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COMPETITION FOR RED BLOOD CELLS CAN ENHANCE *PLASMODIUM VIVAX* PARASITEMIA IN MIXED-SPECIES MALARIA INFECTIONS

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Abstract

We assess the consequences of competition for red blood cells (RBCs) in co-infections with the two major agents of human malaria, *Plasmodium vivax* and *Plasmodium falciparum*, using differential equations to model the population dynamics of RBCs and parasites. *P. vivax* parasitizes only the youngest RBCs, but this can reduce the broader RBC population susceptible to *P. falciparum*. We found that competition for RBCs typically causes one species to suppress the other, depending on their relative reproduction rates and timing of inoculation. However, if the species' reproduction rates are nearly equal, transient increases in RBC production stimulated by the presence of *P. falciparum* may boost *P. vivax* parasitemia above its single-species infection level. Conversely, *P. falciparum* parasitemia is rarely enhanced above its single-species level. Furthermore, transients in RBC production can induce coupled oscillations in the parasitemia of both species. These results are remarkably robust to changes in model parameters.

INTRODUCTION

Malaria infection begins with inoculation of *Plasmodium* parasites from an *Anopheles* mosquito into a host's blood. Parasites penetrate the host's liver cells, multiply there for ~ 1 week, and, as asexual "merozoite" forms, emerge to invade red blood cells (RBCs). The merozoites multiply, burst the RBCs, and release 8–32 merozoites per RBC, which in turn invade more RBCs to continue the cycle. This blood stage asexual replication cycle is associated with exponential growth in parasite numbers and with fever, anemia, and organ complications.

Human malaria is caused by four species of *Plasmodium* that co-exist in various combinations in endemic regions. Although *P. falciparum* is responsible for most of the mortality attributed directly to malaria, *P. vivax* induces enormous morbidity worldwide, despite its virtual absence from sub-Saharan Africa.^{1,2} Mixed *P. vivax*–*P. falciparum* infections in humans can arise

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through sequential bites by singly infected mosquitoes or a single bite by a dually infected mosquito.³ Also possible is concurrent activation of latent *P. vivax* liver stages. Most cross-sectional surveys of human populations have shown deficits of *P. vivax*–*P. falciparum* infections, relative to the frequencies expected if species infections were independent.^{4,5} However, modern polymerase chain reaction (PCR)-based studies^{6,7} and statistical-mathematical analyses^{8,9} suggest that these deficits may be a consequence of infection dynamics: because peaks of parasitemia in a mixed *P. vivax*–*P. falciparum* infection typically alternate between the species,^{10,11} apparent deficits at the population level may reflect the detection thresholds of microscopy and the biologic interactions between parasites in infected individuals. In hindsight, this connection is implicit in classic longitudinal studies.^{12,13} Recent studies confirm that mixed-species infections are far more common than is generally recognized.^{14,15} Perhaps 30–50% of all malaria infections recorded in Thailand are mixed *P. vivax*–*P. falciparum*.^{16,17}

The dynamics of mixed *P. vivax*–*P. falciparum* infections present serious challenges for interventions at the individual and population levels: misdiagnosis and corresponding drug treatment can allow the cryptic species to rebound, with severe clinical consequences.^{18,19} Furthermore, if *P. vivax* infections temper the severity of *P. falciparum* pathology,^{20,21} an anti-*P. vivax* vaccine²² may have unanticipated adverse effects. Hence, it is crucial to move from phenomenological observation to more detailed mechanistic understanding of species interactions.

Although anemia is a common manifestation of malaria and a common cause of death in *P. falciparum* infections, no previous analyses of mixed-species malaria infections have taken into account the dynamics of the host RBC population. RBCs are the substrate on which the blood stages of *Plasmodium* species interact and compete for resources. *P. falciparum* can invade RBCs of all ages, whereas RBC susceptibility to *P. vivax* is restricted to the youngest age class, the reticulocytes.^{1,23} The great disparity in anemia-induced mortality is generally attributed to this distinction.²⁴ Host hematopoietic responses to malaria infection may be critical, but remain poorly understood: they seem to vary between individuals and can include either compensatory RBC production or diserythropoiesis.²⁵

We recently used differential equation models for RBC–*Plasmodium* dynamics to examine the consequences of age-structured RBC invasion and host erythropoietic response for the dynamics of single-species malaria infections.²⁶ Although these models did not include explicit host immune responses, they provided insights into parasite–RBC interactions. For example, we found that without an aggressive host immune response, reticulocyte depletion during *P. vivax* infection chokes off the supply of mature RBCs, producing catastrophic anemia even if the fraction of RBCs infected remains < 1%. This result is in line with a recent report that hemoglobin concentrations in persistent low-level *P. vivax* infections are disproportionately suppressed compared with the percentage of RBCs infected.²⁷ Also, we found that a compensatory response to RBC loss would enhance parasitemia and accelerate anemia by increasing the density of susceptible RBCs. Here we extend our analytic framework to encompass the more complex circumstances of mixed *P. vivax*–*P. falciparum* infections, again with the aim of discovering constraints and imperatives that RBC dynamics impose on malaria parasites and host responses. In particular, we wondered if competition for RBCs in a mixed-species infection could enable one species to facilitate the other.

MATERIALS AND METHODS

Model formulation

We constructed a set of compartmental ordinary differential equations (CODEs) to model RBC dynamics and mixed *P. vivax*–*P. falciparum* infections, as shown schematically in Figure 1.

