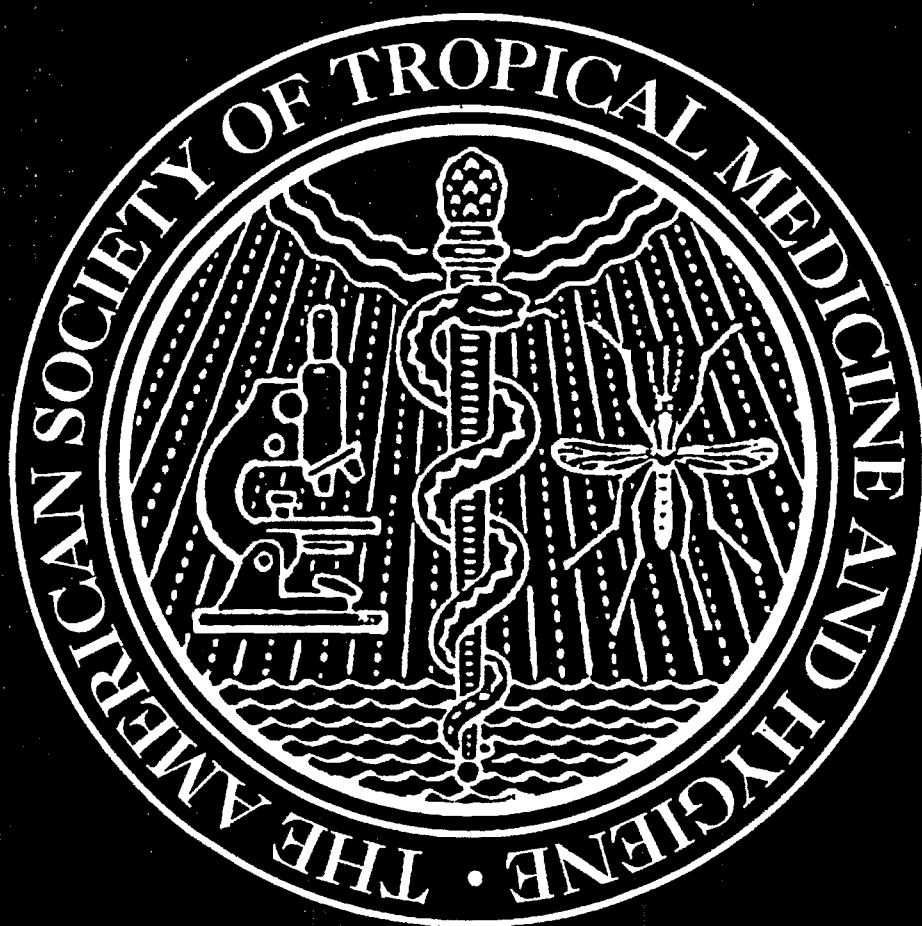
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