Epidemiology of Plasmodium malariae infection in Gambela, Ethiopia

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Abstract
Malaria incidence and prevalence surveys were performed from December, 1967 to February, 1969 among the indigenous Nilotic inhabitants of Gambela, a small administrative centre in the western lowlands of Ethiopia. Entomological data suggested that malaria transmission was seasonal and this was consistent with monthly P. falciparum parasite rates. Monthly P. malariae parasite rates, however, were consistent with an hypothesis of homogeneity. The age-specific incidence of quartan malaria among 26 children zero to 11 years old at the start of study was examined at 28 day intervals over a 15-month period. The resulting data suggested that parasite acquisition was a slow process and an annual P. malariae incidence of 0.17 was derived. This statistic was supported by studies performed five years later: The incidence of P. malariae among 102 infants followed from birth up to 48 months of age was 0.16-0.20. An attempt was then made to account for the prevalence of P. malariae in terms of the entomological conditions observed in Gambela. Macdonald’s formula for the sporozoite rate was used to derive hypothetical relative proportions of P. falciparum and P. malariae among the observed sporozoite-positive mosquito populations. About 4% of the sporozoite challenges were estimated to be of P. malariae. An hypothetical annual entomological P. malariae inoculation rate was then made by multiplying the number of observed sporozoite inoculations per person (approximately 10/year) by the proportion of them estimated to be of P. malariae. The annual P. malariae sporozoite challenge was thus estimated at 0.4 per person, in good agreement with the annual incidence estimates from parasite rates in children.

Disciplines
Community Health and Preventive Medicine | Entomology | Epidemiology | Parasitic Diseases | Public Health

Comments
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EPIDEMIOLGY OF *PLASMODIUM MALARIAE* INFECTION IN GAMBELA, ETHIOPIA

E. S. KRAFSUR * and J. C. ARMSTRONG **

**INTRODUCTION**

Malaria surveys were carried out at 28-day intervals from December, 1967 to February, 1969 among the Nilotic inhabitants of Gambela, a small town in the lowlands of southwestern Ethiopia. It was found that *Plasmodium falciparum* was the most prevalent malaria parasite, followed in frequency by *P. malariae* (Armstrong, 1972). The prevalence of *falciparum* malaria was markedly seasonal while that of *P. malariae* showed no clear seasonal trends (Krafsur and Armstrong, 1978). Maximum *falciparum* parasitaemias occurred late in the season of maximum sporozoite challenge. At these times, December to early February, average *falciparum* parasite rates were 58% among children (< 15 years) and 35% among adults (≥ 15 years). *P. malariae* frequencies of parasitaemia were 16% among the same children and 7% among the adults. Similar values were obtained in years subsequent to 1969 (Armstrong, unpublished).

An attempt was made to relate seasonal prevalence of *falciparum* malaria to corresponding entomologically estimated sporozoite inoculation rates (Krafsur and Armstrong, 1978). It was assumed, for analytical purposes, that all sporozoite inoculations by *Anopheles* were of *falciparum*, an assumption common in field studies of malaria dynamics (Macdonald, 1955; Pull and Grab, 1974; Dietz et al., 1974). But some of the inoculations, measured by entomological methods, must have been *P. malariae*.

The present report examines first the temporal prevalence in man of *P.*

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malariae in Gambela against the risk of an average inhabitant being subjected to one or more sporozoite challenges. The age-specific incidence of detectable infection is then estimated from repeated examinations at 28-day intervals of 26 longitudinally followed children (0-11 yrs.). The resulting approximation of an incidence rate is compared to one estimated from a much larger number of infants studied five years later. An analytical procedure is carried out with the object of estimating the relative proportions of sporozoite-positive Anopheles infected with P. falciparum and with P. malariae. A hypothetical rate of malariae sporozoite inoculation is then derived and compared against the observed age specific incidence of quartan malaria.

Site of investigation and the human population

Gambela, a small administrative centre, lies on the River Baro, a major tributary of the White Nile, in the western lowlands of Ethiopia.

The majority of Gambela's approximately 1600 inhabitants are the Anuak, a Nilotic tribe indigenous to the Western Ethiopian lowlands and Southeastern Sudan. A good description of the area and its people is given by Hutchinson (1971). Major health problems among the Anuak are addressed by Armstrong (1972).

