Malaria in North-East India: Importance and Implications in the Era of Elimination

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Abstract: Worldwide and in India, malaria elimination efforts are being ramped up to eradicate the disease by 2030. Malaria elimination efforts in North-East (NE) India will have a great bearing on the overall efforts to eradicate malaria in the rest of India. The first cases of chloroquine and sulfadoxine-pyrimethamine resistance were reported in NE India, and the source of these drug resistant parasites are most likely from South East Asia (SEA). NE India is the only land route through which the parasites from SEA can enter the Indian mainland. India’s malaria drug policy had to be constantly updated due to the emergence of drug resistant parasites in NE India. Malaria is highly endemic in many parts of NE India, and Plasmodium falciparum is responsible for the majority of the cases. Highly efficient primary vectors and emerging secondary vectors complicate malaria elimination efforts in NE India. Many of the high transmission zones in NE India are tribal belts, and are difficult to access. The review details the malaria epidemiology in seven NE Indian states from 2008 to 2018. In addition, the origin and evolution of resistance to major anti-malarials are discussed. Furthermore, the bionomics of primary vectors and emergence of secondary malaria vectors, and possible strategies to prevent and control malaria in NE are outlined.

Keywords: North-East India; P. falciparum; P. vivax; An. baimaii; An. minimus; artemisinin combination therapy

1. Introduction

Despite large-scale global efforts to eradicate malaria in the last few years, it still remains a major public health burden with an estimated 219 million cases and 435,000 deaths worldwide in 2017 [1]. Even though, there were an estimated 3 million fewer cases than in 2016 (216 million), no significant progress was made in reducing the global malaria burden during 2015–2017 [1]. It is estimated that
India and 15 sub-Saharan African countries were responsible for 80% of the world’s malaria burden [1]. The contribution of India to the world’s malaria cases and deaths is estimated to be 4% [1]. Compared to 2016, India reported a 22% decrease in cases in 2017 [1], and most came from Odisha [2].

Malaria transmission in India occurs pre-dominantly from either *P. falciparum* (*Pf*) or *P. vivax* (*Pv*) [3], however, the prevalence of these species varies greatly across different regions of India. There has been a steady increase in *Pf* cases in the last 4 decades, and as of December 2018, *Pf* was responsible for 48.1% of malaria cases in India [2]. North-East (NE) Indian states along with Odisha, Chattisgarh and Jharkhand contribute most of the *Pf* cases in the country [2]. NE India alone contributes to 12% of India’s *Pf* cases [2].

Assam, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Sikkim, Manipur and Tripura make up the eight states of NE India. The state of Sikkim is mostly malaria free, and the few cases reported are thought to be imported cases, and hence will not be discussed. As of 2018, the seven NE states account for 15.2% of the total malaria cases in India [2]. In India, it is estimated that 162.5 million people live in high-transmission areas [4], which includes many parts of the NE. The hilly and forested areas of NE India are mostly inhabited by the tribal population, and they are at the highest risk of malaria [5]. The distribution of *Plasmodium* species varies among NE states. *Pf* is the predominant species in Assam, Mizoram, Tripura and Meghalaya, while *Pv* is dominant in Nagaland, Arunachal Pradesh and Manipur. Apart from *Pf* and *Pv*, the other human malaria parasites, *P. ovale* and *P. malariae* have also been recorded from Assam and Arunachal Pradesh [6,7]. The hot and humid climate aided by the numerous hill streams and its tributaries in these tribal areas support perennial mosquito breeding. The primary malaria vectors in NE India are *An. minimus* and *An. baimaii*. *An. minimus* is part of the Minimus Complex that also includes *An. harrisoni* and *An. yaeyamaensis*. In NE India, only *An. minimus* has been reported so far [8]. *An. baimaii* is part of the *An. dirus* Complex that includes 7 other sibling species. *An. baimaii* is the most prevalent sibling species found in NE along with a focal presence of another sibling species, *An. dirus X* [9]. Apart from these 2 primary vectors, other Anophelines such as *An. annularis*, *An. phillipinensis/nivipes* and *An. culicifacies* are considered to be secondary vectors of malaria in the region [10]. Due to widespread resistance against both chloroquine (CQ) and sulfadoxine-pyrimethamine (SP), artemisinin-lumefantrine (AL) was introduced as the first line anti-malarial against *Pf* in NE Indian states in 2013 [11,12]. The salient features pertaining to malaria in NE India are outlined in Table 1.

| NE states | Assam, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland and Manipur |
|-----------|---------------------------------------------------------------|
| Geographical Area | 255,128 Km² (consists of hills, valleys and plains) |
| National proportion to land cover | 8% |
| Total Population | 4516111 (2011 census) with an average density of 159 persons/km² |
| Climate | Tropical monsoon humid climate |
| Contribution to nation’s malaria cases | 15.2% (in 2018) |
| Major *Plasmodium* spp. | *P. falciparum* and *P. vivax* |
| Primary malaria vectors | *An. baimaii* and *An. minimus* |

Secondary malaria vectors | *An. annularis*, *An. phillipinensis/nivipes*, *An. culicifacies* etc. |

Treatment for malaria

- *P. falciparum*—Age-specific artemisinin combination therapy (Artemether-lumefantrine) with primaquine @ 0.75 mg/kg body weight on day 2
- *P. vivax*—chloroquine (25 mg/kg body weight divided over three days) and primaquine (0.25 mg/kg body weight daily for 14 days.)

