Reducing the oral quinine–quinidine–cinchonin (Quinimax®) treatment of uncomplicated malaria to three days does not increase the recurrence of attacks among children living in a highly endemic area of Senegal

Christophe Rogier[1], Regis Brau[1], Adama Tall[1], Badara Cisse[1] and Jean-François Trape[2] *[1]Institut Pasteur de Dakar, B. P. 220, Dakar, Sénégal; [2]ORSTOM, B. P. 1386, Dakar, Sénégal*

Abstract

A 3 d shortened course of the quinine–quinidine–cinchonin association Quinimax® was compared to the usual 7 d regimen for routinely treating 462 acute uncomplicated *Plasmodium falciparum* malaria attacks in 72 children under the age of 10 years in Dielmo, a holoendemic village in Senegal. 25 mg/kg Quinimax® salt daily, given in 3 equal doses, improved clinical status in 99.6% of the patients receiving the course and in all of those treated for 7 d. Even if the 3 d course did not systematically eliminate parasitaemia, reducing oral Quinimax® treatment of uncomplicated malaria from 7 to 3 d did not increase the recurrence of attacks, even among the youngest children. Both the quinine sensitivity of the Senegalese strains of *P. falciparum* and the partial acquired immunity of the children were probably responsible for the absence of any difference between the courses. Oral Quinimax® for 3 d is a possible alternative regimen to chloroquine and sulfadoxine–pyrimethamine for treating uncomplicated malaria in highly endemic areas of Africa where clinical resistance to these drugs exists.

Keywords: malaria, *Plasmodium falciparum*, chemotherapy, Quinimax®, children, Senegal

Introduction

Resistance of *Plasmodium falciparum* to chloroquine is now widespread in tropical Africa. Even when chloroquine produces rapid clinical improvement in most patients, chloroquine-resistant *P. falciparum* is responsible for frequent clinical relapses (BLOLAND *et al.*, 1993) and increasing malaria mortality (GREENBERG *et al.*, 1989; CARME *et al.*, 1992), which recently led one African country (Malawi) officially to abandon chloroquine as first-line therapy. Alternative drugs to chloroquine are scarce. Apart from their toxicity, the price of halofantrine, mefloquine and other new antimalarial drugs makes them unaffordable for the population living in malaria-endemic African countries (FOSTER, 1991). Amodiaquine has only a limited therapeutic advantage over chloroquine and its use for repeated treatment is discouraged (WHO, 1990). The pyrimethamine–sulfadoxine association is now recommended in Malawi as first line therapy for uncomplicated malaria. However, this drug is known to induce resistance rapidly and is responsible for rare but possibly fatal reactions. Moreover its extensive use might induce bacterial resistance in areas where this type of drug association is widely use for acute lower respiratory tract infections, another important cause of mortality in children. Quinine, which is classically reserved for the treatment of severe malaria, is in fact widely used in most African countries as first or second line treatment of uncomplicated malaria because of its high efficacy and relatively low cost. However the required 3 daily doses for 7 d of the standard regimen are almost never completed (FELLER-DANSOKHO *et al.*, 1994). The efficacy of a 3 d oral quinine regimen has been demonstrated in several trials involving limited numbers of patients (DELORON *et al.*, 1989; JAMBOU *et al.*, 1990; BJÖRKMAN *et al.*, 1991; KREMSNER *et al.*, 1994). This shortened regimen has not yet been routinely tested. As an adjunct to a cohort study of the natural acquisition of antimalarial immunity (TRAPE *et al.*, 1994), we compared the 3 d and 7 d oral regimens of Quinimax®, an association of quinine, quinidine and cinchonin, for routinely treating acute uncomplicated *P. falciparum* malaria in children from a holoendemic village in Senegal.

Patients and Methods

The study took place from October 1990 to May 1993 in the village of Dielmo, Senegal, where *P. falciparum* is holoendemic and perennially transmitted. The whole population was involved in a prospective study described elsewhere (TRAPE *et al.*, 1994). Briefly, in order to identify all episodes of fever, the 247 inhabitants of Dielmo village were put under daily medical surveillance. At least one physician, a technician or a nurse and 3 medical field workers were present in the village 24 h a day, 7 d a week. Among the population (male:female sex ratio=0·98), 20·4% were children less than 5 years of age and 26·8% were children aged 5–14 years.

