RISK FACTORS FOR GAMETOCYTE CARRIAGE IN GAMBIAN CHILDREN
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Abstract. A widespread reduction in Plasmodium falciparum gametocyte prevalence could reduce malaria transmission. After infection with P. falciparum, a variable proportion of people are found to be gametocytemic. We analyzed risk factors associated with gametocytemia at presentation and 7 days later. We enrolled 1,198 children in 2 antimalarial drug trials between September and December 1998. The children were assigned to 1 of 4 treatment groups: chloroquine only; pyrimethamine-sulfadoxine (PSD) only; PSD combined with 1 dose of artesunate; and PSD combined with 3 doses of artesunate. By the time of enrollment, 200 (17%) of 1,198 children were gametocyte carriers. Three independent risk factors were associated with gametocytemia at enrollment. Children with anemia were more likely to carry gametocytes, whereas children with fever (>37.4°C) or high parasite densities (>100,000 parasites/μL) were less frequently gametocyte carriers. Children with at least 2 of the risk factors were 4 times more likely to be gametocytemic than children with <2 risk factors (odds ratio [OR], 4.4; 95% confidence interval [CI], 2.7–7.1). Seven days after the start of treatment, 355 (37%) of 466 assessable children were found to be gametocyte carriers. Children treated with PSD alone had a significantly higher risk of being gametocytemic by Day 7 compared with children in the other 3 treatment groups. In the subgroup of children who had no detectable gametocytes on enrollment, the effect of treatment with PSD + 3 doses of artesunate was most marked. Nineteen (10%) of 198 children treated with PSD + 3 doses of artesunate became gametocytemic, in contrast to 184 (57%) of 321 children treated with PSD alone (OR, 12.7; 95% CI, 7.3–22.1). Early treatment with highly effective antimalarial therapy has the greatest chance of preventing gametocytemia. The choice of a first-line antimalarial drug for uncomplicated malaria should not only take into consideration the ablation asexual parasitemia but also the suppression of gametocytemia.

INTRODUCTION
Gametocytes, the sexual forms of Plasmodium falciparum, are ingested by female mosquitoes feeding on the human host. In the mosquito gut, male and female gametocytes fuse to form zygotes, which eventually give rise to sporozoites and thus continue the life cycle of the parasite. To ensure parasite survival, each parasite population must produce gametocytes. In the absence of treatment, >90% of immunologically naive people with P. falciparum infections will generate gametocytes within 10–40 days after the onset of patent parasitemia.1 However, at the time of presentation and after treatment, only a fraction of people infected with asexual P. falciparum are gametocytemic. A strategy that avoids identifiable risk factors for gametocytemia may reduce gametocyte prevalence and could contribute to a reduction in malaria transmission. In the current study, we examined risk factors that predict gametocytemia in Gambian children studied at 6 study sites before and after antimalarial treatment.

MATERIALS AND METHODS
A cohort of children enrolled in 2 antimalarial trials was studied. The methodology of the trials has been described in detail elsewhere.2–4 Briefly, the children were recruited in 6 health centers throughout The Gambia, a West African country with holoendemic malaria. Children ≥5 kg body weight and <18 years of age were eligible for enrollment if they were infected with P. falciparum at a density of ≥500 parasites/μL, had a history of fever, and lived within 20 km of a trial center, and if a parent or guardian gave informed consent. Children were excluded if they required parenteral treatment, had been treated within the previous 2 weeks with pyrimethamine-sulfadoxine (PSD), had hematocrit <15%, or had any evidence of chronic disease.

The children in the chloroquine (CQ) alone treatment group received 10 mg of CQ base per kilogram of body weight orally at 0, 24, and 48 hr. Children in the other 3 treatment groups received half a tablet of PSD (12.5 mg pyrimethamine, 250 mg sulfadoxine) if their body weight was <10 kg and an additional quarter tablet for every 5 kg increment in weight. The children in the combination treatment groups received either 4 mg artesunate per kilogram of body weight in the single-dose group, or 12 mg artesunate (divided into three 4-mg doses) per kilogram of body weight in the 3-dose group. A field worker visited the children on Days 7, 14, and 28 to obtain blood films. We used CQ (Alkaloida, Tiszavasvari, Hungary), PSD (Pharmamed, Zejra, Malta), and artesunate (Guilin Pharmaceutical Works, Guilin, China) in the trials.

