Age-structured red blood cell susceptibility and the dynamics of malaria infections

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Malaria parasites and immune responses in an infected human interact on a dynamic landscape, in which a population of replicating parasites depletes a population of replenishing red blood cells (RBCs). These underlying dynamics receive relatively little attention, but they offer unique insights into the processes that control most malaria infections. Here, we focus on the observation that three of the four malaria-parasite species that infect humans are restricted to particular age classes of RBC. We explicitly incorporate this observation in models of infection dynamics to distinguish common from species-specific pressures on host immune responses, and we find that age structuring has profound effects on the course of infection. For all four species conditions exist under which the parasites may persist at low densities, or may clear, even in the absence of an immune response. Catastrophic anemia can occur even with the two species that attack only the youngest RBCs, although only a small fraction of cells are parasitized at any point. Furthermore, with these two, compensatory erythropoietic responses in the host accelerate parasite population growth. A “basic reproduction rate” characterizes these differences in outcomes.

Malaria in a human begins with an inoculum of Plasmodium parasites from an Anopheles mosquito. The parasites penetrate liver cells, multiply, then enter the bloodstream, and invade red blood cells (RBCs), where they again multiply and burst the cells, each releasing 8–32 “merozoites” that invade more RBCs and continue the cycle. Almost all malaria pathology is associated with this blood stage replication cycle; it leads to geometric growth in the parasite population and to fevers, anemia, and sometimes death in the host (1).

Parasite population growth is usually constrained by host immune responses. Accordingly, most mathematical models of within-host dynamics have taken the general form of predator–prey models, with the predator a population of immune agents and the prey a population of Plasmodium (2). A few Plasmodium falciparum models attempt to relate the dynamics of the parasite population to those of its prey, the population of RBCs, but none incorporate RBC aging or age-structured susceptibility (3–7).

RBC age appears to be a strong constraint on malaria parasites, however: susceptibility to Plasmodium vivax or Plasmodium ovale invasion is said to be restricted to the very youngest circulating age class of RBCs, the “reticulocytes,” and Plasmodium malariae invasion to the very oldest (8, 9). P. falciparum, the species responsible for almost all the 1–3 million deaths attributed to malaria each year, seems promiscuous with respect to its RBC targets (10). It is widely assumed that these age constraints explain why counts of parasitized RBCs rarely exceed 25,000 per μl with P. vivax, P. ovale, or P. malariae, but may reach 500,000 and beyond with P. falciparum, and thus, in turn, why fatal anemia occurs only in P. falciparum infections (11).

Most malaria infections are not fatal, but the mechanisms involved in their control are notoriously difficult to comprehend. Here, we use mathematical models to identify critical interactions between Plasmodium and RBC populations and indicate effects that immune responses must add (for instance, to counter the unexpectedly strong demands of P. vivax or P. ovale) if system dynamics are to resemble those observed in actual malaria infections.

The Models

RBCs emerge from bone marrow into the circulation, and in uninfected, healthy adults, they are removed by phagocytosis 120 days later (12). A density of ~5 million RBCs per μl is maintained in adult males. The first 1–2 days of the RBC lifespan are the reticulocyte stage. Compensatory responses to anemia may boost RBC production to several times the basal level (12). We model the rate of RBC production as sensitive to changes in the rate of RBC destruction, with a response time of 48 h, and capped at some maximum given as a parameter value. Here we consider maximum RBC production fixed either at the basal rate [i.e., (5 × 10⁶ RBC per μl)/(120 days) = 1,736 RBC per μl per h] or at twice the basal rate.

Successful invasion of a RBC by a parasite depends on direct contact between the two. We take the contact process itself as random, with contact probabilities proportional to Mer and V, the densities of merozoites and uninfected susceptible RBCs, respectively. On contact, successful invasion depends on the receptor–ligand attachment(s) required for the physical process of invasion, which we model as a binding affinity. Thus, infected RBCs are produced at rate $\xi Mer V$. The details of RBC receptor usage by different Plasmodium species are not yet clear, in particular with respect to RBC age, but P. vivax invasion is limited to RBCs from Duffy-antigen-positive individuals, and P. falciparum can use multiple receptors (13); nothing is known about P. malariae receptors. Nothing is known about receptors for P. ovale except that they must differ from those for P. vivax; given their shared restriction to reticulocytes, however, we treat P. ovale as identical with P. vivax and refer only to the latter.

