An Outbreak of *Plasmodium falciparum* Malaria in U.S. Marines Deployed to Liberia

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Abstract. In 2003, 44 U.S. Marines were evacuated from Liberia with either confirmed or presumed *Plasmodium falciparum* malaria. An outbreak investigation showed that only 19 (45%) used insect repellent, 5 (12%) used permethrin-treated clothing, and none used bed netting. Adherence with weekly mefloquine (MQ) was reported by 23 (55%). However, only 4 (10%) had serum MQ levels high enough to correlate with protection (> 794 ng/mL), and 9 (22%) had evidence of steady-state kinetics (MQ carboxy metabolite/MQ > 3.79). Tablets collected from Marines met USP identity and dissolution specifications for MQ. Testing failed to identify *P. falciparum* isolates with MQ resistance. This outbreak resulted from under use of personal protective measures and inadequate adherence with chemophrophylaxis. It is essential that all international travelers make malaria prevention measures a priority, especially when embarking to regions of the world with high transmission intensity such as west Africa.

“Good doctors are of no use without good discipline. More than half the battle against disease is not fought by doctors, but by regimental officers. It is they who see that the daily dose of mepracine (anti-malarial chemoprophylactic drug used in WW II) is taken…if mepracine was not taken, I sacked the commander. I only had to sack three; by then the rest had got my meaning.”

—Lieutenant General William Slim (1891–1970), Burma Campaign, 1943

INTRODUCTION

Military personnel frequently deploy to regions of the world endemic for tropical infectious diseases. One of the most important tropical diseases, malaria, has had a significant impact on the welfare of U.S. servicemen since the Revolutionary War. Failure to appreciate the importance of malaria led to disastrous results in many units fighting in the South Pacific during World War II. More recently, failure to properly use personal protective measures (i.e., topical insect repellent, bed netting, and permethrin-treated clothing) and chemoprophylaxis contributed to outbreaks of malaria in U.S. servicemen deployed to Somalia and Afghanistan. Physicians reporting these outbreaks concluded that an emphasis on proper adherence to personal protective measures and chemoprophylaxis is essential to prevent malaria during deployments to malaria-endemic regions. In response to civil unrest, 225 U.S. Marines were sent into Liberia in 2003 to 2007 to augment the security of the U.S. Embassy and the major airport outside Monrovia. After deployment, a febrile illness with symptoms that included fever, headache, and diarrhea developed in approximately 80 Marines. After complicated malaria was diagnosed in several Marines, more than half were evacuated to U.S. military hospitals in Germany and Bethesda, Maryland. This report summarizes the clinical, laboratory, and epidemiologic features of an outbreak of malaria that was unique in severity of illness and attack rate after a brief exposure to a locale with high transmission intensity. It also discusses host and parasite factors that contributed to these apparent chemoprophylaxis failures.7