The climate of the study area is quite similar to that observed in the Northern Guinea savannah of West Africa. Rainfall, averaging about 1530 mm per annum, is markedly seasonal, occurring principally from May to October. A severe dry season occurs typically from November to April, when relative humidities, stream levels, Anopheles populations, and sporozoite rates all plunge from their wet season values. The relevant climatological and entomological data are tabulated by Krafsur (1977).

Methods and materials

Parasitological and epidemiological

The subjects were Anuak and indigenous to the study area. At 28-day intervals visits were made to the various Anuak ‘neighbourhoods’ and thin and thick blood smears taken. Attention was concentrated on 72 subjects, 32 of whom were children (< 15 years old), participating in a longitudinal survey of 15 months, from December, 1967 to February, 1969. Numerous villagers not included in the original longitudinal study group were also sampled at these times. Infants born into the Anuak community of Gambela during 1973-1976 were followed longitudinally from birth at 30-60 day
intervals (with a substantial frequency of lapses) up to the age of about four years. This group of 102 subjects provided an independent estimate of *P. malariae* incidence additional to that obtained from the earlier survey.

Access of the Anuak study population to chloroquine, while always a possibility, was reduced by its high cost. Further details of procedures used in selecting subjects for participation in these surveys are offered by Armstrong (1972).

Thick blood smears, stained in Geimsa within 18 hours of preparation, were examined for at least three minutes each. Thin films were used to confirm species diagnosis when necessary. Smears were extensively cross-checked by each of three microscopists.

Entomological procedures

Sampling methods for *Anopheles* mosquitoes included pyrethrum space spray of 24 huts weekly and twice weekly indoor and outdoor all-night man-biting captures. Monthly man-biting rates and sporozoite rates were established for each of the vector species, *A. funestus* Giles, *A. arabiensis* Patton (formerly *A. gambiae* Giles species B), and *A. nili* (Theob.). The man-biting habit was estimated to be 100% among indoor resting specimens of all three species. The entomological investigation was carried out from July, 1967 to February, 1969 and reported in detail (Krafsur, 1970, 1977).

**RESULTS**

Seasonal aspects of *P. malariae* infection

Table 1 shows the monthly proportions of subjects, by age group, among whom *P. malariae* was detected. Corresponding monthly estimates of risk of sporozoite inoculation are also set forth in Table 1. This risk is defined (Krafsur, 1977, Krafsur and Armstrong, 1978) as the probability of an inhabitant receiving one or more sporozoite inoculations (of any *Plasmodium* species) during the course of a month and was estimated by entomological methods. Sporozoite challenge was clearly seasonal, 82% of the annual inoculations occurring in the wet season months of September to December, 1968.

The average monthly prevalence of *P. malariae* parasitaemia was 14.5% among children. No clear seasonal trends were evident and the monthly proportions positive were consistent with a hypothesis of homogeneity ($\chi^2 = 21.1$, 14 d.f., $P = 0.1$) when these were subjected to a variance test.
TABLE 1
Plasmodium malariae parasite rates among indigenous Anuaks in Gambela, December 1967 - February 1969

<table>
<thead>
<tr>
<th>Period</th>
<th>Sporozoite inoculations</th>
<th>Risk $^1$</th>
<th>Proportions positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Nov.</td>
<td>1.30</td>
<td>0.73</td>
<td>-</td>
</tr>
<tr>
<td>Dec.</td>
<td>1.15</td>
<td>0.68</td>
<td>63</td>
</tr>
<tr>
<td>Jan.</td>
<td>0.29</td>
<td>0.25</td>
<td>45</td>
</tr>
<tr>
<td>Feb.</td>
<td>0.59</td>
<td>0.45</td>
<td>59</td>
</tr>
<tr>
<td>Mar.</td>
<td>0</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>Apr.</td>
<td>0.01</td>
<td>0.01</td>
<td>89</td>
</tr>
<tr>
<td>May</td>
<td>0.17</td>
<td>0.16</td>
<td>5-</td>
</tr>
<tr>
<td>Jun.</td>
<td>0.10</td>
<td>0.10</td>
<td>44</td>
</tr>
<tr>
<td>Jul.</td>
<td>0.22</td>
<td>0.20</td>
<td>37</td>
</tr>
<tr>
<td>Aug.</td>
<td>0.46</td>
<td>0.37</td>
<td>107</td>
</tr>
<tr>
<td>Sept.</td>
<td>2.48</td>
<td>0.92</td>
<td>36</td>
</tr>
<tr>
<td>Oct.</td>
<td>2.06</td>
<td>0.87</td>
<td>33</td>
</tr>
<tr>
<td>Nov.</td>
<td>2.23</td>
<td>0.89</td>
<td>38</td>
</tr>
<tr>
<td>Dec.</td>
<td>1.54</td>
<td>0.79</td>
<td>39</td>
</tr>
<tr>
<td>Jan.</td>
<td>-</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>Feb.</td>
<td>-</td>
<td>-</td>
<td>93</td>
</tr>
<tr>
<td>Totals</td>
<td>10.15</td>
<td>&gt;0.99</td>
<td>886</td>
</tr>
<tr>
<td>Means</td>
<td>0.85</td>
<td>0.57</td>
<td>0.145</td>
</tr>
</tbody>
</table>