Therefore, we model age-structured attacks on RBCs as either (i) a P. vivax-like attack on reticulocytes or (ii) a P. malariae-like attack on senescent RBCs, and set the exact duration of RBC susceptibility in each model as a parameter value. For comparison, we model attacks on RBCs that present appropriate receptors at all ages: a fraction $\beta$ of RBCs that emerge from marrow has life-long susceptibility; initially, at the start of infection, a fraction $\beta$ of each RBC age cohort in circulation is susceptible. Hence, we call this attacker a generalist; it is believed $\beta = 1$ for P. falciparum (13).

Fig. 1 gives schematic representations of the models, which we formulated as compartmental differential equation systems (CODEs); CODEs allow us to include uncertainty in the duration $\tau$ of a process, such as RBC aging or parasite development in a RBC. Variance is set by $\tau/F^{1/2}$, where $F$ is the number of compartments used to describe the process; variances are integral to models’ steady-state solutions as well (see supporting information, which is published on the PNAS web site). Each model simulation mimicked 10⁵ h of infection (~11.4 yr), unless

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Abbreviations: CODE, compartmental differential equation system; ODE, ordinary differential equation.

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the density of uninfected RBCs declined to 75% of its initial value, which we set as the threshold for catastrophic anemia, in which case the simulation halted. During the first simulated hour $10^4$ merozoites were infused into a total blood volume of 5 liters to mimic primary release from the liver. We take the nominal parasite count falls to 16/104 merozoites were infused into a total blood volume of 5 liters the density of uninfected RBCs declined to 75% of its initial value (catastrophic anemia). X marks the natural end of the RBC lifespan.

When released from a bursting RBC, a free merozoite will die or be cleared from circulation within a time $\tau_{Mer}$, believed to be minutes (14). Thus, two competing Poisson processes affect the fate of merozoites: (i) they are removed from the blood at rate $1/\tau_{Mer}$; (ii) they attach to RBCs at rate $\zeta V$. The probability that a merozoite infects a RBC rather than being cleared is $\zeta V \tau_{Mer} (1 + \zeta V \tau_{Mer})^{-1}$ (see supporting information). Thus, if $\tau_{Mer} \ll \tau_p$, its average number of descendants after a development cycle is

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

where $p$ is the average number of merozoites released from a bursting RBC. We define $R_0$, the basic reproduction rate, as the value of $R$ at the beginning of the infection. This quantity both determines the initial behavior of an infection and affects its outcome. Nontrivial steady-state solutions exist only if $R_0 > 1$ (see supporting information). Thus, the parasite count ultimately vanishes if $R_0 < 1$, persists at its initial value if $R_0 = 1$, or converges to a larger steady state if $R_0 > 1$, unless the host RBC count is depleted to the point of catastrophic anemia. We stress that $R$ (as opposed to $R_0$) can change during the course of infection as $V$ changes; in particular, $R \to 1$ as a steady state is approached. Note that the existence of a noncatastrophic steady state does not preclude the possibility that catastrophic anemia will occur in the approach.

$$T_{\text{Inf}} = 1/\zeta V = (p - R_0) \tau_{Mer}/R_0$$

The count of vulnerable RBCs for the $P. vivax$ model with reticulocyte stage duration = 36 h; $P. malariae$ with senescent RBC duration = 48 h; $- - -$, generalist with $\beta = 0.05$; $- - -$, generalist with $\beta = 0.99$. (a) Uninfected RBC count. (Inset) The count of vulnerable RBCs for the $P. vivax$, $P. malariae$, and generalist $\beta = 0.05$ models. (b) The fraction of RBCs that are infected. (Inset) Enlargement of the region around 10 $\tau_p$ showing the structure in the "hump." Note the linear (instead of log) vertical scale of Inset.
each model, RBC production was held at the basal rate and \( R_0 \) set to 80/13 (≈6.15). Note that all four models are initially in lockstep, with parasite density growing exponentially for the first 9–12 cycles of \( \tau_f \) (as expected from the \( T_c \) formula). After the first few weeks of the \( \beta = 0.99 \) generalist infection, the density of uninfected RBCs quickly collapses and the fraction of RBCs infected soars. For the \( \beta = 0.05 \) generalist and \( P. \) malariae infections, the density of uninfected RBCs is only mildly affected and the fraction of RBCs infected never exceeds approximately 2%. The \( P. \) vivax infection leads to catastrophic anemia, however, although the maximum fraction of RBCs infected is approximately 3%. The initial fraction of RBCs susceptible to \( P. \) vivax is only 36 h/120 days = 0.0125, but \( P. \) vivax attack has more drastic clinical consequences than the \( \beta = 0.05 \) generalist, because targeted culling of reticulocytes ensures that few remain to mature and replenish the older RBCs.

