The role of submicroscopic parasitemia in malaria transmission: what is the evidence?

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Achieving malaria elimination requires targeting the human reservoir of infection, including those with asymptomatic infection. Smear-positive asymptomatic infections detectable by microscopy are an important reservoir because they often persist for months and harbor gametocytes, the parasite stage infectious to mosquitoes. However, many asymptomatic infections are submicroscopic and can only be detected by molecular methods. Although there is some evidence that individuals with submicroscopic malaria can infect mosquitoes, transmission is much less likely to occur at submicroscopic gametocyte levels. As malaria elimination programs pursue mass screening and treatment of asymptomatic individuals, further research should strive to define the degree to which submicroscopic malaria contributes to the infectious reservoir and, in turn, what diagnostic detection threshold is needed to effectively interrupt transmission.

Active surveillance targeting asymptomatic malaria for elimination

Over the past 5–10 years, increased international funding, political will, and a new generation of malaria diagnostic and treatment tools has reignited efforts to achieve malaria elimination. As of 2012, 34 of the 99 countries where malaria is endemic have adopted strategies to become malaria-free within the next two decades [1]. At the heart of these strategies is a shift in focus from early diagnosis and treatment of those who are symptomatic to active surveillance for and treatment of every case, including those who are asymptomatic [2,3].

Asymptomatic malaria is often not defined rigorously, but generally refers to an individual harboring blood-stage malaria parasites who has not experienced fever or other symptoms that would lead the individual to seek treatment. In most malaria endemic settings, asymptomatic infections outnumber symptomatic infections [4–6]. It follows that the success of elimination strategies relies on the ability to find and treat the asymptomatic reservoir.

However, the lack of rigorous definitions for asymptomatic malaria has clouded research supporting this goal [4,7]. Parasitemia in an asymptomatic infection can be visible by blood smear or submicroscopic and only detectable by molecular methods. The increasing use of methods that detect parasite DNA, including PCR and loop-mediated isothermal amplification (LAMP), has uncovered large numbers of asymptomatic individuals with submicroscopic malaria. It is generally assumed that this translates into a greatly expanded infectious reservoir. But most studies on the reservoir potential of asymptomatics have been done on microscopically patent asymptomatics. Accordingly, the role of submicroscopic parasitemia in ongoing transmission in most settings is unclear.

Here, we examine different lines of research to address the question of whether those with submicroscopic malaria substantially contribute to transmission. We highlight the heterogeneity of the asymptomatic malaria reservoir, what mosquito studies have shown about determinants of human-to-mosquito infectivity, the limited studies on the infectivity potential of submicroscopic malaria, and the lack of data available from ongoing elimination efforts.

A better understanding of the role of submicroscopic malaria as part of the transmission reservoir can shape how active surveillance is used to pursue malaria elimination. For example, recent mass screening and treatment efforts have used rapid diagnostic tests (RDTs) with detection limits similar to microscopy ($\sim$200 parasites/μl) to find and treat asymptomatic cases [8]. When these efforts failed to curb subsequent malaria transmission, it was suggested that more sensitive molecular diagnostics need to be deployed [9,10]. Along these lines, a recent consensus document by the Malaria Eradication Research Agenda consultative group suggests that any parasitemia, no matter how small, represents potential for transmission and therefore is a threat to malaria elimination efforts [11]. We propose that pursuit of the most sensitive diagnostic may be premature. Knowledge gaps are highlighted and recommendations are made for further research to better understand

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Box 1. Heterogeneity of the asymptomatic malaria reservoir

The asymptomatic reservoir is composed of those with submicroscopic and microscopic parasitemia. In both high (Figure IA) and low (Figure IB) transmission settings, asymptomatic infections far outnumber symptomatic infections [4–6]. However, in low transmission settings, most of the asymptomatic reservoir is composed of submicroscopic parasitemia [4,5,21–23]. The relative contribution of submicroscopic and microscopic parasitemia to transmission is unknown.

