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GAMETOCYTEMIA AND FEVER IN HUMAN MALARIA INFECTIONS

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Abstract

We examine the charts of 408 malaria-naïve neurosyphilis patients given malaria therapy at the South Carolina USPHS facility, with daily records encompassing at least 93% of the duration of infection, and focus on the 152 patients infected with the St. Elizabeth strain of *Plasmodium vivax*, 82 with the McLendon strain of *Plasmodium falciparum*, 36 with the USPHS strain of *Plasmodium malariae*, and 15 with the Donaldson strain of *Plasmodium ovale* in whom gametocytes appeared before drug, or other, intervention. In *P. vivax* infections, fever and parasitemia were higher after gametocytes were first detected than before; in *P. malariae* infections, parasitemia was higher. In *P. ovale* infections, fever and parasitemia were similar before and after. In *P. falciparum* infections, fever, parasitemia, and fever frequency were lower after gametocytes were first detected than before. Parasitemia and temperature correlated in *P. vivax* infections, before and after gametocytes were first detected; parasitemia and temperature at first fever were not correlated in infections with any species. Gametocyte density correlated with parasitemia in *P. malariae* and sporozoite-induced *P. falciparum* and *P. vivax* infections. Fevers and detected gametocytemia coincided more often than expected by chance with *P. vivax* and *P. ovale*; fever temperature and gametocyte density were not correlated in infections with any species.

The differing dynamics of gametocytemia in *Plasmodium* species were noted long ago. Boyd and Kitchen (1937) wrote that "in vivax infections … gametocytes are produced at every period of multiplication … [but] falciparum gametocytes are not observed until about 10 days after the first appearance of parasites, and may not be present until after the primary attack subsides." Hackett (1941) added that the first appearance of *Plasmodium malariae* gametocytes "is delayed sometimes for months." Shute and Maryon (1951) observed that gametocytes in *Plasmodium falciparum* infections appeared 8–10 days after a first fever, in *Plasmodium vivax* 6–7 days, and in *Plasmodium ovale* earlier still.

The timing of the first gametocyte detection relative to a first fever or first asexual-form patency follows from the rate at which gametocytes are produced, the multiplication rate of the asexual forms from which gametocytes arise, the extended sequestration of immature *P. falciparum* gametocytes, the dynamics of fever induction, thresholds of parasite detection, and, surely, other factors. Virtually nothing about any part of the process is understood as yet.

In a 3-yr cross-sectional study of adults attending malaria clinics in Peru and Thailand (McKenzie, Wongsrichanalai et al., 2006), we found that *P. vivax* patients with gametocytemia had higher fever and higher parasitemia than those without gametocytemia, while *P. falciparum* patients with gametocytemia had lower fever than those without gametocytemia, but similar parasitemia. Temperature correlated with parasitemia in the gametocytemic *P. vivax* patients and the nongametocytemic *P. falciparum* patients; gametocyte density correlated

with parasitemia in *P. vivax*, but not *P. falciparum*, and correlated with temperature in only 1 of the 8 site-year-species combinations.

In that paper, we also noted that relationships between gametocyte prevalence, gametocyte density, parasitemia, and clinical symptoms remain unresolved for even the best-studied populations, i.e., *P. falciparum*–infected children in sub-Saharan Africa, and that much of the published evidence appears contradictory. Gametocytemia may be associated with higher or lower parasitemia, or with the presence or absence of anemia, in these populations. Over somewhat wider age ranges, some reports indicate that *P. falciparum* gametocyte prevalence among clinic patients declines with age, though gametocyte density does not (Akim et al., 2000), and that young *P. falciparum* gametocyte carriers with fever have lower gametocyte densities, but higher asexual-form densities, than those without fever (Gouagna et al., 2004). The relationships are even more mysterious with other *Plasmodium* species, and in other age groups, across the rest of the malaria-endemic world.

In his study of gametocytemia, Schuffner (1938) posed a classic challenge: “Obviously the collective parasite picture is a composite of numerous individual parasite pictures existing in persons whose state of health, or ill health, differs widely. On the face of it, it would seem impossible to trace any fixed rules in this chaos of unlimited possibilities.” Here, we take advantage of a wealth of longitudinal data on individual patients to investigate how the dynamics of individual infections may relate to static samples, in which data are collected for each patient at a single time point, using the same statistical procedures to address, to the extent possible, the same questions as in our Peru-Thailand study.

MATERIALS AND METHODS

Current knowledge of *Plasmodium* spp. dynamics in infected humans derives largely from 40-yr work with malaria induced to treat neurosyphilis. Many fundamental insights are owed to these malaria therapy patients, to whom we are extremely grateful. Malaria therapy treatment and data collection procedures, including those for the determination of parasitemia, gametocytemia, and patient rectal temperature during each infection, are described in detail elsewhere (Collins and Jeffery, 1999, 2002, 2005; McKenzie et al., 2001, 2002a). The first of these citations also contains extensive information about the participation and treatment of the patient population considered here, and it is accompanied by an explicit, independent analysis of relevant ethical issues.