Differences in model dynamics are also apparent in Fig. 3, which, for a range of \( R_0 \) values > 1, plots the time until the onset of catastrophic anemia as a function of \( f_0 \), the fraction of RBCs initially susceptible (\( \phi \) for the generalist, and the susceptible-stage duration/120 days for the other two models; \( f_0 = 0.0125 \) and 0.0167, respectively, for the \( P. \) vivax 36-h and \( P. \) malariae 48-h examples in Fig. 2). In the generalist and \( P. \) malariae models, the density of uninfected RBCs declines to 75% of its initial value, our threshold for catastrophic anemia, only if \( f_0 > 0.25 \). In the \( P. \) vivax model, however, catastrophic anemia can occur even with \( f_0 = 0.01 \) and \( R_0 \) barely > 1. Even with a reticulocyte duration of just 18 h (\( f_0 = 0.00625 \) and \( R_0 = 10^{0.06} (≈1.15) \)), catastrophic anemia would occur in ≈450 days; the peak infected RBC count would be \( 2.1 \times 10^4 \) per \( \mu \)l, so the fraction of RBCs infected would never exceed \( (2.1 \times 10^4)/(3.75 \times 10^6 + 2.1 \times 10^4) \approx 0.6% \). With a 36-h reticulocyte duration and \( R_0 = 10 \), the fraction of RBCs infected would never exceed \( 3\% \), but catastrophic anemia would occur within 45 days.

\( P. \) vivax model behaviors begin to resemble those of the other two models if the reticulocyte duration expands to encompass > 30% of the RBC lifespan; mature RBCs constitute a smaller fraction of the total RBC count, so choking off their predecessor reticulocytes has less effect. This is especially evident for \( R_0 \) near 1, as can be seen in Fig. 3 and in the steady state (see supporting information).

Fig. 4 shows the peak infected RBC counts, \( I_{PK} \), corresponding to Fig. 3. Age-structured attacks produce striking differences; for a given \( R_0 \) and \( f_0 \), \( I_{PK} \) is higher in \( P. \) vivax and \( P. \) malariae models than in the generalist model. All RBCs emerging from bone marrow reach the stage susceptible to \( P. \) vivax or \( P. \) malariae, whatever the value of \( f_0 \), either immediately (\( P. \) vivax) or after aging (\( P. \) malariae). For the generalist, however, only a fraction \( \beta \) of new RBCs enters the susceptible pool. \( I_{PK} \) curves for the generalist approach those of the other two models as \( f_0 \rightarrow 1 \). Age-structured attacks also differ in their steady-state values (see supporting information).

For \( R_0 < 1 \), all three models effectively have the same stiff but analytically solvable ordinary differential equation (ODE) system. As \( R_0 \rightarrow 1 \), to a very good approximation the time required for the initial parasite population to decrease by \( 1/e \) (the e-folding time) is \( -\tau_f/\ln R_0 \). If \( \tau_f = 48 \) h, the e-folding time is \( 19 \) days for \( R_0 = 0.9 \) and \( 199 \) days for \( R_0 = 0.99 \). Thus nonsustainable infections with \( R_0 \) just below the threshold of persis-
tence could linger, at undetectable densities, with the host vulnerable to increases in $p$ or $\zeta$.