The contribution of the asymptomatic reservoir to malaria transmission is mediated by the duration of infection, incidence of gametocyte carriage, and is ultimately determined by mosquito infectivity. These and other factors are likely to differ in low versus high transmission settings and in microscopic versus submicroscopic parasitemia within these settings (Table I).
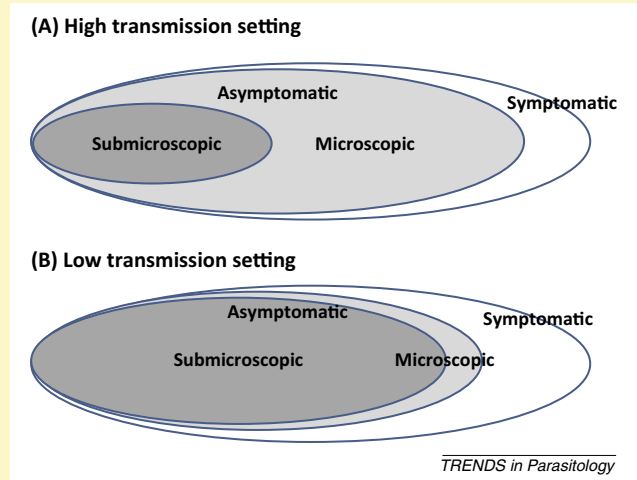

(A) High transmission setting

(B) Low transmission setting

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Figure I. The asymptomatic malaria reservoir.

Table I. The contribution of microscopic versus submicroscopic infections to the asymptomatic malaria reservoir

	High transmission setting			Low transmission setting		
	Microscopic	Submicroscopic	Refs	Microscopic	Submicroscopic	Refs
Persistence of infection[a]	Days to months		[13,14,16]	Days to weeks?[b]		[26–28]
Gametocyte carriage[c]	15–50%	?	[19,20,37,54]	1–50%[b]	?	[29,52,65]
Mosquito infectivity[d]	10–75%	?	[53,54]	3–10%	?	[52,53]
% Mosquitoes infected[e]	8–13%	0.5–3%	[53,54,56,58]	<1% to 1%	<1%[b]	[52,53,55,56]

[a]If untreated.

[b]Studies cited included *P. vivax* in addition to *P. falciparum*.

[c]Gametocytes visible by microscopy.

[d]The proportion of individuals infective to mosquitoes.

[e]The proportion of mosquitoes infected within a study population.

how best to deploy active surveillance methods in the pursuit of malaria elimination.

The asymptomatic reservoir is heterogeneous in terms of persistence and gametocyte carriage

The importance of asymptomatic malaria as a reservoir rests on two key characteristics: duration of infection and gametocyte density. Gametocytes are the sexual stage of the parasite produced in infected human hosts which must be ingested by mosquitoes for transmission to occur. Infections persisting for long periods of time means more opportunities for a malarious individual to infect biting mosquitoes, especially as gametocytes tend to appear late in *Plasmodium falciparum* infection, on the order of 7–15 days after the first appearance of parasites in the blood [12]. Infections with higher gametocyte densities are generally more infectious. Persistence of infection and prevalent gametocyte carriage have long been observed among those with asymptomatic malaria in moderate to high transmission settings in Africa. However, the asymptomatic reservoir in low transmission settings is more heterogeneous and does not necessarily share these same attributes.

In moderate to high transmission settings in Africa, it is common for asymptomatic individuals to harbor microscopically patent (smear-positive) infections that last from weeks to months [13,14] (Box 1). This phenomenon has been explained by a form of acquired immunity that keeps parasitemia and symptoms in check without achieving complete clearance [15]. Longitudinal genotyping of a Ghanian cohort has confirmed that these are persistent infections of the same strains, rather than representing frequent reinfection, and that they persist an average of 194 days [16]. Although gametocytes were not measured in this study, it is likely that these individuals were gametocytemic and thus infectious during much of this time. In fact, asymptomatic microscopically patent infections may be more infectious than clinical malaria. An association between lack of fever and gametocyte carriage has been observed in smear-positive children in The Gambia and Nigeria [17,18]. Additional evidence of the gametocyte production potential of microscopically patent asymptomatics comes from two longitudinal studies in The Gambia and Kenya of untreated asymptomatic infections [19,20]. In these studies, 11–25% were gametocytemic at baseline, whereas approximately 15–20% of microscopically patent asymptomatics without baseline gametocytes became gametocytemic over 4 weeks. Thus, evidence from several studies show that asymptomatic individuals with patent parasitemia are important reservoirs, but none of the studies looked for submicroscopic infections.

