GAMETOCYTAEMIA IN SENEGALESE CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA TREATED WITH CHLOROQUINE, AMODIAQUINE OR SULFADOXINE + PYRIMETHAMINE

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Summary :

Plasmodium falciparum gametocytaemia was studied in 266 Senegalese children (median 4 years, range 0.5-16) with uncomplicated malaria treated with chloroquine (CQ), amodiaquine (AQ) or sulfadoxine+pyrimethamine (SP). The proportion of resistant infections *in vivo* to these drugs was 44 %, 16 % and 7 %, respectively. Gametocytes were counted by microscopy in thick smears on days 0, 4, 7 and 14 after treatment. There was a peak of gametocytaemia one week after treatment; on days 0, 7 and 14 the gametocyte prevalences were 35 %, 73 % and 63 %, and the geometric means of gametocyte densities were 1.3, 12.5 and 5.6/μl of blood. Three factors were found to influence gametocytaemia: treatment, efficacy of treatment, and duration of symptoms before treatment. Gametocyte prevalence and density significantly appeared higher in children treated with SP than with CQ, and higher with CQ than with AQ. Gametocyte prevalence and density were higher in resistant than in sensitive infections. The period between the appearance of the first clinical symptoms and treatment was positively and significantly linked to gametocyte prevalence and density on days 0 and 4. Early treatment with AQ, against sensitive infection, was followed by the lowest gametocytaemia. By contrast, treatment with SP against resistant infection was followed by the highest gametocytaemia. No clear relationship was observed between the density of asexual stages on day 0 and the gametocytaemia at any day between days 0 and 14. The epidemiological significance of post-therapeutic gametocytaemia and its possible role in the spread of resistant parasites are underlined. Solutions are proposed in order to avoid or reduce this gametocytaemia.

KEY WORDS : malaria, *Plasmodium falciparum*, gametocyte, resistance, chloroquine, amodiaquine, sulfadoxine, pyrimethamine, Senegal.

Résumé : LA GAMÉTOCYTÉMIE CHEZ DES ENFANTS SÉNÉGALAIS EN ACCÈS PALUSTRE SIMPLE TRAITÉS À LA CHLOROQUINE, À L'AMODIAQUINE, OU AU SULFADOXINE + PYRIMÉTHAMINE

La gamétocytémie de *Plasmodium falciparum* a été étudiée chez 266 enfants sénégalais (âge médian 4 ans, extrêmes 0.5-16 ans) en accès palustres simples traités soit à la chloroquine (CQ), soit à l'amodiaquine (AQ), soit au sulfadoxine+pyriméthamine (SP). La proportion de résistance *in vivo* pour ces différents traitements a été respectivement de 44 %, 16 % et 7 %. Les gamétocytes ont été dénombrés par observation microscopique de gouttes épaisses réalisées aux jours 0, 4, 7 et 14 après le traitement. Un pic de gamétocytémie a été observé une semaine après le traitement; aux jours 0, 7, et 14, les prévalences gamétocytaires ont été de 35 %, 73 % et 63 %, et les moyennes géométriques des densités ont été de 1.3, 12.5 et 5.6 gamétocytes/μl de sang. Trois facteurs ont influencé la gamétocytémie : le traitement, l'efficacité du traitement, et la durée des symptômes avant le traitement. La prévalence et la densité gamétocytaires ont été significativement supérieures avec le SP qu'avec la CQ, et supérieures avec la CQ qu'avec l'AQ. La prévalence et la densité gamétocytaires ont été significativement supérieures chez les infections résistantes par rapport aux sensibles. Le temps écoulé entre l'apparition des premiers symptômes cliniques et la prise du traitement a été positivement et significativement lié à la prévalence et à la densité gamétocytaires des jours 0 et 4. Un traitement précoce à la CQ contre une infection sensible a été suivi de la plus faible gamétocytémie. Au contraire, un traitement tardif au SP sur une infection résistante, a été suivi de la gamétocytémie la plus élevée. Il n'a pas été observé de relation évidente entre la densité de stades asexués au jour 0 et la gamétocytémie entre les jours 0 et 14. La signification épidémiologique du pic de gamétocytes postthérapeutiques et son rôle éventuel dans la dissémination de la résistance des parasites est soulignée. Des solutions sont proposées pour éviter ou réduire cette gamétocytémie.

