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CAN TREATMENT OF *P. VIVAX* LEAD TO A UNEXPECTED APPEARANCE OF *FALCIPARUM* MALARIA?

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Abstract

Of 994 patients admitted to the Bangkok Hospital for Tropical Diseases for *P. vivax* malaria, 104 (10.5%) experienced appearance of *Plasmodium falciparum* following drug treatment for *P. vivax*. In all patients, *P. falciparum* parasites were not found by microscopic examination upon admission. The mean time for *P. falciparum* appearance was 12.6 days after the commencement of chloroquine treatment. Patients experiencing appearance of *P. falciparum* had significantly lower hematocrit, and greater initial *P. vivax* parasite counts. We use a mathematical model to explore the consequences of chloroquine treatment of such mixed infections. Both clinical results and features of the model suggest that such “hidden infections” may be quite common, and that the appearance of *P. falciparum* may be stimulated by treatment of *P. vivax*.

INTRODUCTION

Plasmodium vivax and *P. falciparum* are the most prevalent of the four species that cause human malaria, and mixed infections of the two are common and frequently recorded in field surveys (for reviews, see Cohen, 1973; Richie, 1988; McKenzie and Bossert, 1997, 1999). Recent studies using acridine orange, nested PCR, and microtiter-plate hybridization methods have indicated that mixed infections are far more prevalent than has been suspected based on conventional microscopy (Brown, *et al*, 1992; Snounou *et al*, 1993; Kawamoto *et al*, 1996; Postigo, 1998; Zhou *et al*, 1998; May *et al*, 1999). It is not surprising, therefore, that numerous hospital studies following patients longitudinally have indicated substantial underreporting of mixed infections in cases thought to be *P. falciparum* alone (Meek *et al*, 1986; Looareesuwan *et al*, 1987; 1994a; 199b; 1997). More recently, we found that 10.5% of patients diagnosed with *P. vivax* alone actually harbored *P. falciparum* as well (Krudsood *et al*, 1999). While the appearance of *P. vivax* following treatment for *P. falciparum* might be attributed to vivax relapse from hypnozoitic forms, the appearance of *P. falciparum* following admission and treatment for *P. vivax* is less easily accounted for, as it is not clear why: (1) such mixed infections are missed during blood examination, and (2) *P. falciparum* appears following the commencement of chloroquine treatment of *P. vivax*.

The dynamics of mixed infections have been explored using models of mixed infection for *P. falciparum* and *P. malariae* (Mason *et al*, 1999) and for *P. vivax* and *P. falciparum* (Mason

and McKenzie, 1999). Both studies suggested that one parasite can greatly affect the dynamic of the second through non-specific and cross-specific immune response, thus leading to significant differences in clinical status. The model of *P. vivax*-*P. falciparum* explored the effects of drug treatment of a mixed infection, and found that mistaken treatment of a mixed infection as a single *P. vivax* infection could lead to a reappearance of *P. falciparum*. Here we discuss the clinical features of *P. falciparum* reappearance after treatment for *P. vivax* with chloroquine and apply our model to suggest a mechanism for this reappearance.

PATIENTS AND METHODS

The study was conducted in concert with a study on chloroquine resistance in Thailand (Looareesuwan *et al* 1999). The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University. Patients older than 12 years who were admitted to the Bangkok Hospital for Tropical Diseases between April 1992 and December 1997 were enrolled if they had acute vivax malaria upon admission, had not received antimalarial treatment before admission, had no patent *P. falciparum* upon admission and signed a consent form. Reasons for exclusion were pregnancy or unwillingness to remain hospitalized for at least 28 days. All patients were treated with a standard regimen of 1,500 mg CQ (chloroquine phosphate from the Government Pharmaceutical Organization of Thailand, in tablets containing 150 mg each) over 3 days: 600 mg base initially (0 hours), followed by 300 mg base at 6, 24, and 48 hours, giving a total dose of approximately 25 mg/kg. *P. falciparum* appearance was defined as the microscopic diagnosis of *P. falciparum* following the commencement of therapy. Standard descriptive and statistical analysis were conducted using version 6.04 of the Epi-Info software (Centers for Disease Control, Atlanta, GA). Comparisons were made using chi-square and Student's *t*-test.