Box 2. *P. vivax* hypnozoites represent a hidden reservoir and obstacle to elimination efforts

The unique biological characteristics of *P. vivax* present even more challenges for elimination [1,74]. Perhaps most important is its propensity to cause relapse. After a *P. vivax*-infected mosquito bites an individual, a proportion of parasites establish a latent liver-stage infection in the form of hypnozoites. Hypnozoites can remain dormant in the liver for weeks or years until unknown triggers cause their reactivation and entry into the bloodstream, where they cause clinical relapse [75]. Remarkably, a single mosquito bite can lead to multiple malaria relapses even when an individual has been adequately treated with drugs that clear blood-stage parasites.

Several other biological characteristics of *P. vivax* make it is plausible that in low transmission settings, many asymptomatic individuals infected with *P. vivax* are gametocytemic and contribute to transmission in a meaningful way. Perhaps due to frequent relapse, most studies show that immunity to *P. vivax* is typically acquired earlier in life than *P. falciparum* in settings where the species are co-endemic [76]. It follows that asymptomatic *P. vivax* infection is relatively common even in low transmission settings [4]. Additionally, in contrast to *P. falciparum* gametocytes, *P. vivax* gametocytes arise early in infection and accompany the majority of symptomatic infections [12,32]. As with *P. falciparum*, submicroscopic gametocyte densities have also been shown to lead to mosquito infection [42].

However, the converse conclusion – that mass screening and treatment efforts can interrupt *P. vivax* transmission – is problematic. This is because of the hidden *P. vivax* hypnozoite reservoir. Excluding the prepatent liver stage of infection, the *P. falciparum* asymptomatic reservoir is composed of those who are either smear-positive or submicroscopic, whereas the *P. vivax* asymptomatic reservoir contains an additional fraction of infected individuals without blood-stream parasites but with dormant liver-stage hypnozoites. These individuals harboring hypnozoites with potential for relapse cannot be identified by any current diagnostic tests. Thus, one-time or even periodic mass screen-and-treatment efforts will only detect infections that have coincidentally reactivated at the time of screening. The size of the hypnozoite reservoir is unknown in all but the most-studied settings, but is likely to be substantial [77]. Perhaps this is why the few examples of successful interruption of *P. vivax* transmission have been associated with mass drug administration (MDA) [78,79] rather than screen-and-treatment efforts.

Research recommendations

Ultrasensitive molecular tests might incrementally detect a larger proportion of submicroscopic and potentially gametocytemic *P. vivax* infections within a population, but their use is perhaps inconsequential for achieving elimination in the face of a much larger hidden hypnozoite reservoir that remains intact. Lines of research that evaluate frequent periodic screening, replace mass screening with MDA with the aid of point-of-care G6PD testing, or uncover triggers of relapse can inform active surveillance strategies for *P. vivax*. However, for most areas where *P. vivax* is endemic, development of a diagnostic test that can detect latent hypnozoite infections in conjunction with a safe single-dose anti-hypnozoite drug and are probably needed in order to make elimination a realistic goal [11,74,80].

The situation in non-African low transmission settings is more complicated. First, in contrast to many African settings, the majority of asymptomatic malaria infections in areas such as the Amazon and Southeast Asia are submicroscopic [4,5,21–23] (Box 1). Second, outside of Africa, *Plasmodium vivax* is the most prevalent malaria species and malarious areas are often co-endemic for *P. falciparum* and *P. vivax*, with *P. vivax* presenting unique challenges to elimination (Box 2). Finally, with lower levels of transmission, acquired immunity is expected to be lower. So at any given time point, an individual may be more likely to be asymptomatic because he/she is either in the process of resolving parasitemia due to treatment or might be in the process of developing a symptomatic infection [4,7,24,25]. Each of these major differences means that the asymptomatic reservoir may vary in different settings with regards to persistence, gametocyte carriage, and hence mosquito infectivity and transmission potential.

