SURVEILLANCE OF IN VIVO RESISTANCE OF \textit{PLASMODIUM FALCIPARUM} TO ANTIMALARIAL DRUGS FROM 1992 TO 1999 IN MALABO (EQUATORIAL GUINEA)

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Abstract. From 1992–1999, we have assessed the therapeutic efficacy of three malaria treatment regimens (chloroquine 25 mg/kg over three days, pyrimethamine/sulfadoxine 1.25/25 mg/kg in one dose, and quinine 25–30 mg/kg daily in three oral doses over a four-, five-, or seven-day period) in 1,189 children under age 10 at Malabo Regional Hospital in Equatorial Guinea. Of those children, 958 were followed up clinically and parasitologically for 14 days. With chloroquine, the failure rate varied from 55% in 1996 to 40% in 1999; the early treatment failure rate increased progressively over the years, from 6% in 1992 to 30% in 1999. With pyrimethamine/sulfadoxine, the failure rate varied from 0% in 1996 to 16% in 1995. The short quinine treatment regimens used in 1992 and 1993 (4 and 5 days, respectively) resulted in significantly higher failure rates (19% and 22%, respectively) than the 7d regimen (3–5.5%). We conclude that: a) failure rates for chloroquine are in the change period (>25%), and urgent action is needed; b) pyrimethamine/sulfadoxine failure rates are in the alert period (6–15%); and surveillance must be continued; and c) quinine failure rates are in the grace period (<6%), so quinine can be recommended.

INTRODUCTION

Malaria caused by \textit{Plasmodium falciparum} remains a major cause of human mortality in the world, especially in the tropics and outstandingly in sub-Saharan Africa, affecting mainly children and pregnant women. A fundamental component of the global strategy for malaria control is the prompt and effective treatment of all cases. Increasing rates of resistance to chloroquine and other antimalarial drugs, such as pyrimethamine/sulfadoxine, have been reported in many sub-Saharan countries and are spreading rapidly.\textsuperscript{1–3} Despite the emergence and spread of resistance, chloroquine and pyrimethamine/sulfadoxine remain the first- and second-line treatments for malaria in most African countries.\textsuperscript{3–6}

Malaria control efforts need to be designed for the specific environment in which they will be used, taking into account the local epidemiology. Also, the drug-resistant strains of malaria are constantly changing in time and space. This makes regular surveillance of utmost importance: following a particular malaria treatment policy for a particular area and determining any changes in the levels of drug resistance so as to introduce alternative drugs when necessary.

The present study was undertaken on Bioko Island in Equatorial Guinea to assess the efficacy of three treatment regimens: chloroquine and the pyrimethamine/sulfadoxine combination (the first- and second-line drugs, respectively, for uncomplicated falciparum malaria in the country), and quinine (the election for treatment in cases of chloroquine and pyrimethamine/sulfadoxine resistance and for severe malaria). The results obtained were compared with those of passive surveys conducted on the island from 1992–1996 to verify the evolution of malaria drug resistance.

MATERIALS AND METHODS

\textbf{Area of study and population.} Equatorial Guinea is a country located on the Gulf of Guinea on the west coast of central Africa. Its overall area of 28,068 km\textsuperscript{2} is divided into an insular and a continental region. The island of Bioko represents the main part of the insular region. Malabo is the principal town on the island and the nation’s capital. Bioko has only two seasons: rainy (May–October) and dry (November–April). Malaria is a hyperendemic disease, and transmission occurs throughout the year, with a slight increase in the rainy season. More than 80% of the malaria infections in the area are caused by \textit{P. falciparum}.\textsuperscript{7}

The study was conducted from May–December of 1999 on children at Malabo Regional Hospital. Patients were selected in accordance with the criteria of the WHO protocol.\textsuperscript{8} Briefly, infection with \textit{P. falciparum} alone, parasitemia >2,000 asexual parasites/μL, age 6–59 months, and no history of previous treatment. The study was approved by the Ethical Committees of the National Malaria Control Program of Equatorial Guinea and the Instituto de Salud Carlos III in Madrid, Spain; informed consent was obtained from the parents of all subjects.

