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Fever in Patients with Mixed-Species Malaria

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Abstract

Background—Clinical symptoms of mixed-species malaria infections have been variously reported as both less severe and more severe than those of single-species infections.

Methods—Oral temperatures were taken from and blood slides were prepared for 2308 adults who presented at outpatient malaria clinics in Tak Province (Thailand) during May–August 1998, May–July 1999, and May–June 2001 with malaria infections diagnosed by 2 expert research microscopists, each of whom was blinded to the other's reports.

Results—In each year, temperatures of patients with mixed *Plasmodium vivax–Plasmodium falciparum* infections were higher than temperatures of patients with *P. vivax* or *P. falciparum* infections. In every mixed-species case, *P. falciparum* parasitemia was higher than *P. vivax* parasitemia, but patient temperature was not correlated with the parasitemia of either species or with the total parasitemia.

Conclusions—Among adults who self-report to malaria clinics in western Thailand, patients with mixed *P. vivax–P. falciparum* infections have higher fevers than patients with single-species infections, a distinction that cannot be attributed to differences in parasitemia. This observation warrants more detailed investigations, spanning wider ranges of ages and transmission environments.

Human malaria can be caused by any of 4 species of *Plasmodium* that occur in various geographically overlapping combinations in regions where they are endemic. *Plasmodium falciparum* is responsible for almost all mortality attributed to malaria, but *Plasmodium vivax* is the source of as much or more morbidity worldwide [1].

Where *P. falciparum* and *P. vivax* are both present, point-prevalence surveys usually report statistical deficits of mixed-species infections in the human population, relative to what would be expected if the species were independent [2,3]. In a mixed *P. vivax–P. falciparum* infection, however, peaks of parasitemia typically alternate between species [4,5]; PCR-based surveys [6,7] and mathematical analyses [8] suggest that the statistical deficits reflect these individual-infection dynamics, because they are mediated by the detection thresholds of microscopy, and the results of classic longitudinal field studies [9–10] seem to be in line with this interpretation.

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One recent estimate is that mixed *P. vivax–P. falciparum* infections make up one-third to one-half of malaria infections in Thailand, rather than the reported 0.3%–0.7% [11].

A case series of adult inpatients in India showed higher frequencies of pernicious syndromes and drug resistance [12], and a review of hospital records in Malaysia found anemia to be more severe and cerebral malaria to be more frequent and more often fatal [13] in mixed *P. vivax–P. falciparum* infections, compared with single-species infections. Field studies in Sri Lanka [14] and Vanuatu [15], however, suggested that *P. vivax* ameliorates subsequent *P. falciparum* infections in children. A study in a refugee camp clinic in northwest Thailand reported lower frequencies of severe clinical outcomes, treatment failures, and anemia in mixed *P. vivax–P. falciparum* infections, compared with single-species *P. falciparum* infections [16–18]. Studies elsewhere in Thailand demonstrated that misdiagnoses of mixed-species infections as single-species infections can lead to the sudden emergence of 1 species as drug treatment clears the other [19], with sometimes fatal consequences when *P. falciparum* is the cryptic species [20].

Thus, to interpret trials of *P. vivax* vaccine candidates [21] and other population-level interventions, it would be helpful to know more about the effects of *P. vivax* on *P. falciparum* infections, and vice versa. For instance, several trials [22,23] of the *P. falciparum* vaccine candidate Spf66 reported increases in the prevalence of *P. vivax* or the incidence of mixed *P. vivax–P. falciparum* infections. In this article, we report results of mixed *P. vivax–P. falciparum* infections in adults from a large, multiyear study in local outpatient malaria clinics in western Thailand.

METHODS

Data were collected during a study of malaria rapid diagnostic devices, the methods for which are fully described elsewhere [24–29]. Briefly, participants presented on their own initiative to existing outpatient malaria clinics operated by local public health authorities at Maesod (Tak Province, Thailand) during 28 May–28 August 1998 or 17 May–9 July 1999. They were ⩾15 years old, with fever (oral temperature, ⩾38°C), headache, or a self-reported history of fever within the previous 72 h. Severely ill patients were immediately referred to district hospitals and were not enrolled. During the study, clinic staff retained full responsibility for patient care; diagnostic and treatment decisions were made independent of the study protocol. The same criteria and protocol held in 2001, but the study was conducted 18 May–29 June, in the Mae Ku (Maesod) and Phob Phra Districts of Tak Province (Thailand), and patients ⩾20 years of age were enrolled. The protocol was reviewed by the Human Use Review Committee (Walter Reed Army Institute of Research), the Human Subjects Research Review Board (US Army Medical Research and Materiel Command), and the Ethical Review of Research Committee (Ministry of Public Health, Thailand).

