CHARACTERISTICS OF PLASMODIUM FALCIPARUM PARASITES THAT SURVIVE THE LENGTHY DRY SEASON IN EASTERN SUDAN WHERE MALARIA TRANSMISSION IS MARKEDLY SEASONAL

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Abstract. We have examined 83 inhabitants of Asar village in eastern Sudan, where malaria transmission lasts approximately 2–3 months each year, for the presence of Plasmodium falciparum during the prolonged dry season. All patients were treated with a standard dose of chloroquine following the first diagnosis, then examined by microscopy and the polymerase chain reaction (PCR) every two weeks for the first two months and subsequently once each month for the next 15 months throughout the dry season until the following transmission season. The PCR primers used amplified polymorphic regions of the merozoite surface protein-1 (MSP-1), MSP-2, and glutamate-rich protein genes. Results show that subpatent and asymptomatic parasitemias persisted in some patients for several months throughout the dry season, often as genetically complex infections. Different genotypes could coexist together in a single infection and the proportions of each could fluctuate dramatically during this period. However, in some individuals, single genotypes appeared to persist for several months. Reappearance of clinical symptoms among patients with chronic infections was often associated with appearance of new alleles, indicating reinfections with parasites of novel genotypes.

In the poor savannah area of eastern Sudan, the malaria transmission season is short and confined to approximately three rainy months of the year. In this region, the epidemiology of malaria is consistent with that of a hypoendemic area, where transmission occurs only following rains. A brief expansion of the mosquito population at this time leads to malaria outbreaks in October and November each year. However, during the long dry season from January to June, apart from a few clinical reports, very little is known about parasite reservoirs, or whether mosquitoes continue to transmit the parasite. A previous entomologic study suggested that the main mosquito vector, Anopheles arabiensis, retracts to scanty breeding sites where it can survive the dry season with continuous feeding activity but an incomplete reproductive cycle.1 Nothing is known about how the long dry season influences the dynamics and the structure of the parasite populations in this region.

During the past seven years, we have carried out regular surveys of the Plasmodium falciparum population in October–November in Asar village in eastern Sudan.2,3 The principal findings have been that 1) a considerable amount of allelic polymorphism exists among the genes of parasites infecting patients at this time of the year, 2) no two patients are infected with identical parasite clones, and 3) most patients are infected with a mixture of parasite clones. A great diversity was found even among parasites that caused the very early malaria cases in the transmission season.2 Such a level of diversity suggests that the parasites that appear at this time of the year may belong to a large parasite reservoir existing prior to the beginning of the rainy season. A recent study in a neighboring village has shown that sub-patent P. falciparum infections occur during the dry season.4 However, it is not known whether these were chronic infections persisting from the previous transmission season, or new infections acquired during the dry season.

In this paper, we attempt to answer the question of the origin of the annually resurgent parasite populations in villages in eastern Sudan. We have closely monitored P. falci-...
parum. Ethical clearance for this study was obtained from The Ethical Clearance Committee of the Ministry of Health of Sudan. For this study, 83 patients belonging to 18 families were recruited in October 1993. The aim and protocol of the study were clearly explained to all participants and informed consent was obtained from the head of each family. Each family was denoted by a letter, and family members by numbers. At the beginning of the study, all patients recruited harbored *P. falciparum* diagnosed microscopically by blood smear. They were all treated with a standard dose of chloroquine or sulfadoxine/pyrimethamine (Fansidar®; F Hoffmann-La Roche, Basel, Switzerland), then examined for parasites and malaria symptoms every two weeks for the first two months and subsequently each month throughout the dry season until the end of the following transmission season in December, 1994. During each visit a blood smear was taken and examined for the presence of malaria parasites, the patient’s clinical condition was monitored, and a fingerprick blood sample (approximately 100–500 µl) was collected. Patients only received chloroquine or sulfadoxine/pyrimethamine when they were shown to have microscopically detectable parasitemia and clinical symptoms. Collected blood samples were separated into red blood cells and plasma by centrifugation and stored in liquid nitrogen for subsequent analysis.

Detection of malaria parasites and identification of polymorphic genes by PCR and oligonucleotide probes. Microscopic detection. Thick and thin blood smears were collected from each patient at each time point. Smears were stained with Giemsa and examined microscopically. A film was declared negative after examination of 100 thick fields, which took approximately 20 min.

