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How complex epidemiology of malaria in India can impact its elimination?

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Abstract

Malaria is a human health hazard in the tropical and subtropical zones of the globe and poised to be eliminated by the year 2030. Despite a decrease in incidence in the last two decades, many endemic countries including India report cases regularly. Epidemiology of malaria in India stands unique due to several features of the *Plasmodium* parasites, *Anopheles* vectors, eco-epidemiological situations conducive for disease transmission and susceptible humans living in rural and forested areas. Limitations in public health reach and poor health seeking behaviour of vulnerable populations living in hard-to-reach areas add to the problem. We herewith have brought all these factors together in a comprehensive framework and opine that in spite of complexities, targeted elimination of malaria in India is achievable with planned programmatic approaches.

Keywords

Malaria; *Anopheles* ; *Plasmodium* ; Epidemiology; Elimination; India

Evolutionary epidemiology of malaria in the context of elimination

Malaria is a vector-borne infectious disease of the tropical and sub-tropical regions of the globe. Malaria has put threat to human lives both in term of mortality and morbidity in all its distribution zones.. As per the World Malaria Report, 2022 released by World Health Organization (WHO), 247 million malaria cases and about 0.619 million deaths were reported worldwide in the year 2021 [1]. Although incidences of malaria have been decreased over past few years [2], if history of malaria resurgence is to be believed, the future havoc of malaria is uncertain [3]. It is a well-known fact that epidemiological outcome of malaria depends on several factors: (i) the biology of parasites, (ii) adaptive behaviour of vectors, (iii) susceptibility of human host and (iv) influence of local

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Declaration of interests

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eco-climatic situations [4]. Following the theory of Darwinian evolution where species populations undergo natural selection and thereby adapt to simultaneous changes in eco-climatic conditions, evidences of natural selection in genes involved in malaria of all the three different organisms in malaria triad (host-parasite-vector) have been detected [5]. For example, both the parasites and vectors have successfully adapted to antimalarials and to insecticides, respectively. This situation ultimately change epidemiological scenarios of malaria in local settings, posing new challenges to control the disease in almost all the endemic countries in the globe. It is therefore considered that malaria epidemiology is dynamic and more of 'local and focal' nature than 'global' [6], needing deep basic understanding on the determinants of such outcome of **evolutionary epidemiology** (see **Glossary**) at the local level. Any successful elimination strategy must therefore rely on such basic evolutionary epidemiological information on malaria in a local setting, and such knowledge must be kept in mind while intervention measures are developed [7].

To this extent, although much of the global malaria mortality and morbidity are contributed by the African countries, the southeast Asian and south American countries contribute substantially [8]. Over the past two decades, incidences of malaria have drastically gone down in the South East Asian countries (from 22.8 million in the year 2000 to about 5.4 million in 2021) with only 2% of the global malaria burden [1]. India accounted for about 79% malaria cases and about 82.4% of all malaria deaths in the WHO South-East Asia Region in the year 2021 [1]. It is argued that epidemiology of malaria in India is quite different from other endemic countries in the globe (including the African countries with high malaria morbidity and mortality) in term of variable complexities [9, 10].

Epidemiological complexities of malaria in India

Distribution, species composition and insecticide resistance of malaria vectors

About 58 morphologically identified species of the genus *Anopheles* are present in India; with six (*An. culicifacies*, *An. fluviatilis*, *An. sundaicus*, *An. stephensi*, *An. baimai* and *An. minimus*) serve as primary and four (*An. annularis*, *An. subpictus*, *An. philippinensis* and *An. jeyporiensis*) as secondary vectors of malaria [11]. Besides *An. stephensi*, other five primary malaria vectors display species complexes [12]. For example, *An. culicifacies*, that contributes to about 65% malaria transmission, form five morphologically indistinguishable species (species A to E). Interestingly, species A is considered to be the most efficient vector of malaria, while species B is a poor or non-vector [13]. Similarly, *An. fluviatilis* (that contributes to about 15% malaria transmission) comprises three sibling species; S, T and U [14], with the possible existence of one additional form in India, provisionally designated *An. fluviatilis* form X [15]. Likewise, the *An. minimus* species complex comprises three sibling species: A, C and E. The species A, formally recognized as *An. minimus sensuo stricto* is distributed in India [16, 17]. *An. baimaii* (a highly anthropophagic vector found in the north-eastern states of India) and *An. elegans* (distributed mainly in Shimoga hills of the Karnataka state) are two of the seven species of the *An. dirus* complexe that are found in India [18]. A new cytotype D has also been reported in *An. sundaicus* in Andaman and Nicobar Island [17, 18]. The distribution of malaria vectors along with members of species complexes in different states of India (Figure 1) indicates widespread and

localized prevalence based on eco-climatic conditions. While *An. culicifacies* (responsible for about 65% malaria transmission) is prevalent in rural and peri-urban areas, *An. fluviatilis* (responsible for about 15% malaria transmission) is predominantly found in forest plains and foothills in India.

Migration of different malaria vectors across nearby locations, ecological successions and colonization to new habitat through evolutionary adaptation to eco-climatic changes have been reported in many instances in India [17]. For example, *An. stephensi* that contributes to about 12-15% of malaria transmission, is widespread and majorly contribute to transmission of urban malaria in India. Interestingly, over the past 50 years, urbanization and associated water storage practices are considered to have helped in extensive range expansions of *An. stephensi* across wide swaths of Asia, into the Middle East, and more recently into Africa [19, 20]. In addition, changes in biting and resting behaviour, host preferences and breeding sites in the primary malaria vectors in India are some of the other complex behavioural features [21]. Change in **vectorial capacity** (from non-vector to secondary vector, as calculated by a formula according to the Ross-Macdonald model) [22] and territory expansion by *An. subpictus* [23] are examples of behavioural and eco-climatic adaptation of malaria vectors in India. Similarly, ecological succession of the principal malaria vector, *An. culicifacies* is also noteworthy. This species, which was very rarely found in the north-eastern states of India previously, now overshadows the known principal malaria vector, *An. minimus* and *An. baimai* [24]. Likewise, *An. minimus*, principally found in the north-eastern states had expanded its range to far places in the southern Indian states (Andhra Pradesh, Tamil Nadu, Kerala and Karnataka) and resurfaced in Uttar Pradesh and Odisha where it was found about three decades ago [25]. This possibly happened in parallel with change in resting behaviour of *An. minimus* (traditionally endophilic to exophilic) for adaptation to new habitat [25].