$^1$ Risk = 1 - $e$-inoculations/person/month.

(Snedecor and Cochran, 1967, p240). Average monthly proportions positive for *P. malariae* were only 3.3 % among the adults and also were consistent with a hypothesis of homogeneity ($\chi^2 = 16.4, 14$ d.f., $P = 0.29$).
Gametocytaemia. The average monthly *falciparum* gametocyte rate in children was 25.5% (114/445), and among adults, 6.8% (37/540). The average monthly *malariae* gametocyte rate in children was 3.4% (15/445) and only 0.18% (1/540) in adults.

Detectability of *P. malariae* infection in blood smears

Temporal homogeneity of *malariae* parasite rates among the examination subjects, even though sporozoite challenge was clearly seasonal, was likely a result of the great persistence and low average densities of parasitaemia (Shute and Maryon, 1951; Covell, 1960). The detectability of *P. malariae* on any one examination was not great due to its low density and to intermittent parasitaemia. Under these circumstances, the proportions of people positive for *P. malariae* on one or more occasions in at least 10 separate blood examinations provides a more realistic index of an infection rate (Table 2). Considering subjects in the longitudinal study group who had been examined at least 10 times, 17 of 30 (57%) children were diagnosed as positive at

<table>
<thead>
<tr>
<th>Age at start of survey</th>
<th>Age midpoint</th>
<th>Subjects positive/examined ²:</th>
<th>Slides positive/total slides ³:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>Proportion ± S.E.</td>
</tr>
<tr>
<td>0-2</td>
<td>1.5</td>
<td>1/5</td>
<td>0.200 ± 0.18</td>
</tr>
<tr>
<td>3-5</td>
<td>4.5</td>
<td>6/11</td>
<td>0.545 ± 0.15</td>
</tr>
<tr>
<td>6-8</td>
<td>7.5</td>
<td>4/5</td>
<td>0.800 ± 0.18</td>
</tr>
<tr>
<td>9-11</td>
<td>10.5</td>
<td>4/5</td>
<td>0.800 ± 0.18</td>
</tr>
<tr>
<td>12-14</td>
<td>13.5</td>
<td>2/4</td>
<td>0.500 ± 0.25</td>
</tr>
<tr>
<td>15-20</td>
<td>18.0</td>
<td>3/11</td>
<td>0.273 ± 0.13</td>
</tr>
<tr>
<td>≥ 21</td>
<td></td>
<td>4/25</td>
<td>0.160 ± 0.07</td>
</tr>
</tbody>
</table>

¹ Reflecting increase in age over duration of survey.
² At least once in 10-16 examinations with exception of 21 year age group which includes an individual 2+ in 3 examinations.
³ No of samples (blood smears) positive of total no of smears from subjects with patent parasitaemia on one or more occasions after the first positive episode.
least once. The detectability of *P. malariae* among these subjects known to be infected may be estimated by taking the number of positive slides *after* the first positive examination as a fraction of the total. Among the 17 children known to have *P. malariae*, 45 of 128 (35.2%) examinations were positive. The frequency of positivity in individuals varied from 15/15 to 0/5. Detectability of *P. malariae* among infected children studied during 1973-76 was 77 positive of 216 (35.6%) examinations. The two estimates are obviously homogeneous. Among the 34 adults examined at least 10 times, seven were positive on one or more occasions (30.6%). The average detectability among the positive adults, measured after the first *P. malariae* diagnosis, was five positive of 38 slides (13.2%).