Computer modeling was conducted using the model for *P. vivax*-*P. falciparum* mixed infections developed by Mason and McKenzie (1999) and is described there in detail. For chloroquine we assumed a parasite reduction rate of 100, based on the published range of 10–1,000 (White, 1997). The pharmacodynamics of chloroquine are notoriously complex, due to a wide range of reported blood half-lives (Desjardins *et al*, 1988), conversion to the active metabolite desethyl chloroquine, and use of multiple doses. Thus, we included a variable representing the time chloroquine remained active (*i.e.*, the time before serum concentrations fell below the minimum inhibitory concentration (MIC)), rather than the value of the MIC itself. The parameter space of immune coefficients, super-inoculation timing, and parasite growth rates was searched for conditions which would result in low *p falciparum* parasitemia relative to *P. vivax* density. In addition to the parameters described in Mason and McKenzie (1999) we tested the model for *P. vivax* erythrocytic schizogony rates of 13 and 18 merozoites/schizont/2-day interval. These parameter sets were then used for simulated chloroquine administration at different *P. falciparum* levels of sensitivity.

RESULTS

Clinical study

Overall, 992 patients were enrolled in the study. Of these 10.5% (104/992) experienced an appearance of *P. falciparum* during their 28 days in the hospital. *P. falciparum* appearance varied from 1 to 28 days following commencement of CQ treatment for *P. vivax*. A summary of days of *P. falciparum* appearance is shown in Fig 1. Due to patients dropping out of the study, charts were available for only 87 of the 104 who experienced an appearance of *P. falciparum*. As can be seen from the graph, the day of appearance was distributed over the entire study time, with the exception of days 24–27. The mean time of appearance was 12.6 days (SD = 6.82). An example of a mixed infection in a patient is given in Fig 2. Briefly, measurements found to be significantly associated with patients experiencing an appearance

of *P. falciparum* were: higher *P. vivax* density on admission, depressed hematocrit, elevated albumin, elevated globulin, and elevated alkaline phosphatase (Krudsood *et al*, 1999).

Application of the model

The structure of the model is described in Mason and McKenzie (1999) which also contains a detailed exploration of parasite equilibria and timing. With regard to the specific questions raised by this study, three conditions were found in which *P. vivax* parasites greatly outnumbered those of *P. falciparum*: (1) *P. falciparum* troughs during out-of-phase *P. vivax*-*P. falciparum* parasitemia oscillation, (2) early phases in a *P. falciparum* superinfection of *P. vivax*, and (3) mixed infections in which *P. vivax* erythrocytic schizogony produced more merozoites than did *P. falciparum* schizogony. An example of each is illustrated in Fig 3. It is important to note the distinction between these figures and the sample patient chart shown in Fig 2: clinically, we could only record parasite density once the patient was admitted; the model gives us insight into the dynamics which preceded and produced the high *P. vivax*/ low *P. falciparum* levels observed in the patient.

Assuming varying degrees of *P. falciparum* chloroquine resistance, treatment in all three scenarios led to rise in *P. falciparum* parasitemia (Fig 4). The length of the period before parasites became patent varied according to *P. falciparum* density at time of treatment (higher density lead to faster recrudescence), immune parameters (stronger immune response, slower recrudescence), and MIC. Parasites with greater MIC reached patency faster, but peak parasitemia was lower due to the persistence of *P. vivax*-raised non-specific immunity.

It is important to note that it is impossible to know which of the three possible conditions illustrated in Fig 3 and discussed in the text produced the low *P. falciparum* density. Rather, clinically, we can only observe the dynamics following treatment, but must rely on the model to suggest dynamic prior to treatment,

DISCUSSION

Although the phenomenon of “hidden mixed infections” has become increasingly familiar, the cause for both the misdiagnosis of mixed-species as single-species infections, as well the rise of *P. falciparum* following chloroquine treatment is less certain. Conducting this study in a hospital setting where malaria cannot be transmitted allowed us to eliminate the possibility that the *P. falciparum* appearance is due to a new infection. And unlike *P. vivax* appearance following treatment which we have documented above, *P. falciparum* does not form hypnozoites; thus such an appearance cannot be due to relapse from the liver stage.