We used CODEs because their solutions closely mimic the mean properties of an ensemble of developing cells.^{28,29} Details of the model and the method of solution are provided in the mathematical appendix below. In the CODEs, an affinity parameter ζ (with different values for different *Plasmodium* species) represents the ability of merozoites to find and bind to a target RBC. We assume that the lifespan of a circulating RBC is 120 days, that the reticulocyte stage spans ~ 36 hours after RBC release from the bone marrow,³⁰ and that *P. vivax* invasion is restricted to reticulocytes.¹ (We also did a small number of simulations with an assumption that *P. vivax* could attack RBCs of up to 14 days of age, based on a report suggesting that young RBCs beyond the reticulocyte stage might still be vulnerable to this species.³¹) Although there are reports that *P. falciparum* shows a preference for young RBCs,³² our earlier results indicate that these data should be interpreted with some caution, because the proportion of RBCs in young age classes always increases as a *P. falciparum* infection proceeds.²⁶ Thus, we followed convention and assumed that *P. falciparum* has the same affinity for RBCs of any age. Note that our model could also be construed as representing dual phenotype *P. falciparum* infections in which one phenotype attacks only reticulocytes.

Based on what is known about the development of *P. vivax* and *P. falciparum*,^{1,2,26} we assumed that after the initial liver stage, $0.002 \mu\text{L}^{-1}$ merozoites are rapidly released into the blood. (The release of merozoites from the liver signals the start of infection in our simulations.) The average development time in infected RBCs is 48 hours. For both species, we take the average clearance time of free merozoites from the blood as $\tau_{\text{mer}} = 6$ minutes.³³ We take $P = 16$ for *P. falciparum* and $P = 16$ or 8 for *P. vivax*. Although multiple infections of RBCs are possible, they are rare³¹; for simplicity, we ignored them. We did not consider the development of merozoites into gametocytes, the non-replicating sexual blood forms that, taken up in an *Anopheles* blood meal, continue the mosquito–human cycle.³⁴ Nor do we consider the possible effects of synchronization among asexual blood forms³⁵ or destruction of uninfected RBCs.³⁶ Recent work suggests that sequestered *P. falciparum*–infected RBCs are capable of wide dissemination of released merozoites³⁷; thus, we ignore the sequestration of infected RBCs, because in our model, merozoite–RBC binding indirectly mediates interaction between the *Plasmodium* species.

The immune responses that controls most malaria infections are not well understood and are likely to be multi-component and sensitive to the developmental stage of the parasite,³⁸ so the model does not attempt to incorporate them directly. Our intent here is to investigate the constraints and imperatives RBC dynamics impose on infection dynamics. (In the Discussion below, we return to the question of the immune effects.) However, the host is not passive in this model. First, the host dies of catastrophic anemia if the RBC count declines to 75% of the basal count. In addition, we examine three models for host response to the added RBC loss because of infection: 1) RBC production *increases* proportionally to the extra rate of RBC loss, up to twice the basal rate (“compensatory” response). 2) RBC production *decreases* proportionally to the infection-induced loss, down to 0.8 times the basal rate (“diserythropoetic” response), and 3) RBC production remains fixed at the basal rate needed to maintain 5×10^6 RBC/ μL in a healthy host ($\sim 1,736 \text{ RBC } \mu\text{L}^{-1}\text{h}^{-1}$).³⁰ For models 1 and 2, the time constant in response changes in the RBC count is 48 hours,³⁰ and reduction in RBC loss allows a return to homeostasis. Details are explained in the mathematical appendix.

Basic reproduction rate

We showed previously by probability arguments that the mean number of descendants produced by an infected RBC is

$$R = p\zeta V\tau_{\text{MER}} (1 + \zeta V\tau_{\text{MER}})^{-1}$$

where V is the density of RBCs susceptible to the infecting species.²⁶ R is a dynamical quantity that changes during the course of an infection (but never exceeds p). In our model, τ_{MER} , p , and ζ are fixed: dynamic changes in R develop from changes in V . (If a dynamic immune response were present, p , τ_{MER} , and ζ could change as well, depending on the component of the response.) The initial value of R at the beginning of infection, R_0 , is of major importance in a single-species infection: if $R_0 < 1$ the parasite does not persist in the host, but if $R_0 > 1$ the parasite population can reach a steady state or produce catastrophic anemia. We refer to R_0 as the basic reproductive rate of a species (or phenotype). We take the initial V for *P. falciparum* as the basal total RBC count ($5 \times 10^6 \mu\text{L}^{-1}$), and for *P. vivax* as the reticulocyte count of a healthy host, $(\tau_{\text{ret}}/120 \text{ days}) \times 5 \times 10^6 \mu\text{L}^{-1}$, where τ_{ret} = duration of the reticulocyte stage in days. (If $\tau_{\text{ret}} = 1.5$ day, $V = 6.25 \times 10^4 \mu\text{L}^{-1}$.) R_{0F} and R_{0V} refer to R_0 for *P. falciparum* and *P. vivax*, respectively. Thus, if $p = 16$ for both species and $\tau_{\text{mer}} = 6$ minutes, $R_{0F} = R_{0V}$ implies that the affinity ζ of *P. vivax* for reticulocytes is > 100 times the ζ of *P. falciparum* for RBCs of all ages.

