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PLASMODIUM MALARIAE BLOOD-STAGE DYNAMICS

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Abstract

We examine the dynamics of parasitemia, fever, and gametocytemia reflected in the preintervention charts of 180 malaria-naïve U.S. neurosyphilis patients infected with the USPHS strain of *Plasmodium malariae*, for malariatherapy, focusing on the 84 charts for which more than 35 days of patency preceded intervention and daily records encompassed 92% or more of the duration of each infection. Inoculum size did not influence any outcome variable. Fevers (days with temperatures $\geq 101^\circ\text{F}$) followed patterns that fit recognized brood structures more often than did our approximations of merogony cycles (via local peaks in parasitemia), but neither closely fit textbook quartan patterns. There were no discernable patterns in gametocytemia. Successful transmission to mosquitoes increased following subcurative drug treatment but did not depend on detectable gametocytemia.

In 1885, Golgi related quartan fever patterns in human malaria directly to the asexual blood-stages of the *Plasmodium malariae* life cycle, postulating that febrile periods coincide with merogony, that all parasites within a brood undergo merogony simultaneously, and that a *P. malariae* infection may contain 1, 2, or 3 broods, i.e., that “simple quartans are determined by a single generation of parasites which develops simultaneously over a period of three days, while the double and triple quartans (daily) are connected with developmental cycles of two or three generations, which mature successively with a day’s interval between them” (Golgi, 1889). Hence, a single-brood *P. malariae* infection is expected to produce fevers and corresponding peaks of asexual parasitemia (in the form of merozoites) every third day, a 2-brood infection to produce peaks on 2 out of 3 days, and a triple-brood infection to produce daily peaks.

Plasmodium malariae asexual blood-stage cycles are authoritatively cited as displaying a period of 72 hr (Garnham, 1966; Coatney et al., 1971; Gilles, 1993; Krogstad, 1995), though it is occasionally remarked that the synchronization of fevers or merogony in any *Plasmodium* species may be imprecise (Lopez-Antunano and Schmunis, 1993). The existence of broods in *Plasmodium* infections is seldom noted in the recent literature, and the processes that regulate their dynamics remain mysterious. Boyd (1940) found that *P. malariae* broods emerge, shift phases, and recede in both blood- and sporozoite-initiated infections. He noted that with either mode of inoculation the initial stages of infection “invariably exhibit a simple quartan pattern,” whereas in later stages the latter “tends to exhibit a simpler pattern”; he developed an elaborate scheme for labeling the “additive,” “subtractive,” and “substitutive” changes in brood structure associated with transformations among single, double, and triple quartan fever cycles over the course of an individual infection. His study remains utterly unique.

Plasmodium malariae infections are characterized by low parasitemias, a trait that has contributed to dramatic underestimates of species prevalence (Snounou et al., 1993; McKenzie

and Bossert, 1997a, 1999; Zhou et al., 1998) and is conventionally attributed to an age-restricted range of target red blood cells, i.e., “*P. malariae* merozoites parasitize senescent erythrocytes” (Reisberg, 1997), and “can invade only aging erythrocytes” (Schmidt and Roberts, 1985). Such statements trace back to a single, problematic study by Kitchen (1939), which, he concluded, “raises the question of whether the predilection of *P. malariae* may not be quite the opposite of that of *P. vivax*.” Though almost all subsequent authors have taken the question as closed, Simpson et al. (1999) have recently found that *P. malariae* red blood cell selectivity could not account for the range of parasitemias observed in their study and, accordingly, have suggested that “factors other than red cell availability limit the expansion of the natural infection.” *Plasmodium malariae* infections typically display low, irregular gametocyte production and unpredictable infectivity (Carter and Graves, 1988). In many cases, *P. malariae* gametocytes are detected only after several months of infection (Boyd and Stratman-Thomas, 1933; Hackett, 1941), yet cross-sectional surveys often find that half or more of *P. malariae* infections include gametocytes (Kligler and Reitler, 1928; Thomson, 1934; Peters, 1957; Panda et al., 1990). Early researchers found it so difficult to infect *Anopheles* with *P. malariae* that other vectors were suggested (Marchoux, 1930). Later, the notion that gametocyte densities determine *P. malariae* infectivity (Kligler and Mer, 1937; Siddons, 1944) was contradicted (Shute and Maryon, 1951; Constantinescu and Negulici, 1967); *P. malariae* infectivity often seemed more a property of the individual infected human than of gametocyte density, the day or hour at which mosquitoes were fed, or any of the several other factors investigated (Young et al., 1948; Young and Burgess, 1961). Hence the dynamics of gametocyte production and its relation to transmission remain as puzzling in *P. malariae* as in its congeners (McKenzie and Bossert, 1997b, 1998).

The present paper takes advantage of a unique opportunity to address several such questions about the dynamics of *P. malariae* parasitemia, fevers, gametocytemia, and infectivity, using a data set collected by expert staff in the same facility, with the same parasite strain, and nominally the same patient population as in the canonical studies of the species (Young, Coatney, and Stubbs, 1940; Young, Stubbs, and Coatney, 1940; Young et al., 1941).