The first possible explanation for a missed diagnosis is that the patient was bitten by a *P. falciparum*-infected mosquito less than 9–10 days (the *P. falciparum* pre-patency period) prior to admission to the hospital for *P. vivax* malaria. However, this could not account for the 59% (51/87) of cases which appeared more than 10 days following chloroquine treatment. Although it is possible that later appearances may be due to either heterogeneity in either the host immune response or the parasites themselves, we think it is unlikely that over half the parasites examined have such widely variant pre-patent periods.

If *P. falciparum* is present in the blood, it may be missed due to low parasitemia. *P. falciparum* is noted for its ability to sequester in organs; thus observed parasite density is may be considerably less than total parasite load (White, 1997). Low parasitemia is especially likely to be missed in the presence of another parasite at greater density. Indeed, the danger of misdiagnosing mixed infections as single infections has been noted since Knowles and White (1930) who described the “flexible stopping rule”, the tendency of workers to stop examining a blood smear once parasites have been found.

Using the mathematical model, we found that *P. vivax* parasitemia will be high relative to *P. falciparum* under three conditions. First, parasite density oscillates due to interspecific suppression, with *P. falciparum* trough densities corresponding to *P. vivax* peaks. Second, in a mixed infection, *P. falciparum* takes a longer time to reach detectable parasitemias (especially if *P. falciparum* is super-inoculated over a standing *P. vivax* infection). Finally, although the original model considered the average *P. vivax* multiplication rate to be 13 merozoites/schizont (Garnham, 1988) in reality this figure is quite variant (Garnham, 1966). According to the model, if *P. vivax* multiplication is greater than 16, *P. falciparum* density levels are pushed even lower.

Even if low *P. falciparum* parasitemia is a product of coinfecting along with *P. vivax* it does not necessarily follow that appearance of *P. falciparum* after treatment for *P. vivax*, is caused by the treatment. Indeed, in two of the three conditions producing low *P. falciparum* levels (oscillation and super-inoculation), *P. falciparum* parasitemia eventually rises to surpass that of *P. vivax* without drug treatment. Nevertheless, the model indicates that the rise of *P. falciparum* can be precipitated following the treatment for *P. vivax*. We suggest the following mechanism: Chloroquine resistance is widespread and well documented in Thailand (Trig and Kondrachine, 1998). Thus chloroquine removes the susceptible *P. vivax*, causing a concomitant fall in non- and cross-specific immune effectors raised by *P. vivax*. Assuming chloroquine levels drop below the MIC before *P. falciparum* is removed, *P. falciparum* will recrudescence.

The time period for *P. falciparum* appearance varied according to *P. falciparum* density at the time of treatment (treatment at periods of higher density led to faster recrudescence), strength of the immune response (a stronger immune response, especially persistent specific immunity, slowed recrudescence), and degree of *Plasmodium* resistance to chloroquine (greater MIC produced faster recrudescence; Fig 3). It is important to note that although it varied in time and peak parasitemia, the surge in *P. falciparum* parasitemia occurred regardless of the complexities of previous parasite dynamics.

P. falciparum liberation from a non- and cross-specific immune response raised by *P. vivax* remains a hypothesis, indeed without being able to compare our cohort with untreated *P. vivax* patients, we cannot be certain whether or not treatment precipitated the rise in *P. falciparum*. Nevertheless, given the severity of *P. falciparum* malaria, the potential for *P. falciparum* appearing following elimination of *P. vivax* must be considered. There is too little data to currently recommend changes in treatment procedure. Physicians should be aware of the risk factors cited above: namely, higher *P. vivax* density on admission, depressed hematocrit, depressed albumin, elevated globulin, and elevated alkaline phosphatase. Patients should be warned to report any reappearance of fever, and follow-up blood checks are highly indicated. Overall, we await other studies from other geographical regions. Indeed, if *P. falciparum* appearance following treatment for *P. vivax* is as common in other areas, the question of whether all vivax malaria should be treated as a mixed infection with *P. falciparum* appears a highly controversial yet intriguing proposal.