Here, we examine the records of adult neurosyphilis patients with no known history of previous malaria infection, treated with the St. Elizabeth strain of *P. vivax*, McLendon strain of *P. falciparum*, USPHS strain of *P. malariae*, or Donaldson strain of *P. ovale* in the U.S. Public Health Service (USPHS) facility in Columbia, South Carolina. All of the *P. malariae* and *P. ovale* infections, most of the *P. falciparum* infections, and roughly half of the *P. vivax* infections considered here were initiated by inoculation of 5 ml of whole blood from a patently infected patient. The remaining *P. falciparum* and *P. vivax* infections were initiated by bites of infectious mosquitoes that previously fed on a patient or by inoculation of glands or sporozoites extracted from infectious mosquitoes. Because our previous analyses showed no notable differences between the latter 2 routes (McKenzie et al., 2002a), we distinguish only “sporozoite-induced” from “trophozoite-induced” infections, in line with our earlier work (Collins and Jeffery, 1999). Because our previous analyses showed differences in dynamics between sporozoite- and trophozoite-induced infections, we analyze those categories separately here (Table I).

Malaria therapy infections generally were allowed to continue as long as possible without intervention, and were then terminated with curative doses of drug. However, at the discretion of medical staff, subcurative doses of drugs, or other interventions, might be given with the

explicit aim of modulating an infection, e.g., to provide a break from fever or to reduce parasitemia. Our analyses include only the parts of charts that precede any such intervention. Further, our analyses include only charts in which extant daily records encompassed at least 93% of the preintervention duration of each infection, i.e., those in which the records were blank on <1 day in each 2 wk, on average.

Temperature was recorded only when ≥101 F (38.3 C), and we adhere to that definition of fever here. Microscopists used the Earle-Perez technique (Earle and Perez, 1932); the threshold of detection for asexual forms or gametocytes was generally 10 per μl.

In most previous analyses, we set the standard time scale for each infection to start with the day of the first detection of asexual blood forms. Here, given our interest in how static surveys reflect parasite dynamics, and the consistency of the interval from asexual-form patency to the first fever in each infection (Table I), we set our initial time point (day 1) as the day of first fever. This provides a practical reference time point for calibrating presentation at a clinic, for instance, as in our recent study in Peru and Thailand (McKenzie, Wongsrichanalai et al., 2006).

Intervention or termination sometimes occurred before, sometimes very soon after, and sometimes 6 mo, or more, after the day of first detection of gametocytes. Because biases would be introduced by considering a few days commensurate to months of gametocytemia, for the purpose of comparing densities, intensities, or even frequencies, we set a standard interval after the day of first detection of gametocytes equal to the interval between the first fever and the first detection of gametocytes, so that in each infection our analyses consider an equal number of days before, and after, the first detection of gametocytes (see the schematic in Fig. 2: interval ab = interval bc). We use the results for these patients (N3 in Table I) as a baseline, but note points at which these results differ from those incorporating charts truncated earlier (N2, ab > bc). We also provide summary statistics for the patients in whom intervention occurred before any gametocytes were detected (N1).

We use the Mann-Whitney *U*-test and Spearman's rank correlation coefficient to compare distributions, giving *P*-values for 2-tailed tests, and use the *G*-test to investigate independence within contingency tables (Sokal and Rohlf, 1981).

RESULTS

Figure 1 shows the daily frequencies of fever and detected gametocytemia, within each species and inoculation mode, with all charts aligned from the first day of asexual-form patency, i.e., the fraction of all patient records that reported fever, or gametocytemia, on each day. The frequency of fever first exceeded 50% on day 2 with *P. falciparum*, day 2–3 with *P. vivax*, and day 7 with *P. ovale*; it reached a maximum 47% with *P. malariae* on day 27. The frequency of gametocytemia first exceeded 50% on day 12–13 with *P. falciparum*, and day 9–10 with *P. vivax*; it reached a maximum of 20% with *P. malariae* on day 23, and 26% with *P. ovale* on day 10. Thus, all else equal, one would not expect to detect gametocytemia in most patients until 10–11 days after the first fever with *P. falciparum*, and 7 days with *P. vivax*, or to detect gametocytemia in most patients on any day with *P. ovale* or *P. malariae*.

Figure 2 shows idealized schematics of the course of individual *P. falciparum* and *P. vivax* infections. Intervention in the infections in group N1 occurred before point b, i.e., there were no gametocytes detected before intervention; in group N2 when interval bc < ab, i.e., there were fewer days after first detection of gametocytes than before; and in group N3 when bc = ab, i.e., the number of days before and after first detection of gametocytes was identical.

We identified the median parasitemia and median fever for each patient before and after the first detection of gametocytes in the infection and used these values as the baseline data for the Mann-Whitney *U*-tests. Table I shows the median and 95% confidence interval for these median values for the N1, N2, and N3 patients, and the results of Mann-Whitney *U*-tests for the N3 patients. Note that the median interval between first fever and first gametocytemia was 9 days for *P. falciparum*, and 5–6 days for *P. vivax*. Figure 3 shows the proportion of patients in whom the median parasitemia or median fever before gametocytes were first detected was higher than the median after, i.e., 58–100% for *P. falciparum* infections, 10–32% for *P. vivax*, 25–33% for *P. malariae*, and 47–60% for *P. ovale*.

The median values observed in a patient had not necessarily occurred on the same day, however. Therefore, for each species and induction mode, we also aggregated all pregametocyte-detection values, and all postgametocyte-detection values, of all patients. The last 4 rows of Table I show the median and 95% confidence interval for these values for the N3 patients. Because the interval between the first fever and the first detection of gametocytes was about 1 wk, on average, these aggregated data included roughly 7 times as many data points as the patient medians. Note that, as expected, the medians are close, but the confidence intervals narrowed relative to those in the rest of Table I. We used these values, pairing same-day same-patient observations, as the baseline data for the Spearman correlation tests.

