CellPress
REVIEWS

Review

Malaria Hotspots: Is There Epidemiological Evidence for Fine-Scale Spatial Targeting of Interventions?

Gillian Stresman,[1,*] Teun Bousema,[2,*] and Jackie Cook[3]

As data at progressively granular spatial scales become available, the temptation is to target interventions to areas with higher malaria transmission – so-called hotspots – with the aim of reducing transmission in the wider community. This paper reviews literature to determine if hotspots are an intrinsic feature of malaria epidemiology and whether current evidence supports hotspot-targeted interventions. Hotspots are a consistent feature of malaria transmission at all endemicities. The smallest spatial unit capable of supporting transmission is the household, where peri-domestic transmission occurs. Whilst the value of focusing interventions to high-burden areas is evident, there is currently limited evidence that local-scale hotspots fuel transmission. As boundaries are often uncertain, there is no conclusive evidence that hotspot-targeted interventions accelerate malaria elimination.

Highlights

Hotspots are an intrinsic part of malaria transmission biology.

The size of hotspots depends on the spatial resolution of the input data with important implications for any resulting inference.

Where an infectious mosquito/human interacts with a susceptible human/mosquito determines the unit of transmission. The underlying transmission unit is currently unclear and may involve complex nested spatial scales.

Infections are more likely to be related when detected closely in space and time; advances in parasite genetic analysis are needed to allow the elucidation of transmission networks at local spatial scales and inform the optimum scale at which interventions need to be undertaken.

Evidence for impact on transmission as a result of hotspot-targeted strategies has been limited but is likely due to confounding factors and an incomplete understanding of spatial transmission dynamics.

Spatially Targeted Interventions

Spatial heterogeneity has been recorded in the majority of disease systems whereby certain areas experience more intense transmission than others [1,2]. For malaria, this phenomenon has largely been attributed to environmental risk factors, at both the macro (e.g., temperature, precipitation) and the micro (e.g., local elevation, land use) spatial scales, linked to the *Anopheles* mosquito vector's preferred habitat and the temperature range that modifies the extrinsic incubation period of the *Plasmodium* parasite [3–8]. However, environmental factors associated with *Plasmodium falciparum* transmission are inconsistent in different settings, likely due to the diversity of vector species, the analytical methods used to identify risk factors, and in some situations, the suboptimal resolution of available spatially referenced data [3,9–11]. The causes and consequences of malaria spatial heterogeneity across the transmission spectrum are current areas of interest both in terms of understanding and monitoring transmission but also for providing an opportunity for more effective control [12–14].

Maps of malaria burden are of value and are increasingly used to prioritize resource allocation [15,16]. In low-transmission settings there is a temptation to spatially target interventions as finely as possible with the objective of targeting residual transmission in order to accelerate the path to elimination [17]. Targeting malaria **foci** (see Glossary) in elimination and postelimination settings has been recommended by the World Health Organization (WHO) [18]. Interventions utilized by National Malaria Control Programmes (NMCPs) that are typically considered for spatial targeting include **reactive case detection (RACD)**, **targeted mass drug administration (tMDA)**, and **targeted indoor residual spraying (tIRS)** [19–21]. In addition to the impact of these approaches within targeted areas, there is an assumption that these '**hotspots**' contribute disproportionately to maintaining transmission and that targeting them will therefore achieve greater impact. Despite the attractiveness and biological plausibility of a spatially targeted approach as a means to interrupt onward transmission in low-transmission settings where **peri-domestic transmission** occurs, it is important to examine whether the available evidence justifies this approach and what knowledge gaps exist to quantify the potential impact of hotspot-targeted interventions on malaria in the wider communities. Therefore, the aim of this work is to undertake a critical review of literature on the spatial dynamics of *P. falciparum* malaria transmission biology to understand if the evidence supports the pursuit of spatially targeted interventions as part of malaria control and elimination programs.

[1]Infection Biology Department, London School of Hygiene and Tropical Medicine, London, UK

[2]Radboud University Medical Centre, Department of Microbiology, HB Nijmegen, The Netherlands

[3]Medical Research Council (MRC) Tropical Epidemiology Group, London School of Hygiene and Tropical Medicine, London, UK

*Correspondence:
Gillian.Stresman@lshtm.ac.uk,
teun.bousema@radboudumc.nl

Evidence for Spatial Heterogeneity of Malaria Infections

The notion of malaria hotspots has been in the literature for the last two decades, with hotspots identified at a variety of spatial scales, and heterogeneity identified within and between communities, politically defined regions, and countries [1,15,22–26]. More recently, the spatial connectedness of malaria, related to travel behavior of (infected) individuals, has been used to identify so-called sources and sinks in different regions [27]. Targeting malaria sources is important but distinct from the topic of the current review that deals with hotspot-targeted interventions that aim to reduce local transmission by targeting those high-burden areas within a locality (hotspots). The term 'hotspot' has been used interchangeably to mean small geographic regions all the way up to country level, with the subsequent interpretation depending on the malaria metric(s) and spatial analytical methods used [28]. The definition of hotspot also tends to vary depending on program objectives, spatial scale, and transmission intensity. For clarity, in this review, the term 'hotspot' is considered to be a subnational area where malaria is higher compared with the surrounding area (in places where peri-domestic transmission occurs). In low-transmission settings, hotspots may be important in sustaining transmission within foci [17,29]. The term 'foci' is here considered to be any area with active transmission in settings with transmission ranging from pre-elimination to elimination, as defined by the WHO [30]. We are considering hotspots to represent any spatial scale which could range from a single household to an entire subnational region, depending on the transmission intensity of the area and what is relevant for the operational aims of the programs (Table 1).

Hotspots in High-Transmission Settings

In higher-transmission areas, where granularity of heterogeneity is of less interest for defining malaria intervention policy, national-level maps are justifiably utilized to prioritize highest risk areas. Identification of the highest risk areas is typically conducted through risk mapping with national or global-level surfaces typically generated with a 1 or 5 km grid cell resolution [11,16,31]. Of note is that environmental suitability alone is not enough to identify high-burden areas, and the models are improved when accounting for nonecological covariates, including intervention coverage, access to health services, and population density [4]. The precision and quality of maps will always be linked to the spatial resolution and quality of the data used to inform predictions. In terms of using these methods to define hotspots, the minimum hotspot size will be restricted to the resolution of the map pixel used. In areas with high transmission, where the objectives are to prioritize areas to target limited resources, the 1 or 5 km resolution is usually sufficient as within-pixel heterogeneity, whilst almost certainly present, is less operationally relevant when transmission is universally high (Table 1) [32].

Hotspots in Low-Transmission Settings

In lower transmission settings, targeting may aim to accelerate the path to elimination in a cost-effective manner. For this, higher spatial resolution is required to increase the degree of heterogeneity detected with the resulting map more intrinsically linked to working with the optimal spatial unit, which is dependent on transmission intensity and programmatic objectives (Table 1). For example, in larger areas with hypo but stable endemic transmission focusing on heterogeneity at the village scale, hotspots have been detected ranging in size from a single community (Bioko Island) to a cluster comprising over 40 villages (Cambodia) [33,34].