Strategy for simulation

Our goal was to comprehensively map the model's behavior in its parameter space. To this end, we simulated 2×10^4 hours of infection (or until the host died) for 26 values of R_0 for each species, with $p = 16$ for both species and with RBCs vulnerable to *P. vivax* for 1.5 days, with R_0 values ranging from $R_0 = 1 + 1/64 = 1.015625$ (barely persistent) to $R_0 = 15$ (extremely pathogenic). The values of R_0 we chose to examine are equally spaced in $\log(R_0 - 1)$ between $\log(1/64)$ and $\log(14)$. With $p = 16$, this range in R_0 corresponds to a 220-fold difference in the size of ζ . In addition, we used the first 23 of these R_0 values (from 1.015625 to ~ 7.19) to investigate the effects of setting $p = 8$ for *P. vivax* (with $p = 16$ for *P. falciparum*). We take ΔST to be the time difference between the time of inoculation of *P. falciparum* and *P. vivax*: $\Delta ST < 0$ means *P. falciparum* infected first, and $\Delta ST > 0$ means *P. vivax* infected first. For all the (R_{0F}, R_{0V}) pairings, for both $p = 16$ and $p = 8$ for *P. vivax*, we did simulations for $\Delta ST = -50, -10, -5, -1, 0, 1, 5, 10$, and 50 weeks. In addition, for each choice of (R_{0F}, R_{0V}) and ΔST , we simulated each of the three types of host erythropoietic response to RBC loss described above. In the text, for simplicity we focus on representative values of ΔST . As stated above, we performed additional simulations for representative values of R_{0F} , R_{0V} and ΔST (with $p = 16$ for *P. vivax*) assuming that τ_{ret} has duration 14 rather than 1.5 days. Finally, we did a number of simulations for values of R_{0F} and R_{0V} not equal to any of the values equally spaced in $\log(R_0 - 1)$.

Comparisons to single-species infections

Many of our results below compare the change in peak infected RBC count, I_{PK} , or the infected RBC count integrated over the course of infection, I_{INT} , for a focal species (*P. vivax* or *P. falciparum*) in a mixed-species infection to the corresponding value in a single-species infection. For the comparisons that involve up or downregulation of RBC production, we obtained the single-species values by running the single-species model with the identical up or downregulation. Thus, as background for our mixed-species results, we briefly discuss single-species infections here. Figure 2 shows the outcome of simulations of single-species infections of *P. vivax* and *P. falciparum*, using our CODE model with the parameters of only one or the other species, and includes many results not shown in our previous work.²⁶ The duration of RBC vulnerability to *P. vivax* is 1.5 days for both *P. vivax* examples shown. The curves for *P. vivax* with $p = 8$ are similar to those with $p = 16$; this would be expected because for fixed R_0 , the ratio of RBC–merozoite binding ζ for $p = 8$ to that for $p = 16$ is $(16 - R_0)/(8 - R_0)$. (Curves for *P. vivax* with $p = 16$ but with an assumption that τ_{ret} is 14 rather than 1.5 days are presented in the supplemental material. These curves are closely similar to those in Figure 2.)

Note particularly that compensatory response to infection-induced RBC loss tends to boost I_{PK} , especially for *P. vivax*, with no gain in host survival, over the values obtained for the same R_0 but with RBC production fixed at the basal rate. This occurs because a boost in RBC production increases the reticulocyte count, thereby increasing the instantaneous R . (Recall that R varies during an infection, whereas R_0 is a fixed quantity.) Even a barely persistent *P. vivax* infection induces catastrophic anemia if the host response to RBC loss is compensatory. However, the opposite effect occurs for a diserythropoietic host response, which tends to reduce I_{PK} and I_{INT} with little cost (or even a benefit) to host survival time for a given R_0 for either species. Note that I_{PK} is not monotonic in R_0 if the host has a diserythropoietic response: the slowdown in RBC production works against the effects of a larger basic reproductive rate. I_{INT} is not monotonic in R_0 in general: the earlier death of the host as R_0 increases works against the higher reproduction rate. Because the simulation outcomes are extremely sensitive to R_{0F} and R_{0V} when both are less than ~ 1.75 , a log scale in $R_0 - 1$ for the horizontal axis (as in Figure 2) gives the best visual representation when results are plotted as a function of R_0 .

RESULTS

Competition for RBCs generally leads to suppression of one of the species

For most (R_{0F}, R_{0V}) combinations, our model predicts that parasitemia of one of the infecting species is reduced below its single-species value, whereas that of the other is barely affected. Figure 3 shows peak parasitemias for both *P. vivax* and *P. falciparum* in (R_{0F}, R_{0V}) parameter space for three values of the relative inoculation time ΔST . (The corresponding integrated parasitemias behave similarly; see supplemental material.) The results plotted in this figure are with the RBC source fixed at the basal rate, and show the typical pattern as ΔST , R_{0F} , and R_{0V} change: the species suppressed and the degree of suppression depend on which species has the higher R_0 value and which infects first; an advantage in one of these factors can often offset a disadvantage in the other. For the examples shown in Figure 3, neither species facilitates the other (i.e., in neither is the parasitemia enhanced above its corresponding single-species infection level). Also shown in Figure 3 is the survival time for the host for the corresponding values of ΔST . Typically, survival time is reduced when the host is infected with both rather than one species.

Figure 4 shows the times series for parasite and RBC counts for two points in (R_{0F}, R_{0V}) space at which I_{PK} and I_{INT} for one species are suppressed, whereas the other is unchanged. For $R_{0F} = 1.741$, $R_{0V} = 1.087$, $\Delta ST = 0$, (Figure 4, right column), *P. vivax* parasitemia is suppressed for the trivial reason that the host dies quickly in the mixed-species infection, before *P. vivax* parasitemia can reach a significant level. For $R_{0F} = R_{0V} = 1.25$, $\Delta ST = -5$ weeks, (left column), a different mechanism causes suppression of *P. falciparum* by *P. vivax*: the culling of reticulocytes by *P. vivax* reduces the total uninfected RBC count and drives the instantaneous R for *P. falciparum* below 1, even though the *P. vivax* inoculation was 5 weeks after that of *P. falciparum*. The data supplement includes an example of system dynamics at a (R_{0F}, R_{0V}) combination that illustrates mutual suppression.

