CellPress

Assessing the infectious reservoir of falciparum malaria: past and future

Will Stone[1], Bronner P. Gonçalves[2], Teun Bousema[1,2], and Chris Drakeley[2]

[1] Radboud University Medical Center, Nijmegen, The Netherlands
[2] London School of Hygiene and Tropical Medicine, London, UK

Renewed interest in malaria eradication has placed greater emphasis on the development of tools to interrupt *Plasmodium* transmission, such as transmission-blocking vaccines. However, effective deployment of such tools is likely to depend on improving our understanding of which individuals transmit infections to mosquitoes. To date, only a handful of studies have directly determined the infectiousness of individuals in endemic populations. Here we review these studies and their relative merits. We also highlight factors influencing transmission potential that are not normally considered: the duration of human infectiousness, frequency of sampling by mosquitoes, and variation in vector competence among different mosquito populations. We argue that more comprehensive xenodiagnostic assessments of infectivity are necessary to accurately quantify the infectious reservoir and better target interventions.

Reducing transmission of malaria: the future of elimination programs

The burden of malaria has declined in many endemic settings in Africa and elsewhere [1]. Local malaria elimination is considered achievable with current control approaches in some of these areas when transmission intensity is low and reintroduction unlikely [2,3]. However, in most endemic areas operational and technical limitations are likely to hinder the complete interruption of transmission [4,5]. New or renewed tools aimed specifically at interrupting the spread of *Plasmodium* species from human to mosquito may therefore be critical for future elimination programs [6,7], especially if transmission efficiency increases as parasite prevalence goes down [8]. Unlike traditional control strategies, which aim to reduce severe morbidity in vulnerable populations, the effectiveness of transmission-reducing interventions (TRIs) hinges on their coverage of individuals responsible for transmission to mosquitoes regardless of their symptomatic status [9]. Despite the central importance of human infectivity for TRI [10], there have been few direct assessments of this. Here we discuss previous studies that aimed to directly assess infectivity at the population level, examine factors necessary to link these controlled transmission experiments with transmission in nature, and advocate the next steps that will provide the key information necessary to better target the human infectious reservoir for malaria.

Quantifying the human infectious reservoir of malaria (1957–2014)

In infected humans, the life cycle of a small portion of the total malaria parasite population culminates the differentiation and maturation into gametocytes (see Glossary). When an anopheline mosquito feeds on blood containing mature gametocytes some may be ingested, which then fuse and undergo sporogonic development making the insect infectious to humans. The human component of the infectious reservoir is the proportion of a population that is capable of infecting mosquitoes [11].

Glossary

Endophilic: arthropod behavior describing a preference for resting indoors after blood feeding.
Exophagic: arthropod behavior describing preference for blood feeding outdoors.
Exophilic: arthropod behavior describing preference for resting outdoors after blood feeding outdoors.
Gametocyte: the sexual stages of the malaria parasite capable of reproduction in the mosquito. Female and male gametocytes circulate in the human peripheral blood, where they may be ingested by blood-feeding *Anopheles* mosquitoes and begin sexual development.
Mosquito feeding assay: xenodiagnostic assays developed to determine the infectiousness of *Plasmodium* gametocytes to *Anopheles* mosquitoes. Mosquito feeding assay may refer to skin feeding assays, in which mosquitoes are allowed to feed directly on a subject's skin, to direct membrane feeding assays, in which mosquitoes feed on venous blood from a subject maintained at body temperature in a membrane feeding device, or to standard membrane feeding assays, in which mosquitoes feed on cultured gametocytes in a membrane-based feeder system.
Perennial transmission: malaria transmission may be described as perennial where environmental and climatic conditions allow parasite transmission throughout the year.
Seasonal transmission: malaria transmission may be described as seasonal where mosquito biting and thus transmission are clustered temporally, generally occurring following predictable periods of rain and subsequently decreasing in periods of drought.
Transmission-blocking immunity: the development of humoral immunity to antigens present during gametocyte development. Antibodies specific to gametocyte antigens may be ingested along with mature gametocytes during blood feeding. If these antibodies target proteins essential for parasite development (e.g., Pfs48/45, Pfs230) and are sufficiently abundant, antibody interaction can prevent parasite development and transmission potential is reduced or entirely blocked. There is a growing body of evidence for the development of transmission-blocking immunity in individuals naturally exposed to malaria infection.
Xenodiagnosis: in this context, is a method to determine the infectiousness of a potentially parasitized host (i.e., malaria-infected humans) by allowing vectors (i.e., mosquitoes) to feed on the subject. Infectiousness is assessed by the infection status of the vector (i.e., mosquito) after a suitable period of parasite development.

Corresponding author: Drakeley, C. (chris.drakeley@lshtm.ac.uk).
Keywords: *Plasmodium falciparum*; gametocytes; infectious reservoir; mosquito; transmission blocking.

