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Parasite Prevalence: A Static Measure of Dynamic Infections

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Abstract

The intensity of malaria transmission is often measured by looking at the fraction of individuals infected at a given point in time. However, malaria infections in individuals are dynamic, leading to uncertainty about whether a cross-sectional survey that represents a single snapshot in time is a useful representation of a temporally complex process. In this analysis, we examine the impact of parasite density fluctuations on the measurement of parasite prevalence. Our results show that parasite prevalence may be underestimated by 20% or more, depending on the sensitivity of parasite detection.

INTRODUCTION

The epidemiology of malaria is characterized using several inter-related measures; the density of competent or infectious mosquito vectors, their human biting rate, the number of infectious bites received on average per person per unit time (the entomologic inoculation rate [EIR]), and the prevalence of bloodstage infection in human hosts. Because entomologic parameters are often much more difficult to accurately measure than human infection, the parasite prevalence (PR), or fraction of hosts with detectable blood stage infections, is often used as a measure of the intensity of malaria transmission. The number of infected individuals is clearly related to the more direct entomologic measurements of malaria endemicity, but the exact relationship cannot be arrived at mechanistically. Therefore, correlations between PR and malaria transmission intensity are empirically derived. Generally, ranges of PR are used to assign qualitative levels of transmission intensity, although recently more sophisticated techniques have been applied to describe the relationship between EIR and PR, particularly for the purposes of comparisons between geographic locations.^{1–4}

During the course of a single infection, parasite density fluctuates daily and even hourly^{5–8} in response to fever, immune mechanisms, sequestration, intra-host competition, antigen-switching, and other events. Densities can fluctuate between undetectable levels and parasitemias of thousands per microliter in a matter of hours and may do so irregularly or with surprisingly regular periodicity if left untreated. Figure 1 gives an example of these fluctuations, as observed in one of the tens of thousands of patients intentionally infected with malaria as a therapy for neurosyphilis in the 1920s–1950s.⁹

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Parasite detection typically depends on examination of a small amount of dried, stained blood by microscopy. Detection of parasites below a certain density is erratic and uncertain^{10–12}; parasites in infected individuals are not always detected, particularly when present at low density. Of particular interest to us was the observation that parasite densities often drop below detectable levels in patients known to be infected and whose parasitemia was measurable on the preceding and subsequent days.

Given that infection is temporally dynamic and detection is imperfect, what does a single, cross-sectional measurement of parasite prevalence mean? Does such a snapshot significantly underestimate the burden of infection due to transiently sub-patent infections? In this simple analysis, we describe the effect of the temporal dynamics of *Plasmodium falciparum* infection on a single cross-sectional measurement of parasite prevalence.

MATERIALS AND METHODS

Daily parasite density measurements were obtained from clinic records of patients with no known previous exposure to malaria who were treated for neurosyphilis in the US Public Health Service (USPHS) facility in Columbia, SC, by infection with either trophozoites (39) or sporozoites (12) of the McLendon strain of *P. falciparum*. The malariatherapy treatment and data collection procedures, including those for the determination of parasitemia during each infection, are described in detail elsewhere.⁹ This reference includes extensive information about the participation and treatment of the patient population considered here and is accompanied by an explicit, independent analysis of relevant ethical issues.

Only the primary infections were considered in the analysis. Fifty-one patients received no drug treatment during the course of the primary infection, and only these were included in the analysis. The Earle and Perez¹³ method for parasite quantification was used, and the threshold of detection for asexual forms was $\sim 10/\mu\text{L}$. The day of first detection of asexual blood forms was designated as Day 1. Patent infections lasted between 10 and 227 days (mean, 88 days). Charts with more than three negative slides after the last positive slide were truncated to include only the last three negative reads; a total of 65 entries in 11 charts were excluded by this criterion. Twelve patients were infected with a second *Plasmodium* species subsequent to the primary *P. falciparum* infection, but the time elapsed between the last positive slide and the new infection was sufficient to ensure that there was no overlap between the two species. Only the *P. falciparum* infection was used in the analysis.

This group of 51 patient histories from individuals known to be infected made up the “test population” with a known prevalence of 1. One observation (i.e., 1 day) during the course of the infection was randomly chosen from each chart, and the observed prevalence was calculated. This procedure was repeated 400 times to simulate daily prevalence measurements in a group of people taken at random times during infection. Repeated simulations generated virtually identical results.