For mixed infections—Age-specific artemisinin combination therapy (Artemether-lumefantrine) for 3 days + primaquine 0.25 mg per kg body weight daily for 14 days.

Despite being a hot bed for malaria transmission, malaria intervention studies in NE India have been very challenging due to inaccessibility, difficult terrains, forested areas, administrative issues in the inter-state and international border areas, frequent flooding, poor roads and inadequate network...
connectivity [5]. Geography and demography of the region, predominance of *Pf* over *Po*, widespread and early emergence of drug resistance to almost all anti-malarials, and the presence of highly anthropophilic malaria vectors like *An. baimaii* and *An. minimus* throughout the region makes it different from the rest of India in terms of malaria control and elimination. In early 2016, the Indian Government announced the national framework for malaria elimination (2016–2030) [13,14]. This is in line with the larger Global Technical Strategy of the World Health Organization (WHO) [15] and Asia Pacific leaders to eliminate malaria by 2030 [16]. Successful malaria intervention strategies in the NE are critical for India’s target to eliminate malaria by 2030.

2. Geography of North-East (NE) States

The NE is largely a mountainous territory with two-thirds of its area occupied by hilly and mountainous terrain with heights ranging from 50 m in Brahmaputra valley to 7000 m above mean sea level in the Himalayan borderland [17]. The states are between 22°00' to 29°30' N and 90°40' to 97°25' E, and cover an area of 255,128 km². The NE shares about 4600 Km of international borders with China (on North), Myanmar (on East), Bangladesh (on South) and Bhutan (On West) (Figure 1). The general climate of the NE is tropical monsoon humid climate. The average temperature during winter is 16 °C, and summer is 30 °C. There is a significant climatic contrast between the valleys and the mountainous region. The NE region receives heavy to very heavy rainfall during the South-West Monsoon season (June to September), and rains peak in June. The average annual rainfall is 2000 mm with local variations (1500 to 12,000 mm) [18]. Except for Assam, more than half the geographical area of all NE states is forest. The NE is also one of the two biodiversity hotspots in India [19]. The total population of the region is 45,161,611 (2011 census) and the distribution is not homogeneous. The most densely populated parts of NE region are the plains of Brahmaputra, Barak rivers in Assam, the Imphal plain in Manipur and the western part of Tripura [20].

Assam, located in the central part of NE is considered to be the gateway to NE India as it connects the NE to the Indian mainland by a 22 km strip, called the ‘Chicken’s Neck’, near Siliguri district in West Bengal. Mizoram, Nagaland and Arunachal Pradesh share their international borders with Myanmar (Burma). South East Asia (SEA) is considered to be the hotspot for the generation of drug-resistant parasites. Myanmar is the only land route through which the drug-resistant parasites of SEA can enter India. Thus Mizoram, Nagaland and Arunachal Pradesh, the states bordering Myanmar are strategically important as they can serve as a conduit for the drug-resistant parasites from Myanmar to rest of India.

The NE is inhabited by many tribal groups with unique cultural, social and occupational behavior which have a direct role in malaria transmission. Based on topography and socio-demographic conditions, malaria in the NE region can be classified as tribal malaria, forest malaria, border malaria, and organized sector malaria [21].
3. Malaria Epidemiology (2008 to 2018)

The NE is co-endemic for both *Pf* and *Pv* with a low-to-moderate transmission intensity resulting in intermediate to stable malaria in the whole region [22]. During the eradication era (late 1950s to early 1960s), the NE along with the other states of India witnessed a steady decline in malaria cases. However, the situation reversed and malaria made a comeback in 1966, and peaked in 1976 with 10.2% slide positivity rate (SPR) (Figure 2) (See Box A1 for definition of epidemiological parameters) [21]. The surge in malaria cases was due to the emergence of chloroquine resistance (CQR) in *Pf* and its widespread therapeutic failure all over the NE. Since then, both the SPR and slide *falciparum* rate (SFR) of the NE remained more or less constant until 2008 when CQ was discontinued, and was replaced by artemisinin combination therapy (ACT) as the first line of anti-malarial treatment. Prior to 2008, some major peaks were also observed, and were mostly due to the failure of SP, and emergence of multi-drug resistant parasites in the NE (Figure 2) [11].

![Figure 1](image1.png)

**Figure 1.** Location map of North-East (NE) India. Red line shows the boundaries of the seven states of NE India.

![Figure 2](image2.png)

**Figure 2.** Slide positivity rate (SPR), slide *falciparum* rate (SFR) and emergence of drug resistance in NE states during 1961–2018. Adapted from Mohapatra et al., 2014 [11].
Malaria epidemiology data (Table 2) obtained from National Vector Borne Disease Control Program (NVBDCP) website from 2008 to 2018 for 7 NE states are shown spatio-temporally in Figure 3. The State Vector Borne Disease Control Program (SVBDCP) unit will collect information of all malaria cases from block primary health centers (PHCs), sub-centers and private hospitals and will send it to NVBDCP, New Delhi, every month. From 2008 to 2012, Assam reported the maximum number of malaria cases, followed by Meghalaya. The maximum number of cases (91,413, Pf-66,557) in the study period was observed in Assam followed by Meghalaya (76,759) in 2009. In Assam, from 2009 onwards, there has been a steady decline in malaria cases, and in 2018, the number of cases recorded were 3816 (Pf-2859), below Tripura (13,079) and Meghalaya (6394). In Assam, from a high of 25,815 Pv cases in 2008, there has been a continuous decrease, and in 2018, only 957 cases were reported. As of 2018, Pf and Pv contribute 74.9 and 25.1% of the cases in Assam. Malaria cases in proportion to population is given in Table 3. Among the NE states, Assam has the highest population density (397 persons/sq km). However, based on the cumulative number of cases during the study period (2008–2018), the number of cases per year (0.11%) is comparatively low to the total population.
Table 2. Distribution of malaria cases in 7 NE states from 2008 to 2018 (Source: National Vector Borne Disease Control Program (NVBDCP), India).

