THE EPIDEMIOLOGY OF PLASMODIUM FALCIPARUM MALARIA IN TWO CAMEROONIAN VILLAGES: SIMBOK AND ETOA

ISABELLA A. QUAKYI, ROSE G. F. LEKE, ROSA BEFIDI-MENGUE, MARTIN TSAFACK, DENNIS BOMBA-NKOLO, LUCIEN MANGA, VIVIANE TCHINDA, EMMANUEL NIEUNGUE, SAMUEL KOUONTCHOU, JOSEPHINE FOGAKO, PHILOMENA NYONGLEMA, LUCY THUTA HARUN, ROSINE DJOKAM, GRAACE SAMAA, ANA ENO, ROSETTE MEGNEKOU, SIMON METENOU, LEOPOLD NDOUTSE, ALBERT SAME-EKOBO, GRACE ALAKE, JEAN MELI, JULIA NGU, FELIX TIETECH, JEANNE LOHOUE, JOE LOUIS MVONDO, EMMANUEL WANSI, ROBERT LEKE, ALAIN FOLEFACK, JUDE BIGOGA, CECILE BOMBA-NKOLO, VINCENT TITIANI, ANNIE WALKER-ABBEY, MICHAEL A. HICKEY, ARMEAD H. JOHNSON, AND DIANE WALLACE TAYLOR

Abstract. In support of ongoing immunologic studies on immunity to Plasmodium falciparum, demographic, entomologic, parasitologic, and clinical studies were conducted in two Cameroonian villages located 3 km apart. Simbok (population = 907) has pools of water present year round that provide breeding sites for Anopheles gambiae, whereas Etoa (population = 485) has swampland areas that dry up annually in which A. funestus breed. Results showed that individuals in Simbok receive an estimated 1.9 and 1.2 infectious bites per night in the wet and dry season, respectively, whereas individuals in Etoa receive 2.4 and 0.4 infectious bites per night, respectively. Although transmission patterns differ, the rate of acquisition of immunity to malaria appears to be similar in both villages. A prevalence of 50–75% was found in children < 10 years old, variable levels in children 11–15 years old, and 31% in adults. Thus, as reported in other parts of Africa, individuals exposed to continuous transmission of P. falciparum slowly acquired significant, but not complete, immunity.

INTRODUCTION

Malaria is endemic throughout Cameroon.1–4 Due to increasing drug resistance in Plasmodium falciparum parasites and lack of adequate vector control measures,5–9 the prevalence, endemicity, and severity of malaria appear to be increasing in Cameroon.10 Since malaria is a major threat to children, pregnant women, and the aged, intervention strategies to reduce malaria transmission are clearly needed. Currently, vector control measures and impregnated bed nets are useful. Ongoing studies exploring the potential of developing a vaccine for malaria hold promise for the future.

Before a successful vaccine can be developed, however, a better understanding of the components of naturally acquired immunity is needed. This information can only be obtained by conducting immunologic studies at well-characterized field sites. The most important parameter known to affect the acquisition and maintenance of immunity to P. falciparum is the intensity of malaria transmission throughout the year, including the number and species of vectors present, species of circulating malaria parasites, environmental conditions, distribution of annual rainfall, and human population density.11–13 The level of malaria transmission is not uniform in endemic areas and may even differ among locations within a single village.14–16 Thus, it is important to monitor malaria transmission patterns while conducting immunologic studies, since the quantitative dynamics of transmission must be known to interpret immunologic data on the acquisition of immunity to malaria.

In September 1994, we initiated longitudinal field studies in two malaria-endemic villages in Cameroon, Simbok and Etoa. These rural villages are located near the capital city of Yaounde, Cameroon (Figure 1). Results from entomologic, parasitologic, and clinical studies are described in this report. These results provide a baseline for future epidemiologic studies and for the interpretation of ongoing research on immunologic responses to possible vaccine candidate antigens.
brackish water form all year round as a result of the rains and human activities. In contrast, Zones 3 and 4 in Simbok are located at a higher level within the forest with fewer pools of standing water. The village of Etoa has a higher density of houses that are surrounded by a smaller amount of foliage (Figure 2). Zones 1 and 2 are located in relatively flat areas where water from the two surrounding rivers form an intricate pattern of grassy marshes and swamps. These wetlands dry up during the dry season. Zone 3 is located on a plateau away from the influence of the rivers.