Labored methods. Thick blood films were left to dry for a minimum of 24 hr and stained with Giemsa. Parasite density was determined on the basis of the number of parasites per 200 leukocytes on a thick film, assuming a total leukocyte count of 8.0 × 109/L. Parasites other than P. falciparum were not detected. If gametocytes were seen, the gametocyte count was extended to 1,000 leukocytes. Each slide was read by 2 experienced microscopists, and discrepancies were reviewed by a third senior microscopist. Hematocrit was measured by microhematocrit centrifugation.

Analysis. Analysis was based on the prevalence of gametocytes at enrollment and during follow-up. Risk factors were determined by using combined data from the 6 study sites and were evaluated for each study site independently. Results of the combined data are presented unless indicated otherwise. Gametocyte prevalence was expressed as the proportion of children with patent gametocytemia. Anemia was defined as a hematocrit <33%. Hyperparasitemia was defined as a parasite density >100,000 parasites/μL. Children with a temperature above 37.4°C were considered febrile. Normally distributed data were compared by Student’s t-test.
test or analysis of variance; homogeneity of variance was tested by the F-test. Discrete data were compared by Kruskal-Wallis test, Fisher’s exact test, or the chi-square test with Yates’ correction, as appropriate. A multivariate logistic regression model was used to test the association between binary outcomes (gametocytemia yes/no) and independent variables that were statistically significant on univariate analysis. A final model employed for the analysis of risk factors for gametocytemia at enrollment included the variables anemia, fever, and hyperparasitemia. The model of risk factors for gametocytemia on Day 7 included the variables gametocytemia at enrollment and antimalarial treatment. All statistically significant relationships were 2-tailed and evaluated at $P < 0.05$. Analyses were conducted by Stata version 6 (Stata, College Station, TX).

Informed consent was obtained from parents or guardians of all children. The studies were approved by the ethics review boards of The Gambian Government/Medical Research Council Laboratories and the London School of Hygiene and Tropical Medicine.

RESULTS

Between September and December 1998, a total of 1,198 children were enrolled in the study. Characteristics of the children at enrollment are described in Table 1. A blood film was obtained 967 (81%) of the 1,198 children on Day 7. A peak in gametocyte prevalence was observed 7 days after treatment; 272 (91%) of 299 children with patent gametocytemia during the follow-up period were gametocyte carriers on Day 7.

**Gametocytes at presentation.** At the time of enrollment, 200 (17%) of 1,198 children were found to be gametocyte carriers. The geometric mean gametocyte density was 397 gametocytes/μL (95% confidence interval [CI], 326–483). After adjustment for fever, anemia, and age, the risk for gametocytemia at enrollment was found to be similar at 5 study sites. At one study site, Nguyen Sanjal, children were significantly less likely to be gametocyte carriers. The odds ratio (OR) for children in Nguyen Sanjal to be gametocyte carriers compared with children in the other 5 sites was 0.4 (95% CI, 0.2–0.9).

Anemia, the absence of fever, and low-density parasitemia were independently related to gametocyte prevalence (Table 2). Children with at least 2 of the risk factors were 4 times more likely to be gametocyte carriers than children with $<2$ risk factors (OR, 4.4; 95% CI, 2.7–7.1).

The children enrolled during September or October had a lower gametocyte prevalence (37 of 337; 11%) compared with 163 (19%) of 861 of children enrolled during November or December ($P = 0.001$). The OR adjusted for anemia, fever, and hyperparasitemia was 1.5 (range, 0.99–2.2). Children aged $<5$ years were more frequently gametocyte carriers (114 of 601; 19%) than older children (86 of 596; 14%) ($P = 0.035$). After adjustment for anemia, fever, and hyperparasitemia, the OR for gametocytemia in children aged $<5$ years compared with older children was 1.1 (range, 0.8–1.6). There was no significant difference in gametocyte prevalence between ethnic groups or sex.