In *P. vivax* and *P. malariae* infections, fever and parasitemia were higher after gametocytes were first detected than before. Results for *P. vivax* did not differ when the N2 patients were included, but the difference in fever for *P. malariae* was diminished (Mann-Whitney *U*-test, $P = 0.03$). In *P. ovale* infections, fever and parasitemia before gametocytes were first detected were indistinguishable from fever and parasitemia after ($P > 0.83$). In trophozoite-induced *P. falciparum* infections, fever and parasitemia were lower after gametocytes were first detected than before; in sporozoite-induced infections, only fever was lower. When the N2 patients were included, all of these differences for *P. falciparum* were diminished ($P > 0.04$). The results for differences in fever frequency (Table I) did not change when the N2 patients were included, however, for any species or induction mode.

Parasitemia and temperature correlated in *P. vivax* infections, both trophozoite- and sporozoite-induced, both before and after gametocytes were first detected (Spearman $P < 0.0003$). Parasitemia and temperature also correlated in *P. malariae* and sporozoite-induced *P. falciparum* infections before gametocyte detection ($P < 0.0006$), and in trophozoite-induced *P. falciparum* infections after gametocyte detection ($P = 0.001$). The results did not change when the N2 patients were included. Parasitemia and temperature at first fever were not correlated in infections with any species, induction mode, or patient group (N3, N2 + N3, N1 + N2 + N3; $P > 0.10$).

Gametocyte density correlated with parasitemia in *P. malariae* and sporozoite-induced *P. falciparum* and *P. vivax* infections ($P < 0.00003$). Gametocyte density and temperature were not correlated in any infections ($P > 0.09$). However, detected gametocytemia and fever (after the first gametocyte detection) coincided more often than expected by independence in *P. vivax* and *P. ovale* infections (*G*-test, $P < 0.003$; vs. $P > 0.18$ for *P. malariae* and *P. falciparum*). The results did not change when the N2 patients were included.

We examined the resilience of our conclusions both by comparing our baseline results with the N3 patients to results when the N2 patients were included, as noted above, and by applying the Spearman and Mann-Whitney *U*-tests to the patient-median and aggregated data, respectively (see Table I and Appendix). Concurrence across these 4 different sampling and statistical procedures was in most respects greater than we had expected. Overall, our

conclusions for *P. vivax* and *P. ovale* appeared less sensitive to the inoculation mode and the sample (of patients and/or time points) than those for *P. malariae* and *P. falciparum*.

DISCUSSION

Our results were generally in accord with our results from the Peruvian and Thai clinics (McKenzie, Wongsrichanalai et al., 2006), i.e., the *P. vivax* patients with gametocytemia had higher fever and higher parasitemia than those without gametocytemia, and the *P. falciparum* patients with gametocytemia had lower fever than those without gametocytemia. However, in Peru and Thailand, parasitemia was generally indistinguishable between *P. falciparum* patients with and without gametocytemia, while here we found *P. falciparum* parasitemia lower after gametocytes were first detected than before. We can simulate the Peru-Thailand parasitemia result here by including the N2 patients, which serves to lower the median parasitemia before and raise the median parasitemia after gametocytes were first detected (Table I). Including the N2 patients negates the fever result, however.

Considering the idealized schematics in Figure 2, the nominal effect of including the N2 patients is to narrow the sampling interval after gametocytes were first detected by moving point c closer to point b (the first gametocyte detection). Since including these patients also alters the “typical” parasitemia curve and fever intensity for *P. falciparum* infections (top panel), features presumably related to the earlier intervention, and shifts point b slightly closer to point a, i.e., to slightly earlier gametocytemia, this suggests that the relationship between parasitemia and fever relative to the onset of gametocytemia differed between the populations.

Our correlation results seem to support this interpretation and were unaffected by including the N2 patients. The data for the sporozoite-induced infections were consistent with those from the Peruvian and Thai clinics in that temperature and parasitemia correlated in nongametocytemic, but not in gametocytemic, *P. falciparum* patients. With trophozoite-induced *P. falciparum* infections, the pattern was reversed, in that temperature and parasitemia correlated in gametocytemic, but not nongametocytemic, patients. Here, as in the Peruvian and Thai clinics, fever temperature and gametocyte density were not correlated in infections with either species, whether sporozoite- or trophozoite-induced. Thus, the remaining points of partial disagreement are that, here, temperature and parasitemia correlated in nongametocytemic as well as gametocytemic *P. vivax* infections, whether sporozoite- or trophozoite-induced, and that gametocyte density and parasitemia correlated in sporozoite-induced infections with *P. falciparum* as well as *P. vivax* (but not in trophozoite-induced infections with either species).

