Seasonal fluctuation of Plasmodium falciparum gametocytemia.

Seasonal fluctuation of the number of Plasmodium falciparum gametocytes in human blood is described.
Seasonal fluctuation of *Plasmodium falciparum* gametocytaemia

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**Abstract**

Two numerically minor components of *Plasmodium falciparum* prevalence—gametocytaemia and trophozoite densities >99/500 white blood cells—displayed an annual cycle that reflected the seasonal abundance of infective *Anopheles dirus* at a hyperendemic focus in Thailand, even though the gross monthly prevalence for combined ages remained stable. Gametocyte prevalence rose more than 300% within 30 d after the capture of the dry season's first infective mosquito, remained at about 8% until the beginning of the monsoon 7 months later, then fell within 60 d to about 2%. The number of cases with a high density of trophozoites behaved similarly. These periodic fluctuations represented changes in incidence, at least half of which appeared to be due to superinfection. Almost 49% of all gametocyte carriers were older than 14 years, but nearly all gametocyte densities >20/500 white blood cells were in children. These observations, as well as the calculated efficiency of human infectivity, imply that superinfection of adults may contribute significantly to transmission in semi-immune populations.

**Introduction**

*Plasmodium falciparum* is usually first detected in the peripheral circulation of immunologically naive humans as trophozoites 7–11 d after a successful sporozoite inoculation. The number of parasites multiplies exponentially at each 48-h schizogonic cycle, reaching its highest density 5–8 d after initial patency. After this asexual peak has subsided, trophozoites behave similarly. These periodic malaria epidemiology, but one which is difficult to quantify. Naturally acquired immunity to malaria greatly mitigates the risk of being ill from the disease, but does not prevent reinfection, which may occur before parasites from a previous infection have disappeared. The greater the ratio of incidence to rate of recovery, the greater the chance of superinfection; in some hyperendemic communities many children seem to be continuously infected. Above a threshold determined by variables specific to the situation, increasing incidence no longer increases prevalence, yet there is evidence that it does affect rates of mortality, morbidity, splenomegaly, and parasite density (MacDonald, 1951). We have conjectured that differences in disease patterns between some Asian and African examples of hyperendemicity are due to differences in inoculation rates and size of incula (Rosenberg et al., 1990a).

A hyperendemic community in Thailand had a gross monthly prevalence of *P. falciparum* infection that was essentially stable (Rosenberg et al., 1990b), even though the abundance of infective *Anopheles dirus*, the predominant vector, was seasonal (Rosenberg et al., 1990a). In this report we show how seasonal oscillations of gametocytaemia and high trophozoite densities reflected incidence, some of which may have been due to superinfection, and how the timing of these oscillations was related to changes in vectorial capacity. We discuss how increased numbers of gametocyte carriers at the time when vector survival was longest may have influenced transmission.

**Methods**

**Site**

The study area, part of Ban Phluang village, was in south-eastern Thailand. The inhabitants (approximately 250) were relatively prosperous cultivators of rubber, fruit trees and rice, and seldom left the settlement. Houses were in or near orchard and scrub, and intense malaria transmission occurred in the village itself, despite the presence in most houses of...
mosquito nets. Almost all malaria was transmitted during the November–May dry season. About half those older than 12 years spent at least one night per week during the dry season tapping rubber near the village; tapping was done between midnight and dawn, when vector activity was high (ROSENBERG et al., 1990a). A more detailed description of the topography, demography, and economy of the area is given by ROSENBERG et al. (1990b).

**Blood collection and examination**

Thick and thin blood films were collected for 24 consecutive months from nearly all inhabitants; data were analysed from those contributing at least 8 specimens per year, 176 people in the first year and 186 in the second. Thick films were stained with Giemsa’s stain and the volume containing 500 white blood cells (WBC) was examined at a magnification of 1000 x, a procedure requiring about 10–15 min per slide by a single microscopist; there were approximately 5500 WBC/mm² of a stained thick blood film (ROSENBERG et al., 1990b). Thick films made in April 1984 were accidently fixed and no gametocyte rate or parasite densities are available for that month. The 36% of cases with the highest densities—which contained more than 76% of all densities >99,500 WBC—were treated monthly. *P. vivax* was treated with chloroquine and primaquine; *P. falciparum* was treated in the first year with quinine, tetracycline and primaquine and in the second year with mefloquine + sulfadoxine + pyrimethamine and primaquine. Mefloquine does not affect the viability of gametocytes present at the time of treatment or sporogonic development from them (HARINASUTRA et al., 1987); nor do sulphonamides and pyrimethamine seem to have any gametocidal or sporontocidal activity at concentrations typically found in humans treated for malaria (SCHOLER et al., 1984). Details of examination and treatment are given in a previous report (ROSENBERG et al., 1989b).