Polymerase chain reaction (PCR) detection and characterization of *P. falciparum* genes. Allelic variants of three *P. falciparum* genes, merozoite surface protein-1 (MSP-1),6 MSP-2,7 and glutamate-rich protein (GLURP),8 were investigated by PCR. Plasmidial DNA was first extracted from 10–20 µl of each blood sample using a resin buffered suspension matrix (Instagene Matrix) as described by the manufacturer (Bio-Rad, Hemel Hempstead, United Kingdom). The PCR amplification of each gene was then carried out using six pairs of primers, one pair for the initial round of amplification and one pair for the nested PCR to enhance the sensitivity of detection of subpatent infections.9,10 The primers amplified respectively a polymorphic region (block 2) of MSP-1,11 most of MSP-2,12 and the central region of the GLURP gene.8

To characterize alleles of these genes, the PCR-amplified fragments were separated by electrophoresis on 1.6% agarose gels, their sizes were calculated, and they were then transferred onto nylon membrane by Southern blotting. Variations in the length of the amplified fragments of each gene were identified as size differences of the PCR products. Due to the continuous nature of size variation of the amplified fragments of the two genes, alleles with similar sizes, within 20-basepair (bp) differences, were grouped into discrete bins around an estimated average size.13 In addition, three sequence classes of the amplified block 2 of the MSP-1 gene, denoted K1, MAD20, and RO33,14 and two of block 3 of the MSP-2, denoted IC1 and FC27, were identified by sequence-specific probes hybridized to Southern blots of PCR-amplified fragments.15 In addition, a probe was used that recognizes alleles of the FC27 form of MSP-2 that lacks the 12 amino acid repeat16 denoted SUD (5’-GTTCA-AGTTCTGGCAATGCA-3’).

RESULTS

At the start of the study, 75 of the 83 patients were found to be infected with *P. falciparum* and eight with a mixture of *P. falciparum* and *P. vivax*; their initial parasitemias ranged from 360 parasites/µl of blood to 264,000 parasites/µl of blood. Seventy-four patients completed the longitudinal follow-up period from October–November 1993 to December 1994.

Pattern of *P. falciparum* infection in patients over a period of 15 months. The PCR characterization of *P. falciparum* was carried out on all samples collected from each individual throughout the study period. Complete characterization of detected parasites was successfully achieved for 65 of the 74 patients who completed the study. Nineteen of these patients cleared their primary *P. falciparum* infection following treatment in October–November 1993, and were not reinfected during the transmission season of 1994 (Figure 1A). Twenty patients cleared their primary infection, some of whom showed a delayed parasite clearance, and became infected again during the transmission season of 1994 (Figure 1B). Twenty-six patients did not clear their infection, and maintained parasitemias that fluctuated between patent and subpatent levels throughout the follow-up period (Figure 1C). Figure 2A summarizes the frequencies of these three types of infections among the studied patients.

The ages of all patients who cleared their primary infections, whether or not they became reinfected during the transmission season of 1994, ranged between seven and 60 years (average = 17.3 years). The age of patients who had chronic asymptomatic infections ranged between six and 22 years (average = 11.6 years). No difference in the influence of age on the pattern of infection was observed (P > 0.05).

Reproducibility of the PCR. All samples that gave no PCR product for all three genes examined were repeated twice with a bigger volume of DNA and in all cases no PCR product could be obtained. We classified these samples as negative although it is possible that in some cases parasites may be present but sequestered outside the peripheral blood circulation at the time of sampling. Moreover, approximately 10% of the samples that gave PCR products were checked twice. In all of these samples discrepancies in PCR results were not observed.

Characteristics of initial *P. falciparum* isolates (October–November 1993). Extensive allelic polymorphisms were observed among the parasites that caused the initial infections in the 65 patients examined. At least 20 alleles of MSP-1, 23 of MSP-2, and 21 of GLURP were identified. One rare allele (650-bp/SUD type) of MSP-2 was identified in a single patient, which was found in this patient throughout the study period as a chronic infection.

Each of the 65 patients was found to harbor different parasites, and this was the case even among members of the same family. Table 1 shows the characteristics of the *P. falciparum* infections of family L, in which each family member was infected with different parasites characterized by
FIGURE 1. Alleles of the merozoite protein-2 gene in *Plasmodium falciparum* infections detected by the polymerase chain reaction among patients in Asar village, Sudan. **A,** an example of a patient found to be infected in October 1993 (lane 2). The parasites were cleared following chloroquine administration, and no parasites were detected in blood samples collected between October 1993 and December 1994 (lanes 3–16).