When malaria data with spatially referenced households are available, additional layers of heterogeneity become apparent with identified hotspots being as small as 120 meters in diameter, or even single households, which becomes increasingly relevant in pre-elimination settings [35]. Individual and household-level heterogeneity in malaria risk has been well documented. The majority of infections are routinely identified in less than half of all households within villages with significant differences in malaria risk between households [36–40]. Between-household variability has also been observed when exposure to the malaria vector was measured. Spatial variations in *Anopheles* densities have been observed, with a few households experiencing a disproportionate fraction of the overall mosquito burden [41,42]. Combining parasitological and entomological data, evidence supports the notion that, in areas with peri-domestic transmission, households may be the relevant spatial unit of transmission and would therefore be the optimal spatial scale for delineating hotspots.

Glossary

Foci: any area with active malaria transmission: may consist of a cluster of a few houses to entire countries or regions.
Force of infection: the rate at which individuals are exposed to malaria infections, as either new or incident infections or the number of new malaria parasite clones acquired.
Hotspot: household or cluster of households within an area of ongoing transmission that have a higher burden than the surrounding area and may seed transmission.
Long-lasting insecticide-treated bednet (LLIN): netting impregnated with durable insecticide formulations placed over sleeping spaces, providing a protective barrier to mosquito vectors.
Peri-domestic transmission: malaria transmission occurs around the household, therefore the household location is a reasonable proxy for the location where transmission has occurred.
Reactive case detection (RACD): the practice where a programmatically detectable confirmed malaria infection (e.g., a clinical case) is traced back to their place of residence to either test for, or presumptively treat, household members and sometimes neighbors under the assumption that additional infections will be detected in proximity to the index case.
Reproductive rate, basic (R_O): the number of secondary infections resulting from a single infected individual in a naïve population.
Reproductive rate accounting for malaria control interventions (R_C): the number of secondary infections resulting from a single infected individual in a population implementing control interventions that change the rate at which an infection will generate secondary cases , for example, in malaria, use of LLINs or indoor residual spraying will reduce the potential for vectors to contract and transmit the parasite.
SatScan: software for cluster detection that employs Kuldorff's scanning statistic comparing the point data inside a window with the global study area. Options are available for binary or count data, circular or elliptical shaped

Hotspots in Elimination/Prevention of Reintroduction Settings

In areas where the objective is to achieve or maintain malaria elimination, taking a spatially defined approach could be important to target the remaining vestiges of transmission, to maximize the sensitivity of surveillance activities, and to confirm the absence of infections [30]. In settings aiming to achieve elimination, the evidence to support the concept of hotspot-targeting is mixed. For instance, the assumption that an index infection provides a source of parasites that spreads in the surrounding population has fueled the use of RACD strategies [20]. However, the resource-intensive RACD can yield few additional infections [e.g., 9 rapid diagnostic test (RDT) positives of 1898 screened in a recent study evaluating RACD in Cambodia [43]], suggesting that there may be a threshold in transmission where investigating hotspots yields too few additional infections to be of value [21,43]. Alternatively, RACD strategies may be of most value where population levels of protective immunity are sufficient and asymptomatic infections are likely. In an immunologically naïve population, as would be expected once transmission has been interrupted for a sufficient period of time, secondary infections resulting from onwards transmission of the index case are likely to result in clinical symptoms and, if access to care is adequate, makes reactive testing and/or treatment activities less important [20].

How Stable Are Hotspots Over Time?

There are two distinct spatiotemporal dynamics that have been associated with hotspot transmission in the literature: stable and unstable. Stable hotspots are consistent with the classic definition of hotspots in the sense that they are persistent areas of higher burden within an area with overall lower levels of transmission intensity [17]. Several studies have identified hotspots that are temporally stable and range in size from single households to several villages [34,44,45]. Characteristics of stable hotspots include having a greater prevalence of asymptomatic infections and a younger average age of clinical infections [45,46]. These findings are consistent with the development of protective immunity resulting from higher levels of exposure in these populations. Due to their persistence over time, stable hotspots have been found to be predictive of future malaria incidence, with one study suggesting that they may seed transmission in the surrounding area [47–49].

windows, or adjusting for covariables known to impact the disease.

Targeted indoor residual spraying (tIRS): a campaign where the inside of houses in a specified area is sprayed with a long-lasting insecticide that will both kill and repel the mosquito vector, effectively reducing the vectorial capacity.

Targeted mass drug administration (tMDA): a campaign where entire populations are given a curative dose of malaria medication that also ideally has a long prophylactic period to both cure any current infection and protect from reinfection.

Transmission network: linking parasite strains in space and time to trace the source and subsequent spread of an infection in a population.

Transmission intensity[a]	Relevant spatial scale for interventions	Relevant definition of hotspot	Programmatic objective for spatially targeted intervention	Targeted intervention options available[b]
High/hypo/hyper endemic; PfPR >50%	National or subnational areas (e.g., districts)	Areas of higher burden	Control; prioritize resources to highest burden areas	IRS; LLIN; case management
Stable/meso endemic; PfPR ~ 10.1–50%	Subnational high-burden areas (e.g., districts or villages)	Areas of higher burden	Control; prioritize resources to highest burden areas	IRS; LLIN; case management
Unstable/hypo endemic; PfPR ~ 1–10%	High-burden foci (e.g., individual villages or parts of villages)	Areas of high burden that may serve as a source to maintain transmission	Transmission reduction	RACD; tMDA; tIRS; case management
Unstable, nonendemic; pre-elimination; PfPR <1	Small-scale hotspots (within village foci or high-burden households)	Areas of high burden that may serve as a source to maintain transmission	Transmission interruption	RACD; tMDA; tIRS; case management
Elimination/ prevention of reintroduction	At-risk households or individuals	Households with evidence of infection or exposure that may reinitiate transmission	Prevention of transmission resurgence	RACD; tMDA; tIRS; case management

Table 1. Approaches for Targeting High-Risk Populations in Different Transmission Settings

[a]Transmission intensity was defined according to the strata outlined in [108].

[b]Abbreviations: IRS, indoor residual spraying; LLIN, long-lasting insecticide-treated bednets; PfPR. *Plasmodium falciparum* parasite rate; RACD, reactive case detection; tIRS, targeted indoor residual spraying; tMDA, targeted mass drug administration.

In contrast, unstable hotspots have also been identified in the literature; they exhibit characteristics similar to epidemics and tend to be characterized at more granular spatial resolution in low-transmission settings. Unstable hotspots typically emerge during the peak transmission season and are not necessarily sustained throughout the year; this is consistent with epidemics dying out [46,50]. Unstable hotspots by nature will not be identified in the same place over time, so they are poorly predictive of future malaria risk [35,51,52]. Unstable hotspots are typically associated with more symptomatic malaria in older age groups, consistent with expected lower levels of population immunity in areas not experiencing continuous levels of exposure [53]. The presence of these unstable hotspots suggests that malaria cannot be sustained in these populations without an introduced infection. The frequency of unstable hotspots may potentially be a function of the broader transmission intensity in the area and local population movement [54].

Both the presence and absence of spatiotemporal stability associated with detected hotspots in low-transmission areas suggest that hotspots are a fundamental, yet stochastic, part of malaria-transmission biology. In the next section we examine the evidence for whether these hotspots are responsible for propagating transmission into broader areas.