| States          | Assam | Arunachal Pradesh | Meghalaya | Mizoram | Nagaland | Manipur | Tripura |
|-----------------|-------|-------------------|-----------|---------|----------|---------|---------|
| Year            |       |                   |           |         |          |         |         |
| 2008            | 83,939| 58,124            | 25,815    | 29,146  | 8217     | 20,927  | 39,616  |
| 2009            | 91,413| 66,557            | 24,856    | 22,066  | 6602     | 15,464  | 41,642  |
| 2010            | 68,353| 48,330            | 24,856    | 17,946  | 17,940   | 6,602   | 41,642  |
| 2011            | 47,397| 34,707            | 12,690    | 12,690  | 13,950   | 9,094   | 41,642  |
| 2012            | 29,999| 20,579            | 8,368     | 24,856  | 24,856   | 12,690  | 41,642  |
| 2013            | 19,542| 14,969            | 6,398     | 12,690  | 12,690   | 6,602   | 41,642  |
| 2014            | 14,540| 11,210            | 6,082     | 12,690  | 12,690   | 6,602   | 41,642  |
| 2015            | 15,557| 11,675            | 6,082     | 12,690  | 12,690   | 6,602   | 41,642  |
| 2016            | 7,826 | 5686              | 6,082     | 12,690  | 12,690   | 6,602   | 41,642  |
| 2017            | 5,281 | 3,949             | 6,082     | 12,690  | 12,690   | 6,602   | 41,642  |
| 2018            | 3,816 | 2,859             | 6,082     | 12,690  | 12,690   | 6,602   | 41,642  |

Table 3. Malaria cases in proportion to population.

| State            | Pf cases | Pv cases | Total cases (2008–2018) | Density (2011) Population (2011) Average cases/year (2008–2018) Average cases to population Total cases to population |
|------------------|----------|----------|-------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Arunachal Pradesh| 35,648   | 78,693   | 114,341                 | 17                                                            | 1,382,611                                                                                     | 10,394.63                                                                                     | 0.75%                                                                                     | 8.3%                                                                                     |
| Assam            | 278,190  | 109,473  | 387,663                 | 397                                                           | 31,169,272                                                                                   | 35,242.09                                                                                   | 0.11%                                                                                     | 1.2%                                                                                     |
| Manipur          | 2176     | 2212     | 4388                    | 122                                                           | 2,721,756                                                                                   | 398.90                                                                                      | 0.01%                                                                                     | 0.2%                                                                                     |
| Meghalaya        | 349,961  | 24,526   | 374,487                 | 374                                                           | 14,418                                                                                       | 3,734                                                                                       | 0.15%                                                                                     | 12.6%                                                                                   |
| Mizoram          | 116,876  | 15,301   | 132,177                 | 132                                                           | 14,418                                                                                       | 3,974                                                                                       | 0.15%                                                                                     | 12.6%                                                                                   |
| Nagaland         | 9602     | 22,261   | 31,863                  | 119                                                           | 1,980,602                                                                                   | 2896.63                                                                                     | 0.15%                                                                                     | 1.6%                                                                                     |
| Tripura          | 207,962  | 14,120   | 222,082                 | 350                                                           | 3,671,032                                                                                   | 20,189.27                                                                                   | 0.55%                                                                                     | 6.0%                                                                                     |
Fascinatingly, in Meghalaya, the cases nearly doubled from 39,616 (Pf-36,301) in 2008 to 76,759 in 2009 (Pf-74,251). From a peak of 76,759 cases in 2009, there was a gradual decrease in cases until 2012 (20,834, Pf-19,805). From 2013 to 2015, there was a gradual increase in cases (24,727, Pf-22,885 to 48,603, Pf-43,828), and this was followed by a steady decline until 2018 (6394 cases, Pf-6065). Interestingly, Pf cases gradually dropped from 2008 (3315) until 2012 (1029), and was followed by an increase in cases from 2013 (1842) until 2015 (4775), and again was followed by a steep decline until 2018 (329). In Meghalaya, as of 2018, Pf and Pv contributed 94.8 and 5.2% of the cases. Of the 7 NE states, Meghalaya reported the highest proportion of malaria cases (1.15%/year) to the total population.

In the state of Tripura, there was a steady decline in malaria cases from 2008 (25894, Pf-23,588) to 2013 (7396, Pf-6998). However, there was a huge malaria epidemic in 2014, and there was a 6.9 fold rise in malaria cases (51240, Pf-49,653). Even though there was a gradual decrease from 2015 (32,525, Pf-30,074) to 2017 (7051, Pf-6571), malaria cases almost doubled in 2018 (13,079, Pf-12,600). Pf is the leading cause of malaria in Tripura, and contributes to over 90% of the cases. In 2018, Pf contributed to just 3.6% of the cases. Tripura’s proportion of malaria cases per year to the total population is 0.55%, and it ranks fourth in malaria endemity in the 11 year study period.