Considering controlling vector populations as the most effective measure of malaria control, insecticides are of common use for preventing man-mosquito contacts either by indoor residual spraying (IRS) or insecticide treated nets (ITNs). Long use of insecticides had created evolutionary pressure for adaption to the insecticides used in programmatic mode in India by the Anopheles mosquitoes. As a result, **insecticide-resistant malaria vectors** are widely populated in India. For example, *An. culicifacies* is reported to display complete resistance to the insecticide, dichloro-diphenyl trichloroethane (DDT), and also is mostly resistant to malathion. Resistance to multiple insecticides in a single *An. Culicifacies* mosquito had also been reported in India [26]. Similarly, the other three primary vectors of malaria; *An. fluviatilis*, *An. sundaicus* and *An. stephensi* are also resistant to DDT and malathion [27]. Distribution of different species of *Anopheles* with current status of resistance to different insecticides in different malaria endemic states of India is depicted in Table 1. Interestingly however, *An. minimus*, apart from some degree of tolerance to DDT [28] is still susceptible to almost all the insecticides, although cases of behavioural avoidance to some insecticides have been reported [29].

Distribution, pattern of infection and drug resistance in Indian malaria parasites

Complementing to the intricacies in malaria vectors, malaria parasites in India too pose high degree of complex features and are distinct in many respects in comparison to other endemic countries [10]. All the five known human malaria parasites of the genus *Plasmodium* (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malaria* and *P. knowlesi*) are present either in single or in mixed infections (as inferred with PCR diagnosis) in different states of India (Figure 2) [10]. The two globally distributed major species of malaria parasites (*P. vivax* and *P. falciparum*) are distributed in almost all the endemic regions and the proportion of malaria caused by *P. falciparum* to *P. vivax* is about 49:51 [30]. Interestingly, **population genetic structure** of these two malaria parasites inferred with evolutionarily neutral genetic loci seem to be quite opposite. While local and geographically close populations are genetically identical in *P. falciparum* [31] (Figure 3A), genetic similarity in geographically distant populations are features of Indian *P. vivax* [32] (Figure 3B). Furthermore, human infections of *hitherto* neglected malaria parasites (*P. ovale* and *P. malarie*) are majorly confined to Odisha state that contribute highly to malaria incidences [33]. This fact has recently been confirmed with detection of infections with substantial frequencies (14.1% for *P. ovale* and 10.3% for *P. malarie*) in Odisha [34]. Similarly, *P. knowlesi* has so far been reported in five different Indian states/union territories, majorly in mixed infection with *P. falciparum* [35].

Malaria epidemiological intricacies are further convoluted by the recent finding on the presence of a novel *P. falciparum*-like parasite in Indians (*PfIndia**) that is considered to be a **missing-link** during the course of **host-switching** of *P. falciparum* [10, 36]. Further, detection of *P. falciparum* in rhesus and bonnet monkeys of India [37] provides evidence on the ongoing exchange of *P. falciparum* isolates between Indians and Indian non-human primates. Interestingly, whereas malaria caused by *P. vivax* are considered to be benign with generally favourable treatment outcome, finding of (i) quick relapse pattern in *P. vivax* infections is suggested to be attributed to Chesson strain type [38]. Furthermore, with (ii) several reports of *P. vivax* infections displaying severe malaria outcome [39, 40], the future of *P. vivax* malaria in India remains concerning. On the other hand, *P. falciparum* seems to be evolving to demonstrate no acute clinical symptoms (**asymptomatic/sub-microscopic infections**), generally correlating with low density infection in high endemic areas [41], although some proportion of these asymptomatic infection may display symptoms and cause mortality and morbidity. Interestingly, prevalence of asymptomatic infection in the community has been found to be in the range of 5-50% in different malaria transmission settings in India [41, 42, 43]. Moreover, infections by *P. falciparum* and *P. vivax* in a single individual (mixed infections) that was thought to be negligible before, have come out to be as high as 13% [30], 30% [33] and 45% [44] at different timepoints and in different malaria epidemiological settings in India. Interestingly, the two neglected malaria parasites, *P. ovale* and *P. malarie* seem to use the infection pathways of either *P. vivax* and *P. falciparum* [33], and such incidences can be up to 75.8% for *P. ovale* [34] in India.

Malaria treatment relies on antimalarials; with chloroquine (CQ) as the principal first-line drug in the 20th century that had sustained for the longest duration. However, continuous and irrational use of CQ had led to evolution of **drug-resistant malaria parasite**, *P. falciparum* [45] that is now spread to all the *P. falciparum* malaria endemic regions of the

globe [10]. Distributional prevalence and the patterns of drug-resistance in *P. falciparum* add further to complexity of malaria epidemiology in India. Field surveillance with molecular markers had provided evidence of absolute resistance to chloroquine (CQ) [10, 46] (Figure 3C) and great degree of resistance for sulfadoxine and pyrimethamine (SP) in *P. falciparum* isolates from almost all over India [47]. Similarly, large-scale resistance to SP in Indian *P. vivax* had also been reported [48]. Moreover, higher diversities in genes providing resistance to antimalarials (CQ and SP) in Indian *P. falciparum* than African isolates had been detected [49, 50]. Interestingly, except single reports each in eastern [51] and southern Indian isolates [52], Indian *P. falciparum* remains grossly susceptible to artemisinin. However, recently, emergence of reduced sensitivity to artesunate-sulfadoxine-pyrimethamine combination drug in central Indian *P. falciparum* isolates had been reported [53]. It had also been suggested that artemisinin-resistance in Indian *P. falciparum* might be kelch13-independent [53]. To this extent, *P. vivax* in India remains largely susceptible to CQ [54], although sporadic cases of resistance to this drug had been reported [55].

Do socio-behavioural and cultural aspects in communities influence malaria intervention plans?