A preliminary parasitologic survey, funded by the National Epidemiological Board of Cameroon and carried out in February 1992, showed that in Simbok 75% of the children less than 16 years old had malaria towards the end of the dry season. The village of Etoa is located 3 km from Simbok. Etoa is better developed and has a small clinic that provides minimal health services, a village water tank, and electricity. These support resources are not available in Simbok.

Study design and field procedures. Informing the population about the study. A series of meetings were held in Simbok and Etoa to ensure that individuals residing in these villages understood the project. The mode of malaria transmission, the need for a longitudinal epidemiologic study (objectives, protocols, anticipated results), and the possibility of future control measures were discussed. It was made clear that participation in the project was voluntary, that all members of the villages were eligible for enrollment in the project, and that free basic health care services would be provided to everyone, including those who did not volunteer. After reaching agreements with the village chiefs, section leaders, and the population, details of field procedures were discussed, including how the census, entomologic, and malaria prevalence studies would be conducted, the use of the questionnaire to establish information related to malaria morbidity, and the nature of clinical and laboratory investigations.

Demography. Mapping and census taking were carried out between September and December 1994 in Etoa, and in June 1997 in Simbok. Twelve village helpers were identified who functioned as census registrars. Initially, hand-drawn maps of the villages were prepared (Figure 2) and all houses were given unique survey numbers. Both villages had preexisting geo-political divisions known as Zones. It was important that each village helper, assigned to a specific Zone, was a resident of that area. Each census was completed within one to two weeks. Subjects in each household were registered and information, including name, sex, age, and relationship to the head of the household, was recorded. The head of the household (the patriarch/matriarch) was the principal respondent and provided information on the relatedness of all individuals in the household. Visits were in the afternoon or evening to include children attending school and adults absent from the home during the day. Each member of a study village was given a unique number for the project so that at the end of the census all demographic and clinical information could be coded.
Entomologic studies. Entomologic surveys were carried out in Simbok and Etoa in September–November 1994, towards the end of the wet season, and in December–February 1994/1995, during the dry season. Briefly, houses with human volunteers were selected from each village. These houses were scattered throughout the village to be representative. On two consecutive nights, indoor all-night catches on human volunteers were conducted between 6:00 PM and 6:00 AM. All night catches were repeated two weeks later. Mosquitoes were classified to species. Previous studies have shown that essentially all A. gambiae s.l. in the forest zone of Cameroon are A. gambiae sensu stricto (s.s.) (Manga L., unpublished data). Immediately after capture, anopheline mosquitoes were dissected and the salivary glands were examined for the presence of sporozoites.

Parasitologic survey of malaria in Simbok and Etoa. Simbok is large and spread out; therefore, central locations were identified for each zone where villagers assembled for the surveys. Overall, cooperation in Simbok has been very high and longitudinal studies are currently ongoing. Etoa is a small village. This made it possible to conduct a house-to-house survey with the help of local community health nurses. Cooperation in Etoa proved to be poor and the study was suspended in late 1996. Thus, most of the data presented herein are from Simbok. Results from Etoa are presented to illustrate the differences in epidemiologic data that can exist between two villages located only 3 km apart.

Persons who volunteered in the survey were given a clinical examination by a project physician and axilla temperatures were recorded with digital thermometers using disposable plastic covers. After informed consent from consenting adults and parents of children was obtained, thick and thin smears were made from stick blood. Determinations were performed within 10 hr of determination by age and sex, based on information collected in August 1997, the prevalence of illness in Simbok due to malaria was assessed at free clinics held three times a week. A person was diagnosed as having malaria by the attending physician using a set of algorithms developed by the National Epidemiology Board of Cameroon for malaria case management. The algorithms have been extensively validated throughout the country and have been shown to be highly efficient in diagnosing clinical malaria. Thick and thin blood smears were collected and examined for malaria parasites from patients who had fevers (i.e., a temperature of ≥ 37.5°C) and from patients who reported having had a fever during the past 24 hr. It should be noted that one needs to take several blood smears, and not just a single smear, from a patient to accurately diagnose malaria because it is not unusual for a patient to have a negative smear. All patients with slide-positive malaria were treated. Chloroquine was given at a dose of 25 mg/kg over a three-day period. For villagers with non-malarial diseases, physicians provided free diagnosis and a prescription for treatment.

