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Discrete-Event Models of Mixed-Phenotype *Plasmodium falciparum* Malaria

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Abstract

We extend our basic discrete-event model of *Plasmodium falciparum* malaria to encompass circumstances in which multiple phenotypic variants of the parasite circulate within interacting human and mosquito populations, and we compare a version in which variants behave independently to one in which they interact through shared host immune responses. Relative to the standard hypothesis of statistical independence, frequencies of mixed-phenotype infection in humans were as expected in the independent-immunity version and much less than expected in the cross-immunity version; in both versions, however, such frequencies in mosquitoes were much greater than expected.

Keywords

Epidemiology; infectious disease; malaria; population biology; prevalence; transmission; vector

1. Introduction

Public-health policymakers who cannot predict with reasonable confidence the epidemiological consequences of interventions may have little rational basis for choosing among or combining available alternatives, evaluating outcomes or allocating resources for further research and development. Becker [1], among others, has argued that the efficiency of statistical inference in infectious-disease epidemiology could be markedly improved by consistently applying our biological understanding of transmission processes to the construction of more specific distribution-based models. With malaria transmission, the dynamic relationships among the parasite populations within an individual human or mosquito and those within a human or mosquito population are so intricately intertwined that biology-based epidemiological models may prove unusually helpful not only in statistical analyses but in empirical investigations of underlying mechanisms and relationships. The outstanding recent example is a model proposing that *P. falciparum* populations are structured as independently-transmitted, antigenically-based “strains” [2]. This model stimulated much of the subsequent empirical research indicating that “strains” may in fact interact in ways that affect malaria dynamics [3–6]. Such results may temper the initial conclusion that “malaria may be easier to control by mass vaccination than expected” [7], but should lead to more sophisticated and successful intervention strategies.

In introducing a discrete-event approach to the modeling of malaria dynamics [8], we followed a number of the conventions of the classic differential-equation models of malaria, including the treatment of the parasite *P. falciparum* as a biologically homogeneous, single-genotype, single-phenotype entity. That is, although the existence of distinct “strains” or “races” of *P. falciparum* was recognized long ago [9], most previous modelers who considered these at all considered them as completely mutually-independent entities, while in some manner implicitly allowing their dynamics to be subsumed within those of a uniform species [10]. Molecular

methods have now revealed immense diversity within *P. falciparum*, at the level of genotypes (potential recombinants) and at the level of phenotypes—concordant variants in terms of gametocyte production, antigenic profile or drug resistance, for instance [11,12]—and, as noted above, have suggested that such entities may interact.

Accordingly, we have extended our basic discrete-event model to encompass the dynamics of multiple *P. falciparum* phenotypes. Here we distinguish a “phenotype” strictly in operational terms, as a labeled entity corresponding to a set of parameter values distinct from that of any other such entity, and in the context of perennial transmission we investigate two contrasting versions of the extended model: one in which two phenotypes operate in literal independence, with no interactions or effects of one on the other, and another in which the phenotypes clearly interact, through their interactions with a shared human immune response. For both the independent-immunity and cross-immunity versions, we again compare the influence of vector mortality on malaria prevalence in humans to that of the durations of host infectivity and host immunity to reinfection, and we then focus on frequencies of mixed-phenotype infections.

2. Methods

We coded host and vector states for each of two phenotypes, “A” and “B”—e.g., as “infected but not yet infectious” with A, or with B, or with both or with neither, “(infected and) infectious” with A, B, both or neither—and each corresponding state transition, so as to incorporate all possibilities for simultaneous infections and superinfections in each possible human and mosquito state and state transition. For each value of each parameter for each phenotype, we repeated all runs used to evaluate the single-phenotype model [8]. With the independent-immunity model, this confirmed that the two phenotypes behaved independently, without implementation artifacts, by demonstrating that (1) variation in each parameter of a focal phenotype had effects on its own dynamics identical to those in the single-phenotype model, and (2) variation in each parameter of a focal phenotype had no effect on the dynamics of the other.