Is There Evidence That Local-Scale Hotspots Amplify Transmission?

Malaria interventions have been found to influence the **force of infection** in neighboring, untargeted communities [55,56]. This diffusion of infection has also been observed in E-Swatini, an area with very low transmission, whereby additional infections clustered around detectable infections [21]. However, other settings have demonstrated that the expected pattern of infections spreading from high-burden households/areas to surrounding ones does not occur and that household-level burden is more stochastic in nature [39]. Some of the inconsistencies in the evidence may be explained by vector behavior. For an extreme example, if 100% of mosquitoes are consistently biting the same 20% of the population this would effectively create a closed circuit with limited spreading of infections to the wider community [57,58]. In reality, it is likely that hotspots are both self-contained transmission units while also serving as the source of infections outside of the hotspot contributing to the stochastic patterns observed and fueling unstable hotspots via human and/or mosquito movement [54]. The implications of this potential dynamic are that it will be harder to disentangle any intrinsic between household transmission. Evidence focusing on **transmission networks** and any factors associated with any subsequent diffusion of parasites between mosquitoes and people could answer this question directly.

Following the parasite genetic signature to link infections in time and space could confirm the presence of hotspots and quantify the extent to which they seed transmission outside hotspot boundaries [59]. Parasite genetic tools have been able to identify the source and routes of propagation of malaria between communities, particularly in relation to population movement [60,61]. However, to address the question of hotspots, transmission dynamics within villages are most relevant, ideally capturing both local human movement and the time and location of all new parasites' genetic barcode [62].

Evidence from some settings has been consistent with parasite transmission networks between neighboring households or within households when samples are identified closely in time [63,64]. For example, studies in Colombia and Cameroon found that there was a high probability of sampling-related parasites in different residents of the same and neighboring households [65,66]. Yet, there are also reports that have identified limited spatiotemporal correlation in parasite strains, consistent with local transmission networks being more stochastic in nature [67,68]. For instance, work in Senegal found that parasites with similar genetic barcodes were not clustered within households [69]. The lack of a clear spatial clustering of genetically related parasites in these studies may be due to technical limitations in data analytical methods, study designs used not employing optimal sampling methods both in terms of space and time to capture all transmission events, or transmission being too high within the broader foci to discern a signal. In addition, the presence of undetected asymptomatic and/or polyclonal infections that are poorly amplified with current technologies, or challenges with identifying key parasite genetic markers within each setting that are relatively

conserved but able to determine heredity, may play a role and blur patterns, thereby yielding insufficient discriminative power to identify related infections (see Box 1 for further discussion) [70,71].

Evidence of the Impact of Spatially Targeted Interventions

To reduce malaria transmission, a strategy must target and eliminate enough of the parasite reservoir in humans and/or mosquitoes before it can be replenished either by infections missed by the campaign or from human movement bringing in parasites from the surrounding areas [19,43]. The impact of hotspot-targeted interventions for reducing transmission in low-transmission settings, where hotspot dynamics are more relevant, has been mixed. In settings where village-level interventions, including tMDA and/or tIRS, have been employed, transmission is often temporarily reduced, but gains are not always sustained [19,20]. Several factors likely impact the efficacy of hotspot-targeted interventions to date, including not having sufficient control populations with which to evaluate efficacy, incomplete intervention coverage, not sustaining interventions long enough to interrupt transmission, or not accounting for population movement reintroducing parasites into the population postintervention. Also, when community-based hotspot-targeted activities have been attempted in the research context, hotspots are typically defined in one transmission season, with targeted interventions often applied in the subsequent season: effectively assuming that all hotspots identified are stable and correctly delineated. Between the time when an area is labelled as 'hot' and deploying interventions the hotspot location and/or size may have changed, which could limit any expected impact [72].

In terms of RACD-type interventions, where households and/or neighbors of index cases are targeted, there is currently no evidence that this results in transmission reduction [21,39]. The lack of impact may be due to suboptimal implementation or evaluation, as described above. It is more plausible that the proportion of infections that are targeted is insufficient to result in a sustained decrease in transmission: one third to one half of infected individuals and high-burden households may evade detection by current methods (Figure 1) [35,73–75]. Furthermore, the time between detection and response may be an important confounder. Strategies using a clinical-based reactive strategy (i.e., RACD) attempt to respond to the households to be targeted within a week of the case being reported, and this may be too long to prevent subsequent transmission. Similarly, for RACD to be considered as a transmission-reduction strategy, one would have to assume that all 'hotspots' will

Box 1. Considerations for Data Collection with the Aim of Teasing Apart Malaria-Transmission Networks

- A sufficient proportion of all prevalent infections should be mapped in space and time, and parasite genotypes determined with markers related to parental lineage targeting conserved regions that are able to link parasite generations, accounting for the high rates of parasite recombination. All subsequent incident infections should be captured, and parasites similarly typed.
- The detection limit of genotyping tools must ensure that low-density infections can be characterized and mapped.
- Multiclonal infections must be accounted for and included in any analysis, something not easily achieved with current genetic tools. In other words, when an individual is infected with more than one parasite, the parasite barcode for each unique clone must be identified with high degrees of confidence.
- Analytical methods to determine if infections are related must account for the sexual recombination and the extrinsic incubation period of the *Plasmodium* parasites.
- Local movement of humans (e.g., whether they spend the night in other houses within a village or travel that otherwise incurs exposure risks) must be measured to determine the appropriate unit of transmission for each infection.
- To identify parasite strains that can be considered as the parent infection, it may maximize the likelihood of detecting a network if all prevalent infections are cleared prior to the study, or if the study begins during the low-transmission season.

have a symptomatic case [74,76,77]. In elimination settings that are immunologically naïve, this assumption is likely valid, and RACD strategies could help to contain transmission as part of outbreak responses. However, in pre-elimination settings, limited impact on transmission is expected.

Before it will be possible to confirm or disprove the theory that local-level hotspots in low-transmission settings fuel or sustain transmission, it will be important to address the potential confounding factors that may obscure our ability to implement locally targeted intervention strategies to achieve maximum impact.

Confounding the Hotspot Issue: Why Evidence of Impact is Elusive

Several factors may be masking our ability to detect the hypothesized trends. The inconsistent or lack of impact may be partly due to our incomplete understanding of the transmission biology in terms of relevant spatial units and a lack of a clear standard for delineating and defining hotspots appropriately.

