Long-Term Asymptomatic Carriage of *Plasmodium falciparum* Protects from Malaria Attacks: A Prospective Study among Senegalese Children

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**Background.** In areas of seasonal malaria transmission, long-term asymptomatic carriage of *Plasmodium falciparum* throughout the dry season has been primarily studied in terms of the parasites, and the clinical consequences of persistent parasite carriage are unknown.

**Methods.** A prospective study was conducted in Senegal, from 2001 through 2003 among 1356 children living in areas where malaria is endemic, with seasonal transmission occurring from August through December. Cross-sectional parasitological measurements and detection of active malaria attacks were performed. A malaria attack was defined as an axillary temperature ≥37.5°C, associated with a parasite density ≥2500 trophozoites/μL. Children harboring *P. falciparum* in June who did not have clinical signs were defined as asymptomatic carriers. The association of asymptomatic carriage with parasite densities and with the occurrence of malaria attacks during the rainy season were analyzed separately for the years 2002 and 2003, taking into account potential confounding covariates and use of antimalarial drugs.

**Results.** The prevalence of asymptomatic carriage was 32% (332 of 1025 persons) in June 2002 and 23% (208 of 912 persons) in June 2003. Asymptomatic *P. falciparum* carriers had a significantly higher mean parasite density and a significantly lower probability of developing a malaria attack during the subsequent rainy season than did noncarriers (adjusted odds ratio in 2002, 0.56; *P* < .01; adjusted odds ratio in 2003, 0.50; *P* < .01).

**Conclusions.** These results suggest that in areas of seasonal transmission, asymptomatic carriage of *P. falciparum* may protect against clinical malaria. Further studies are needed to understand the immune effectors and host susceptibility that could be involved in this phenomenon.

Malaria is a major cause of illness and death in children in Africa [1]. In areas of seasonal transmission, outbreaks of *Plasmodium falciparum* malaria occur after the beginning of the rainy season, whereas, during the dry season, reports of clinical cases are rare. However, long-term asymptomatic carriers of *P. falciparum* can be found in the population throughout the dry season, despite a very low level of transmission [2–5]. Long-term parasite carriage can be considered to be critical for parasite survival, because the infected individuals may constitute a major reservoir in the absence of transmission [3]. However, whether such infections are chronic infections persisting from the previous transmission season or new infections acquired during the dry season is not clear.

Thus far, this phenomenon has been studied primarily in terms of the parasites. Results from surveys conducted in areas of markedly seasonal transmission reveal that asymptomatic parasitemias persisting during the dry season are often genetically complex infections, with different genotypes coexisting together in a single infection [2, 3, 6]. Multiplicity of infection (MOI) has particularly been explored, and in asymptomatic children, MOI may reflect acquired immunity or premunition [7] and may influence the risk of subsequent malaria attacks (MAs). However, discordant results exist on the clinical consequences of MOI and persistent parasite carriage in asymptomatic individuals [8–18], and the role of age has also been underlined [19]. Hennig et al. [20] revealed that, among individuals aged...
PATIENTS AND METHODS

Study Site

The study was conducted in 2 villages (Toucar and Diohine) in the Niakhar region of Senegal; the region is 150 km southeast of Dakar. Malaria is endemic in this area, with a distinct transmission season occurring almost exclusively during the rainy season (approximately from August through December), and it is caused exclusively by the complex *Anopheles gambiae* s.l. The entomological inoculation rate was estimated to be 9–12 bites per person yearly [22]. Data on recent mosquito catches confirmed a homogeneous distribution of malaria vectors in the study villages (C. Sokhna, personal communication).

Study Population and Design

This prospective community study was performed during the 2002 and 2003 transmission seasons. Our cohort consisted of 1356 Senegalese children living with their 2 parents who agreed to participate in our study (with signed, informed consent provided by the parents).

In June 2002, the children were asked to attend the health center, where a systematic thick blood smear was performed. During the following transmission season, thick blood smear samples were obtained by finger prick for all of the children during the 4-day cross-sectional surveys in each village that were performed in September, October, and November 2002. In addition, children were asked to provide urine samples for detection of chloroquine metabolites, which were assessed by a colorimetric method (Haskins modified by Mount II) [23]. Positive results (chloroquine level, ≥3 μg/mL) gave information about previous intake of chloroquine. All children had free access to health services and treatment. All parents were instructed to bring any child with a clinical symptom and/or sign of malaria to the dispensary.