MOTS CLÉS : paludisme, *Plasmodium falciparum*, gamétocyte, résistance, chloroquine, amodiaquine, sulfadoxine, pyriméthamine, Sénégal.

INTRODUCTION

Gametocyte is a fascinating stage, responsible for the passage of the malaria parasite from man to anopheline mosquitoes. Its consideration is

not purely biological but can have implications in case management, transmission control, and limiting the spread of resistant parasites.

Parasite commitment to the gametocyte development pathway is irreversible and occurs prior to the merozoite stage developing into the gametocyte (Bruce *et al.*, 1990). The factors that trigger and regulate this switch from asexual to sexual development remain largely unknown (Lobo & Kumar, 1998). According to what is observed in continuous culture, these factors refer to genetic mechanisms (Graves *et al.*, 1984) and

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to environmental mechanisms, especially when conditions deteriorate (Alano & Carter, 1990). Completion of *Plasmodium falciparum* gametocytogenesis, from merozoite to morphologically mature gametocyte, takes 10-12 days *in vivo* (Thomson, 1911; Sinden *et al.*, 1996), an estimation in accordance with the 10 days observed *in vitro* (Smalley, 1976). In the blood stream, gametocyte has a half-life of 2.4 days and one gametocyte wave may persist up to three weeks (Smalley & Sinden, 1977). *Plasmodium falciparum* gametocytogenesis occurs in the marrow of bones, and consequently the gametocytes commonly observed in the peripheral circulation are morphologically mature.

Recent publications have emphasised that the effect of antimalarials on gametocytes and infectivity for vector mosquitoes requires consideration (Butcher, 1997; Handunnetti *et al.*, 1996; Hogh *et al.*, 1998; Jones, 1997; Robert *et al.*, 1996; Robert & Trape, 1998). Chloroquine (CQ), amodiaquine (AQ), sulfadoxine (S) and pyrimethamine (P) are schizonticides, are active against young gametocytes in the marrow of bones, and have no effect on the survival of mature gametocytes in the peripheral blood-stream (Smalley & Sinden, 1977; Butcher, 1997). Induction of gametocytogenesis was demonstrated with subcurative doses of CQ *in vivo* for *P. chabaudi* (Buckling *et al.*, 1997), and *in vitro* for *P. falciparum* (Buckling *et al.*, 1999).

We have performed a study in children of an endemic area, presenting with uncomplicated malaria, and treated with three antimalarials, which are among the most used in Africa: CQ, AQ and SP. The main objective of this study is to identify the variables linked with the post-therapeutic gametocytaemia.

PATIENTS AND METHODS

STUDY AREA

The study was carried out in the village of Diohine (about 110 km East of Dakar, Senegal, West Africa), part of the Niakhar area in which the population has been followed for decades for demography and health (Delaunay, 1998). In this area of mesoendemic malaria, transmission occurs from August to October with an annual entomological inoculation rate of 10 (Robert *et al.*, 1998). The parasite rate reaches 80 % in children at the end of the transmission season. Chloroquine resistance has emerged in this area in 1992 and has previously increased the following years. *P. falciparum* resistance *in vivo* to chloroquine at RII-RIII levels at day 7 was 10 %, 15 % and 17 % in 1993, 1994 and 1995, respectively (Sokhna *et al.*, 1997). The emergence of chloroquine resistance

has been associated with a dramatic increase of malaria mortality in the studied population. During the period 1992-1995, malaria mortality averaged 8.2 per thousand per year for children under ten (Trape *et al.*, 1998).