Laboratory tests. Blood samples were analyzed for malaria and other blood parasites, PCV, Hb, Hb polymorphism, and ABO blood groups. Plasmodium genotyping, HLA typing, and antibody titers to vaccine candidate antigens were also carried out.

Malaria parasite density was assessed using thin and thick smears. Dried thin smears, prepared in the field, were fixed with methanol (only thin smears were fixed), then stained with Diff-quick (Baxter International, Deerfield, IL). Stained slides were analyzed for malaria and other blood parasites by two microscopists. A person was considered to be malaria-positive if malaria parasites were detected in the blood smear. A blood smear was considered negative if parasites were not detected after examination of 200 oil-immersion fields of the thick smear. Thin films were used to determine the malaria parasite species. When malaria parasites were demonstrable in a blood film, the parasitemia (parasite density) was determined using one of two approaches: 1) on thin smears, the number of parasites present was counted per 10,000 red blood cells, or 2) on thick smears, the number of parasites present was counted per 200 leukocytes. To arrive at an approximate parasite count per microliter of blood, the number of malaria parasites counted on the thick film was multiplied by 40, based on the assumption that the average leukocyte count was 8,000/µl of blood.

The PCV, determined by microcentrifugation of heparinized microcapillary tubes, was obtained directly from finger stick blood. Determinations were performed within 10 hr of blood collection. For hemoglobin (g/dl), the cyanmethemoglobin method was used.

Anemia was defined as a PCV < 33% and/or Hb level < 11 g/dl. The determination of HbS was carried out by electrophoresis using a commercially available kit (Helena Laboratories, Beaumont, TX). The ABO blood typing was carried out using commercial antisera (Transclone; Sanofi Diagnostic Pasteur, Paris, France).

Statistical analysis. Data from pre-coded forms from the census were checked for validity and entered into the computer. The contents of the computer files were then checked against the original pre-coded data sheets for errors or omissions. All statistical analyses were performed using Statistical Analysis Systems (SAS Institute, Inc., Cary, NC).

RESULTS

Results from Simbok Village. Census results showed that 907 people live in Simbok in 160 households. The number of residents per household ranged from two to 20 persons. Males and females were registered in nearly equal numbers: 477 females and 430 males. The distribution of the population by age and sex, based on information collected in August 1997, is shown in Figure 3A.

Entomologic studies. During the wet season and the dry season of 1994–1995, 1,592 mosquitoes were captured and analyzed. The principal potential vectors present were A. gambiae (62.9%), A. moucheti (23.4%), A. nili (8.3%), A. funestus (5.1%), and A. paludis (0.3%). The sporozoite index was 4.6% in A. gambiae and 2.7% in A. moucheti. None of the other mosquitoes collected in Simbok during this survey were sporozoite-positive. The parity rates of A. gambiae and A. moucheti were 54.6% and 62.9%, respectively. The average mosquito biting rate (expressed as the number of bites...
per person per night [b/p/n]) was 53 b/p/n. The average inoculation rate (number of infectious bites received per person per night [ib/p/n]) was 1.2 ib/p/n during the dry season and 1.9 ib/p/n during the wet season, with 82% due to \textit{A. gambiae s.l.} and 18% due to \textit{A. moucheti}. Thus, the range in transmission rates did not vary greatly between the wet and dry seasons.

**Malaria prevalence.** Malaria prevalence studies were conducted in November 1996, 1997, and 1998, with 28%, 35%, and 41% of the population participating in each survey, respectively. \textit{Plasmodium falciparum} (100%) was the predominant species. Mixed infections ranged from 2% to 5% with \textit{P. falciparum} and \textit{P. malariae} and 2–3% with \textit{P. falciparum}, \textit{P. malariae}, and \textit{P. ovale}. The parasite rate, defined as the proportion of the survey population with patent \textit{P. falciparum} parasitemia, was 41%, 45%, and 55% in 1996, 1997, and 1998, respectively.

Table 1 shows the level of \textit{P. falciparum} infection by age between 1996 and 1998. During this three-year period, parasite rates in infants less than six months (0.5 years) ranged from 40% to 67%. In general, the highest parasite rates were found in the 0.6 through 5-year age groups (range = 64–83%). Parasite rates were variable in the 6–10-year age group and began to decrease as children reached 11–15 years of age. By 16 years of age, parasite rates appeared to have stabilized at ~30%. When the 16–65-year age group was further stratified to 16–35, 36–45, 46–60, and > 60 years, there was no significant difference in annual parasite rates among the groups. Thus, in subsequent analyses, these groups were pooled.