### Integrated Parasite Count

As a proxy for the cumulative number of parasites produced in an infection, we define the integrated infected RBC count $I_{\text{INT}}$ (average infected RBC count)/(duration of simulated infection). Fig. 5 plots $I_{\text{INT}}$ vs. $I_{\text{PK}}$ for key values of $f_0$, $R_0$ varies from $10^{0.01}$ to $10^{1.1}$ along each curve, tracking $I_{\text{PK}}$. The maximum $I_{\text{INT}}$ for each model occurs at a distinct combination of $R_0$ and $f_0$ values, e.g., a relatively small $R_0$ (1.17) and large $f_0$ (0.99) for the generalist, large $R_0$ (>12) and intermediate $f_0$ (0.25) for $P. \text{malariae}$. With small $f_0$ values in the generalist or $P. \text{malariae}$ models, $I_{\text{INT}}$ is monotonic in $I_{\text{PK}}$, but large $f_0$ values lead to catastrophic anemia: $I_{\text{INT}}$ abruptly collapses with increased $I_{\text{PK}}$ as $f_0$ exceeds 0.25. Again, the $P. \text{vivax}$ model differs dramatically: the $I_{\text{INT}}$ vs. $I_{\text{PK}}$ curve is relatively insensitive to reticulocyte duration, and the largest values of $I_{\text{INT}}$ occur with $I_{\text{PK}} < 30,000$ per $\mu l$, an order of magnitude lower than with the $P. \text{malariae}$ or generalist models.

### Compensatory Responses to Anemia

The preceding results assume that RBC production remains fixed at the basal rate throughout a malaria infection; in fact, significant RBC loss usually stimulates enhanced or accelerated production. The particular mechanisms involved are still poorly understood (17), but one recent analysis reported an average 37% increase in RBC production in adult first-time $P. \text{falciparum}$ patients (18). Here, we allow RBC production to match RBC depletion, up to a maximum of twice the basal rate (Eq. 5). Fig. 6 shows the effects of compensatory responses, with varying $R_0$, in four model situations: $P. \text{vivax}$ with 36-h reticulocyte duration,

P. $\text{malariae}$ with 48-h senescent RBC duration, and the generalist with $\beta = 0.05$ or 0.99.

In the $P. \text{vivax}$ model, the host gains little or no benefit from compensatory response. For $R_0 < 10^{0.05} \approx 4$, increased RBC production actually speeds the onset of catastrophic anemia. In contrast, compensation slightly delays the onset in the $\beta = 0.99$ generalist model. Compensation boosts peak parasite counts in all models, but most dramatically in the $P. \text{vivax}$ model for $R_0 < 10^{0.6} \approx 2.5$. Consider the $P. \text{vivax}$ model with $R_0 = 10^{0.6} \approx 1.122$, for instance: doubling the rate of RBC production quickly doubles the reticulocyte count, the instantaneous reproduction rate $R$ becomes nearly twice $R_0$ (2.097; Eq. 1), and a nonlethal $P. \text{vivax}$ infection becomes lethal. Compensation also boosts the integrated count, with the notable exception of $P. \text{vivax}$ with $R_0$
catastrophic anemia with \textit{P. vivax} reduces lethal. If infection reduces RBC production rates, the onset of catastrophic anemia with \textit{P. vivax} is delayed, and \( I_{PK} \) decreases, consistent with a reduction of \( R \) (see supporting information).

**Discussion**

Our results show that age-restricted RBC susceptibility strongly influences the dynamics of \textit{Plasmodium} infection and thus the imperatives for host immune response. They conform to clinical observations: if we limit susceptibility to the small fraction of RBCs that are senescent, infections are relatively benign, as is \textit{P. malariae}. They suggest that compensatory RBC production usually aggravates infections; in fact, it may be functionally suppressed in chronically exposed patients (17). However, our results argue against the belief that, because only a small fraction of RBCs are reticulocytes, \textit{P. vivax} and \textit{P. ovale} are intrinsically less dangerous than \textit{P. falciparum}; attacks on reticulocytes choke off the supply of mature RBCs and lead to catastrophic anemia; control demands aggressive reactions.

Insights into RBC dynamics should aid in assessing interventions, most obviously exchange transfusion (19), but also drugs or potential vaccines. With age-restricted susceptibility, uninfected RBCs can graduate into or out of a susceptible class, but depletion of that class depletes older ones. Future work must identify more precise age-associated RBC characteristics that govern susceptibility, however. Because circulating RBCs are anucleate and cannot synthesize new surface molecules, receptors that decay cannot be replaced; reticulocyte susceptibility to \textit{P. vivax} may be related to higher Duffy-antigen density, for instance (20). If RBC susceptibility to \textit{P. malariae} is related to senescence markers, the accelerated senescence of uninfected RBCs reported with \textit{P. falciparum} (21) should greatly affect mixed-species infections (22). The common portrayal of \textit{P. falciparum} as a generalist is complicated by evidence of a seeming preference for young RBCs (23), but more detailed resolution is needed here as well: our model shows that the proportion of RBCs that is in young age classes increases as an infection proceeds.