Since local eco-climatic factors drive malaria transmission in a defined zone, malaria is considered as a ‘local and focal’ disease. As a result, socio-behavioural and cultural aspects of local communities contribute to the net malaria outcome in the concerned malaria endemic zone. Apart from the fact that malaria epidemiological outcome depends on the level of community awareness to follow preventive measures (*e.g.*, minimizing man-mosquito contact), health seeking behaviour and treatment adherence are also considered equally important [56]. Availability of healthcare facility in locations that can easily accessed by the local communities also plays a major role. Furthermore, in tribal areas in India, where malaria is highly endemic, easy access and faith on the ‘traditional tribal healers’ also minimize access to public health facilities by the tribal communities [57]. Therefore, whether decisions taken by the government on malaria control/elimination are effectively implemented in the field due to various social, cultural, and behavioural factors associated with specific areas, to be regularly assessed and if required, to be set right [58]. To this extent, bringing public health facilities on malaria close to communities living in high malaria endemic regions and generating awareness among the local communities through counselling and advocacy for utilization of the public health facilities could help [59]. Such complexities due to socio-behavioural and cultural aspects put an additional layer of complexity to the malaria elimination plan in India. Social mobilization to shift ownership of health including malaria to the local communities [60] with public health support could be a sustainable solution in term of early screening, prompt treatment, drug adherence and prevention of malaria in India.

How might malaria complexities impact elimination in India?

Undoubtedly, there exists multifaceted epidemiological complexities of malaria in India (Box 1). This is reflected by several unique features of the malaria parasites, vectors and eco-climatic situations conducive for malaria. In this regard, (i) **range expansion** and

disappearance of species of *Anopheles* with high transmission capacity is of high values in term of **malaria elimination**. Further, (ii) dominance of *An. culicifacies* that is responsible for 60-65% malaria transmission in India in the north-eastern states and (iii) range expansion of *An. minimus* to four south Indian states will surely put a challenge in malaria elimination efforts. Moreover, (iv) high degree of resistance to multiple insecticides in *An. culicifacies* will add the problem. Similarly, (v) change in vectorial capacity (from non-vector to vector) by *An. subpictus* will put further challenge. Elimination program in India should therefore consider these changes in vector behaviour and integrate appropriate measures to deal with these complexities while framing elimination strategies.

Multidimensional complexities in malaria parasites and malaria epidemiological outcome in India can also put further challenges to malaria elimination efforts in India. Apart from distribution of all the five human malaria parasites in defined eco-climatic setups, infection of multiple parasites in a single individual as mixed infection might jeopardize treatment outcomes and will have far-reaching impact in elimination program. This is true, as both *P. falciparum* and *P. vivax* have undergone **demographic expansion** over the years and are currently growing in population size [31, 32]. Further, high genetic diversity in the drug-resistant genes in *P. falciparum* indicating genetic reconstruction [49] might attract natural selection for better adaptation to the existing drug and evolve resistance to new drugs as well [61]. To this respect, evolution of *P. falciparum* isolates to the working antimalarial artemisinin with involvement of some other genetic determinant for artemisinin resistance [53] is daunting. Furthermore, range expansion of the two neglected malaria parasites (*P. ovale* and *P. malarie*) [33], high asymptomatic infections of *P. falciparum* [42] might pose great hindrance in malaria elimination drive. This is because, some proportion of asymptomatic infection, with time, may become symptomatic causing mortality and morbidity. Moreover, evolutionary connection of *P. falciparum* with non-human primates [36, 37], reports of Chesson strain of *P. vivax* [38] might enhance zoonotic transmission of malaria to an unimaginable extent that have not been considered by the elimination plans. Apart from this, differential genetic structure displayed by *P. vivax* and *P. falciparum* in India (Figure 3) is quite intriguing. Observation of genetic similarities among physically close populations (in *P. falciparum*) that are quite genetically distinct from other such local populations (Figure 3A) indicates that one single elimination program might not be effective in whole of India. Alternatively, tailored-made, locally suitable, customized and sustainable management and elimination plans might work for that particular locality [62]. Therefore, India needs several such different local and focal *P. falciparum* malaria elimination plans to reach the goal by the year 2030. Conversely, *P. vivax* showing no population genetic structure (Figure 3B) with high genetic differentiation among local populations and high similarities with geographically distant population in India will require thorough genetic mapping for similarities/differences, on which basis further elimination strategies of *P. vivax* malaria can be built up.

Indian malaria elimination initiatives and lessons learnt

In order to eliminate malaria nationally and contribute to improved health, quality of life and alleviation of poverty in India, the Indian government has framed a guideline called the “National Framework for Malaria Elimination 2016-2030” (NFME) [63]. Developed

in line with the WHO global technical strategy for malaria and the malaria elimination roadmap of Asia Pacific Leaders Malaria Alliance (APLMA), the primary aims of the NFME are to (i) eliminate malaria (zero indigenous cases) in the country by the year 2030, (ii) maintain malaria-free status where transmission has been interrupted and (iii) prevent reintroduction of malaria. In line of this, and to develop models for elimination of malaria in the most affected areas and populations (tribal populations and hard-to-reach areas), two independent malaria elimination initiatives had been undertaken in India in two highly endemic states with different malaria epidemiological scenarios. While (i) the Malaria Elimination Demonstration Project (MEDP) was undertaken in a tribal district (Mandla) in the state of Madhya Pradesh covering 1233 villages consisting of a population of >1.15 million under a public-private-partnership (PPP) mode [64], (ii) the ***D****urgama* ***A****nchalre* ***Ma****laria* ***N****irakaran* (DAMaN) (malaria elimination in hard-to-reach areas) project was undertaken by the government of Odisha state in difficult-to-access areas covering about 5000 different villages/hamlets of Odisha [65]. These two independent intervention projects followed close-to-similar strategies in many instances, but demand-based unique action plans were also followed in each. For example, both the projects were implemented in high malaria endemic settings and malaria interventions were provided in addition to the regular interventions through programmatic approach by the respective state governments. The MEDP relied upon (i) both active and passive case detection, (ii) mass screening and treatment (MSaT) to identify asymptomatic cases, (iii) monitoring and supervising the ongoing vector control activities, (iv) sentinel surveillance of cases treated by the private practitioners (v) providing information, education and communication (IEC) at communities and school levels and (vi) building capacities in health functionaries [66]. Robust accountability, reviews, monitoring and feedback at each level also constituted the backbone of MEDP. At the same time, the DAMaN initiative was taken up (i) to provide early diagnosis and complete treatment in a camp-based approach (with about 200 population in each camp). Simultaneously, (ii) intensive vector control approaches for blocking malaria transmission were also implemented. After about three years of project implementation, reduction of indigenous malaria cases was achieved about 91% in the MEDP and about 88% in the DAMaN initiatives [66]. These two implementation programs have not only yielded success in demonstrating malaria reduction in the respective endemic areas, but also opened new directions for similar, large-scale programs to be taken up for malaria elimination on programmatic modes. It appears that not only robust surveillance and management of malaria (early diagnosis, prompt treatment, regular treatment follow-up, *etc.*) and intervention with regard to vector control by long lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) were essential, but sensitization to the affected community through appropriate behaviour change communication (BCC) strategies were equally important [66]. The national program should not only take these learnings and implement in other similar endemic areas of India, but also ensure sustainability of successful demonstration of malaria elimination by MEDP and DAMaN initiatives.