In addition, an active clinical survey aimed at detecting any asymptomatic carriage in June. Asymptomatic carriage of *P. falciparum* during the dry season was defined as presence of parasitemia in June, without any clinical sign, fever, or history of fever reported during the month. To ensure the inclusion of only asymptomatic children, children who attended the dispensaries for any reason in June were excluded from the asymptomatic carrier group.

**MPD.** Analyses of the 3 systematic parasite densities measured during the rainy season were conducted using a logarithmic transformation based on log (parasite density + 1) to allow for a 0 count. Because some children may not have been present at each visit, we considered only the children who had at least 2 measurements obtained. During the follow-up visits, the log-transformed parasite density (LPD) varied significantly with time (P<.001), consistent with the variability of the transmission rate during the rainy season. Thus, individual LPDs were adjusted for the seasonal effect by subtracting the mean LPD of the corresponding visit from each individual LPD. Individual LPDs were similarly adjusted for chloroquine intake and co-infection with other species (e.g., *Plasmodium malariae* and *Plasmodium ovale*); the mean LPD calculated for the children with chloroquinuria and the mean LPD calculated for...
to age, male-to-female sex ratio, and village of residence between the group of children surveyed and the overall cohort.

In June 2002, only 1025 of 1356 children attended the health center to have samples obtained. Children absent from the survey in June 2002 were significantly older than the children who were present (10.9 ± 0.12 years vs. 8.5 ± 0.23 years; \( P < .001 \)). Activities, such as farm work or tending to livestock, may explain this difference. The male-to-female sex ratio did not differ between the children present and not present in June 2002. In June 2003, the same pattern of comparison was observed between the 912 children who attended the health center and the children who did not attend the health center.

**MAs during the rainy season.** A total of 517 and 503 consultations for fever occurred from August through December in 2002 and 2003, respectively. A thick blood smear was performed for each visit, and malaria was confirmed in 205 (40%) of 517 patients in 2002 and 263 (52%) of 503 patients in 2003. No MAs were reported from March through June 2003. The MPD in persons experiencing MAs was 56,938 trophozoites/\( \mu \)L (range, 3136–533,277 trophozoites/\( \mu \)L) in 2002 and 56,218 trophozoites/\( \mu \)L (range, 2517–399,251 trophozoites/\( \mu \)L) in 2003. Urine metabolite levels were undetectable in 82%, 77%, and 85% of the children in September, October, and November 2002, respectively, and in 81%, 83%, and 96% of the children during the same respective months in 2003.

**Asymptomatic carriage of** *P. falciparum* **during the dry season.** In June 2002, 332 (32%) of 1025 children had *P. falciparum* parasitemia without fever or any clinical sign and were considered to be asymptomatic *P. falciparum* carriers. The prevalence of asymptomatic carriage was 23% (208 of 912 children) in June 2003.

Among children harboring *P. falciparum* in June, the MPD was 12.2 (± 32.3) parasites per 100 leukocytes in 2002 and 8.5 (± 19.2) parasites per 100 leukocytes in 2003. Age and sex did not differ significantly between asymptomatic carriers and noncarriers in June 2002 (\( P = .3 \) and \( P = .8 \), respectively) and June 2003 (\( P = .8 \) and \( P = .1 \), respectively).

Effect of asymptomatic carriage of *P. falciparum on MPD.*
The unadjusted MPD during the transmission season was 25 parasites per 100 leukocytes (range, 0–1520 parasites per 100 leukocytes) in 2002 and 56 parasites per 100 leukocytes (range, 0–3435 parasites per 100 leukocytes) in 2003. The results of the multivariate linear regression analysis are shown in table 1. Asymptomatic *P. falciparum* carriers and male patients had a significantly higher MPD during the rainy season, compared with noncarriers and female patients, respectively. There was no effect of age or village of residence on MPD. The results during the 2003 season were consistent with those during the 2002 season. Furthermore, asymptomatic carriers seemed to be significantly more frequently infected during the transmission season than noncarriers. The relative risk for an asymptomatic
carrier to maintain asymptomatic carrier status at each measurement during the transmission season (September, October, and November) was 2.4 (P = .002) in 2002 and 1.6 (P < .001) in 2003.