**Annual incidence of *P. malariae* in children**

Table 2 shows the proportions of subjects, by age group, who were positive for *P. malariae* on at least one sampling occasion during the 15 month study period. The age specific period prevalence, among 30 children examined 10-16 times each, increases progressively up to the age of puberty, when a marked decline occurs. These data allow an estimate of annual incidence.

Rates of becoming positive for *P. malariae* are best calculated for yearly time units because sporozoite challenge was clearly seasonal. Also, *P. malariae* positivity of children was determined by any one or more of 10-16 separate examinations made over a continuous 15-month period.

Suppose that (i) *P. malariae* sporozoite challenge was fairly constant annually, (ii) the subjects of parasitological examination resident in Gambela all of their lives, (iii) recovery from primary infection nil over the first 7-10 years of childhood, (iii) these children all being susceptible to infection, and (v) the parasitological test adequate to detect all those who experienced a primary infection. The cumulative incidence $x_t$ of *P. malariae* infection, under the foregoing assumptions, is

$$x_t = 1 - e^{-\alpha t}$$ (Macdonald, 1950, Draper et al., 1972),

where $\alpha$ is the annual incidence rate, and $t$ is age in years. This is the same as the one-way catalytic model of Muench (1959), who sets forth its derivation and discusses its assumptions.

Substituting the observed frequencies of parasitaemia, $x_t$, for the age class midpoints in which they occur and solving for $\alpha$, find

$$\alpha = -\log_e(1 - x_t)/t.$$. 
Epidemiology of P. malariae

An age of nine is used as the midpoint for the 6-8 and 9-11 year age, these two classes showing similar frequencies of parasitaemia:

<table>
<thead>
<tr>
<th>Midpoint age (t, in years)</th>
<th>P. malariae frequency, ( x_t )</th>
<th>Annual rate, ( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>0.20</td>
<td>0.159</td>
</tr>
<tr>
<td>4.5</td>
<td>0.545</td>
<td>0.175</td>
</tr>
<tr>
<td>9.0</td>
<td>0.80</td>
<td>0.179</td>
</tr>
</tbody>
</table>

Mean \( \alpha = 0.17 \)

Further parasitological surveys carried out during 1973-1977 support the foregoing estimate. The incidence of *malariae* was recorded among 102 infants born over this four year interval and followed longitudinally. Incidence rates were estimated for successive three month periods among subjects never before found positive. There were one to three blood examinations per child per 91 day interval. The incidence of primary *malariae* infections is additive. There was a sum of 31 primary detections among 804 susceptible infant-periods. A mean annual infective inoculation rate \( \alpha \) is obtained as before:

\[
\alpha = \frac{-\log_e(1 - x)}{t},
\]

where \( x \) is the proportion of infants found positive for the first time and \( t \) is the unit of time in which incidence was measured, 91 days (0.25 yr.). Substituting,

\[
\alpha = \frac{-\log_e(1 - 31/804)}{0.25}
\]

\( \alpha = 0.158. \)

Children less than six months old suffered an incidence of *malariae* significantly less than the older subjects. This may well have been due to presence of maternal antibodies and a lesser risk of infants to *Anopheles* (Port et al., 1980). Excluding these newborns, we have

\[
\alpha = \frac{-\log_e(1 - 29/600)}{0.25}
\]

\( \alpha = 0.198. \)

Both of these estimates are comfortably close to the approximation of \( \alpha \) for subjects of a much broader age distribution studied in 1967-1969.

**Estimation of a yearly mean P. malariae sporozoite inoculation rate**

It was not possible to determine in the field which species of *Plasmodium* might be represented by the sporozoites found in an infected mosquito.
Hypothetical estimates may be made, however, of the average relative frequencies of *P. falciparum* and *P. malariae* sporozoite infections if the duration of the extrinsic incubation period (*n* days) of each species, the probability of a mosquito acquiring a *Plasmodium* infection at a single random blood meal (*x₆*), and the survival rates of the mosquito species (*p*) were well approximated. Also required is an estimate of the average daily frequency of feeding on man (*a*).