**B,** an example of a patient found to be infected in October 1993 (lane 2). The infection lasted for at least two weeks (lane 3) following chloroquine treatment. No parasites were detected in blood samples collected between November 1993 and October 1994 (lanes 4–13). However, parasites appeared in samples collected in November and December 1994, indicating a reinfection (lanes 14–16).

**C,** an example of a patient found to be infected in October 1993 who did not clear his infection despite the administration of chloroquine (lanes 2–16). However, this patient remained asymptomatic during the follow-up period. Lanes 1 and 17 contain molecular size markers. bp = basepairs.

Unique alleles of MSP-1, MSP-2, and GLURP identified by size and/or probe hybridization. Even family members L1 and L2, who came to the clinic on the same day, harbored different parasites, as did members L4 and L5. Similar patterns of diversity were observed among other families.

Multiclonal infections, identified by the presence of at least two alleles of at least one of the studied genes in a single patient, occurred at a frequency of 75% among the initial samples in October–November 1993. Subsequent sampling revealed more multiclonal infections among in-
affected individuals, and the overall frequency increased to 91%.

**Characteristics of *P. falciparum* causing chronic infections.** Twenty-one of the 26 patients with persisting parasitemias had initial infections containing multiple genotypes. The other five patients had single clonal infections, one of whom maintained the same clone throughout the study period. The remaining four in this group were found to harbor multiple clones in subsequent samplings (Figure 2B). In the patients with multiclonal infections, the combination of different alleles of the studied genes changed over time, even during the dry season. In six patients, the rate of change was remarkable, with different combinations of alleles of MSP-1, MSP-2, and GLURP occurring at every sampling point. An example of such a patient (C3) is shown in Table 2. However, in 13 patients the same combinations of these genes persisted for 2–3 months and then changed. In six patients, the same combinations of alleles of each gene were maintained for periods of 5–7 months (Table 3). This group included two patients with multiclonal infections at the start, but in whom only a single clone persisted during the dry season (Figure 3).

![Figure 2](image)

**Table 1.** MSP-1, MSP-2, and GLURP alleles in initial *Plasmodium falciparum* infection among five members of family L*

<table>
<thead>
<tr>
<th>Code</th>
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<th>MSP-2</th>
<th>GLURP</th>
</tr>
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<td>+480</td>
</tr>
<tr>
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<td>+(580)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L3</td>
<td>11/8/93</td>
<td>+(480)</td>
<td>+540</td>
<td>+480</td>
</tr>
<tr>
<td>L4</td>
<td>11/24/93</td>
<td>+540</td>
<td>+540</td>
<td>+480</td>
</tr>
<tr>
<td>L5</td>
<td>11/24/93</td>
<td>–</td>
<td>+520</td>
<td>+480</td>
</tr>
</tbody>
</table>

* MSP-1 = merozoite surface protein-1; MSP-2 = merozoite surface protein-2; GLURP = glutamate-rich protein; – = absence of an allele; + = presence of an allele. Values in parentheses are estimated sizes of alleles in basepairs.

All 26 patients who harbored chronic infections had clinical *P. falciparum* malaria when they were recruited into this study in October–November 1993. Some of them also experienced successive clinical episodes following the initial attack (Table 4). However, as the dry season progressed, clinical episodes among these 26 patients reduced to four; four, two, and three during December 1993; January, February, and March 1994, respectively. Between March and September 1994, all of these infected individuals remained asymptomatic, except for one who had fever in July 1994.

Six of the 26 patients with chronic *P. falciparum* infections experienced fresh clinical episodes during the transmission season of 1994 (September to December). In four individuals these episodes were clearly associated with the appearance of genotypes differing from those in the preceding months (Figure 3). Table 4 shows an example of data on clinical episodes and parasite genotypes obtained from a nine-year-old girl who was found to harbor a chronic *P. falciparum* infection. She experienced two successive episodes of malaria in October and November 1993, each of which was distinguishable by different *P. falciparum* clones. Furthermore, she had two malaria episodes in February and at the end of September 1994, with the latter episode occurring during the transmission season. The episode that occurred during the dry season in February 1994 was not associated with new clones, although her fever in September 1994 clearly was associated with new distinct clone(s) (Table 4 and Figure 3).

The one patient (F3) who appeared to have been infected with a single clone, which persisted between November 1993 and December 1994 (Table 5), experienced a clinical malaria episode in December 1993 associated with an increase in parasitemia to a level detectable by microscopy.

