

Artesunate/Amodiaquine Malaria Treatment for Equatorial Guinea (Central Africa)

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Abstract. The objectives of this study were: 1) to evaluate the safety and efficacy of combination artesunate (AS)/amodiaquine (AQ) therapy, and 2) to determine the difference between recrudescence and resistance. An *in vivo* efficacy study was conducted in Equatorial Guinea. A total of 122 children 6–59 months of age from two regional hospitals were randomized and subjected to a 28-day clinical and parasitological follow-up. A blood sample on Whatman paper was taken on Days 0, 7, 14, 21, and 28 or on any day in cases of treatment failure, with the parasite DNA then being extracted for molecular analysis purposes. A total of 4 children were excluded, and 9 cases were lost to follow-up. There were 17 cases of late parasitological failure, 3 cases of late clinical failure, and 89 cases of adequate clinical and parasitological response. The parasitological failure rate was 18.3% (20 of 109) and the success rate 81.70% (95% confidence interval [72.5–87.9%]). After molecular correction, real treatment efficacy stood at 97.3%. Our study showed the good efficacy of combination AS/AQ therapy. This finding enabled this treatment to be recommended to Equatorial Guinea's National Malaria Control Program to change the official treatment policy as of March 2008.

INTRODUCTION

Currently, malaria is one of the most important public health problems in African countries, not only in terms of morbidity and mortality, but also because of its socio-economic impact and repercussions on human development. Overall, in sub-Saharan countries, a family spends around 25% of its income on the control and prevention of malaria.¹

It is estimated that every year malaria gives rise to ~247 million clinical cases and as many as 881,000 deaths (91% of which are in sub-Saharan Africa).²

In areas of Africa with stable malaria transmission, such as Equatorial Guinea (EQG), the principal symptoms of malaria are connected with anemia and the presence of parasites in the placenta. Malaria is estimated to cause 10,000 maternal deaths per annum and be responsible for 8–14% of all low birth weight and 3–8% of all infant deaths.³ Indeed, it contributes to the death of 75,000 to 200,000 children/year.⁴ The World Health Organization (WHO) estimates that the probability of dying among children under 5 years of age in EQG is 205 of 1,000 live births, with 24% of this total being attributable to malaria.

The impact of malaria is the result of a variety of factors, such as the species of parasite, the distribution and efficiency of the vector, and human immunology. One of the most pressing problems to be tackled in Africa is the rapid spread of anti-malarial drug resistance. During recent decades, *Plasmodium falciparum* has developed resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) in most African countries, two drugs that were both financially affordable and easy to take.⁵

The above was shown graphically by different *in vivo* studies undertaken in EQG. From 1992 to 1999, the Carlos III Institute of Health (Madrid, Spain), working in collaboration with Equatorial Guinea's Ministry of Health, conducted a study on Bioko Island and concluded that resistance to CQ was over 40% and resistance to SP was around 25% among children under 5 years of age. On the mainland, studies undertaken in 2001 yielded the same results.⁶ In 2005, following

the WHO recommendation to use combination therapy for malaria treatment, a trial was performed in Equatorial Guinea to evaluate the efficacy of two combinations, namely, artesunate + sulfadoxine-pyrimethamine and amodiaquine + sulfadoxine-pyrimethamine, with cure rates of 97.5% and 97.3%, respectively.⁷

In 2006, the country's National Malaria Control Program (NMCP) began the process of changing its malaria policy. Based on the experience of other infectious diseases, the WHO guideline states that a combination therapy should be used in malaria-endemic countries. In this context, "combination therapy" should be construed as the simultaneous use of two or more blood schizontocidal drugs having independent modes of action and different biochemical targets in the parasite.⁸ Accordingly, the NMCP and its partners decided to use artesunate (AS) + amodiaquine (AQ) as a first line of treatment of non-severe falciparum malaria.

When a country like EQG is situated in a high malaria-incidence area with a high transmission rate, it is necessary to establish a method to differentiate recrudescence from re-infection in an *in vivo* study. Methods such as polymorphic gene (*msp-1* and *msp-2*) analysis⁹ or microsatellite analysis are useful for this purpose.¹⁰

The objectives of this study were: 1) to evaluate the efficacy of the AS/AQ combination as a first line of treatment of uncomplicated malaria in Equatorial Guinea, and 2) to determine the difference between recrudescence and resistance and furnishing real data about resistance levels in the country.