In the cross-immunity model we added an explicit inter-phenotype interaction, such that the two phenotypes share every aspect of a host’s immune-response dynamics except, in general, the phenotype-specific value of the host-immune-decay parameter IM (see below). As in the single-phenotype [8] and independent-immunity models, the probability that a human exposed to an infectious bite can become infected with a phenotype with which he has not previously been infected is 1, the probability that he can become infected with a phenotype with which he is currently infected is 0, and, following clearance of a single-phenotype infection, the probability that he can become re-infected with that same phenotype increases as $1 - e^{-IM(h+WN)}$. However, in the cross-immunity model, in any interaction between a randomly-selected mosquito and a randomly-selected human [8], if that mosquito is infectious for either or both phenotypes, we compared the probability that that human could become infected with phenotype A against the probability that he could become infected with phenotype B and applied the lower probability to that interaction. Hence once a human has been infected by either phenotype, that host’s immunity to re-infection also applies to subsequent infection by the other, with each sequential infection potentially re-setting and “boosting” a host’s immunity against both phenotypes. We tagged and tracked individual records to confirm that the model behaved as intended with this added feature.

We again focused on the influence of vector survivorship (VS), the duration of host infectivity (WN) and the duration of host immunity (IM) on the prevalence of *P. falciparum* infection, using parameter ranges and initial conditions as in the single-phenotype model [8], here with the 25% of hosts initially infected selected at random, with replacement, for infection with phenotype A or phenotype B. We again generated response surfaces of 231 prevalence points

for each pairwise combination of the parameters VS, WN and IM at five levels of the third parameter, with each point an average of 100 simulation runs, with a standard deviation of 0.01 or less. We again calculated best-fit planes to allow comparisons among the average effects of each parameter, and we completed analyses corresponding to those for the single-phenotype model, with all conditions set as in that model except that while the two phenotypes shared common values of the parameter VS, values of their parameters WN and IM varied independently over the same ranges as in the single-phenotype model. We also compared the prevalence of mixed-phenotype infections to the product of the prevalences of each phenotype, the standard statistical hypothesis of independence [13], i.e., for each set of results we compared $P(AB)$ to $P(A)P(B)$, where $P(X)$ represents the prevalence of phenotype X.

3. Results

For comparable parameter sets the overall prevalence of malaria in humans and in mosquitoes was highest with the independent-immunity model and lowest with the single-phenotype model. Prevalence results with the cross-immunity model were intermediate, with typical ratios of 1.4 independent-immunity: 1.1 cross-immunity: 1.0 single-phenotype.

As expected from our previous results [8], for the independent-immunity model, the influence of the shared parameter VS on overall *P. falciparum* prevalence in humans was of similar magnitude to that of the two phenotypic WN values, varied jointly, and greater than that of the two phenotypic IM values, varied jointly. The influence of the parameters VS, WN and IM all declined relative to the single-phenotype model, but declined roughly in proportion; hence the relative effects of the parameters were much as in the single-phenotype model. For the cross-immunity model, the influence of WN declined only slightly less than in the independent-immunity model. The average influence of IM increased, and that of VS was unchanged relative to the single-phenotype model, but specific gains and losses in the influence of each of these parameters interacted with each other and with levels of WN. Relative to the independent-immunity model, however, the influence of VS and IM on overall *P. falciparum* prevalence in humans consistently increased.

For comparable parameter sets the prevalence of mixed-phenotype infections in humans and in mosquitoes was much lower with the cross-immunity model than with the independent-immunity model, and this relative decrease was much greater than the corresponding decrease in overall *P. falciparum* prevalence. With the cross-immunity model, the influence of VS, WN and IM on mixed-phenotype prevalence in humans each declined relative to the independent-immunity model, and declined roughly in proportion; hence the relative effects of the parameters were similar. Though the influence of VS, WN and IM on mixed-phenotype prevalence in mosquitoes increased with the cross-immunity model, the relative effects of VS and WN declined in comparison to IM.

As Figure 1 illustrates, with the independent-immunity model, the prevalence of mixed-phenotype infections in humans was generally as expected under the standard statistical hypothesis of independence [13], and in the cross-immunity model generally much less than expected by the same criterion. In both models the prevalence of mixed-phenotype infections in mosquitoes was much greater than expected; see Figure 2.

4. Discussion

None of the specific new results presented here seems particularly surprising, at least post hoc. This is encouraging, given that we expect to use a series of such incremental or modular additions in developing transparent logical connections from the most abstract and conceptual to the most detailed and specialized discrete-event models of malaria. In more general terms,

however, our results here indicate that there may be substantial differences between the absolute and relative influences of variation in some parameters, and among the effects of such variation on overall and mixed-phenotype prevalence in humans and mosquitoes. As discrete-event models become capable of addressing the complex effects of real-world interventions, such differences should be of particular help in evaluating outcomes of field trials.