MATERIALS AND METHODS

In August 2003, 225 Marines with the 26th Marine Expeditionary Unit aboard U.S. Navy ships were deployed to Roberts International Airport outside the coastal city of Monrovia. Liberia has a tropical climate that includes frequent rainstorms (average rain fall is 78 cm/month) and daytime high temperatures average approximately 27°C in the summer months. Additionally, years of conflict and lack of a functioning government has led to the neglect of disease and vector control programs. As a result, Liberia is holoendemic for malaria and has an annual *Plasmodium falciparum* entomologic inoculation rate, defined as the product of the mosquito biting-rate times the proportion of mosquitoes carrying sporozoites in their salivary glands, as high as 62.05 infectious bites per year in some areas. Deployed Marines worked and slept in an abandoned airport warehouse that had standing water and was infested with rats and mosquitoes (Figure 1). They had already been at sea for several months, most serving briefly in Iraq (April) and Djibouti (July) before arriving in Liberia in August. For malaria chemoprophylaxis during deployment, Marines were issued a supply of generic mefloquine (MQ) hydrochloride (250 mg tablets; Geneva Pharmaceuticals, Dayton, NJ), which were foil-wrapped in blister packs. Each Marine was given several blister packs before going ashore in the Middle East and Africa. Adherence with weekly MQ was based on the honor system, not directly observed therapy (DOT). None of the Marines used doxycycline or atovaquone/proguanil as chemoprophylaxis. The unit spent 10 days in Liberia. Within one day after returning to their ships, a febrile illness developed in the first of what amounted to 80 Marines.
In 2003, 44 U.S. Marines were evacuated from Liberia with either confirmed or presumed Plasmodium falciparum malaria. An outbreak investigation showed that only 19 (45%) used insect repellent, 5 (12%) used permethrin-treated clothing, and none used bed netting. Adherence with weekly mefloquine (MQ) was reported by 23 (55%). However, only 4 (10%) had serum MQ levels high enough to correlate with protection (> 794 ng/mL), and 9 (22%) had evidence of steady-state kinetics (MQ carboxy metabolite/MQ > 3.79). Tablets collected from Marines met USP identity and dissolution specifications for MQ. Testing failed to identify P. falciparum isolates with MQ resistance. This outbreak resulted from under use of personal protective measures and inadequate adherence with chemophrophylaxis. It is essential that all international travelers make malaria prevention measures a priority, especially when embarking to regions of the world with high transmission intensity such as west Africa.
The broad differential diagnosis of a febrile illness in these individuals was narrowed when shipboard physicians identified *P. falciparum* in peripheral blood smears of several Marines. Considering the limited diagnostic resources available to rapidly evaluate a large number of patients, the remainder of the febrile Marines were presumed to have malaria and empirically treated with oral quinine sulfate and doxycycline until supplies of atovaquone/proguanil could be flown to the ship (Figure 2). However, the possibility of other febrile diseases associated with such an outbreak in the tropics (e.g., leptospirosis, rickettsial diseases, or Lassa fever) remained a concern.

Because of the severity of symptoms in the first Marines in which illness developed, 40 were evacuated to the National Naval Medical Center (NNMC) in Bethesda, Maryland, and 4 to Landstuhl Regional Medical Center in Germany. Remaining Marines with presumptive malaria were in a sufficiently stable condition to receive treatment on ship. Knowing that Liberia is endemic for Lassa fever, there was a possibility

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**Figure 1.** Warehouse that housed U.S. Marines at Roberts International Airport in Monrovia, Liberia demonstrating numerous risk factors for contracting an arthropod-borne illness including broken windows, persons sleeping on the roof, standing water, and tall grass in the surrounding area, for contracting an arthropod-borne illness. This figure appears in color at www.ajtmh.org.

**Figure 2.** Flow diagram of U.S. Marines ashore in Liberia. † Doxycycline administered for malaria treatment and for possible co-infection with leptospirosis or rickettsial disease.
some Marines were infected, or co-infected, with Lassa virus after exposure to rat excreta and as a safety measure, they were initially cared for with contact and droplet isolation precautions.\textsuperscript{12-14}

Upon admission, each Marine answered a standard outbreak questionnaire (not anonymous) to obtain data on symptoms and adherence to personal protection measures and chemoprophylaxis. Clinical data, which included physiological examination findings and admission laboratory results, were recorded. Thick and thin peripheral blood smears were prepared on admission (approximately 48 hours after initiation of treatment with antimalarial drugs while aboard ship). To facilitate rapid diagnosis of malaria in an outbreak setting, the NOW ICT Malaria P.f/P.v test (Binax, Inc., Portland, ME) cards were concurrently used for the first 32 Marines arriving at NNMC. At the time of this outbreak, this test was not yet approved by the Food and Drug Administration (FDA) (Silver Spring, MD) and was used only in conjunction with standard microscopy.\textsuperscript{15}