RESULTS

Using 51 charts, which represent 51 individual patients known to be infected with *P. falciparum*, we calculated the parasite prevalence that would have been observed in this population based on the parasite densities recorded in their charts for a randomly selected day. We repeated this sampling 400 times for random days. Although the true prevalence was known to be 1, the mean observed prevalence was 0.8, with a range of 0.64–0.92. Figure 2 gives the frequency distribution of the observed prevalence.

True and observed prevalence were compared for several values of true PR by replacing some charts with uninfected charts (setting all density measurements to zero). Random numbers between 1 and 51 were generated, and charts corresponding to those numbers were reassigned as uninfected. Five, 10, 15, or 25 charts were reassigned to give a true PR of 90%, 80%, 70%, and 50%, respectively, and observed PR was calculated as before. A plot of observed versus true PR shows a linear relationship between them (Figure 3); observed PR was ~79% of the true PR. That is, on any particular day, an average of 21% of true infections was subpatent.

We determined the effect of sensitivity of detection of parasitemia on the observed prevalence in our test population. The lower limit of detection previously reported for these data was ~10 parasites/ μ L. Indeed, there were only two densities < 10/ μ L reported in all 51 charts. We set every reported density of 10/ μ L or less to zero, to simulate a limit of detection of 11/ μ L, and calculated the observed PR for 400 random days. We extended this procedure to zero out all data points $\leq$ 50/ μ L and $\leq$ 100 / μ L (Figure 4). The observed PR decreased from 0.8 to 0.57, relative to a true PR of 1, as the limit of detection increases to 101/ μ L.

DISCUSSION

The endemicity of malaria is often characterized by the prevalence of infection in a cross-section of the exposed population. However, point-prevalence surveys cannot capture or account for the dramatic fluctuations in parasite density in infected individuals, which can periodically fall below detectable levels. How important is this missing information when characterizing malaria prevalence and endemicity in a population?

Here, we examined the consequences of taking a static snapshot of dynamic processes, by constructing test populations of infected individuals from daily charts of observations in individuals undergoing malaria therapy for neurosyphilis, and measuring prevalence on random days. The prevalence in the test group was known to be 1, but fluctuations in detectable parasitemia led to a > 20% underestimation of parasite prevalence. When the test population was altered to include uninfected individuals, the underestimation of true PR scaled linearly with true PR. As expected, declining sensitivity of detection leads to greater underestimation of PR.

The natural history of infection in an individual depends on previous exposure. Therefore, the types and distribution of dynamic profiles of infection in a population will be related to the local transmission intensity. Certainly, our results depend very strongly on the amount of time that parasite densities remain at or near the limit of detection during an infection. Individuals with little or no prior exposure to malaria, either very young children or people living in low-transmission areas, are more likely to experience high parasitemias with large fluctuations, similar to the individuals in our test population who were experiencing their first malaria infection. Thus, this test population is an appropriate comparison for studies in which PR in children younger than 10 years of age is used to characterize transmission. Persistent, low-density infections are more likely to occur in semi-immune adults and older children. Our results suggest that PR measured in semi-immunes may be underestimated to an even greater extent if they do indeed maintain very low-density infections. On the other hand, in areas where re-infection is occurring faster than the fluctuations of parasitemia in a single infection (new infections emerging every 5–10 days), observed prevalence may be closer to one than in our theoretical population.

It should be noted that the limit of detection for microscopy reported for these data of 10/ μ L, if accurate, represents very good microscopy. In general, field microscopy in epidemiologic surveys will have a much lower sensitivity.¹⁴ Prevalence detected by polymerase chain reaction (PCR) may capture more of the infections that have temporarily dropped to low levels

that are undetectable by microscopy. Alternatively, the slope of the decline in parasite densities with age in a community may capture more of the dynamic nature of infection and allow better comparisons between transmission areas. New techniques for describing transmission that exploit the dynamics of development of immunity in response to exposure are being developed and offer a very promising alternative to parasitologic and entomologic measures.¹⁵

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Figure 1.

A typical profile of parasite density during the natural course of *P. falciparum* infection. Daily parasite density measurements are plotted over the course of an infection in a previously malaria-naïve patient deliberately infected with *P. falciparum* for treatment of tertiary syphilis. Arrows indicate periods during which the parasite density temporarily dropped below the limit of detection.

Figure 2. Frequency distribution of the observed parasite prevalence (PR) measured in the test population of 51 individuals known to be infected with *P. falciparum*.

Figure 3.
True prevalence plotted vs observed prevalence in the test population.

Figure 4. Box plot of observed prevalence measured on 400 random days when the limit of detection is varied from 10 to 100/ μ L.