**Mosquito infection**

Man-biting *Anopheles* were collected outdoors from 1900 h to 0450 h for 7 consecutive nights per month for 2 years by 2-man teams working simultaneously at each of 2 unchanging sites within the village (ROSENBERG et al., 1990a). Collections began the night following each month’s blood collection. An extraordinary collection was made between 2 and 7 November 1983. After species identification, *Anopheles* were dissected to determine parity and midgut and salivary gland infection rates. The species of sporozoites found was determined using indirect fluorescent antibody or enzyme-linked immunosorbent assays. In this report only mosquito infectivity (the presence of sporozoites in glands) is considered, and no distinction is made between sites or hours of capture.

**Results**

In the first year 5·3% of *P. falciparum* infections included, or were composed solely of, gametocytes; in the second year, the figure was 4·2%. Most gametocyte cases (88·3%) also had trophozoites. In the first year 96 cases with gametocyaemia occurred in 65 people (36·9% of the study population), 18 of whom accounted for 51·0% of the cases by being positive more than once; in the second year, 83 cases occurred in 65 people, 11 of whom accounted for 34·9% of the cases. Of gametocytaemias found in individuals, only 12 cases in the first year and 8 in the second occurred consecutive to each other, suggesting that many represented fresh rather than recurrent infections. Gametocyte densities ranged from 1 to 2952 per 500 WBC, or about 12–35424 per mm²; the geometric mean for all ages was 3·8/500 WBC, or about 46·mm².

The number of people showing *P. falciparum* gametocytes in their blood increased more than 300% each December (Figure, A) or within 30 d after catching the first infective mosquito of the transmission season (Figure, C). The number of gametocyte carriers remained high throughout the dry months, November to May, but then steeply declined soon

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**Table 1. Seasonal distribution by age of *Plasmodium falciparum* gametocyte carriers**

<table>
<thead>
<tr>
<th>Age group*</th>
<th>Wet season (June–October)</th>
<th>Dry season (November–May)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year 1</td>
<td>Year 2</td>
</tr>
<tr>
<td>≤14 years</td>
<td>5·7% (263)</td>
<td>0·9% (216)</td>
</tr>
<tr>
<td>&gt;14 years</td>
<td>0·8% (509)</td>
<td>1·3% (462)</td>
</tr>
<tr>
<td>Total</td>
<td>2·5% (772)</td>
<td>1·2% (678)</td>
</tr>
</tbody>
</table>

*All comparisons within age groups are significant (t test, P<0·05), except for age group >14 years in wet season; all comparisons between age groups are significant (t test, P<0·05), except for totals in dry season.

*bNumbers in parentheses are numbers of specimens examined including negative slides.
after the last infective mosquito was found, about the time the annual monsoon began (Figure, A). A disproportionately high number of gametocyte carriers \( G = 7.4 \), \( P < 0.01 \) (SOKAL & ROHLF, 1981) was found between November and May (Table 1); gametocyte densities were also higher then.

Persons aged more than 14 years comprised 67% of the population and harboured 49% of the gametocytæmas; but 84-6% of gametocyte densities >20/500 WBC were in those aged 14 years or less. Dry season prevalence was 2.6 times higher than wet season prevalence in children, compared to 8.0 times higher in those over 14 years old, a relationship that has also been found in Nigeria (MOLINEAUX & GRAMICIA, 1980), and which may reflect more conscientious treatment of children. The second year the inoculation rate fell to about one-third of that in the first year (ROSENBERG et al., 1990a), and the proportion of the population aged 14 years or less first year and 60% in the second. Lower survival rates after the last infective mosquito was found, about the time the annual monsoon began (Figure, A). A

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\]

If it is assumed that vectorial capacity \( C \) (GARRETT-JONES & SHIDRAWI, 1969) is unchanged, then the prevalence of infective mosquitoes \( y \) needed to account for a given inoculation rate \( h' \) can be estimated from \( y = h'/C \) (DIETZ et al., 1974). This has been done in Table 2 for both dry seasons using \( C \) calculated from the mean observed parous rate of An. dirus (ROSENBERG et al., 1990a) and from its 95% confidence limits (MEILLON et al., 1967). A comparison of the observed gametocyte prevalences of 0.076 in the first year and 0.065 in the second with the range of calculated human infectiousness (Table 2) suggests a high level of gametocyte infectivity. If daily mosquito survival is assumed to have been at the upper end of the confidence limit—only slightly greater than that calculated from mean parity—and the infectiousness of people with negative blood films is disregarded, then infectivity was nearly 68% in the first year and 60% in the second. Lower survival rates would require higher, and increasingly unlikely, efficiency of transmission (COVELL, 1960).