Each villager was visited daily at home. Three thick blood films and medical examinations were made for each illness episode. One of the blood films was Giemsa-stained without previous dehaemoglobinization and examined immediately so that a decision regarding treatment could be made; the other 2 were dehaemoglobinized, stained and stored. The criteria for uncomplicated malaria treatment was based on age and a parasite:leucocyte ratio of 2 or more (TRAPE *et al.*, 1985). Children under 10 years of age with uncomplicated malaria were treated with daily oral doses of 25 mg/kg Quinimax® (Quinimax® contains 71·4% quinine-resorcin bichlorhydrate, 18·6% quinidine-resorcin bichlorhydrate, 5% cinchonin-resorcin bichlorhydrate and 5% cinchonidin-resorcin bichlorhydrate) divided into 3 equal doses administered every 8 h by a medical field worker or a physician. With the informed consent of parents, the duration of the Quinimax® course was randomly allocated by drawing from a box containing 4 7-d and 6 3-d papers. In the absence of clinical and parasitological improvement after a 3 d course, it was planned to continue treatment for 7 d. The children were observed for 15 min after each dose; if vomiting occurred they were re-treated. Clinical signs and symptoms (hot sweats, shivering, asthenia, vomiting, diarrhoea and headache) were recorded and the axillary or rectal temperature was taken every 8 h for 7 d. Thick blood films were prepared between 3 and 10 d after the beginning of treatment to check its parasitological efficacy. These blood films were also dehaemoglobinized, stained and stored. All thick blood film examinations were standardized (TRAPE, 1985). A total of 200 microscope oil-immersion objective fields was examined on each slide (about 0.5 μL of blood) by the same experienced technician at the end of the study. The parasite counts used for the analysis were those obtained from this independent examination. Finally, the malaria attacks were defined as fever (rectal temperature $\geq$38·5°C or axillary temperature $\geq$38·0°C) or reported fever associated with a parasite:leucocyte ratio above an age-dependent pyrogenic threshold previously identified in this population by ROGIER *et al.* (in press) as 2·45 before the age of 12 months, 2·7 between 12 and 23 months, 2·4 at 2 years, 2·0 at 5 years, and 1·6 at 9 years.

The population was asked not to use any drug without informing the team, and urine tests were regularly car-

ried out (on average once a month) to detect the presence of antimalarial drugs, using the modified Saker–Solomons test (MOUNT *et al.*, 1989). As a result, no antimalarial self-treatment was recorded during the study period.

For this analysis, we took into consideration the children aged 9 years or less who continued to live in Dielmo during the follow-up period (maximum absence 10 d) and met the following criteria: (i) at least 50% of their life since birth spent in Dielmo or an area of high malaria endemicity; (ii) continued presence in the village or a maximum absence of 30 d during the 6 months preceding the study period; and (iii) continued presence in the village or a maximum absence of one year during the 3 years preceding the study.

The clinical recovery time was calculated as the period between the first dose of Quinimax® and the time when all symptoms disappeared, estimated as the median time between the last symptomatic visit and the first visit free of any symptom. The parasitological efficacy was assessed by the percentage of thick blood films free of asexual parasites between 3 and 10 d after the beginning of treatment. When several blood films were made, we took the latest into account. We recorded the frequency of new malaria attacks during the 60 d following the end of the treatment; any new malaria attacks occurring during the 28 d following the end of treatment were considered to be possible recrudescences.

We used a random-effect logistic regression model (STIRATELLI *et al.*, 1984) to test proportional data, taking into account the interdependence of successive treatments in the same child. With this model, the estimated odds ratio can be considered as estimations of individual relative risk of treatment failure associated with the duration of the cure (ZEGER *et al.*, 1988). It was also possible to take into account the effect of age, season, and delay between the start of treatment and the parasitological examination. The odds ratios (ORs) were compared to one by the likelihood ratio test (HOSMER & LEMESHOW, 1989); 95% confidence intervals (CI) were calculated.