Serum levels of MQ and its carboxy metabolite (MMQ) were measured using previously described methods in 41 of the 44 Marines by the Centers for Disease Control and Prevention (Atlanta, GA).\textsuperscript{16,17} The serum level of MQ estimated to prevent clinical illness (breakthroughs in MQ chemoprophylaxis) is based on observations in U.S. Peace Corps volunteers serving in west Africa in the early 1990s.\textsuperscript{18} In that report, a mean (SD) whole blood MQ level of 384 (174) ng/mL was seen in 25 persons in whom levels were obtained. Probit analysis predicted 99% prophylactic efficacy could be achieved at a blood MQ concentration of approximately 915 ng/mL, 95% efficacy at a concentration of 620 ng/mL, and 90% efficacy at a concentration of 462 ng/mL.\textsuperscript{18} The serum to whole blood MQ ratio is 1.28.\textsuperscript{16} Therefore, protection (95% efficacy) against malaria is associated with serum MQ levels > 794 ng/mL (this value was determined by multiplying the prophylactic efficacy of MQ in whole blood [620 ng/mL] by the MQ serum-to-whole blood ratio of 1.28).\textsuperscript{16} A ratio of MMQ:MQ in serum greater than 3.79 (95% confidence interval = 3.48–4.10) represents prior dosing associated with steady-state kinetics but does not correlate with protection.\textsuperscript{16}

To ensure that the tablets provided to the Marines were actually composed of MQ hydrochloride and met industry standards for identity and dissolution, five tablets in blister packs collected from the pockets of Marines with complicated malaria were sent to an FDA reference laboratory in St. Louis, Missouri, for analysis.

To evaluate the possibility of MQ-resistant malaria, the Walter Reed Army Institute of Research (Silver Spring, MD) cultured isolates of \textit{P. falciparum} from 10 Marines. Parasites were expanded in culture in human erythrocytes, and MQ susceptibility testing was performed by using \textsuperscript{3}H-hypoxanthine incorporation assays using standard methods.\textsuperscript{19} Assays were performed in duplicate and results reflected mean 50% inhibitory concentration (IC\textsubscript{50}) values of at least three separate assays performed on different dates using control clones from west Africa and Indochina.\textsuperscript{20} In addition, we performed polymerase chain reaction (PCR) for these parasites to detect an increase in copy number of the \textit{P. falciparum} multidrug resistance 1 (\textit{pfmdr}1) gene according to standard methods.\textsuperscript{21}

With the concern for Lassa virus infection or co-infection, enzyme-linked immunosorbent assays for IgM and IgG against Lassa virus and PCR were performed according to an established method on serum samples from the first 32 Marines arriving in Bethesda at the U.S. Army Medical Research Institute of Infectious Diseases.\textsuperscript{22,23}

**RESULTS**

Complete clinical, laboratory, and epidemiologic data were available for 32 of the 44 Marines. The mean (SD) time spent ashore was 9.9 (2.6) days, and the mean time for development of symptoms was 15.4 days after arriving in Liberia (Figure 3).

![Figure 3](image-url)  
**Figure 3.** Epidemic curve showing the onset of symptoms in days after first possible exposure in Liberia. † The symptoms this U.S. Marine reported on day 1 in Liberia were not considered related to malaria.
All patients were men (age range = 19–43 years, median age = 24 years). Anemia, an increase in the level of alanine aminotransferase, and prolonged prothrombin and partial prothrombin times were the most common laboratory findings (Table 1). Fever, headache, and diarrhea were the most common symptoms. Stool studies (culture and examination for ova and parasites) were performed for five Marines with continued complaints of diarrhea on admission in Bethesda; one patient (who also had a negative blood smear for malaria) had a positive stool culture for *Salmonella* spp. Group D. Hospital admission days ranged from 1 to 25 days.

Of the 44 Marines, 14 (32%) were confirmed to have malaria by demonstration of asexual parasites in erythrocytes on peripheral blood smears after arrival at Landstuhl or NNMC (Table 2). Ten (31%) of the initial 32 Marines evaluated at NNMC showed positive results for the NOW ICT test.