**Gametocyte prevalence on Day 7.** Seven days after the start of treatment, 355 (37%) of 966 children were gametocyte carriers. The geometric mean of their gametocyte density was 812 gametocytes/μL (95% CI, 683–966). Antimalarial treatment group and gametocytemia at enrollment were found to be associated with gametocytemia on Day 7 (Table 3). Hyperparasitemia, anemia at enrollment, age, season, study site, splenomegaly, ethnicity, and sex were not independently associated with gametocytemia.

Gametocytemia was most frequently observed after treatment with PSD alone (Table 3). The CQ and PSD + 1 dose or 3 doses of artesunate had an intermediate effect on gametocyte prevalence. There was no significant difference between the effect of CQ PSD + 1 dose or + 3 doses of artesunate on gametocyte prevalence. We observed a difference in the effect of antimalarials on emerging compared with preexisting gametocytes. One hundred ten (68%) of 161

**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), median (IQR)</td>
<td>5.0 (3.1–7.5)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>642/1,198 (54%)</td>
</tr>
<tr>
<td>Asexual parasite density (parasites/μL)</td>
<td>17,005 (15,405–18,770)</td>
</tr>
<tr>
<td>Gametocytemia</td>
<td>200/1,198 (17%)</td>
</tr>
<tr>
<td>Gametocyte density (gametocytes/μL)</td>
<td>397 (326–483)</td>
</tr>
<tr>
<td>Hyperparasitemia (&gt;100,000 parasites/μL)</td>
<td>145/1,144 (13%)</td>
</tr>
<tr>
<td>Fever (&gt;37.4°C)</td>
<td>769/1,198 (64%)</td>
</tr>
<tr>
<td>Anemia (hematocrit &lt;33%)</td>
<td>777/1,145 (68%)</td>
</tr>
<tr>
<td>Treatment (number enrolled)</td>
<td></td>
</tr>
<tr>
<td>CQ</td>
<td>135</td>
</tr>
<tr>
<td>PSD</td>
<td>476</td>
</tr>
<tr>
<td>PSD + 1 dose artesunate</td>
<td>313</td>
</tr>
<tr>
<td>PSD + 3 doses of artesunate</td>
<td>274</td>
</tr>
</tbody>
</table>

* CI = confidence interval; CQ = chloroquine; GM = geometric mean; IQR = interquartile range; PSD = pyrimethamine-sulfadoxine.

**Table 2**

<table>
<thead>
<tr>
<th>Status at enrollment†</th>
<th>Gametocytes present at enrollment</th>
<th>Crude OR (95% CI)</th>
<th>$P$</th>
<th>Adjusted OR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemic ($n = 777$)</td>
<td>163 (21%)</td>
<td>1</td>
<td>&lt;0.001</td>
<td>3.0 (1.8–4.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Not anemic ($n = 368$)</td>
<td>27 (7%)</td>
<td>3.4 (2.2–5.2)</td>
<td>&lt;0.001</td>
<td>0.6 (0.5–0.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>Febrile ($n = 768$)</td>
<td>103 (13%)</td>
<td>0.5 (0.4–0.7)</td>
<td>&lt;0.001</td>
<td>0.2 (0.1–0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Afebrile ($n = 429$)</td>
<td>97 (23%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperparasitemic ($n = 145$)</td>
<td>6 (4%)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not hyperparasitemic ($n = 999$)</td>
<td>194 (19%)</td>
<td>0.2 (0.1–0.4)</td>
<td>&lt;0.001</td>
<td>0.2 (0.1–0.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* CI = confidence interval; OR = odds ratio.
† Anemia defined as hematocrit < 33%; febrile defined as a temperature $>37.4°C$; hyperparasitemia defined as a parasite density $>100,000$ parasites/μL.
children with gametocytemia at enrollment remained gametocytemic after 7 days. In contrast, only 245 (30.4%) of 806 children without gametocytes in the peripheral circulation at enrollment became gametocytemic ($P < 0.001$; Figure 1). In the subgroup of children who had no detectable gametocytes at enrollment, the effect of treatment with PSD + 3 doses of artesunate was most marked. Nineteen (10%) of 198 children treated with PSD + 3 doses of artesunate became gametocytemic, in contrast to 184 (57%) of 321 children treated with PSD alone ($OR, 12.7; 95\% CI, 7.3–22.1$).