Results

During the study period, 482 uncomplicated malaria attacks were treated with Quinimax®. Among them, 20 cases, 8 of 285 3-d courses and 12 of 197 7-d courses were excluded from the analysis either because the children left the village for several days during treatment or the medical files were incomplete.

The analysis therefore involved 277 3-d and 185 7-d treatments in 72 children. In 22 cases (9 3-d and 13 7-d courses) the dose given was lower than the planned figure because of vomiting without re-treatment or absence from the village for a few hours. In these cases, the given dose was on average 88·5% and 93·6% of the planned dose of the 3 d and 7 d courses, respectively. These cases were maintained in the analysis.

In one case of the 3 d treatment group (0·4%), the persistence of symptoms and parasitaemia at the end of the course justified the prolongation of the Quinimax® treatment for 7 d. For the assessment of parasitological efficacy, we took into consideration the positive blood film on the fourth day after the start of treatment.

There was no significant difference in distribution of the 3 d and 7 d courses according to age, initial body temperature, vomiting frequency and parasite density before treatment (Table 1).

In 19·9% of the 462 cases, the symptoms disappeared between the last visit before treatment and the start of the Quinimax® course (median time: 5 h, interquartile interval (25–75%) 2–11 h). The frequency of spontaneous clinical recovery was similar before both the 3 d and 7 d courses (20·2% and 18·9%). Among the 371 children who were still symptomatic at the beginning of Quinimax® treatment, the median clinical recovery time was 13 h (interquartile interval (25–75%) 8–22 h). The clinical recovery rate before the twelfth hour after starting Quinimax® was similar in both courses (Table 2; OR=0·91; 95% CI 0·57–1·46). The probability of clinical recovery before the twelfth hour was higher among the older children; it inceased by a factor of 1.3 per year of age (OR=1·32; 95% CI 1·16–1·5). The frequency of vom-

Table 1. Distribution of Quinimax® course duration according to age, initial body temperature, vomiting before treatment and initial malaria parasite density

	Duration of course	
	3 d	7 d
No. of treatments	277	185
Age (years)		
<1	25 (9%)	21 (11%)
1	52 (19%)	46 (25%)
2	67 (24%)	39 (21%)
3–4	72 (26%)	51 (28%)
5–9	61 (22%)	28 (15%)
Rectal temperature °C		
<38·5	32 (12%)	18 (10%)
≥38·5<39	93 (34%)	55 (30%)
≥39	152 (55%)	112 (60%)
Vomiting before treatment	83 (30%)	65 (35%)
Parasitaemia[a]	5·98 (5·74–6·23)	6·48 (6·11–6·76)

[a]*P. falciparum* parasites per leucocyte (geometric mean; 95% confidence interval in parentheses).

Table 2. Clinical and parasitological efficacy of Quinimax® according to course duration

	Duration of course	
	3 d	7 d
No. of treatments	277	185
Clinical recovery time		
<12 h	87 (39%)	59 (39%)
≥12 h	134 (61%)	91 (61%)
No. cured	221 (100%)	150 (100%)
No. clinically recovered before treatment	56	35
Day 3		
Without parasitaemia	51 (50%)	20 (59%)
With parasitaemia	52 (50%)	14 (41%)
Total blood films	103 (100%)	34 (100%)
Days 4–5		
Without parasitaemia	38 (78%)	27 (82%)
With parasitaemia	11 (22%)	6 (18%)
Total blood films	49 (100%)	33 (100%)
Days 6–10		
Without parasitaemia	65 (89%)	78 (98%)
With parasitaemia	8 (11%)	2 (2%)
Total blood films	73 (100%)	80 (100%)
No blood film 3–10 d after start of treatment	52	38
Recurrence before day 28		
Yes	117 (48%)	79 (47%)
No	126 (52%)	89 (53%)
No. of cases followed-up to day 28	243 (100%)	168 (100%)
Excluded cases[a]	34	17

[a]Absent for 3 or more days, or incorrect chemotherapy.

iting during the course was similar with both treatments (12·5% and 15·1%).

