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Research Focus

Transmission and immunity: the importance of heterogeneity in the fight against malaria

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The complex relationship between transmission and parasite prevalence in humans is an important issue. Using a large dataset matching estimates of malaria transmission and *Plasmodium falciparum* prevalence in African children, a stimulating study published in *Nature* provides evidence that heterogeneity in susceptibility crucially determines the prevalence of infection. Moreover, it suggests that children who clear infections are not immune to new infections, irrespective of the amount of transmission. It is important to question the relevance of such results based on mathematical models when discussing host–parasite interactions, especially their implications for public health interventions.

Antimalarial immunity in question

Malaria transmission and parasite prevalence are usually measured using the entomological inoculation rate (EIR; the number of bites of infected mosquitoes received per person per year) and the parasite ratio (PR; the proportion of *Plasmodium falciparum*-positive individuals in a random sample of the population). Taking a dataset consisting of 91 studies matching EIR and PR in African children under 15 years, and after controlling for uneven quality of microscope screening and age-bias of individual datasets, D. Smith, J. Dushoff, R. Snow and S. Hay [1] derived several mathematical models, each fitting the observed distribution to varying extents. They focussed on the best fit model, written as a simple formula, featuring variability among individuals in terms of transmission intensity or susceptibility to infection (Box 1). From this process of testing alternative hypotheses, the authors conclude that immunity, defined as a temporary state of resistance to new infections, does not have an important role in determining PR during childhood. A second conclusion supports the fears of scientists that substantial reductions in EIR will give modest reductions in PR. Finally, 20% of individuals gather 80% of new infections, the numbers of Pareto's principle. As a consequence, this pattern might be the guideline for antimalaria campaigns specifically targeting individuals at risk. These conclusions generally confirm accepted views in malariology, except for the provocative statement on the development of antiparasite immunity in childhood. Indeed, the classical understanding is that immunisation against peripheral parasitaemia takes place after repeated infections [2,3]. There is considerable evidence to suggest that acquired immunity increases during childhood. For instance, age has been identified as a crucial factor in the rate of re-infection (the median times before reappearance of parasites in the peripheral blood following successful drug treatment were 22 days in 1–6-year-old children compared with 39 days in 7–14-year-old children [4]).

The article by Smith *et al.* [1] presents three points for further debate: (i) mathematical models for the relationship between EIR and PR; (ii) methods usually employed to measure malaria transmission; and (iii) the definition of immunity in the field of malaria.

Susceptibility and variability: all roads do not lead to Rome

Smith and colleagues [1] have taken great care in data acquisition and selection. Careful scrutiny of the values and controlling for the presence of outliers leads to the conclusion that there is indeed some type of heterogeneity in this dataset. Certainly, the conclusions drawn are correct from a mathematical point of view and the absence of immunity in African children is consistent with the observed heterogeneity in the framework of the best fit model.

Notwithstanding, conclusions drawn from mathematical models remain surmises. Obviously, the fact that a model fits the data is not evidence that the model reflects real life. Usually hypotheses are difficult to test in practice (otherwise the model would not be necessary) and, regardless of mathematical correctness, several hypotheses can usually account for any pattern emerging from data.

Smith *et al.* clearly ruled out the existence of immunity in children when it is defined as a temporary state of resistance to new infections. Most importantly, the study does not fully exclude the acquisition of immunity when considered as a long-term phenomenon whereby regular confrontation with the parasite leads to increased resistance to new infections (Box 1). For Smith *et al.*, intrinsic heterogeneity between individuals could be the key element to account for the impact of EIR on PR. From

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Box 1. Decreased susceptibility or individual variation?