Concluding remarks

Clearly, malaria epidemiology in India stands apart in term of complexity in comparison to other global endemic counties, including Africa. New malaria epidemiological studies

are also adding up *hitherto* unknown epidemiological facts that further contribute to the complexities. Enormous diversity in term of species of malaria vectors, genetic structure and expanding parasite populations, high frequencies of mixed parasite infection, emergence of asymptomatic *P. falciparum* infections, and consistent evolution of resistance to insecticides by Anopheline mosquitoes and drug resistance by malaria parasites, *etc.*, are some of the hallmarks to malaria outcome in India. Epidemiological complexities caused due to range expansions by the species of malaria vectors and parasites, human migration and porous political borders with endemic countries are supposed to further complicate the issues. Apart from epidemiological complexities, challenges pose by false-negative diagnosis of *P. falciparum* through rapid diagnosis test (RDT) due to deletion in the histidine-rich-protein-2 (*hrp2*) region, that is widely used in the field [67] are of primary concern in malaria public health in India. In addition, with no clear-cut treatment guidelines for mixed species malaria infection and especially of single *P. malarie* and/or *P. ovale* infection, and considering the fact that these two species have expanded range in recent times [33], management of malaria involving these two species in India will be under great threat in future. Also, there is a chance that unreported mixed malaria parasite species infection might increase *P. vivax* malaria [68]. Moreover, rising evidence of asymptomatic infection of *P. falciparum* in recent times in India [41] can contribute to silent malaria transmission in populations and increase hushed burden of malaria. In the absence of defined treatment for asymptomatic and mixed malaria infections, the epidemiological outcome of *P. falciparum* malaria might remain elusive in future. While high genetic diversity in drug-resistant genes provide evidence for massive genetic reconstructions [49], high diversity in functionally inert genes indicated demographic expansion in both *P. falciparum* and *P. vivax* in India [31, 32]. Considering malaria in India is principally confined to economically weak and underprivileged rural and tribal populations that remain inaccessible by public health system in major places, and the health seeking behaviour of these vulnerable populations is considered to be poor, measures must be in place to alleviate the underprivileged communities from malaria burden. More importantly, the NFME in India [63], developed following recommendations of WHO and of Southeast Asian countries seems to be quite limiting, as the epidemiological complexities, as discussed here have been grossly overlooked.

In addition to the research already conducted and knowledge generated on various aspects of malaria epidemiology in India, further research involving multidirectional and multidisciplinary approaches will help in devising implementable research towards elimination of malaria (see Outstanding Questions). To this extent, a six-point agenda has recently been suggested [69]. In addition, and in complementation to these suggestions, additional six points may be considered and composite plans of action to eliminate malaria in India be prepared. Since majorities of malaria burden comes from Indians living in hard-to-reach areas (rural, forested and tribal areas), where public healthcare facilities are scarce, (i) clear-cut and defined elimination plans for eliminating malaria in such specific vulnerable populations shall have to be prepared. This must include (ii) estimation of realistic malaria burden in defined habitat and communities (forest, rural, urban, industrial, tribal, *etc.*) that remained foci of malaria since time immemorial. Undoubtedly, this will help in unravelling hidden epidemiological complexities. Whereas (iii) intensified case detection through mass screening approaches followed by prompt treatment have been proved to

reduce malaria burden significantly in the two malarial elimination initiatives (see above), (iv) sustainability of malaria reduction in these areas is to be ensured. Future elimination strategies (v) must integrate community participation under stakeholder model, not as beneficiaries. Moreover, in the absence of an effective vaccine for malaria, (vi) sensitization in local community to follow preventive measures against mosquito breeding and biting, promptness in getting tested in case malaria symptoms appear and strict drug compliance in patients under treatment, *etc.* could prove as boons in eliminating malaria from India (Figure 4, Key Figure). Such a '**social vaccine**' could prevent malaria infection locally and ultimately contribute to a great extent in achieving the targeted elimination in India by the year 2030.

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Glossary

Asymptomatic/sub-microscopic infections
the presence of asexual parasites in blood, without symptoms of illness. In this case, malaria parasites are not identified by microscopy (microscopy negative) but con be found to be positive by PCR analysis.

Demographic expansion
spatial expansion of population when a species spreads outward to fill vacant ecological space or to overthrow resident species, resulting in increased geographic range.

Drug-resistant malaria parasite
genetic adaptation in the malaria parasite that reduces the effectiveness of an antimalarial used to treat malaria.

Evolutionary epidemiology
study involving epidemiology and evolution of rapidly evolving infectious disease agents. Usually, such studies are performed conjointly to be properly understood. For example, during the course of an epidemic, mutations are generated *de novo* and can spread in the population, creating a reciprocal link between the polymorphisms of the pathogen and its propensity for onward transmission in the host population over time.

Host-switching
an evolutionary change of the host specificity of a pathogen.

Insecticide-resistant malaria vectors
genetic adaptation in the malaria vectors that reduces the effectiveness of an insecticide used to kill/suppress mosquito vectors.

Malaria elimination
deliberate efforts with continued intervention resulting in reduction of the incidence of malaria to zero in a defined geographical area.

Missing link
transitional morphologies, or forms. Any organism in possession of in-between evolutionary properties of both the ancestors' original traits and the traits of the evolved descendants, showing a clear connection between the two.

Population genetic structure
study of genetic variation in time and space. Assessment of population genetic structure informs on the dispersal of species, mating behaviours and the delimitation of species and population boundaries.

Range expansion
events when a population expands into space that was previously unoccupied by that population.

Social vaccine
refers to actions addressing social mobilizations in society, which can act as a precursor to the public health problem being addressed.

Vectorial capacity
is a measure of transmission potential of a vector-pathogen system, classically known as the Ross-Macdonald model. The classical formula for calculating vectorial capacity (V) is (i) the parasite's extrinsic incubation period (EIP, n days), (ii) the ratio of mosquitoes to humans (m), (iii) mosquito survival through one day (p) and (iv) human biting rates (a).

$$V = \frac{ma^2 p^n}{-\ln(p)}$$

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Box 1.