At least one blood film was made in 372 of the 462 cases (80·5%), 225 cases on a 3 d course and 147 on a 7 d course. Whatever the duration of the Quinimax® course, the frequency of blood films free of parasites increased from day 3 to day 10 after the start of treatment (Table 2). Taking into account the effect of the time of making the blood film in a logistic regression model, there was no significant difference in parasitological clearance rate between the 3 d and 7 d courses at day 3 (OR=1·4, 95% CI 0·5–3·7) or at days 4 or 5 (OR=1·9, 95% CI 0·5–6·6). However, the blood films made between days 6 and 10 after starting treatment had a 5·9 times higher probability of being free of parasites after a 7 d than a 3 d course (OR=5·9, 95% CI 1·1–30·6). The probability of parasitological clearance was higher among the older children, increasing by a factor of 1·6 per year of age (OR=1·6; 95% CI 1·3–2·0). There was no significant interaction between the effect of age and duration of the course.

The Figure shows a Kaplan–Meier estimate of the probability of occurrence of a malaria attack as a function of time and duration of the Quinimax® course. Whatever the group, the risk of a new malaria attack was similar during the 60 d following the end of Quinimax® treatment.

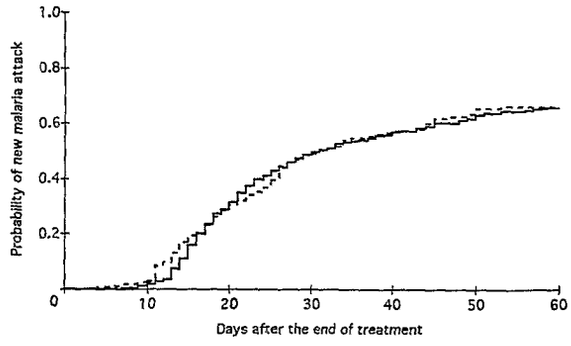

Figure. Incidence of new *P. falciparum* malaria attacks after oral quinine–quinidine–cinchonin (Quinimax®) treatment according to the duration of the course: 3 d (continuous line, $n=277$) and 7 d (broken line, $n=185$). Dielmo, Senegal, October 1990–May 1993, 72 children aged less than 10 years.

In 51 cases, children left the village for more than 3 d or received unplanned antimalarial treatment (due to overestimation of the parasitaemia at the emergency examination) during the 28 d following the end of the Quinimax® treatment. These cases were as frequent after a 3 d course as after a 7 d course (11·9% and 9·6%), and were excluded from the subsequent analysis. The frequency of recurrence before day 28 after the end of treatment was lower among the older children; it was decreased by a divisor of 1·2 per year of age (OR=1·22; 95% CI 1·06–1·40). Adjusting for the effect of age and the seasonal malarial transmission rate by a logistic regression model, the recurrence rate before day 28 was similar after both courses (Table 2; OR=0·97; 95% CI 0·62–1·52). There was no significant interaction between the course duration effect and the age or seasonal transmission effects.

Discussion

Reducing the oral Quinimax® treatment of uncomplicated malaria from 7 to 3 d did not increase the recurrence of attacks, even among the youngest children. This has not previously been demonstrated by trials which involved a limited number of subjects (15–35 individuals per group) who were asymptomatic (BJÖRKMAN *et al.*, 1991) or attending an outpatient clinic (DELORON *et al.*, 1989; JAMBOU *et al.*, 1990; KREMSNER *et al.*, 1994). In only one of these studies (BJÖRKMAN *et al.*, 1991) was the shortened course compared to the standard 7 d regimen. In the present study, the efficacy was assessed in routine practice at the population level while the absence of self treatment was continuously controlled. Moreover, the severity of symptoms and the parasite density before treatment, as well as the clinical recovery rate before the twelfth hour and the parasitological clearance rate at day 3, testify to the similarity of both course groups before and after the randomization.