At enrollment, patients were asked how long they had felt ill; responses were recorded as 1–8 days, with “8 days” representing any duration 17 days. They were also asked whether they had taken antimalarial drugs within the previous 2 weeks. Those who gave positive responses were not enrolled in 1998, but were enrolled and noted as such in 1999 and 2001. This analysis excludes 21 otherwise-eligible enrollees (none with mixed-species infections) who gave positive responses. Males comprised 66%–70% of all participants, 61%–63% of uninfected patients, 77%–81% of patients infected with *P. falciparum*, 70%–75% of patients infected with *P. vivax*, and 80%–92% of patients with mixed-species malaria. Log-linear analysis [30] shows a statistical interaction in each year between sex and malaria infection ($P < .0001$), but it also shows that male overrepresentation in the infected categories applies independently to each species and that the statistical interactions between *P. falciparum* and *P. vivax* infection are sex-independent. That is, the male bias among patients with single-species *P. falciparum* and

P. vivax infections accounts for the male bias among patients with mixed-species infections. No patients <18 years old were enrolled in 1999. We removed the 83 enrollees <18 years old from the 1998 data (none of whom had mixed-species infections), rendering the 1998 and 1999 age distributions indistinguishable ($P = .07$, by the Mann-Whitney U test). The median age of participants was 25 years for each year, but the 2001 age distribution differed from the age distributions for 1998 and 1999 ($P < .001$), reflecting the absence of 18–19-year-old participants. Patients with mixed-species infections were not distinguishable from patients infected with *P. falciparum* or *P. vivax* on the basis of age ($P > .41$) or number of days reported as being ill ($P > 0.13$). Also, they were not distinguishable by calendar date of enrollment in 1998 or 1999 ($P > .26$), but in 2001, patients with mixed-species infection were enrolled earlier than patients with *P. falciparum* or *P. vivax* infection (median time of enrollment: patients with mixed-species infection, 2 June; patients with single-species infection, 9 or 11 June [$P = .018$ or $P = .007$]).

The oral temperature of each patient was taken immediately before blood was drawn. Approximately 2 μL of venous blood was drawn from each individual into an EDTA-filled tube for blood films and an automated blood count. Precise volumes of well-mixed whole blood were micropipetted and prepared as thick and thin smears on each of 3 precleaned slides by well-trained technicians following standardized procedures. One slide was used promptly by clinic staff for diagnosis and medical intervention, if such action was indicated. The remaining 2 slides were held overnight, then stained with 3% Giemsa. Each of 2 expert microscopists, blinded to the other's interpretation, read the same one of these slides. Two hundred oil-immersion high power fields on the thick film were read before any blood smear was interpreted as showing negative results; the thin film was used only for species determination. Species identifications and density estimates were based solely on asexual blood forms. If, after 200 WBCs were counted, ⩾10 asexual parasites had been counted, the total number was recorded. If asexual parasites were present but numbered <10 per 200 WBCs, the microscopists continued examining the smear and counting parasites until 500 WBCs had been counted.

Blood tubes were promptly transported on ice to a field laboratory, where cell counts were performed using a Coulter automated cell counter (Beckman-Coulter). The extensive quality-assurance procedures employed are described elsewhere [24–29]. As expected, WBC counts had skewed distributions [30]. Detailed hematological data were collected from the patients enrolled in 2001 [27] but did not provide any basis for distinguishing patients with mixed-species infections from patients with *P. falciparum* or *P. vivax* infections ($P > .085$).

The 2308 malaria-infected and 2747 uninfected patients considered here include 1925 patients enrolled in 1998, 1118 patients enrolled in 1999, and 2012 patients enrolled in 2001; excluded are 109 patients, because microscopists disagreed regarding the presence or species of parasites. The ratio of *P. falciparum*–infected patients to *P. vivax*–infected patients was 1.02 in 1998, 0.97 in 1999, and 0.62 in 2001. The 36 cases for which 1 microscopist reported a mixed-species infection and the other microscopist reported a single-species infection were considered separately. The parasite densities used here are the averages of the 2 microscopists' reports. We used the Mann-Whitney U test and Spearman's rank correlation coefficient on distributions and the G-test on categorical data [31]. P values given are for 2-tailed tests.