Twenty-nine (3%) of the 966 children had a second episode of asexual parasitemia 7 or 14 days after enrollment. These parasitological failures were most frequently seen in children treated with CQ (18 of 90; 20%), but also in 8 (2%) of 375 children after treatment with PSD alone, 2 (1%) of 262 after treatment with PSD + 1 dose of artesunate, and 1 (0.4%) of 239 after PSD + 3 doses of artesunate. The subgroup of children who had a second episode of parasitemia after treatment with CQ were 4 times more likely to be gametocytemic 7 days after treatment (adjusted $OR, 4.3; 95\% CI, 1.0–18.8$).

**Gametocyte prevalence after Day 7.** By Day 14, gametocyte prevalence had dropped to 253 (29%) of 876, and by Day 28, it dropped to 76 (9%) of 872. The mean geometric gametocyte density dropped to 505 gametocytes/μL (95% CI, 427–597) by Day 14 and to 222 gametocytes/μL (95% CI, 167–296) by Day 28. Children who had not been treated with PSD + 3 doses of artesunate or had gametocytes at enrollment remained at a significantly higher risk of being gametocytemic on Day 14 (OR, 12.4; 95% CI, 6.0–25.7) and on Day 28 (OR, 11.2; 95% CI, 2.7–45.9). Anemia or treatment failure did not predispose to gametocytemia ≥ 14 days after treatment.

**DISCUSSION**

In the current study, the presence of anemia and the absence of fever and high parasite densities were identified as independent risk factors for gametocyte carriage before treatment. Gametocytemia at the time of presentation, and treatment other than PSD + 3 doses of artesunate were risk factors for gametocytemia after treatment. Fever and high parasite densities are the hallmarks of an acute malaria infection resulting in an immune response. The delay in gametocyte release 10–14 days after patent parasitemia allows gametocytes to avoid exposure to high levels of

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**TABLE 3**

<table>
<thead>
<tr>
<th>Status at enrollment</th>
<th>Gametocytes present at Day 7</th>
<th>Crude OR (95% CI)</th>
<th>$P$</th>
<th>Adjusted OR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gametocytes present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>($n = 161$)</td>
<td>110 (68%)</td>
<td>1</td>
<td></td>
<td>8.4 (5.4–13.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No gametocytes present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>($n = 806$)</td>
<td>245 (30%)</td>
<td>4.9 (3.4–7.1)</td>
<td>&lt;0.001</td>
<td>0.12 (0.06–0.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treated with:</td>
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<tr>
<td>PSD ($n = 376$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CQ ($n = 90$)</td>
<td>235 (63%)</td>
<td>1</td>
<td></td>
<td>0.11 (0.07–0.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSD + 1 dose of artesunate ($n = 262$)</td>
<td>58 (22%)</td>
<td>0.17 (0.12–0.24)</td>
<td>&lt;0.001</td>
<td>0.09 (0.06–0.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSD + 3 doses of artesunate ($n = 239$)</td>
<td>45 (19%)</td>
<td>0.14 (0.08–0.24)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CI = confidence interval; CQ = chloroquine; OR = odds ratio; PSD = pyrimethamine-sulfadoxine.
cytokines, which may be lethal to gametocytes. It has been shown that extracellular factors can kill Plasmodium cynomolgi gametocytes. The correlation between anemia and gametocytemia may be related to the presence of cell debris associated with hemolysis and the reticulocytosis in response to ensuing anemia. It has been noted that hemolysis of infected erythrocytes can induce gametocytogenesis and that gametocytes are more frequently in reticulocytes than in normochromatophores.