MATERIALS AND METHODS

Study area and population. The EQG is situated in Central Africa, in and on the Gulf of Guinea. It is divided into two regions: the mainland area, called Rio Muni, lying between Cameroon and Gabon; and the island region (Bioko, Annobón, and Corisco Bay). Bioko, the largest island, lies 40 km from the Cameroon coast and Annobón. Something like 75% of EQG's total population—estimated at around 484,000—lives on the mainland.

Malabo, the country's capital, is situated on Bioko Island, which has a tropical climate, with a rainy season from May to

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October, and a dry season from December to March. This is a mesoendemic area, documented as having a *Plasmodium* index of 26% in 2006.¹¹ Bata is the most important city on the mainland. It too has a tropical climate, and is a meso-hyperendemic area with a *Plasmodium* index of 41–75% among children under 5 years of age (unpublished study based on a 2006 National Prevalence Survey: Ministry of Health and Carlos III Institute of Health).

Patient recruitment. The *in vivo* study was conducted from June to December 2006 at the two regional hospitals of Malabo and Bata, using the WHO methodology. All cases included for assessment were drawn from the hospitals' respective pediatric outpatient departments. The study comprised a prospective evaluation of the clinical and parasitological response to an antimalarial AS/AQ combination therapy. Because there was no evidence of treatment failure, the minimum sample size for ensuring 10% precision with a 95% confidence level (95% CI) was 96 patients.¹²

All potentially eligible children were medically screened, applying the following inclusion criteria: age 6 to 59 months; no signs of severe malnutrition; slide-confirmed monospecific *P. falciparum* infection; parasite density of 2,000 to 200,000 asexual parasites/microliter; axillary temperature > 37.5°C; easy access to hospital; absence of history of hypersensitive reaction to either of the drugs being evaluated; and informed consent of parents or caregivers. Before being enrolled, each child's medical history was obtained and he/she had to undergo a clinical examination by a physician. The following exclusion criteria were applied, i.e., any danger signs of severe malaria, such as inability to drink or breastfeed, vomiting, recent history of convulsions, lethargy, and inability to sit or stand up.

Treatment and follow-up. The clinical and parasitological levels of all the children were evaluated for a period of 28 days. Treatment was administered during the first 3 days under direct observation. Drugs were dissolved in water with sugar and given with a spoon. Children were observed for 30 minutes after treatment to monitor for side effects. The dosage given was as follows: artesunate 50 mg (Action Medeor Lot 055585; expired 11/2008) as a single dose of 4 mg/kg/day for 3 days; and amodiaquine 150 mg (Holden Medical, Cameroon; set 04L02; expired 11/2007) as a single dose of 10 mg/kg/day for 3 days. The rescue treatment was oral quinine 200 mg (Holden Medical; Lot MFF 371/05 A 01; expired 12/2007), with a dosage of 10 mg of body weight every 8 hours for 7 days.

We used a record form that included patients' age, sex, and address, the name of the caregiver, a contact telephone number (where possible), and the following indicators: temperature over 6 days; drug dosages on Days 0, 1, and 2; parasitemia on Days 0, 1, 2, 3, 7, 14, 21, and 28, or whichever day the child was brought to the hospital; hematocrit on Days 0 and 28; filter paper on Days 0, 14, 21, and 28 or any day in cases of treatment failure. To avoid as many losses to follow-up as possible, all the children were accompanied to their homes on the first day to ascertain where they could be found/collected and given the treatment if they failed to appear at the hospital on one of the days of the protocol.

Giemsa-stained blood films were made and parasitemia was quantified by a standard approximation method (number of asexual parasites per 200 white blood cells per 40 on thick film). A positive smear was defined as the presence of at least

one asexual form seen after examining 100 thick smear fields. Slide quality control was conducted by re-reading 10% of slides selected randomly, and discordant results were referred to a third microscopist. Hematocrit was measured by microhematocrit centrifugation on Days 0 and 28.

Classifications of treatment outcomes. Efficacy was measured on the basis of the parasitological cure rates on Day 28. Criteria to determine treatment failure are described below.¹²

Early treatment failure (ETF) was deemed to be 1) development of severe signs of or severe malaria on Days 1, 2, or 3 in the presence of parasitemia; 2) parasitemia on Day 2 higher than on Day 0, regardless of temperature; 3) parasitemia on Day 3, with axillary temperature > 37.5°C; or, 4) parasitemia on Day 3 of over 25%.