During the three-year period, parasite rates peaked in children in different age groups (Table 1). For example, in 1996 and 1997, parasite rates were highest in the 0.6–1-year age group. In 1998, the peak parasite rate was observed in children between the ages of one and 10 years. A significant difference in the parasite rate was seen in the 6–10-year age group during the three-year period, varying from 33% in 1996 to 71% in 1998 (\( P < 0.0004 \)). The rate in the 11–15-year age group also varied among the three years (\( P < 0.018 \)) (Table 1).

**Parasite rates within Simbok.** Since different micro-environments within the village might influence malaria transmission and prevalence within the village, parasite rates were compared among the zones. Although variation in parasite rates were found among the zones, there was no statistically significant difference.

**Malaria parasite density in asymptomatic carriers in Simbok.** During the 1997 survey, 139 individuals were iden-
tified who were blood-smear positive, but had no clinical symptoms of disease (i.e., were asymptomatic). Parasite density in these individuals was subdivided into five categories and the proportion of the population within each category by age is shown in Table 2. The highest parasite density (60,880 parasites/μl of blood) and the highest mean parasite density 10,240 parasites/μl were found in the 0.6–1-year age group. Mean densities, thereafter, slowly decreased with age. Most people (58%) had parasitemias between 500 and 4,999 parasites/μl of blood. Overall, only 14.4% of the asymptomatic carriers had parasite densities greater than 5,000 parasites/μl of blood, and 5% (7 of 139) had parasite densities ≥ 10,000 parasites/μl. Of those individuals with high parasitemias > 10,000 parasites/μl, 57% were in the 1–5-year age group.

Clinical malaria infections in Simbok. Three times per week, physicians examined all children and adults who came to the clinic. The patients were ill with a variety of diseases, including malaria. Axillary temperatures were taken at the time of presentation, as well as information determining if malaria. Axillary temperatures were taken at the time of presentation. Approximately half of the patients with clinical malaria in a total of 208 individuals (Table 3). Approximately half of the patients with clinical malaria were less than 16 years old and half were older. Based on axillary temperatures taken at the time of presentation, ~54% of the children less than 11 years of age had fevers, whereas only 28% of patients ≥ 11 years old had fevers. Results showed that 100% of the slide-positive children less than one year of age had fever. In addition, the mean temperature of all children less than one year of age was higher than that of the other groups (Table 3). These observations contrast sharply with slide-positive children between the ages of one and 10 years, of which only 57% had detectable fevers when they were examined in the clinic (Table 3). Accordingly, an association between fever and malaria was found in children less than one year of age (odds ratio = 1.44), suggesting that fever is a predictor of disease in children less than one year of age, but is a less useful predictor in older children.

Parasite densities in children less than one year of age averaged 7,280 parasites/μl of blood, whereas higher densities were found in children between one and five years old (mean = 33,640 parasites/μl of blood) (Table 3). Since 100% of slide-positive children less than one year of age had fever compared with only 56% and 57% of those in the 1–5- and 6–10-year age group, respectively, the data suggest that children in the latter group had acquired some level of anti-disease immunity.

The majority, approximately 73%, of children less than 10 years old with clinical malaria were anemic, indicating that Plasmodium falciparum infection was a strong risk factor for anemia in children ≤ 10 years old (Table 3). The influence of the sickle cell trait (Hb AS) on prevalence of clinical malaria was also evaluated. Results showed that among individuals with clinical malaria the AS trait was present in 0% of children in the 0.6–5-year age group, 47% in the 6–10-year group, 27% in the 11–15-year group, and 14% in the 16–65-year group. Thus, presence of the sickle cell trait altered the risk of clinical disease in children less than five years of age.