Not all (R_{0F}, R_{0V}) combinations follow this pattern. Especially when the host's RBC production can increase in response to the infection-induced loss of RBCs, *P. vivax* parasitemia in the mixed-species infection is enhanced over its corresponding *P. vivax*-only value. These enhancements tend to be along a "transition boundary" in (R_{0F}, R_{0V}) space, where the identity of the suppressed species changes. Figure 5 shows the peak *P. vivax* parasitemia in (R_{0F}, R_{0V}) space, with a compensatory RBC response, for three values of ΔST and three different combinations of p for *P. vivax* and τ_{ret} . (For *P. falciparum*, $p = 16$ in all three cases.) Even if *P. falciparum* infects many weeks before *P. vivax*, there are regions in (R_{0F}, R_{0V}) space in which peak *P. vivax* parasitemia is enhanced. [The integrated count for *P. vivax* is enhanced in a small region in (R_{0F}, R_{0V}) space in which the host survives for 20,000 hours; we found

almost no enhancement for *P. falciparum*. See supplementary materials.] Although proportionally greater for values of R_{0V} near 1, some enhancement of the maximum *P. vivax* parasitemia in mixed-species infections occurs even for values of R_{0V} near P . Surprisingly, an enhancement of both peak and integrated *P. vivax* parasitemia can occur when the host has a *diserythropoetic* response, and *P. falciparum* infects many weeks before *P. vivax*, as shown in Figure 6. We found no values of (R_{0F}, R_{0V}) for which I_{PK} or I_{INT} are appreciably enhanced for both species together: that is, this model predicts little interspecific synergy. We now examine mechanisms driving facilitation of *P. vivax* by *P. falciparum*.

Increased RBC production rates can enhance *P. vivax* parasitemia, with little benefit to *P. falciparum*

If the host can compensate for RBC loss, some enhancement of I_{PK} for *P. vivax* would be expected because of its ability to rapidly exploit an expanded reticulocyte population. However, what could account for an enhancement with a *diserythropoetic* response? Figure 7 shows times series for parasite and RBC counts for three (R_{0F}, R_{0V}) combinations for which the *P. vivax* I_{PK} is enhanced. (For the three examples, $\tau_{ret} = 1.5$ days.) The figures in the left column are for $R_{0F} = 1.5$, $R_{0V} = 3.0$, $\Delta ST = -10$ weeks (*P. vivax* infects 10 weeks after *P. falciparum*), and a compensatory response in RBC production to infection-induced RBC loss. The figures in the middle column are for $R_{0F} = 1.1137$, $R_{0V} = 1.061$, $\Delta ST = 0$ (simultaneous inoculations), and a compensatory response in RBC production to infection-induced RBC loss. The figures in the right column are for $R_{0F} = 1.087$, $R_{0V} = 1.320$, $\Delta ST = -50$ weeks, and a *diserythropoetic* response. For the two examples shown with compensatory response, the peak *P. vivax* parasitemia is 11% greater in the dual-species infection than in the corresponding *P. vivax*-only infection, although the integrated *P. vivax* parasitemia is suppressed in the dual-species infection. For the example with *diserythropoetic* response, the peak *P. vivax* parasitemia is 150% greater in the dual-species infection than the *P. vivax*-only infection, and the integrated *P. vivax* parasitemia is enhanced in the dual-species infection by 79% over the *P. vivax*-only value.

For the *P. vivax*-only infections shown in the left and middle columns of Figure 7, the increased RBC production is not enough to counteract the relentless consumption of reticulocytes (Figure 7C), and thus the host dies. However, in the corresponding dual-species infections, the *P. falciparum* infection triggers a boost in the reticulocyte count before the reticulocyte consumption by *P. vivax* becomes critical. For the example in the left column, *P. falciparum* infected first. For the example in the middle column, the growth rate of *P. falciparum* is higher than that of *P. vivax*, so its depletion of RBCs of all ages starts the boost in RBC production before *P. vivax* has consumed its prey population. Interestingly, in the later infection, the two species suppress each others' parasitemias below the corresponding single-species values, after their peaks in the dual-species infection (Figure 7A and B), and the host survives longer than in the *P. vivax*-only infection (Figure 7D).

The right column plots in Figure 7 show catastrophic anemia produced by a *P. vivax* phenotype with a relatively high R_0 superinfecting a *P. falciparum* infection that otherwise would have been controlled by the host's *diserythropoetic* response. The *P. vivax* inoculation suppresses the *P. falciparum* I_{INT} . However, the *diserythropoesis* triggered by the pre-existing *P. falciparum* infection slows the growth rate of *P. vivax* (Figure 7A) until homeostasis returns, with the lessening of RBC loss caused by *P. falciparum*. The superinfecting *P. vivax* takes advantage of the boost in reticulocyte count (Figure 7C), which greatly amplifies its growth rate and enhances its I_{PK} over the single-species infection value. Even if we add a further 6-month delay to the recovery of RBC production, the *P. vivax* I_{PK} in the mixed-species infection is higher than in the single-species infection (see data supplement).