The cross-immunity model provides a complementary bounding case for the independent-immunity model, contrasting a complete absence of superinfection to unconstrained superinfection in humans. One consequence is that in the cross-immunity model, mixed-phenotype infections in humans cannot arise from the sequential bites of singly-infectious mosquitoes, but only from the bites of dually-infectious mosquitoes; hence the corresponding frequencies in humans are much lower than those expected under the standard hypothesis of statistical independence [13]. The higher-than-expected frequencies of mixed-phenotype infections in mosquitoes in both models are very much in line with our earlier analyses of mixed-species *Plasmodium* infections in *Anopheles* [14], however, and again point to possible difficulties in applying conventional statistical null models to human malaria epidemiology [15–17].

The most interesting implications of the disproportionate decline in mixed-phenotype prevalence in the cross-immunity model relative to the independent-immunity model may be those with respect to the probabilities of *P. falciparum* genetic recombination events, e.g., those involving drug-resistant or vaccine-evading variants. Our preliminary results with an extended model that incorporates the possibility of recombination between phenotypes—i.e., a model of genotype interaction—suggest that typical time intervals until the appearance of the first recombinant form in the cross-immunity model may be several orders of magnitude greater than in the independent-immunity model [18].

Drug-based, vaccine-based and other interventions will inevitably alter the dynamics of malaria transmission, but not necessarily in a readily predictable manner. Our capacity for prediction should increase as our models more fully apply our biological understanding of the multiple processes that influence transmission. In particular, Dietz et al. [19] pointed out that “the effect of immunity on transmission... may be relatively unimportant for a theory of eradication, but is crucial for a theory of control where the endpoint is a new balance of the host and parasite populations.” Halloran and colleagues [20–22] emphasized the importance of the relationship between the duration of natural immunity and the duration of vaccine effectiveness in human populations, and suggested that the effective life of a prospective anti-asexual-form malaria vaccine might be extended by incorporating explicit transmission-blocking components. These relationships involve areas in which our discrete-event models can and must be vastly improved, by incorporating intermediate levels of phenotypic cross-immunity, parasite antigenic variation as well as antigenic polymorphism [23] and, eventually, explicit, detailed mechanisms of innate and acquired immune responses in individual humans.

We previously demonstrated a general congruence, and several specific discrepancies, between our single-phenotype discrete-event model and dynamic models in classic form, using familiar, shared metrics such as the overall prevalence of malaria in a closed human population [8]. We expect to relate field results to results from our model much as we relate them to results from dynamic models in classic form or familiar statistical models in biology and epidemiology, i.e., using goodness-of-fit, maximum-likelihood and time-series methods, in conjunction with Monte Carlo procedures, to select among alternative models (including those in classic form) and estimate model parameters. Techniques for model validation and selection at this level of resolution are well established [1,24–26], and can be adapted to studies of phenotypes defined by standard measures of differential gametocyte production, antigenic profile or drug resistance, for instance [7,11,12,29,30]. We are involved with longitudinal field studies that

will yield appropriate data; such comparisons should more fully address the general utility of our discrete-event models and provide further insight into the relative areas of strength of discrete-event and classic approaches to malaria modeling.

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Figure 1.

The ratios of observed to expected prevalences of dual-phenotype infections in human populations in the independent-immunity (Part a) and cross-immunity (Part b) models, with parameters and initial conditions as in Figure 4 in [8], but with vector half-life values shared by both phenotypes, host immunity half-life for both phenotypes fixed at 100 days, and host infectivity interval for one phenotype fixed at 20 days while the other ranges freely. For each point on each surface the observed mixed-phenotype prevalence $P(AB)$ is divided by the expected prevalence $P(A)P(B)$, where $P(X)$ represents the prevalence of phenotype X . The best-fit planes describe these ratios as: (a) $1.006 - [0.002/(1-VS)] + 0.00002WN_1$ and (b) $0.147 - [0.003/(1-VS)] - 0.00001WN_1$.

Figure 2.

The ratios of observed to expected prevalences of dual-phenotype infections in the mosquito population in the independent-immunity model, interacting with the human population in Part (a) of Figure 1. The best-fit plane describes these ratios as $1.611 - [0.021/(1-VS)] + 0.00255WN_1$. Similar results obtained for the cross-immunity model (not shown), described by the corresponding best-fit plane as $1.642 - [0.020/(1-VS)] - 0.00286WN_1$.