Patients enrolled in the study were initially examined for \textit{P. falciparum} infection. Name, age, weight, hemoglobin and glycemia data, temperature, and clinical findings were recorded. Scheduled visits were made on days 1, 2, 3, 7, and 14. All visits including the taking of axillary temperature and a brief physical examination. Parents were encouraged to return at any time for additional examination if the child was ill.

Capillary blood was collected for thick and thin blood films on days 0, 1, 2, 3, 7, and 14 or any other day the child was ill. They were stained with Giemsa, and the parasite density was determined by counting asexual malaria parasites against 200 leukocytes, assuming a total white cell count of 8,000 per μL of blood.

Several \textit{in vivo} tests had been conducted at Malabo Regional Hospital from 1992–1996 on children who met the inclusion criteria in brief, younger than age 10, a blood film positive for asexual forms of \textit{P. falciparum} alone, and parasite count >1,000/μL of blood. Patients were monitored voluntarily in the hospital for 7 days after treatment and then, on an outpatient basis, on days 10 and 14. Each case also was followed up clinically and parasitologically as mentioned before.

\textbf{Drug prescriptions.} During the trial in 1999, children were assigned on day 0 to receive either chloroquine phosphate 100
or 150 mg (Pharmamed Ltd., Malta) 25 mg/kg over 3 days; pyrimethamine 25 mg/sulfadoxine 500 mg (Pharmamed Ltd.) 1.25/25 mg/kg in one dose; or quinine sulfate 200 or 300 mg (Pharmamed Ltd.) 25 mg/kg daily in three oral doses over a seven-day period. Chloroquine and pyrimethamine/sulfadoxine were given under direct supervision. Patients with complicated malaria cases were hospitalized and treated with quinine dihydrochloride 300 mg/mL (Pharmamed Ltd.) by intravenous injection. All patients who did not respond to treatment were cured with second- or third-line treatment: pyrimethamine/sulfadoxine, quinine, or Artesunate® (Guilin Pharmaceutical Works, Guangxi, China) plus mefloquine (Eloquine®, Medochemie Ltd., Cyprus).

From 1992–1996, the following treatment regimes were used: chloroquine phosphate (Pharmamed Ltd.) 100 or 150 mg, 35 mg/kg over three days; quinine sulfate (Pharmamed Ltd.) 200 or 300 mg; and pyrimethamine 25 mg/sulfadoxine 500 mg (Pharmamed Ltd.) 1.25/25 mg/kg in one dose. (The quinine sulfate dosage in 1992 of 30 mg/kg daily for four days was extended to five days in 1993 and has been seven days since 1994.)

Clinical and parasitologic responses to therapy were classified according to the WHO criteria:

- Early treatment failure (ETF) was defined as: 1) danger signs within 72 h in the presence of parasitemia, 2) axillary temperature ≥37.5°C after 48 h with parasitemia greater than that on day 0 or after 72 h with parasitemia, and 3) parasitemia after 72 h ≥25% of that on day 0.
- Late treatment failure (LTF) was defined as danger signs or temperature ≥37.5°C with parasitemia during days 4–14 without meeting the criteria for ETF.
- Adequate clinical response (ACR) was defined as no parasitemia or axillary temperature <37.5°C with parasitemia without meeting the criteria for ETF.
- Adequate clinical response (ACR) was defined as no parasitemia or axillary temperature <37.5°C with parasitemia at follow-up on day 14, without meeting the criteria for ETF or LTF.

Before 1996, the response to treatment was classified according to the previous WHO guidelines in four categories: S, RI, RII, and RIII. This classification did not consider the clinical aspects. To compare the results from the studies of both periods, data from 1992–1996 have been reclassified as follows: RI, RII = LTF; RIII = ETF; S = ACR.

**Data analysis.** Data were analyzed using Epi-Info v.6 software. Differences in proportions were tested with the \( \chi^2 \) test, and a value of \( P < 0.05 \) was considered significant.

## RESULTS

A total of 1,189 children were enrolled in the studies, and 958 completed follow-up for 14 d (477 in the chloroquine, 204 in the pyrimethamine/sulfadoxine, and 277 in the quinine groups). Studies of some patients could not be completed because of abandonment before day 14, taking other antimalarials during the study, and the death of one patient in the chloroquine group on day 5 in 1999, from unknown causes but with a lower parasitemia than observed on day 0.