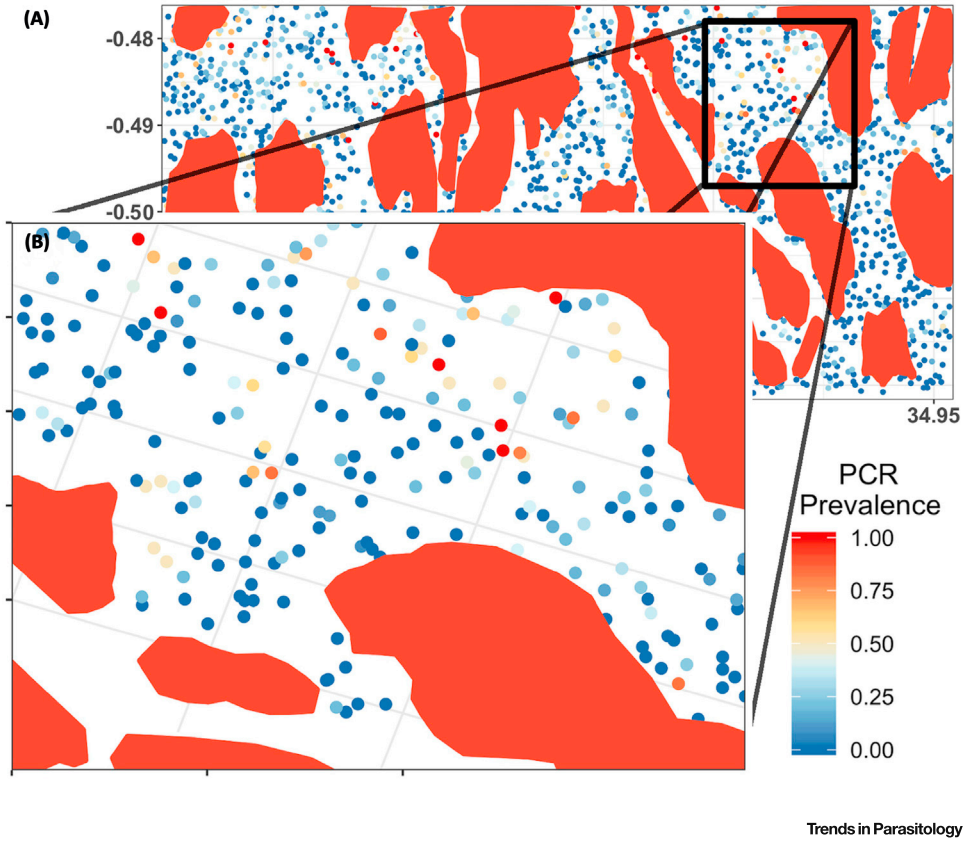

Figure 1. High-Burden Households Consistently Located Outside of 'Hotspots'.
Current malaria metrics used to measure, and spatial methods used to delineate, hotspots consistently miss some high-burden households. This measurement bias may be why hotspot-targeted approaches within villages show limited impact on sustained reductions in transmission. The plot shown is of the data collected in the Western Kenyan highlands (A) as has been described [72], with areas identified as hotspots delineated by SatScan shown in red. The sampled households are shown as the circles, with the color denoting the prevalence of malaria within the household, with a section zoomed in (B) to provide an example of the number and distribution of households not included as part of a hotspot. In this setting, of the 3204 households sampled, 778 of 1866 households with zero infections detected (46.1%) were within the hotspot boundaries, and 47 of 121 (38.8%) high-burden households (PCR prevalence >70%) were not included within hotspot boundaries.

Unit of Transmission

In higher transmission settings, programs typically target interventions based on a convenient administrative unit that may not reflect the relevant spatial unit of transmission. To date, studies have defined the unit of spatial analysis opportunistically based on the spatial resolution of the available data, including politically defined units (e.g., districts), health facility catchment areas, village-, household-, and pixel-level with the size depending on the resolution of the input raster layers [78–82]. In settings where transmission intensity is higher, whereby a health facility catchment or entire village is the logical spatial unit to receive interventions, or interventions are most effective when there is an expected community effect, working at this resolution appears appropriate.

However, the base unit of transmission likely exists at the intersection of two nested components: one being the area over which the mosquito vector travels from a specific breeding site to feed, and the second being where humans interact with the vector (Figure 2, Key Figure) [83,84]. The role of the vector habitat in driving hotspots is supported by ecological factors conducive to mosquito breeding and measures of vector density consistently associated with higher risk of malaria [85,86]. The second relevant spatial scale, where the infectious mosquito comes into contact with the human, is also intuitive and is consistent with spatial analysis consistently using the household location as the proxy for the location of transmission [6]. This assumption is not valid in settings where transmission occurs in nondomestic settings, that is, the forest or other ecological niches [29]. Translating these nested spatial scales, which may consist of a single household or two households linked by the distance between where a mosquito ingests gametocytes, becomes infectious, and where that infection was subsequently retransmitted into a hotspot that can be detected by spatial algorithms, will be required for any spreading effect to be observed. Identifying these potentially nested spatial scales becomes even more difficult in practice when also accounting for the heterogeneities and nuances within transmission systems, including mosquito vector dynamics, human genetic factors modifying susceptibility (Box 2), short- or long-distance movement of individuals, and the expected lag-time between related infections [87].

Delineating Hotspots: Implications for Choice of Hotspot Detection Method

Statistically, 'hotspots' within foci have been delineated using different methods, including cluster detection algorithms and geostatistical models [88,89]. Spatial clustering detection algorithms generally involve comparing the density of points (i.e., malaria infections) within a defined area with the distribution of all sampled points (both negative and positive cases) in the entire study area, with Kulldorf's spatial scanning statistic (**SatScan**) and Getis Ord-Gi* being the most regularly applied tools [31,90]. Spatial prediction algorithms, using model based geostatistics, kriging, or regression trees, for example, are used to estimate the malaria burden at unsampled locations informed by the available malaria data and any relevant covariable information available such as **long-lasting insecticide-treated bednet (LLIN)** coverage, elevation, or land use to help predictions. Hotspots are then considered as those areas where the predicted burden is at or above a predefined threshold with a given degree of certainty [91]. Analytically, each of these methods is different, with the appropriateness of the tool depending on the specific question of interest, the data available, and any underlying spatial dynamics present. Therefore, it is unsurprising that the precise boundaries delineated are sensitive to the methods used to define them [92]. Until there is a standard for what constitutes a hotspot, the optimum method or minimum data required for delineating it cannot be effectively determined, and the impact of a targeted strategy cannot be fairly ascertained. Furthermore, for spatial analysis to be meaningful, the spatial unit used to represent the location of transmission should be representative of the expected epidemiological dynamics, with the household location being relevant only where peri-domestic transmission occurs.

The sample size used to inform the spatial algorithm also impacts how hotspots are delineated [93]. For example, a study in Kenya demonstrated that, as the spatial denominator became more granular, the resulting areas considered to be hotspots were refined [53]. If the extent of any identified hotspots depends on the spatial resolution of the input data and spatial method employed, to what extent do any identified hotspots reflect areas that maintain transmission vs. a spatial process reflecting the

Key Figure

Schematic to Represent the Unit of Malaria Transmission.