Late clinical failure (LCF) was deemed to be 1) development of severe malaria or danger signs after Day 3, with parasitemia but without previous criteria of ETF; or 2) parasitemia and temperature > 37.5°C on any day between Days 4 and 28, without previous criteria of ETF.

Late parasitological failure (LPF) was deemed to be 1) presence of parasitemia on any day between Days 7 and 28, with temperature < 37.5°C, without previous criteria of ETF or LCF.

Adequate clinical and parasitological response (ACPR) was defined as absence of parasitemia on Day 28, regardless of axillary temperature, without the patient meeting ETF, LCF, or LPF criteria.

Other outcomes that were deemed not to constitute treatment failure were withdrawal (e.g., children's caregivers who decided not to continue with the study); or cases where a child could not be located, whether at the hospital, in the community, or in the study area.

DNA extraction and molecular study. The DNA was extracted from the blood sample and spotted onto Whatman paper. A circle of paper (4 mm) with dried blood was cut out and template DNA was then extracted using the SpeedTools Tissue DNA kit (Biotools, B&M Laboratories, S.A., Madrid, Spain). This DNA was used for the respective molecular assays, namely; 1) semi-nested multiplex PCR for diagnosis of malaria and confirmation of the *Plasmodium* species¹³; and 2) PCR analysis of *P. falciparum* microsatellite loci.¹⁰ To distinguish cases of recrudescence from those of re-infection in the *in vivo* study, *P. falciparum* microsatellite loci were analyzed by PCR. Recrudescence was defined as any case in which the same populations of parasites appeared on Day 0 and on another Day (7, 14, 21, or 28). In such cases, the sample was classified as treatment failure. When it had been indicated in the field as treatment failure, re-infection was then defined as any case in which a new population, not detected on Day 0, appeared. After PCR, the result was visualized by electrophoresis on agarose gel (3%) (Metaphore) stained with ethidium bromide. This agarose has a high resolution, which enables amplification fragments with little difference in the basepairs to be distinguished (the microsatellite PCR protocol has been published by Mwangi and others¹⁰).

Ethical considerations. This study was reviewed and approved by the health ethics committee of the EQG Ministry of Health and Social Welfare and the NMCP. Informed verbal and written consent to participate was obtained from the children's mothers and caregivers.

Statistical analysis. The aim of this study was to evaluate the efficacy of the drug combination in ensuring parasite

clearance with no recrudescence during the 28 days of follow-up. Data were transferred from a daily record form to the WHO excel sheet¹⁴ and analyzed using Epi Info 3.4 (CDC, Atlanta, GA). Therapeutic efficacy was calculated by dividing the number of cases of ACPR by the number of patients evaluated on Day 28; and the 95% CIs for efficacy were likewise calculated.

RESULTS

From June to October 2006, 122 children from the outpatient departments of two regional hospitals (45 from Malabo and 77 from Bata) were selected to evaluate the efficacy of AS/AQ treatment. Demographic and laboratory details are in Table 1.

Of the 122 children enrolled in the study, three were excluded on Day 0: one presented with danger symptoms 9 hours after initiating treatment, and the other two had parasitemia of over 200,000 trophozoites per microliter. The first was hospitalized and administered quinine by perfusion, and the other two continued with the same combination, with good results.

There were 10 children lost to follow-up, i.e., 2 on Day 2, 1 on Day 3, 2 on Day 4, 1 on Day 8, 2 on Day 14, and 2 on Day 21. All of these had negative parasitemia. The follow-up rate was 10.7% (13 of 122), which is below the 20% mark recommended by the WHO in cases where follow-up is conducted over 28 days (Figure 1).

The rate of *P. falciparum* reappearance with the combination therapy was as follows: 3 of 109 cases registered as LCF, 2.8% (95% CI: [0.60–7.80%]); and 17 of 109 cases registered as LPF, 15.6% (95% CI: [9.40–23.80%]). The ARCP rate was 81.70% (95% CI: [72.5–87.9%]). No deaths were reported.

At enrollment, 75% of the children had mild-moderate anemia, with hematocrit counts of between 24 and 33. Children with hematocrit counts below this level were excluded. By Day 28, children with anemia had declined to 50% among those who completed the follow-up.