These foregoing variables are discussed in the appendix, and their numerical values are set out in Table 3. The method adopted is to evaluate separately hypothetical sporozoite rates for *P. malariae* and *P. falciparum* with respect to *n*, *x₆*, *p*, and *a*. The relationship was defined by Macdonald (1952),

\[ s = p^a x₆ / (a x₆ - \log p). \]

There are two principal assumptions: (i) The probability of mosquito survival *p* is held to be independent of age and (ii) *Anopheles* with sporozoites in their salivary glands remain infected and infective for life. Results of the calculations are shown in Table 3.

A difference of one day in the duration of the extrinsic cycle (*n*) of *P. malariae* made little difference in its hypothetical sporozoite rate, in the absolute sense or relative to *P. falciparum*. Theoretical estimates of a *P. malariae* sporozoite rate, when *p* = 0.85 and *x₆* = 0.0114, vary little more than a factor of two when *n* is taken as a short 15 days (*s* = 0.2 %) or a long 20 days (*s* = 0.09 %). No substantial error in approximating *s* for *P. malariae* is likely to result in the present treatment from an inaccurate specification of *n*.

A two or a three day interval between successive blood meals on man (*a*) did not greatly influence hypothetical sporozoite rates.

Sporozoite rates, where *p*, *n*, and *a* were held constant, were directly proportional to the chance of an anopheline mosquito acquiring an infectious blood meal, *x₆*. But the relationship is not linear and the slope of log *s* on log *x₆* is less than unity (cf Macdonald, 1952, p580).

The sporozoite rate was clearly most sensitive to *p*, the average daily chance of survival among *Anopheles* populations. A substantial difference in hypothetical sporozoite rates is obtained when *p* is changed from 0.85 to 0.89, given constant values of *x₆*, *a*, and *n*. In this respect, *s* for *P. malariae* is more sensitive than it is for *P. falciparum*. Gillies and Wilkes (1965) demonstrated that the rate of mortality accelerates by the third week of life among *A. gambiae* s.l. and *A. funestus* in Northeastern Tanzania. An increasing risk of mortality with age would more strongly reduce the calculated
TABLE 3

HYPOTHETICAL P. malariae AND P. falciparum SPORozoite rates s, AS DETERMINED BY THE PROBABILITY OF MOSQUITO SURVIVAL THROUGH ONE DAY p, THE DURATION IN DAYS OF THE EXTRINSIC CYCLE n, THE DAILY FREQUENCY OF FEEDING ON MAN a, AND THE PROBABILITY OF ACQUIRING INFECTION FROM MAN ON ANY ONE FEEDING xg. Sporozoite rate, s = p^n x_g/ax_g - log_e p (MACDONALD, 1952).

<table>
<thead>
<tr>
<th>Survival p</th>
<th>Frequency of feeding a</th>
<th>P. malariae ( n = 17 \ days )</th>
<th>P. falciparum ( n = 11 \ days )</th>
<th>Proportion of sporozoite infections comprised of malariae ( n = 12 \ days )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.79</td>
<td>1/2</td>
<td>0.0114</td>
<td>0.043%</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.79</td>
<td>1/2</td>
<td>0.0057</td>
<td>0.022</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0114</td>
<td>0.144%</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0057</td>
<td>0.072</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.89</td>
<td>1/2</td>
<td>0.0144</td>
<td>0.572%</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.89</td>
<td>1/2</td>
<td>0.0057</td>
<td>0.279</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.89</td>
<td>1/3</td>
<td>0.0114</td>
<td>0.400</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.89</td>
<td>1/3</td>
<td>0.0057</td>
<td>0.197</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.89</td>
<td>1/3</td>
<td>0.0028</td>
<td>0.094</td>
<td>0.624</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0028</td>
<td>0.240</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0114</td>
<td>0.122</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0057</td>
<td>0.062</td>
<td>0.1248</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0114</td>
<td>0.122</td>
<td>0.624</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0057</td>
<td>0.062</td>
<td>0.624</td>
</tr>
<tr>
<td>0.85</td>
<td>1/3</td>
<td>0.0028</td>
<td>0.031</td>
<td>0.624</td>
</tr>
</tbody>
</table>

1 Age weighted prevalence of gametocytaemia was, \( P. malariae = 0.0114, P. falciparum = 0.1248 \)
2 Proportion of malariae sporozoite infections = \( s \) (malariae)/s (falc.) + \( s \) (mal.).
proportion of *Anopheles* harbouring *P. malariae* sporozoites than the proportion harbouring *P. falciparum*.