The quinine–quinidine–cinchonin association is known to be more effective *in vitro* than quinine alone (DRUILHE *et al.*, 1988). This type of association makes our results difficult to compare with those assessing the oral efficacy of quinine given alone (BJÖRKMAN *et al.*, 1991; KREMSNER *et al.*, 1994). Furthermore, we used a daily dose of 25 mg/kg of the quinine–quinidine–cinchonin salt, which is the most widely used (FELLER-DANSOKHO *et al.*, 1994) and was recommended by the manufacturers of Quinimax® (Sanofi). This is equivalent to 16 mg/kg of quinine base daily, much less than the usual dosage (24–30 mg/kg quinine base) used in the previous studies. This could explain the low parasitological clearance rate we observed (89%) between days 6 and 10 after the start of the 3 d treatment. This rate, on days 6 or 7, varied from 97% to 100% in the previous studies. On the other hand, we determined the parasite density by counting the number of parasites per 200 microscope fields, corresponding to about 3200 leucocytes or 0·5 μL of blood. This represents a substantially longer scrutiny of each slide than that in some of the previous studies (DELORON *et al.*, 1989; JAMBOU *et al.*, 1990; BJÖRKMAN *et al.*, 1991). Thus, the low parasitological clearance rates we observed are not surprising. Despite the lack of parasitological efficacy, the absence of any difference between the 3 d and 7 d courses suggests that new malaria attacks occurring after the end of treatment were most probably due to new infections. This is also suggested by the molecular typing of 28 *P. falciparum* isolates collected during successive clinical malaria episodes in 10 children treated in Dielmo by 3 d courses of Quinimax® (CONTAMIN *et al.*, in press). Comparison of the genotypes of the parasites showed that the parasite populations were different in each clinical episode. Furthermore, the 3 d Quinimax® treatment almost always resulted in the disappearance of the alleles identified during the clinical attack. The single case in which the genotypes of 2 successive episodes could not be distinguished was a recrudescence due to obviously incomplete treatment; the delay between the 2 clinical attacks was 16 d.

The children enrolled in the present study were in the process of acquiring natural protective immunity (ROGIER & TRAPE, 1993), resulting in a marked influence of age on parasitological and clinical recovery. Even among the youngest children, not every infective bite or malaria infection necessarily entails the occurrence of a malaria attack (TRAPE *et al.*, 1994). We also observed a significant proportion of spontaneous clinical recoveries before the start of treatment. It is obviously possible that, in the absence of antimalarial treatment, these recoveries would have been followed by some relapses or intermittent fever. However, natural protective immunity may have been responsible for the absence of a difference between the 3 d and 7 d courses, even if they did not invariably eliminate parasitaemia. Moreover, it is not certain that eradication of infection is desirable in children and adults living in highly endemic areas, since an appreciable degree of immunity develops, and this could be maintained by the stimulus of persistent infection (WILSON, 1939).

Investigations *in vitro* in Dielmo during the study period showed good sensitivity of *P. falciparum* to quinine (WHO microtest of inhibition of schizont maturation: mean 50% inhibitory concentration [IC]=1·02 μmol/L of blood medium; mean 90% IC= 4·03 μmol/L; and mean 100% IC=5·10 μmol/L [I. B. Bah, personal communication]). This is probably another explanation of the absence of difference between the 3 d and 7 d

courses. However, the widespread use of insufficient dosages is likely to encourage the development of quinine–resistant *P. falciparum* strains, which have already been reported in some African countries (BRANDICOURT *et al.*, 1986; BRASSEUR *et al.*, 1992).

Since the other drugs are unaffordable by most of the populations in tropical Africa, the pyrimethamine–sulfadoxine association is the only alternative drug to chloroquine available for treatment of chloroquine-resistant malaria. However, it is responsible for infrequent but severe adverse reactions like Stevens–Johnson syndrome or agranulocytosis (SALAKO, 1984). Above all, resistance to sulfadoxine–pyrimethamine has been reported in Africa and the rapid development of resistance to this association when it is widely used has already been observed in Asia and South America (BJÖRKMAN & PHILLIPS-HOWARD, 1990).