Similarities and differences in immune response to different \textit{Plasmodium} species remain obscure partly due to preoccupation with \textit{P. falciparum}. Duffy, sickle-cell and other RBC polymorphisms indicate that malaria has shaped human immunity in more than the usual, proximate sense of the term, however. The four species that infect humans diverged long ago, their histories and non-human hosts differ, and comparing their traits should contribute to anemia (17). Although we do not explore any instance (20). If RBC susceptibility to \textit{P. vivax} tors that decay cannot be replaced; reticulocyte susceptibility to \textit{P. falciparum} with non-human hosts differ, and comparing their traits should indicate that malaria has shaped human immunity in more than the usual, proximate sense of the term, however. The four species that infect humans diverged long ago, their histories and non-human hosts differ, and comparing their traits should contribute to anemia (17). Although we do not explore any instance (20). If RBC susceptibility to \textit{P. vivax} tors that decay cannot be replaced; reticulocyte susceptibility to \textit{P. falciparum}.

We do not consider parasite synchronization, which is likely immune-mediated (27), but note that it would probably benefit the host of a \textit{P. vivax} infection were reticulocyte susceptibility <48 h. We also do not consider the blood stages transmissible to mosquitoes; their production entails complex trade-offs between density, persistence, pathogenesis, and transmission (28, 29), but these should not alter the qualitative conclusions given here. Here, any correlates of the peak infected RBC count would be maximized simply by maximizing \( R_0 \) and \( I_0 \); larger \( R_0 \) values also lead more quickly to catastrophic anemia, however, and correlates of the integrated infected RBC count would be maximized by species-specific balances between \( R_0 \) and \( I_0 \). Finally, although our results are scaled on a per-microbiter basis, we note that any given number of parasites represents a higher proportion of the RBC total in a child than in an adult, which may add to response imperatives.

**Mathematical Formalism**

We model the infection dynamics with systems of CODEs. The notation is as in Fig. 1. The CODEs for the infected RBCs are:

\[
\begin{align*}
\frac{dI_n}{dt} &= \xi Mer(t) - I_{n-1},
1 < n \leq FI
\end{align*}
\]

where FI is the number of compartments and \( \xi = I_1/t_\tau \). Given its short duration, we use a single ODE for the merozoite.

\[
\frac{dMer}{dt} = p\xi Mer(t) - \xi Mer(t) - Mer/(\tau Mer + L(t)).
\]

\( V \) is the total count of vulnerable RBCs. \( L(t) \) describes the primary infusion of merozoites from the liver into the blood, the details of which are unknown beyond that it occurs quickly (<1 h), with just \( 10^4-10^5 \) parasites released (1). We took a tent form for \( L(t) \), which allows for some control in the time profile of release:

\[
L(t) = \frac{c - d^*(t-t_{MX})}{c - d^*(t-t_{MX}) + d_{TX} - t_{TX}}
\]

\[
L(t) = \begin{cases} 
\frac{c - d^*(t-t_{MX})}{c - d^*(t-t_{MX}) + d_{TX} - t_{TX}} & \text{for } t_{MX} < t < t_{MX} + t_{D} \\
0 & \text{otherwise}.
\end{cases}
\]

The release is at its maximum rate at \( t = t_{MX} \) and concludes at \( t = t_{TX} \). Parameters \( c, d, \) and \( d \) are fixed uniquely for a given \( t_{MX} \) and \( t_{D} \) by requiring \( L(t) \) to be continuous with time integral equal to \( 10^4 / (5 \times 10^4 \text{ mm)} \). Details of \( L(t) \) had little effect on model dynamics, because the number released is small and \( t_{D} \) is no more than a few hours (results not shown). For simulations reported here, \( t_{MX} = 0.5 \) h and \( t_{D} = 1 \) h.

The RBC source has its own ODE:

\[
\frac{dr(t)}{dt} = \lambda_{s0}(s_0 - U_T - r(t)), s_0 - U_T < s_{MX} = \lambda_{s0}(s_{MX} - s(t)), s_0 - U_T > s_{MX}.
\]

The release is at its maximum rate at \( t = t_{MX} \) and concludes at \( t = t_{TX} \). Parameters \( c, d, \) and \( d \) are fixed uniquely for a given \( t_{MX} \) and \( t_{D} \) by requiring \( L(t) \) to be continuous with time integral equal to \( 10^4 / (5 \times 10^4 \text{ mm)} \). Details of \( L(t) \) had little effect on model dynamics, because the number released is small and \( t_{D} \) is no more than a few hours (results not shown). For simulations reported here, \( t_{MX} = 0.5 \) h and \( t_{D} = 1 \) h.