Hypothetical *P. falciparum* sporozoite rates agreed well with those observed in the field when $p$ was taken as 0.85 and $x$ as the age-weighted gametocyte rate, for example among *A. arabiensis* in the wet season of 1968 ($73 + /2526$ dissections, $s = 2.89 \%$, Krafsur, 1977). Under the same general suppositions of $p = 0.85$, $a = 1/3$ and $x$ a simple multiple ($1/3X, 1/2X, X, 2X$) of the age-weighted gametocyte rate, we obtain hypothetical *P. malariae* sporozoite rates of 0.03 to 0.24 % (Table 3). These approximations for *P. malariae* are 2-7 % of the total theoretical sporozoite-positive *Anopheles*.

Having estimated, however roughly, the relative specific *Plasmodium* components of the sporozoite-bearing mosquito population (ignoring *P. vivax* and *P. ovale*, both rarely detected), we may now calculate hypothetical *P. malariae* sporozoite inoculation rates. The annual total sporozoite inoculations per person $a$ in Gambela estimated by entomological methods, was approximately 11.6 in 1967 and 10.2 in 1968 (Krafsur, 1977). Two to seven percent of these probably were *P. malariae*, and an annual mean inoculation rate $a$ approximates to 0.2-0.7 when annual sporozoite challenge is taken as ten/person. In terms of risk, the proportion $x$ of the human population receiving one or more *P. malariae* sporozoite challenges in time $t$ years is,

$$x = 1 - e^{-a t}.$$

The principal assumptions are that sporozoite inoculations fall randomly among Gambela inhabitants and annual challenge is constant. The time $t$, in years, for half the population at risk to experience at least one inoculation is, where $a = 0.2$, $t = 3.47$ (1265 days), and where $a = 0.7$, $t = one year$. The median (and we think best approximation) estimate of annual sporozoite challenge, $a = 0.4/person$, would require 1.73 years (632 days) to subject 50 % of the residents to *P. malariae*. These minimum and maximum estimated rates of exposure suggest that acquisition of *malariae* in Gambela is a slow process.

**DISCUSSION**

One of the assumptions adopted in estimating an inoculation rate for *P. malariae* among children, was that of life-long residency in Gambela. The Anuak are highly mobile (though children are less so) and it seemed that a high proportion of those in Gambela had relatives in villages further to the west on the River Baro and to the south, on the Rivers Gilo and Akobo.
Entomological studies in villages on the Baro suggested rates of sporozoite challenge some nine to ten-fold greater than in Gambela (Krafsur, 1977) and this admits the possibility that a fraction of the *P. malariae* infection detected in Gambela were contracted elsewhere. The uncertainty is greatest for the study group of 1967 because of its wider age distribution. We were unable with confidence to exclude from enumeration those subjects in the 1973-76 study group who may have spent significant periods away from Gambela. Travel throughout the region is very difficult, however, during the wet season when most sporozoite inoculations are delivered. Moreover, the risk of *P. malariae* acquisition during the dry season is held to be slight. The attenuated survival rates in *Anopheles* thought to prevail in the desiccating dry season environment (Krafsur, 1970) suggest that *P. malariae* sporozoite rates were apt to be quite small. This view is consistent with the sporozoite rates observed during the dry season of 1968, when only 8 out of 2184 (0.37\%) *A. arabiensis* and 4 of 1916 (0.21\%) *A. funestus* were sporozoite positive.

Table 3 shows that *P. falciparum* sporozoite infections among *Anopheles* are very likely to outnumber greatly those of *P. malariae*. Their frequencies are very sensitive to the survival rate of their mosquito hosts and *P. malariae* is the most sensitive of the two because of its longer extrinsic period *n*. Hypothetical sporozoite rates will prove too high (given the accuracy of *x*_g_ and *n*) when the mosquito survival rate decreases with calendar age (Gillies and Wilkes, 1965) and *P. malariae* will be more strongly affected than *P. falciparum*. This means that the present hypothetical *P. malariae* sporozoite rates are likely to be more exaggerated in magnitude than the *P. falciparum* estimates.