MATERIALS AND METHODS

Malariatherapy

Most current knowledge of the dynamics of *Plasmodium* infections derives from 40 yr of work with induced malaria as a treatment for neurosyphilis (Winckel, 1941; Covell and Nicol, 1951; Miller et al., 1994); during that era, it is said, “malariatherapy was less expensive and produced clinical improvement more frequently and more rapidly than did the best drug treatments” (Chernin, 1984). Malariatherapy treatment procedures in general and at the USPHS facility in Columbia, South Carolina, in particular have been described by Mayne and Young (1941), Becker (1949), Glynn et al. (1995), and Collins and Jeffery (1999); the last of these contains extensive information about the participation and treatment of the patient population considered here and is accompanied by an explicit, independent analysis of relevant ethical issues (Weijer, 1999). The present study and many others would not have been possible without the participation of hundreds of malariatherapy patients, to whom we are extremely grateful.

The *P. malariae* strain most widely used in the U.S.A. was isolated in 1932 and was used continuously at the South Carolina USPHS facility until it closed in 1963. Through at least the first 8 yr of its use there, it was characterized as gametocyte-producing, highly synchronous (“seldom breaks up into more than one brood”; Young, Stubbs, and Coatney, 1940), and unchanging in clinical presentation (Coatney and Young, 1941); at the end of this period, while treating 4 patients in the South Carolina facility with this USPHS strain, Young, Stubbs, and Coatney (1940); Young, Coatney, and Stubbs (1940); and Young et al. (1941) conducted their benchmark study of *P. malariae* merogony, associated fevers, and parasitemia peaks.

The present paper reviews the charts of 180 adult neurosyphilis patients, with no known history of previous malaria infection, whose infections began with intravenous inoculation of 5 ml of whole blood from a patient patently infected with the USPHS strain of *P. malariae*. Blank daily records in patient charts reflect occasional, isolated absences, or omissions by staff, and 1 major change in personnel and procedures in November–December 1949. For the 96 charts prepared between May 1940 (patient S-218) and December 1949 (patient S-1014), there were daily records of parasitemia, gametocytemia, and/or fever for 40–63% (mean 45%) of the days of each infection, following initial patency; for the 84 charts prepared between January 1950 (patient S-1020) and September 1958 (patient S-1318), there were daily records for 92–100% (mean 99%). The present paper focuses on the charts from 1950 through 1958. Fever records were absent in 20 of the 1950–1958 charts, 14 of them in the 19 charts from 1952 to 1954.

Parasitological and clinical data collection

During each infection, parasitemia and gametocytemia (microgametocytes only) were determined each morning by thick-film and thin-film microscopy, respectively, and recorded on the chart on a per-cubic-millimeter (mm^3) basis. Staff used the Earle and Perez (1932) technique with thick films but often converted gametocyte counts per 200 white blood cells (WBC) on thin films by assuming an average 5,000 WBC/ mm^3 . Inoculum size was calculated from the recorded parasitemia of the donor patient and set day 1 of infections as the first day of patent (detected) parasitemia, in effect, the day on which observed asexual-form parasite density reached 10/ mm^3 .

Patient rectal temperature was taken every 4 hr throughout an infection, and hourly during fevers, i.e., temperatures of 101 F or higher. Only the highest temperature each day was recorded on the chart and then only in the event of a fever. Fevers were recorded in increments of 0.2 F. Because extended series of high fevers and other clinical manifestations of malaria were considered beneficial, infections were generally allowed to continue as long as possible without intervention. At the discretion of the medical staff, subcurative doses of amodiaquine, quinine, bismuth thioglycolate, or other drugs were given to provide temporary breaks from fevers or other symptoms; usually, a dose of 1,500 mg of chloroquine base was administered over 3 days to terminate an infection. In some cases, intervention consisted of reinoculating a patient with *Plasmodium* of the same or a different species or strain. Intervention was by drug for 90 of the 96 patients from the 1940s, and for 79 of the 84 from the 1950s (11 after spontaneous clearance). Only those parts of charts that preceded any intervention were included in analyses.

Mosquitoes were fed on 10 of the 96 South Carolina USPHS *P. malariae* patients in the 1940s (17 feeds total) and on 19 of the 84 patients in 1950–1958 (133 feeds total); results reported below hold for both sets. Young and Burgess (1961) describe procedures by which cages of 25–35 *Anopheles* were fed and transmission success determined (as the proportion of fed mosquitoes found with oocysts when dissected 10 days later) at the South Carolina USPHS facility in this period, but they do so without reference to gametocyte densities. Any instance in which mosquitoes were judged infected, over a reported range of 3.3–62.5%, was defined as successful.