STUDY DESIGN

Patients were recruited from September to November 1996 when presenting at the dispensary. Children (six months-16 years) were eligible to join the study if they were suffering from uncomplicated *P. falciparum* malaria (> 5,000 parasites/ μ L of blood), living in the Niakhar area, not using any specific antimalarial drug for the current period of illness, and if their parents gave informed verbal consent for them to participate in the study. The duration of symptoms before treatment was recorded by asking the children or their parents for how many days the current symptomatic period had lasted. Those with cerebral/complicated malaria or severe anaemia (with packed cell volume < 17 %, measured with a centrifuged micro-haematocrite tube) were excluded from the study. The study protocol was approved by the Senegalese Ministry of Health.

TREATMENT REGIMENS

The children were allocated into three oral treatment groups following the order of inclusion in the study. The treatments were chloroquine (CQ) (chloroquine phosphate, SIPOA Senegal, 25 mg/kg body weight given over three days: 10 mg/kg on days 0 and 1 and 5 mg/kg on day 2), amodiaquine (AQ) (Camoquin[®], Parke-Davis[™], 25 mg/kg body weight given over three days: 10 mg/kg on days 0 and 1 and 5 mg/kg on day 2), or sulfadoxine + pyrimethamine (SP) (Fansidar[®], Laboratoire Roche[™], 25 mg/kg sulfadoxine + 1.25 mg/kg pyrimethamine given in a single dose). All children were provided with antipyretics (paracetamol tablets, 30 mg/kg/day) on days 0 and 1. Children were observed one hour after treatment, and those who vomited during this period were excluded from the study.

FOLLOW UP OF CHILDREN

Surveillance was carried out during 14 days after treatment. Thick smears were performed on days 0, 4, 7 and 14. Parents were advised to return children to the dispensary at any time if symptoms deteriorated. During the follow-up period if severe malaria and/or clinical failures occurred, a second line treatment was administered, i.e. PS for those initially treated with CQ or AQ, and quinine for those initially treated with SP. At the end of the study, all children who had asexual *P. falciparum* parasitaemia received a second-line treatment.

MICROSCOPIC OBSERVATIONS

Thick blood films were Giemsa-stained and 200 microscopic oil-immersion fields were systematically examined. For each thick film, the mean number of leucocytes per field were evaluated on a sample of fields, gametocyte and asexual parasite densities were calculated assuming an average number of 8,000 leucocytes/ μ L of blood. Microscopic examinations were performed blind to treatment regimens.

DATA ANALYSIS

Geometric means of trophozoites and gametocytes densities were calculated using the geometric mean of Williams [exponential (arithmetic mean ($\log_e (x_i + 1)$)) - 1]. Discrete data were compared between groups using either the chi-square test or the Fisher's exact test. Differences between group means were analysed using non parametric Mann-Whitney *U*-test or Kruskal-Wallis test. Various gametocyte prevalences depending on the duration of symptoms before treatment were compared using the tendency chi-square test.

RESULTS

A total of 319 children with uncomplicated malaria were included in the study on day 0 and allocated into the three treatment groups ($n = 107$ with CQ; $n = 106$ with AQ, and $n = 106$ with SP). Fifty three children (17 %) were lost to follow-up or excluded: 20 vomited, 11 travelled, seven experienced clinical failures and received second line treatment, seven refused a blood test, five had an illegible thick smear at either days 4, 7 or 14, and three had no measure of haematocrite. Twenty-three among them had been treated with CQ, 19 with AQ, and 11 with SP. Hence the results presented here involve 266 children with a mean age $\pm$ SD = 5.09 ± 3.54 (median = 4; range = six months-16 years) distributed as 84 CQ, 87 AQ and 95 SP. At day 0, the children in these three treatment groups did not differ for mean age, duration of symptoms before treatment, trophozoite density, gametocyte density, sex-ratio or gametocyte prevalence ($0.14 < P < 0.74$).