1471-4922/
 http://dx.doi.org/10.1016/j.pt.2015.04.004

Box 1. Demography and the malaria infectious reservoir

In previous surveys, the 'bottom-heavy' age structure typical of malaria endemic regions has acted to boost the contribution of more infectious children to the total malaria infectious reservoir, while less infectious adults, representing approximately half of most populations, generally contributed to a lesser extent. Recent demographic estimations show that Sub-Saharan African populations are still disproportionately young: approximately 17% and 27% of the population are aged 0–4 and 5–14 years, respectively (http://esa.un.org/wpp/). The influence of demography on patterns of transmission needs to be considered in light of the numerous other factors affecting the likelihood of infectious individuals contributing to mosquito infections. Although infants and children are overrepresented in endemic areas they may be the age group least available and, perhaps, least attractive to mosquitoes (see 'Determinants of human transmission potential: the need for data'). Conversely, older children and adolescents may represent a privileged group for human–mosquito transmission; individuals in this age group possess moderate gametocyte densities (approximately double the infectivity of individuals >15 years), lack the severe symptoms that prompt treatment, and appear comparatively vulnerable to mosquito biting (see Figure 1 in main text).

Women in pregnancy have been observed to attract more than twice as many anophelines as non-pregnant women over short distances [21]. However, such observations must be weighed against behavioral and demographic parameters unique to this group. Census and fertility rate data show that pregnant women comprise a small proportion of African populations. In Zambia, 8.7% of women of child-bearing age were pregnant at any one time in 2013, which equates to 2–2.5% of the entire population (http://www.dhsprogram.com/pubs/pdf/FR304/FR304.pdf). Pregnant women are also preferentially targeted by net and drug treatment campaigns, so the high attractiveness to mosquitoes of this small proportion of the population may be balanced by interventions.

In community surveys, data from microscopy typically show that children and infants harbor *Plasmodium* gametocytes more commonly and in greater numbers than older age groups [12]. Such observations have long galvanized belief among the scientific community that young individuals represent the main source of infection for mosquitoes [12]. However, in the late 1940s and early 1950s xenodiagnostic assessments revealed that the presence of gametocytes in blood films was not a prerequisite for onward transmission [12–15]. The first population-based assessment of human infectivity to mosquitoes was conducted in rural Liberia, where individuals living in an endemic region were recruited for mosquito feeding experiments regardless of their parasite status [16]. While this study found that young children were much more infectious to mosquitoes, it also showed that, when estimates were adjusted for the demographic composition of the population, all age groups were relevant contributors to malaria transmission (Box 1). In nearly 60 years since this study, a handful of similar surveys have been reported (Tables 1 and 2). In general, these studies show that while young children are consistently more likely to be infectious *per se* [17,18], the contribution of older age groups is by no means negligible; in previous xenodiagnostic surveys that were able to sample individuals of all ages, individuals >15 years old constituted between 21.9% and 40.7% of the total infectious reservoir [16–19]. Several studies have assessed human–mosquito transmission using entomological parameters [20]. However, these studies provide only broad population estimates of infectiousness and they are neither able to describe the distribution of the reservoir among different members of the population nor allow an examination of the role of parasite density and infection dynamics.

Ultimately, determining the infectiousness of different age groups relative to their representation in the population is just one necessary step in characterizing the infectious reservoir. Contribution to transmission is influenced not only by intrinsic parasite and human determinants of infectivity, reflected in the outcome of mosquito feeding assays, but also by various additional factors that make the capacity to infect mosquitoes in controlled experiments distinct from the probability of onward transmission in nature (e.g., human exposure to mosquito biting) (Figure 1). These factors are not commonly taken into account and are not uniform across time or space or between individuals of different ages. To more accurately reflect transmission in its epidemiological context, these elements need to be considered and incorporated into future assessments.

Determinants of human transmission potential: the need for data

Duration and dynamics of infectivity

The likelihood of transmission to *Anopheles* mosquitoes is determined primarily by gametocyte density, fitness, and circulation time. The nonlinear relationship between gametocyte density and mosquito infection risk has been described several times [21–23]. Yet, because of the relatively high proportion of low-density parasitemias and gametocytemias in natural infections, there is increasing focus on how important these often subpatent infections are for the maintenance of transmission [24,25]. While the limited sensitivity of microscopy to detect gametocytes is well established [21], many claims of submicroscopic gametocyte densities contributing to transmission are based on microscopy-positive asexual parasite carriers with accompanying submicroscopic gametocyte densities. This is an important difference when considering how current diagnostics may capture the human infectious reservoir [26,27]; infections with submicroscopic gametocytes that accompany patent asexual parasites are detectable by conventional diagnostics while infections with no microscopically detectable parasite stages require more sensitive diagnostics [28].

Because of their cross-sectional nature, previous xenodiagnostic surveys have not formally examined the duration of infectiousness, another key factor influencing transmission potential. Data from malaria therapy studies suggest that the infectiousness of an individual infection can last for many months [29]. However, it is difficult to extrapolate the observations from a limited age range of malaria-naïve individuals to endemic populations. Broadly, in endemic settings children (possessing limited immunity) are likely to have more acute, higher-density infections that often require treatment [30]. This may result in cross-sectional surveys missing recently treated infections and identifying only a limited number of highly infectious children without recent treatment. Conversely, adults and older children (often semi-immune, and presenting few clinical symptoms) would have longer, chronic