Data presentation

As expected with data from truncated time series, plots of summary statistics for each chart indicated that values of some timing-related variables depended strongly on the number of days of preintervention infection observed. No preintervention peak could occur on day 12 (or later) in the briefest of the 1950s patient charts, for instance, because only 11 days of patent infection preceded intervention. The transition at day 36 was the most pronounced overall (Fig. 1): linear regression (Sokal and Rohlf, 1981) of the day of peak parasitemia on the number of days

observed before intervention gives an r^2 value of 0.11; for patient charts with 11–35 days preintervention the r^2 value is 0.81 but only 0.52 if the next 2 charts (each at 37 days) are added. For the day of peak fever, the r^2 value is 0.21: 0.71 for charts with 11–35 days but only 0.46 if the next 2 charts (mean 37 days) are added. We concluded that the relevant analyses should distinguish patient charts with more than 35 days of patency before intervention from those with 35 days or less (Tables I, II). Note that there is no such sharp transition associated with the day of peak gametocytemia; the r^2 value is 0.44 overall and 0.51 for charts with 11–35 days before intervention. The r^2 values for peak densities and temperatures are less than 0.05.

Brood structures in these infections cannot be assessed directly in terms of merogony, because charts do not provide stage-specific densities of asexual forms. Therefore, we made a rough approximation, under the assumption that asexual-form densities within a single brood are highest at merozoite release. A local peak in parasitemia was defined as a day on which the asexual-form counts both on the day immediately previous and the day immediately following were lower by at least $20/\text{mm}^3$, and potential local peaks adjacent to blank daily records were excluded. Counts within $10/\text{mm}^3$ on consecutive days were considered equal; a 1-day interval between peaks was defined as an instance in which counts on the day before and day after equal counts were each lower by at least $20/\text{mm}^3$. This approximation located 1,507 local peaks in preintervention parasitemias in the 84 patient charts from 1950 to 1958, with a mean interval of 3.9 days between peaks. Subsequent analyses considered only the 77 cases with 4 or more such local peaks in parasitemia, and thus 3 or more intervals between peaks; similarly, among the 64 cases with fever records, only the 56 with 4 or more fevers (days with temperatures $\geq 101^\circ\text{F}$) were considered. These intervals were defined such that none spanned a blank daily entry: the last peak preceding a blank was considered the (prospective) endpoint of an interval and the first peak after a blank the start of the next interval. The first and the last interval in each chart were then removed from consideration, such that in each chart each remaining interval both followed and preceded an interval (unless adjacent to a blank entry). The frequency distributions of these middle intervals (Table III) were indistinguishable from those of the full set of intervals.

For the local peaks in parasitemia and the presence/absence of fever (binary data) we also calculated 4×4 matrices representing the frequencies of transitions between particular intervals, matrices in which the first row represents the frequencies at which a 1-day interval was followed by a 1-day interval (first column), a 1-day interval by a 2-day interval (second column), and so forth. Here, had every infection consisted of a single brood with a precisely quartan cycle, and were the approximations perfect, the entry in the third row, third column of the transition matrix would be a frequency near 1 and all the rest near 0. Had every infection consisted of 2 such broods, the entries in row 1, column 2, and row 2, column 1 would each be near 0.5. Had every infection consisted of 3 such broods, the entry in the row 1, column 1 would be a 1.

Statistical procedures

For the 1950–1958 patient charts with 11–35 days of preintervention patency, those with more than 35 days, and overall, we calculated pairwise linear regressions with the 31 variables as given in Tables I and II, log-transformed values for the 13 density variables, and patient identification numbers (assigned in order of admission to the facility). In only 14 of these pairs did an r^2 value exceed 0.33, and 9 of these were solely in the 11–35-day group; below, the remaining pairs are reported and should be interpreted with great caution. Further analyses split each of the 3 groups (11–35 days, >35 days, and overall) into subgroups: (A) peak parasitemia, $<10,000/\text{mm}^3$ or $\geq 10,000/\text{mm}^3$; (B) peak fever, $<105^\circ\text{F}$ or $\geq 105^\circ\text{F}$; and (C) gametocytes, detected or not, and with each remaining variable used the Mann–Whitney test to examine distributional differences. Below, the only 2 instances with a P value <0.01 are

reported. Investigations of independence within contingency tables, for drug response and infectivity, relied on *G*-tests (for 2×2) and log-linear models (for $2 \times 2 \times 2$), again with *P* values <0.01 marking significant differences (Sokal and Rohlf, 1981).

We calculated autocorrelation coefficients and plotted correlograms for the raw per-cubic-millimeter parasitemia data, for the local peaks in parasitemia (binary data, as defined above), for the fever data binned (<101 , $101\text{--}101.8$, $102\text{--}102.8$, $103\text{--}103.8$, $104\text{--}104.8$, $105\text{--}105.8$, or $106\text{--}106.8$ F), and in binary form (<101 or ≥ 101 F), and for the (binary) data on gametocyte presence/absence. The autocorrelation coefficient extends the familiar correlation coefficient (Sokal and Rohlf, 1981); it provides similar information about internal relationships among the elements of a stationary time series by measuring correlation between observations separated by a particular interval or time lag. A correlogram plots serial correlation within a time series as a function of the time lag between observations, by plotting values of autocorrelation coefficients for each period (Chatfield, 1989; Dunstan, 1993). For example, were only single broods with exactly quartan cycles present in an infection, and were the local peak approximations in perfect correspondence with merogony, there would be positive values of the autocorrelation coefficients for periods of 3, 6, and 9 days, and negative values for the other periods.