Prevalence of sickle cell trait (AS) and ABO blood groups in the general population in Simbok. Based on

### Table 2

Summary of prevalence and parasite density of asymptomatic malaria in Simbok, Cameroon (based on data collected 1997)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n</th>
<th>Range (parasites/μl of blood)</th>
<th>Mean</th>
<th>SEM</th>
<th>Percent (%) Plasmodium falciparum positive within each age group (parasites/μl of blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.5</td>
<td>8</td>
<td>80–34,000</td>
<td>5,715</td>
<td>±4,092</td>
<td>13 (100) 25 (100–499) 38 (500–999) 13 (&gt;1,000)</td>
</tr>
<tr>
<td>0.6–1</td>
<td>7</td>
<td>560–60,880</td>
<td>10,240</td>
<td>±8,451</td>
<td>0 (100) 0 (100–499) 86 (500–999) 0 (&gt;1,000)</td>
</tr>
<tr>
<td>1–5</td>
<td>33</td>
<td>200–19,280</td>
<td>3,754</td>
<td>±877</td>
<td>0 (100) 21 (100–499) 58 (500–999) 9 (&gt;1,000)</td>
</tr>
<tr>
<td>6–10</td>
<td>30</td>
<td>120–6,680</td>
<td>2,051</td>
<td>±589</td>
<td>0 (100) 27 (100–499) 60 (500–999) 13 (500–999)</td>
</tr>
<tr>
<td>11–15</td>
<td>20</td>
<td>240–11,440</td>
<td>2,884</td>
<td>±721</td>
<td>0 (100) 30 (100–499) 45 (500–999) 20 (500–999)</td>
</tr>
<tr>
<td>≥16</td>
<td>41</td>
<td>80–11,680</td>
<td>1,237</td>
<td>±294</td>
<td>2 (100) 32 (100–499) 64 (500–999) 2 (500–999)</td>
</tr>
</tbody>
</table>

### Table 3

Summary of prevalence of fever, parasitemia, and anemia in individuals with symptomatic malaria in Simbok, Cameroon

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>% presenting with fever</th>
<th>% slide-positive with fever</th>
<th>Mean temperature (°C)</th>
<th>Slide-positive for Plasmodium falciparum</th>
<th>Mean ± SD PCV</th>
<th>Mean ± SD hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.5</td>
<td>6</td>
<td>67</td>
<td>100</td>
<td>38.1</td>
<td>67% 12,160</td>
<td>27 ± 5.6</td>
<td>9 ± 1.8</td>
</tr>
<tr>
<td>0.6–1</td>
<td>6</td>
<td>50</td>
<td>100</td>
<td>38.1</td>
<td>50% 770</td>
<td>35 ± 9.2</td>
<td>11 ± 1.7</td>
</tr>
<tr>
<td>1–5</td>
<td>35</td>
<td>43</td>
<td>56</td>
<td>37.4</td>
<td>38% 33,640</td>
<td>32 ± 6.3</td>
<td>11 ± 2.1</td>
</tr>
<tr>
<td>6–10</td>
<td>37</td>
<td>62</td>
<td>57</td>
<td>37.3</td>
<td>60% 3,600</td>
<td>33 ± 4.2</td>
<td>11 ± 1.4</td>
</tr>
<tr>
<td>11–15</td>
<td>22</td>
<td>32</td>
<td>38</td>
<td>37.4</td>
<td>30% &lt;217</td>
<td>36 ± 4.3</td>
<td>12 ± 1.4</td>
</tr>
<tr>
<td>≥16</td>
<td>102</td>
<td>23</td>
<td>221</td>
<td>36.9</td>
<td>19% 3,508</td>
<td>38 ± 5.5</td>
<td>13 ± 1.8</td>
</tr>
</tbody>
</table>

a Percentage of individuals with temperatures ≥37.5°C at the time of presentation.

† Mean temperature of individuals with temperatures >37.5°C.

‡ % of individuals with a packed cell volume (PCV) <33% of a hemoglobin level <11 g/dl. Based on data collected in Zone 1 in 1996.
results from the prevalence surveys, the prevalence of Hb AS was 29% in the general population in Simbok (estimated from 317 children and adults). In addition, two children were found to be homozygous for the S allele (one month old with a PCV of 43% and seven months old with a PCV of 32%). In contrast to clinical malaria, there was no relationship between Hb AS, PCV, anemia, and parasite density observed in asymptomatic malaria carriers.

The prevalence of ABO blood groups in Simbok was as follows: group O, Rhesus positive (O+) 53.5%, B+ 22.2%, A+ 20.5%, AB+ 2.7%, and O− 1.1%. No correlation was found between blood group type and parasite density or Hb S status.

Results from Etoa Village. The 1994 census survey showed that there were 457 individuals residing in Etoa, of which 203 are male and 254 female (ratio = 1:1.25). Residents lived in 92 households with the number of individuals per household varying from one to 25 persons. The distribution of the population by age is shown in Figure 3B.