**Plasmodium falciparum** infections in the transmission season of 1994. All 26 patients with chronic infections had some or all of the original *P. falciparum* clones encountered in 1993 during the next transmission season in 1994. However, some of these patients showed an evidence of infection with new clones during that period. Moreover, among patients who cleared their infection of 1993, 20 were reinfected during the transmission season in 1994. All of them were infected with *P. falciparum* clones that were different from those of 1993.

**DISCUSSION**

The primary purpose of the current study was to attempt to define the source of the highly diverse *P. falciparum* in-
defects that cause the annual outbreaks of malaria in Asar village in eastern Sudan, following the brief rainy season.\textsuperscript{2} We have shown that many people who become infected at this time continue to harbor chronic infections throughout the ensuing dry season, many despite drug treatment. These infections frequently contain a multiplicity of genetically distinct clones that undergo a turnover in composition in some individuals, while in others single clones may persist for several months. While we cannot rule out human or vector immigration as a possible source of new parasites into the village, we consider that these persisting infections form the most likely reservoir of the following season’s infection.

This work is the first to reveal the diversity of \textit{P. falciparum} clones in the inhabitants of a village monitored regularly over a whole year. Our findings extend those of others who have carried out similar studies over shorter periods, and in areas of differing malaria transmission intensity. In the village of Darawish near Asar, Roper and others\textsuperscript{5} carried out surveys in April and June, and observed the presence of \textit{P. falciparum} infections in some inhabitants with PCR primers detecting ribosomal DNA. In an area of unstable transmission in Senegal, Daubersies and others\textsuperscript{17} found that some asymptomatic individuals maintained similar \textit{P. falciparum} clones over a five-week period in the dry season. However, these investigators also showed that in Dielmo village in Senegal, in which there is year round \textit{P. falciparum} transmission, a turnover of diverse clones occurred in patients sampled over a three-month period. In the same village, Contamin and others\textsuperscript{18} examined parasites collected every two weeks from 10 children over a three-month period, and showed that new clinical malaria episodes were associated with the appearance of new genotypes and/or an increase in parasitemia. In one study in a highly endemic area of Tanzania, daily samples revealed that different \textit{P. falciparum} clones appeared on successive days,\textsuperscript{19} presumably due to sequestration of different parasite cohorts every 24 hr.

Asymptomatic carriage of \textit{P. falciparum} infections among clinically immune inhabitants of areas with year-round transmission is well known.\textsuperscript{20,21} \textit{Plasmodium falciparum} infection can persist asymptotically in semi-immune individuals for more than 18 months, in conditions where the possibility of reinfection is excluded.\textsuperscript{22} The current work has revealed a

\begin{table}
\centering
\caption{MSP-1, MSP-2, and GLURP alleles of \textit{Plasmodium falciparum} isolates collected from patient C3 (female/six years of age) in Asar village, Sudan between November 1993 and December 1994\textsuperscript{*}}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Date of collection & K1 & MSP-1 & RO33 & IC1 & MSP-2 & GLURP \\
\hline
11/22/93 & + (480) & + (580, 500) & + (480) & + (500) & + (560) & + (620, 540) \\
12/1/93 & - & + (580) & + (480) & + (540) & - & + (540) \\
12/20/93 & + (480) & - & + (500) & + (480) & + (780, 540) \\
1/4/94 & + (480, 560) & - & + (500) & - & + (520) \\
1/26/94 & + (480, 560) & + (480) & + (500) & - & + (520) \\
3/1/94 & + (560) & + (480) & - & + (540) & - & + (920, 520) \\
5/1/94 & + (560) & - & - & + (520) & - & + (520) \\
6/3/94 & + (560) & + (480) & - & + (520) & - & + (520) \\
6/27/94 & - & + (480) & - & + (620) & - & + (560) \\
7/30/94 & + (560) & + (480) & - & +(540, 480) & + (540) & + (520) \\
8/30/94 & - & + (560) & - & + (540) & + (480, 540) & + (520) \\
9/28/94 & - & + (560) & - & + (540) & + (480, 535) & + (520) \\
10/20/94 & - & + (480) & - & + (540) & (480, 540) & + (920) \\
11/27/94 & - & + (560) & - & + (540) & - & + (540) \\
12/27/94 & - & - & - & + (540) & - & + (480) \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{MSP-1, MSP-2, and GLURP alleles of \textit{Plasmodium falciparum} isolates collected from patient T4 (female/17 years of age) in Asar village, Sudan between November 1993 and December 1994\textsuperscript{*}}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
Date of collection & BF & Treatment & Fever\textsuperscript{†} & K1 & MSP-1 & MAD20 & RO33 & IC1 & MSP-2 & FC27 & GLURP \\
\hline
11/20/93 & P.f. & CQ & + & + (560) & + (480) & + (480) & + (540) & + (560) & + (560) & + (840) \\
\hline
\end{tabular}
\end{table}