RESULTS

The microscopists agreed on 15 mixed *P. vivax–P. falciparum* infections in 1998, 6 mixed infections in 1999, and 13 in 2001. In every case, *P. falciparum* parasitemia was higher than *P. vivax* parasitemia (table 1 and figure 1; $P < .00001$, by the Mann-Whitney U test); the median *P. falciparum*–to–*P. vivax* parasitemia ratio was 36 in 1998, 89 in 1999, and 316 in 2001. In 1998 and 2001, *P. vivax* parasitemia was lower among patients with mixed-species infections

than among patients with single-species infections ($P < .00001$), but *P. falciparum* parasitemia among patients with mixed- and single-species infections was indistinguishable ($P > .15$). In 1999, *P. vivax* parasitemia among patients with mixed- and single-species infections was indistinguishable ($P = .07$), but *P. falciparum* parasitemia was higher among patients with mixed-species infections than among patients with single-species infections ($P = .0055$). In each year, the total parasitemia among patients with mixed-species infections was higher than the total parasitemia among patients with single-species *P. vivax* infections ($P < .0005$); in 1999, the total parasitemia among patients with mixed-species infections was higher than the total parasitemia among patients with single-species *P. falciparum* infections ($P = .005$), but in 1998 and 2001, parasitemias among patients with both types of infections were indistinguishable ($P > .14$).

Among patients with mixed-species infections, 12% of patient temperatures were <38°C and 24% of patient temperatures were >140°C. The comparable figures were 52% and 9%, respectively, for patients with single-species *P. vivax* infections and 40% and 12%, respectively, for patients with single-species *P. falciparum* infections (table 2). In each year, patient temperatures among patients with mixed-species infections were higher than those among uninfected patients ($P < .00009$, by the Mann-Whitney *U* test), *P. vivax*–infected patients ($P < .002$), or *P. falciparum*–infected patients ($P = .029$ in 1998, $P = .009$ in 1999, and $P = .027$ in 2001). In contrast to single-species infections [28], in the mixed-species infections, there was not a correlation between temperature and *P. falciparum* parasitemia ($P > .35$, by Spearman's rank correlation coefficient), *P. vivax* parasitemia ($P > .70$), total parasitemia ($P > .35$), or the *P. vivax*–to–*P. falciparum* parasitemia ratio ($P > .65$).

Overall, the microscopists agreed on the presence or absence of gametocytes in 88% of patients in whom they had agreed on the presence and species of asexual parasites. Among these patients, the microscopists agreed that *P. vivax* gametocytes were present in 19% and *P. falciparum* gametocytes were present in 8% of patients with mixed-species infections, including 1 patient with gametocytes of both species. The comparable figures are that gametocytes were present in 57% of patients with *P. vivax* and 8% of patients with *P. falciparum* single-species infections; these few cases suggest a decreased incidence of *P. vivax* game-tocytemia but not of *P. falciparum* gametocytemia among patients with mixed *P. vivax*–*P. falciparum* infections [32].

WBC counts among patients with mixed-species infections varied widely, and in each year, WBC counts were intermediate between and statistically indistinguishable from WBC counts among patients with single-species infections with either *P. vivax* or *P. falciparum* ($P > .25$, by the Mann-Whitney *U* test). There was no correlation among patients with mixed-species infections between WBC count and *P. vivax* parasitemia, *P. falciparum* parasitemia, combined parasitemia, or patient temperature ($P > .88$, by Spearman's rank correlation coefficient). In 29 of the 34 patients with mixed-species infections, the WBC count was <8000 cells/μL [28].

For an additional 36 patients, 1 microscopist reported a mixed-species and the other microscopist reported a single-species infection. Median temperature and total parasitemia were indistinguishable from those of the 34 patients for whom both microscopists reported a mixed-species infection ($P > .17$, by the Mann-Whitney *U* test). For 23 patients, the single species reported was *P. falciparum*, and among all 23 patients, the other microscopist reported that *P. falciparum* parasitemia was higher than *P. vivax* parasitemia. Among 8 of the 13 patients for whom the single infecting species reported was *P. vivax*, the other microscopist reported that *P. falciparum* parasitemia was higher than *P. vivax* parasitemia. Patient temperatures among patients in these various categories were indistinguishable ($P > .55$). If these 36 patients are added to the 34 patients agreed to have mixed-species infections, temperatures among infected patients were higher than those among uninfected ($P < .0000001$), *P. vivax*–infected

($P < .0000001$), or *P. falciparum*–infected patients ($P = .00015$). For 35 of these 36 patients, a third microscopist examined the slides, and for 5 patients the microscopist reported a mixed-species infection, for 15 patients the microscopist reported a *P. vivax* infection, and for 15 patients the microscopist reported a *P. falciparum* infection; the third microscopist disagreed with both initial microscopists regarding infections in 3 of 35 patients.