RESULTS

Tables I and II give summary statistics for the 57 USPHS *P. malariae* patient charts from the 1950s and 74 from the 1940s with more than 35 days of observation prior to intervention (see Materials and Methods). Figure 2 summarizes daily infection dynamics in all 84 patient charts from 1950 to 1958; Figure 3 depicts the individual charts of 10 infected patients from the 1950s.

Parasitemia

Inoculum size did not influence duration of prepatency (Fig. 4A), initial asexual-form density (Fig. 4B), or other key variables (Tables I, II) in these patient charts. Rates of response to the standard curative drug regime (1,500 mg of chloroquine base administered over 3 days) did not differ between the relevant charts from the 1940s and those from the 1950s; parasites were cleared after a mean of 4.8 and 4.2 days, with standard deviation 4.7 and 3.0 days, respectively. Prior treatment did not influence rates of response.

Not surprisingly, the correlogram of the raw per-cubic-millimeter parasitemia data in the 84 patient charts from 1950 to 1958 shows a smooth tailing-off of positive autocorrelation coefficients, which indicates that these data lack a dominant frequency or period and display only serial day-to-day correlation of densities above or below the mean value. However, the correlogram of the binary local peaks in parasitemia (Fig. 5A) indicates a mild degree of periodic oscillation, albeit not in an expected pattern. There is a positive value for period 3 (and for 2, 5, and 6), but the strongest positive correlation is for period 8, i.e., if there was a local peak at day *t*, then there was likely to be another peak at day *t* + 8, and if there was no peak at day *t*, then there was likely to be no peak at day *t* + 8.

Table IVA gives the transition matrix for the 1,186 intervals between these local peaks in parasitemia. The 3 textbook possibilities together account for only 15.4% of the transitions; if the ≥ 4 -day categories are eliminated, for instance, this figure rises only to 30%. Even allowing for what must be enormous error in the local peak approximations, this suggests that brood structures changed during the course of an infection, differed between infections, or both; this could account for more than half of the local-peak transitions.

A priori expectations for longer sequences of intervals can easily be calculated from the frequency distribution. Such calculations (not shown) suggest that consecutive 3-day intervals

occur less often than would be expected, and that when consecutive 3-day intervals do occur they are bounded by 2-day intervals more often than would be expected.

Fevers

The 64 patient charts from 1950 to 1958 with fever records reported a total of 858 preintervention days with at least 1 fever; 14% of the recorded fevers were 101–101.8 F, 5% were 106 F or higher, and the remaining 81% were approximately evenly distributed among the 4 intervening 1 F bins. In all 64 cases, parasitemia was patent on or before the day the first fever was reported. The first fevers were higher, by a mean of 1 F, in charts with peak parasitemias $\geq 10,000/\text{mm}^3$. The number (and proportion) of febrile days was greater, by a mean 11 days (0.19), in charts with peak fevers ≥ 105 F. Febrile days were more frequent earlier in infections than later (Fig. 2). A general positive correlation between the level (F) of peak fevers and the number (and the proportion) of days with fevers appeared in r^2 values of 0.36 (0.34) for the 1950–1958 charts overall and 0.35 (0.33) for those with 11–35 days before intervention.

The correlogram of the fever data, binned and in binary form, is given in Figure 5B. It indicates a strong degree of periodic oscillation, much of it in the pattern expected for a single brood. However, there are unexpected positive values of the autocorrelation coefficient for period 5, and, as with the local peaks in parasitemia, period 8.

Table IVB gives the transition matrix for the 677 intervals between these fevers. Here, the 3 textbook possibilities together account for 47.7% of the transitions; eliminating the ≥ 4 -day categories would increase this figure to 58%. This is much higher than with the local peaks in parasitemia but again suggests that brood structures may have changed during the course of infections, differed between infections, or both; this could account for more than 80% of the fever transitions.

Again, a priori probabilities for various sequences of intervals within the middle 677 intervals can easily be calculated from the frequency distribution. These calculations suggest that consecutive 3-day intervals, alternating 1- and 2-day intervals, and consecutive 1-day intervals occur more often than would be expected; as with the local peaks, consecutive 3-day intervals are bounded by 2-day intervals more often than would be expected.

Parasitemia and fevers

In only 4 of the 64 patient charts with fever records did the peak fever and the peak parasitemia occur on the same day, and in 16 more they occurred within 1 day of each other. In 37 charts, the peak fever preceded and in 23 followed the peak parasitemia. The peak fever and 1 of the next 2 highest parasitemias cooccurred in 10 charts; the peak parasitemia and 1 of the next 2 highest fevers cooccurred in 6 charts. In 5 charts, the peak fever coincided with a local peak in parasitemia; in 30 charts, the peak parasitemia coincided with a fever.