Dynamical response from the RBC source can lead to coupled, long-term oscillations in the parasitemia of both species

Our model predicts that if the RBC source is allowed to respond to the infection-induced cell loss, rather than remaining at a fixed rate, the parasitemia of the two species in a dual-species infection will tend to undergo long-term, coupled oscillations, provided that the host survives. Figure 8 shows this long-term behavior by presenting the results of simulated infections for up to 200-week duration. (For the three examples, $\tau_{\text{ret}} = 1.5$ days.) The figures in the left column are for $R_{0F} = 1.181$, $R_{0V} = 1.087$, $\Delta ST = +50$ weeks (*P. vivax* infects 50 weeks before *P. falciparum*), with the RBC production rate fixed at the basal rate. The figures in the middle column are for the same R_{0F} , R_{0V} , and ΔST , but with a diserythropoetic response by the RBC source. The figures in the right column are for $R_{0F} = 1.125$, $R_{0V} = 1.0442$, $\Delta ST = -10$ weeks (*P. vivax* infects 10 weeks after *P. falciparum*), with a compensatory response to infection-induced RBC loss.

For the example with the fixed RBC source rate (left column), the parasitemias and blood counts converge to steady-state values, perhaps after undergoing some damped oscillations. However, the middle and right columns show more interesting behaviors. For $R_{0F} = 1.181$, $R_{0V} = 1.087$, $\Delta ST = +50$ weeks with a diserythropoetic response (middle column), not only does the presence of the earlier-infecting *P. vivax* prevent *P. falciparum* from killing the host, but the introduction of *P. falciparum* modifies the amplitude and phase of the oscillations of the *P. vivax* parasitemia. In the mixed-species infection, the oscillations of the two parasitemias become coupled, with *P. vivax* counts reaching their maxima a few weeks before *P. falciparum* counts. For both species, the maxima and minima in the counts differ by factors of 10–100. The parasitemia oscillations are coupled to those of the reticulocyte population. Oscillatory behavior is not so regular in the times series in the right column, for a case with compensatory response to infection-induced RBC loss; nonetheless, the presence of the earlier-infecting *P. falciparum* induces more frequent oscillations in the *P. vivax* parasitemia, and *P. vivax*, in turn, suppresses the *P. falciparum* parasitemia. There is a complex series of oscillations in the reticulocyte count.

Reductions in a susceptible RBC population can boost the integrated parasitemia of a superinfecting species by extending the host's lifespan

The example in the middle column of Figure 8 ($R_{0F} = 1.181$, $R_{0V} = 1.087$, $\Delta ST = +50$ weeks, diserythropoetic response) shows a second mechanism of enhancement of one species' parasitemia by the presence of another: the reduction of the RBC population by one species can prevent a later-infecting species from killing the host, thus increasing the integrated parasitemia of the later-infecting species. In this example, an initial *P. vivax* infection reduces the overall RBC count and prevents a subsequent *P. falciparum* infection from inducing catastrophic anemia. However, this second mechanism of facilitation occurs over regions of (R_{0F} , R_{0V}) space that are tiny compared with those in which *P. vivax* is enhanced through increasing transients of RBC production.

DISCUSSION

The argument that RBC supply is not a limiting factor for parasitemia in *P. falciparum*-only infections fits the available data.^{39–41} Our previous work showed that the common argument that *P. vivax*-only infections exhibit limited anemia and lethality simply because *P. vivax* attacks only a small fraction of RBCs is flawed.²⁶ Here we have shown that RBC population dynamics may dramatically affect the outcome of mixed-species infections as well. The typical outcome predicted by our model in *P. vivax*-*P. falciparum* infections is asymmetric suppression: competition for RBCs suppresses the parasitemia of one species and leaves the other unaffected, even if the anemia is only partially determined by parasite destruction of

RBCs (as in the case of the diserythropoetic response of the host to the infections). However, with some combinations of RBC affinities, inoculation times, and host erythropoetic responses, the simulations also suggest mechanisms by which competition for RBCs may lead one species to facilitate the other's parasitemia, especially *P. falciparum* facilitating *P. vivax*. Thus, because interactions between these species are likely to be mediated by their interactions with RBCs, at least in part, constraints and imperatives for immunologic response must be intertwined with those for erythropoetic response. Even if $R_0 \sim p$, as it is believed to be in some malaria-naïve patients,³⁹ immune effectors might reduce ζ or p or both: we would expect a dynamic immune response to reduce the instantaneous growth rates R (not R_0) of the two parasites to values near 1 much more quickly than in our model (in which R can only be reduced from R_0 by a reduction in the RBC population vulnerable to the species) and to start the chronic phase of the disease within a matter of weeks. The long-term model behavior for R_{0F} and R_{0V} less than ~ 1.75 may still be relevant for understanding real infections. The key point suggested by our results is that a transient rise in RBC production could boost *P. vivax* parasitemia, whether that transient is a direct response to the extra RBC loss because of infection or arises from a return to homeostasis with the control of a *P. falciparum* infection. There is no reason *a priori* why the host's immune response should be expected to obliterate this effect of RBC competition.

Because *P. vivax* and *P. falciparum* diverged long ago, the challenges they present a common host should be similar but not identical. Salient differences should be reflected in host responses. For instance, *P. vivax* induces fever at a much lower parasitemia than does *P. falciparum*, suggesting that their interactions may involve a difference in the production of pyrogenic cytokines.^{42–44} It is clear that innate and acquired immune responses differ in their regulatory effects on parasite dynamics in *P. vivax*–*P. falciparum* infections,^{9,45} and it seems likely that most malaria infections are controlled by combinations of species- and phenotype-specific responses along with more general ones. Our results here suggest that these various distinctions should be examined more closely with regard to the erythropoetic involvement, especially as the qualitative behavior of the model is remarkably robust to changes in model parameters.