The adult neurosyphilis patients considered here differed from the adult patients at the Peruvian and Thai malaria clinics in many ways. For instance, whatever concurrent infections may have been present in the latter, they were less microbiologically homogeneous and less clinically severe; however, it is much more likely that they included cryptic infections with other *Plasmodium* species, which can affect gametocytemia, fever, and parasitemia (Price et al., 1999; McKenzie et al., 2002b; McKenzie, Smith et al., 2006). Furthermore, previous malaria exposure must have greatly affected infections in the Peruvian and Thai patients by altering immune responses. Infections in some neurosyphilis patients were affected by the transfers of trophozoites in whole blood, rather than of sporozoites passaged through mosquitoes. The differences between the induction modes noted above may have arisen from the relative timing of the blood-stage dose, the mixtures of antigenic material present in whole blood, synchronization of antigenic-variant expression by mosquito passage (Peters et al., 2002), or other unknown factors. The correlation of gametocyte density with parasitemia in the sporozoite-induced, but not the trophozoite-induced *P. falciparum* and *P. vivax* infections, seems particularly intriguing in this regard.

These and other differences between the populations make the similarities of their malaria infections still more remarkable (Winckel, 1941) and reinforce the point that such combinations of similarities and differences can be used to guide studies at different levels of resolution (Talman et al., 2004). Much the same holds for comparisons across *Plasmodium* species, i.e., there is no reason to assume that the factors that regulate gametocyte production, or stimulate fever, are either exactly the same or entirely different across the 4 species investigated here.

The timing of gametocytemia in relation to fever and parasitemia in any of these species may be only an epiphenomenon, marking elapsed time in an infection, or it may reflect underlying connecting mechanisms. It is conceivable, for instance, that high temperatures alter parasite or host metabolism in some way that affects gametocyte production, sequestration, decay, immune response, or some other factor that affects gametocytemia, but to the best of our knowledge there is no evidence to support or refute any such speculation. The crucial role of gametocytes in *Plasmodium* spp. epidemiology, evolution, transmission, and recombination would seem to argue for some link, and recent studies of gametocyte production in mixed-genotype *P. falciparum* infections (Abdel-Wahab et al., 2002; Sutherland et al., 2002; Nassir et al., 2005) have begun to suggest a complexity of dynamics at levels previously examined only in theoretical work (McKenzie, Ferreira et al., 2001; McKenzie, Killeen et al., 2001). The forces driving observed population-level differences in gametocyte prevalence and density with respect to age, season, and transmission intensity (Covell, 1960; Rosenberg et al., 1990; Luxemburger et al., 1996; Drakeley et al., 2000) remain mysterious at the level of individual *P. falciparum* infections and unexamined in other species; we look forward to their resolution.

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APPENDIX

These day-by-day data from hundreds of patients provided an opportunity to examine the sensitivity of our conclusions, through the inclusion or exclusion of a particular subset of patients (N2), and through testing both the single median observations for each variable from each patient and roughly 1 wk of observations for each, aggregated for all patients, before and after the first detection of gametocytemia.

We performed Mann-Whitney *U*-tests on the aggregated data as well as the patient-median data (see Table I), and, as expected, the results were closely similar. The 3 disagreements involved the *P. malariae*, *P. ovale*, and sporozoite-induced *P. falciparum* infections, the categories with the smallest sample sizes. Here, in the *P. ovale* and sporozoite-induced *P. falciparum* infections, parasitemia was lower after gametocytes were first detected than before. With *P. malariae*, the difference in fever was greatly diminished ($P = 0.33$). When the N2 patients were included, the only change was that the difference in fever in the sporozoite-induced *P. falciparum* infections became indistinguishable ($P = 0.11$).

We performed Spearman tests on the patient-median data, as well as the aggregated data, pairing same-patient observations, and found an unexpected similarity in the results. With the N3 patients, parasitemia and temperature again correlated in all *P. vivax* infections; when the N2 patients were included, the correlations for *P. malariae* and *P. falciparum* appeared as well. Gametocyte density and temperature were again not correlated in any infections ($P > 0.17$). Gametocyte density correlated with parasitemia only in the trophozoite-induced *P. vivax* infections, however ($P = 0.01$); when the N2 patients were included, a correlation appeared in the *P. malariae* infections ($P = 0.0009$). Thus, the parasitemia-gametocytemia results were the most sensitive to day-day pairing.

With any of these species, the gametocytes detected on a given day did not necessarily arise from the same generation as the asexual-form parasites present on that day or any particular number of days before. Gametocytes were detected on most but not all days after gametocytes were first detected (Fig. 1; Table I). Unlike fevers, which can be taken as relatively discrete events on a day-to-day basis, gametocyte densities involve decay as well as production rates (Eichner et al., 2001), and gametocyte detection involves many further complications (McKenzie et al., 2003;O'Meara et al., 2005) on which a future paper will focus. Here, though it is hazardous to equate "after gametocytes were first detected" with "detected gametocytemia," we do so in comparing our results to those from other studies, including our own from Peru and Thailand.