The Center for the Study of Complex Malaria in India (CSCMi)

The Center for the Study of Complex Malaria in India (CSCMi; http://malariacenterindia.org) is one of ten International Centers of Excellence in Malaria Research (ICEMRs) funded by the United State National Institute of Allergy and Infectious Diseases/National Institutes of Health [70]. Active since 2010 and led by faculty at New York University and the London School of Hygiene and Tropical Medicine, the overall goal of the center is to address gaps in knowledge on the epidemiology, transmission and pathogenesis of malaria in India, and to help train the next generation of malariologists through capacity building and transfer of technology. The CSCMi is a close partnership between Indian and international researchers, clinicians and public health workers at a variety of private, public, and government institutions, including the Indian Council of Medical Research (ICMR)-National Institute for Research in Tribal Health, Indian Institute for Public Health-Shillong, Ispat General Hospital, Community Welfare Society Hospital, and ICMR-National Institute for Malaria Research.

The focus of CSCMi is to understand the complexity of malaria in India [71–72]. Considering the fact that research and surveillance of malaria in India must include studies on *P. falciparum* and *P. vivax*, less prevalent *P. malariae* and *P. ovale*, and ten different Anopheles species known to be vectors [10], studies have been conducted at six different field sites in the states of Meghalaya, Odisha, Gujarat, Tamil Nadu, Madhya Pradesh, and the National Capital Territory of Delhi (Figure I). Following are the key findings:

1. Malaria epidemiological studies in four Indian states revealed high burdens of asymptomatic and sub-microscopic *P. falciparum* infections that may act as a reservoir, with implications for malaria elimination [73–75].
2. Testing of novel surveillance tools, such as reactive case detection [76], and sero-epidemiological ones [74, 77].
3. Studies on severe *P. vivax* as well as severe *P. falciparum* malaria, informed first report of posterior reversible encephalopathy syndrome [78] in *P. falciparum*, and clinical characterization of *P. vivax* [79].
4. Generation of the first reference genomes of Indian *P. vivax* and *P. falciparum* that indicated greater genetic diversity in *P. vivax* than *P. falciparum* [80, 81].
5. Anopheles surveillance studies confirmed (i) decline in *An. baimaii* and *An. minimus* populations in the northeast India [73], (ii) shift in *An. fluviatilis* to more zoophilic behaviour warranting redirecting control efforts towards the zoophilic cycle [82], and (iii) identification of preference to cattle shed by *An. stephensi* over human dwellings [83].

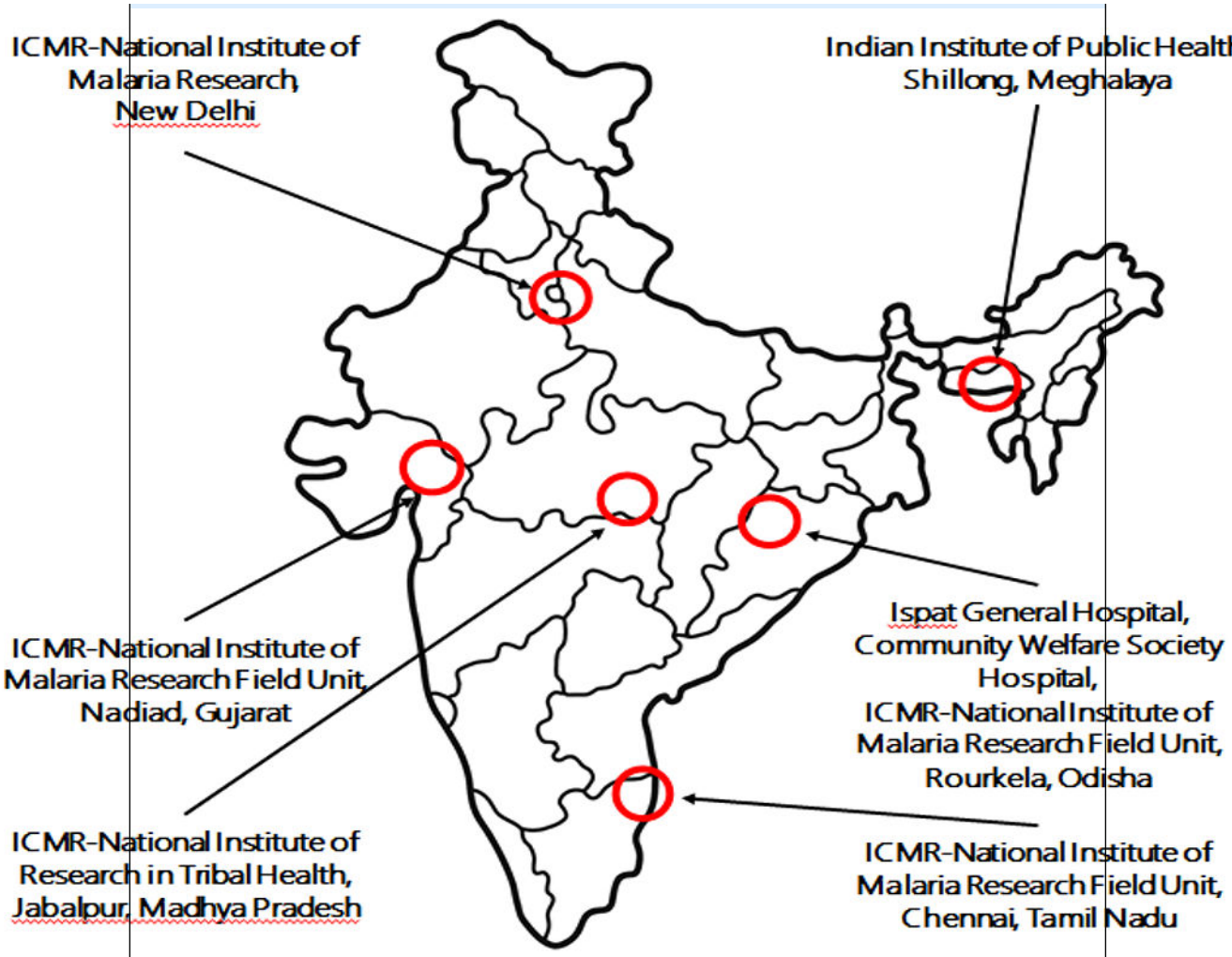

Figure I. Map of the six sites involved in CSCMi studies since 2010 to till date.
The sites are indicated with red circles and the names of the institutions involved in the study are indicated in addition.