In Mizoram, the cases increased from 2008 (7361, Pf-6172) to 2010 (15,594, Pf-14,664), followed by a steep decline in 2011 (8861, Pf-8373). From 2012, the cases started increasing again, and a peak of 28,593 cases (Pf-24,602) was recorded in 2015. There is a decline from 2016 (7583, Pf-5907) to 2018 (4296, Pf-3937). In Mizoram, Pf predominates, and was responsible for 91.6% of the cases. In Mizoram, the population density is 52 persons per sq km, but the number of cases reported is proportionately very high. Nearly, 1.1 percent of the total population is affected on an average per year, and is the second most malarious state.

The states of Arunachal, Nagaland and Manipur have reported a steady decline in malaria cases from 2009 until 2018, and in 2018, the number of cases reported were 625 (Pf-154, Pv-471), 113 (Pf-24,
*Pv*-89) and 12 (*Pf*-3, *Pv*-9) respectively. Fascinatingly, in Arunachal and Nagaland, *Pv* predominates, and in 2018, *Pv* contributed 75.3 and 78.7% of cases respectively. In Arunachal, the average number of cases to population is 0.75%, and is the third highest among all NE states. In Manipur, over the years, both *Pf* and *Pv* have contributed equally to malaria infections. Manipur has the lowest incidence of malaria as the average number of cases to population is just 0.01%.

*Pv* was more in Arunachal Pradesh, Nagaland and Manipur, the states that border Myanmar, while *Pf* predominated in Meghalaya, Tripura, Mizoram and Assam, the states that bordered Bangladesh. A declining trend of malaria was observed in Assam, Arunachal Pradesh, Nagaland and Manipur, the states with higher proportion of *Pv* cases. A cyclical trend was observed in Meghalaya, Tripura and Mizoram dominated by *Pf*.

4. Drug Resistance

Historically, SEA has been a hotspot for the generation of drug-resistant parasites. In the last 50 years, SEA has been a fertile ground for developing resistance against pyrimethamine, CQ, SP, quinine, mefloquine, and most worryingly, artemisinins [23]. The only land route through which the parasites of SEA can enter India is through Myanmar. The 4 NE states of India: Mizoram, Manipur, Arunachal and Nagaland share their international borders with Myanmar. Most likely, the drug-resistant parasites originating in SEA would have entered the 4 NE states through Myanmar, and subsequently should have spread to the Indian mainland. CQR originated in the late 1950s in SEA [24], and was first reported in Myanmar (Burma) in 1969. In India, CQR was first reported in the Karbi Anglong district of Assam in 1973 [25]. Following the initial reports of CQR, 13 regional teams were set up in 1978 to monitor anti-malarial resistance through in vivo-efficacy trials [26]. From 1978 to 2007, 54 CQ efficacy studies were carried out in the NE involving 2541 patients, and the treatment failure was 35% (893) [26]. Across India, CQ treatment failure was observed most in NE, and is the probable locus from which CQR would have spread to eastern, followed by western, parts of India [26]. In 1982, a national drug policy was implemented to improve the management of malaria cases, and established SP as the treatment in CQ-resistant regions [26]. In 2005, for CQ treatment failures, artesunate plus SP replaced SP alone as the second-line of treatment, and in regions with documented drug resistance, artesunate plus SP was recommended as the first-line of treatment [26]. In 2007, for regions with documented CQ resistance and high-risk districts, artesunate plus SP was selected as the first-line anti-malarial [26]. From 2010 onwards, artesunate plus SP has been the recommended first-line anti-malarial across India [27]. In 2009, nation-wide sentinel sites were set up to monitor anti-malarial drug resistance [28].

The first report of SP resistance was again in Karbi Anglong district of Assam in 1979 [29]. Drug-resistance studies carried out in Assam during 2006 and 2007 identified 12.6% treatment failure in SP, accompanied by resistant mutations in *dhfr* and *dhps* [11]. Artesunate plus SP efficacy studies carried out in three NE states (Mizoram, Tripura and Arunachal Pradesh) in 2012 revealed treatment failure rates of >10%, necessitating a change in drug policy [12]. From 2013 onwards, artesunate plus SP was replaced by AL as the first line anti-malarial in NE states, while in the rest of the country, artesunate plus SP continues to remain as the first line of treatment [11,12]. CQ and SP drug-resistance studies carried out in NE are given in Table 4.

The first therapeutical efficacy study on ACT was carried out by Indian Council of Medical Research (ICMR) Regional Medical Research Centre, NE Region, Dibrugarh during 2004–2006 in 4 sites along the Indo-Myanmar border. This is long before ACT was introduced as a first line anti-malarial for treating *Pf* cases in NE. The study recorded 95.5% Adequate Clinical and Parasitological Response (ACPR) of ACT with mean parasite clearance time of 55:17±14:26 h. It is significant that 4.5% of the cases failed ACT (late clinical failure: 2.1%, and late parasitological failure: 2.4%) [11]. Later studies have identified the failure in ACT is due to the resistance to the partner drug, SP [11,12]. The genetic determinant of artemisinin (ART) resistance has been linked to mutations in the propeller domain of the kelch protein (*Pfkelch13* gene) [30]. Molecular screening of *Pfkelch13* by ICMR National Institute of Malaria Research (NIMR) has identified one F446I (n =85) and one R561H (n =24) validated ART resistance marker mutations in the Changlang district of Arunachal Pradesh that borders
Myanmar [31,32]. In addition, few non-/synonymous mutations (K189T, A578S, and G533A) have also been reported in NE states [31,32]. Both the K189T and A578S mutations were observed in Lunglei district, Mizoram [31]. However, all the reported mutations were not associated with any decline in the clinical efficacy of ACT [31–33].