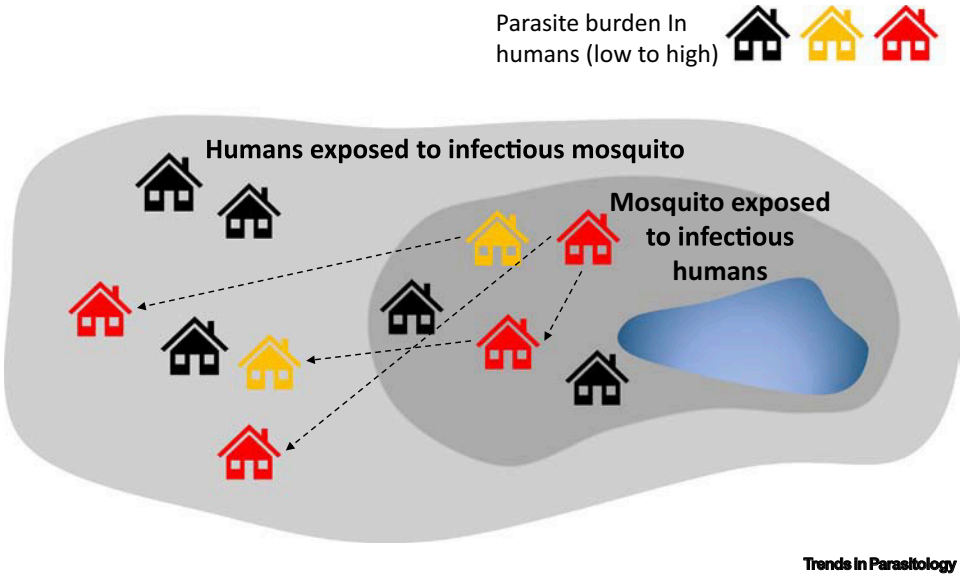

Figure 2. Malaria transmission requires the interface between an infectious *Anopheles* mosquito vector and a susceptible human host. In areas with transmission expected to occur around the household, the unit of transmission is likely to be the nested space where the mosquito interacts with the infectious human, and the human subsequently interacts with the infectious mosquito. These units are not necessarily static over time and space and could consist of a single household or two households connected by the plausible flight range of the mosquito. The dark grey area is shaded to represent where the mosquito is expected to be infected by humans, with the broader light-grey area representing the area where humans could become infected by an infectious mosquito. Houses are colored to represent the parasite burden in the humans, with the arrows denoting possible routes for how parasites move between households (with transmission also expected to occur within the household). Adapted from [17].

quality of the data available and algorithms employed? If the former is true, then the evidence supports the use of these methods to initiate hotspot-targeted strategies where the aim is to reduce transmission. If the latter explanation is closer to reality, either hotspot-targeted strategies with the expectation that efforts will result in a sustained decrease in transmission are not justified or approaches should be refined to better reflect the nuances in spatially delineated transmission.

Delineating Hotspots: Implications for Choice of Malaria Metric

Malaria transmission is ideally measured in a way that accounts for all components of the parasite lifecycle. The **basic reproductive rate (R_O)**, or **reproductive rate accounting for malaria control interventions (R_C)**, which provide a direct measure of transmission efficiency could be used, but this is difficult to measure in practice [94]. Instead, proxies of transmission measuring a single component of the parasite lifecycle, such as prevalence of infection in the human population or entomological inoculation rate, are typically used [32]. In practice, different metrics tend to identify different areas as hotspots and it is often unclear how best to operationally use the information garnered [28,95]. Alternative approaches that may lead to more accurate hotspots could be to consider combined metrics that account for different measurable components of transmission or to account for the nested and overlapping factors that enable exposure, although this increases the logistical expense.

Box 2. Unquantified Impacts of Vector Behavior and Human Genetics on Hotspots

Vector Ecology

Interaction between the human and the mosquito vector is a necessary component for malaria transmission to take place. In areas with peri-domestic transmission, this is assumed to take place within or around the household as *Anopheles* mosquitoes are typically active at night. However, transmission can also take place in forest or other settings, depending on the ecology of the specific vector species and risk factors for infection (e.g., hot-pops) [29,99]. Transmission hotspots could therefore be considered as the areas within both the normal flight distance from the vector breeding site, the density of mosquitoes that are old enough to have survived the extrinsic incubation period, and the availability of suitable hosts for blood meals [22]. In settings where breeding sites are not static and are strongly influenced by (seasonal) rains, areas to be considered as a 'hotspot' may change accordingly and account for the degree of stability/instability of hotspots observed in the field. Other potentially modifying factors include wind speed and direction, and availability of alternative species providing an acceptable bloodmeal source, and distribution of vector-control interventions, none of which are static factors [57].

Heterogeneity of exposure to mosquito vectors will vary at the household and individual level. Mosquito abundance and subsequent risk of malaria will be modified by household factors, including the presence of containers providing a source of stagnant water, house construction, use of vector-control interventions such as insecticide residual spraying or the use of LLINs, as some examples [100,101]. Individual-level factors may also confound hotspot analysis. Exposure to mosquitoes can still be substantially different within the same household, with mosquitoes exhibiting preferential biting behavior towards specific individuals based on their attractiveness or who is not protected by LLINs [41,102]. For example, adults tend to be bitten more frequently than children [103,104]. Generally, vector ecology, and how it relates to malaria-infectious individuals, may drive hotspot transmission dynamics, confound efforts to understand and delineate them, or a combination of both. Teasing apart this complex interaction would lead to being able to better map transmission between individuals and identify who contributes disproportionately to onward transmission.

Human Genetics

Human genetic diversity may also modify malaria hotspot dynamics. It has been suggested that approximately 25% of the variation in individual susceptibility is due to host genetic factors [105,106]. Several genetic factors, including the sickle-cell trait, α-thalassemia, gucose-6-phosphate dehydrogenase deficiency, and having the O blood group, offer protection against infection, clinical symptoms, or severe disease [66,107]. An individual's attractiveness to mosquitoes has also been identified as a heritable trait, with those least attractive protected from malaria and those most attractive being most at risk [98,104]. When genetically similar groups cluster together in space, as happens with a family unit in a household, or genetically similar populations in villages, it could impact the observed spatial patterns of malaria but may not be connected with hotspot transmission dynamics. Although it may not be practical to measure all of these characteristics, which are likely secondary in importance when compared to environmental characteristics, the human genetic component and their relative attractiveness to mosquitoes adds another layer of complexity to delineating hotspots of malaria transmission.

Ultimately, the malaria metric and spatial method used will impact the feasibility and sensitivity of hotspot detection [96,97]. Regardless of which method and malaria metric are used, some areas/households will always be identified as 'hot' but there will also be households missed or erroneously included (Figure 1) [98]. For example, if malaria is measured by RDT alone, any resulting hotspot will not account for infections with parasite densities below the limit of detection of the tool. Similarly, if a cluster detection algorithm is used, assuming the household is the location of transmission, it will identify areas which can be a group of houses or a single house that has higher transmission than the surrounding area. Some houses may not meet criteria to form part of a statistically significant hotspot but are nevertheless characterized by above-average malaria exposure and may contribute to sustaining transmission. As transmission becomes very low, and only key foci sustaining transmission remain, their delineation may become consistent irrespective of the metric used [93]. However, being able to identify the 'truth' for what constitutes a hotspot, if they are truly present within the investigated locality, is required before the optimal metric(s) and method can be identified and any associated measurement biases can be accurately ascertained.

Concluding Remarks

The decision to undertake a spatially targeted approach for malaria control is ideally made based on the available evidence that justifies its use. Hotspots have been identified in many settings, across all transmission intensities, using different malaria metrics and spatial statistical methods. Defining hotspots and a relevant spatial scale based on transmission intensity and program objectives provides a consistent framework in which hotspot theory should be applied. In high-transmission settings, identifying that smallest geographical unit of transmission is less relevant given that a large proportion of the population is infected, meaning that a uniformly applied strategy, as is currently being advocated, is most appropriate. In areas where transmission occurs in the forest or other nondomestic settings, spatially delineated targeted approaches should not be employed, with a targeted approach to high-risk individuals being more appropriate.