\textsuperscript{*} BF = blood film; P.f. = infection with \textit{P. falciparum}; CQ = chloroquine treatment. For definitions of other abbreviations and other information, see Table 1. \\
\textsuperscript{†} + and - indicates presence and absence of malaria symptoms.
FIGURE 3. Alleles of the merozoite protein-2 gene of *Plasmodium falciparum* in patient J2 who was found to maintain a chronic asymptomatic infection, as detected by the polymerase chain reaction (PCR) and hybridization with allele-specific probes. **A**, PCR-amplified fragments separated by electrophoresis. **B** and **C**, Southern blots of these fragments hybridized with probes IC1 and FC27, respectively. **D**, interpretation of results. Lane 1 contains a molecular size marker; lanes 2 and 3 contain control *P. falciparum* clones 3D7 and HB3, respectively; lanes 4–18 are *P. falciparum* isolates collected between October 1993 and December 1994. Note the size and sequence variation between isolates. Some isolates have two alleles differing by sequence (lane 4) or size (lane 5). bp = basepairs. Also note the that changes in parasite genotype during the next transmission season in September 1994 (lane 15) were associated with a clinical episode (see Table 4).
great proportion of inhabitants carrying prolonged \textit{P. falciparum} infection in this area of seasonal transmission. Considerable genetic complexity was observed among the parasites in these patients in the dry season in Asar. The principal questions that arise from these findings are 1) what is the mechanism by which a given clone can persist for several months in a patient, 2) what is the explanation for the appearance of parasites with novel genotypes during the dry season, 3) what is the cause of the clinical episodes occurring in some patients during this period, and 4) what is the source of the gametocytes giving rise to the epidemic type of \textit{P. falciparum} that occurs in October–November each year?

With regard to the persistence of a given clone, a simple explanation might be due to drug resistance. Previous work in Asar showed clearly that clones with a wide range of response to chloroquine and pyrimethamine coexisted in this village. There are also cases of clinical failure to sulfadoxine/pyrimethamine (Babiker HA, unpublished data). Assuming that only drug-resistant clones survive the dry season to initiate the next transmission, then effective selection may result in steady increase in frequency of resistant parasites.\textsuperscript{23,24} However, longitudinal studies indicated stable frequency of chloroquine-resistant parasites in this area\textsuperscript{3} (Satti G, unpublished data). This suggests that not only drug-resistant \textit{P. falciparum} clones, but sensitive ones, as well, are persisting during the dry season as asymptomatic infections, unexposed to drug pressure.

The role of antigenic variation in maintaining asymptomatic infections must be considered. Recently, it was shown that the \textit{P. falciparum} genome contains a large family of genes (\textit{var} genes), some of which encode variant antigens known as \textit{P. falciparum} erythrocyte membrane proteins that undergo differential expression.\textsuperscript{25,26} These antigens are thought to act as sequestration ligands for various receptors on human endothelial cells.\textsuperscript{25,26} It has been hypothesized that variation in the expression of members of the \textit{var} gene family helps the parasite to escape the host immune response. The presence of chronic infections of single clones in some patients in Asar gives support to such an idea, and provides suitable material to test this hypothesis. Variations in antigen gene expression may allow the parasite to escape the cumulative immune response in patients with chronic infection and thus lead to their survival in the human host until transmission becomes possible.

The fluctuations of different parasite clones in a single patient during the dry season could have several causes. It is possible that some limited mosquito transmission of \textit{P. falciparum} continues at a local level during this time, and thus the appearance of novel genotypes could be due to superinfection. However, surveys of mosquitoes by spray catches during the dry season in Asar have shown that their

### Table 4

<table>
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<tr>
<th>Date of collection</th>
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<th>MSP-1 K1</th>
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<th>MSP-2 IC1</th>
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\*G = gametocyte stages. For definitions of other abbreviations and other information, see Tables 1 and 3.