The conclusions drawn by Smith *et al.* [1] rely on rigorous mathematical analysis of their dataset. They built a series of models by progressively adding in parameters, fitted them individually and finally chose the model corresponding to the best fit with the smallest number of parameters. This was how they included individual variability, summarized by their parameter *k*, the estimated value of which in the best overall model predicts that 20% of the individuals gather 80% of new infections. Crucially, they also added to all the models two parameters representing the human error in the detection of parasites. This can help cope with poor diagnostic quality but it is a double-edged sword because poor models can be explained by 'blaming' the experimenter (i.e. the intrinsic lack of fit of the model is interpreted as inaccurate blood smear readings).

Their methodology essentially raises two comments. First, Smith *et al.* interpret their parameters in terms of the logic that led to their inclusion in the model. Thus, the parameter *k*, representing individual variability, is eventually used to draw the 20:80 figure. Yet a different logic could lead to the incorporation of such a parameter. For instance, one could assume that susceptibility (defined by Smith *et al.* as a measure of the real infection rate of a person) decreases as EIR increases and, for the sake of boundary conditions, define the functional relationship as $s = ln\,(1+k\varepsilon)/k\varepsilon$ (where s is susceptibility, ε is EIR and k is a scale parameter). This gives exactly the same model as that of Smith *et al.*, but now k is interpreted in terms of the impact of transmission on mean susceptibility.

The second comment is that Smith *et al.* did not consider the possibility that EIR might impact on susceptibility to new infections. Under this assumption, there is no immunization, rather a long-term decrease in susceptibility to new infections in endemic regions. A way of putting this in the equation is to assume that susceptibility decreases as a power function of EIR. Fitting this new model with the method used by Smith *et al.* gives: $\exp\{-b\varepsilon^{0.33}/r\}$, where (following Smith *et al.*) b is the transmission efficiency, $1/r$ is the expected time to clear each infection and ε is EIR. The prediction of this model is plotted along with that of the best model of Smith *et al.* in Figure I. According to Akaike's criterion, this model is not as good ($\Delta AIC = 54$) but the two error parameters seem somewhat more plausible. Indeed, Smith *et al.* claim that 12% of the parasite-free individuals are deemed infected and only 4% of the infected individuals are deemed clear, whereas our proposed model gives 0% and 20% for these errors estimates, respectively. Without claiming that this model is better than that of Smith *et al.*, we propose that the decreased susceptibility hypothesis (considered as another interpretation of the model of Smith *et al.* or as a new model) is a serious alternative interpretation of the data that should have been taken into consideration.

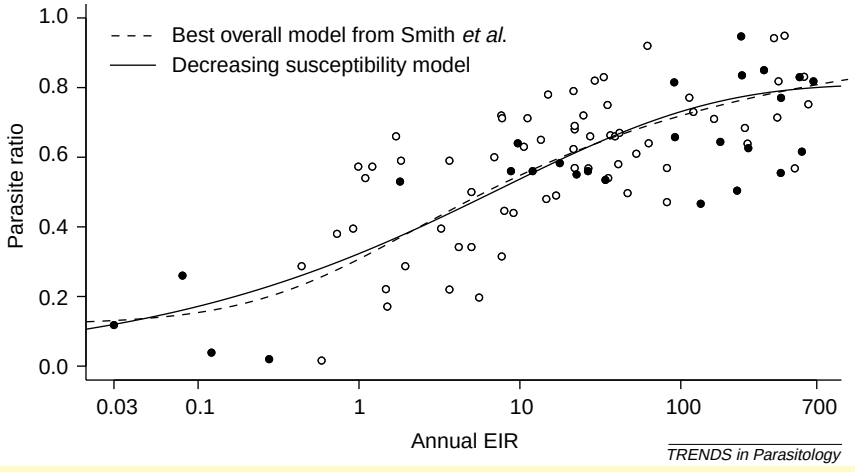

Figure I. The relationship between parasite ratio (PR) and entomological inoculation rate (EIR). The data from Smith *et al.* [1] have been plotted as black circles when the PR was measured on >500 children and as open circles when measured on <500 children. The broken line is the best overall model of Smith *et al.* and the unbroken line is our decreased susceptibility model.

our point of view, the pattern could similarly stem from differences between individuals or alternatively from the progressive immunisation that occurs with increasing EIR. Probably both processes coexist and participate to different extents in malaria epidemiology. If high levels of transmission promote resistance to new infections, however, it is irrelevant to calculate a variability index of the type 20:80 because this is meaningful only if the infection rate is homogeneous.