The prevalence of \( P. falciparum \) trophozoite densities higher than 99/500 WBC (about 1200/mm³) showed a seasonal pattern similar to that of gametocytæma: only 5-8% of high densities occurred during June-August 1983, March-April, May, and June-July 1984. The high level of gametocyte infectivity (Table 2) suggests that those over 14 years of age were also acquiring infections at some place where the risk was greater than in their homes.

Table 2. Observed prevalences of infected humans compared with theoretical prevalences calculated from parous rates and their 95% confidence limits

<table>
<thead>
<tr>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitism (%)</td>
<td>0.70 (0.65-0.74)</td>
</tr>
<tr>
<td>Daily survival (p)</td>
<td>0.89 (0.87-0.91)</td>
</tr>
<tr>
<td>Vectorial capacity (C)</td>
<td>1.28 (0.79-1.92)</td>
</tr>
<tr>
<td>Infective prevalence (y)</td>
<td>0.078 (0.13-0.05)</td>
</tr>
<tr>
<td>Gametocyte prevalence</td>
<td>0.016 (0.006-0.05)</td>
</tr>
</tbody>
</table>

*Parasitism of \( P. falciparum \) and \( P. vivax \) combined, \( p = parasitism \). \( C = (ma)ap, - \ln(1-0)', where \( m = 0.03 \) and \( a = 0.33 \); days between blood meals \( n = 3 \). \( \gamma = y = h'/C \). \( h' = 0.03; f = 0.33 \). \( \beta = 108; \gamma = 10, \beta = 108; \gamma = 10 \). \( \gamma = 1 \) for \( C = 1 \). \( \gamma = 0 \) for \( C = 0 \). \( \gamma = 0 \) for \( C = 0 \).

Table 3. Seasonal fluctuation of prevalence of \( P. falciparum \) asexual forms by age

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Jun.</td>
<td>57.6</td>
<td>37.3</td>
<td>20.3</td>
<td>63.6</td>
<td>60.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Jul.</td>
<td>67.6</td>
<td>41.9</td>
<td>25.7</td>
<td>48.6</td>
<td>38.9</td>
<td>9.7</td>
</tr>
<tr>
<td>Aug.</td>
<td>45.9</td>
<td>30.8</td>
<td>15.1</td>
<td>50.0</td>
<td>40.8</td>
<td>9.2</td>
</tr>
<tr>
<td>Sep.</td>
<td>45.9</td>
<td>36.4</td>
<td>9.5</td>
<td>44.1</td>
<td>27.2</td>
<td>16.9</td>
</tr>
<tr>
<td>Oct.</td>
<td>43.2</td>
<td>31.6</td>
<td>11.6</td>
<td>48.6</td>
<td>43.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Nov.</td>
<td>27.8</td>
<td>30.0</td>
<td>-2.2</td>
<td>43.8</td>
<td>38.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Dec.</td>
<td>36.1</td>
<td>29.8</td>
<td>6.3</td>
<td>43.6</td>
<td>24.7</td>
<td>8.9</td>
</tr>
<tr>
<td>Jan.</td>
<td>27.5</td>
<td>35.1</td>
<td>-7.6</td>
<td>40.0</td>
<td>40.6</td>
<td>-0.6</td>
</tr>
<tr>
<td>Feb.</td>
<td>57.1</td>
<td>24.4</td>
<td>32.7</td>
<td>60.0</td>
<td>33.1</td>
<td>26.9²</td>
</tr>
<tr>
<td>Mar.</td>
<td>44.1</td>
<td>27.1</td>
<td>17.0</td>
<td>56.1</td>
<td>38.2</td>
<td>17.9²</td>
</tr>
<tr>
<td>Apr.</td>
<td>48.4</td>
<td>37.9</td>
<td>10.5</td>
<td>41.0</td>
<td>36.2</td>
<td>2.8</td>
</tr>
<tr>
<td>May</td>
<td>57.6</td>
<td>37.3</td>
<td>20.3</td>
<td>63.6</td>
<td>60.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

¹Adult prevalence subtracted from child prevalence.
²Difference greater than expected (\( \chi^2 \) test, \( P < 0.05 \)).
occurred among 40 people. Gross P. falciparum prevalence dropped from 12.8% in the first year to 7.2% in the second; 93.8% of blood films containing gametocytes also included trophozoites. Gametocyte densities ranged from 1 to 75/500 WBC (median = 2.5).

