

## MONITORING FOR MEFLOROQUINE-RESISTANT *PLASMODIUM FALCIPARUM* IN AFRICA: IMPLICATIONS FOR TRAVELERS' HEALTH

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**Abstract.** The effectiveness of mefloquine to prevent malaria caused by *Plasmodium falciparum* is influenced by the sensitivity of the malaria parasites to this drug. Concern has been raised that resistance to mefloquine may develop in sub-Saharan Africa as has been observed in Southeast Asia. Case reports, along with blood smears to confirm the diagnosis and blood samples to determine the mefloquine concentration, were provided on any Peace Corps volunteer serving in sub-Saharan Africa who was diagnosed with malaria. We defined prophylaxis failures probably due to mefloquine resistance as patients with *P. falciparum* malaria confirmed at the Centers for Disease Control and Prevention, reported compliance with prophylaxis, no ingestion of mefloquine between date of illness onset and date of blood drawing, and a mefloquine level  $\geq 620$  ng/ml in blood drawn within five days of onset of illness. Between January 1, 1991 and September 6, 1996, 44 (31%) of 140 volunteers with confirmed *P. falciparum* had blood drawn within five days of onset of illness. Twenty-nine (66%) had not fully complied with prophylaxis. Five of 15 prophylaxis failures in four countries had mefloquine levels  $\geq 620$  ng/ml. Failure of mefloquine prophylaxis is primarily due to noncompliance. Evidence of probable resistance to mefloquine among strains of *P. falciparum* was found in five Peace Corps volunteers in sub-Saharan Africa. Clusters of well-documented prophylaxis failures need to be followed-up by therapeutic *in vivo* studies to document parasite resistance to mefloquine. Reduced sensitivity to mefloquine does not (yet) appear to be a significant problem in sub-Saharan Africa.

Malaria continues to cause great morbidity and mortality throughout the world. One of the reasons for this persistent threat is drug resistance, which has rendered chemotherapy and chemoprophylaxis difficult in sub-Saharan Africa and Southeast Asia.<sup>1</sup> Monitoring drug resistance is particularly important among travelers, expatriates, personnel on humanitarian missions, and others who plan temporary residence in highly malarious environments.

The emergence of chloroquine-resistant *Plasmodium falciparum* in the 1980s in West Africa, with its intense malaria transmission, demonstrated the importance of detecting resistance early. Among Peace Corps volunteers taking chloroquine with or without concurrent proguanil for chemoprophylaxis in West Africa, the incidence of *P. falciparum* increased from 9.3 cases per 100 in 1986 to 42 cases per 100 in 1989. Because of this rapid increase, chloroquine-based regimens were recognized as ineffective. In response, mefloquine was made available to Peace Corps volunteers in September 1989,<sup>2,3</sup> and the incidence of *P. falciparum* decreased to pre-epidemic levels within two years (Figure 1).

Several large studies have demonstrated that mefloquine is the most effective chemoprophylactic agent for visitors to sub-Saharan Africa.<sup>4–6</sup> Nevertheless, because of the concern that resistance to mefloquine could develop in Africa, much as it has done in Southeast Asia,<sup>7,8</sup> adequate surveillance for the emergence of mefloquine resistance is essential.

Drug resistance of malaria is defined as the ability of a malaria parasite to multiply or survive in the presence of drug concentrations that normally destroy or prevent the multiplication of parasites.<sup>9,10</sup> These drug concentrations are based on dosing for treatment, not dosing for prophylaxis. Resistance has traditionally been monitored using *in vitro* or therapeutic *in vivo* studies. These studies have been used to track chloroquine resistance, but they cannot detect the early emergence of drug resistance.<sup>11</sup> They rely on samples from relatively small populations in limited geographic areas, and

tend initially to detect only high-level resistance.<sup>12,13</sup> In addition, the value of *in vitro* assays to detect mefloquine resistance is unknown because the correlation between *in vitro* and *in vivo* resistance is poor.<sup>14</sup>

Monitoring prophylaxis failures, i.e., the incidence of laboratory-confirmed malaria among individuals who are using chemoprophylaxis, is an effective surveillance tool for detecting the early emergence of drug resistance. This method can detect low-level resistance, and can be applied to large populations or large geographic areas. It is also a more rational approach to detecting parasite resistance to mefloquine, a drug used primarily for prophylaxis, not treatment. While prophylaxis failures in persons using chemoprophylaxis consistently and correctly cannot prove resistance, since the definition of resistance is based on treatment doses of mefloquine, it can document probable resistance, which then needs to be confirmed by further studies.