Most patients received a preventive medicine briefing before arriving in Liberia, which outlined the measures necessary to avoid malaria (Table 1). However, only 19 (45%) used insect repellent on a regular basis, 5 (12%) applied permethrin to clothing, and no one used bed netting. Only 23 (55%) claimed adherence to weekly MQ dosing on the questionnaire. Poor adherence was attributed to forgetfulness.

Serum MQ levels (determined approximately 25 days after arrival in Liberia and 10 days after onset of symptoms) sufficient to provide 95% prophylactic efficacy (MQ > 794 ng/mL) were detected in 4 (10%) Marines, and 9 (22%) had a MMQ:MQ > 3.79 (Table 1). All MQ tablets analyzed met parameters for identity and dissolution as specified in Abbreviated New Drug Application 76.175. The MQ IC₅₀ values for *P. falciparum* isolates ranged from 3.8 ng/mL to 17.6 ng/mL, which indicated that all isolates were susceptible to the drug (MQ resistance is associated with an IC₅₀ > 45 ng/mL) (Table 2).

TABLE 1

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<th>Characteristic</th>
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| Laboratory abnormalities (n = 44) | No. (%)
| Anemia (hematocrit < 42%) | 35 (80)
| Alanine aminotransferase elevation (> 56 U/L) | 22 (50)
| Prothrombin time prolonged (> 14 seconds) | 20 (45)
| Partial prothrombin time prolonged (> 42 seconds) | 18 (41)
| Thrombocytopenia (< 150,000 cells/µL) | 19 (43)
| Hypoglycemia (glucose < 76 mg/dL) | 12 (27)
| Leukopenia (< 4 cells/µL) | 11 (25)
| Leukocytosis (> 10.5 cells/µL) | 3 (7)
| Acute renal failure (creatinine > 1.4 mg/dL) | 1 (2)
| Serum mefloquine measurements (n = 41) | 4 (10)
| Mefloquine > 794 ng/mL | 9 (22)
| Clinical symptoms (n = 42) | 35 (83)
| Fever (> 38°C) | 26 (62)
| Headache | 16 (38)
| Diarrhea | 14 (33)
| Vomiting | 3 (7)
| Abdominal pain | 1 (2)
| Preventive measure (n = 42) | 39 (93)
| Attended preventive medicine briefing | 19 (45)
| Regularly used insect repellent | 5 (12)
| Wore permethrin-treated clothing | 0 (0)
| Used bed nets | 14 (33)
| Adherence to weekly mefloquine | 23 (55)
| Reason for poor adherence with mefloquine (n = 19) | 19 (100)
| Forgetfulness | 0 (0)

**Abbreviations:** MQ = mefloquine; MMQ = monomethylated mefloquine; MMQ:MQ = ratio of mefloquine to monomethylated mefloquine.

DISCUSSION

The 14 confirmed and 30 presumptive, cases of malaria among the 44 Marines presented represent the largest single outbreak of *P. falciparum* malaria in the U.S. Military since Operation Restore Hope in Somalia during 1993–1994. If the additional 36 presumptive cases that did not require evacuation from the ship are included, this outbreak in 80 patients was the largest cluster of malaria cases in the U.S. Military since the Vietnam Conflict. We now summarize key clinical findings and review host and parasite factors identified in our outbreak investigation that contributed to this event.