The overall prevalences of *in vivo* resistant infections were 44 %, 16 % and 7 % at day 14 after treatment with CQ, AQ and SP, respectively (Table I). The efficacy of treatment was not linked with the age of children (the means of ages with sensitive infections versus resistant infections were not different; Mann-Whitney *U*-test $P = 0.67$ for the three treatments; $P = 0.81$ for CQ; $P = 0.49$ for AQ; $P = 0.35$ for SP).

Considering the total number of observations ($n = 266$) the gametocytaemia presented a peak after treatment. At days 0, 4, 7, and 14 the gametocyte prevalences were 35 %, 72 %, 73 % and 63 %; the geometric means of gametocyte densities were 1.3, 7.4, 12.5 and 5.6/ μ L of blood.

EFFECT OF TREATMENT ON GAMETOCYTAEMIA

Post-therapeutic gametocytaemia significantly differed between treatments. It was higher with SP than with CQ, and higher with CQ than with AQ (Fig. 1). Gametocyte prevalences peaked at 59 % and 69 % on day 4 for AQ and CQ, respectively, and 94 % on day 7 for SP. Geometric mean densities of gametocytes/ μ L of blood peaked at 3.6 on day 4 for AQ, and at 10.1 and 43.8 on day 7 for CQ and SP, respectively. General differences in gametocyte densities were observed between treatment groups at each follow-up times (by Kruskal-Wallis test with $df = 2$, P always $< 10^{-4}$ on days 4, 7 and 14). Areas under these curves of densities between days 0 and 14 are 2.03 times higher with CQ than AQ, and 7.90 times higher with SP than AQ.

EFFECT OF PARASITOLOGICAL EFFICACY OF TREATMENT ON GAMETOCYTAEMIA

The parasitological response of infections after treatment was another factor influencing gametocytaemia. Gametocyte prevalence was higher in resistant infection than in sensitive ones; this was systematically observed on days 0, 4, 7 and 14 for all three treatments (Table II) and significant differences were observed with CQ and AQ on day 7. When considering only children without gametocyte on day 0, significant differences were observed between sensitive and resistant infections with CQ on day 7 and with AQ on days 4, 7 and 14 (Fig. 2) (P always < 0.03 by Fisher's

Treatment	Number of infections							% R
	N	Sensitive	RI day 7	RI day 14	RII	RIII	Total R	
CQ	84	47	2	12	23	0	37	44.0
AQ	87	73	0	6	8	0	14	16.1
SP	95	88	0	4	3	0	7	7.4
Total	266	208	2	22	34	0	58	—

Table I. – Number of *P. falciparum* infections, according to treatments (CQ = chloroquine, AQ = amodiaquine, SP = sulfadoxine + pyrimethamine) and to parasitological responses (R = resistant).

Fig. 1. – Prevalence (%) and geometric mean of Williams of *Plasmodium falciparum* gametocytes for patients in simple malaria access treated with different drugs (8 patients with chloroquine CQ, 87 patients with amodiaquine AQ, and 95 patients with sulfadoxine plus pyrimethamine SP). Above spots of AQ and SP curves, one asterisk indicates a P value < 0.05 and two asterisks indicate a P value < 0.005 , by comparing against CQ on the same day with Fisher's exact test (prevalence) or Mann-Whitney U -test (density). Bars indicate the 95 % confidence interval.

Fig. 2. – Prevalence of *P. falciparum* gametocytes for patients in simple malaria access, without gametocytes at day 0, treated with different drugs (57 patients with chloroquine, 58 patients with amodiaquine, and 58 patients with sulfadoxine plus pyrimethamine). Above spots of resistant (R) infections, one asterisk indicates a P value < 0.05 and two asterisks indicate a P value < 0.005 , by comparing against sensitive (S) infections on the same day with Fisher's exact test. Bars indicate the 95 % confidence interval.

exact test). The prevalence of gametocytaemia was not significantly different between the resistance levels RI or RII (results on day 7 are presented in Table III; results on days 4 and 14 were similar but data are not shown).