To date, strict criteria have not been used to study mefloquine prophylaxis failures prospectively in a large cohort of highly exposed, nonimmune individuals. Since the weekly mefloquine prophylaxis dosage schedule was established in 1990, the Peace Corps Office of Medical Services (OMS) and the Centers for Disease Control and Prevention (CDC) have monitored *P. falciparum* infections among Peace Corps volunteers taking mefloquine in sub-Saharan Africa. We sought to document whether individuals who were reported to have failed prophylaxis indeed had malaria. If an individual had malaria, we sought to determine whether *P. falciparum* developed because the mefloquine levels were inadequate or because the parasite had probably developed resistance to the drug.

### METHODS

**Case reporting.** Most Peace Corps volunteers serving in areas with chloroquine-resistant *P. falciparum* are advised to take mefloquine, 250 mg, once per week. As part of Peace

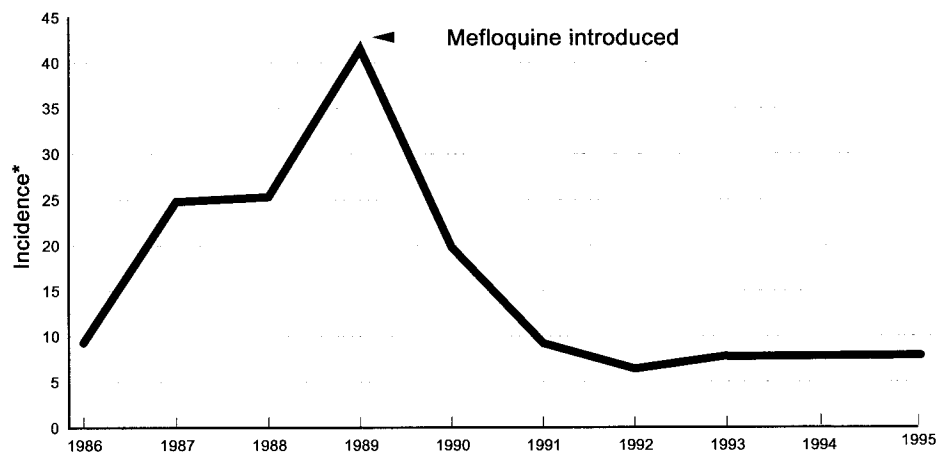


FIGURE 1. Incidence of *Plasmodium falciparum* in U.S. Peace Corps volunteers, in West Africa, 1986–1995. \*Number per 100 volunteers per year.

Corps' epidemiologic surveillance system, Peace Corps medical officers (PCMOs) provide case reports to the CDC on any volunteer diagnosed with malaria (regardless of species) in sub-Saharan Africa while taking mefloquine prophylaxis. These reports document when the symptoms began, when the diagnosis was made, and who made the diagnosis. For each patient, PCMOs provide thick and/or thin blood smears as well as whole blood or serum samples to determine the concentration of mefloquine. This study analyzed data collected between January 1, 1991 and September 6, 1996.

**Data management and case definitions.** All laboratory evaluations were performed at the CDC. Blood smears were classified as unsatisfactory for evaluation, no parasites found, or malaria with species identified. Levels of mefloquine were determined using high-performance liquid chromatography on whole blood or serum samples.<sup>15</sup> Serum levels were converted to whole blood levels by dividing the drug concentration by 1.28.<sup>16</sup>

Patients were classified as prophylaxis failures probably due to drug resistance if they met the following criteria: *P. falciparum* malaria confirmed at CDC, a history of compliance with prophylaxis, blood drawn within five days of onset of illness (blood sample for breakthrough mefloquine level), no ingestion of mefloquine between date of illness onset and date of blood drawing, and breakthrough mefloquine levels  $\geq 620$  ng/ml. Because the drug has a half-life of 21 days, we assumed that a mefloquine concentration in blood taken within five days of onset of symptoms would provide a good approximation of the level present when the volunteer first developed parasitemia. Patients were considered compliant if they reported never missing a dose of mefloquine during prophylaxis. A mefloquine level  $\geq 620$  ng/ml was estimated to provide 95% effective protection against *P. falciparum* in Africa,<sup>4</sup> and the development of malaria in this setting is best explained by reduced sensitivity of the parasite to mefloquine.