Entomologic studies. During the wet season and the dry season of 1994, 783 mosquitoes were captured and analyzed. The predominant species was A. funestus (89.5%) followed by A. gambiae s.l. (15%), A. moucheti (0.9%), and A. paludes (0.2%). A parity rate of 89% was established for A. funestus. Due to sample size, parity rates for the other vectors were not determined.

Individuals residing in Etoa received an estimated 47 mosquito b/p/n throughout the year. They received an estimated 65 b/p/n from A. funestus during the wet season and 18 b/p/n during the dry season. Since the sporozoite index was 3.7% in the wet season and 2.3% in the dry season, the transmission rate was estimated to be 2.4 infectious bites/person/night (ib/p/n) in the wet season, but only 0.4 ib/p/n in the dry season. In Etoa, A. funestus was clearly the major vector.

Malaria prevalence. Prevalence studies were conducted in October 1994 and April/May 1995. In 1994, 43% of the 377 people examined were slide-positive for malaria parasites with 56% (105 of 189) of children 15 years and younger and 30% of those >16 years old being positive. In 1995, 40% of the population (80 of 200) tested were slide-positive for malaria, with 52% of the children less than 15 years old and younger and 31% of those >16 years old positive. Plasmodium falciparum was the predominant species in both years. Mixed P. falciparum and P. malariae infections were detected in 1% and 2% of the population in 1994 and 1995, respectively.

Malaria prevalence in different age groups is shown in Table 1. The highest parasite rate was in children in the 0–0.5-year age group (78%). Parasite prevalence ranged from 58% to 67% in children 0.6–10 years old, and then decreased to 34% in children 11–15 years old. The lowest rate (30%) was found in adults 16–65 years old.

Prevalence of malaria in different zones in Etoa. The percentages of people residing in Zones 1 and 2 who were slide-positive for malaria are shown in Figure 4. Data from Zone 3 were limited (n = 65 people) and thus not included in this analysis. Clearly, the pattern of malaria prevalence differed among different age groups living in Zones 1 and 2. For example, 30–50% of the children less than one year of age living in Zone 1 were infected compared with 100% of those in Zone 2 (P = 0.001). However, between the ages of one and 10 years, 65–75% of the children living in both Zone 1 and 2 were slide-positive. It appears that children living in Zone 2, where there are more A. funestus breeding sites, are infected more frequently at a young age, but that transmission is sufficiently high in both zones so that by 11–15 years of age, levels of immunity similar to those found in adults, were obtained.

Prevalence of sickle cell trait (AS) in Etoa. In Etoa, the overall prevalence of Hb AS was 28% (based on results from 395 children and adults). No relationship between Hb AS, PCV, anemia, and parasite density was observed in asymptomatic individuals.

Discussion

In 1994, longitudinal studies were initiated to follow changes in cellular and humoral immune responses in children and adults in Simbok and Etoa and to determine how these changes correlated with the acquisition of immunity to malaria. To interpret the immunologic results, information on the level of transmission and the prevalence of malaria in these two villages was needed. Results reported herein show that P. falciparum is transmitted throughout the year and is stable in both villages. However, differences in environmental conditions result in different patterns of malaria transmission in the two villages. Small pools of water are present throughout the year in Simbok that provide breeding sites favored by A. gambiae s.l. Entomologic results estimate that individuals in this village received approximately 1.9 and 1.2 ib/p/n in the wet and dry seasons, respectively. In contrast, marshes and grassy bays in the Mefou and Biyeme Rivers make A. funestus the dominant vector in Etoa. Since these marshes dry up during the dry season, individuals in Etoa received approximately six times more infectious bites during the wet than the dry season (e.g., 2.4 versus 0.4 ib/p/n). In Cameroon, there are two wet/dry seasons annually, during which approximately six months are wet and six months are dry. Therefore, we estimate that individuals in Simbok received ~550 ib/p/year and those in Etoa receive ~520 ib/p/year. This estimate agrees well with that which has been reported previously for Etoa (475 ib/p/year).38 Thus, even though the major vector species and transmission patterns differ between the two villages, individuals in both villages received similar exposure to malaria on an annual basis.