**Effect of asymptomatic carriage on occurrence of MA.**
Table 2 shows the results of the multivariate logistic regression analysis of factors associated with MA. Compared with non-carriers, asymptomatic carriers of *P. falciparum* in June had a significantly lower probability of developing an MA during the following rainy season (adjusted OR in 2002, 0.56; P = .01; adjusted OR in 2003, 0.50; P = .01). Older children also had a lower risk of developing an MA (adjusted OR, <1; P < .001). There was no statistically significant relationship between MA and sex or village of residence. The probability of developing an MA during the transmission season according to age and carriage status and predicted with the logistic model including age as a quantitative variable is shown in figures 1 and 2.

**DISCUSSION**
We found that asymptomatic children harboring *P. falciparum* infection during the dry season had a significantly higher parasite load during the following rainy season and a lower probability of developing an MA than did non-carriers. We defined asymptomatic carriage as the microscopic detection of *P. falciparum* in a thick blood smear sample from asymptomatic children in June. Considering that we used microscopic detection, which has low sensitivity, compared with a more sensitive test, such as PCR, we may have underestimated the prevalence of asymptomatic carriage [2, 28, 29]. However, PCR amplification, based on msp2 genotyping, was performed for a sample of 414 specimens from individuals in June 2002. When we defined asymptomatic carriage of *P. falciparum* using a microscopic or PCR method, 62 new asymptomatic carriers were detected and similar results were obtained.

Our study revealed that sex could be a significant factor for the mean level of infection. The role of sex hormones has been identified to explain differences in the evolution of parasite densities between male and female individuals [30]. Kurtis et al. [31] revealed that dehydroepiandrosterone sulfate and testosterone were significant independent predictors of resistance to *P. falciparum* parasitemia, even after adjusting for age, suggesting the complexity of the involvement of host development in the acquisition of immunity.

Although age is certainly one of the most important factors involved in the acquisition of immunity against *P. falciparum* parasitemia, our results reveal that age has no effect on MPD. This absence of effect has been previously described in the same region [32], and a significant but very weak effect was described in this region during a previous transmission season [25]. These results are consistent with the low level of transmission and, probably, the complex effect of age on the acquisition of immunity in this population [33].

In the rural Sahelian regions of Senegal, the period of malaria transmission is confined to the rainy season [22], with the expansion of the mosquito population. Although vectors were observed throughout the year in the study region, their densities were very low during the dry season, and there was no evidence of transmission during this period [22]. The same results were obtained by entomological surveys performed in regions of the Sudan with seasonal transmission [4]. Our study revealed that a noteworthy proportion of children in this region were long-term carriers of *P. falciparum* during the transmission-free season, with a prevalence of asymptomatic carriers of 32% in June 2002 and 23% in June 2003. Such a discrepancy between the existence of asymptomatic carriers and a quasi-absence of vectorial transmission in regions of seasonal transmission has been previously described [2–6, 34, 35] and suggests that these chronic infections result from persistence of the original infecting parasites, although we cannot exclude that some limited mosquito transmission of *P. falciparum* may continue during the dry season.

The phenomenon of asymptomatic carriage of *P. falciparum* has been studied primarily in terms of the parasites [2, 3, 6]. Different genotypes could coexist together in an infection, and