**Statistical analysis.** Statistical analysis was performed using Epi-Info version 6.4 (Centers for Disease Control and Prevention, Atlanta, GA). Univariate analysis was performed using the chi-square test and Fisher's exact test. Confidence intervals (CIs) around risk ratios (RRs) that excluded one

were considered to be statistically significant. Yates'-corrected P values  $\leq 0.05$  were considered statistically significant.

#### RESULTS

Between January 1, 1991 and September 6, 1996, we received reports on 261 volunteers in 38 countries who had been diagnosed with malaria in sub-Saharan Africa while taking mefloquine for chemoprophylaxis.

To document which individuals failed prophylaxis, we first needed to confirm the diagnosis of malaria. Of 242 patients who had blood smears sent for CDC confirmation, 16 (7%) were unsatisfactory for evaluation because of the slides' poor quality. Of the remaining 226, 141 (62.4%) were confirmed to have malaria (140 *P. falciparum* and one *P. vivax*), and 85 (37.6%) were not confirmed. The positive predictive value for a diagnosis of malaria in sub-Saharan Africa (CDC-confirmed slides divided by total number of satisfactory slides sent to CDC) was found to be 62.4% (95% CI = 55.7, 68.7).

We found that the likelihood of a correct laboratory diagnosis in Africa depended on which type of laboratory examined the slide. Laboratories associated with Peace Corps or U.S. Embassy Health Units were three times more likely (RR = 2.9, 95% CI = 1.8, 4.7,  $P < 0.001$ ) to make the correct diagnosis compared with other laboratories (Table 1).

Breakthrough blood samples were received at CDC for 44 of the 140 volunteers confirmed to have *P. falciparum*. These volunteers were not demographically different (age, sex, country) than the 96 who did not supply breakthrough blood samples. Twenty-nine (66%) of the 44 volunteers reported not to have fully complied with prophylaxis. Among the 15 patients who had complied with mefloquine prophylaxis, the mean mefloquine concentration was 554.9 ng/ml (SD = 351.3) with a range of 50 ng/ml to 1,275 ng/ml. Ten of these 15 prophylaxis failures had mefloquine levels  $< 620$  ng/ml, possibly due to decreased absorption or increased elimination.

Thus, the remaining five patients met our case definition of a prophylaxis failure probably due to drug resistance (Table 2). Two of these became ill in 1995 and 1996, respec-

TABLE 1  
Proportion of confirmed diagnoses among Peace Corps volunteers diagnosed with malaria in Africa by blood smear examination\*

		Diagnosis confirmed by CDC		% Confirmed (95% CI)
		Yes	No	
Malaria in Africa	PC/EHU	99	34	74.4 (66.2, 81.6)
diagnosed by	Local laboratory	13	38	25.5 (14.3, 39.6)

\* CDC = Centers for Disease Control and Prevention; CI = confidence interval; PC = Peace Corps; EHU = Embassy Health Unit.

tively, while stationed in Cameroon. The other three were from Niger (1991), Sierra Leone (1993), and the Central African Republic (1994). Four patients were treated with quinine and tetracycline and one was treated with halofantrine. All recovered uneventfully, continued their mefloquine prophylaxis, and did not experience further episodes of malaria.

#### DISCUSSION

The risk of falciparum malaria in sub-Saharan Africa is a serious health threat for all travelers, but it is especially high for expatriates such as Peace Corps volunteers and missionaries who are highly exposed over long periods of time and who may not have ready access to adequate medical care. Mefloquine and doxycycline are effective drugs for prophylaxis in sub-Saharan Africa but doxycycline has a short half-life and its usefulness is limited because of the need for daily dosing<sup>6</sup> and its contraindication in pregnant women and young children. In addition to chemoprophylaxis, Peace Corps volunteers are encouraged to use protective measures to reduce contact with mosquitoes.

Our surveillance of volunteers diagnosed with malaria in Africa indicates the importance of independent confirmation of malaria diagnosis. We found that laboratories used by Peace Corps volunteers in sub-Saharan Africa tend to overdiagnose malaria by blood smear examination. In no type of laboratory was the positive predictive value greater than 85%. Nevertheless, laboratories associated with Peace Corps and Embassy Health Units demonstrated a three-fold higher rate of correct diagnoses than other laboratories. The accurate diagnosis of malaria remains a problem in many countries, many laboratories tend to overdiagnose. Combined with empiric treatment with antimalarial drugs on clinical suspicion of malaria, this practice probably reduces malaria morbidity at the expense of drug side effects and delays in diagnosis of nonmalarial illnesses. In addition to seeking the best available medical care while abroad, individuals visiting sub-Saharan Africa would be well advised to request the blood slides used for malaria diagnosis to bring back to their

home country. Organizations responsible for travelers' health should evaluate the quality of local laboratories in their region and develop standards for quality control. Physicians and traveler's health clinics should advise their patients about how to seek out facilities with well-trained personnel and of the importance of bringing back a slide for independent confirmation of any diagnosis of malaria.