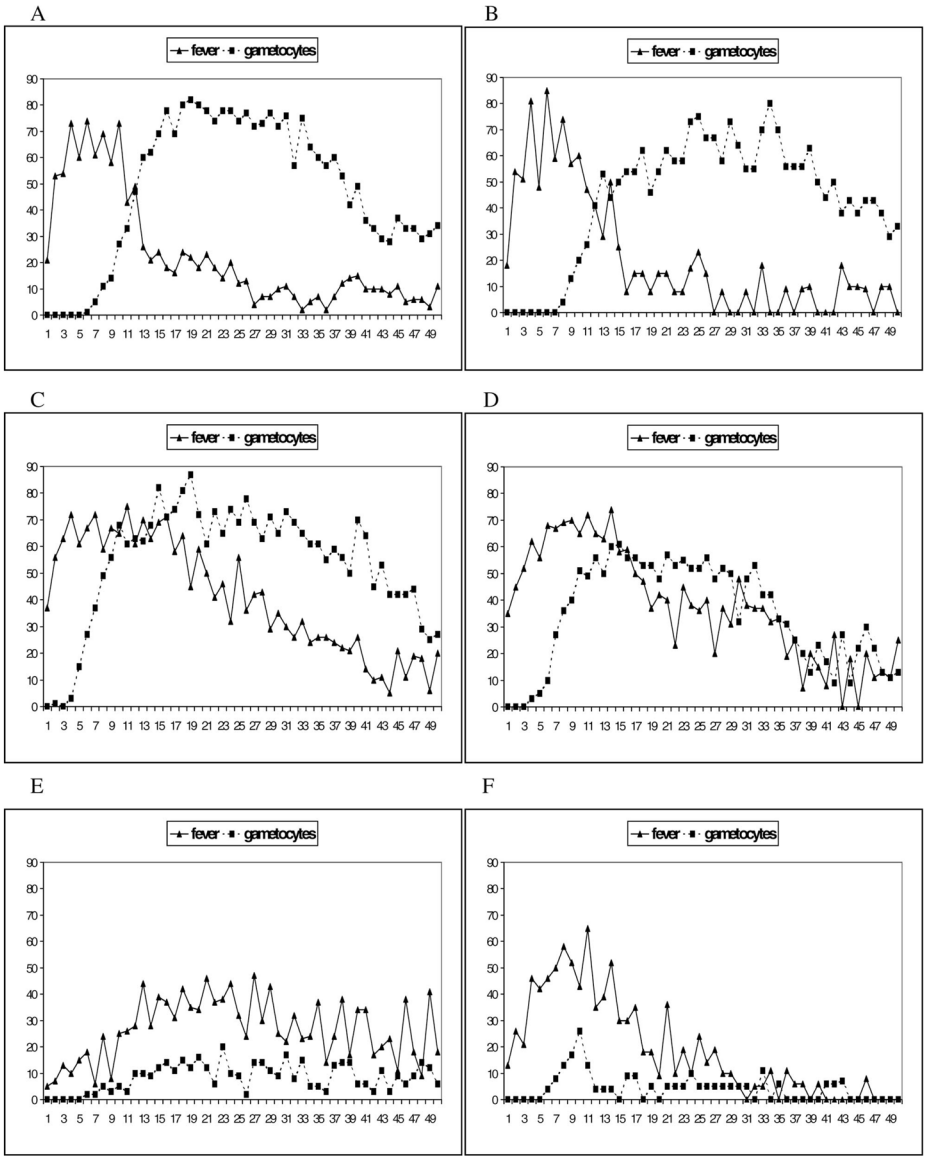

Figure 1.

Day-by-day frequencies (percentage of patients; vertical axis) of fever or gametocytemia, from the first day of patency (as day 1; horizontal axis). Top panels: *P. falciparum*, trophozoite-induced (left, **A**) and sporozoite-induced (right, **B**). Middle panels: *P. vivax*, trophozoite-induced (left, **C**) and sporozoite-induced (right, **D**). Bottom panels: *P. malariae* (left, **E**) and *P. ovale* (right, **F**), both trophozoite-induced.