In 33 patient charts, the number of days with fevers was greater than the number of local peaks in parasitemia, in 5 the 2 were equal, and in 26 the number of days with fevers was less than the number of local peaks. Only 176 of the 858 fever days coincided with 1 of the 1,115 local peaks in parasitemia; another 502 occurred within 1 day of a local peak in parasitemia. That is, the correlograms in Figure 5 show striking similarities between the periodicities of local peaks and fevers, but on a chart-by-chart basis the local-peak and fever intervals rarely coincided.

Parasitemia and gametocytemia

As with the per-mm³ parasitemia data, the correlogram of the binary data on gametocyte presence/absence in the 57 patient charts from 1950 to 1958 in which gametocytes were reported before intervention indicates that these data lack a dominant frequency or period and display only serial day-to-day correlation of presence or absence.

In 4 patient charts, the peak gametocytemia and peak parasitemia occurred on the same day and, in 3 more, occurred within 1 day of each other. In 24 charts, the peak gametocytemia preceded and in 29 followed the peak parasitemia. Of the 363 days on which gametocytes were reported, 114 coincided with a local peak in parasitemia, and another 119 occurred within 1 day of a local peak in parasitemia. In 25 charts, the peak gametocytemia coincided with a local peak in parasitemia; in 13 charts, the peak parasitemia coincided with a gametocytemic day.

Gametocytes and infectivity

Gametocytes were recorded before intervention in 57 patient charts from the 1950s, on a total of 363 days. Seven of these charts reported only 1 day of gametocytemia, 8 only 2, and 7 only 3; in 6 patient charts, gametocytes were detected only after interference. Not only were gametocytes rarely detected (Fig. 2), but, when they were detected, their densities were generally low: just over half of the days recorded only 10 (male) gametocytes/mm³; only 10% recorded $\geq 50/\text{mm}^3$.

Four of the 17 mosquito feeds conducted in the 1940s led to successful transmission; each successful feed was on a different patient and, in each instance, was 1 of 2 feeds conducted on that patient. Twenty-seven of the 133 mosquito feeds in the 1950s were successful; these 27 feeds were on 8 different patients, representing 4–67% of the feeds conducted on those patients.

Mosquito feeds conducted after administration of subcurative drugs more often led to mosquito infection than did those conducted before ($P < 0.001$). The predrug and postdrug days on which mosquitoes were fed did not differ with respect to the presence or absence of gametocytes ($P > 0.5$). Successful feeds occurred earlier in infections, by a mean of 9 (predrug) or 23 (postdrug) days, but the distribution of the (elapsed) day of infection on which mosquitoes were fed did not differ between successful (mean day 59) and unsuccessful (mean day 61) feeds overall. The presence or absence of gametocytes as detected on feeding days did not determine whether or not a feed was successful ($P > 0.5$). However, among feeds at which gametocytes were detected, gametocyte density was higher in successful (mean 43/mm³) than in unsuccessful (mean 21/mm³) feeds.

Homologous reinfection

Previously malaria-naïve patients were occasionally infected twice in succession with the USPHS strain of *P. malariae* in the South Carolina facility; both charts are available for 6 such patients. With 2 of these patients, the first and second infections both occurred in the 1940s, with intervals between the final parasite-positive day of the first and the day of inoculation with the second of 22 and 60 days. With 2 others, the first infection occurred in the 1940s and the second in the 1950s, with intervals of 431 and 1,838 days. For the remaining 2, the first and second infections both occurred in the 1950s, with intervals of 4 and 7 days.

With all 6 patients, in the second infection the inoculum size, mean fever, and peak fever were lower, and the day of the first fever earlier, than in the first. With 5 patients, in the second infection the mean asexual density, peak asexual density, and number of days of fever were all lower; with 5, the day of peak fever was earlier and with 5, intervention occurred later; in each case a different patient showed the reverse relationships. For patients with shorter interinfection intervals, prepatency was shorter, the day of peak asexual density later, and the

first fever lower in the second infection than in the first; the reverse relationships held for those with the longest interinfection intervals. There were no clear patterns with respect to initial asexual-form density or to any of the gametocyte measures.

DISCUSSION

We have taken advantage of a unique opportunity to examine the preintervention blood-stage dynamics of *P. malariae* infections in a population of malaria-naïve U.S. neurosyphilis patients infected with a well characterized strain of the parasite, in circumstances nominally identical to those of the canonical studies of the species (Young, Stubbs, and Coatney, 1940; Young, Coatney, and Stubbs, 1940; Young et al., 1941). Thus, in many respects, our most striking results here are negative ones and are puzzling.