Although the infamous persistence of *P. vivax* infections has generally been attributed to its latent liver stages, PCR-based studies have begun to confirm suspicions that *P. falciparum* infections also persist much longer than is commonly recognized.^{46–48} (As far back as 1951, Eyles and Young reported that parasitemia detectable with the methods of the time can last more than a year in neurosyphilis patients infected with *P. falciparum*.⁴⁹) Thus, in the context of our model of mixed-species infections, it is interesting that both a variable host erythropoetic response and a limitation of susceptible RBCs can alter R for one or both species so as to delay or prevent catastrophic anemia, and/or generate long-term coupled oscillations of the parasitemia of the two species with a period on the order of weeks to months. As the host's immune response was almost certainly a significant determinant of the coupled oscillatory behavior of the *P. vivax* and *P. falciparum* parasitemia in dually infected neurosyphilis patients,⁹ our results cannot be directly compared with the patterns seen in these or other patients. Our model indicates that competition for RBCs may have a role in inducing or otherwise contributing to such behavior, however.

Where *P. vivax* and *P. falciparum* co-occur, their ongoing evolution must be influenced by competition for RBCs, in part by affecting the dynamics of their gametocytes. It is gametocytes, ingested by a mosquito, that can recombine and propagate parasite genes. However, because gametocyte production trades replicating, non-transmissible forms for non-replicating, transmissible forms, it reduces the rate of RBC destruction.⁵⁰ Thus, it is intriguing that *P. falciparum* gametocytemia is increased in the reticulocyte-rich blood of sickle-cell patients⁵¹ and decreased, in density and frequency, in *P. vivax*–*P. falciparum* infections.⁵² Gametocyte production and transmission remain poorly understood, and our model cannot

directly address these observations, but, if the probability that a species' gametocytes are transmitted is proportional to that species I_{PK} or I_{INT} , our results suggest that in most situations the species that has the higher R_0 or infects earlier would have an advantage, because RBC competition generally suppresses both I_{PK} and I_{INT} for the other species. However, the seemingly small regions of (R_{0F}, R_{0V}) space in which one species would facilitate I_{PK} or I_{INT} (and thus transmission) of the other could be important in selecting for traits that encourage or discourage co-infections. They may be important in selection of traits in the host as well. How the host's immune response would affect the size and boundaries of those regions in (R_{0F}, R_{0V}) space is an important question for study.

MATHEMATICAL APPENDIX

We model the parasite and RBC dynamics with systems of CODEs, in a manner closely similar to our previous work on single-species infections.²⁶ Notation is as in Figure 1. To model an ensemble of cells undergoing a specific process of development that requires duration τ on average with standard deviation sd , the number of compartments F is set so that $sd = \tau F^{-1/2}$. In a sense, the compartments are an abstraction, but the duration and standard deviation of the development process are the tangible quantities.

The CODEs for the development chain of infected RBCs are

$$\begin{aligned} dI_{1,sp}/dt &= \zeta_{sp}\mu_{sp}V_{sp} - \kappa_{sp}I_{1,sp} \\ dI_{n,sp}/dt &= \kappa_{sp}(I_{n-1,sp} - I_{n,sp}), 1 < n < F_{sp} + 1, \end{aligned}$$

where subscript sp is v for *P. vivax* or f for *P. falciparum*, F_{sp} is the number of compartments in the development chain for the given species, and $\kappa_{sp} = F_{sp}/\tau_{sp}$. μ_f and μ_v are the merozoite counts for the two species. V_{sp} is the total count of vulnerable RBCs for a given species: $V_v =$ total reticulocyte count, and $V_f = E_T$, the total uninfected RBC count. Because we took $\tau_f = \tau_v = 48$ hours, with a variance of 4.8 hours, $F_f = F_v = 100$. Because of their short duration in the blood, we used just one compartment for the merozoite stage:

$$d\mu_{sp}/dt = p_{sp}\kappa_{sp}I_{F_{sp},sp} - \zeta_{sp}\mu_{sp}V_{sp} - \mu_{sp}/\tau_{\mu,sp} + L_{sp}(t)$$

In our simulations, we took $p_f = 16$, $p_v = 8$ or 16 , and $\tau_{\mu,f} = \tau_{\mu,v} = 0.1$ hour. $L_{sp}(t)$ is the primary infusion of merozoites of the given species from the liver into blood, which is believed to happen quickly and involve 10^4 – 10^5 merozoites after the liver stage of the parasite develops for ~ 1 week. For simplicity, we took $L_{sp}(t) = 0$, except for a 1-hour period:

$$L_{sp}(t) = 10^4 / (5 \times 10^6 \text{ mm}^3/\text{h}), \\ 168 \text{ hours} < t + t_{0,sp} < 169 \text{ hours}$$

where $t_{0,sp}$ is the time of initial inoculation with the given species. Use of other functional forms for $L_{sp}(t)$ changed the outcome of test simulations little. For our simulations, the initial time ($t = 0$) corresponds to the release of the first parasite from the liver.