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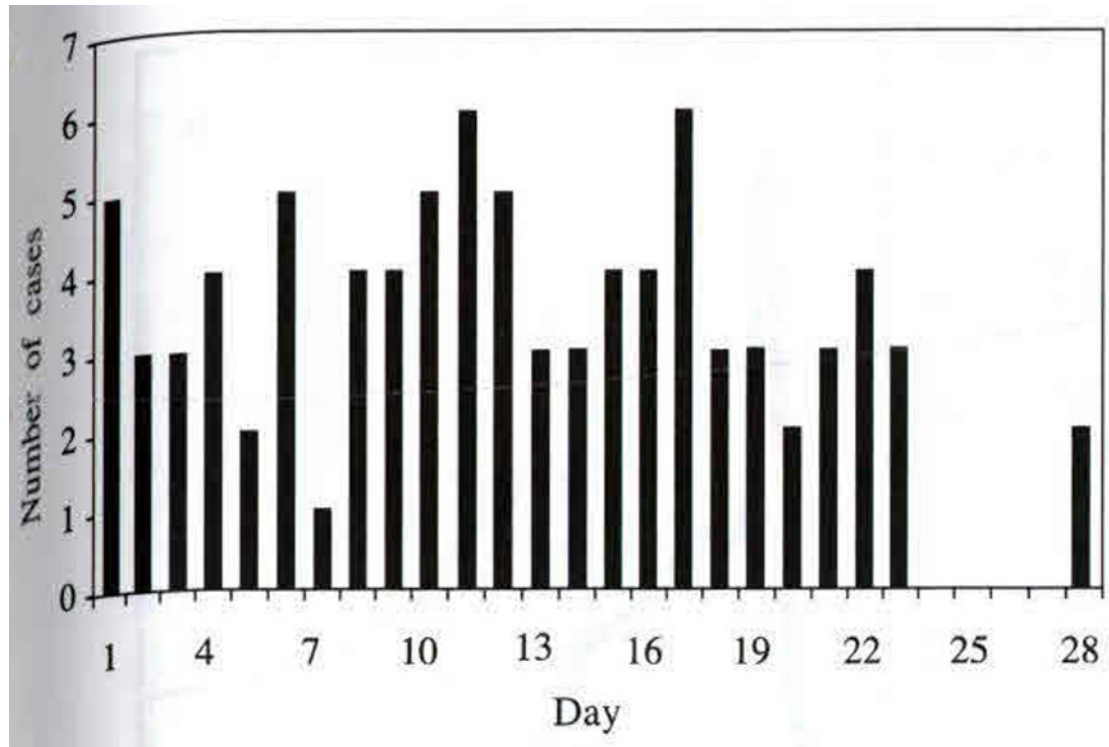


Fig 1. Number of cases of *P.falciparum* appearance by day, following the commencement of chloroquine treatment for *P. vivax* infection.