The canonical studies of this species generally supported the “common knowledge that single infections of *P. malariae* result in paroxysms every third day and ... the paroxysms are associated with the segmentation of the parasites,” but also noted that while “in certain patients the parasites move through successive life cycles with great precision ... in others ... the individual life cycles may be longer or shorter by several hours” (Young, Stubbs, and Coatney, 1940), and that cycles can shift in response to external conditions (Young, Coatney, and Stubbs, 1940). Boyd (1940) and Tiburskaja and Vrublevskaja (1965) found *P. malariae* fevers strongly periodic in some neurosyphilis patients but highly variable in others; Thayer (1897) and James (1910) remarked on the frequent discordance of fever and parasite development cycles. These authors, and others (Ciuca et al., 1934, 1964; Covell and Nicol 1951; Lupasco et al., 1968; Collins et al., 1973), reported dramatic variation among individual infections in the timing and magnitude of first fevers, peak fevers, first patent parasitemia, and peak parasitemia. Hence, case-to-case variation is a recognized, if often neglected, feature of *P. malariae* infections.

The variability reflected in our results seems exceptional, however; in particular, the parasitemia and fever dynamics in these patient charts only rarely correspond to conventional, textbook descriptions of quartan patterns. We found no strong relationships between measures of parasitemia and fever, in timing or magnitude (Tables I, II). Even the overlaps in periodicities of local peaks in parasitemia and of fevers (Fig. 5) are belied by chart-by-chart examination (Fig. 3). In several charts, for instance, 2 or more of the tertian–quartan–tertian sequences of local-peak intervals noted above interlocked (e.g., Fig. 3H), and in several charts 2 or more tertian–quartan–tertian sequences of fever intervals interlocked (e.g., Fig. 3G); this broader-scale pattern appeared both for local peaks and fevers in only 2 charts (e.g., Fig. 3A), however, and in neither did the patterns coincide. We cannot explain such absences of association.

Published records of daily *P. malariae* parasitemia are extremely rare, and as is the case here, none provide actual stage-specific asexual-form densities, hence we cannot gauge how well local peaks approximate merozoite release and thus brood structures in terms of merogony. Among published *P. malariae* fever charts (Boyd, 1940; Young, Coatney, and Stubbs, 1940; Young, Stubbs, and Coatney, 1940; Kitchen, 1941; Mackerras and Ercole, 1948), noting that the 2 charts given by Young and Burgess (1961) are included in the present study, only 1 (Kitchen, 1949) is accompanied by an associated parasitemia record. Furthermore, it is seldom clear when or if drug intervention occurred in these earlier charts or in other published data; this omission may be particularly significant for our purposes here, because a frequent byproduct of subcurative drug treatment in malariatherapy, and sometimes its aim (Becker, 1949), was to induce synchrony and reduce frequency in fevers.

Several differences between the patient charts from 1950 to 1958 and those from the 1940s are apparent (Tables I, II). Daily records doubled in frequency, but the fraction of recorded days with fevers or gametocytes declined. It may be that the decline in fever frequencies reflects

some change in intervention criteria, perhaps associated with the change in record-keeping procedures, or that, by chance or design, the 1940s procedures disproportionately recorded febrile and gametocytemic days. None of the magnitude-related aspects and only 1 timing-related aspect (the first fever day) of the fevers changed dramatically, but, because these were used as key indicators and objectives in malariatherapy, this is hardly surprising. Asexual-form and gametocyte densities seemed to decline by almost all measures, however. Perhaps repeated passage during the 1940s led to changes in the parasite that were not evident during the 1930s or, according to our regression results, the 1950s, but this is simply speculation; it is not difficult to censor half the daily records in the 1950s charts so as to reproduce the 1940s statistics, nor is it valuable to undertake formal resampling procedures, given the enormous variation within the 1950s patient charts.

There may be alternatives, or additions, to our suggestion that brood structures changed during the course of infections, differed between infections, or both. For instance, despite the canonical studies, it seems possible that the periodicity of a *P. malariae* brood is not strictly 72 hr, at least with this strain, and, similarly, that the correspondence of fevers to its merogony may be approximate rather than exact. Some portions of some charts can be fit by arbitrarily positing asexual blood-stage cycles with a fixed period between 2 and 3 days, particularly given that observation times may have varied by a few hours each day. This scheme can be elaborated by positing a multitude of broods, each with slightly different periods and asexual replication rates, and this might account for even more of the data; however, each such elaboration risks improving the fit simply by increasing degrees of freedom.

Almost nothing is known about antigenic or other polymorphisms in *P. malariae* (Tahar et al., 1998), and we do not know whether it exhibits antigenic variation. If *P. malariae* does in fact exhibit antigenic variation, then this might be intimately connected with its brood structures, somehow mediated by a network of immunological agents, though the connection would be far from immediate or transparent. We hope that future research will resolve these anomalies and will clarify the biological nature of broods.

Effects of inoculum size have previously been examined for only a few *P. malariae* infections, with conflicting results (Glynn, 1994). Our negative results with respect to inoculum size are in accord with analyses of malariatherapy charts from infections with *P. falciparum* (Glynn et al., 1995) and *P. ovale* (Glynn and Bradley, 1995a), and differ from the corresponding analyses of *P. vivax* infections (Glynn and Bradley, 1995b). Similarly slow rates of *P. malariae* response to drug treatment have been noted elsewhere (Collins et al., 1973).