In low-transmission settings where a more granular spatially targeted approach becomes attractive, the evidence is mixed. This review suggests that hotspots are an intrinsic part of malaria-transmission biology, with the household being the smallest operational unit capable of sustaining transmission (where peri-domestic transmission occurs). However, there is no conclusive evidence as to whether hotspots fuel transmission to an extent that justifies their preferential targeting. This lack of evidence may in part stem from factors confounding our ability to accurately measure hotspots and transmission from hotspots (see Outstanding Questions). The pressing need is to ensure that a robust approach is developed, ideally one that enables an accurate delineation of parasite transmission networks within and between households (Box 1). Improvements in the available tools and analytical techniques to map parasite lineage and to enable tracking of sexually recombining *Plasmodium* parasites between individuals (via the mosquito vector) may provide the required evidence to confirm to what extent high-burden households seed transmission and how this can be most effectively delineated with available spatial tools and malaria data [59]. Until this evidence is available, a hotspot-targeted strategy with reactive drug or vector-control strategies targeting the household, or entire communities, is justified once transmission becomes sufficiently low and focal but alone may not lead to a sustained reduction in transmission outside of the targeted area.

Outstanding Questions

What is the geographical unit of malaria transmission in different epidemiologies?

How does short-distance population movement affect this unit of transmission?

What metric(s) and spatial method(s) can most accurately delineate hotspots of malaria transmission?

How can we map transmission networks, ensuring the largest possible fraction of infections in humans linked to its parent infection?

When mapping transmission networks, do you gain extra resolution by including entomological assessments whereby parasite genotypes are determined in infected/infectious mosquitoes?

What is the relevant temporal window between detected infections whereby they are likely to be linked transmission events?

What is the optimum duration of hotspot-targeted interventions to observe an effect in the wider community?

Acknowledgments

We would like to thank the reviewers for their helpful comments that no doubt strengthened this work. Funding for this work was provided as part of the Wellcome Trust Sir Henry Wellcome Fellowship (204693/Z/16/Z) awarded to G.S.

Author Contributions

Conceived the paper: G.S., T.B., J.C.; conducted the literature review and wrote the first draft: G.S.; contributed to the writing of the manuscript: G.S, T.B., J.C.; agree with the manuscript's results and conclusions: G.S., T.B., J.C. All authors have read, and confirm they meet, International Committee of Medical Journal Editors (ICMJE) criteria for authorship.

References

1. Woolhouse, M.E.J. *et al.* (1997) Heterogeneities in the transmission of infectious agents: Implications for the design of control programs. *Proc. Natl. Acad. Sci. U. S. A.* 94, 338–342
2. Rothman, K.J. (1990) A sobering start for the cluster busters' conference. *Am. J. Epidemiol.* 132, S6–S13
3. Canelas, T. *et al.* (2016) Systematized literature review on spatial analysis of environmental risk factors of malaria transmission. *Adv. Infect. Dis.* 06, 52–62
4. Stresman, G.H. (2010) Beyond temperature and precipitation: ecological risk factors that modify malaria transmission. *Acta Trop.* 116, 167–172
5. Manjurano, A. *et al.* (2011) Association of sub-microscopic malaria parasite carriage with transmission intensity in north-eastern Tanzania. *Malar. J.* 10, 370
6. Thomas, C.J., and Lindsay, S.W. (2000) Local-scale variation in malaria infection amongst rural Gambian children estimated by satellite remote sensing. *Trans. R. Soc. Trop. Med. Hyg.* 94, 159–163
7. Snow, R.W. *et al.* (1993) Periodicity and space-time clustering of severe childhood malaria on the coast of Kenya. *Trans. R. Soc. Trop. Med. Hyg.* 87, 386–390
8. Cohen, J.M. *et al.* (2010) Local topographic wetness indices predict household malaria risk better than land-use and land-cover in the western Kenya highlands. *Malar. J.* 9, 328