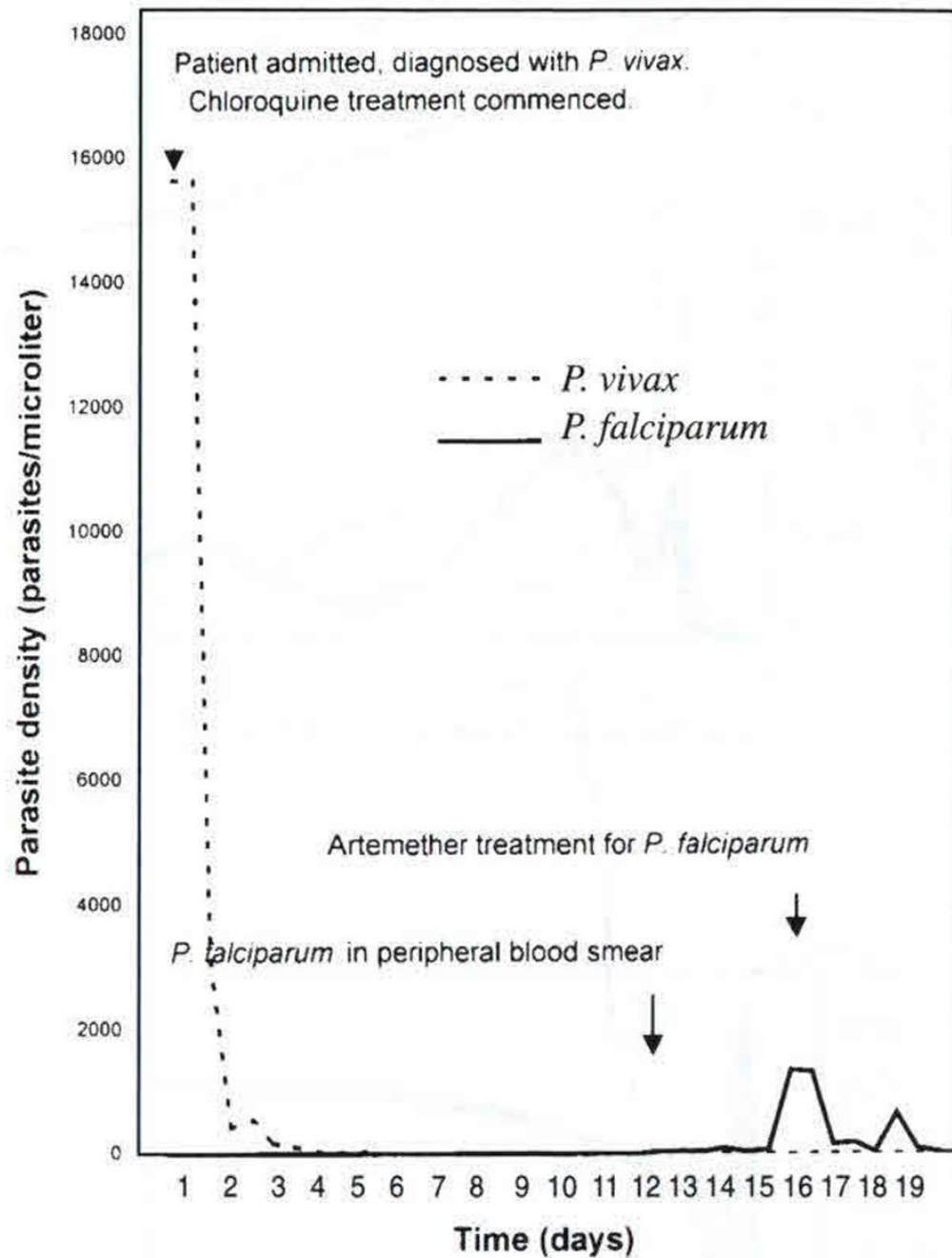


Fig 2. Sample parasite density chart of patient 58, a 20-year old man admitted with *P. vivax* malaria on day 1, and treated with chloroquine on day 1. *P. falciparum* appeared in the peripheral blood smear on day 13, and was treated with artemether on day 18.