In vitro ring stage survival assay (RSA) carried out on 12 NE isolates obtained from Assam, Arunachal Pradesh and Tripura found that 8 out of 12 (66%) had a greater than 1% survival rate [34]. One of the NE isolates from Assam carried an A675V mutation, a candidate/associate marker, but was not associated with the slow clearance phenotype [34]. In addition, 8 of the 12 isolates had an Asn-Asn (NN) insertion between codon 142 and 143 in the Pf kelch13 gene. Worryingly, the first report of partial ART resistance has been in the Purulia, Bankura and Kolkata regions of West Bengal [35], a state that lies in close proximity to Assam. The widespread ART in SEA necessitates the continuous monitoring of the Indo-Myanmar border to aid malaria control and prevention strategies.

Doxycycline has been recommended by NVBDCP as a short-term prophylaxis (less than 6 weeks) for selective groups from non-endemic areas such as military, para-military forces and travelers [36]. However it is rarely used, and no resistance has been observed so far.

The summary of key events related to malaria in the NE is given in Figure 4.

### Figure 4. Summary of key events related to Malaria prevention and control in the NE (DDT: Dichlorodiphenyltrichloroethane, NMEP: National Malaria Eradication Program, GFATM: Global Fund to Fight AIDS, Tuberculosis and Malaria, LLIN: Long-lasting insecticide nets, ACT: Artemisinin-based combination therapy, RDT: Rapid diagnostic kit, ACT-AL: ACT Artemether-lumefantrine, NFME: National Framework for Malaria Elimination).
Table 4. Drug-resistance studies in NE India.

| Year | Anti-malarial Drug | Assay Method | State/Area | Outcome | Ref No. |
|------|--------------------|--------------|------------|---------|---------|
| 1973 | CQ                 | Therapeutic efficacy | Assam      | RI level of resistance in 52.5% and RII level in 22.5% of cases* | [25] |
|      | CQ (1500 mg)       | 28 days *in vivo*  | Garohills, Meghalaya | RI-33%, RII-9.5%, RIII-12.5% | [37] |
| 1974 | Quinine + pyrimethamine | Therapeutic efficacy | Assam      | RI-24% | [38] |
| 1975 | CQ (1500 mg)       | 28 days *in vivo*  | Burnihat, Meghalaya | RI-78.3%, RII-4.3% | [37] |
| 1976 | CQ (1500 mg)       | 7 days *in vivo*   | Assam (different districts) | 0–33.4% resistance | [39] |
|      | CQ (900 mg) + Pyrimethamine (50 mg) | 7 days *in vivo* | Nagaland | 47% resistance | [40] |
|      | CQ (600 mg) + Pyrimethamine (50 mg) | 7 days *in vivo* | Bama Bazar, Garohills, Meghalaya | S/RI-82.2%, RII-18% | [37] |
|      | CQ (600 mg) + Pyrimethamine (50 mg) | 28 days in vivo | Miao, AP | 29.9% resistance | [39] |
|      | CQ (600 mg) + Pyrimethamine (50 mg) | 28 days in vivo | North Tripura | No resistance observed | [41] |
|      | CQ (900 mg) + Pyrimethamine (50 mg) | 28 days in vivo | Aizawl, Mizoram | 29% resistance | [37] |
|      | CQ (600 mg) + Pyrimethamine (50 mg) | 28 days in vivo | Jiribam, Manipur | No resistance observed | [37] |
| 1978 | CQ (1500 mg)       | 7 days *in vivo*   | Karbi Anglong, Assam | 66.6% resistance | [39] |
| 1979 | CQ (1500 mg)       | 28 days *in vivo*  | Mendipalchar, Garohills, Meghalaya | RI-50% | [37] |
| 1995 | CQ                 | 3 days *in vivo* test | Assam | S/RI-85%, RII-7%, RIII-3%, RII-5% | [42] |
|      | Alpha-Beta Arteether | 28 days therapeutic efficacy | Dibrugarh, Assam | No resistance observed | [43] |
| 1999 | CQ                 | 28 days *in vivo*  | Jirampur, AP (Indo-Myanmar Border) | ETF-60.4%, LTF-22.6% | [44] |
|      | SP                 | do             | do | ETF-32.6%, LTF-11.6% | |
|      | quinine            | 7 days *in vivo* | do | 15.8% treatment failure observed | |
| 2001 | CQ                 | 42 days prospective randomized non-blinded trial | Sonitpur, Assam | RI-53%, RII-2.2%, RIII-8.9% | [45] |
|      | SP                 | 42 days prospective randomized non-blinded trial | | RI-29%, RII-3.8%, RIII-5.8% | |
|      | Mefloquine         | 42 days prospective randomized non-blinded trial | | RI-4.4% | |
|      | Mefloquine+artesunate | 42 days prospective randomized non-blinded trial | | RII-8.7%, RIII-2% | |
|      | CQ                 | 42 days prospective randomized non-blinded trial | | RI-37.5%, RII-29%, RIII-29% | |
|      | SP                 | 42 days prospective randomized non-blinded trial | | RI-36.7%, RII-14.3%, RIII-6.1% | |
| 2002–2003 | CQ | 28 days *in vivo* | AP | ETF-23.8%, LCF-14.3%, LPF-10.7% | [46] |
| 2005 | CQ                 | 35 days *in vivo* | Assam | RI-13%, RII-4%, RIII-Nil | [47] |
**SP** | **Molecular (Nested PCR)** | **Sonitpur, Assam** | **pfcrt K76T-72.13%, pfmdr1 N86Y-41.79%, K76T+N86Y-32.7%** | **No resistance**
--- | --- | --- | --- | ---
2007–2010 | CQ | Molecular (Nested PCR) | Sonitpur, Assam | [48]
2010 | CQ | In vivo therapeutic efficacy | Tripura (Indo-Bangladesh Border) | ~30% ETF, 5% LTF | [49]
2011 | CQ | 28 days *in vivo* | Tripura | ETF-32.5%, LCF-35% | [50]