Outstanding questions

- Multiple species complexes in malaria vectors. Why malaria vectors present multiple species complexes? Does this due to high adaptabilities of Indian Anopheles to changing eco-climatic conditions, or largely due to resource partitioning to reduce competition?
- Insecticide susceptibility in *An. minimus.* While almost all the Indian malaria vectors are resistant to insecticides in use, why *An. minimus* is still susceptible to all the insecticides in spite of regular exposure to different insecticides? In spite of its susceptibility to insecticides, it is still a successful species.
- *An. subpictus* as an emerging vector of malaria. *A* malaria vector in Sri Lanka, Malaysia and Maldives, this species has emerged as an important vector of malaria transmission in Odisha and coastal areas of south India. How to prevent further spreading of this species to other places in India needs to be established.
- Host-switching of *P. falciparum.* Preliminary results on the presence of common mitochondrial DNA mutations in *P. falciparum*-like malaria parasites in Indian rhesus monkey and *P. falciparum* infecting Indians suggest that *P. falciparum* might have switched its host from non-human parasites to human in India. What are the clinical and epidemiological relevance of this new parasite (*PfIndia**) in malaria outcome in India?
- Growing prevalence of asymptomatic *P. falciparum* infections. Why *P. falciparum*, known for severe malaria outcome, has evolved to 'asymptomatic' from and spreading rapidly in India? Is this species following a similar strategy as *P. vivax*? If so, what is the mechanism of its survival in a low density parasitemia in human host? Which kind of evolutionary changes did *P. falciparum* make in its genome?
- Contrasting population genetic structures in *P. vivax* and *P. falciparum.* Despite living in sympatry in India by both the malaria parasites (*P. falciparum* and *P. vivax*), why local populations of the former species display genetic similarities, whereas geographically far away populations are genetically similar in the latter?

Highlights

- Malaria epidemiology in India is quite complex due to wide distribution of all the five species of human malaria parasites of the genus *Plasmodium* and 10 different species of malaria vectors of the genus *Anopheles*.
- Presence of species complexes in *Anopheles* vectors, mixed *Plasmodium* species infections in a single human, rising cases of asymptomatic *P. falciparum* cases and host-switching of *P. falciparum* from Indian rhesus monkey to Indians make malaria epidemiology further complex.
- Wide distribution of drug-resistant *P. falciparum* parasite, insecticide-resistant *Anopheles* vectors and malaria susceptible humans create conducive environment for malaria transmission in India.
- Two malaria elimination models were demonstrated in recent past in highly endemic tribal and inaccessible areas in India, indicating malaria can be eliminated with planned and concerted efforts.

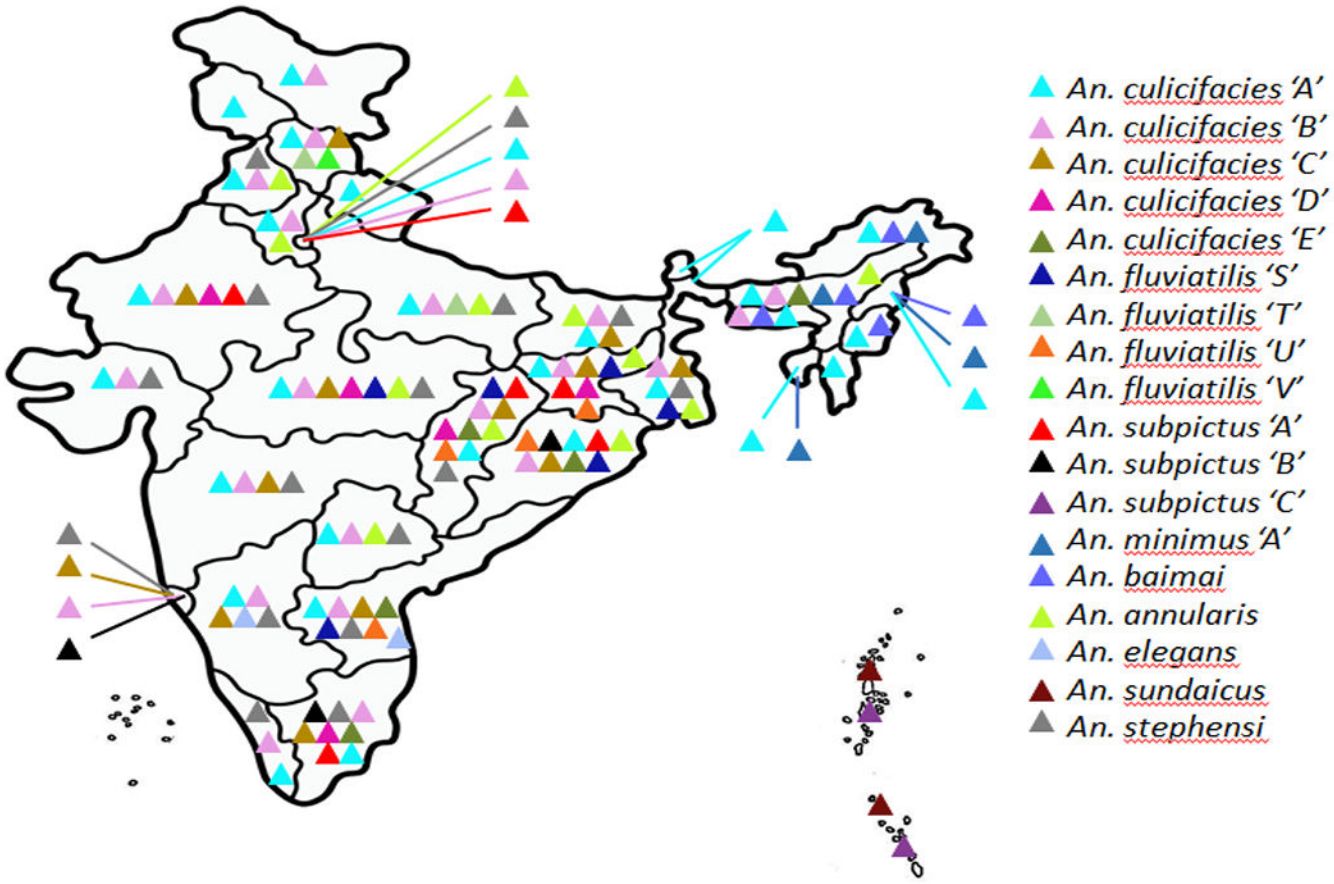

Figure 1. Map of India showing distribution of different species (and sibling species) of the malaria vector, *Anopheles*.