The duration of infection among asymptomatics in low transmission settings remains elusive (Box 1). The very limited evidence suggests that a minority can persist for several weeks, but the majority spontaneously clear. In Brazil, 16 submicroscopic PCR-positive individuals with asymptomatic *P. vivax* infection were followed for 30 days; three-quarters were PCR-negative at 2 weeks, whereas the remaining four remained PCR-positive at 30 days, with none developing symptoms [26]. In serial cross-sectional studies conducted in Colombia and Peru, approximately half of smear-positive asymptomatic infections spontaneously cleared within 14 days in Colombia (13 out of 24) and within 7 days in Peru (16 out of 30) [27–29]. As for the rest, the majority remained asymptomatic at follow-up, with half of those in the Peru study showing decreased parasitemia at day 7, and 25% of those in the Colombian study remaining parasitemic at day 14. Overall, the duration of untreated submicroscopic infections in low transmission areas seems to be much shorter than asymptomatic *P. falciparum* infections in Africa.

Even less is known about gametocyte carriage among asymptomatic infections in low transmission settings. One study in Peru limited to smear-positive malaria found that an impressive 50% of *P. falciparum* and 22% of *P. vivax* infections identified by active case detection harbored gametocytes [29]. However, this was in the context of an overall malaria prevalence of 63% in the at-risk zones that were targeted. Otherwise, studies of gametocyte carriage have mostly been limited to those enrolled in clinical trials. Studies in Thailand have found that the greatest risk factor for *P. falciparum* gametocyte carriage was visible gametocytemia at admission [30], present in just 2.4% of patients. Meanwhile, the average duration of gametocytemia post-treatment in Thailand is significantly shorter than in The Gambia [31]. For *P. vivax* patients in Thailand and Indonesia, gametocyte carriage mirrored asexual parasitemia, with high asexual parasitemia associated with increased risk of gametocytemia during follow-up [32]. These studies perhaps indirectly suggest that submicroscopic asymptomatic malaria does not have the same infectious potential as asymptomatic malaria in Africa.

Studies of mosquito infectivity indicate a gametocyte threshold detectable by microscopy

Gametocytes serve as an imperfect surrogate marker of transmission potential. Experimental studies whereby mosquitoes are fed blood-stage parasites, either by direct skin feeding or via a membrane-feeding apparatus, and subsequently dissected to look for malaria parasites (oocysts in the mosquito midgut), represent a more direct way for assessing human-to-mosquito infectious potential [33–36].
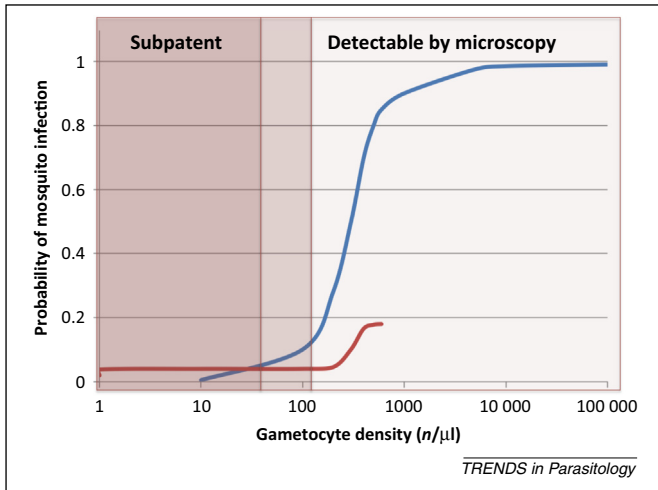
Figure 1. Submicroscopic gametocytemia and the mosquito infectivity threshold. Mosquito-feeding assays, whereby *Anopheles* mosquitoes are fed blood-stage parasites, either by direct skin feeding or via a membrane-feeding apparatus, and subsequently dissected to look for malaria parasites, represent the standard way of assessing human-to-mosquito infectious potential [33–36]. Depicted are approximate curves of human-to-mosquito infectivity as a function of density of gametocytes in the circulating blood drawn from data depicting natural human malaria infections in West Africa (blue) [39,40] and children post-treatment in Burkina Faso and Kenya (red) [41]. Although the range of the older series is much greater, both demonstrate that the probability of mosquito infection is very low until corresponding asexual parasitemias are detectable by microscopy.