**Table 1. Factors associated with mean parasite density: multivariate linear regression analysis.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>2002 Season</th>
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<th>2003 Season</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>P</td>
<td></td>
<td>Coefficient (95% CI)</td>
<td>P</td>
<td>---</td>
</tr>
<tr>
<td>Asymptomatic carriage of <em>P. falciparum</em> in June</td>
<td>0.28 (0.14–0.42)</td>
<td>&lt;.001</td>
<td>0.28 (0.05–0.51)</td>
<td>.02</td>
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<tr>
<td>Age (quantitative)</td>
<td>0.01 (–0.01 to 0.03)</td>
<td>.31</td>
<td>–0.02 (–0.04 to 0.01)</td>
<td>.24</td>
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<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Female</td>
<td>Reference</td>
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<td>Reference</td>
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<tr>
<td>Male</td>
<td>0.15 (0.02–0.28)</td>
<td>.02</td>
<td>0.24 (0.05–0.44)</td>
<td>.02</td>
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<tr>
<td>Village</td>
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<tr>
<td>Diohine</td>
<td>Reference</td>
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<td>Reference</td>
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<tr>
<td>Toucar</td>
<td>0.06 (–0.08 to 0.19)</td>
<td>.40</td>
<td>–0.16 (–0.37 to 0.04)</td>
<td>.12</td>
<td>---</td>
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</tbody>
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Table 2. Factors associated with malaria attacks occurring during the subsequent rainy season: multivariate logistic regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>2002 Season Adjusted OR (95% CI)</th>
<th>2003 Season Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic carriage of</td>
<td></td>
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<tr>
<td>Plasmodium falciparum</td>
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<tr>
<td>No</td>
<td>1</td>
<td>1</td>
<td></td>
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<tr>
<td>Yes</td>
<td>0.56 (0.37–0.86) .01</td>
<td>0.50 (0.29–0.86) .01</td>
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<tr>
<td>Age, years</td>
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<tr>
<td>&lt;5</td>
<td>1</td>
<td>1</td>
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<tr>
<td>6–10</td>
<td>0.48 (0.29–0.78) .20</td>
<td>0.20 (0.07–0.56)</td>
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<tr>
<td>&gt;10</td>
<td>0.32 (0.19–0.54) !.001</td>
<td>0.14 (0.05–0.39) .001</td>
<td></td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.20 (0.83–1.74) .34</td>
<td>1.27 (0.79–2.04) .32</td>
<td></td>
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<tr>
<td>Village</td>
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<tr>
<td>Diohine</td>
<td>1</td>
<td>1</td>
<td></td>
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<tr>
<td>Toucar</td>
<td>1.30 (0.90–1.88) .16</td>
<td>0.75 (0.46–1.19) .22</td>
<td></td>
</tr>
</tbody>
</table>

the proportion of each could fluctuate substantially, reflecting complex dynamics of multiple-clone infection during chronic asymptomatic carriage [2, 3, 6]. Several studies have found relationships between MOI and the risk of malaria-associated morbidity, but the results were discordant. Studies involving children in Tanzania [8, 9], Ghana [10], the Sudan [12], and Mozambique [11] found that MOI was a risk factor for MAs, whereas other studies in Tanzania and Papua New Guinea revealed opposite results [13–18]. Cross-protection against clinical malaria by MOI may be characteristic of the semi-immune hosts; whether occurring in children with less immunity or little exposure, each additional infection adds to the risk of a clinical attack. This hypothesis underlines the role of host characteristics in this phenomenon.

It has been proposed that high parasite densities increase the probability of detecting concurrent clones in an individual [36, 37]. In our study, long-term asymptomatic carriers had a higher MPD than noncarriers, which could be related to newly acquired parasite strains during the rainy season. Surprisingly, long-term asymptomatic carriers developed significantly less MAs than did noncarriers, and this point provides further evidence of a major involvement of host characteristics, together with parasite diversity and vectorial transmission pattern, in the control of malaria parasitemia and tolerance.

Other results of our study suggest the role of the host in the control of parasite persistence. Asymptomatic carriers in June 2002 were more likely to be asymptomatic carriers in June 2003 (relative risk, 1.8; P<.001). Of the asymptomatic carriers in 2002, 30% were still asymptomatic carriers during the subsequent dry season. There were no significant differences with regard to sex (P = .93), village of residence (P = .20), and age (P = .25) among the 84 children who were asymptomatic carriers during both seasons and the children who were either noncarriers or asymptomatic carriers during only 1 season. Furthermore, the geographical distribution of those who were asymptomatic carriers during 1 season and those who were asymptomatic carriers throughout both seasons did not reveal any geographical correlation (data not shown). Among the host characteristics, genetic factors could be involved in the control of parasite infection. Several studies have focused on the relationship between host genetic factors and susceptibility and/or resistance to malaria [38]. However, there is only a paucity of information concerning the role of host genetic factors in asymptomatic malaria. Recently, Mombo et al. [39] found that G6PD A and TNF-238 polymorphisms could be involved in
the control of asymptomatic malaria. Further studies are ongoing in the Senegalese population to explore the role of host genetics in this particular aspect of malaria infection.

In terms of public health, treatment of patients with asymptomatic long-term parasitemia may be important for the control of malaria in regions of endemicity. Treatment of asymptomatic individuals, regardless of their malaria infection status, with regularly spaced therapeutic doses of antimalarial drugs has been proposed as a method to reduce malaria-associated morbidity and mortality [40]. However, the results of our study advocate caution in introducing chemoprophylaxis or intermittent treatment in children who are asymptomatic *P. falciparum* carriers, because clearance of asymptomatic parasitemia in such children may increase their subsequent risk of clinical malaria.

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