Triangles with different colours represent to a species of *Anopheles* (and sibling species, under inverted coma). Data source: [17].

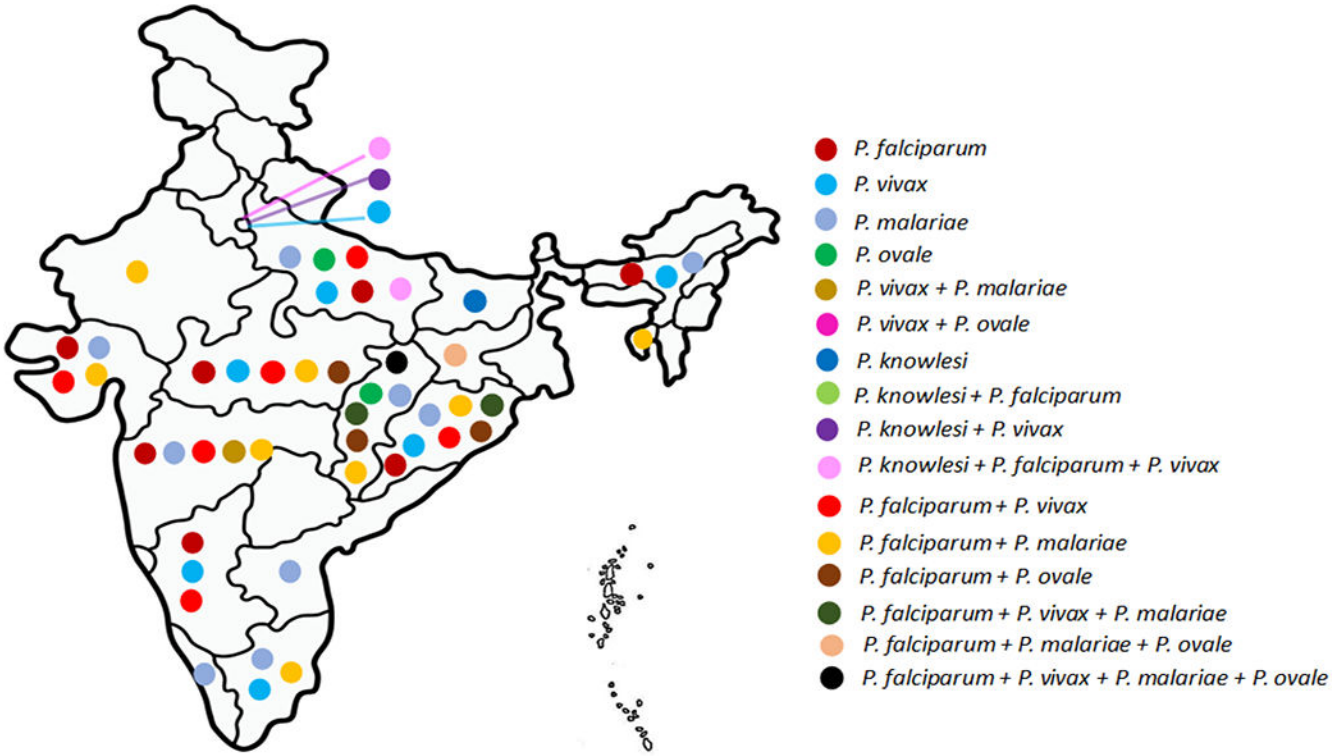

Figure 2. Distributional prevalence of the five human malaria parasites in India as revealed with PCR diagnosis.

Dots of different colors depict different malaria parasites either in mono or mixed infections.

Data source: [30, 35].

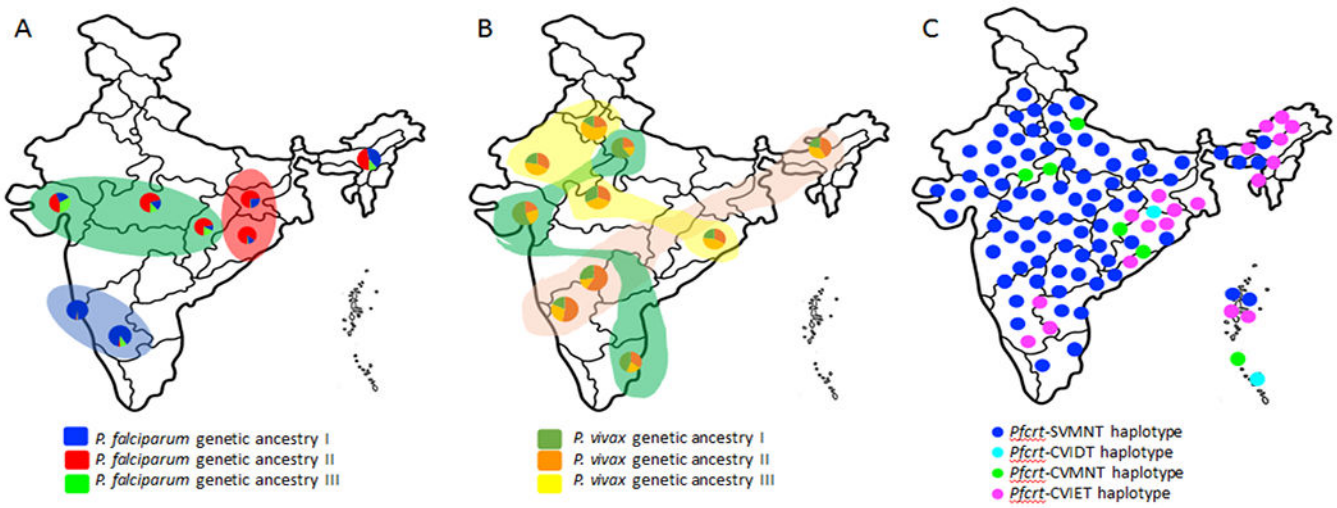

Figure 3. Differential population genetic structure of the two widely distributed malaria parasites (*P. falciparum* and *P. vivax*) in India inferred with neutral DNA sequence polymorphisms (A and B) and distributional prevalence of four different haplotypes of the *Pfcrt* associated with CQ resistance (C).