Individuals with higher gametocytemia are generally more infectious to mosquitoes than those with low gametocytemia. Although children in Africa without smear-detectable gametocytes are capable of infecting mosquitoes, infections occur in only a small minority of mosquitoes [37,38]. By contrast, evidence from many studies suggests a threshold of ~100 gametocytes/µl, above which individuals more consistently achieved infection in the majority of mosquitoes, and cause mosquito infections with higher oocyst burdens [39,40] (Figure 1). More recent data based on modeling of mosquito membrane-feeding studies in Burkina Faso and Kenya similarly found that although 4% of mosquitoes are infected at one gametocyte/µl, this low infectivity rate does not increase meaningfully until ~200 gametocytes/µl are present [41] (Figure 1).

Comparable studies for *P. vivax* are few in number. Although they suggest a positive correlation between gametocyte density and frequency of mosquito infection, an appreciable gametocyte threshold above which infection is likely has not been defined [35,42,43].

Because the density of gametocytes relative to asexual parasites in *P. falciparum* infection is usually low – fewer than 5% in most settings [44] – a gametocyte threshold of ~100 gametocytes/µl approximately translates into an overall parasitemia of 2000 parasites/µl, well into the range of parasitemia detectable by microscopy. There may be parasite and host factors beyond gametocyte density that are important determinants of human-to-mosquito infectivity [12,45–47], and membrane-feeding assays may still be an imperfect way of measuring human infectiousness to mosquitoes [48]. But overall, these data argue that submicroscopic infections may not substantially contribute to the reservoir.

The potential role of submicroscopic malaria in transmission has only been indirectly studied in high transmission settings with prevalent gametocyte carriage

Should all parasitemic individuals be considered current or future gametocyte producers and, by implication, treated [12,49]? Studies supporting this rationale have been largely confined to children in Africa [37,38], a group where gametocyte carriage is expected to be more prevalent. Further, although microscopically positive gametocytemic individuals are clearly infectious to mosquitoes, some studies highlighting the infectious potential of submicroscopic gametocytemia can be difficult to interpret because they do not specify whether such subjects also had submicroscopic malaria (i.e., they could have had detectable asexual stages).

Among a random sample of 80 children in Burkina Faso, approximately twice as many of those with microscopically detectable gametocytes were infectious to mosquitoes compared with those with gametocytes detectable only by molecular methods: 68% with smear-detectable gametocytes infected on average 13% of mosquitoes, whereas 32% with submicroscopic gametocytes infected on average 2.3% of mosquitoes [37]. Based on these numbers, it was estimated that those with submicroscopic gametocytes contributed to 24.2% of overall transmission because of their greater prevalence in the population. However, <20% of children in this study had submicroscopic malaria, so the majority with submicroscopic gametocytemia probably still had asexual parasites visible by smear. Another study in Western Kenya also found that submicroscopic gametocytemia frequently resulted in mosquito infection [38]. However, the study recruited children who were 14 days post-treatment, mostly with sulfadoxine–pyrimethamine-containing regimens, which have been associated with increased post-treatment gametocytemia [31,50,51].

Human-to-mosquito infectivity studies among asymptomatics in Western Thailand also found individuals harboring submicroscopic gametocytemia that were infectious to mosquitoes, but these were among microscopically patent infections. Village-wide screening by microscopy resulted in 3.3% of individuals infected with *P. falciparum* by microscopy, 80% of whom were asymptomatic [52]. Among the 51 infected individuals in whom gametocytes were not visible, five (9.8%) still infected mosquitoes, yielding low oocyst burdens (average 1.2 oocysts/mosquito). Meanwhile, 13.5% of the *P. vivax* patients without smear detectable gametocytes also infected mosquitoes. Overall, 10% of microscopically identified *P. falciparum* infections transmitted their infection to mosquitoes in a membrane-feeding assay, a rate similar to that seen in asymptomatics found by active surveillance in two villages in Cameroon (9–13%) [53], but much lower than that observed in a village sample in Burkina Faso (>50%) and treated children in Western Kenya (62%) [38,54]. These studies suggest that the underlying gametocyte prevalence in different settings can vary widely and has an impact on the size of the infectious reservoir [12,31].