In this study, most individuals who developed malaria appear to have done so because they did not take mefloquine consistently. Previous observation of Peace Corps volunteers indicates that mefloquine is well-tolerated by this population and suggests that noncompliance is unlikely to be due to side effects.<sup>4</sup> Compliance with malaria prophylaxis is more problematic among long-stay travelers than tourists.<sup>17</sup>

Over a period of nearly six years, our monitoring of *P. falciparum* in Peace Corps volunteers in sub-Saharan Africa detected only five cases that suggested the presence of probable mefloquine resistance. Identification of clusters of prophylaxis failures due to probable mefloquine resistance should lead to therapeutic *in vivo* studies to assess its significance and distribution. It is not known why 10 (66%) of 15 compliant patients had mefloquine levels < 620ng/ml. Pharmacokinetic studies have demonstrated a wide range of variability of mefloquine levels at steady state even with the same dosage and same formulation of drug.<sup>18</sup> Further research is needed to explain why some individuals do not develop protective mefloquine levels even when they comply.

Reports of mefloquine resistance in Africa have come from nine countries based on *in vitro* assays, three countries based on *in vivo* assays, and eight countries based on prophylaxis failure. *In vitro* data is extremely difficult to interpret because of the poor correlation between *in vitro* and *in vivo* resistance. The *in vivo* data includes only one report on a series of patients. Brasseur and others found that 13% (six of 46) of asymptomatic *P. falciparum* carriers in northern Cameroon showed moderate-to-high levels of resistance (RII-RIII) when treated with 25 mg/kg of mefloquine.<sup>19</sup> Of our 44 possible prophylaxis failures, six were stationed in Cameroon. Two of these met the criteria for prophylaxis fail-

TABLE 2  
Characteristics of Peace Corps volunteers with prophylaxis failures\*

Country	Age (years)	Sex	Date onset of illness	Mefloquine concentration (ng/ml)
Niger	31	M	September 16, 1991	830
Sierra Leone	22	M	December 29, 1993	744
Cameroon	30	M	October 4, 1995	1,275
CAR	26	F	August 31, 1994	1,149
Cameroon	25	M	March 28, 1996	962

\* Probably due to drug resistance. CAR = Central African Republic.

ures probably due to drug resistance. Further surveillance of prophylaxis failures among expatriates in Cameroon is necessary to confirm the possible emergence of drug resistance.

Only two *in vivo* studies of mefloquine prophylaxis failure have been conducted in sub-Saharan Africa. One of these studies did not use breakthrough mefloquine levels and did not use accurate data about the level of mefloquine required to suppress parasitemia.<sup>20</sup> The other series is the only report that has used valid and reliable criteria to confirm the diagnosis of malaria, document breakthrough levels, and assess which patients had drug levels sufficient for prophylactic effectiveness.<sup>6</sup> This study, however, evaluated only a select group of persons considered true prophylaxis failures among U.S. military personnel in Somalia.

Our study is limited primarily by sampling bias. Although we received blood samples for 87.1% (122) of the patients, only 31.4% (44 of 140) of the confirmed *P. falciparum* cases met our strict criteria, making it possible that we missed some cases of probable drug-resistant *P. falciparum*. Although we may have excluded some true prophylaxis failures, we did not want to overestimate the degree of drug resistance. Because the number of effective anti-malarial drugs is so small and the risk of resistance so large, it would be unwise to discard an effective medication such as mefloquine without accurate data on the prevalence and degree of resistance.

The strength of this study lies in the development and use of strict criteria to evaluate a heretofore largely anecdotal phenomenon, i.e., prophylaxis failure, among a large cohort of long-term travelers to sub-Saharan Africa. Wider application of the criteria used in this study will help in developing uniform and comparable data on emerging mefloquine resistance, the prevalence of which appears low at this time in sub-Saharan Africa.