9. Noor, A.M. *et al.* (2008) Spatial prediction of *Plasmodium falciparum* prevalence in Somalia. *Malar. J.* 7, 159
10. Khattak, A.A. *et al.* (2013) Prevalence and distribution of human *Plasmodium* infection in Pakistan. *Malar. J.* 12, 297
11. Pfeffer, D.A. *et al.* (2018) malariaAtlas: an R interface to global malariometric data hosted by the Malaria Atlas Project. *Malar. J.* 17, 1
12. Paull, S.H. *et al.* (2012) From superspreaders to disease hotspots: linking transmission across hosts and space. *Front. Ecol. Environ.* 10, 75–82
13. Reid, H. *et al.* (2010) Baseline spatial distribution of malaria prior to an elimination programme in Vanuatu. *Malar. J.* 9, 150
14. Reiner, R.C. *et al.* (2015) Mapping residual transmission for malaria elimination. *eLife* 4, e09520
15. Hay, S.I. *et al.* (2009) A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Med.* 6, 3
16. Gething, P.W. *et al.* (2011) A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar. J.* 10, 378
17. Bousema, T. *et al.* (2012) Hitting hotspots: spatial targeting of malaria for control and elimination. *PLoS Med.* 9, e1001165
18. Magalhaes, R.J. *et al.* (2012) Finding malaria hotspots in northern Angola: the role of individual, household and environmental factors within a meso-endemic area. *Malar. J.* 11, 385
19. von Seidlein, L. *et al.* (2019) The impact of targeted malaria elimination with mass drug administrations on falciparum malaria in Southeast Asia: A cluster randomised trial. *PLoS Med.* 16, e1002745
20. DePina, A.J. *et al.* (2019) Spatiotemporal characterisation and risk factor analysis of malaria outbreak in Cabo Verde in 2017. *Trop. Med. Health* 47, 3
21. Sturrock, H.J. *et al.* (2013) Reactive case detection for malaria elimination: real-life experience from an ongoing program in Swaziland. *PLoS One* 8, e63830
22. Clements, A.C.A. *et al.* (2013) Further shrinking the malaria map: How can geospatial science help to achieve malaria elimination? *Lancet Infect. Dis.* 13, 709–718
23. Elyazar, I.R. *et al.* (2011) *Plasmodium falciparum* malaria endemicity in Indonesia in 2010. *PLoS One* 6, e21315
24. Kazembe, L.N. *et al.* (2006) Spatial analysis and mapping of malaria risk in Malawi using point-referenced prevalence of infection data. *Int. J. Health Geogr.* 5, 41
25. Kleinschmidt, I. *et al.* (2000) A spatial statistical approach to malaria mapping. *Int. J. Epidemiol.* 29, 355–361
26. Raso, G. *et al.* (2012) Mapping malaria risk among children in Cote d'Ivoire using Bayesian geo-statistical models. *Malar. J.* 11, 160
27. Wesolowski, A. *et al.* (2012) Quantifying the impact of human mobility on malaria. *Science* 338, 267–270
28. Lessler, J. *et al.* (2017) What is a hotspot anyway? *Am. J. Trop. Med. Hyg.* 96, 1270–1273
29. Sturrock, H.J. *et al.* (2013) Targeting asymptomatic malaria infections: Active surveillance in control and elimination. *PLoS Med.* 10, e1001467
30. WHO. (2018) *Malaria Surveillance, Monitoring and Evaluation: a Reference Manual*, WHO
31. Sturrock, H.J. *et al.* (2014) Fine-scale malaria risk mapping from routine aggregated case data. *Malar. J.* 13, 421
32. Tusting, L.S. *et al.* (2014) Measuring changes in *Plasmodium falciparum* transmission: precision, accuracy and costs of metrics. *Adv. Parasitol.* 84, 151–208
33. Cook, J. *et al.* (2010) Using serological measures to monitor changes in malaria transmission in Vanuatu. *Malar. J.* 9, 169
34. Heng, S. *et al.* (2017) Passive case detection of malaria in Ratanakiri Province (Cambodia) to detect villages at higher risk for malaria. *Malar. J.* 16, 104
35. Platt, A. *et al.* (2018) Dynamic malaria hotspots in an open cohort in western Kenya. *Sci. Rep.* 8, 647
36. Pongvongsa, T. *et al.* (2016) Household clustering of asymptomatic malaria infections in Xepon district, Savannakhet province, Lao PDR. *Malar. J.* 15, 508
37. Mwandagalirwa, M.K. *et al.* (2017) Individual and household characteristics of persons with *Plasmodium falciparum* malaria in sites with varying endemicities in Kinshasa Province, Democratic Republic of the Congo. *Malar. J.* 16, 456
38. Rodriquez-Barraquer, I. *et al.* (2016) Quantifying Heterogeneous Malaria Exposure and Clinical Protection in a Cohort of Ugandan Children, JID ePub.
39. Stresman, G.H. *et al.* (2018) Do hotspots fuel malaria transmission: a village-scale spatio-temporal analysis of a 2-year cohort study in The Gambia. *BMC Med.* 16, 160
40. Mwesigwa, J. *et al.* (2017) Residual malaria transmission dynamics varies across The Gambia despite high coverage of control interventions. *PLoS One* 12, e0187059
41. Guelbeogo, W.M. *et al.* (2018) Variation in natural exposure to anopheles mosquitoes and its effects on malaria transmission. *eLife* 7, e32625
42. Cottrell, G. *et al.* (2012) Modeling the influence of local environmental factors on malaria transmission in Benin and its implications for cohort study. *PLoS One* 7, e28812
43. Hustedt, J. *et al.* (2016) Reactive case-detection of malaria in Pailin Province, Western Cambodia: lessons from a year-long evaluation in a pre-elimination setting. *Malar. J.* 15, 132
44. Coulibaly, D. *et al.* (2017) Spatio-temporal dynamics of asymptomatic malaria: bridging the gap between annual malaria resurgences in a Sahelian environment. *Am. J. Trop. Med. Hyg.* 97, 1761–1769
45. Bejon, P. *et al.* (2010) Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. *PLoS Med.* 7, e1000304
46. Zhou, G. *et al.* (2015) Active case surveillance, passive case surveillance and asymptomatic malaria parasite screening illustrate different age distribution, spatial clustering and seasonality in western Kenya. *Malar. J.* 14, 41
47. Noe, A. *et al.* (2018) Mapping the stability of malaria hotspots in Bangladesh from 2013 to 2016. *Malar. J.* 17, 259
48. Mosha, J.F. *et al.* (2014) Hot spot or not: A comparison of spatial statistical methods to predict prospective malaria infections. *Malar. J.* 13, 53
49. Parizo, J. *et al.* (2016) Spatiotemporal analysis of malaria in urban Ahmedabad (Gujarat), India: identification of hot spots and risk factors for targeted intervention. *Am. J. Trop. Med. Hyg.* 95, 595–603
50. Wangdi, K. *et al.* (2011) Spatio-temporal patterns of malaria infection in Bhutan: a country embarking on malaria elimination. *Malar. J.* 10, 89
51. Ihantamalala, F.A. *et al.* (2018) Spatial and temporal dynamics of malaria in Madagascar. *Malar. J.* 17, 58
52. Soto-Calle, V. *et al.* (2017) Spatio-temporal analysis of malaria incidence in the Peruvian Amazon Region between 2002 and 2013. *Sci. Rep.* 7, 40350
53. Bejon, P. *et al.* (2014) A micro-epidemiological analysis of febrile malaria in Coastal Kenya showing hotspots within hotspots. *eLife* 3, e02130