Recently, annual entomologic inoculation rates across Africa have been compared.49 Based on results from 159 distinct sites in 16 different countries, the mean annual inoculation rate was found to be 121 infected bites, with a range from 0 to 884. Rates were higher in rural areas where the mean rate of 146 ib/p/year (range = 0–884) was reported. Thus, the level of malaria exposure individuals in Simbok and Etoa receive annually (~520–550 ib/p/year) is clearly very high, but falls within the range reported in other African countries. High transmission rates have also been reported for other rural villages in Cameroon, e.g., 182 ib/p/year in villages near the Sanaga River (1989), 200 ib/p/year in Mbebe (1989), and 355 ib/p/year in Ebogo (1991).35–37 Since the early 1990s, a number of malaria control measures have been reduced and transmission of malaria appears to
be on the increase in Cameroon.\textsuperscript{10} Thus, the level of malaria exposure in Simbok and Etoa is representative of that found elsewhere in the country.

A picture of acquisition of immunity to \textit{P. falciparum} in Simbok begins to emerge by examining the data on prevalence of asymptomatic malaria (Table 1), parasite density by age (Table 2), and clinical malaria disease in Simbok (Table 3). During 1996–1998, \textasciitilde{}46\% of children less than six months of age were slide-positive for malaria and this figure increased to 73\% in children 0.6–1 year of age (Table 1). It is well established that children less than six months old are partially protected due to passively acquired maternal anti-malarial antibodies, as well as other non-immunologic factors (e.g., \textit{p}-aminobenzoic acid-deficient milk diet, fetal hemoglobin, and decreased exposure to mosquitoes).\textsuperscript{18} Clearly, infants in Simbok less than six months old quickly became infected with malaria as soon as maternal antibodies waned. During their first year of life, infants were at a high risk of both infection and disease, in that 100\% of slide-positive infants with clinical disease also had fever (Table 3) and they also had the highest parasitemias (Table 2). On average, 73\% of the children between one and five years old had asymptomatic malaria at the end of the wet season. In this age group, the children were beginning to gain some immunity, as shown by the decreased mean parasite levels (Table 2) and decreased incidence of fever in association with slide-positivity (Table 3). Between six and 15 years of age, children showed a further decrease in the prevalence of asymptomatic infections, decreased mean parasitemias, and lower levels of anemia. By 16 years of age, individuals reached “adult” levels of immunity. Thus, during the first 16 years of life, children residing in Simbok slowly gained immunity to this parasite. However, an average of 32\% of the adults still had asymptomatic malaria at the end of the wet season and 23\% presented at the clinic with malaria disease (Table 3).

A similar picture was observed in Etoa (Table 1). At the end of the wet season in 1994, more than 60\% of the children less than 10 years old had asymptomatic malaria. The prevalence decreased to 32\% in children between 11 and 15 years old, which was similar to that seen in adults (Table 1). Because of significant annual variation in prevalence, especially in the 11–15-year age groups (Table 1), one cannot conclude that there is a difference in the rate of immune acquisition in children living in these two villages. It is clear, however, that in both settings children less than 10 years old were at a high risk of infection, that some anti-parasite immunity was achieved by 11–15 years of age, and that significant protection was reached by 16 years of age. However, adults still remained susceptible to asymptomatic malaria and clinical disease. Thus, individuals in Simbok and Etoa slowly acquire immunity to disease and asymptomatic infections. Data from ongoing studies comparing antimalarial immune responses in children and adults living in the two villages will help to determine if differences in transmission patterns influence the type of immune responses that are induced.

A number of studies have compared entomologic inoculation rates and prevalence of asymptomatic \textit{P. falciparum} infection in the population. Recently, Beier and others\textsuperscript{49} compared data from 31 sites throughout Africa, using the maximum prevalence for any designated age group as an indicator of prevalence. They reported that when annual inoculation rates were comparable to that in Simbok and Etoa (i.e., between 101 and 1,000 per year), the prevalence of malaria at different sites averaged 83.2\% (range = 70.0–94.5\%). Thus, results from the current study (67\%–83\%) agree with data from other African countries. The classical definition of malaria endemicity is based on the prevalence of malaria in children less than 10 years of age.\textsuperscript{40,41} When the prevalence is between 50\% and 75\%, malaria is considered to be hyperendemic. Thus, malaria is hyperendemic in these villages. Results from ongoing immunologic studies in Simbok (and completed studies in Etoa) should be applicable to other hyperendemic African sites with equivalent inoculation and prevalence rates.