(CQ: Chloroquine, SP: Sulfadoxine/Pyrimetamine, *Classification of anti-malarial treatment types can be found in Box A2*)
5. Vectors of NE India

The tropical climate provides a fertile breeding ground for varied mosquito fauna [51]. In India, 61 species of anophelines have been recorded within the subgenera Anopheles and Celia, and there is a possibility of this number increasing with identification of new species under several groups or complexes [52]. Among the 61 Anopheles spp., 9 have been incriminated as malaria vectors in India [53,54]. NE India is part of the “Indo-Burma hot-spot” [55], and is home to diverse fauna and flora. In the early 1990s, Malhotra and Mahanta reported 37 anophelines from NE India [56]. A later survey in early 2000s documented 45 anophelines from this region [P. Dutta, Regional Medical Research Centre, Dibrugarh personal communication]. Anopheles dirus and An. minimus complex mosquitoes are regarded as the main malaria vectors in NE India [8], and these mosquitoes inhabit distinct ecological niches.

The Minimus complex includes three formally named species: An. minimus (earlier species A), An. harrisoni (earlier species C) and An. yaeyamaensis (earlier species E). Present day taxonomy of the An. minimus complex has been resolved by painstaking work of several groups [57–60]. An. minimus was considered to be the most important malaria vector in the entire sub-Himalayan belt. Due to the large-scale application of dichlorodiphenyltrichloroethane (DDT) under the National Malaria Eradication Program (NMEP), this species was thought to have disappeared from this region. However, it re-emerged in the 1980s and was incriminated as a malaria vector in Assam [61,62], Arunachal Pradesh [63], Nagaland [64] and Mizoram [65]. In NE India, only An. minimus s.s. (species A) has been reported [8]. An. minimus is found throughout the year with higher densities between March and August, and is endophilic, endophillic and highly anthropophilic. It bites humans throughout the night, and the peak biting period is between 01:00 to 04:00AM. It breeds in slow-flowing streams with grassy banks [66], and is susceptible to DDT [67]. An. minimus is a highly efficient malaria vector and is responsible for focal disease outbreaks characterized by high Pf infections and death in forest fringe and foot hills areas of NE. Even though it is susceptible to DDT, behavioural resistance and avoidance of both DDT and long-lasting insecticide nets (LLIN) have been observed [68].

The Minimus and Fluviatilis complexes are phylogenetically [69] and morphologically closely related and are often misidentified [70]. Originally identified as An. fluvialitis in NE India, it is now recognized as a hyper-melanin form of An. minimus (species A) based on molecular data [70]. Both An. minimus and its hyper-melanic form were found in sympatry during winter season, and were also incriminated in malaria transmission [68].

An. baimaii is the widely prevalent species of An. dirus complex mosquitoes in the entire NE, along with a focal presence of An. dirus X (another unnamed species in the complex) in North Cachar [9]. An. baimaii is a forest-dwelling species found in deep forested areas and in forest borders. High anthropophilicity along with its capacity to bite equally both indoor and outdoor, and resting behavior (outside houses) makes it one of the most efficient primary malaria vectors in SEA including NE [71]. Even though the peak biting time of An. baimaii has been estimated to be from 22:00 to 02:00 h, in the NE it has been observed that human feeding starts from 18:00 h, and an estimated 13% of biting occurs in the 1st quarter of the night alone [71,72]. An. baimaii inhabits most of the hilly and forested areas of NE bordering Myanmar and Bangladesh, and most of the drug resistant cases have been recorded from these areas. Literature search shows that CQ resistant Pf has been commonly associated with An. dirus s.l. [65,73–75]. In NE, An. baimaii is susceptible to many insecticides. Due to its exophilic nature, conventional use of indoor residual insecticides is ineffective, which is also the major reason for sustained transmission of malaria in An. baimaii-dominated areas. Lack of an effective control strategy against An. baimaii is the major reason for its large population size (Ne), high genetic diversity and lack of population bottleneck in its evolutionary history in the NE [76]. The distribution of primary malaria vectors (An. baimaii and An. minimus) of NE is given in Figure 5.

Bionomics of primary malaria vectors are given in Table 5.
Figure 5. Distribution of primary and secondary malaria vectors in NE India.