We found no strong relationships between measures of gametocytemia and parasitemia, in timing or magnitude (Tables I, II). Most infections produced detectable gametocytes at some point but at low and seemingly erratic daily densities, as in previous studies of *P. malariae* (Basu, 1947; Constantinesco and Negluci, 1967; Collins et al., 1973). In many of the 1950–1958 patient charts, in particular, gametocyte densities fluctuated around the threshold of detection, often on a day-to-day basis. Transmission success related directly to gametocyte density only among mosquito feeds conducted on days with detectable gametocytemia and did not depend on the detected presence of gametocytes. Infectivity increased after subcurative drug administration, reminiscent of recent findings with *Plasmodium falciparum* (Chutmongkonkul et al., 1992; Robert et al., 2000). Overall, the timing of mosquito feeds had little, if any, effect on infectivity, and in this respect our results differ from those of the Collins et al. (1973) study of volunteers infected with Nigerian and Philippine strains, in which all 10 of the (83 total) mosquito feeds that proved infectious took place between days 14 and 24 of patent parasitemia. More generally, our results resemble those from a recent field study in Burkina Faso (Boudin et al., 1993), and those of Shute and Maryon (1951), who remarked that batches of mosquitoes often failed to become infected by feeding on patients with high *P. malariae* gametocyte counts

and that “batches became infected by feeding on patients where no gametocytes were found. This is quite beyond our understanding.”

Seventy years ago, Knowles and White (1930) wrote that *P. malariae* “is now senescent, and is gradually disappearing.” But *P. malariae* still exists, and its blood-stage dynamics still offer unique, valuable perspectives on the phenomena of human malaria. Though the premise of James (1910) may be improved, his conclusion stands: “Since the quartan is by far the rarest type of malaria ... it is not of as much practical and clinical importance to the physician ... yet the species ... will yield an abundant profit to one who carefully studies it.”

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Figure 1.

Plots, for the 96 patient charts from 1950 to 1958, of the day of patent infection on which the peak gametocyte density (A), peak fever (B), and peak asexual-form density (C), occurred. The arrow indicates day 35 of patency (see text).

Figure 2.

Patent gametocyte frequency (**A**; binary, in all 84 charts), fever frequency (**B**; binary, in the 64 charts with fever records), and asexual-form density (**C**; as the mean of the log₁₀ parasitemia, in all 84 charts), for each day of patent, preintervention infection in the 1950s charts with more than 35 days of observed preintervention patency.

Figure 3.

Examples of 1950–1958 charts with more than 35 days of preintervention patency. For each chart, the bar graph shows fevers (as $F - 100$), the line graph log 10 parasitemia (as circles) and gametocytemia (as diamonds) per cubic millimeter of blood, plotted from the first day of patent parasitemia to the last day prior to intervention. Patient identification numbers and days plotted are: (A) S-1175, 132 days; (B) S-1078, 38 days; (C) S-1109, 114 days; (D) S-1054, 48 days; (E) S-1290, 108 days; (F) S-1215, 55 days; (G) S-1268, 65 days; (H) S-1223, 62 days; (I) S-1277, 199 days; (J) S-1052, 196 days. Single quartan intervals between local peaks in parasitemia are most evident in parts A (days 70–82 and 84–90) and H (days 34–40 and 42–48). Single quartan intervals between fevers (i.e., days ≥ 101 F) are most evident in parts A (days 4–13, 39–51, 53–59, 72–78, and 83–89) and G (days 20–29, 31–37, 39–45, 47–56, 58–64). The patient in part J was treated with 1 g of terramycin daily on days 38–41 and reinoculated with USPHS *P. malariae* on day 72.

Figure 4.

The duration of prepatency (**A**) and density of the first patent parasitemia (**B**) plotted against the log 10 inoculum size in the 80 (of the 84 total with more than 35 days of preintervention patency) 1950s patient charts for which this information was available.

Figure 5.

The correlograms of local peaks in parasitemia (A; binary) and fevers (B; binary as solid line, binned as dotted line) in the 77 and 56 patient charts from the 1950s, respectively, with 4 or more fevers or local peaks.

Summary statistics for the 57 charts from 1950 to 1958 (top) and the 74 from the 1940s (bottom); for variables showing substantial differences) with more than 35 days of observed preintervention patency.*