Gametocyte density was higher in resistant infections than in sensitive ones; this was systematically observed on days 4, 7 and 14 with the three treatments and significant differences were observed with CQ and AQ on days 7 and 14 (Table IV). Furthermore, gametocyte density was higher in RII than in RI infections although this difference was not significant (Table III). After a Log (x + 1) transformation of trophozoite and gametocyte densities, trophozoite density on day 4 was positively correlated with the gametocyte density on day 7

($r = 0.151$, $n = 266$, $P = 0.013$) and day 14 ($r = 0.168$, $n = 266$, $P = 0.006$); such a correlation was also observed between trophozoite density on day 7 and gametocyte density on day 14 ($r = 0.137$, $n = 266$, $P = 0.026$).

EFFECT OF DURATION OF SYMPTOMS BEFORE TREATMENT ON GAMETOCYTAEMIA

The median of the duration of symptoms reported by the children or their parents at the beginning of treatment (day 0) was three days (mean $\pm$ SD = 3.45 ± 4.26 ; range: 0-60). The mean ($\pm$ SD) duration of symptoms before treatment among children with gametocytes on day 0 was 4.85 ± 6.68 ; among children without gametocytes on day 0 it was 2.70 ± 1.57 ($P < 10^{-4}$, Mann-Whitney *U*-test); these differences were observed in the

Treatment	Parasitological response	n	Gametocyte prevalence at							
			day 0	P	day 4	P	day 7	P	day 14	P
CQ	S	47	29.8	0.64	61.7	0.15	55.3	0.009	48.9	0.07
	R	37	35.1		78.4		83.8		70.2	
AQ	S	73	32.9	0.99	54.8	0.57	49.3	0.02	40.0	0.08
	R	14	35.7		78.6		85.7		64.3	
SP	S	88	38.6	0.99	86.4	0.99	93.2	0.99	86.4	0.99
	R	7	42.9		85.7		100.0		85.7	

Table II. – *P. falciparum* gametocyte prevalence (%), according to treatments (CQ = chloroquine, AQ = amodiaquine, SP = sulfadoxine + pyrimethamine) and to parasitological responses (S = sensitive, R = resistant). The *P* values were obtained by the Fisher's exact test.

Treatment	Type of resistance	n	Gametocyte			
			Prevalence (%)	P	Density	P
CQ	RI	14	13/14 (93)	0.17	21.4	0.66
	RII	23	18/23 (78)		25.1	
AQ	RI	6	4/6 (67)	0.38	12.3	0.89
	RII	8	8/8 (100)		16.8	
SP	RI	4	4/4 (100)	0.99	72.8	0.99
	RII	3	3/3 (100)		150.6	

Table III. – Comparison of *P. falciparum* gametocyte prevalence and density for resistant infections, on day 7 after treatment, according to the type of resistant infections (RI = resistant infection, level 1; RII = resistant infection, level 2). The *P* values were obtained by Fisher's exact test (prevalence) or Mann-Whitney *U* test (density).

Treatment	Parasitological response	n	Gametocyte density at							
			day 0	P	day 4	P	day 7	P	day 14	P
CQ	S	47	1.0	0.51	5.4	0.26	4.9	0.0039	2.0	0.0094
	R	37	1.6		8.9		23.6		8.3	
AQ	S	73	1.6	0.84	3.3	0.27	2.4	0.027	1.0	0.0056
	R	14	0.6		5.6		14.7		7.3	
SP	S	88	1.2	0.91	14.6	0.93	44.1	0.24	17.8	0.10
	R	7	0.7		15.2		99.5		48.1	

Table IV. – Geometric mean of *P. falciparum* gametocyte density, according to treatments (CQ = chloroquine, AQ = amodiaquine, SP = sulfadoxine + pyrimethamine) and to parasitological responses (S = sensitive, R = resistant). The *P* values were obtained by the Mann-Whitney *U*-test.