The mean age of the children in each treatment group varied from year to year, with significant differences in some cases (in the pyrimethamine/sulfadoxine group, for example, 94.8 months in 1994 and 24.1 in 1996). One could expect lower treatment failure rates in groups with a higher mean age because of a more efficacious response due to greater immunity to *P. falciparum*. But in analysis of the different study years, age did not seem to have great influence on the failure rate. In the pyrimethamine/sulfadoxine group in 1996, for example, the mean age was the lowest (24.1 months) but there were no failures. In 1999, the quinine group had a significantly lower mean age (23.8 months) than in previous years but the failure rate was similar.

The therapeutic response to chloroquine during the 14d follow-up is shown in table 1. The failure rate with chloroquine varied from 55% in 1996 to 40% in 1999, but the differences are not statistically significant (\( P = 0.5 \)). Nevertheless, ETF rates increased progressively over the years, from 6% in 1992 to 30% in 1999, a significant difference (\( P = 0.009 \)).

The results of the pyrimethamine/sulfadoxine trials are presented in table 2. The failure rate varies from 0% in 1996 to 16% in 1995, but the difference is not statistically significant (\( P = 0.1 \)). ETF and LTF do not vary significantly over the years, with a mean value for both of 5% in 1999.

Table 3 summarizes the results of the *in vivo* tests for sensitivity to quinine. The short treatment regimen (4–5 days) in 1992 and 1993 resulted in significantly (\( P = 0.0003 \)) higher failure rates (19% and 22%, respectively) than the 7d regimen (3–5.5%). All failures were LTFs except for one case in 1993.

## DISCUSSION

The present survey shows the problem of drug-resistant *falciparum* malaria in Equatorial Guinea. However, the

### Table 1

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<tr>
<td>No. enrolled</td>
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<td>125</td>
<td>160</td>
<td>73</td>
<td>36</td>
<td>19</td>
</tr>
<tr>
<td>Mean age (months)</td>
<td>46.8</td>
<td>52.8</td>
<td>51.6</td>
<td>27.1</td>
<td>22</td>
<td>24.9</td>
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<tr>
<td>Enrollment GMPD*</td>
<td>24,003</td>
<td>11,512</td>
<td>14,048</td>
<td>10,520</td>
<td>16,120</td>
<td>20,428</td>
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<tr>
<td>ACR</td>
<td>38%</td>
<td>59%</td>
<td>51%</td>
<td>47%</td>
<td>50%</td>
<td>60%</td>
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<tr>
<td>LTF</td>
<td>22</td>
<td>49</td>
<td>53</td>
<td>19</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>ETF</td>
<td>35%</td>
<td>39%</td>
<td>33%</td>
<td>26%</td>
<td>27.5%</td>
<td>10%</td>
</tr>
<tr>
<td>Failure rate</td>
<td>4%</td>
<td>12%</td>
<td>17%</td>
<td>20%</td>
<td>10%</td>
<td>6%</td>
</tr>
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</table>

* GMPD, geometric mean parasite density, expressed as the number of asexual parasites/μL.

ACR: adequate clinical response.

ETF: early treatment failure.

LTF: late treatment failure.

ETF: early treatment failure.

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\( P \)
prevalence, intensity, and evolution of drug resistance is not uniform for the different antimalarial drugs tested.

Data reveal an unacceptably high degree of therapeutic failure after chloroquine treatment of uncomplicated childhood malaria. Although there was not a significant change in the global failure rate until 1999, the high level of resistance we found since 1992 (40–55%) represents a serious problem. A clinical failure rate of 25% within 14 days after therapy has been proposed as the upper limit above which treatment should be changed.10 The failure rate in Malabo has been above 25% since 1992.

Another important point regarding chloroquine resistance is the large increase in the ETF rate and its clinical consequences. A very interesting study11 concluded that when chloroquine treatment is associated with a 20% ETF rate, the hemoglobin levels are markedly reduced after 72h and recovery is unsatisfactory, even in children with ACR. These are two reasons anemia increases in children despite chloroquine treatment. The ETF rate for chloroquine in Malabo has risen from 6% in 1992 to 30% in 1999, with the treatment not only proving ineffective but also making matters worse in a relatively uncomplicated case of malaria. A change in the recommended first-line drug should be seriously considered.