The mean onset of symptoms in this cohort was only 15.4 days after initial exposure in Liberia. This time was significantly shorter than a mean of 28 days recorded among British soldiers deployed to Sierra Leone in 2000. In addition, our cohort contracted malaria after only 10 days ashore, which is a considerably shorter duration of exposure compared that in British and French soldiers in whom malaria developed after months in either Sierra Leone or Côte d’Ivoire. We observed a malaria attack rate of 196 cases/1,000 persons/10 days when considering this cohort of 44 Marines and 356/1,000 persons if the entire group of 80 febrile Marines is included. This rate was significantly higher than a previously reported attack rate of 5.1 cases/1,000 persons per month in British travelers returning from Nigeria, Ghana, and the Gambia, and 53 cases/1,000 persons/month (during the rainy season) in French soldiers deployed to Sierra Leone. In comparison, U.S. servicemen in Somalia had an attack rate that peaked at 30 cases/1,000 persons/per week, and U.S. servicemen in Afghanistan had an attack rate of 52 cases/1,000 persons. We suspect that the markedly higher attack rate in our cohort and the decrease in time to contract disease and time to onset of symptoms were a reflection of high local transmission intensity and the minimal use of malaria preventative measures.

Five Marines met the World Health Organization criteria for the definition of complicated malaria with parasitemia levels ranging from 2% to 17% combined with pulmonary and/or cerebral complications (Table 2). All five required monitoring in an intensive care unit, mechanical ventilation (range = 4–9 days), and vasopressor support. The remaining 39 Marines with uncomplicated malaria were treated with atovaquone/ proguanil per guidelines of the Centers for Disease Control and Prevention. All 44 showed complete recoveries.

Fever and headache were the most common initial symptoms in this cohort. Loose stools, a less frequently recognized but common symptom of *P. falciparum* infection, were observed in 62% of the Marines. Of interest, severe diarrhea was a prominent initial symptom among several of the first Marines in which illness developed. This symptom mis-
led shipboard physicians to initially diagnose them with travelers’ diarrhea and treat them empirically with ciprofloxacin while their conditions continued to worsen. In retrospect, the failure to recognize diarrhea as a symptom of malaria led to a delay in diagnosis and prompt initiation of correct treatment.

The use of malaria rapid testing (NOW ICT test) proved to be essential in expeditiously evaluating the first 32 Marines who arrived at NNMC. Because rapid test kits offer speed and accuracy when compared with traditional microscopy, these tests are critical in making a timely diagnosis of malaria in resource-limited environments or in the setting of an outbreak of a febrile illness in travelers returning from the tropics. As a case in point, in the chaotic setting of preparing and reading blood smears for the first 32 Marines arriving at NNMC, one patient was originally reported as having a negative thin blood smear although the results of the NOW ICT test were positive. After further review of the thin blood smear, P. falciparum malaria was confirmed.

This outbreak of malaria was the result of inadequate use of personal protective measures and poor adherence with chemoprophylaxis. The use of topical insect repellents, permethrin-treated clothing, and bed nets are effective measures in decreasing malaria transmission and have been incorporated into military deployment doctrine. However, because of the urgency of the deployment, bed nets were not brought ashore. Despite receiving a preventive medicine brief, less than half of the Marines consistently used insect repellent. Permethrin was not frequently used because shipboard stocks had been depleted by treatment of desert uniforms used in Iraq and Djibouti, whereas jungle uniforms were required for Liberia. Reviewing factors associated with poor adherence to personal protective measures highlights the challenges of rapidly deploying large numbers of troops in a short period.

According to White, antimalarial drug failures result from four main host and parasite factors; compliance (or adherence), pharmacokinetics, pharmacodynamics, and resistance. Good adherence to chemoprophylaxis prevents infection with malaria. Contrary to standard U.S. Navy/Marine Corps procedures, Marines were issued MQ without an established plan for DOT, which undoubtedly contributed to only a 55% self-reported adherence rate. Of note, the only explanation reported for missed doses was forgetfulness and not a concern about the highly publicized neuropsychiatric side effects related to MQ (this deployment occurred one year after reports of severe adverse events in soldiers that were initially linked to MQ). The adherence rate to chemoprophylaxis in our cohort was similar to that noted in U.S. service members infected with malaria after deployments to Somalia and Afghanistan.