Discussion
The perennial, monsoon-generated cycle of malaria incidence at Ban Phluang was more evident in the seasonal fluctuation of high trophozoite densities and of gametocyte prevalence than in the commonly used index of gross parasite prevalence. We observed a temporal relationship between transmission, high densities, and gametocytaemia that was consistent with the known biology of P. falciparum. WILSON (1936) interpreted his African data as showing that gametocyte prevalence peaked after transmission had stopped, leading him to conclude that the 2 events were only indirectly related; he may have been misled by inconsistencies in his study design, as his collection of mosquitoes and blood data were neither synchronized nor always regular, making precise alignment of data difficult. Nonetheless, the pattern we observed in Thailand was strikingly similar to that which WILSON (1936) described for Tanzania 50 years earlier; the similarity is all the more remarkable as the 2 sites have virtually no features in common other than seasonally intense transmission.

Except in infants, reinfection and superinfection accounted for all malaria incidence at Ban Phluang. Discussion of how to understand and estimate the influence of superinfection on the chronicity of infection is problematical when low densities make the identification of cases uncertain (EARLE et al., 1939; MILLER, 1958), but it appears that at least half of the densities >99/500 WBC were superimposed on current, low-level infections. One effect of superinfection should be to decrease the recovery rate (ARON & MAY, 1980), explaining why prevalence remains high during brief periods of low transmission. Superinfection also explains why at least some infection was manifested by a rise in the numbers of gametocyte carriers but not in trophozoite prevalence. It is less clear why reinfections, which may have accounted for half of the incidence, did not infect prevalence soon after transmission recommenced. Although annual P. falciparum incidence was at least 100% (ROSENBERG et al., 1990b), fewer than one-third of the population were ever found to have an elevated trophozoite density or a gametocytaemia, and it is possible that many reinfections may have been initially subpatent.

The influence of superinfection on the chronicity of hyperendemic malaria has been an important component in attempts to understand the epidemiology of the disease (BAILEY, 1982). It should also be considered in quantifying transmission if, as seems evident from our data, it alters the infectiousness of man to vector. But the most ambitious transmission model yet tested is in part on the explicit assumption that 'superinfection prolongs parasitaemia, without effect on infectivity' (DIEZ et al., 1974). Although seasonal incidence at Ban Phluang disproportionately increased the prevalence of gametocytaemia in adults, any increase in gametocyte prevalence, even at the low densities typical of semi-immunes, almost certainly favoured parasite dissemination.

The contribution to transmission made by low density infections in adults has been controversial. It was once presumed to be insignificant (MACDONALD, 1951), but it has been proved that even sub-patent infections can infect mosquitoes (COVELL, 1960). Except for the 13% of the population aged below 5 years, gametocyte densities at Ban Phluang were the same in all age groups (ROSENBERG et al., 1989b) and it seems probable that, as in Africa (MURRHEAD-THOMSON, 1957), the proportionately large number of semi-immunes played a crucial part in transmission. The actual efficiency of human infectivity was unlikely to have been as high as we calculated using the Diez-Molineaux-Thomas model (Table 2); considering the large number of approximations used and the relatively small sample sizes, it may be that the validity of the equation cannot be evaluated without feeding uninfected mosquitoes on a representative sample of the population.

Each year transmission began during the last weeks of the annual monsoon as unknown factors, probably micro-climatic, suddenly began to favour prolonged survival of the vector (ROSENBERG et al., 1990a). For a period of less than 30 d high survival rates coincided with high adult emergence. The annual explosive increase in vectorial capacity at that initial point gametocyte prevalence was low, but so presumably was immunity to reinfection. The great rapidity with which gametocytes and high densities ascended to something of a plateau suggests that incidence also rapidly rose and levelled. This implies that the basic reproductive rate was high and that the susceptible population was sufficiently large to provide the critical size necessary to maintain a stable prevalence (ARON & MAY, 1980). To what extent increased gametocytenosis enhanced transmission after mosquito survival improved is unclear, but the large drop in vectorial capacity after the initial increase (ROSENBERG et al., 1990a) did not lower gametocyte prevalence, suggesting that compensation occurred.

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References


Schiffner, W. A. P. (1938). Two subjects relating to the epidemiology of malaria. *Journal of the Malaria Institute of India*, 1, 221-256.


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