Some of the hypothetical *P. falciparum* sporozoite rates approximated well the observed sporozoite rate in the field, and this may be taken as presumptive evidence that parameter estimation was fairly accurate. But it is estimation of the relative proportions of the observed sporozoite-positive *Anopheles* populations comprised of *P. falciparum* and *P. malariae* that are of present concern. And the critical assumption in such estimation is equating age-weighted *P. malariae* gametocytaemia, or a simple multiple thereof, to *x*_g_, the probability of a mosquito acquiring an infection at a single blood meal. *P. malariae* gametocytaemia was hard to measure at low densities typical of the parasite. The problem was compounded by difficulties in distinguishing gametocytes from trophozoites, and by inevitable losses in dehaemoglobinizing them (Dowling and Shute, 1966). It is therefore likely that *P. malariae* gametocytaemia was underestimated. When *x*_g_ is postulated to equal twice the observed age-weighted *P. malariae* gametocyte rate, c. 7\% of the annual ten sporozoite inoculations/person are composed theoretically of *P. malariae* spo-
rozoites. Laboratory studies however do not support the hypothesis that patients lacking identifiable gametocytes are very infective to experimentally applied Anopheles. The most likely trial value of $x_g$ for $P. malariae$, we think, is the age-weighted gametocyte rate (0.0114).

The hypothetical annual $P. malariae$ sporozoite challenge prevailing in Gambela was estimated to be 0.2-0.7 inoculations/person. The mean annual rate of children becoming positive for quartan malaria corresponds to an infective annual inoculation rate of 0.17 per child. The correspondence of the two estimates, parasitological and entomological, is satisfactory.

APPENDIX

Parameters relating to sporozoite rates among Anopheles mosquitoes

1. The extrinsic cycle $n$ of $P. falciparum$ is about 12 days in the wet season, when mean 24 hour temperatures were c. 27°C, and 11 days during the dry season with its mean temperatures of c. 28.5°C. The rate of extrinsic development of $malariae$ is less clear, but the literature (see Boyd, 1949, pp. 97 and 616; Shute and Maryon, 1951 for reviews) suggests that its cycle requires more time, at any particular temperature, than does $P. falciparum$. Plainly the difference in rate of extrinsic development tends to converge with increasing temperature. Shute and Maryon (1952) observed that an 11-12 day period was required for $P. falciparum$ to develop to maturity in $A. stephensi$ at 25°C, and a 15-21 day period was necessary for $P. malariae$ under similar circumstances. The difference between the two species was 4 to 9 days, with a median of 6.5 days. $A. freeborni$ fed on $Aotus$ monkeys infected with $P. malariae$ required 18-20 days at 25°C to become infectious (Collins et al., 1973). Young and Burgess (1961) recorded a range of 19-23 days at 24.4°C for sporogony of $P. malariae$ in four new world species of Anopheles. Although temperatures prevailing in Gambela were somewhat higher than 25°C, we adopt values of $n$ of 12 days for $P. falciparum$ and 17 or 18 days for $P. malariae$, recognizing that the rate at which $P. malariae$ matures increases with temperature faster than $P. falciparum$.

2. Probability of Anopheles acquiring a Plasmodium infection at a single random blood meal. It has been demonstrated, in well-designed experiments, that a positive correlation may be found between the density of $P. falciparum$ gametocytes of infected subjects and the proportion of Anopheles subsequently becoming infected (Macdonald, 1952 and Covell, 1960 for reviews; Draper, 1953; Jeffery and Eyles, 1955; Rutledge et al., 1969). Relationships between $P. malariae$ gametocyte densities and infectiousness to Anopheles are
much less clear (Covell, 1960) although it has been remarked that subjects with detectable microgametocytaemia seem more infective than subjects apparently free of gametocytes (Young et al., 1948; Bray, 1960; Collins et al., 1973). Correlations between gametocyte density in peripheral circulation and infectivity to *Anopheles* are tenuous, however, at the low gametocyte densities typical of *P. falciparum* parasitaemia among chronically infected subjects and almost unpredictable when dealing with *P. malariae*. It seems that *Anopheles* may be more successful in finding *P. falciparum* and *P. malariae* gametocytes than experienced microscopists (Shute and Maryon, 1951; Muirhead-Thomson, 1957; Young and Burgess, 1961). The best working hypothesis to use, in light of the foregoing, is that the probability of a mosquito acquiring a *P. malariae* or a *P. falciparum* infection from a blood meal taken at random ($x_g$) is directly proportional to the gametocyte frequencies of the two parasite species among the human community at large. On age-weighting gametocyte prevalence, we obtain

$$12.48\% \text{ } P. \text{ falciparum} \text{ and}$$
$$1.14\% \text{ } P. \text{ malariae}.$$ 