The CODEs for the susceptible (R) and nonsusceptible (M) RBCs in the \textit{P. vivax} model are:

\[
\begin{align*}
\frac{dR}{dt} &= s(t) - \kappa_R R - \xi Mer(t) R \\
\frac{dR}{dt} &= \kappa_R R_{n-1} - R_n - \xi Mer(t) R_n, 1 < n \leq FR \\
\frac{dM}{dt} &= \kappa_M R_{FR} - \kappa_M M, \\
\frac{dM}{dt} &= \kappa_M (M_{n-1} - M_n), 1 < n \leq FM,
\end{align*}
\]

We do not consider parasite synchronization, which is likely immune-mediated (27), but note that it would probably benefit the host of a \textit{P. vivax} infection were reticulocyte susceptibility <48 h. We also do not consider the blood stages transmissible to mosquitoes; their production entails complex trade-offs between density, persistence, pathogenesis, and transmission (28, 29), but these should not alter the qualitative conclusions given here. Here, any correlates of the peak infected RBC count would be maximized simply by maximizing \( R_0 \) and \( I_0 \); larger \( R_0 \) values also lead more quickly to catastrophic anemia, however, and correlates of the integrated infected RBC count would be maximized by species-specific balances between \( R_0 \) and \( I_0 \). Finally, although our results are scaled on a per-microbiter basis, we note that any given number of parasites represents a higher proportion of the RBC total in a child than in an adult, which may add to response imperatives.
where $\kappa_B = FR/\tau_B$ and $\tau_B$ is the average reticulocyte duration. Based on what is known about RBC development (12), we took $FR = 21$ so that the variance in the reticulocyte stage duration is $\sim 0.22\tau_B$ (or $\sim 8$ h if $\tau_B = 36$ h), $\kappa_M = FM/\tau_M$, and $\tau_M$ is the nominal duration of the nonvulnerable stage (120 days $- \tau_B$). We choose $FM$ so that $1/\kappa_M = 18$ h. The total count of the susceptible cells is $V = \sum_n = 1, FR S_n$.

The CODEs in the $P. malariae$ model are:

$$dM_1/dt = s(t) - \kappa_M M_1$$
$$dM_n/dt = \kappa_M (M_{n-1} - M_n), 1 < n \leq FM$$
$$dS_1/dt = \kappa_E (M_1 - S_1) - \xi \text{Mer}(t) S_1$$
$$dS_n/dt = \kappa_E (S_{n-1} - S_n) - \xi \text{Mer}(t) S_n, 1 < n \leq FS \quad [7]$$

$FM/\kappa_E$ is the nominal duration of the nonvulnerable RBC stage, and $FS/\kappa_E$ is the nominal duration of the senescent stage. We chose $FM + FS$ so that $1/\kappa_E = 12$ h, for a RBC lifespan of 120 days. Here $V = \sum_n = 1, FR S_n$.

The CODEs in the generalist model are:

$$dV_1/dt = \beta(t) - \kappa_E V_1$$
$$dV_n/dt = \kappa_E (V_{n-1} - V_n) - \xi \text{Mer}(t) V_n, 1 < n \leq FE$$
$$dN_n/dt = (1 - \beta(t)) - \kappa_E N_n$$
$$dN_n/dt = \kappa_E (N_{n-1} - N_n), 1 < n \leq FE \quad [8]$$

We took $1/\kappa_E = 12$ h for both nonsusceptible and susceptible cells, and $V = \sum_n = 1, FR V_n$.

If at any point the merozoite count or the infected, susceptible or nonsusceptible RBC total drop to $<1$ for the whole volume of blood (here $5 \times 10^8 \mu l$), all the compartments that contribute to that particular total are reset to zero. All ODE systems were solved by using the fifth-order Runge–Kutta–Fehlberg algorithm, which incorporates an embedded fourth-order Runge–Kutta algorithm for adaptive stepsize control of the time integration (30, 31).

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