To investigate further the relationship between transmission and parasite ratio is a daunting task. One might question the relevance of PR, which is by nature a cross-sectional measure and usually less informative than longitudinal observations repeated on the same individuals, especially when the aim is to highlight continuous processes that can develop slowly over a relatively long period of time (e.g. acquisition of immunity during childhood) [5]. Moreover, using PR omits two essential variables – parasite density and the genetic structure of parasites – that are central in host–parasite relationships [6,7]. Concerning transmission, more detailed measures of infectious bites are required on individual, local and regional scales. As far as we know, such distributions are unknown.

EIR does not mean malaria transmission

The intensity of human malaria parasite transmission varies from zero (for those who remain in malaria-free zones and would never be infected during their lifetime) to ~100 000 (for those who would receive the maximum of 1000 infected bites per year during 100 years [8].

The classical method to measure EIRs is to count the number of aggressive mosquitoes using landing catches on the bare legs of adult people. Within this

anthropophilic sample of anophelines, infectivity can be estimated using various methods (microscopy for examining sporozoites in salivary glands, ELISA for detecting circum-sporozoite protein in the head and thorax, and PCR for detecting parasite DNA sequences in the head and thorax). EIR is, by definition, calculated as the product of human biting rate and anopheline infectivity. This index is considered the best proxy for malaria transmission, yet it suffers from many weaknesses (Box 2). Despite all its misgivings, EIR has proven to be a reliable and robust measure of transmission intensity, and is a central parameter in epidemiology. There is no doubt that EIR will continue to be used to measure transmission because of its workable advantages in epidemiological studies.

Concluding remarks on heterogeneity and immunity

Heterogeneity lies at the core of current research to understand the dynamics of transmission. Smith *et al.* [1] suggest that 20% of people receive 80% of all infections. This pattern might hold globally within their dataset because of heterogeneity between sites and between children but, as they stressed, might fail to occur on a local scale in human populations. In addition, this figure might be the average of a spectrum of different situations and, therefore, might itself be variable from one site to another.

Heterogeneity is not only pivotal in understanding transmission patterns but also impacts on vaccine strategy. Smith *et al.* propose to focus on the 20% gathering most of the infections, which would require that these sensitive individuals exist and can be readily detected. Heterogeneity in transmission will also result in a variation in the challenge to the immune system. Natural infections following vaccination can either reinforce the immunity or overwhelm it. Therefore, vaccination strategies should be tailored according to variation in natural transmission intensity.

Much of the interpretation and discussion hinges on what is meant by immunity. Smith *et al.* defined immunity as a temporary state of resistance to new infections. This definition, although perfectly admissible, is restrictive and draws a narrow view of the complex relationship between malaria transmission and the immune response. Traditionally, malaria is defined as a disease for which there is a slow acquisition of non-sterilising immunity against blood parasites [2]. Even once attained, persistent and/or intermittent infection is required to maintain such a state of protection. In other contexts, immunity is considered with respect to antibodies, parasite density, malaria attacks, malaria mortality, and so on. All are acceptable but have to be carefully defined.

The heterogeneity in malaria transmission still leaves the scientific community disarmed. In this context of temporal and spatial variation, the epidemiology of malaria cannot be homogeneous. Smith and colleagues bravely attempt to tackle this complexity, but their conclusion might be the tip of the iceberg. Their great merit was to have reminded the scientific community that the question of how immunity to malaria is acquired remains unsolved.