While *P. falciparum* (**A**) displays geographically structured populations (data source: [31]), no such structure could be seen in Indian *P. vivax* (data source: [32]). (**B**). The colors in the pie charts (both in A and B) refer to presence of different genetic ancestries of the malaria parasite *P. falciparum* (A) and in *P. vivax* (B) in different Indian populations. (**C**) Graphical representation on the distribution of four major haplotypes of the *Pfcrt* gene (SVMNT, CVIDT, CVMNT and CVIET) in different regions of Indian *P. falciparum* [10]. Data not on scale.

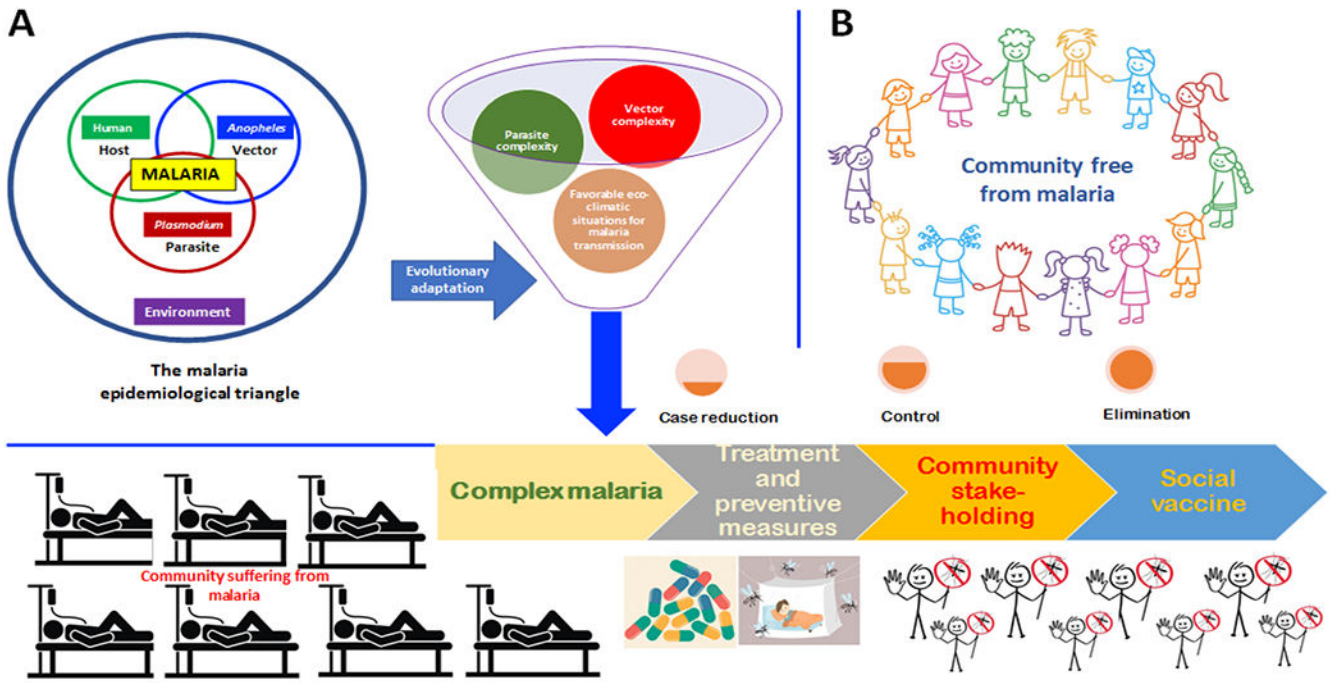

Figure 4, Key Figure. A model describing the process from evolution of malaria complexity to malaria elimination.

(**A**) the evolution of malaria complexity out of differential competition among host (human), vector (Anopheles) and parasite (*Plasmodium*) in the presence of changing environment that makes the community sick of malaria. (**B**) Malaria complexities can be tackled with treatment and preventive measures (case reduction) keeping community as stakeholders (control) and shifting the onus to the community as a part of social vaccine to bring back the community free from malaria.

Table 1.

Status of resistance to different insecticides by different species of *Anopheles* that are vectors (both primary and secondary) of malaria parasites in India.

Malaria vectors of Anopheles species	Resistance status of different insecticides in different Indian states		
	Organochlorine (DDT)	Organophosphate (Malathion)	Pyrethroid (Deltamethrin)
An. culicifacies (Primary vector)	Andhra Pradesh, Assam, Chhattisgarh, Delhi, Gujarat, Haryana, Jharkhand, Maharashtra, Gujarat, Karnataka, Madhya Pradesh, Odisha, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal	West Bengal, Uttarakhand, Telangana, Andhra Pradesh, Odisha, Maharashtra, Madhya Pradesh, Karnataka, Jharkhand, Haryana, Gujarat, Chhattisgarh	Andhra Pradesh, Assam, Chhattisgarh, Gujarat, Madhya Pradesh, Odisha, Tamil Nadu, Telangana
An. fluviatilis (Primary vector)	Chhattisgarh, Himachal Pradesh, Jharkhand, Karnataka, Maharashtra, Tamil Nadu, Uttarakhand	Jharkhand, Maharashtra	Maharashtra
An. sundaicus (Primary vector)	Andaman and Nicobar Islands	No report available	No report available
An. minimus (Primary vector)	Assam, Odisha, Tripura	No report available	No report available
An. stephensi (Primary vector)	Delhi, Goa, Gujarat, Karnataka, Rajasthan, Maharashtra, Uttar Pradesh, West Bengal	Delhi, Goa, Gujarat, Karnataka, Rajasthan, West Bengal	Gujarat, Karnataka, Rajasthan
An. baimai (Primary vector)	No report available	No report available	No report available
An. subpictus (Secondary vector)	Gujarat, Rajasthan	Gujarat, Punjab, Rajasthan	Gujarat, Punjab, Rajasthan
An. annularis (Secondary vector)	Assam, Jharkhand, Maharashtra, Odisha, Rajasthan	Maharashtra, Rajasthan	Assam, Maharashtra
An. philippinensis (Secondary vector)	No report available	No report available	No report available
An. jeyporiensis (Secondary vector)	No report available	No report available	No report available

Updated published literature have been used to gather information on the three insecticides that are used in the field in programmatic mode in India.