To explain such poor adherence, it is important to note that these Marines were issued MQ while deployed in Iraq and Djibouti in the months before deployment to Liberia and, regardless of adherence in those settings, malaria did not develop in any of them. Poor adherence in regions with low transmission intensity often has no negative consequences, which may have led the Marines to believe that malaria preventative measures in general are unnecessary. This outbreak accentuates the challenge military and civilian providers face when determining the true need for chemoprophylaxis because malaria transmission intensity varies significantly throughout the world. In addition, it emphasizes the importance of DOT in an operational setting.

Barriers to attaining serum MQ levels high enough to kill blood-stage parasites include poor absorption because of vomiting and increased clearance secondary to diarrhea (an interruption in enterohepatic cycling leads to increased clearance). Although several Marines had loose stools as an initial symptom, none had gastrointestinal symptoms before becoming ill, which suggests that altered absorption (or pharmacokinetics of MQ) and resultant decreased serum levels were factors in this outbreak.

Low serum levels may have resulted from deficiencies in the MQ tablets. However, at the time of admission, all blister packs examined were intact and none had exceeded the stamped expiration date. Although most Marines carried the tested MQ in their pockets for several weeks under a variety of climactic conditions (desert, ship board, and jungle...
immune travelers with therapeutic serum levels of MQ have throughs of prophylaxis. Occasional breakthroughs in non-adherence with weekly dosing.

Finally, despite the FDA-approved regimen of taking MQ at least one week before entering a malarious area, it can take up to 7–9 weeks to reach protective serum concentrations. However, an inadequate lead time was not an explanation for subtherapeutic levels because these Marines had been issued MQ for their brief deployment in Djibouti at least a month before entering Liberia. Because MQ regimens also require taking the drug for four weeks after leaving the malarious area, if the Marines were adherent with their chemoprophylaxis, they should have had protective levels upon arriving in Liberia. Consequently, it appears that low serum MQ levels in these Marines were unlikely to be a reflection of a pharmacokinetic problem and most likely resulted from inadequate loading-dose regimens of MQ achieve protective concentrations, but using MQ in this manner is not FDA approved. 

Third, adherence rates to malaria preventative measures were self-reported and based on a non-anonymous questionnaire, which raises questions concerning the true validity of marine responses. We suspect compliance with malaria preventative measures may have been even lower than reported because some Marines may have overestimated their adherence for fear of administrative sanctions. As seen in the past, this most recent outbreak of malaria in military members resulted from poor adherence with personal protective measures and chemoprophylaxis. In the current political climate, we must assume that U.S. servicemen will be called on again to deploy to regions of the world in which malaria is endemic. Clearly, military members and their leadership must ensure that malaria prevention measures are viewed as equally importantly as other key elements of deployment, e.g., supplies and communications. Additionally, chemoprophylaxis should be given by DOT and, as advocated by Lieutenant General William Slim after witnessing the effects of malaria on British soldiers in World War II, leadership must be held accountable when service-men do not adhere to DOT. Pre-travel discussions with all travelers should address differences in malaria transmission intensity in various regions of the world to guard against complacency with preventive measures and pill fatigue. Providers should use rapid diagnostic tests (e.g., NOW ICT test) and have a low threshold to initiate empiric treatment of malaria when evaluating a febrile patient returning from a tropical environment.

Ultimately, the development of new chemoprophylactic agents and an effective malaria vaccine will aid in the prevention of malaria in all travelers at risk, military and civilian alike. Until then, for the next precipitous deployment into a high transmission area, such as these Marines experienced in Liberia, the use of a drug that is dosed weekly, can take up to 7–9 weeks to achieve protective concentrations, and requires four weeks of post-exposure dosing is clearly suboptimal. Loading-dose regimens of MQ achieve protective drug levels in four days, but using MQ in this manner is not FDA approved. 

Therefore,
prescribing a loading dose of MQ cannot be used operationally by the U.S. military. The use of chemoprophylactic drugs such as atovaquone/proguanil or doxycycline, which provide protection after the first dose, would offer more rapidly attainable malaria chemoprophylaxis in deployment circumstances as those encountered by the 26th Marine Expeditionary Unit.

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