54. Ruktanonchai, N.W. *et al.* (2016) Identifying malaria transmission foci for elimination using human mobility data. *PLoS Comput. Biol.* 12, e1004846
55. Hawley, W.A. *et al.* (2003) Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. *Am. J. Trop. Med. Hyg.* 68, 121–127
56. Parker, D.M. *et al.* (2019) Herd protection against *Plasmodium falciparum* infections conferred by mass antimalarial drug administrations and the implications for malaria elimination. *eLife* 8, e41023
57. Smith, D.L. *et al.* (2004) The risk of a mosquito-borne infection in a heterogeneous environment. *PLoS Biol.* 2, e368
58. Smith, D.L. *et al.* (2007) Revisiting the basic reproductive number for malaria and its implications for malaria control. *PLoS Biol.* 5, e42
59. Wesolowski, A. *et al.* (2018) Mapping malaria by combining parasite genomic and epidemiologic data. *BMC Med.* 16, 190
60. Cook, J. *et al.* (2016) High heterogeneity of malaria transmission and a large sub-patent and diverse reservoir of infection in Wusab As Safil district, Republic of Yemen. *Malar. J.* 15, 193
61. Simpalipan, P. *et al.* (2014) Diversity and population structure of *Plasmodium falciparum* in Thailand based on the spatial and temporal haplotype patterns of the C-terminal 19-kDa domain of merozoite surface protein-1. *Malar. J.* 13, 54
62. Redmond, S.N. *et al.* (2018) *De novo* mutations resolve disease transmission pathways in clonal malaria. *Mol. Biol. Evol.* 35, 1678–1689
63. Omedo, I. *et al.* (2017) Geographic-genetic analysis of *Plasmodium falciparum* parasite populations from surveys of primary school children in Western Kenya. *Wellcome Open Res.* 2, 29
64. Niang, M. *et al.* (2017) Spatio-temporal analysis of the genetic diversity and complexity of *Plasmodium falciparum* infections in Kedougou, southeastern Senegal. *Parasit. Vectors* 10, 33
65. Gomez, M.K. *et al.* (2017) Characterizing the malaria rural-to-urban transmission interface: The importance of reactive case detection. *PLoS Negl. Trop. Dis.* 11, e0005780
66. Roman, D.N.R. *et al.* (2018) Role of genetic factors and ethnicity on the multiplicity of *Plasmodium falciparum* infection in children with asymptomatic malaria in Yaounde, Cameroon. *Heliyon* 4, e00760
67. Nkhoma, S.C. *et al.* (2018) Intra-host dynamics of co-infecting parasite genotypes in asymptomatic malaria patients. *Infect. Genet. Evol.* 65, 414–424
68. Omedo, I. *et al.* (2017) Micro-epidemiological structuring of *Plasmodium falciparum* parasite populations in regions with varying transmission intensities in Africa. *Wellcome Open Res.* 2, 10
69. Daniels, R. *et al.* (2013) Genetic surveillance detects both clonal and epidemic transmission of malaria following enhanced intervention in Senegal. *PLoS One* 8, e60780
70. Barry, A.E. *et al.* (2013) High levels of genetic diversity of *Plasmodium falciparum* populations in Papua New Guinea despite variable infection prevalence. *Am. J. Trop. Med. Hyg.* 88, 718–725
71. Rice, B.L. *et al.* (2016) Genetic evidence that the Makira region in northeastern Madagascar is a hotspot of malaria transmission. *Malar. J.* 15, 596
72. Bousema, T. *et al.* (2016) The impact of hotspot targeted interventions on malaria transmission in Rachuonyo South district in the western Kenyan Highlands: A cluster-randomized controlled trial. *PLoS Med.* 13, e1001993
73. Mosha, J.F. *et al.* (2013) Epidemiology of subpatent *Plasmodium falciparum* infection: implications for detection of hotspots with imperfect diagnostics. *Malar. J.* 12, 221
74. Stresman, G.H. *et al.* (2015) Focal screening to identify the subpatent parasite reservoir in an area of low and heterogeneous transmission in the Kenya Highlands. *J. Infect. Dis.* 212, 1768–1777
75. Bjorkman, A. *et al.* (2017) Spatial distribution of falciparum malaria infections in Zanzibar: Implications for focal drug administration strategies targeting asymptomatic parasite carriers. *Clin. Infect. Dis.* 64, 1236–1243
76. Bejon, P. *et al.* (2011) Serological evidence of discrete spatial clusters of *Plasmodium falciparum* parasites. *PLoS One* 6, e21711
77. Baidjoe, A.Y. *et al.* (2016) Factors associated with high heterogeneity of malaria at fine spatial scale in the Western Kenyan highlands. *Malar. J.* 15, 307
78. Myers, W.P. *et al.* (2009) Micro-geographic risk factors for malarial infection. *Malar. J.* 8, 27
79. El Hassan, I.M. *et al.* (2015) Progress toward malaria elimination in Jazan Province, Kingdom of Saudi Arabia: 2000–2014. *Malar. J.* 14, 444
80. Chourasia, M.K. *et al.* (2017) Additional burden of asymptomatic and sub-patent malaria infections during low transmission season in forested tribal villages in Chhattisgarh, India. *Malar. J.* 16, 320
81. Qayum, A. *et al.* (2015) Socio-economic, epidemiological and geographic features based on GIS-integrated mapping to identify malarial hotspots. *Malar. J.* 14, 192
82. Alegana, V.A. *et al.* (2016) Advances in mapping malaria for elimination: fine resolution modelling of *Plasmodium falciparum* incidence. *Sci. Rep.* 6, 29628
83. Carter, R. *et al.* (2000) Spatial targeting of interventions against malaria. *Bull. WHO* 78, 1401–1411
84. Bannister-Tyrrell, M. *et al.* (2017) Defining micro-epidemiology for malaria elimination: systematic review and meta-analysis. *Malar. J.* 16, 164
85. Wanjala, C.L. *et al.* (2011) Identification of malaria transmission and epidemic hotspots in the western Kenya highlands: its application to malaria epidemic prediction. *Parasit. Vectors* 4, 81
86. Durnez, L. *et al.* (2013) Outdoor malaria transmission in forested villages of Cambodia. *Malar. J.* 12, 329
87. Marshall, J.M. *et al.* (2016) Key traveller groups of relevance to spatial malaria transmission: a survey of movement patterns in four sub-Saharan African countries. *Malar. J.* 15, 200
88. Giorgi, E. *et al.* (2018) Using non-exceedance probabilities of policy-relevant malaria prevalence thresholds to identify areas of low transmission in Somalia. *Malar. J.* 17, 88
89. Kabaghe, A.N. *et al.* (2017) Adaptive geostatistical sampling enables efficient identification of malaria hotspots in repeated cross-sectional surveys in rural Malawi. *PLoS One* 12, e0172266
90. Pullan, R.L. *et al.* (2012) Spatial parasite ecology and epidemiology: a review of methods and applications. *Parasitology* 139, 1870–1887
91. Jackson, M.C. *et al.* (2009) Comparison of tests for spatial heterogeneity on data with global clustering patterns and outliers. *Int. J. Health Geogr.* 8, 55
92. Aamodt, G. *et al.* (2006) A simulation study of three methods for detecting disease clusters. *Int. J. Health Geogr.* 5, 15
93. Stresman, G.H. *et al.* (2017) Impact of metric and sample size on determining malaria hotspot boundaries. *Sci. Rep.* 7, 45849
94. Azman, A.S., and Lessler, J. (2015) Reactive vaccination in the presence of disease hotspots. *Proc. Biol. Sci.* 282, 20141341
95. Cohen, J.M. *et al.* (2017) Mapping multiple components of malaria risk for improved targeting of elimination interventions. *Malar. J.* 16, 459

96. Zemene, E. *et al.* (2018) Detection of foci of residual malaria transmission through reactive case detection in Ethiopia. *Malar. J.* 17, 390
97. Tambo, M. *et al.* (2018) Evaluation of loop-mediated isothermal amplification as a surveillance tool for malaria in reactive case detection moving towards elimination. *Malar. J.* 17, 255
98. Kangoye, D.T. *et al.* (2016) Malaria hotspots defined by clinical malaria, asymptomatic carriage, PCR and vector numbers in a low transmission area on the Kenyan Coast. *Malar. J.* 15, 213
99. Thanh, P.V. *et al.* (2015) Epidemiology of forest malaria in Central Vietnam: the hidden parasite reservoir. *Malar. J.* 14, 86
100. Musoke, D. *et al.* (2018) Malaria prevention practices and associated environmental risk factors in a rural community in Wakiso district, Uganda. *PLoS One* 13, e0205210
101. Bennett, A. *et al.* (2014) A methodological framework for the improved use of routine health system data to evaluate national malaria control programs: evidence from Zambia. *Popul. Health Metrics* 12, 30
102. Karl, S. *et al.* (2016) Spatial effects on the multiplicity of *Plasmodium falciparum* infections. *PLoS One* 11, e0164054
103. Goncalves, B.P. *et al.* (2017) Examining the human infectious reservoir for *Plasmodium falciparum* malaria in areas of differing transmission intensity. *Nat. Commun.* 8, 1133
104. Fernandez-Grandon, G.M. *et al.* (2015) Heritability of attractiveness to mosquitoes. *PLoS One* 10, e0122716
105. Mackinnon, M.J. *et al.* (2005) Heritability of malaria in Africa. *PLoS Med.* 2, e340
106. Bousema, T. *et al.* (2010) Identification of hot spots of malaria transmission for targeted malaria control. *J. Infect. Dis.* 201, 1764–1774
107. Mpimbaza, A. *et al.* (2018) Associations between erythrocyte polymorphisms and risks of uncomplicated and severe malaria in Ugandan children: A case control study. *PLoS One* 13, e0203229
108. Hay, S.I. *et al.* (2008) Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infect. Dis.* 8, 369–378