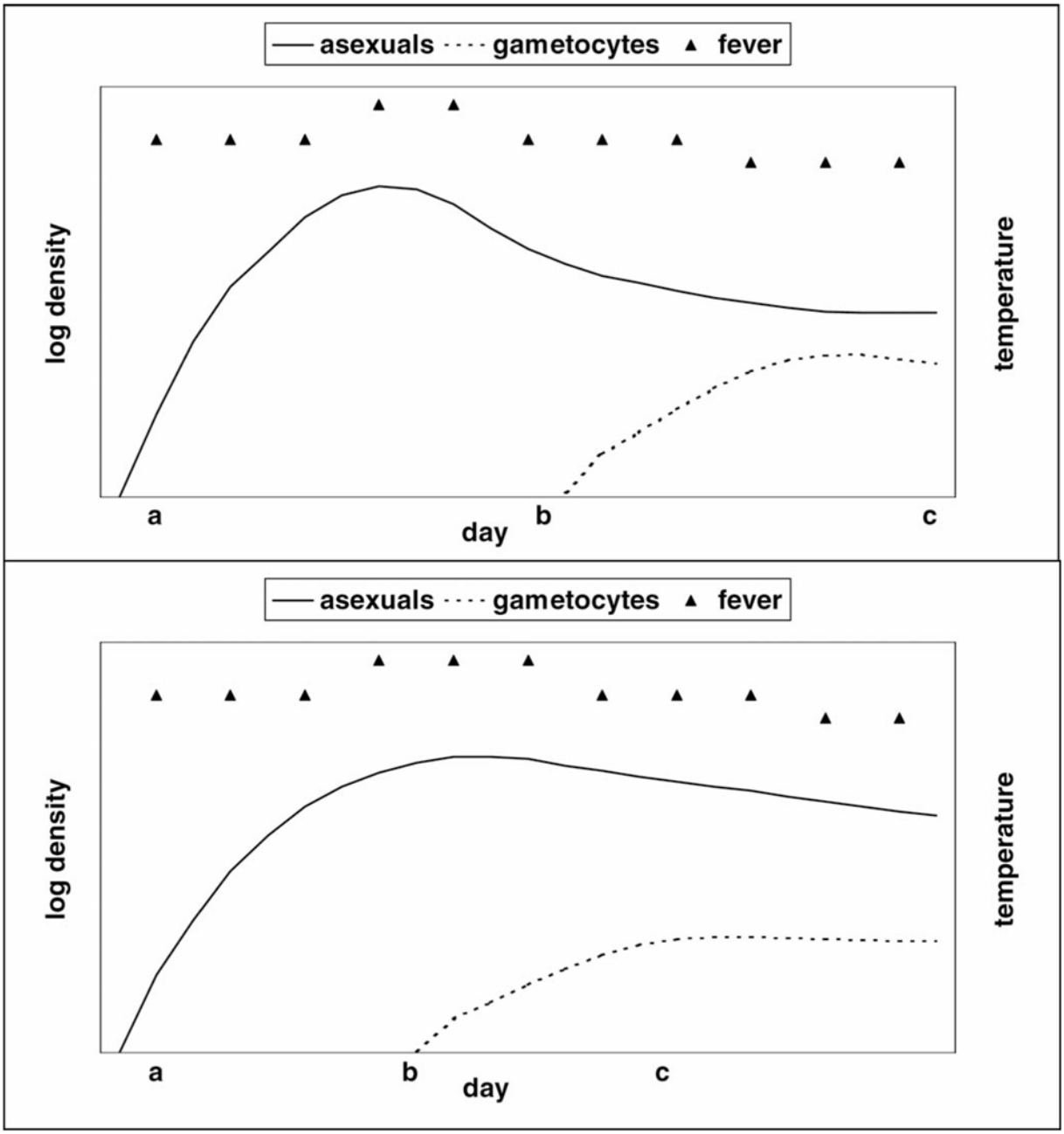

Figure 2.
Schematics of idealized *P. falciparum* (top panel) and *P. vivax* (bottom panel) infections. The first fever occurs on day a, the first gametocytemia on day b; day c is set such that interval bc = ab (group N3; see text).