6. Role of Secondary Vectors

Apart from these 2 primary vectors, *An. philippinensis*, *An. nivipes*, *An. annularis*, *An. culicifacies* and *An. vagus* have been found to play an important and interesting role in malaria transmission, especially in the winter seasons [77]. Sporadic reports of parasite positivity in these species have been reported in the NE ever since 1941, when Anderson and Viswanathan [78] first incriminated *An. culicifacies* mosquitoes as a malaria vector in Assam. Similarly, *An. philippinensis* has been incriminated as a malaria vector from Burnihut area of Meghalaya during 1968–1969 [79]. Since then, many other anophelines have been incriminated as malaria vectors in this region (Table 6), however, their actual role in transmission of malaria is still not clear. The distribution of secondary malaria vectors are shown in Figure 5. Another important aspect of these secondary vectors is that most of them display a wide spectrum of metabolic, physiological and behavioral resistance against many insecticides [80–82]. Due to deforestation, shift in agricultural activities, urbanization and climate change, many natural habitats of established malaria vectors are shrinking, and their place has gradually been taken over by other invading species such as *An. culicifacies* in NE. Therefore, it is important to monitor the vectorial competency of these secondary vectors under changing climate to inform and aid the malaria control program.
Table 5. Bionomics of major malaria vectors in NE India.

| Species Complexity | Sibling species Present | Range | Larval habitat | Resting habitat | Feeding habit | Peak biting activity (hours) | Susceptibility status to insecticides | Disease Transmission Relationships |
|--------------------|-------------------------|-------|----------------|-----------------|--------------|---------------------------|--------------------------------------|-----------------------------------|
| An. dirus complex  | An. baimaii (Dirus D) [8,9] | All NE Indian states except for Sikkim [9] | Water pits, elephant footprints, rock bed ravine, mud pools etc. | Outdoor | Highly anthropophilic, both indoor and outdoor biter [72] | 21:00–03:00 | Susceptible [83] | 5.78# (1986–1988, Arunachal Pradesh) [84]; 0.39* (1989–1990, Assam) [66]; 5.03* (2000–2001, Assam) [85]; 1.54# (2007–2008, Tripura) [49]; 4.0–6.0* (2012, Tripura) [83]; 4.0* (2014, Tripura) [86]; 0.97% (1986–1988, Arunachal Pradesh) [84]; 1.6% (Aug 1995–July 1996, Assam) [87]; 3.2% (June 1999 to May 2000, Assam) [88]; 0.7% sporozoite rate (2000, Assam) [85]; 1.9% (1995–2000, Assam) [87]; 11% (2014, Tripura) [86] |
| An. minimus complex | An. minimus (Sibling Sp. A) [8] | All NE Indian states except for Sikkim | Perennial foothill seepage water streams | Human-dwellings indoors | Highly anthropophilic | 01:00–04:00 | Susceptible [83] | 1.86# (1986–1988, Arunachal Pradesh) [84]; 0.4* (2014, Tripura) [86]; 0.40% (1986–1988, Arunachal Pradesh) [84]; 0.05% (2012, Tripura) [83]. |

* Per person per night, #—Trap density per night
Table 6. Secondary malaria vectors in NE India.

| Sl. No. | Year of Incrimination | Month/Season   | Place                | Species                  | Total Positivity | Method of Incrimination | Reference |
|---------|------------------------|----------------|----------------------|--------------------------|------------------|-------------------------|-----------|
| 1       | 1941                   | Not Known      | Assam                | An. culicifacies         | 0.56%            | Dissection              | [78]      |
| 2       | 1968                   | August         | Burnihat, Meghalaya  | An. philippinensis       | 1 no             | Dissection              | [79]      |
| 3       | 1969                   | July           | Burnihat, Meghalaya  | An. aconitus             | 3.95%            |                         |           |
|         |                        |                |                      | An. annularis            | 5.80%            |                         |           |
|         |                        |                |                      | An. hyrcanus             | 0.48%            |                         |           |
|         |                        |                |                      | An. jeyporiensis         | 6.25%            |                         |           |
|         |                        |                |                      | An. kochi                | 1.28%            |                         |           |
|         |                        |                |                      | An. philippinensis/nivipes | 0.94%        |                         |           |
|         |                        |                |                      | An. tesellatus           | 6.67%            |                         |           |
|         |                        |                |                      | An. varuna               | 3.23%            |                         |           |
|         |                        |                |                      | An. vagus                | 3.87%            |                         |           |
| 4       | 2001–2002              | Throughout the year | Jorhat, Assam       | An. philippinensis/nivipes | 1.20%            | CSP ELISA               | [77]      |
| 5       | 2000–2002              | Throughout the year | Jairampur, AP       | An. philippinensis/nivipes | 2.60%            | CSP ELISA               | [89]      |
|         |                        |                | Soraipung, DBR, Assam |                         |                  |                         |           |
|         |                        |                | Titabor, Assam       |                         |                  |                         |           |
| 6       | 2008–2009              | April-October  | Jorhat, Assam        | An. nivipes              | 0.70%            | PCR                     | [10]      |
|         |                        |                | Dimapur, Nagaland    | An. nivipes              | 0.95%            |                         |           |
| 7       | 2013                   | Pre & Post monsoon | Meghalaya           | An. culicifacies         | 6.40%            | CSP ELISA               | [90]      |
|         |                        |                | Manipur              | An. culicifacies         | 1.80%            |                         |           |
|         |                        |                | Manipur              | An. culicifacies         | 6.60%            |                         |           |
|         |                        |                | Manipur              | An. culicifacies         | 3.50%            |                         |           |
|         |                        |                | Manipur              | An. culicifacies         | 9.50%            |                         |           |
| 8       | 2013                   | March-August   | Orang, Assam         | An. vagus                | 0.56*            | CSP-ICT                 | [91]      |
|         |                        |                | Orang, Assam         | An. annularis            | 0.22*            |                         |           |
|         |                        |                | Balipara, Assam      | An. vagus                | 0.13*            |                         |           |

(* Minimum infection rate, CSP: Circumsporozoite protein, ELISA: Enzyme-linked immunosorbant assay, PCR: Polymerase chain reaction, ICT: Immunochromatographic test)
7. Conclusions and Future Directions

Malaria in the NE is highly complex and multi-faceted due to its unique climatic and ecological conditions and, topographic and socio-demographic heterogeneity. In the past, NE has witnessed decline and surge in malaria cases. The significant decline in malaria cases is usually associated with the introduction of new drug or intervention strategies. Also, the emergence of drug resistance, especially to \( P_f \), has led to a resurgence of malaria in this region. First resistance to CQ and SP in India have been reported from NE, and the origin of these resistant parasites can be traced to SEA. With the threat of ART resistance looming in India, it is critical to step up active surveillance in NE states, especially those that border Myanmar. Strict adherence and compliance to the existing drug policy is critical in preventing and controlling drug resistance in the region.