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#### REFERENCES

1. Olliaro P, Cattani J, Wirth D, 1996. Malaria, the submerged disease. *JAMA* 275: 230–233.
2. Lobel HO, Bernard KW, Williams SL, Hightower AW, Patchen LC, Campbell CC, 1991. Effectiveness and tolerance of long-term malaria prophylaxis with mefloquine: need for a better dosing regimen. *JAMA* 265: 361–364.
3. Centers for Disease Control, 1990. Revised dosing regimen for malaria prophylaxis with mefloquine. *MMWR Morb Mortal Wkly Rep* 39: 630.
4. Lobel HO, Miani M, Eng T, Bernard KW, Hightower AW, Campbell CC, 1993. Long-term malaria prophylaxis with weekly mefloquine. *Lancet* 341: 848–851.
5. Steffen R, Fuchs E, Schildknecht J, Naef U, Funk M, Schlaggenhauf P, Phillips-Howard P, Nevill C, Sturchler D, 1993. Mefloquine compared with other malaria chemoprophylactic regimens in tourists visiting East Africa. *Lancet* 341: 1299–1303.
6. Wallace MR, Sharp TW, Smoak B, Iriye C, Rozmajzl P, Thornton SA, Batchelor R, Magill AJ, Lobel HO, Longer CF, Burans JP, 1996. Malaria among US troops in Somalia. *Am J Med* 100: 49–55.
7. White N, 1994. Mefloquine in the prophylaxis and treatment of *falciparum* malaria. *Br Med J* 343: 286–287.
8. Mockenhaupt FP, 1995. Mefloquine resistance in *Plasmodium falciparum*. *Parasitol Today* 11: 248–253.
9. Bruce-Chwatt LJ, Black RH, Canfield CJ, Clyde DF, Peters W, Wernsdorfer W, 1981. *Chemotherapy of Malaria*. Geneva: World Health Organization, 205–210.
10. Lobel HO, Campbell CC, 1986. Malaria prophylaxis and distribution of drug resistance. *Clin Trop Med Dis* 1: 225–242.
11. Moran JS, Bernard KW, 1989. The spread of chloroquine-resistant malaria in Africa: implications for travelers. *JAMA* 262: 245–248.
12. Lewis AN, Dondero TJ Jr, Ponnampalam JT, 1973. *Falciparum* malaria resistant to chloroquine suppression but not sensitive to chloroquine treatment in West Malaysia. *Trans R Soc Trop Med Hyg* 67: 310–312.
13. Spencer HC, Masaba SC, Kiaraho D, 1982. Sensitivity of *Plasmodium falciparum* isolates to chloroquine in Kisumu and Malindi, Kenya. *Am J Trop Med Hyg* 31: 902–906.
14. Hatin I, Trape JF, Bauchet J, LeBras J, 1992. Susceptibility of *Plasmodium falciparum* strains to mefloquine in an urban area of Senegal. *Bull World Health Organ* 70: 363–367.
15. Bergquist Y, Hellgren U, Churchill F, 1988. High performance liquid chromatographic assay for the simultaneous monitoring of mefloquine and its acid metabolite in biological samples using protein precipitation and ion-pair extraction. *J Chromatogr* 432: 253–263.
16. Todd GD, Hopperus Buma APCC, Green MD, Jaspers CAJJ, Lobel HO, 1997. Comparison of whole blood and serum levels of mefloquine and its carboxylic acid metabolite. *Am J Trop Med Hyg* 57: 399–402.
17. Lobel HO, Phillips-Howard PA, Brandling-Bennett AD, Steffen R, Campbell CC, Huong AY, Were JBO, Moser R, 1990. Malaria incidence and prevention among European and North American travellers to Kenya. *Bull World Health Organ* 68: 209–215.
18. Pennie RA, Koren G, Crevoisier C, 1993. Steady state pharmacokinetics of mefloquine in long-term travelers. *Trans R Soc Trop Med Hyg* 87: 459–462.
19. Brasseur P, Kouamou J, Moyou-Somo R, Druilhe P, 1992. Multi-drug resistant *falciparum* malaria in Cameroon 1987–1988. II. Mefloquine resistance confirmed *in vivo* and *in vitro* and its correlation with quinine resistance. *Am J Trop Med Hyg* 46: 8–14.
20. Raccurt CP, Dumestre-Toulet V, Abraham E, Le Bras M, Brachet-Liermain A, Ripert C, 1991. Failure of *falciparum* malaria prophylaxis by mefloquine in travelers from West Africa. *Am J Trop Med Hyg* 45: 319–324.