### Table 5

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<th>Date of collection</th>
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<td>+ (500)</td>
<td>-</td>
<td>+ (800)</td>
</tr>
<tr>
<td>5/1/94</td>
<td>Pf.</td>
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<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>1/26/94</td>
<td>Pf.</td>
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<tr>
<td>2/28/94</td>
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<td>-</td>
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<td>-</td>
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<td>+ (500)</td>
<td>-</td>
<td>+ (800)</td>
</tr>
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<td>-</td>
<td>+ (800)</td>
</tr>
<tr>
<td>5/1/94</td>
<td>Pf.</td>
<td>-</td>
<td>-</td>
<td>+ (600)</td>
<td>-</td>
<td>-</td>
<td>+ (500)</td>
<td>-</td>
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</tr>
<tr>
<td>6/2/94</td>
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<td>-</td>
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<td>-</td>
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<td>+ (500)</td>
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<td>+ (800)</td>
</tr>
<tr>
<td>9/27/94</td>
<td>Pf.</td>
<td>CQ</td>
<td>+</td>
<td>+ (500)</td>
<td>-</td>
<td>-</td>
<td>+ (620)</td>
<td>+ (1,030)</td>
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</tr>
<tr>
<td>10/27/94</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (500)</td>
<td>-</td>
<td>-</td>
<td>+ (620)</td>
<td>+ (1,030)</td>
<td>-</td>
</tr>
<tr>
<td>11/26/94</td>
<td>Pf.</td>
<td>-</td>
<td>-</td>
<td>+ (500)</td>
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<td>-</td>
<td>+ (620)</td>
<td>+ (1,030)</td>
<td>-</td>
</tr>
<tr>
<td>12/27/94</td>
<td>Pf.</td>
<td>-</td>
<td>-</td>
<td>+ (500)</td>
<td>-</td>
<td>-</td>
<td>+ (620)</td>
<td>+ (1,030)</td>
<td>-</td>
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\* For definitions of abbreviations and other information, see Tables 1 and 3.
numbers decrease dramatically after the rains, with none being caught between April and June (Karrar A, 1996, Seasonality of Malaria Transmission in Asar and Daraweesh Villages [Gedaref State, Eastern Sudan], M. Sc. Thesis, University of Khartoum, Khartoum, Sudan). In the absence of mosquito transmission, the newly appearing clones could only have originated from parasites already present in the patient, but undetectable at the earlier times of sampling. Such parasites could have been sequestered outside the peripheral circulation at this time, or have been at levels too low to have been detectable. If so, the reasons for such variations can only be the subject of speculation. Oscillation of clones might be driven by variations in the sequestration pattern of existing clones.\(^{17,29}\) Varying levels of strain-specific immunity during the dry season could presumably be involved, but nothing is known of this subject in natural infections.

With regard to new episodes of malaria symptoms, a current view is that they may be due to infection with a novel strain.\(^{27}\) This is very likely to be a cause of many of the symptomatic infections seen in October–November each year following the rains. Most patients with persisting parasitemias remained asymptomatic during the dry season, but 23% of them reported fever after the start of malaria transmission in 1994. We have found that two-thirds of these episodes were associated with the appearance of new P. falciparum clones, a finding similar to that of Contamin and others\(^{19}\) in Senegal discussed above. A factor to be considered in this regard is that following the resurgence of mosquitoes after the rains, mixed clones from patients with persisting parasitemias taken up by the mosquitoes could undergo cross-mating to generate new P. falciparum genotypes not previously present in the village. Also, one person in Asar is likely to encounter very few infective mosquitoes each transmission season,\(^3\) and therefore would experience only a limited number of the many different clones in circulation in the village. Assuming that protective immune responses are clone-specific, an individual would thus be susceptible to the majority of clones existing in the village. However, this explanation cannot account for the clinical episodes in a few people who apparently harbored one and the same type clone, or a group of identical clones, before and after each clinical episode.

Previous mathematical modeling of malaria indices has estimated parasite rates in excess of that detected by microscopy.\(^{28}\) The PCR diagnosis has supported such estimates and highlighted a great proportion of subpatent P. falciparum infections not revealed by microscopy.\(^{29,30}\) This study has extended these findings and highlighted the nature of chronicity of P. falciparum infection in an area of unstable malaria and the multiplicity of clones in people during the dry season. Some of these chronic infections showed gametocytes on the rare occasions when they became patent. In the absence of molecular techniques for gametocyte detection, it is impossible at present to assess the gametocyte rate among subpatent infections. However, such knowledge is clearly important and could lead to a method of malaria control by targeting individuals harboring gametocytes using appropriate chemotherapy.

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