Should PCR be used to screen for the transmission reservoir? Epidemiologic data pertaining directly to this question are scarce. In one study in the Brazilian Amazon, 15 asymptomatic carriers (11 *P. vivax*, three *P. falciparum*,

one mixed P. *falciparum*/*vivax*) detected in a mass survey who were microscopy-negative but PCR-positive were recruited for mosquito-feeding experiments 2 months after PCR diagnosis. Of 294 mosquitoes fed on their blood by both direct and membrane feeding, two mosquitoes became infected with one *P. vivax* oocyst each, yielding an infection rate of 0.7% [55]. Comparing this to a mosquito infection rate of 22% among 17 symptomatic carriers with microscopically detectable parasitemia in the same study yields an approximately 300-fold difference in infectivity between submicroscopic asymptomatics and microscopic symptomatics in this setting.

Further epidemiological data gleaned from three human-to-mosquito infectivity studies were recently cited to compare mosquito infectivity between submicroscopic and microscopic asymptomatics. These showed between a 4- and 16-fold difference in % mosquitoes infected among submicroscopic versus slide-positive *P. falciparum* infections [56]. However, one of these studies involved African–American malariotherapy patients with neurosyphilis who were partially treated [57], another represents unpublished data from Burkina Faso, and in the third study from South Carolina in 1948 [58], the prevalence of submicroscopic carriers had to be estimated. All three studies appear to be drawn from populations where the underlying gametocyte prevalence is high: slide-detectable gametocyte prevalence was ≥68% in the malariotherapy study and 64% in the South Carolina study [57,58]. Gametocyte prevalence in areas where malaria is now hypoendemic is expected to be much lower. Thus, these 4- to 16-fold infectivity ratios are only approximate estimates. These estimates were used to suggest that submicroscopic carriers are the source of 20–50% of all human-to-mosquito transmissions when transmission reaches very low levels [56]. This conclusion, although based on the best available evidence, underscores the need for more epidemiological data, especially from low transmission settings where malaria elimination efforts are underway.

Sparse data exist from elimination efforts using active surveillance to guide practice

As countries pursue malaria elimination, interventions ranging from focal or mass screening and treatment to mass drug administration to targeted populations are being tried [3]. Reactive case detection, whereby households within an established radius surrounding a symptomatic positive index case are screened, is also increasingly being used as a strategy that bridges passive and active surveillance [59–61]. Many of these efforts are applying molecular methods to find and treat asymptomatic infections [23,62–64]. But we found only two attempts to measure gametocytes in such studies. In a school- and population-based survey in the Melanesian Islands where an overall malaria point prevalence of 2–3% was found by both microscopy and PCR, gametocytes were detected in 15% of the *P. falciparum* positives and 4% of the *P. vivax* positives [65]. In northwest Thailand, PCR of 475 individuals in two villages as part of a mass screening revealed eight cases with low density parasitemia later confirmed by expert microscopists. None of these carried gametocytes visible by smear, but submicroscopic gametocytes were not

measured [66]. Because infected individuals are treated, the duration of these submicroscopic cases is also unknown. The possibility exists that the submicroscopic infections found by such surveillance efforts might not significantly contribute to the transmission reservoir [3].

Research strategies

Because of the logistical difficulties of studying asymptomatic infections, much of what is known about asymptomatic malaria in African settings has been extrapolated to settings with much lower transmission where the asymptomatic reservoir mostly consists of those with submicroscopic malaria. Studies of duration of infection, submicroscopic gametocyte carriage, and gametocyte production potential need to be carried out in submicroscopic infections in low transmission settings where elimination is being considered (Box 3). This can be done, either by retrospective molecular analysis of prior cohort studies where only microscopy was used, or prospectively, in the context of cohort studies and national surveys, in which microscopy-negative but PCR-positive individuals are followed and not treated unless they become symptomatic or develop patent parasitemia. These studies are made more feasible by available methods to measure submicroscopic gametocytemia from blood spots collected for PCR [67–71] but require longitudinal follow-up, which can be challenging.

Going one step further, human-to-mosquito infectivity studies can provide more direct information about the transmission potential of submicroscopic infections (Box 3). Ideally, serial mosquito membrane-feeding studies using local mosquito vectors would be conducted in those with asymptomatic submicroscopic malaria identified by current mass screening and treatment efforts. Although challenging and tedious, these would more directly measure the potential impact that submicroscopic cases uncovered by active surveillance have on transmission. Because such studies still do not measure mosquito-to-human transmission or overall malaria incidence, cluster randomized trials of different interventions, for example, mass screening and treatment versus mass drug administration versus vector control, remain important [72]. Incorporating different types of molecular diagnostics (RDT, PCR, and LAMP) into such studies would allow better definition of a diagnostic threshold for elimination below which transmission is unlikely, a practical goal that could guide allocation of resources for diagnostics in malaria elimination programs.