DISCUSSION

The statistical deficit of mixed-species infections reported here is in accord with most previous point-prevalence surveys from Asia [2,3]. If the species were statistically independent, the expected number of mixed *P. vivax–P. falciparum* infections would be 268. Based on the "antagonism" between these species noted above [4,5], one or the other species would be expected to dominate parasitemia at most points during a mixed infection. In each of the 34 patients with mixed-species infections reported here, *P. falciparum* parasitemia was much higher than *P. vivax* parasitemia. Patient temperatures were higher in patients with mixed *P. vivax–P. falciparum* infections than in patients with single-species infections. However, in contrast to patients with single-species infections [28], among patients with mixed-species infections, there was no correlation between patient temperature and parasitemia, for either or both species. Despite the complexity of fever curves, synchronization, and (*P. falciparum*) sequestration in malaria infections, the distinctions were seen at presentation. These are striking results, but they call for cautious interpretation.

First, reports that include data regarding frequencies of mixed-species infections seldom include data regarding the species densities of those infections, so it is possible that, in this regard, our results are not as unusual as they appear to be. In the only data of which we are aware, from a clinic in rural Pakistan, *P. vivax* parasitemia was higher than *P. falciparum* parasitemia in 14% and similar in 38% of patients with mixed *P. vivax–P. falciparum* infections. That study showed no statistical deficit of mixed-species infections [33] and a similar prevalence of mixed-species infections among patients younger and older than 13 years [34].

Second, despite our extraordinarily rigorous procedures, it is almost certain that some mixed-species infections went undetected. Detection is generally an inverse function of parasite densities, and with microscopy, the fields examined are a sample of uneven distributions of parasites across blood films [26,29]. It is not clear to us, for instance, how to interpret the 36 cases in which only 1 of the microscopists reported a mixed-species infection, but a similar study supplemented by quantitative PCR detection techniques could be informative, perhaps even with respect to whether our seemingly robust results regarding patient temperatures indicate some potential diagnostic value in raising or lowering suspicion of a cryptic mixed-species infection.

Third, it is not clear whether higher fevers, per se, indicate greater clinical severity or more-effective immune responses, hence, whether our results conflict with those that suggest mixed-species infections ameliorate clinical malaria. As noted above, for instance, we found no evidence that the frequency of anemia differed between patients with mixed-species infections and patients with single-species infections in 2001. It is possible that in mixed *P. vivax–P. falciparum* infections in adults, fever does not directly correspond to parasite density but is high when *P. falciparum* parasitemia is greater than *P. vivax* parasitemia and low when *P. vivax* parasitemia is greater than *P. falciparum* parasitemia. If adults with lower temperatures are less likely to self-report to clinics, a study based on passive case detection would be biased accordingly.

Effective immune responses against *P. vivax* are thought to develop after fewer infections than those against *P. falciparum* [35–37], hence, all else being equal, at younger ages. Parasitemia "thresholds" for fever are thought to be lower with *P. vivax* than with *P. falciparum* [38–39], although with *P. falciparum*, parasitemia thresholds may decrease with age [37]. Thus, mixed-species infections may appear more often in younger age groups, perhaps with higher densities of *P. vivax* and different patterns or intensities of fever. Parasitemia and fever in *P. vivax, P. falciparum*, and mixed-species infections are likely to peak—and ameliorative effects are likely to be most apparent—at different ages. For instance, although the refugee camp clinic study cited above [16–18] did not report patient temperatures, parasitemia levels, or age-specific protective effects for patients with mixed-species infections, in the camp population, the incidence of *P. vivax* infection peaked during ages 0–4 years, the incidence of *P. falciparum* infection peaked during ages 20–29 years, and the incidence of mixed-species infection peaked during ages 4–15 years [38].