As there are few therapeutic options for treating malaria, the second-line pyrimethamine/ sulfadoxine can be an alternative. This drug is still very effective; its failure rate was 10% in 1999 and has not increased significantly since 1992. But a generalized use of the drug could quickly increase the levels of resistance, as has happened in Malawi, Tanzania, and Kenya.12–14 Although resistance may be inevitable, it probably can be slowed down. WHO recommends an antimalarial combination therapy based on the synergistic or additive potential of two or more drugs to improve therapeutic efficacy and also delay the development of resistance to the combination’s individual components.15 Some combinations with pyrimethamine/sulfadoxine and, recently, chlorproguanil—inhibitor of dihydrofolate reductase—and sulfone dapsone (LAPDAP, GlaxoSmithKline, Tanzania, Kenya, Malawi, Botswana and South Africa) are recommended as alternatives for first-line treatment in sub-Saharan countries.16 A decision to change the first-line antimalarial has to consider different aspects of both the country (epidemiologic and economic) and the drugs (efficiency, affordability, acceptability).

Quinine remains an effective drug against *P. falciparum* malaria in Equatorial Guinea. The global failure rates have been stable since 1994 (but were probably higher in 1992 and 1993 because of a shorter drug regimen), and data are not alarming. Furthermore, it is very probable that treatment failures resulted in part from patients’ lack of adherence to the regimen because of adverse reactions and the prolonged dosage (three times a day for seven days), as nearly all the failures were LTFs. Therefore, the failure rate could be even lower. Quinine, because of its unfavorable dosage and annoying side effects, cannot be considered an alternative for

### Table 2

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<tbody>
<tr>
<td>Dosage: mg/kg × days</td>
<td>30 × 4</td>
<td>30 × 5</td>
<td>30 × 7</td>
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<tr>
<td>Mean age (months)</td>
<td>51.6</td>
<td>97.2</td>
<td>94.8</td>
<td>39.2</td>
<td>24.1</td>
<td>30.9</td>
</tr>
<tr>
<td>Enrollment GMPD*</td>
<td>19,439</td>
<td>5,390</td>
<td>22,496</td>
<td>10,421</td>
<td>5,631</td>
<td>19,204</td>
</tr>
<tr>
<td>ACR</td>
<td>57</td>
<td>36</td>
<td>24</td>
<td>21</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>LTF</td>
<td>97%</td>
<td>95%</td>
<td>89%</td>
<td>84%</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>ETF</td>
<td>3%</td>
<td>5%</td>
<td>11%</td>
<td>12%</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Failure rate</td>
<td>0%</td>
<td>1%</td>
<td>4%</td>
<td>4%</td>
<td>0%</td>
<td>10%</td>
</tr>
</tbody>
</table>

* GMPD, geometric mean parasite density, expressed as the number of asexual parasites/μL.
ACR: adequate clinical response.
LTF: late treatment failure.
ETF: early treatment failure.

### Table 3

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<td>19,204</td>
</tr>
<tr>
<td>ACR</td>
<td>48</td>
<td>65</td>
<td>21</td>
<td>36</td>
<td>20</td>
<td>52</td>
</tr>
<tr>
<td>LTF</td>
<td>81%</td>
<td>78%</td>
<td>95.5%</td>
<td>97%</td>
<td>95%</td>
<td>94.5%</td>
</tr>
<tr>
<td>ETF</td>
<td>19%</td>
<td>21%</td>
<td>4.5%</td>
<td>3%</td>
<td>5%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Failure rate</td>
<td>19%</td>
<td>22%</td>
<td>4.5%</td>
<td>3%</td>
<td>5%</td>
<td>5.5%</td>
</tr>
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* GMPD, geometric mean parasite density, expressed as the number of asexual parasites/μL.
ACR: adequate clinical response.
LTF: late treatment failure.
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first-line treatment of uncomplicated malaria. It will remain the drug of choice for first-line treatment of severe malaria or for cases of multidrug resistance.

Based on the WHO classification of the clinical failure rates,\textsuperscript{17} and in accordance with the results obtained in our surveys on Bioko Island, we can conclude that: a) failure rates for chloroquine are in the change period (>25%), and urgent action is needed; b) pyrimethamine/sulfadoxine failure rates are in the alert period (6–15%), and surveillance must be continued; and c) quinine failure rates are in the grace period (<6%), and the drug can be recommended without hesitancy about its efficacy.

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REFERENCES