	Min	Max	Median	Mean	SD
1950s charts					
Inoculum ($\times 1,000,000$)	0.1	86	12.5	19.8	19.2
Prepatency (days)	1	33	10	13.2	9.6
Preintervention (days)	37	264	74	91.1	48.7
Gametocytes rec (days)	0	27	5	7.4	6.3
% days gam recorded	0	33	6	9	8
Fevers recorded (days)	1	43	20	17.4	10.4
% days fever recorded	1	47	23	22	15
First asexuals (per mm^3)	10	950	20	44.6	126.8
First asexual peak (day)	2	14	4	4.9	2.4
First gametocytes (day)	4	60	23	26.8	15.3
First gam (per mm^3)	8	50	23	18.6	11.9
First fever (day)	1	68	10	12.3	14.5
First fever (F)	101	104.6	102.2	102.4	1.1
Peak asexuals (day)	11	76	27	31.4	14.6
Peak asexuals (per mm^3)	140	26,500	6,925	7,370	4,882
Peak gametocytes (day)	13	116	41	46.6	23.9
Peak gam (per mm^3)	10	200	30	49.3	40.9
Peak fever (day)	5	74	24	31.1	18.7
Peak fever (F)	101	106.6	105.6	105.1	1.4
1940s charts					
Gametocytes rec (days)	0	36	8	10.7	9.1
% days gam recorded	0	31	11	12	8
Fevers recorded (days)	4	51	27	26.7	11.0
% days fever recorded	6	70	32	33	16
First gam (per mm^3)	6	160	49.5	58.4	38.7
First fever (day)	1	30	5	6.9	5.4
Peak asexuals (per mm^3)	190	30,700	11,184	10,688.9	5,883.8
Peak gam (per mm^3)	8	330	117.5	123.8	78.9

* Values of magnitude variables are given as absolute numbers (inoculum size: $\times 1,000,000$), per millimeter densities (per mm^3), or degrees fahrenheit (F); values of the timing variables (days) are given as relative to the first day of patency (day 1). If 2 or more prospective peaks of equal magnitude occurred in a chart, the timing is considered to correspond to the earliest. Abbreviations: minimum, Min; maximum, Max; standard deviation, SD; percent, %; recorded, Rec; gametocytes, gam; not available, na.

Table II

Summary statistics for the 57 1950s and 74 1940s charts, as in Table I, showing arithmetic means of the asexual form and gametocyte densities (no./mm³) and fevers (i.e., $\geq 101^\circ\text{F}$), between the first day of patency and the timing points given in Table I.*

	Min	Max	Median	Mean	SD	Mean CV
1950–1958 charts						
Mean no. asexuals/mm ³ , to						
First asexual peak	10.0	1,449	73.6	147.2	229.2	1.13
Peak asexuals	46.0	5,689	1,783.0	1,908.0	1,232.0	1.05
First gametocytes	4.0	4,625	108.4	364.8	773.2	0.99
Peak gametocytes	56.0	4,624	1,649.0	1,725.5	1,282.9	0.95
First fever	57.4	6,288	997.8	1,476.2	1,406.5	1.0
Peak fever	57.4	6,288	1,883.0	2,093.9	1,414.1	0.87
Intervention	36.0	6,338	1,998.0	1,995.0	1,428.0	0.98
Mean no. gameto/mm ³ , to						
Peak gametocytes	10	91.2	22	26.9	17.14	0.47
Intervention	10	86.7	19.3	22.6	14.77	0.52
Mean fever ($\geq 101^\circ\text{F}$), to						
Peak fever	101	105.3	103.5	103.4	0.96	0.40
Intervention	101	104.8	103.4	103.3	0.94	0.35
1940s charts						
Mean no. asexuals/mm ³ , to						
Peak asexuals	104.0	14,716.0	3,645.0	3,687.3	2,066.5	0.99
First gametocytes	10.0	2,918.0	98.3	259.8	478.2	0.79
Peak gametocytes	16.7	10,199.0	3,378.0	3,475.7	2,097.7	0.95
First fever	341.0	6,687.0	2,664.0	2,652.8	1,643.5	1.02
Peak fever	346.0	9,718.0	3,450.0	3,575.3	1,755.1	0.86
Intervention	62.3	10,005.0	3,511.5	3,549.4	2,292.5	0.92
Mean no. gameto/mm ³ , to						
Peak gametocytes	14.4	172.0	65.0	69.3	36.5	0.45
Intervention	13.7	140.0	56.7	64.5	34.7	0.46

* The mean CV column gives the arithmetic mean of the coefficient of variation (calculated as standard deviation/mean) for each chart, which provides a measure of within-chart variation. Other conventions and abbreviations as in Table I.

Table III

Frequency distributions for the intervals between local peaks in parasitemia, in 77 patient charts, and between fevers, in 56 patient charts (see text).

Interval/frequency	Local peaks (1,186)	Fevers (677)
1 day	0.029	0.240
2 days	0.328	0.264
3 days	0.365	0.394
≥4 days	0.278	0.102

Table IV
Transition matrices for the intervals (A) between local peaks in parasitemia, and (B) between fevers, in 77 and 56 patient charts, respectively (see text).

Transition	To 1 day	To 2 days	To 3 days	To ≥ 4 days
A)				
From 1 day	0.004	0.009	0.003	0.008
From 2 days	0.009	0.080	0.141	0.095
From 3 days	0.007	0.129	0.132	0.094
From ≥ 4 days	0.009	0.104	0.077	0.099
B)				
From 1 day	0.106	0.078	0.041	0.015
From 2 days	0.104	0.032	0.106	0.021
From 3 days	0.027	0.138	0.189	0.040
From ≥ 4 days	0.007	0.022	0.046	0.028