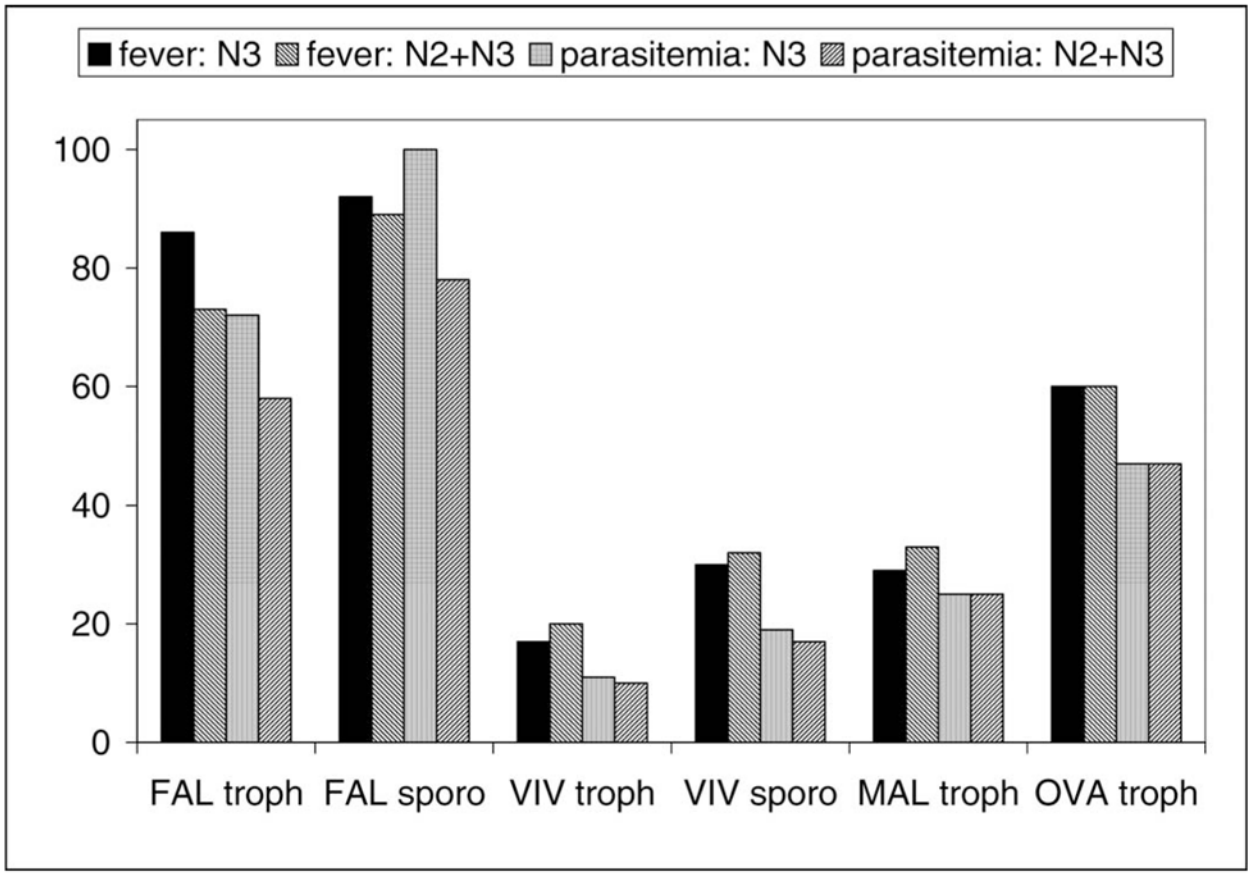

Figure 3.
The proportion of patients (%; vertical axis) in whom the median parasitemia or median fever before gametocytes were first detected was higher than the median after, in *P. falciparum* (FAL), *P. vivax* (VIV), *P. malariae* (MAL), and *P. ovale* (OVA) infections, induced by trophozoites (troph) or sporozoites (sporo), in the N2 or N2 + N3 patients (see text).

Table I

Median values (95% confidence limits) for patient medians; for the N1, N2, N3 patients; and for the aggregate values for the N3 patients (see text). Results of Mann-Whitney *U*-tests, for comparisons of values before and after the first detection of gametocytes are denoted * $P < 0.01$, $^{**}P < 0.0002$, both for the N3 patients and the N2 + N3 patients (denoted in the N2 rows). Columns are for patients infected with *P. falciparum* (FAL), *P. vivax* (VIV), *P. malariae* (MAL), or *P. ovale* (OVA). Rows show log 10 per-μl parasitemia or gametocytemia (gam), patient fever (fvr) in degrees C or daily frequency (/d), the number of days between the first fever and intervention (1st fvr–end), the first fever and first gametocytemia (1st fvr–1st gam), or the first gametocyte and intervention (1st gam–end). Thus, rows are for parasitemia at the first fever (1st fvr), temperature of the first fever, day of the first fever (with the first day of patency as day 1), day of the first gametocytemia (with the first day of patency as day 1), parasitemia before and after the first gametocytemia, fever before and after the first gametocytemia, fever frequency per day (/d) before and after the first gametocytemia, the difference in fever frequency (before–after), and the daily frequency of gametocytemia (/d).