Using the notation in Figure 1, the CODEs for the RBC development chains are

$$\begin{aligned} dR_1/dt &= \sigma(t) - \kappa_R R_1 - (\zeta_v\mu_v + \zeta_f\mu_f)R_1 \\ dR_n/dt &= \kappa_R (R_{n-1} - R_n) - (\zeta_v\mu_v + \zeta_f\mu_f)R_n, \\ &1 < n < FR + 1 \\ dM_1/dt &= \kappa_R R_{FR} - \kappa_M M_1 - \zeta_f\mu_f M_1 \\ dM_n/dt &= \kappa_M (M_{n-1} - M_n) - \zeta_f\mu_f M_n, 1 < n < FM + 1 \\ dS_1/dt &= \kappa_M M_{FM} - \kappa_S S_1 - \zeta_f\mu_f S_1 \\ dS_n/dt &= \kappa_S (S_{n-1} - S_n) - \zeta_f\mu_f S_n, 1 < n < FS + 1 \end{aligned}$$

Here, $\kappa_R = \tau_R/FR$, $\kappa_S = \tau_M/FM$, and $\kappa_S = \tau_S/FS$, where τ is the duration of the respective blood development stages. For reticulocytes, we took $\tau_R = 36$ hours with $sd = 6$ hours, so that $FR = 36$; for mature-stage RBCs, $\tau_M = 2,796$ hours with $sd = 168$ hours so that $FM = 276$; for senescent-stage RBCs, $\tau_S = 48$ hours with $sd = 12$ hours so that $FS = 16$.

The model for the RBC marrow source depends on how the source responds to change in the count of uninfected RBCs. For compensatory erythropoiesis,³⁰ we take

$$\begin{aligned} d\sigma/dt &= \lambda_\sigma [\sigma_0 - dE_T/dt - \sigma(t)], \sigma_0 - dE_T/dt < \sigma_{MX} \\ &= \lambda_\sigma [\sigma_{MX} - \sigma(t)], \sigma_0 - dE_T/dt > \sigma_{MX} \end{aligned}$$

Here, σ_0 is the basal RBC production rate, σ_{MX} is the maximum allowed RBC production rate, which we took as $2\sigma_0$; $1/\lambda_\sigma$, the response time to changes in E_T , was set to 48 hours. For diserythropoiesis, we assume that $\sigma(t)$ is driven towards a floor value $\sigma_{MN} = 0.8\sigma_0$ instead:

$$\begin{aligned} d\sigma/dt &= \lambda_\sigma [\sigma_0 + dE_T/dt - \sigma(t)], \sigma_0 + dE_T/dt > \sigma_{MN} \\ &= \lambda_\sigma [\sigma_{MN} - \sigma(t)], \sigma_0 + dE_T/dt < \sigma_{MN} \end{aligned}$$

Again, $1/\lambda_\sigma = 48$ hours.

If at any point the merozoite count for a given species, the total infected RBC count for a given species, or the total uninfected RBC count drops to < 1 in a total blood volume of $5 \times 10^6 \mu\text{L}$, the values of all the compartments that contributed to that particular count were reset to zero. The CODE system was solved using the fifth-order Runge-Kutta-Fehlberg algorithm with adaptive stepsize control for time integration^{53,54} so that the difference between the fourth- and fifth-order solutions for each component of the CODE systems was less than one part in 10^6 .

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Supplementary Material

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Figure 1.

Schematic of model dynamics. The bone marrow source of new RBCs is labeled as $\sigma(t)$. RBC stages R_1 – R_{FR} are the reticulocytes, M_1 – M_{FM} are the mature stages, and S_1 – S_{FS} are the senescent stages (with X marking the natural end of the RBC lifespan). Only reticulocytes are vulnerable to *P. vivax*, but RBCs of all ages are assumed to be susceptible to *P. falciparum*. I_{v1} – I_{vFv} and I_{f1} – I_{fFF} are the RBCs infected with the developing stages of *P. vivax* or *P. falciparum*, respectively; μ_v and μ_f are the corresponding free merozoite stages. RBC production is regulated by the rate at which RBCs are lost through both normal senescence and infection; 2×10^4 hours of infection are simulated in each run of the model unless the uninfected RBC count falls below 75% of its initial value (catastrophic anemia).

Figure 2.

Outcomes for single-species infection as a function of the basic reproduction rate R_0 . For the $p = 8$ and $p = 16$ *P. vivax* curves, the duration of RBC vulnerability to infection is 1.5 days.

(A) Time of onset of catastrophic anemia vs R_0 with a single-species *P. vivax* or *P. falciparum* infection. Data points on the top “plateau” show where host survival exceeded the simulated duration of infection (~ 833 days). (B) Peak counts of infected RBCs, I_{PK} , vs R_0 . (C) Counts of infected RBCs integrated over the course of the infection, I_{INT} , vs R_0 . In each panel, the simulated data points are labeled as follows: (○) RBC production fixed at the basal rate, (Δ) compensatory response in RBC production, up to two times the basal rate, and (▽) diserythropoietic response in RBC production, down to 0.8 times the basal rate.

Figure 3.

Peak parasitemia and host survival, varying with basic reproduction rates R_{0V} and R_{0F} and relative inoculation time ΔST . RBC production is fixed at the basal rate. Contours for I_{PK} for *P. vivax* are spaced $10^4 \mu\text{L}^{-1}$ apart; contours for I_{PK} for *P. falciparum* are spaced $7.5 \times 10^4 \mu\text{L}^{-1}$ apart. Those for the time of onset of catastrophic anemia are spaced 25 days apart. (A) $\Delta ST = +10$ weeks. (B) $\Delta ST = 0$. (C) $\Delta ST = -10$ weeks. The gray shading from dark gray to white is to guide the eye from minimum to maximum values. The darkest gray shows values between 0 and the first contour. Black shading shows where the host dies before the second infection begins. For both *P. vivax* and *P. falciparum*, $p = 16$, and the duration of RBC vulnerability to *P. vivax* is 1.5 days. Because of the sharp sensitivity of host survival time to

R_{0F} or R_{0V} when R_{0F} and $R_{0V} \sim 1.13$, the contour lines in the charts for host survival are bunched around the white plateau where the host has survived for 2×10^4 hours (~ 833 days).