In addition to transmission, Hb polymorphisms are known to provide partial protection and influence the acquisition of immunity to malaria.\textsuperscript{42–47} The frequency of the Hb S allele in Simbok and Etoa is high, being 29\% and 28\%, respectively, for AS. This frequency is at the upper limit that can be obtained for a lethal allele in a population.\textsuperscript{49} It is therefore likely that Hb S is providing some protective advantage. Results from the current study found no association between presence of Hb AS and parasite rates in asymptomatic individuals. However, none of the children less than five years old who came to the clinic in Simbok with clinical disease carried the Hb S phenotype, even though the incidence in the population was 29\%. It appears that Hb S has a protective influence against disease but not infection. The Hb S allele is known to have a detrimental effect on \textit{P. falciparum}
by altering both invasion and parasite growth within erythrocytes. Although the sickle cell trait clearly reduces the risk of dying of malaria, the precise protective mechanism is not well understood. A mechanism of resistance-promoting modulation of the innate system by malaria-infected HbS variant cell has been proposed, in which variant infected cells undergo modification to their antigenicity, a reduction in sequestration and cytoadherent properties, and alterations in antigen processing, presentation, and recognition, which leads to accelerated and early removal of parasites. Fewer parasites reach schizogony and disease-causing eoxantigen release is reduced. In this study, the potential influence of ABO blood group polymorphism was also evaluated. No significant difference in malaria prevalence, parasite density, and/or presence of anemia was found. Despite an extensive literature on possible protective role(s) for ABO blood groups in malaria, data remain inconclusive.

In conclusion, results from the current study describe the malaria transmission and prevalence picture present in two rural Cameroonian villages. In Simbok and Etoa, malaria is hyperendemic and transmission in both villages is perennial and stable. Inoculation rates are high, and 50%–75% of children less than 10 years of age have asymptomatic parasitemias. Between the ages of 11 and 15 years, children begin to show signs of acquisition of both anti-disease and anti-parasite immunity. As in other African countries, immunity is never completely established since ~30% of the adults were blood-smear positive. Because the picture of malaria in Simbok and Etoa is similar to that found at many other African sites, information from ongoing immunologic studies should be applicable to individuals residing in rural villages throughout Africa.

Acknowledgments: We are indebted to the Chiefs and villagers of Simbok and Etoa for their participation, and especially for the continued collaboration of the Simbok population with the project. Without the approval of the Ministry of Health of the United Republic of Cameroon, the Ethical Committee of the Faculty of Medicine and Biomedical Sciences (FMBS), and continued support of Professor Maurice Sosso (Dean of FMBS, University of Yaounde I), and Professor Mimpfoundi (Director of the Biotechnology Center), this work would not have been possible. Support from the National Epidemiology Board of Cameroon (Yaounde) is gratefully acknowledged. We also acknowledge with grateful thanks the continued support of Dr. Joseph Neale (Chair, Department of Biology) for providing additional support funds. We also thank Drs. Patricia Romans, Gordon Wallace, Nancy Mendell, and Allan Saul for critical review and helpful discussions.

Financial support: Primary support for this work was provided by grant U01-AI-135839 from the National Institute of Allergy and Infectious Diseases (National Institutes of Health), with supplemental support from grant U01-AI-43888.

Authors’ addresses: Isabella A. Quakyi, Lucy Thuita Harun, Annie Walker-Akker, Michael A. Hickey, and Diane Wallace Taylor, Department of Biology, 406 Reiss Science Center, Georgetown University, Washington, DC 20057. Rose Leke, Gordon Wallace, Nancy Mendell, and Allan Saul for critical review and supplemental additional support funds. We also acknowledge with grateful thanks the continued support of Dr. Joseph Neale (Chair, Department of Biology) for providing additional support funds. We also thank Drs. Patricia Romans, Gordon Wallace, Nancy Mendell, and Allan Saul for critical review and helpful discussions.

Financial support: Primary support for this work was provided by grant U01-AI-135839 from the National Institute of Allergy and Infectious Diseases (National Institutes of Health), with supplemental support from grant U01-AI-43888.

Authors’ addresses: Isabella A. Quakyi, Lucy Thuita Harun, Annie Walker-Akker, Michael A. Hickey, and Diane Wallace Taylor, Department of Biology, 406 Reiss Science Center, Georgetown University, Washington, DC 20057. Rose Leke, Gordon Wallace, Nancy Mendell, and Allan Saul for critical review and supplemental additional support funds. We also thank Drs. Patricia Romans, Gordon Wallace, Nancy Mendell, and Allan Saul for critical review and helpful discussions.