Box 2. EIR: misuses and abuses

- EIR is averaged over one year, which unfortunately erases any seasonal variation for the mid-range values of EIR, and such seasonal variation is of paramount importance for the development of immunity. Biological responses are radically different for individuals receiving 12 infected bites in one month (for instance, in the Sahelian part of Africa during the short rainy season) or one infected bite each month during the full year (for instance, in evergreen forests). In both cases, however, the annual EIR is 12. The duration of the non-transmission season is crucial.
- EIR counts the bites of anophelines with sporozoites in their salivary glands, so-called 'infected bites'. But when considering infections in humans, the right variable would be the 'infectious bites' (i.e. those among the infected ones that generate an infection in humans). Information is scarce but one infected bite is thought to be infectious half of the time in immunologically naïve people [9]. This ratio seems to decrease with the level of endemicity. Calculations of *b* (parameter describing this from the Ross Macdonal model) in, for example, Bekessy *et al.* [10] also suggest that *b* changes with age.
- Up to four human *Plasmodium* spp. might infect mosquitoes and these species are often entangled in the calculation of EIR, inducing loss of precision in terms of the relative contribution of each plasmodial species.
- EIR is measured in the absence of personal protection against mosquito bites and anti-vectorial measures, situations that nowadays seem particularly irrelevant in natural settings.
- EIR is a mean, calculated from a sample of capturers and mosquitoes. However, major differences in biting rate are documented (individual behaviour, individual attractiveness, habitat type, proximity with breeding larval places, and so on). For example, the attractiveness of pregnant women and gametocyte carriers to mosquitoes is increased [11,12].
- The number of bites received might, to some extent, be age dependent. It is commonly admitted that adults are bitten twice as frequently as children, and children twice as frequently as infants [13,14].
- EIR is measured for non-sleeping individuals at night and any differences in mosquito attractiveness of awake versus sleeping people are totally ignored.

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Letters

Drug resistance and drug tolerance in parasites

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In their recent *Trends in Parasitology* article on tolerance and antimalarial drug resistance, Hastings and Watkins [1] made the extremely important point that resistance is not an all-or-nothing effect, and that several mutations can occur before the malarial parasite fails to respond to the full therapeutic treatment in the patient. Clearly, this must be taken into account in any modelling on the development of resistance. The authors use the word 'tolerance' to describe the intermediate state between susceptibility and complete drug failure. We made the same mistake in work carried out in Kenya in the 1980s, which showed the 'resistance' of *Schistosoma mansoni* to oxamniquine in an article entitled 'Tolerance of Kenyan *Schistosoma mansoni* to oxamniquine' [2]. It should have been entitled 'resistance'.

To try and correct this misunderstanding, we defined resistance in *Schistosoma* as the following [3]: 'A population of *Schistosoma* is resistant when either a susceptible population shows a significant decrease in response to a schistosomicide or is significantly less responsive than a fully susceptible population…It is complete when the maximum dose of drug tolerated by the host has no effect on the parasite population.' As used by Fallon *et al.* [4] and discussed in our previous letter [3], 'tolerance is an innate insusceptibility of a parasite to a drug, even before the parasite has been exposed to the drug.' We cited the example of oxamniquine, which does not work against *Schistosoma haematobium*. Tolerance can be total or it can be stage-specific; for example, ivermectin does not kill inhibited cyathostomins in horses but is extremely effective against the lumenal stages [5]. There is a similar confusion by farmers when they think anthelmintics are working adequately but low levels of resistance are detected on the farm. This low-level resistance will not necessarily show up as lost productivity but serves as a warning that future problems will develop if changes are not made in the use of that anthelmintic. This slightly lowered efficacy is not tolerance, but resistance.

I believe it is important to use the same terminology when discussing drug resistance, whether it is in malaria, nematodes or any other parasites. I would, therefore, like to suggest that the definition we proposed in 1997 [3] is adopted by all parasitologists, and that 'tolerance' is not used to describe intermediate levels of resistance but is confined to the innate inactivity of a drug towards a stage or a particular species of parasite, in which activity might be expected as a result of drug efficacy against a closely related species.

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