Although no severe reactions were recorded among the group of children as a whole, adverse effects were present in a low percentage. A total of five children (4.5%; 5 of 109)

TABLE 1
Baseline characteristics at enrollment*

	Artesunate plus amodiaquine
Patients	122
% male	63.4 (78)
Age (months)	
Mean (SD)	29.3 [13.9]
Range	6–58
Weight (kg)	
Mean (SD)	12.4 [3.09]
Range	6–21
Temperature	
Mean (SD)	37.6 [1.07]
Range	36.3–38.1
Parasite/microliter	
Median	25.96
IR†	12,480–38,359
Gametocyte %	5.7 (7)

*Together children from Bata and Malabo Hospitals.

†IR = interquartile rank.

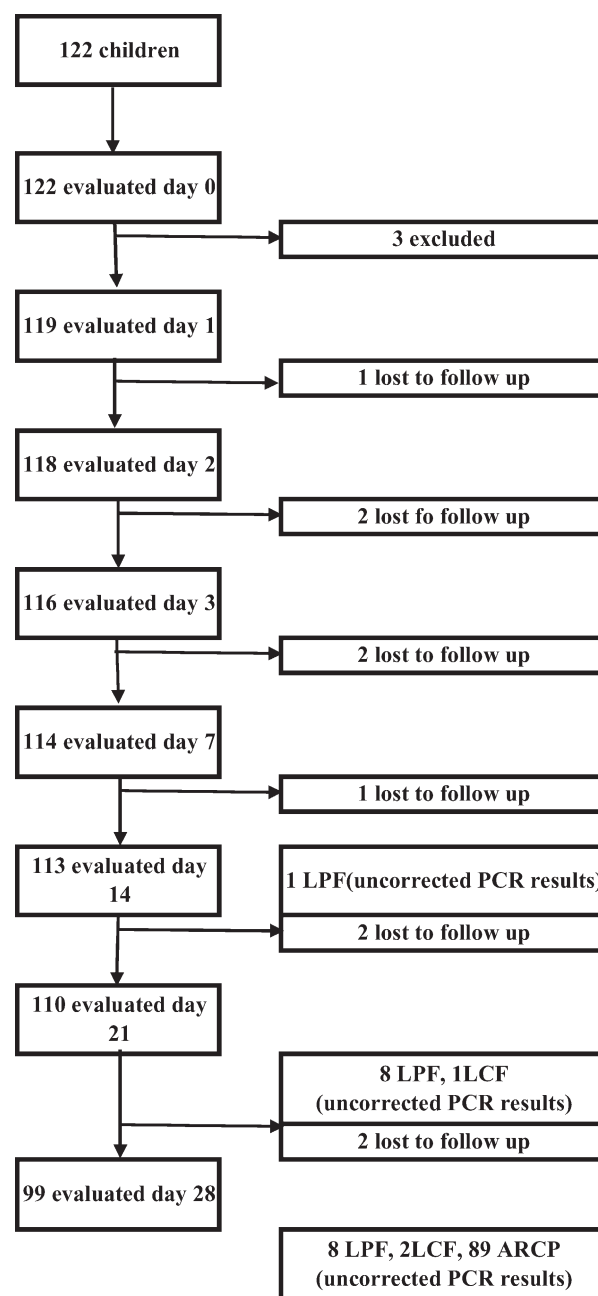


FIGURE 1. Trial profile.

presented with vomiting but they were all retreated and the follow-up did not have to be halted.

The combination showed itself to be extremely expeditious in terms of parasite clearance: by Day 1, 45.6% (50 of 109) were parasitemic, by Day 2, 0.8% was parasitemic (1 of 109), and by Day 3 the result was negative. This trend can be clearly seen in Figure 2.

Gametocyte carriage was low with this combination, proving undetectable at baseline and persisting from Day 1 (5.8%) to Day 14 (2.5%). No gametocytes were detected on Days 21 and 28.

Molecular correction was performed to distinguish recrudescence or re-infection from 20 cases of resistance detected in the field. Following molecular assay by means