	FAL trophozoite	FAL sporozoite	VIV trophozoite	VIV sporozoite	MAL trophozoite	OVA trophozoite
N1 Patients	27	20	14	27	26	9
1st fvr parasitemia	2.76 (1.78–3.48)	2.19 (1.78–2.88)	2.15 (1.00–2.91)	1.66 (1.00–2.30)	2.88 (2.43–3.41)	2.84 (1.00–3.61)
1st fvr temperature	39.6 (38.9–40.0)	39.8 (38.9–40.6)	39.2 (38.6–40.0)	39.1 (38.7–39.6)	38.9 (38.3–39.4)	40.1 (38.3–40.8)
1st fvr day	2 (1–4)	2 (1–4)	2 (1–3)	2 (1–3)	12.5 (6–15)	3.5 (1–8)
Parasitemia	3.88 (3.32–4.57)	3.58 (3.11–4.15)	3.49 (2.48–3.91)	3.01 (2.52–3.53)	3.29 (2.28–3.57)	2.06 (1.00–2.90)
Fever	40.1 (39.6–40.6)	40.4 (40.2–40.6)	40.1 (39.3–40.7)	40.4 (39.7–40.8)	39.4 (39.0–40.0)	39.7 (38.8–40.8)
Fever (/d)	0.85 (0.67–1.0)	0.81 (0.67–0.86)	0.82 (0.67–1.0)	0.77 (0.47–0.88)	0.37 (0.06–0.53)	0.19 (0.11–0.75)
1st fvr–end (d)	5 (3–8)	6 (3–7)	6 (4–7)	8 (7–12)	42 (10–67)	43 (8–64)
N2 Patients	14	6	20	22	8	
1st fvr parasitemia	2.94 (1.00–3.41)	2.16 (1.30–4.35)	1.69 (1.00–2.57)	1.53 (1.00–2.08)	2.08 (1.00–3.47)	
1st fvr temperature	39.6 (38.6–40.8)	39.9 (39.2–40.6)	39.5 (38.6–40.0)	39.1 (38.3–39.8)	38.9 (38.3–39.9)	
1st fvr day	2.5 (1–4)	2 (1–4)	1 (1–3)	1 (1–3)	6 (1–12)	
1st gam day	8.5 (7–11)	12 (8–21)	9.5 (7–12)	10 (7–13.5)	29 (8–60)	
1st fvr–1st gam (d)	6 (5–9)	9 (6–19)	7.5 (3–10)	8 (5–10.5)	21 (5–48)	
Parasitemia before	3.28 (2.63–3.82)	3.71 (3.04–4.39)	3.02 (2.55–3.62)	3.26 (2.68–3.61)	3.23 (1.85–3.74)	
Parasitemia after	4.16 (3.82–4.55)	4.10 (1.60–4.69)	4.05 (3.59–4.16)**	3.88 (3.46–4.02)**	3.36 (2.92–3.99)*	
Fever before	40.3 (39.3–41.1)	40.0 (39.4–41.1)	40.3 (39.4–40.6)	40.3 (38.9–40.6)	39.8 (38.6–40.6)	
Fever after	40.8 (40.2–41.1)	40.2 (38.9–41.1)	40.6 (39.9–41.0)**	40.6 (39.9–40.8)**	39.8 (38.9–40.8)	
Fever (/d) before	0.69 (0.50–0.80)	0.71 (0.47–1.0)	0.69 (0.62–0.78)	0.72 (0.60–0.85)	0.38 (0.20–0.45)	
Fever (/d) after	1.0 (0.67–1.0)**	0.67 (0.50–1.0)*	0.93 (0.67–1.0)	1.0 (0.53–1.0)	0.48 (0.09–1.0)	
Difference (/d)	−0.2 (−0.50–0.0)	0.04 (−0.33–0.50)	−0.09 (−0.33–0.03)	−0.22 (−0.36–0.07)	−0.09 (−0.57–0.40)	
Gam (/μl)	1.40 (1.00–2.07)	1.68 (1.00–3.10)	1.61 (1.20–1.94)	1.52 (1.00–1.85)	1.07 (1.00–1.65)	
Gam (/d)	1.0 (1.0–1.0)	0.83 (0.33–1.0)	1.0 (0.75–1.0)	1.0 (0.63–1.0)	0.33 (0.08–1.0)	
1st gam–end (d)	2 (1–3)	2.5 (1–6)	3 (2–4)	3 (1–5)	5 (1–8)	
N3 Patients	50	12	63	47	28	15
1st fvr parasitemia	2.59 (2.08–3.36)	2.41 (1.00–3.02)	2.05 (1.60–2.32)	1.68 (1.48–2.00)	2.33 (1.30–2.74)	2.10 (1.48–3.04)
1st fvr temperature	39.3 (38.9–39.8)	38.9 (38.3–40.6)	39.3 (38.9–39.7)	39.2 (38.9–39.4)	39.1 (38.6–39.7)	39.4 (38.6–40.1)
1st fvr day	2 (2–4)	2 (2–6)	2 (1–2)	2 (2–3)	6 (3–10)	4 (2–5)
1st gam day	12 (11–14)	12 (9–24)	8 (6–8)	8 (7–10)	16 (13–25)	10 (7–19)
1st fvr–1st gam (d)	9 (7–11)	9 (6–18)	5 (4–7)	6 (4–9)	9 (4–21)	7 (2–15)
Parasitemia before	3.60 (3.27–3.96)	3.82 (2.26–4.36)	3.07 (2.90–3.24)	3.09 (2.81–3.25)	2.83 (2.41–3.12)*	3.04 (2.40–3.34)
Parasitemia after	3.12 (2.54–3.28)**	2.68 (1.30–3.45)	3.80 (3.72–3.92)**	3.68 (3.46–3.82)**	3.28 (2.91–3.53)	3.08 (2.14–3.45)
Fever before	39.8 (39.6–40.0)	39.7 (39.0–40.3)	40.1 (39.7–40.4)	40.0 (39.6–40.4)	39.2 (38.8–40.0)*	39.9 (38.6–40.6)
Fever after	39.1 (38.9–39.4)**	38.9 (38.3–39.6)*	40.7 (40.4–40.8)**	40.6 (40.1–40.8)**	40.1 (39.4–40.5)	40.2 (38.5–40.8)
Fever (/d) before	0.67 (0.50–0.75)	0.56 (0.20–0.82)	0.68 (0.63–0.83)	0.63 (0.56–0.67)	0.40 (0.25–0.50)	0.50 (0.33–0.67)
Fever (/d) after	0.27 (0.10–0.40)**	0.07 (0–0.29)**	0.60 (0.55–0.69)	0.57 (0.50–0.71)	0.34 (0.25–0.50)	.33 (0–0.50)
Difference (/d)	0.35 (0.20–0.55)	0.34 (0.13–0.82)	0 (0–0.15)	0 (0–0.19)	0 (−0.11–0.17)	0.07 (−0.07–0.50)
Gam (/μl)	2.03 (1.18–2.24)	1.90 (1.00–2.60)	1.50 (1.28–1.77)	1.72 (1.34–1.85)	1.08 (1.00–1.30)	1.00 (1.00–1.18)
Gam (/d)	1.0 (0.73–1.0)	1.0 (0.09–1.0)	0.90 (0.73–1.0)	0.82 (0.57–1.0)	0.30 (0.17–0.55)	0.20 (0.08–1.0)

	FAL trophozoite	FAL sporozoite	VIV trophozoite	VIV sporozoite	MAL trophozoite	OVA trophozoite
Aggregated (N3)						
Parasitemia before	3.63 (3.49–3.81)	3.75 (3.08–3.97)	3.08 (2.95–3.24)	3.02 (2.89–3.17)	3.00 (2.85–3.11)	3.13 (2.96–3.31)
Parasitemia after	3.09 (2.96–3.16)**	2.92 (2.54–3.15)**	3.74 (3.66–3.79)**	3.51 (3.38–3.61)**	3.27 (3.17–3.34)**	2.51 (2.30–2.74)**
Fever before	39.8 (39.6–40.0)	39.7 (39.2–40.0)	40.2 (40.0–40.6)	40.2 (40.0–40.4)	39.7 (39.4–40.0)	40.1 (39.4–40.4)
Fever after	39.1 (38.9–39.4)**	39.1 (38.3–39.4)*	40.7 (40.6–40.8)*	40.6 (40.3–40.8)*	40.1 (39.8–40.3)	39.4 (38.6–40.6)

*$P < 0.01$,

**$P < 0.0002$.