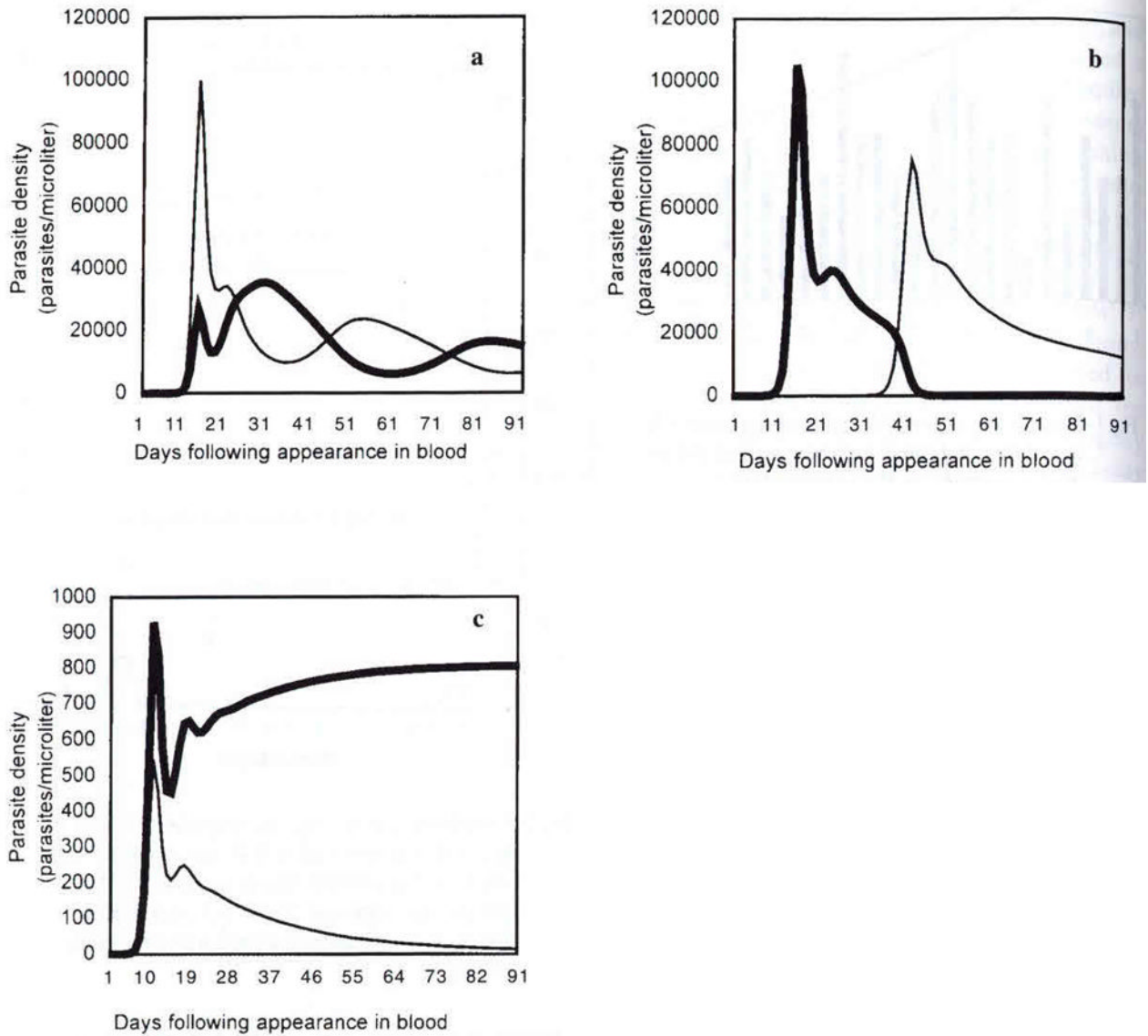


Fig 3. Conditions resulting in low *P. falciparum* para-sitemia, relative to *P. vivax* density. *P. vivax* density is shown as a heavy line, *P. falciparum* as a thin line

3a. *P. falciparum* and *P. vivax* oscillate out of phase with each other. Troughs in *P. falciparum* correspond to peaks in *P. vivax* parasitemia. (parameters set at $c=0.001$; $cn=0.01$; $s=0.001$; $sn=0.001$; $x=y=0.0$).

3b. *P. falciparum* superinfection occurs 15 days after that of *P. vivax*. *P. falciparum* does not reach patency until day 30, and is outnumbered by *P. vivax* until day 38. ($c=0.001$; $cn=0.01$; $s=0.001$; $sn=0.001$; $x=y=0.1$).

3c. *P. vivax* strain with greater than average multiplication rate (= 18 merozoites per schizont). *P. falciparum* approaches equilibrium at subdetectable levels. ($c=0.001$; $cn=0.01$; $s=0.001$; $sn=0.1$; $x=y=0.1$) (Note different y-axis scales, due to variations in immune coefficients).