Figure 4.

Time series of parasitemia and RBC count for two examples in which one species is suppressed and the other is unaffected. All series are plotted against the time since the host was inoculated with the initial infecting species. Results in the right column are for $R_{0F} = 1.741$, $R_{0V} = 1.087$, $\Delta ST = 0$ (simultaneous inoculation), and in the left column for $R_{0F} = R_{0V} = 1.25$, $\Delta ST = -5$ weeks (inoculation of *P. vivax* 5 weeks after *P. falciparum*). RBC production is fixed at the basal rate for both simulations. T_{INC} is the time since the first inoculation. (A) Time series for the count of *P. vivax*-infected RBCs per microliter. (B) Time series for the count of *P. falciparum*-infected RBCs per microliter. (C) Time series for the ratio of the uninfected reticulocyte count to the basal reticulocyte count. (D) Times series for the ratio of the total

uninfected RBC count to the basal RBC count. Solid black curves show results in the mixed-species infection, dotted curves (---) show the *P. vivax*-only infection with the corresponding R_{0V} , gray curves show the *P. falciparum*-only infection with the corresponding R_{0F} . Where a mixed-species infection curve coincides with a single-species infection curve, only the mixed-species infection curve is shown. “x” marks the onset of catastrophic anemia. For $R_{0F} = R_{0V} = 1.25$, the count of RBCs infected with *P. falciparum* is completely eliminated after 108 weeks.

Figure 5.

Peak *P. vivax* parasitemia, varying with basic reproduction rates R_{0V} and R_{0F} and relative inoculation time ΔST , with a compensatory RBC source. Contours for I_{PK} for *P. vivax* are spaced $10^4 \mu\text{L}^{-1}$ apart for $\tau_{\text{ret}} = 1.5$ days and $2 \times 10^4 \text{ mL}^{-1}$ apart for $\tau_{\text{ret}} = 14$ days. (A) $\Delta ST = +10$ weeks. (B) $\Delta ST = 0$. (C) $\Delta ST = -10$ weeks. The gray shading from dark gray to white is to guide the eye from lowest to maximum values. The darkest gray shows values between 0 and the first contour. Black shading is where the host dies before the second infection begins. For the simulations depicted, $p = 16$ for *P. falciparum*.

Figure 6.

P. vivax parasitemia and time of onset of catastrophic anemia, varying with basic reproduction rates R_{0V} and R_{0F} , with a diserythropoietic RBC source and *P. falciparum* infecting 50 weeks before *P. vivax* ($\Delta ST = -50$). The gray shading from dark gray to white is to guide the eye from lowest to maximum values. The darkest gray shows values between 0 and the first contour. Black shading is where the host dies before the second infection begins. (A) I_{PK} for *P. vivax*. Contours are spaced $10^4 \mu\text{L}^{-1}$ apart for $\tau_{\text{ret}} = 1.5$ day and $2 \times 10^4 \mu\text{L}^{-1}$ apart for $\tau_{\text{ret}} = 14$ day. (B) I_{INT} for *P. vivax*. Contours are spaced $2.5 \times 10^5 \mu\text{L}^{-1}$ apart for all values of p and τ_{ret} . (C) Time of onset of catastrophic anemia with first species. Contours are spaced 25 days apart. For simulations depicted, $p = 16$ for *P. falciparum*.

Figure 7.

Time series of parasitemia and RBC count for three examples in which *P. falciparum* facilitates *P. vivax* through transient surges in the reticulocyte count. All series are plotted against T_{INC} , the time since the host was inoculated with the initial infecting species. The left column shows system dynamics for $R_{0F} = 1.5$, $R_{0V} = 3$, with a compensatory response and $\Delta ST = -10$ weeks (*P. falciparum* infects 10 weeks before *P. vivax*). The middle column shows system dynamics for $R_{0F} = 1.1137$, $R_{0V} = 1.061$, with a compensatory response and $\Delta ST = 0$ (both species inoculated at the same time). The right column shows system dynamics for $R_{0F} = 1.087$, $R_{0V} = 1.320$, with a diserythropoietic response and $\Delta ST = -50$ (*P. falciparum* infecting 50 weeks

before *P. vivax*). Panels and all symbols are as in Figure 4. For the three simulations, $\tau_{\text{ret}} = 1.5$ days.

Figure 8.

Time series of parasitemia and RBC count for three examples showing long-term behavior of the model (up to 200 weeks of simulated infection). All series are plotted against T_{INC} , the time since the host was inoculated with the initial infecting species. The left column is for $R_{0F} = 1.181$, $R_{0V} = 1.087$, $\Delta ST = +50$ (*P. falciparum* infecting 50 weeks after *P. vivax*), with the RBC rate of production fixed at the basal rate. The middle column is also for $R_{0F} = 1.181$, $R_{0V} = 1.087$, $\Delta ST = +50$ but with a diserythropoietic response. The right column is for $R_{0F} = 1.125$, $R_{0V} = 1.0442$, $\Delta ST = -10$ (*P. falciparum* infecting 10 weeks before *P. vivax*) with a compensatory response to the loss of RBCs caused by infection. Panels and all symbols are as in Figure 4.