Furthermore, given that *P. falciparum* and *P. vivax* typically co-occur in regions with seasonal transmission, in which their prevalences peak at different points in the year, we note that in some previous studies, cases of *P. falciparum* infection greatly outnumbered cases of *P. vivax* infection [15,33,36]. In the Thai refugee camp clinic [16], and in our 1998 and 1999 study populations, the species frequencies were nearly identical, although cases of *P. vivax* infection outnumbered cases of *P. falciparum* infection in our 2001 data (in accordance with national trends). A mixed-species infection in a human can result from a single bite by a mosquito infected with multiple species or from multiple bites by mosquitoes infected with single species. The relative frequency of these events depends on the age distribution in the vector population, which changes during a season and year [40]; the order in which the *Plasmodium* species infect may be critical to their dynamics in the human [8]. Thus, the reported frequencies of mixed-species infections are likely to vary by season and by the particular time during a season at which a study occurs. Though we found no association between the slightly earlier calendar dates of enrollment each year (median enrollment times: 14 July in 1998, 22 June in 1999, and 11 June in 2001), the increase in relative *P. falciparum* parasitemia among patients with mixed-species infections, the decrease in relative *P. falciparum* frequency overall, or changes in other variables, we recognize that calendar dates do not necessarily reflect critical environmental and entomological variables.

We expect that critical distinctions between the species, with respect to stimulating fever, in single- and mixed-species infections, in relation to parasite densities, can be discovered through careful studies that integrate molecular, clinical, and field studies of malaria parasites, hosts, and vectors. Most helpful would be studies that encompass wider age spans and >1 time point during each infection, coordinated with active detection of asymptomatic infections in catchment areas.

In closing, we note that results based even on highly expert microscopy may differ by an order of magnitude from those based on PCR [11], a gap similar to that in our data between the number of mixed-species infections observed and the number expected on the basis of statistical independence between the species. Clearly, if the true number of mixed-species infections was close to the expected number and if the distribution of temperatures was close to the distribution of temperatures observed, our major conclusion here would be even more striking. We previously suggested that frequencies of detection represent an intersection of biological phenomena and methodological shortcomings [2]. If biological interactions between species are such that one may substantially exacerbate or ameliorate the effects of another, it is imperative that the technical obstacles that block our comprehension of the biology be addressed and overcome.

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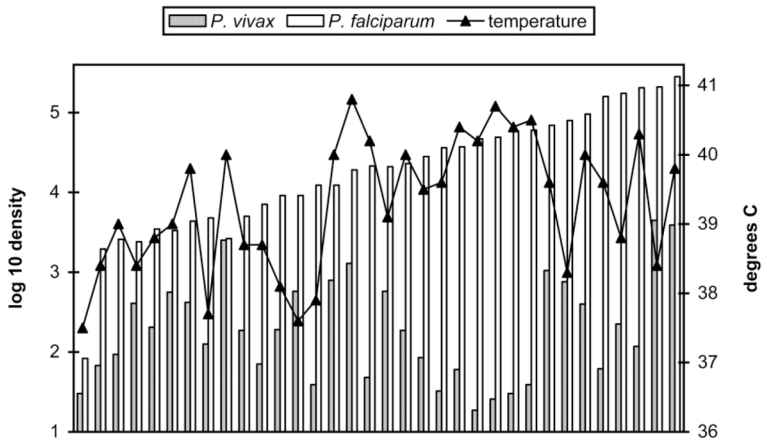

Figure 1.
Per-μL densities of asexual *Plasmodium vivax*, *Plasmodium falciparum*, and body temperatures in 34 patients with mixed *P. vivax–P. falciparum* infections, by total asexual density (left to right).

Table 1

Body temperature and asexual *Plasmodium vivax* and *Plasmodium falciparum* parasitemia in patients with single-species and mixed-species infections, by infection status.

Infection status	No. of patients	Body temperature, median °C (95% Cl)	Parasitemia $\log_{10}$ parasites/μL (95% Cl),
Uninfected	2747	37.4 (37.3–37.4)	…
Infeclted			
P. vivax only	1263	37.9 (37.7–38.0)	3.51 (3.42–3.58)
P. falciparum only	1011	38.3 (38.1–38.5)	4.00 (3.92–4.09)
Mixed-species	34	39.5 (38.4–40.0)	2.27 (1.78–2.75)[a] 4.32 (3.68–4.77)[b]

[a] P. vivax.

[b] *P. falciparum.*

Table 2

Distribution of patient temperatures.

Type of infection	<38°C	38°C–40°C	140°C
Plasmodium vivax only	52	39	9
Plasmodium falciparum only	40	48	12
Mixed-species	12	64	24

NOTE. Data are % of patients.