These estimates of gametocytaemia are used as trial values of $x_g$. An additional value is employed for *P. falciparum*, one-half the estimated prevalence of gametocytaemia, or 6.24%. Further trial values of $x_g$ for *P. malariae* are also employed, 2.28% and 0.57%, respectively twice and one-half the estimated prevalence of gametocytaemia. The higher trial value of the probability of *Anopheles* acquiring a *P. malariae* infection in a single random feed is evaluated because *P. malariae* gametocytaemia may well have been underestimated, identification of gametocytes being difficult (Shute and Maryon, 1951; Bray, 1960). The lesser trial value of $x_g$ is evaluated because the density of *P. malariae* is normally very much less than *P. falciparum*, and subjects with quartan malaria have, in general, proved less infective to experimentally applied *Anopheles* than have subjects with *P. falciparum* malaria (Garnham, 1966).

3. The average daily survival rates of the vector mosquito species $p$, and their mean daily frequency of feeding on man, $a$ (Macdonald, 1952). Estimates of $p$ for *A. arabiensis* and *A. funestus* in Gambela were made on the basis of the proportions parous, ratios of immediate to delayed sporozoite rates, and analysis of seasonal differences in sporozoite rates (Krafsur, 1970). Values obtained for both species were,

$$p = 0.89 \text{ wet season}$$
$$p = 0.79 \text{ dry season}.$$
The frequency of feeding was held to occur, on average, once every two days, but this was supported by entirely circumstantial evidence. The man biting habit was taken to approximate unity (Krafsur, 1977) and, based on the foregoing, it was suggested that \( a \) was equal to 0.5. Compelling experimental evidence from Northeastern Tanzania, however, demonstrated that there were three day intervals between successive feeds \( (a = 0.333) \) among the closely-related \( A. gambiae \) species \( A. \) (Gillies and Wilkes, 1965) and \( A. funestus \) (Gillies and Wilkes, 1963); the average daily rate of survival in each mosquito species, as shown by mark, release, and recapture coupled with Polovodova’s age grouping method, was very close to 85%. To our knowledge, Gillies and Wilkes’ findings are the only successful application of a direct method of age grading to African \( Anopheles \) and may be indicative of the situation existing in Gambela. Their values are therefore used in addition to our own.

**Summary**

Malaria incidence and prevalence surveys were performed from December, 1967 to February, 1969 among the indigenous Nilotic inhabitants of Gambela, a small administrative centre in the western lowlands of Ethiopia. Entomological data suggested that malaria transmission was seasonal and this was consistent with monthly \( P. falciparum \) parasite rates. Monthly \( P. malariae \) parasite rates, however, were consistent with an hypothesis of homogeneity. The age-specific incidence of quartan malaria among 26 children zero to 11 years old at the start of study was examined at 28 day intervals over a 15-month period. The resulting data suggested that parasite acquisition was a slow process and an annual \( P. malariae \) incidence of 0.17 was derived. This statistic was supported by studies performed five years later: The incidence of \( P. malariae \) among 102 infants followed from birth up to 48 months of age was 0.16-0.20. An attempt was then made to account for the prevalence of \( P. malariae \) in terms of the entomological conditions observed in Gambela. Macdonald’s formula for the sporozoite rate was used to derive hypothetical relative proportions of \( P. falciparum \) and \( P. malariae \) among the observed sporozoite-positive mosquito populations. About 4% of the sporozoite challenges were estimated to be of \( P. malariae \). An hypothetical annual entomological \( P. malariae \) inoculation rate was then made by multiplying the number of observed sporozoite inoculations per person \( (\sim 10/\text{year}) \) by the proportion of them estimated to be of \( P. malariae \). The annual \( P. malariae \) sporozoite challenge was thus estimated at 0.4 per person, in good agreement with the annual incidence estimates from parasite rates in children.
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LITERATURE CITED