Gametocyte prevalence at	Beginning of the symptomatic period				P
	Days 0 or - 1	Day - 2	Day - 3	≤ days - 4	
Day 0	3/28 (11 %)	26/87 (30 %)	24/76 (32 %)	46/75 (61 %)	< 10 ⁻⁵
Day 4	13/28 (46 %)	72/87 (83 %)	61/76 (80 %)	60/75 (80 %)	0.028

Table V. – *P. falciparum* gametocyte prevalence (%) at days 0 and 4 related to the duration of symptoms before treatment for the whole 266 children of the study. The *P* values were obtained by the chi-square test for linear trend.

three treatment groups (*P* always < 0.003). The gametocyte prevalence increased with duration from zero to four days (Table V, in which duration ≥ 4 days were presented together because a plateau was observed for such durations of symptoms; data not shown) but not on days 7 and 14 (*P* = 0.14 and *P* = 0.27, Mann-Whitney *U*-tests).

Gametocyte densities on days 0 and 4 were correlated with the duration of symptoms before treatment (*r* = 0.131, *n* = 266, *P* = 0.03 on day 0; *r* = 0.145, *n* = 266, *P* = 0.02 on day 4); such a relationship was not observed on days 7 and 14 (*P* = 0.12 and *P* = 0.81, respectively). On day 4, geometric mean of gametocyte density calculated with the positive values only increased from 11.1/μL when the duration of symptoms was 0 or 1 day to 30.6/μL when the duration of symptoms was ≥ 4 days, i.e. a 2.8 fold increase.

LACK OF EFFECT OF OTHER VARIABLES ON GAMETOCYTAEMIA

Asexual parasite density on day 0 was linked with the gametocyte prevalence on day 0: geometric mean of asexual parasites on day 0 was 47,893/μL for children without gametocyte on day 0 versus 34,534 for children positive for gametocytes (*P* = 0.002; Mann-Whitney *U*-test). This relationship was not observed between asexual parasite density on day 0 and gametocyte prevalences on day 4 (*P* = 0.43), day 7 (*P* = 0.64) and day 14 (*P* = 0.08). Asexual parasite density on day 0 was not correlated with the gametocyte density on day 0: (*r* = 0.048, *n* = 266, *P* = 0.43), day 4 (*r* = - 0.076, *P* = 0.22), day 7 (*r* = - 0.036, *P* = 0.56) and day 14 (*r* = - 0.010, *P* = 0.86). Such analysis, stratified either in relation to therapeutic efficacy (sensitive versus resistant, whatever treatment performed), or in relation to treatment and therapeutic efficacy, led to the same conclusions (*P* always > 0.05); a Log transformation of gametocyte density did not change these results.

The ratio of circulating sexual-to-asexual-form densities on day 0 was not significantly correlated with host age (*r* = - 0.113, *n* = 266, *P* = 0.065).

Haematocrit on day 0 was negatively correlated with gametocyte density on day 0 (*r* = - 0.255, *n* = 266, *P* < 10⁻⁴) as well as with duration of symptoms before treatment (*r* = - 0.270, *n* = 266, *P* < 10⁻⁴).

DISCUSSION

After treatment of children with uncomplicated malaria, a wave of gametocytes has been observed. In our study, three factors were found to influence gametocytaemia: the treatment, the efficacy of treatment, and the duration of symptoms before treatment. Firstly, gametocyte prevalence and density significantly appeared higher in children treated with SP than with CQ, and higher with CQ than with AQ. These results agree with previous studies comparing SP and CQ (Putu & Manyando 1997; Hogg *et al.*, 1998) and highlight the advantage of AQ in this aspect. Secondly, gametocyte prevalence and density were higher in resistant than in sensitive infections. That was verified for the three treatment regimens and on any day after treatment. Even on day 0 gametocyte prevalence was found to be higher in resistant infections than in sensitive ones; although statistically non-significant, this observation has previously been made twice in Senegal (Robert *et al.*, 1996; Robert & Trape, 1998). Unlike to what was observed by Hogg *et al.* (1998) the gametocytaemia in SP-resistant infections was higher than in -sensitive ones. Thirdly, the delay between the first appearance of clinical symptoms and treatment was positively and significantly linked to gametocyte prevalence and density. This result strongly supports the idea that an early treatment with an efficient antimalarial greatly reduces gametocytaemia, and hence could reduce the transmission. Unlike to previous study (Robert *et al.*, 1996), no significant differences between gametocyte prevalence and density were observed between RI and RII; this may be due to low number of observations (Table III).