Our observations suggest that the shortened quinine–quinidine–cinchonin regimen is safe, effective and well accepted in treating chloroquine-resistant *P. falciparum* malaria. However, there is evidence in the literature for an association between the wide use of quinine and the occurrence of blackwater fever (BRUCE-CHWATT, 1987). It has been emphasized that African children who escape blackwater fever under natural conditions may become susceptible to this acute haemolysis when they are submitted to quinine treatment (MANSON-BAHR, 1946). If the shortened oral quinine regimen is used as widely as chloroquine is now used in tropical Africa, blackwater fever could become again a fearsome threat. This must encourage reserving the oral use of quinine for malaria which is resistant to chloroquine and sulfadoxine–pyrimethamine.

Acknowledgements

We are grateful to the villagers of Dielmo for their active participation and continuing collaboration in the project. We thank Dr L. Ravinet (Sanofi) for providing Quinimax® and Dr I. B. Bah (University of Dakar) for the *in vitro* microtests. We also thank Dr P. Druilhe (Institut Pasteur, Paris), Dr O. Puijalon (Institut Pasteur, Paris) and Dr V. Robert (ORSTOM, Dakar) for reviewing the manuscript. This work was supported by grants from the Ministère de la Recherche et de l'Enseignement Supérieur and the Ministère de la Coopération et du Développement (Paris).

References

Björkman, A. & Phillips-Howard, P. A. (1990). The epidemiology of drug-resistant malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 177–180.

Björkman, A., Willcox, M., Marbiah, N. & Payne, D. (1991). Susceptibility of *Plasmodium falciparum* to different doses of quinine *in vivo* and to quinine and quinidine *in vitro* in relation to chloroquine in Liberia. *Bulletin of the World Health Organization*, **69**, 459–465.

Bloland, P. B., Lackritz, E. M., Kazembe, P. N., Were, J. B. O., Steketee, R. & Campbell, C. C. (1993). Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infectious Diseases*, **167**, 932–937.

Brandicourt, O., Druilhe, P., Diouf, F., Brasseur, P., Turk, P. & Danis, M. (1986). Decreased sensitivity to chloroquine and quinine of some *Plasmodium falciparum* strains from Senegal in September 1984. *American Journal of Tropical Medicine and Hygiene*, **35**, 717–721.

Brasseur, P., Kouamouo, J., Brandicourt, O., Moyou-Somo, R. & Druilhe, P. (1992). Multidrug resistant falciparum malaria in Cameroon. I. Stable figures of prevalence of chloroquine and quinine resistant isolates in the original foci. *American Journal of Tropical Medicine and Hygiene*, **46**, 1–7.

Bruce-Chwatt, L. J. (1987). Quinine and the mystery of blackwater fever. *Acta Leidensia*, **55**, 181–196.

Carme, B., Yombi, B., Bouquety, J. C., Plassard, H., Nzingnula, S., Senga, J. & Akani, I. (1992). Child morbidity and mortality due to cerebral malaria in Brazzaville, Congo. A retrospective and prospective hospital-based study 1983–1989. *Tropical Medicine and Parasitology*, **43**, 173–176.

Contamin, H., Fandeur, T., Rogier, C., Bonnefoy, S., Konaté, L., Trape, J. F. & Mercereau-Ouijalon, O. (in press). Molecular typing of *Plasmodium falciparum* isolates collected during successive clinical malaria episodes in Senegalese children. *American Journal of Tropical Medicine and Hygiene.*

Deloron, P., Lepers, J. P., Verdier, F., Chougnet, C., Remanan-mirija, J. A., Andriamangatiana-Rason, M. D., Coulanges, P. & Jaureguiberry, G. (1989). Efficacy of a 3-day oral regimen of a quinine–quinidine–cinchonin association (Quinimax®) for treatment of falciparum malaria in Madagascar. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **83**, 751–754.