Although we observed variation in gametocyte prevalence at the time of presentation between the 6 study sites, we found that the risk factors for gametocytemia did not vary between study sites, perhaps indicating a more universal applicability. Risk factors for gametocytemia have recently been studied on the western border of Thailand and in southeastern Tanzania. Although the gametocyte prevalence before treatment was higher in our study population (17%) compared to Tanzania (8%) or Thailand (2%), the risk factors were remarkably similar. Price and others identified the absence of fever and high parasite density, the presence of anemia and the patent gametocytemia at enrollment as independent risk factors in Thailand. Akim and others identified young age to be a risk factor for gametocytemia in Tanzania. We found that age was inversely related to gametocyte prevalence. However, young age was not a significant risk factor in a multivariate model adjusted for anemia status.

Fever and hyperparasitemia are likely to be markers for an acute malaria episode. In contrast, anemia in a malaria-endemic setting frequently results from hemolysis secondary to a persistent Plasmodium falciparum infection. Therefore, an afebrile child with low-density parasitemia is likely to be infected for longer than a child with acute fever, high-density parasitemia, and a normal hematocrit. The number of days with patent asexual parasitemia may determine the presence of gametocytes in the peripheral circulation at the time of presentation.

The significantly lower gametocyte prevalence at one study site, Nguyen Sanjai, may be explained by the ease of access to a clinic that has a dispensary and therefore on the clinic. Similarly, the lower prevalence of gametocytemia in Thailand may be due to earlier presentation of P. falciparum-infected people compared with people in The Gambia. The time between onset of patent asexual parasitemia and presentation may also account for the higher gametocyte prevalence during the second half of the malaria season. Children who present toward the end of the malaria season may have had a previous infection that resulted in gametocytemia. In addition, it is likely that the enthusiasm of parents to bring frequently sick children for medical attention wanes during the season, and clinic visits are thus postponed.

Children who had gametocytes at the time of enrollment were significantly more likely to be gametocytemic 7 days later than children without gametocytemia, irrespective of which treatment regimen they received. This finding suggests that gametocytes circulate peripherally, are metabolically relatively inactive. The drugs under investigation had only a limited effect on this gametocyte population. We found more significant differences in the drugs efficacies on younger gametocytes, which could not be detected in the peripheral circulation at the time of enrollment but were likely sequestered. After treatment with PSD alone, 57% of children initially free of detectable gametocytes became gametocytemic. Only 15% of children treated with PSD + 1 dose of artesunate and 10% of children treated with PSD + 3 doses of artesunate became gametocytemic. Therefore, PSD by itself has little effect on young gametocytes, although our data cannot exclude the possibility that PSD triggers the premature release of immature gametocytes. In contrast, artemisinin derivatives had an effect against the most immature gametocytes that was amplified by giving repeated doses. However, data from concurrent membrane feeding experiments do not support the suggestion that 1 or 3 doses of artesunate are gametocytocidal.

Paradoxically, CQ treatment, which is significantly less effective than PSD in the treatment of asexual parasitemia in our study population, resulted in a lower gametocyte prevalence overall. We observed higher gametocyte prevalence in people with recrudescent infections. Similar observations have been made in Senegal, Thailand, and Tanzania. Resistance of asexual parasites is therefore a risk factor for gametocytemia, but susceptibility of asexual parasites does not predict susceptibility of gametocytes.

To have an effect on malaria transmission, case management has to reduce gametocyte prevalence. Early treatment with highly effective antimalarial drugs has the greatest likelihood of preventing gametocytemia. None of the drugs under investigation ablated gametocytemia 7 days after the onset of treatment. The only drugs with a proven gametocytocidal effect are 8-aminoquinolines, such as primaquine, which have limited usefulness because they can induce hemolysis. New, safe gametocytocidal drugs are therefore needed. In addition, most malaria infections in sub-Saharan Africa are subclinical, and only a fraction of Plasmodium falciparum infections are treated early. To control malaria, new strategies are required to treat subclinical infections.

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