The policy implications are important: if individuals found by active surveillance to have submicroscopic malaria turn out to have minimal transmission potential, perhaps ultrasensitive PCR methods are not as important as an emphasis on better coverage and follow-up of targeted populations. If partially treated or presymptomatic individuals make up a significant part of the asymptomatic reservoir, intensifying malaria treatment efforts to involve periodic follow-up post-treatment as well as enhancing early detection might be more cost-effective than screening large populations.

Concluding remarks

As resources are allotted to elimination efforts, further research is critical to better characterize the asymptomatic

Box 3. Outstanding questions and possible strategies to answer them

Research question	Possible study designs
Submicroscopic parasitemia	
What proportion of untreated/unrecognized submicroscopic infections persist asymptomatically?	Retrospective molecular analysis of prior cohort studies where only microscopy was used.
What proportion go on to become microscopically patent and/or symptomatic?	
How often do treated patients develop subsequent submicroscopic parasitemia?	Retrospective molecular analysis of therapeutic efficacy studies.
Transmission potential of submicroscopic infections	
What proportion of submicroscopic infections harbor or produce gametocytes, and at what density?	Prospective molecular analysis incorporated into cohort studies or national surveys.
Besides gametocyte density, what parasite or human host factors affect human-to-mosquito infectivity?	*In vitro* membrane-feeding assays.
How commonly does submicroscopic versus microscopic malaria in asymptomatics successfully infect mosquitoes? How does this vary in different transmission settings and in different mosquito vectors?	Vectorial capacity studies, estimates of entomological inoculation rate (EIR), mosquito membrane-feeding studies *in vitro*, and from patients in surveys and/or cohorts.
Diagnostic tests to detect submicroscopic parasitemia and estimate transmissibility	
How sensitive does a diagnostic test need to be to identify all those in the infectious reservoir?	Comparative analysis of diagnostic tests incorporated into cohort studies and mass surveys, paired with malaria seroprevalence surveys.
How large is the hypnozoite reservoir in *P. vivax*-endemic areas, and how can it be measured?	Studies to identify surrogate markers of latent *P. vivax* infection.

reservoir in settings where elimination is being pursued and the factors that may or may not make submicroscopic infections important for continuing transmission. In particular, a key operational question is, 'how sensitive does a diagnostic test need to be to identify all of those who make up the infectious reservoir?' Because a gametocyte–mosquito infectivity threshold has been established for *P. falciparum* in African settings, this can act as a guide. Nevertheless, determining the gametocyte carriage potential of individuals followed without treatment is also key, especially for those with submicroscopic parasitemia. With regards to infection with *P. vivax*, the present inventory of tools is inadequate to achieve elimination without a diagnostic marker for latent hypnozoite infection or introduction of a universally safe antimalarial that can clear hypnozoites.

Presently, the lack of evidence for or against a substantive role of submicroscopic malaria for transmission makes the ethics of treating asymptomatic and submicroscopic parasitemia unclear and dependent on the risk–benefit ratios of the treatment and/or prevention strategies being implemented. Untreated individuals may go on to develop clinical disease at rates that are currently unpredictable and infect others in their households or communities. Alternatively, infection could clear irrespective of treatment that might cause adverse effects in patients. If the treatment is ineffective, they may continue to harbor persistent, increasingly drug-resistant parasites post-treatment. The spread of infection, although thought by some to be from a widespread reservoir of asymptomatic individuals, may in fact be limited to only a relatively small pool of patients with microscopically patent infection. The sporadic and focal nature of malaria transmission argues for the latter hypothesis. However, even if transmission is focal, and limited to a few carriers, the potential beneficial effects of mass treatment to provide prophylaxis and disrupt transmission must be carefully considered [73]. Longitudinal studies that incorporate molecular analyses and mosquito membrane-feeding techniques will help tease out some of these outcomes and contribute to our understanding of how best to target the asymptomatic malaria reservoir.

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