Druilhe, P., Brandicourt, O., Chongsuphajaisiddhi, T. & Berthe, J. (1988). Activity of a combination of three cinchona bark alkaloids against *Plasmodium falciparum in vitro*. *Antimicrobial Agents and Chemotherapy*, **32**, 250–254.

Feller-Dansokho, E., Ki-Zerbo, G. & Badiane, S. (1994). Prise en charge diagnostique et thérapeutique de l'accès palustre simple dans la région de Dakar, Sénégal. *Annales de la Société Belge de Médecine Tropicale*, **74**, 291–300.

Foster, S. D. (1991). Pricing, distribution and use of antimalarial drugs. *Bulletin of the World Health Organization*, **69**, 349–363.

Greenberg, A. E., Ntumbanzondo, M., Ntula, N., Mawa, L., Howell, J. & Davachi, F. (1989). Hospital-based surveillance of malaria related paediatric morbidity and mortality in Kinshasa, Zaire. *Bulletin of the World Health Organization*, **67**, 189–196.

Hosmer, D. W. & Lemeshow, S. (1989). *Applied Logistic Regression*. New York: Wiley-Interscience.

Jambou, R., Abissegue, B., Gelas, H., Ghipponi, P. M., Farinotti, R., Le Bras, J. & Hengy, C. (1990). L'accès palustre simple en zone de haut niveau de résistance à la chloroquine. I. Evaluation d'un traitement court par la quinine. *Bulletin de la Société de Pathologie Exotique*, **83**, 46–52.

Kremsner, P. G., Winkler, S., Brandts, C., Neifer, S., Bienzle, U. & Graninger, W. (1994). Clindamycin in combination with chloroquine or quinine is an effective therapy for uncomplicated *Plasmodium falciparum* malaria in children from Gabon. *Journal of Infectious Diseases*, **169**, 467–470.

Manson-Bahr, P. E. C. (1946). Blackwater fever. *Manson's Tropical Diseases*, 12th edition. London: Cassell, pp. 72–80.

Mount, D. L., Nahlen, B. L., Patchen, L. C. & Churchill, F. C. (1989). Adaptations of the Saker–Solomons test: simple, reliable colorimetric field assays for chloroquine and metabolites in urine. *Bulletin of the World Health Organization*, **67**, 295–300.

Rogier, C. & Trape, J.-F. (1993). Malaria attacks in children exposed to high transmission: who is protected? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 245–246.

Rogier, C., Commenges, D. & Trape, J. F. (in press). Evidence for an age-dependent pyrogenic threshold of *Plasmodium falciparum* parasitemia in highly endemic populations. *American Journal of Tropical Medicine and Hygiene.*

Salako, L. A. (1984). Toxicity and side-effects of antimalarials in Africa: a critical review. *Bulletin of the World Health Organization*, **62**, supplement, 63–68.

Stiratelli, R., Laird, N. M. & Ware, J. H. (1984). Random-effects models for serial observations with binary response. *Biometrics*, **40**, 961–971.

Trape, J. F. (1985). Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 181–184.

Trape, J. F., Peelman, P. & Morault-Peelman, B. (1985). Criteria for diagnosing clinical malaria among a semi-immune population exposed to intense and perennial transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 435–442.

Trape, J. F., Rogier, C., Konate, L., Diagne, N., Bouganali, H., Canque, B., Legros, F., Badji, A., Ndiaye, G., Ndiaye, P., Brahimi, K., Faye, O., Druilhe, P. & Pereira da Silva, L. (1994). The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *American Journal of Tropical Medicine and Hygiene*, **51**, 123–137.

WHO (1990). *Practical Chemotherapy of Malaria*. Geneva: World Health Organization, Technical Report Series, no. 805.

Wilson, D. B. (1939). Implications of malarial endemicity in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **32**, 435–465.

Zeger, S. L., Liang, K. Y. & Albert, P. S. (1988). Models for longitudinal data: a generalized estimating equation approach. *Biometrics*, **44**. 1049–1060.

Received 21 June 1995; accepted for publication 8 August 1995