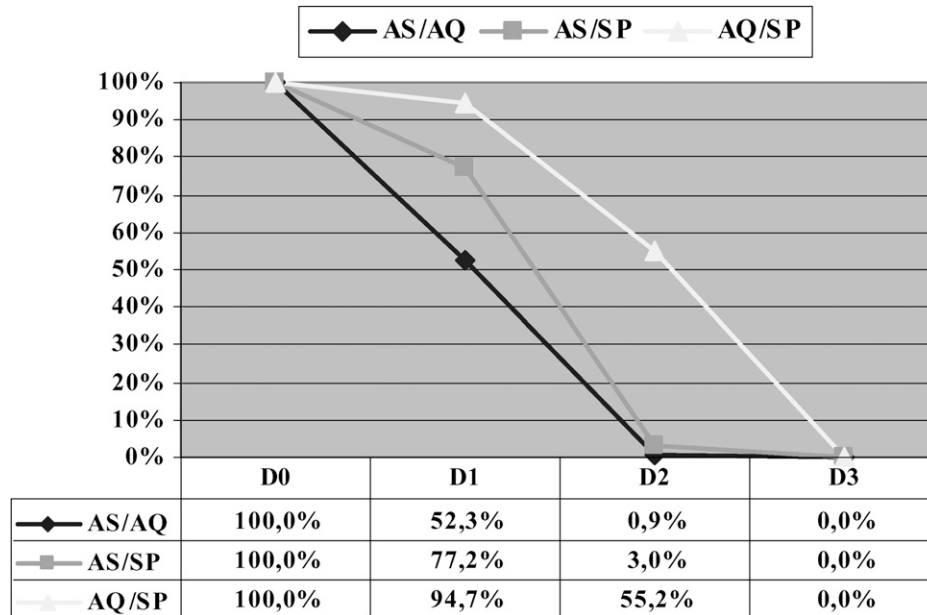


FIGURE 2. Parasite clearance by day of follow-up, comparing this study with another one carried out the previous year, using the same methodology. Clearance rates were faster in the AS/AQ group (efficacy of Artesunate + Sulfadoxine-Pyrimethamine [AS + SP] and Amodiaquine + Sulfadoxine-Pyrimethamine [AQ + SP]) for uncomplicated falciparum malaria in Equatorial Guinea (Central Africa), Charle and others.⁷

of microsatellite analysis, all resistant cases detected in Malabo (6) were classified as cases of re-infection. In Bata, 14 cases of resistance were detected, and with the help of molecular analysis, 11 were classified as re-infection and 3 as real cases of resistance.

DISCUSSION

The use of an efficacious treatment with artemisinin derivatives is one of the WHO's most preferable recommendations for decreasing the impact of malaria in sub-Saharan Africa. A number of meetings were held in EQG between the NMCP management and its partners, namely, the WHO, the Carlos III Institute of Health, Medical Care Development International (MCDI), physicians from the public and private sector, and other actors such as pharmacists and the like. The aim was to change the current malaria CQ treatment protocol and decide on the most appropriate combination for treating uncomplicated falciparum malaria in the country. The three possible combinations to be used in Africa are artesunate + sulfadoxine-pyrimethamine (AS/SP), AS/AQ, and artesunate + lumefantrine (AS/LMT; Coartem). Of these three, the AS/AQ combination was chosen, based on the following considerations: 1) it goes some way to prevent development of resistance to SP, since the latter is the only safe drug for use as intermittent preventive treatment of pregnant women and children. Furthermore, SP is best not used in combination for treatment but rather left as a prevention strategy; 2) in the case of AQ/AS, adherence is said to be better than in that of other combinations with SP, because the former has already been marketed as a fixed-dose combination in daily doses (Coarsucam), with different dosages for children and adults. This formula reduces the number of tablets to be taken¹⁵; 3) the price of AS/AQ is lower than that of Coartem, the other fixed-dose combination¹⁶ that also guarantees adherence; and 4) EQG's neighbors have adopted this AS/AQ combination

in their malaria policies, e.g., Gabon in 2003, Cameroon in 2004, Congo Brazzaville in 2006, and Chad in 2005 (together with Coartem).¹⁷ It is recommended that the same treatment be adopted at a regional level, as a means of dealing with cross-border population movement and preventing possible development of multi-drug resistance.

The aim of this study was to provide the NMCP with enough information to enable it to know the baseline efficacy of the combination to be used across the country and so monitor any possible development of resistance following the introduction of the new policy. Children were selected from both the Bata and Malabo hospitals. The number of children from Malabo was lower compared with Bata caused by the decrease of prevalence; the strategy of indoor residual spraying implemented by the Ministry of Health in collaboration with Medical Care Development International (MCDI). Malabo also has a lower *Plasmodium* index, thereby making it more difficult for children who met the inclusion criteria to be found at this hospital. Nevertheless, no statistical differences in resistance were detected between the two areas.