The relationship between density of asexual stage and subsequent gametocytaemia is unclear. No correlation was observed between asexual parasite density on day 0 and gametocyte density on day 0 nor gametocyte prevalence or density at any other day up to day 14. The actual correlation coefficients between trophozoite density (on day 4 or 7) with gametocyte density (on days 7 or 14) are all small (< 0.17) and do not suggest a major relationship between trophozoite density and subsequent gametocyte densities. Our data are consistent with the idea that gametocytogenesis and asexual growth operate as two distinct pathways with

very few communication. This situation is puzzling because each gametocyte is always generated from an asexual parasite; it could be a consequence of numerous interactions such as relative timing and longevity of gametocytes, symptomatic patients or immune responses, and mixed infections with variable production of gametocytes relative to asexual stages in each infection.

What is the impact of the post-therapeutic gametocytaemia in terms of transmission? Higher gametocyte densities are strongly associated with greater infectivities to mosquitoes (Carter & Graves, 1988). Antimalarial drugs often have an effect (positive or negative) on gametocyte infectivity and/or parasite development in the anophelines: pyrimethamine is clearly sporonticidal, and there are some indications of such an effect for sulfadoxine; the latter might increase gametocytogenesis in drug-resistant lines of *P. gallinaceum* (Butcher, 1997); CQ enhances gametocyte infectivity, whilst SP reduces it (Hogh *et al.*, 1998). We investigated infectivity of gametocytes of patients taken at day 7 after chloroquine treatment (Robert *et al.*, 2000): the relative risk for patients with chloroquine-resistant infection to infect anophelines was 4.07 higher than with sensitive infection. This result demonstrated the importance of the peak of gametocytaemia after chloroquine treatment and its consequence in the increase of transmission.

In Niakhar area as in other parts of the Sahel, anophelines vectors are abundant only a few weeks each year. As a consequence, transmission is relatively low and strictly seasonal. Protective immunity is slowly acquired and a high proportion of new infections leads to clinical attacks. In such context, post-therapeutic gametocytaemia may represent a high proportion of the parasites reservoir. Furthermore, due to the dramatic differences in post therapeutic gametocytaemia between CQ sensitive and CQ resistant strains, drug pressure is likely to rapidly increase both the prevalence of *P. falciparum* resistant strains, the sporozoite rate of anopheline vectors and thus the level of malaria transmission. The most severe consequences of this succession of events are expected to occur in areas where malaria is unstable and only a fraction of the population is infected every year. A dramatic increase in malaria mortality has been documented through hospital and dispensary records in recent years in Saint-Louis region, in Northern Senegal. Here we hypothesise that this could be due to an increase in malaria transmission related not only to high rains and water management schemes, but also to an increase in the prevalence of post-therapeutic gametocytaemia caused by the spread of chloroquine resistance. Chloroquine resistance increases the fatality rates of malaria attacks, as observed since 1992 in Niakhar area (Trape *et al.*, 1998). Its maximum impact is

however expected to occur in areas of even lower transmission since it is only for very low values of the entomological inoculation rate that the incidence of malaria attacks is function of transmission intensity (Trape & Rogier, 1996).

We believe that our study highlights the importance of preventing post-therapeutic gametocytaemia both for preserving drug efficacy and avoiding an increase in malaria transmission in areas with low transmission. The combined therapy strategy using artemisinin and its derivatives which are strongly effective to reduce both gametocytaemia and malaria transmission would be particularly relevant in this region of Africa (White, 1998; von Seidlein *et al.*, 2001; Targett *et al.*, 2001).

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