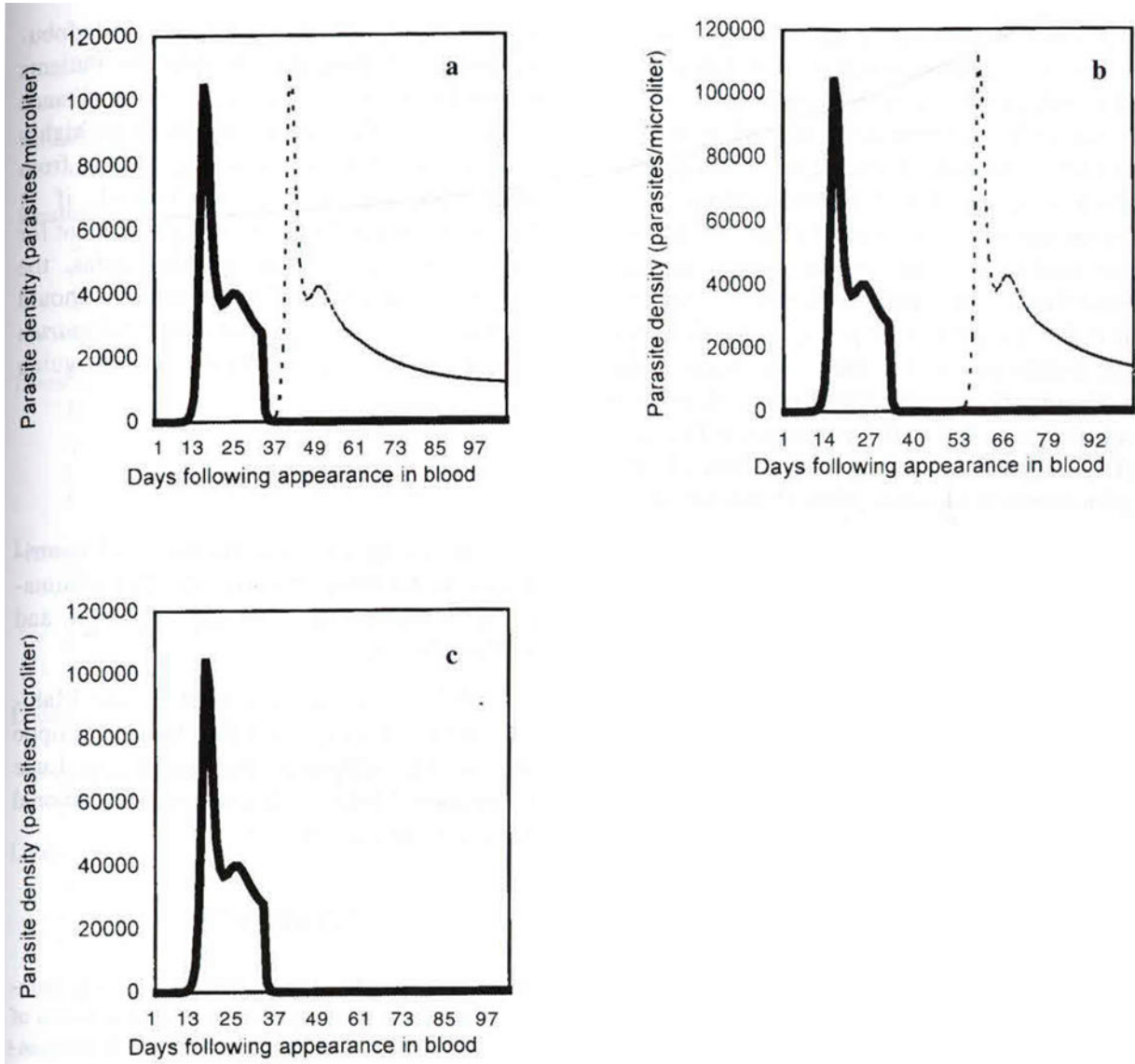


Fig 4. Parasite dynamics of a mixed *P. vivax*- *P. falciparum* mixed infection misdiagnosed as *P. vivax* only. In this example, the patient presented within 30 days following the appearance of *P. vivax* in the blood, and was treated immediately with chloroquine. *P. falciparum* infection occurred 15 days after *P. vivax* infection, and was not yet patent at the time of microscope analysis. *P. vivax* density is shown as solid line, *P. falciparum* a dashed line. Graphs indicate differences in *P. falciparum* density for varying periods of chloroquine activity against *P. falciparum*. (4a) *P. falciparum* is completely chloroquine resistant (RIII) (MIC > initial dose). (4b) The MIC is reached in 10 days. *P. falciparum* is RI resistant, decreases slightly and recrudesces to patent levels after 18 days. (4c) *P. falciparum* is sensitive to chloroquine. The MIC is reached in 20 days, by this point the sensitive *P. falciparum* has been eliminated from the blood. (cs=0.001, cn=0.01, ss=0.001, sn=0.001, x=y=0.1, PRR=100).