Our study showed that the AS/AQ combination is efficacious for use as a first-line treatment in EQG. The ACPR rate after PCR correction was 97.3%. This result is similar to those obtained in many African countries. A study conducted in the Sudan, which compared the efficacy of AS/AQ to that of AS/SP, reported an ACPR of 95.2% with AS/AQ.¹⁸ Similarly, the PCR-corrected efficacy of AS/AQ was 100% in another efficacy study with a 28-day follow-up, which targeted children in Nigeria.¹⁹ Finally, a study undertaken in Tanzania to ascertain the baseline resistance of two different combinations, namely, Coartem and AS/AQ, reported the efficacy of the latter as being 93.8%.²⁰

The use of microsatellites helped detect the number of genuine cases of resistance. Of a total of 20 cases detected in the field, only three were classed as genuinely resistant; the remaining 17 were cases of reinfection, a very common phenomenon in a

country such as EQG, with a high level of transmission. The use of molecular analysis of, for example, *msh-1* and *msh-2* genes or, in this particular case, of microsatellite loci of *P. falciparum*, is invaluable for the purposes of such differentiation, and we highly recommend that it be used in *in vivo* studies conducted in countries having a high transmission rate.

Parasite clearance was extremely swift, with around 50% of the children testing negative after receiving the first dose. By Day 1, half the children, and by Day 2, < 1% was parasitemic. This rapid and substantial reduction in parasite biomass is caused by the artemisinin derivatives. Unlike other antimalarial treatments, this drug kills several blood stages of the parasite. Comparison of these results to those of the study carried out 1 year earlier in EQG using another two combinations—AS/SP and AQ/SP—showed that in these cases parasite clearance had been slower, i.e., by Day 1, two-thirds and over 90%, and by Day 2, 3% and half the children were positive, using AS/SP and AQ/SP, respectively (Figure 2). Other studies conducted in different African countries yielded results similar to ours. In a study undertaken in Uganda in 2007, the difference in parasitemia clearance using combinations with and without artemisinin derivatives was described: by Day 2, just 3% in the first group as opposed to 48% in the second group were positive.²¹ In that same year, another study in Senegal, using five different combination therapies, showed a parasitemia of 5% by Day 2 with AS/AQ. Clearance was even faster using Coartem.²²

The combination was well tolerated, no severe reactions were recorded, and in no case did the treatment have to be halted. The principal adverse effects were gastrointestinal disorders, with 4.5% of children presenting with vomiting on Day 1 of follow-up. These data are similar to another study conducted in Senegal using the same combination, where 4.7% of children presented with vomiting.²³ Some caregivers reported that children also suffered from asthenia. Insofar as this symptom is concerned, it is difficult to determine whether it is caused by the malaria treatment or is related to the disease itself. No severe reactions linked to amodiaquine use as a prophylactic, such as leukopenia or agranulocytosis, was recorded.

The results of our study have already had important implications on policies in relation to the fight against malaria:

1. A recent WHO recommendation envisages the use of combination therapy for treating uncomplicated falciparum malaria, with an efficacy of over 95%. This good result in terms of AQ/AS efficacy and safety furnishes the NMCP with a baseline against which to monitor any possible development of resistance.
2. EQG plans to start using this new combination during the coming months, as its main method of treatment of combating malaria. Thereafter, efficacy will have to be re-evaluated every 2 years to be able to change policy, before drug resistance has a chance to rise to unacceptable levels.

Received May 8, 2012. Accepted for publication November 30, 2012.
Published online March 25, 2013.

Acknowledgments: We are grateful to all the mothers and caregivers of the children of Equatorial Guinea. We thank that country's National Malaria Control Programme and its Programme Director at the Ministry of Health, Gloria Nseng, for her unstinting assistance and support throughout the study. Special thanks must go to labora-

tory technicians, Jacqueline Obono, Magdalena Lwanga, Natividad Nlang, Araceli Nchama, Catalina Mangue, Anastasio Micha, and Natividad Nsee for the responsible, patient, and cheerful way in which they have been doing this type of work for so many years. Finally, we are indebted to Jonathan Mwangi and Lisa Ranford-Cartwright at the Division of Infection and Immunity (University of Glasgow, Scotland) for their kind help.

Financial support: This study received financial support from Spain's International Development Aid Agency (*Agencia Española de Cooperación Internacional para el Desarrollo - AECID*) and the National Centre of Tropical Medicine at the Carlos III Institute of Health. Cooperative Research in Tropical Diseases (CRTD), Project: RD06/0021/0000.

Disclaimer: The authors declare that they have no competing interests. All authors have read and approved the manuscript.

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