Epidemiological trend indicates that there has been a gradual decrease in cases over the past 11 years in NE. This decline in malaria cases can be attributed to the combined effect of newer and effective drugs (Artemisinin), effective diagnostic system (use of bivalent RDTs) and implementation of effective mosquito control strategies such as LLINs. Even though the cases have decreased, Assam, Arunachal Pradesh, Tripura and Mizoram continue to be malaria-endemic states. As part of the control strategy, high transmission zones or malaria hot-spots in these regions need to be mapped, and have to be continuously monitored. Malaria hotspots from 2013 to 2016 based on annual parasite incidence (API) at district and PHC level in Assam mapped by Geographic Information system (GIS) are shown in Figure 6. Malaria intervention strategies have to be focused on these mapped high-transmission settings. Similar hot spot mapping have to be carried out in other NE states for effective malaria control and elimination in this region.

![Figure 6](image_url)

**Figure 6.** District and primary health center (PHC)-level malaria hotspot in Assam from 2013 to 2016. Malaria hotspots were calculated based on the annual parasite incidence (API) of the respective districts and PHCs.

The true burden of asymptomatic malaria carriers have to be carefully ascertained in these malaria endemic states. Asymptomatic carriers are individuals who carry the malaria parasites without manifesting any clinical symptoms. However, they remain infectious to mosquitoes and continue to spread the disease [92], and they are potential threat to malaria elimination efforts [93]. It
is estimated that 20%–70% of the infections cannot be detected by conventional microscopy and RDTs [94]. Active surveillance using molecular tools and treatment of asymptomatic carriers should be given high priority as part of malaria elimination efforts in this region.

Even though *Pf* is the major cause of malaria in the malaria-endemic states of Tripura, Meghalaya, Assam and Mizoram, a significant number of cases of *Pv* are reported every year. Furthermore, in the states of Arunachal and Nagaland, *Pv* is the leading cause of malaria. In these states, *Pv* cases were recorded throughout the year with a peak during the rainy season and children less than 5 years old were more affected [22]. It is important to understand the relapse pattern and transmission dynamics of *Pv* in different eco-epidemiological settings for effective control and elimination of *Pv* in the NE. Also, the status of CQR in *Pv* has to be continuously monitored in all NE states.

Most of the vector biology studies in the NE have focused around *An. baimaii* and *An. minimus*, the major malaria vectors. Climate change, deforestation, urbanization and migration have had a great impact on the current habitat of these established malaria vectors. The natural habitat of the primary vectors are shrinking, and in parallel, this has created habitats for new and emerging malaria vectors. Several studies have reported the emergence of secondary malaria vectors in the NE. The vectorial capacity of other *Anophelines* in different ecological and epidemiological settings of the NE have to be studied to ascertain their role in malaria epidemiology in this region. *An. subpictus* incriminated as a major malaria vector in Western India [95] has been identified in mosquito epidemiology studies in Mizoram [96]. Substantial focus has to be given to understanding the emergence of new vectors and vector parasite relationships for formulating effective vector-control strategies in this region.

Overall, malaria control and elimination in the NE will be an important step in India’s efforts to eradicate malaria in the next decade.

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Appendix A

Box A1. Definition of epidemiological parameters used in malaria.

| Parameter                                      | Definition                                                                 |
|------------------------------------------------|---------------------------------------------------------------------------|
| Annual parasitic incidence (API)               | Number of new infections per year per 1000 population (Total no. of positive slides for parasite in a year × 1000/Total population × (ABER’TSPR)/10 |
| Incidence                                      | Number of newly diagnosed malaria cases during a defined period in a specified population |
| Malaria mortality rate                         | Number of deaths from malaria per unit of population during a defined period |
| Prevalence                                     | Proportion of a specified population with malaria infection at one time    |
| Slide positivity rate (SPR)                    | Proportion of blood smears found to be positive for *Plasmodium* among all blood smears examined (Total positive × 100/Total slides examined) |
| Slide *falciparum* rate (SFR)                   | Proportion of blood smears found to be positive for *P.* among all blood smears examined (Total positive Pf × 100/Slides examined) |

Box A2. Classification of responses to anti-malarial treatment [97].

RI: Recrudescence of the infection between 7 and 28 days after completion of treatment following initial resolution of symptoms and parasite clearance.

RII: Reduction of parasitaemia by >75% at 48 h but failure to clear parasites within 7 days.

RIII: Parasitaemia does not fall by >75% within 48 h

Early treatment failure (ETF):
- Danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
- Parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
- Parasitaemia on day 3 with axillary temperature ≥37.5 °C; and
- Parasitaemia on day 3 ≥25% of count on day 0.

Late clinical failure (LCF):
- Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure; and
- Presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure (LPF):
- Presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature <37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response (ACPR):
- Absence of parasitaemia on day 28 (day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

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